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Eimeria nebulosa n. sp. and *Klossia pachyleparon* n. sp. from the monitor lizard *Varanus nebulosus* in Malaysia

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Summary.

Eimeria nebulosa n. sp. and *Klossia pachyleparon* n. sp. are described from the monitor lizard *Varanus nebulosus* in Malaysia. The flask shaped oocysts of *E. nebulosa* average 20.7 by 12.6 μm. The oocyst wall is composed of a single layer. There is a single polar granule but no residuum. Ellipsoidal sporocysts average 11.1 by 5.6 μm. A sporocyst residuum is present. Endogenous stages develop in the epithelial cells of the small intestine. The spherical oocysts of *K. pachyleparon* average 33.6 μm in diameter. The wall is about 2.5 μm thick and is composed of 3 layers. The spherical sporocysts average 10.8 μm in diameter. Sporocysts contain 4 sporozoites and residuum. Developmental stages were not observed.

Résumé.

***Eimeria nebulosa* n. sp. et *Klossia pachyleparon* n. sp. parasites du varan *Varanus nebulosus* en Malaisie.**

Eimeria nebulosa n. sp. et *Klossia pachyleparon* n. sp. sont décrites chez le varan *Varanus nebulosus* en Malaisie. Les oocystes d'*E. nebulosa*, de forme ovoïde avec un pôle conique, mesurent en moyenne 20,7 μ × 12,6 μ. La paroi de l'oocyste est simple. Celui-ci contient un granule polaire unique et pas de résidu oocystique. Les sporocystes ellipsoïdaux

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mesurent en moyenne 11,1 μ × 5,6 μ. et contiennent un résidu sporocystique. Les formes de multiplication se développent dans les cellules épithéliales de l'intestin grêle. Les oocystes sphériques de *K. pachyleparon* ont un diamètre moyen de 33,6 μ. Leur paroi, épaisse de 2,5 μ, est formée par 3 enveloppes. Les sporocystes sphériques ont un diamètre moyen de 10,8 μ. Ils contiennent 4 sporozoïtes et un résidu sporocystique. Les formes tissulaires n'ont pas été observées.

During a survey of parasites of Malaysian reptiles one of 7 monitor lizards (*Varanus nebulosus*) were found to be infected with an *Eimeria* species and one with *Eimeria* and *Klossia* species. The sporulated oocysts and endogenous stages are described here.

Materials and Methods

Feces containing coccidial oocysts were crushed and placed in a thin layer of 2,5 % potassium dichromate solution in a petri dish. Oocysts were allowed to sporulate 1 week at room temperature (approximately 30 C) and the stored at 4 C until examination.

After sugar flotation, the sporulated oocysts were studied a Leitz Ortholux microscope with apochromatic objectives and measured with an ocular micrometer. The composite drawing is based on freehand sketches and photomicrographs. Measurements are in micrometers. The range is followed by the mean in parentheses.

The lizard positive for *Eimeria* and *Klossia* spp. was killed and impression smears were made from the small and large intestine, liver, lungs, kidney, spleen and brain. Smears were fixed with absolute methanol and stained with Giemsa. Additional tissue from the alimentary tracts was fixed in Carnoy, embedded in paraffin and sectioned. Slides were stained with collophonium-Giemsa.

Results

Eimeria nebulosa n. sp.

Description of oocysts (*fig. 1, 5*). Oocysts ellipsoidal with a constriction at one end so that the overall shape is flask-like. Oocyst wall smooth composed of a single yellowish layer, approximately, 1 μm thick. No micropyle. Thirty sporulated oocysts 19-23 by 11-14 um (20.7 by 12.6). Length-width ratios 1.5-1.8 (1.64). Sporulated oocyst with a single polar granule but no residuum.

Sporocysts ellipsoidal to slightly ovoid. Stieda body absent. Twenty five sporocysts 10-13 by 5-6 µm (11.1 by 5.6). Length-width ratios 1.8-2.3 (1.96). Sporocyst residuum coarsely granular. Sporozoites comma-shaped, lying lengthwise "head to tail" in sporocysts. A refractile globule is present at each end of the sporozoite.

Endogenous stages. All endogenous stages were in epithelial cells of the small intestine. Heaviest infection was seen in the duodenum. Trophozoites 3-5 µm in diameter were seen above the host cell nuclei (*fig. 2*). Schizonts 5-7 µm in diameter were also observed (*fig. 3*). Schizonts had up to 12 nuclei. Micro and macrogametrocytes 7-12 µm in diameter were observed above or beside host cell nuclei (*fig. 4*).

Remarks. The shape of the oocyst of *E. nubulosa* is markedly different from all other *Eimeria* species described from lizards to date (Pellerdy, 1965, 1969 ; Bovee and Telford, 1965 ; and Cannon, 1969). The only other lizard eimerian with flask-shaped oocysts is *E. hemadactyli* from the gekko *Hemadactylus flavaridis* (Knowles and Das Gupta, 1935).

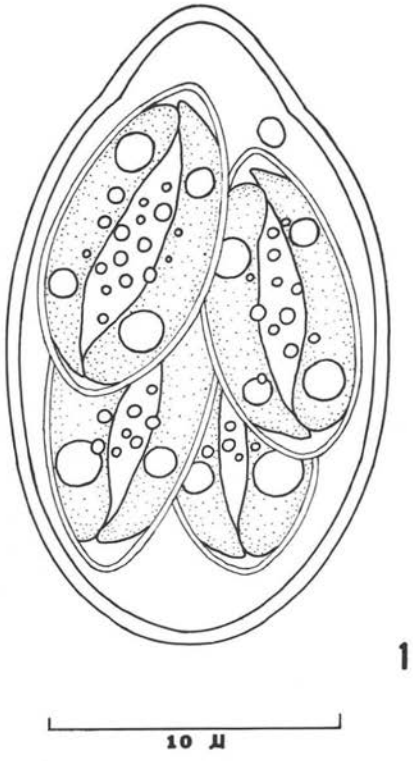


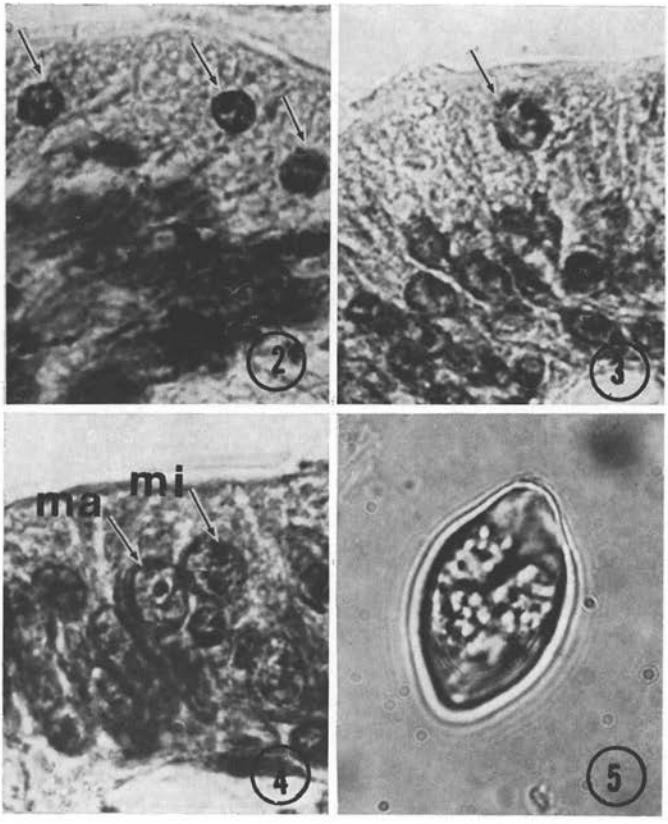
FIG. 1. — Sporulated oocyst of *Eimeria nebulosa* sp. n.

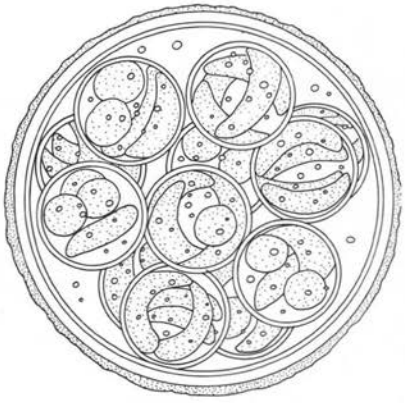
The oocyst of *E. nebulosa* differs from that of *E. hemadactyli* as follows. In having an average length-width ratio of 1.64 rather than 1.23 and in having more elongate sporocysts with an average length-width ratio of 1.96 rather than 1.5.

On the basis of host and morphological differences we feel that *E. nebulosa* should be considered a separate species.

Klossia pachyleparon n. sp.

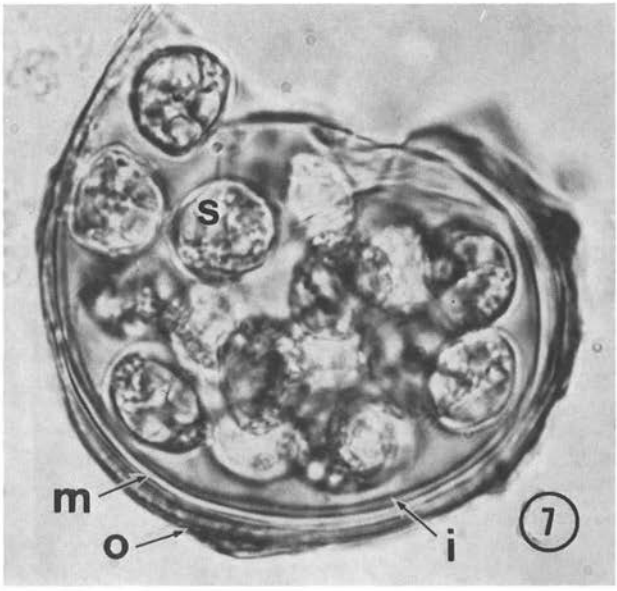
Description of oocysts (*fig. 6, 7*). Oocysts spherical. Oocyst wall composed of 3 layers; a rough, dark brown outer layer 1 µm thick, a yellowish middle layer 1 µm thick and a colorless inner layer 0,5 µm thick (verified by crushing oocysts). No micropyle. Twelve sporulated oocysts 29-40 µm in diameter (33.6). Scattered oocyst residuum present.





↑ FIG. 6. — Sporulated oocyst of *Klossia pachyleparon* sp. n.

← FIG. 2 à 5. — Endogenous stages of *E. nebulosa* in intestinal epithelial cells of *Varanus nebulosus*. Hematoxylin-eosin. All figures × 1,500. *Fig. 2.* Trophozoites (arrows) lying above host cell nuclei ; *fig. 3.* Schizont (arrow) ; *fig. 4.* A macrogamete (*ma*) and microgametocyte (*mi*) lie in adjacent host cells ; *fig. 5.* Sporulated oocyst of *E. nebulosa* showing typical flask shape. Unstained. × 1,500.



← FIG. 7. — Sporulated oocyst of *K. pachyleparon* showing the outer (*o*), middle (*m*) and inner (*i*) layers of the wall. Note the spherical sporocysts (*s*) within the oocyst. Unstained. × 1,500.

The oocyst is packed with sporocysts. Oocysts with 8, 9, 11, 16, 17, and 18 sporocysts were observed. Twenty sporocysts 9-11 μm in diameter (10.8). Scattered sporocyst residuum present. Each sporocyst has 4 sausage-shaped, curved sporozoites.

Remarks. Levine et al. (1955) described *K. perplexans* from a mouse deer and *K. variablis* from a bat in North America. The oocyst of *K. pachyleparon* differs from these in being spherical rather than ellipsoidal and in having 3 rather than 2 layers in the wall. Mullin and Colley (1972) reported *Klossia* sp. from a rat in Borneo. *K. pachyleparon* differs from this species in having a thick 3-layered wall rather than a membranous single-layered one.

To our knowledge *Klossia* has not been previously reported in a reptile. Most members of this genus have been reported as parasites of invertebrates and its presence in vertebrates may be spurious. Levine et al. (1955) discuss this possibility and review previous work on *Klossia* and related genera.

K. pachyleparon is the only *Klossia* species described to date which has a 3-layered oocyst wall. On this basis we feel that *K. pachyleparon* should be considered a separate species.

The name *pachyleparon* is Greek, meaning thick wall.

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