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INTESTINAL INFECTIONS BY *EIMERIA* (S. L.) *VANASI* N. SP. (EIMERIIDAE, APICOMPLEXA, PROTOZOA) IN CICHLID FISH

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SUMMARY. *Eimeria* (s. l.) *vanasi* n. sp. is described from the intestine of a variety of cichlids in Israel and South Africa. Merogony and gamogony stages are both intra- and epiepithelial. Sporogony is exogenous with young zygotes being released from the fish in the faeces. Fully developed sporocysts are ellipsoid and apparently lacking both a Stieda body (characteristic of *Eimeria* s. st. and *Epieimeria* species) and a suture line (characteristic of *Goussia* species). In view of the present controversial generic status of piscine coccidia, the species is tentatively designated as *Eimeria* (s. l.) *vanasi* n. sp. (Eimeriidae, Apicomplexa, Protozoa).

Key-words: *Eimeria*. Coccidia. New species. Intestine. Fish. Cichlid. Exogenous sporogony.

Infections intestinales par *Eimeria* (s. *l.*) *vanasi* n. sp. (Eimeriidae, Apicomplexa, Protozoa) chez les Poissons Cichlidés.

RÉSUMÉ. *Eimeria* (s. l.) *vanasi* n. sp. est décrite à partir des stages trouvés dans l'intestin d'un certain nombre de Cichlidés d'Israël et d'Afrique du Sud. La mérogonie et la gamogonie sont à la fois intra- et épiépithéliales. Puisque la sporogonie est exogène, les jeunes zygotes sont excrétés avec les fèces du poisson. Les sporocystes mûrs sont ellipsoïdes et apparemment dépourvus de corps de Stieda (caractéristiques des *Eimeria* s. st. et des *Epieimeria*) et de sutures (caractéristiques de *Goussia*). En raison des controverses sur le statut générique des Coccidies de Poisson, l'espèce est provisoirement désignée comme *Eimeria* (s. l.) *vanasi* n. sp. (Eimeriidae, Apicomplexa, Protozoa).

Mots-clés: *Eimeria*. Coccidie. Espèce nouvelle. Intestin. Poisson. Cichlidés. Sporogonie exogène.

Introduction

Recent studies on the coccidian parasites of cichlid fish have reported on a new species of *Goussia* found in the swim bladder (Landsberg and Paperna, 1985). Presented here is a description of another new species which parasitises the intestine.

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Materials and methods

Study material examined included cichlid fish (of the genera *Oreochromis*, *Sarotherodon*, *Tilapia* and *Pseudocrenilabris*) from both Israel and South Africa *(Table III)*. Visceral organs from autopsied fish were examined in fresh squash preparations by Nomarski interference optics; from stained smears and from histological preparations. Intestinal smears were air dried, fixed in methanol and stained in Giemsa (1 : 4, pH 6.8) for 20 minutes. Histological preparations made from intestines and whole fish fry were fixed in cold 10 % (v/v) buffered neutral formalin and embedded in glycol methacrylate (Lulham, 1979). 3-6 μm sections were cut by a Sorval JB-4 glass knife microtome and were stained either by Mayer's haemalum (counterstained with eosin alone or initially with phloxine) or by Giemsa and PAS methods adapted to methacrylate sections (Du Pont, 1981). Faecal casts containing young zygotes were placed in tap water at approximately 21° C and examined over a three day period to study sporogony.

Results

1 — Description of developmental stages

Merogony, gamogony and early sporogony stages were only found in or attached to the intestinal epithelium. They were not observed in any other visceral organs. *Table I* gives measurements and details of these different stages.

Merogony

Several types of merozoite formations within parasitophorous vacuoles were observed *(Table I; Pl. I a-d, m; Pl. II a)*. Formations contained between 2-32 merozoites. The size of individual merozoites was not related to the total number per formation. Some free merozoites, as well as both merozoites of some of the two cell formations contained two nuclei. In histological sections the merozoite formations were found either within *(Pl. I m)* or attached to the surface of the mucosal epithelial cells *(Pl. I n)*. No early predifferentiated meronts were seen.

Plate I. — Preparations of intestinal stages: *a*-L. (scale: 5.0 μm), stained with Giemsa; *m* (scale: 20.0 μm), *n* (scale: 7.0 μm), histological sections stained with Mayer's haemalum and eosin.

a. Formation of two merozoites.
b-d. Formations of 4, 10 and 12 merozoites.
e-h. Formation of early stage gamonts.
i. Formation of 2 early gamonts (arrow).
j-k. Differentiating macrogamonts (arrows).
l. Macrogamont (arrow).
m. Merozoite rosettes (M, large arrows) and macrogamonts (Ga, small arrows) within the mucosa epithelium.
n. Epiepithelial merogony.

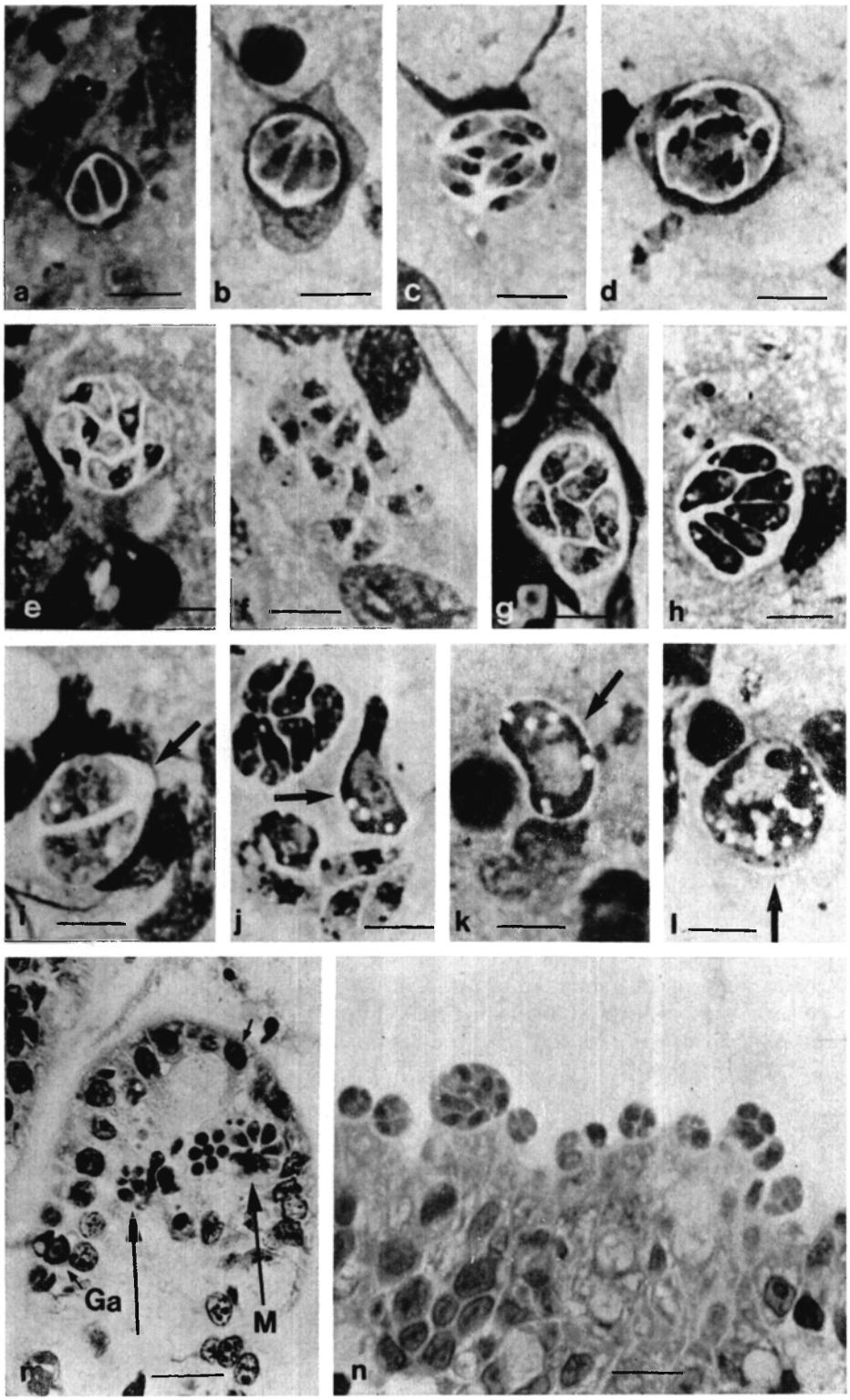

PLATE I

TABLE I. — Measurements of intestinal stages of *E. vanasi* from *Oreochromis aureus* × *O. niloticus* in Israel. (Taken from histological sections except for #: Giemsa stained smears; *ie*: intraepithelial; *ee*: epiepithelial; *ec*: extracellular; *pv*: parasitophorous vacuole.)

Stage	Type	Diameter of *pv* (μm) ($n = 10$)	Location	Number	Individual size (μm) ($n = 10$)
Merogony	Scattered	4-13×4-18	*ie*	4-32	1- 5× 5- 8
	Rosette (I)	8-14×8-14	*ie*	4-16	3- 4× 4- 6
	Rosette (II)	8-10×8-10	*ie*	4-16	1- 2× 4- 5
	Regular (I)	3- 6×3- 8	*ee*	2, 4, 8, 16	1- 4× 4- 6
	Regular (II)	6- 8×6- 8	*ee*	4,8	1- 4× 4- 6
Individual	Merozoites	—	*ie/ee/ec*	1	1- 5× 4- 9
Gamogony		5-11×6-11	*ie*	4-16	3- 4× 3- 4
Immature		—	*ie*	1- 2	3- 4× 4- 6
					# 4- 6×10-11
		—	*ee*	1, 2, 4	3- 7× 4-10
Mature					
Microgamonts:					
Early		—	*ie/ee*	—	5- 8× 6-10
					# 9×12
Intermediate		—	*ie/ee*	—	6×10
Mature		—	*ie/ee*	—	5-10× 5-10
Macrogamonts	Mature	—	*ie/ee*	—	8-14× 8-14
Zygote	Early	—	*ie/ee/ec*	—	10-14×10-14

Early merozoites were spindle shaped and contained a nucleus of dense chromatin *(Pl. I a-d)*. When located within the epithelial layer, the host cell nucleus was hypertrophic and vesiculate *(Pl. I b, d)*. In histological sections rosette formations of 4-16 merozoites were seen attached to a residual body *(Pl. II a)*.

Gamogony

Measurements of gamonts are given in *Table I*.

In smears, the products of final merogony which developed into gamont stages were club-shaped, with a large, eccentric heterochromatic nucleus. The cytoplasm

PLATE II. — Histological sections of intestinal stages (scale: 10.0 μm) stained with Mayer's haemalum and eosin.

a. Merozoite rosettes connected to residual bodies.
b. Final intraepithelial merogony yeilding gamont stages (open arrows).
c. Mature microgamont (open arrow).
d-f. Differentiating (closed arrows) and mature (open arrows) epiepithelial macrogamonts (Ga), intraepithelial zygote (Z).
g. Intraepithelial macrogamont in parasitophorous vacuole.
h. Early stage zygote in the intestinal lumen. Note many pyknotic sloughed cells.

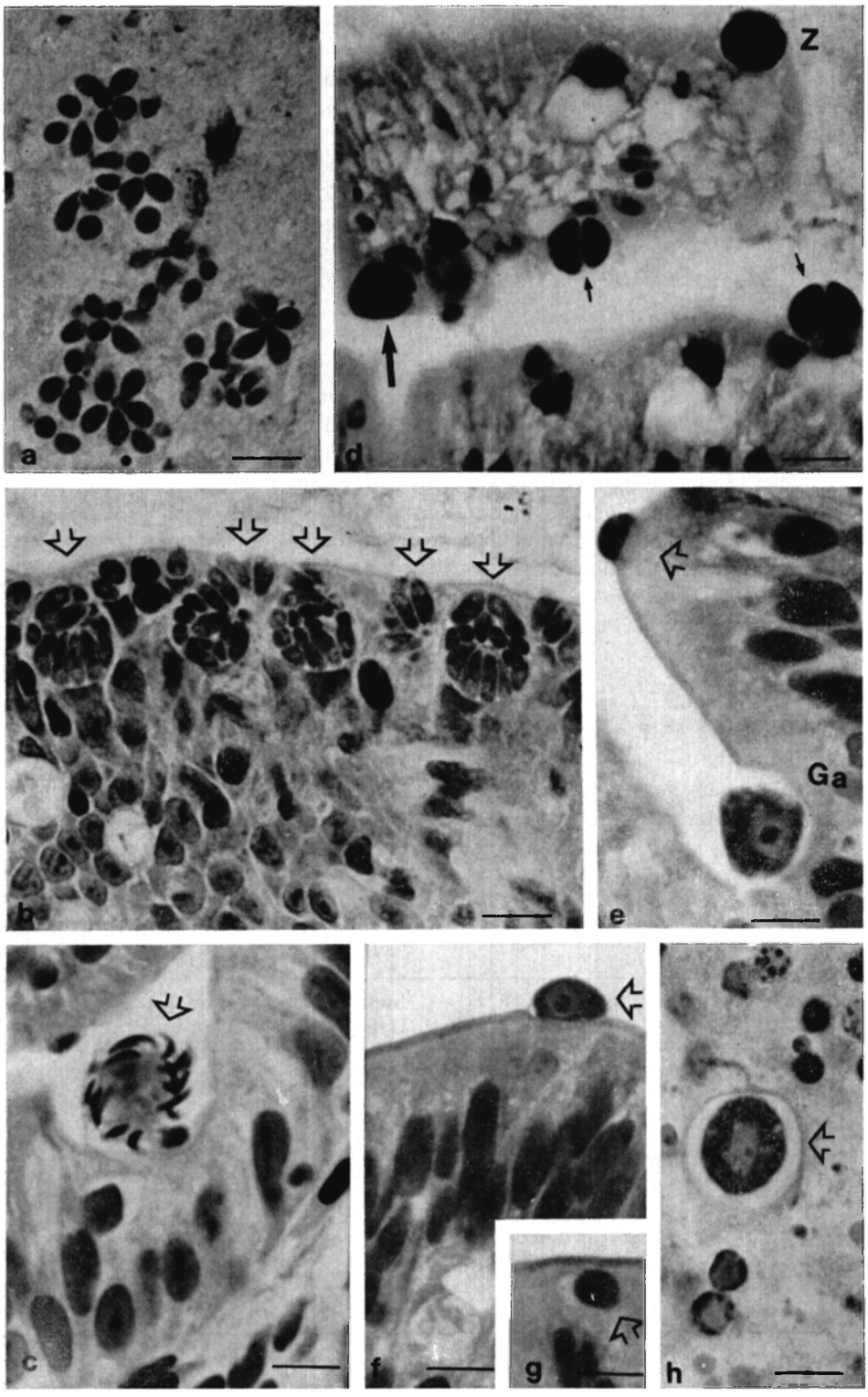

PLATE II

contained a few transparent vacuoles *(Pl. I e-j)*. Merogony yielding 2-16 gamonts occurred either within the superficial cells of the mucosal epithelium (with progeny of 4-16, *Pl. II b*) or on their surface (with progeny of 2-8 gamonts, *Pl. II d*).

Immature gamonts were recognized by having a dense cytoplasm and a vesiculated nucleus. In macrogamonts, the nucleus contained a large, distinct round nucleolus. The number of transparent vacuoles in the cytoplasm increased with the maturation of the gamonts *(Pl. I j-l)*.

Macrogamonts occurred singly within the epithelial cells as well as attached to their surface *(Pl. I m; Pl. II e-g)*.

Fertilized macrogamonts, still located at the mucosal surface or in the intestinal lumen had an extremely vacuolated cytoplasm *(Pl. II e, h)*. Wall formation had occurred before zygotes were excreted from the fish *(Pl. II e, h)*.

Early differentiating microgamonts were distinguishable by the presence of peripheral pockets of scattered chromatin which further developed into 8 peripheral nuclei. Some contained a small central vacuole. Later stages demonstrated approximately 16 peripheral nuclei. A gradual budding of the peripheral nuclei into the parasitophorous vacuole indicated the start of microgametogenesis. Fully differentiated microgamonts had 16-32 flagellated microgametes and a residual body *(Pl. II c)*.

Sporogony

Measurements of sporogony stages are shown in *Table II*.
Young zygotes with the cytoplasm tightly packed against the cell wall were excreted in the faeces. Sporulation occurred externally and was usually completed within 72 hours (water temperature approximately 21° C). Within 12 hours, the oocyst diameter had increased and the sporont cytoplasm became contracted

TABLE II. — Measurements of sporulating stages excreted from *O. aureus* × *O. niloticus*, incubated *in vitro*. (Time in hours, measurements in μm; *n*: sample size; *m*: mean; *sd*: standard deviation; *r*: size range).

Stage	Time	Oocyst (diameter)	Sporoblast (diameter)	Sporocyst	Sporozoite
Immature ($n = 10$)	24	12-19×12-19	6-8×6-8	—	—
	24-48	12-19×12-19	6-8×10-12	—	—
	48-72	12-19×12-19	4-6×12-14	—	—
Mature (Type I)	72 +	*m* : 16.2×13.3 *sd* : ±0.99×0.57 *r* : 14.0-18.0 ×12.0-15.0 ($n = 60$)	—	12.7×5.2 ±1.32×0.46 11.0-15.0 ×4.0-6-0 ($n = 30$)	11.8×3.2 ±1.24×0.55 8.0-14.0 ×2.0-4.0 ($n = 30$)
(Type II)	72 +	10.0×10.0 ($n = 5$)		7.5×4.5 ($n = 14$)	—

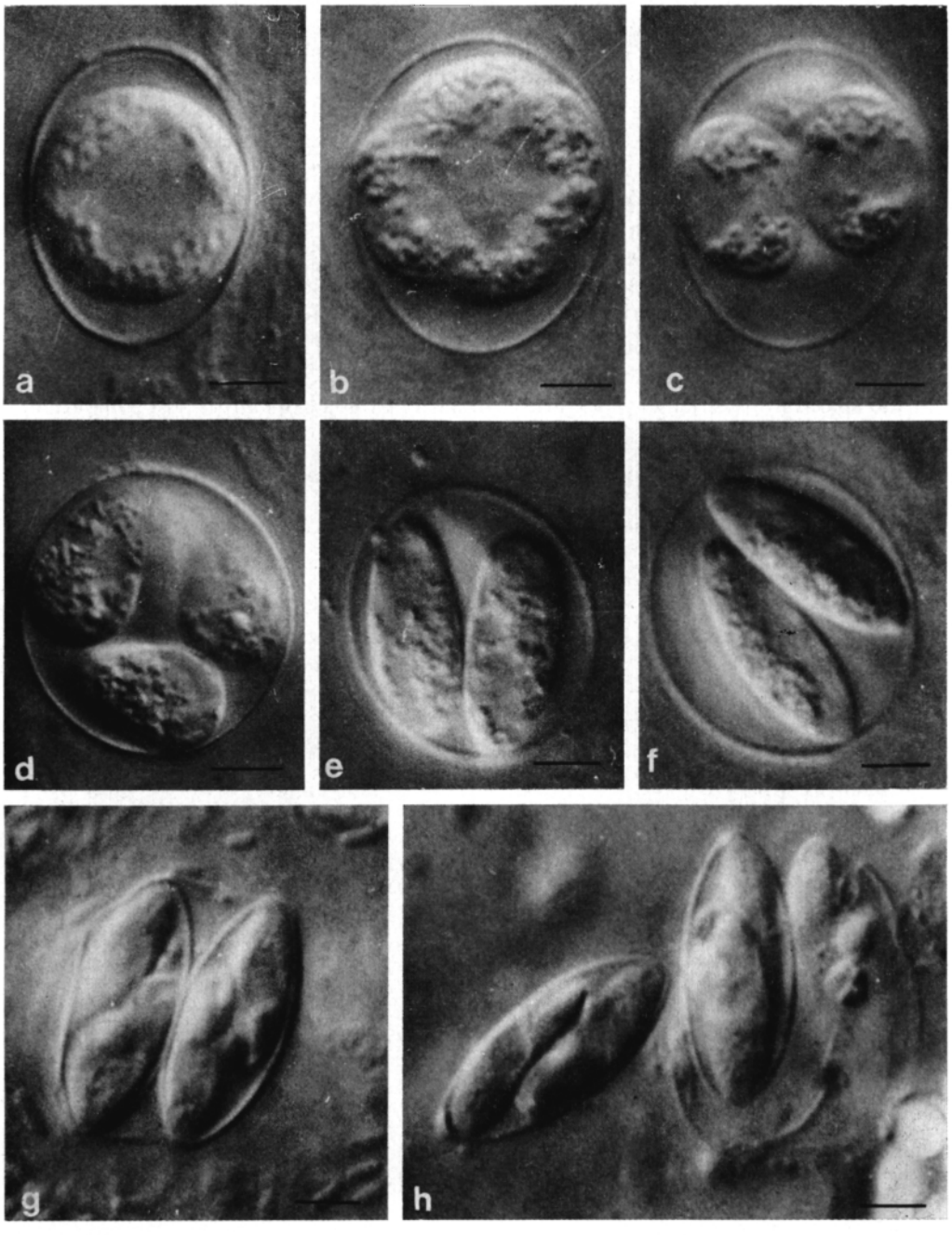

PLATE III. — Stages of sporogony
(fresh oocysts, Nomarski interference optics) (scale: 4.0 μm).

a. Undivided oocyst 12 h post excretion.
b-d. Division leading to formation of sporoblasts between 24-48 h post excretion.
e, f. Formation of sporocysts between 48-72 h post excretion.
g, h. Formation of sporozoites within the sporocysts 72 h post excretion.

away from the cell wall *(Pl. III a)*. By 24 hours sporoblast formation had begun forming 4 circular sporoblasts occupying 2/3 the volume of the oocyst *(Pl. III b, c)*. Between 24-48 hours, the sporoblasts gradually extended from one end becoming pear-shaped *(Pl. III d)*. During 48-72 hours, the sporoblasts eventually assumed an ellipsoid shape before sporozoite formation began *(Pl. III e, f)*.

When fully mature, sporozoites were laterally displaced within the sporocyst *(Pl. III g, h)* with the anterior ends of the sporozoites tightly folded over against the rest of the sporozoite. Mature sporocysts did not apparently have a suture line, bivalved spores or any obvious Stieda or sub-Stieda bodies. Mature fully sporulated oocysts were subcircular and the sporocysts were ellipsoid, usually lying 2 + 2 within the oocyst. Another population of smaller oocysts (Type II, *Table II*) rarely occurred with the common larger type (Type I, *Table II*). Sporocysts and sporozoites were morphologically similar to those of the larger size population.

2 — Host range, distribution and site of infection

Species of cichlids found infected and their localities are listed in *Table III*.

Table III. — Hosts and localities of *E. vanasi* in Israel and South Africa

Host	Length (mm)	Locality	Habitat
Israel:			
Oreochromis aureus (Steindachner) × *Oreochromis niloticus* (L.)	10-20 (fry) 21-50 (fingerlings) 51-110 (young-of-the-year)	Fish ponds and hatcheries in the Jordan Valley	Brackish water (1.6 p. p. t.)
Sarotherodon galilaeus (L.)	30-110	Ginnosar fish ponds western shore of lake Kinneret	Fresh water
South Africa:			
Oreochromis mossambicus (Peters)	28-40	Amalynda farm, near East London, South East Cape	Fresh water
	50-130	Kowie River estuary, South East Cape	Sea water (35 p. p. t.)
	50-130	Cultivated in Rhodes University, Grahamstown, origin Kowie River	Fresh water
	90-150	Orlando Dam, Soweto, Transvaal	Heated effluent
Tilapia sparrmanii (Smith)	30-45	Rand University Dam, Johanessburg	Fresh water
Pseudocrenilabris philander (Weber)	20-30	Rand University Dam, Johanessburg	Fresh water

In fry, infections were extremely heavy and often coincided with widespread emaciation, growth retardation and occasional heavy mortalities. The various stages and types of meronts and gamonts in these fish occurred throughout the digestive tract in a distinct segregated pattern. Intraepithelial meronts occurred in the anterior intestine *(Pl. I m)*; intraepithelial final stage merogony yielding gamont stages occurred in the anterior and mid intestine *(Pl. II b)*, while epiepithelial meronts and gamonts occurred in the posterior intestine *(Pl. I n)*. Mature gamonts were rarely seen. In fish other than fry, all meront and gamont stages were confined to the anterior intestine. In fingerlings and larger juveniles, the incidence of infection was usually moderate to low with one exception; juveniles of *Oreochromis mossambicus* (up to 60 mm in length), had heavy infections in fish maintained at low temperatures ($< 13^{o}$ C) during winter.

Discussion

The formation of paired merozoites as well as binucleate merozoites suggests that binary fission or endodygeny occurred prior to, or concurrently with, other merogonous divisions. Finding pairs of immature gamonts suggests that binary division/endodygeny also occurred during the final merogony which yields gamonts. Endodygeny was confirmed in EM studies (Paperna and Landsberg, unpublished). In some species of *Eimeria*, sporozoites may divide several times before transforming into uninucleate, spheroid meronts (Chobotar and Scholtyseck, 1982). Endodygeny is a type of asexual reproduction characteristic of *Toxoplasma* (Hammond, 1973). Endodygeny and possibly also binary fission as suggested by the finding of binucleate zoites, were also reported in several species of *Isospora* infecting both host tissue and cultured cells (Frenkel and Dubey, 1972; Fayer and Thompson, 1974; Daly and Markus, 1981). This type of division has not, to our knowledge, previously been reported in fish coccidia.

E. vanasi is host specific to african cichlids and appears to follow the geographical distribution range of its hosts. The merogony and gamogony stages described show both intra- and epiepithelial forms as well as two distinct size groups of mature oocysts. This could be suggestive of mixed infections of two parasite species. Nevertheless, infections in both South African and Israeli cichlids usually consisted of both epiepithelial and intraepithelial forms, while small size oocysts were extremely rare. Furthermore, recent fine structural studies have demonstrated the successive development of epiepithelial stages into intraepithelial forms (Paperna and Landsberg, unpublished).

Dyková and Lom (1981) considered an epicellular mode of development in fish coccidia to be a distinguishing generic characteristic. Accordingly, they classified *Eimeria anguillae* Léger and Hollande 1922 as the type species of their new genus *Epieimeria.* Structural similarities are apparent between epiepithelial stages of *E. vanasi* (Paperna and Landsberg, unpublished) and *Epieimeria anguillae* (Molnár and Baska, 1986). Epiepithelial stages of *Goussia cichlidarum*, however, have

entirely different fine structural features (Paperna, Landsberg and Feinstein, 1986).

Eimeria (s. l.) *pigra* (Léger and Bory, 1932) Dyková and Lom, 1981 is known to have epiepithelial and (rarely) intracellular merogony and gamogony. It also has exogenous sporogony and a sporocyst lacking a Stieda body similar to that noted in *E. vanasi*. These characteristics seen in both species may be of generic significance. In species of *Epieimeria*, however, the sporocysts have a Stieda body and sporogony is usually endogenous (Dyková and Lom, 1981; Lom and Dyková, 1981). True exogenous sporogony has also been reported in *Eimeria dingleyi* (Davies, 1978), *E. raiarum* (Van Den Berghe, 1937), *E. squalli* (Fitzgerald, 1975) and *E. aurati* (Hoffman, 1965). With the exception of the latter (which is unknown), only intracellular merogony and gamogony has so far been reported in these species. These coccidia do not have the characteristic sporocyst structure known for *Eimeria* (s. st.) (Dyková and Lom, 1981). Sporogony in a few *Eimeria* species may be both endogenous and exogenous (Mandal and Chakravarty, 1965; Marinček, 1973; Molnár and Hanek, 1974; Molnár, 1981). As pointed out by Molnár (1981), heavy infections and the resulting increased sloughing of host mucosal cells may consequently release young zygotes which are then excreted in a non-sporulated state. This phenomenon apparently leads to exogenous sporogony in species which otherwise develop endogenously. In *E. vanasi*, the excretion of non-sporulated oocysts occurred in fish at all intensities of infection and therefore did not appear to result from cellular changes induced by hyperinfection. It may thus be regarded as a true specific characteristic.

Eimeria (s. l.) *vanasi* n. sp.

Differential diagnosis: intra- and epiepithelial merogony and gamogony. Sporogony exogenous. Sporocysts without suture line, Stieda or sub-Stieda body (viewed by light microscope).

Location in the host: intestine.

Type specimens: syntypes deposited in the National Zoological Collection at the Hebrew University of Jerusalem, Israel.

Type host and locality: hybrids of *Oreochromis aureus* (Steindachner) × *O. niloticus* (L.) (Cichlidae, Pisces) from hatcheries in the Jordan Valley, Israel.

Remarks: This species is named in honour of Professor J. G. Van As, Research Unit for Fish Biology, Rand Afrikaans University, Johanessburg, South Africa.

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