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A survey of coccidian infections of freshwater fishes of Peninsular Malaysia, with descriptions of three species of *Goussia* Labbé, 1896 (Apicomplexa: Eimeriidae)

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Abstract

Ninety-five specimens of 14 freshwater fish species from small streams in the Kuala Terengganu district and the Lake Kenyir Reservoir, Malaysia, were surveyed for coccidian infections. Six fish species proved to be infected with apicomplexans belonging to the genus *Goussia*. In all of these fishes *Goussia* species were found in unsporulated and semisporulated stages. Oöcysts of four species inhabiting the intestinal epithelium became sporulated in tap-water within 24 hours. In two fish species sporulation failed and only unsporulated oöcysts were recorded in the intestine. Three of the intestinal species finishing sporulation proved to be new to science and were described as *Goussia malayensis* n. sp., *G. bettae* n. sp. and *G. pogonognathi* n. sp. from *Apocheilus panchax, Betta splendens* and *Hemirhamphodon pogonognatus*, respectively. The fourth species, found in *Trichogaster pectoralis*, was identified as *G. trichogasteri* Székely & Molnár, 1992, a species known from aquarium-cultured *T. trichopterus*.

Introduction

Little is known of the coccidian infections of tropical freshwater fishes. Some data are available from Brazil, where *Calyptospora serrasalmi* Cheung, Nigrelli, & Ruggieri, 1986 and *C. tucunarensis* Békési & Molnár, 1991 infect the black piranha *Serrasalmus niger* Schomburgk and tucunare *Cichla ocellaris* Bloch & Schneider. From the tropical zone of India two freshwater coccidians, *Eimeria glossogobii* Mukherjee & Haldar, 1980 from *Glossogobius giuris* (Hamilton) and *E. notopteri* Chakravarty & Kar, 1944 from *Notopterus notopterus* (Pallas) were mentioned by Mukherjee & Haldar (1980). In southern China coccidian infections have been recorded in *Channa argus* (Basilewsky) and *C. maculata* (Lacepède), and *Eimeria kwangtungensis* Chen, 1973 and *E. ophiocephalae* Chen & Shieh, 1960 have been described (Chen, 1973). The coccidian fauna of Malaysian fishes has not previously been studied, except for the marine species *E. quentini* Boulard 1977 which was mentioned by Boulard (1977) from the Malacca Straits off the western coast of Malaysia.

This paper describes a survey of coccidian infections of fishes from small channels and creeks on the eastern coast of the Malaysian Peninsula and in the Tasik Kenyir Reservoir, which resulted in the finding of six species of *Goussia* Labbé, 1896, three of which are described here as new to science.

Materials and methods

Altogether 266 fish species are known from the freshwaters of peninsular Malaysia (Mohsin & Ambak, 1983). During the present survey fish were collected from two biotopes during May, 2001 and February, 2002. Sixty-one specimen of six species [*Hemirhamphodon pogonognatus* (Bleeker) (23), *Apocheilus panchax* (Hamilton) (15), *Betta splendens* Regan (5), *Oxyleotris marmoratus* (Bleeker) (3), *Trichogaster pectoralis* Regan (19) and *Monopterus albus* (Zuiew) (6)] were collected from small streams in the Campus of Kolej Universiti Sains & Teknologi Malaysia

(KUSTEM), while 26 specimen of eight species [*Chela oxigastroides* (Bleeker) (7), *Rasbora sumatrana* (Bleeker) (5), *Puntius schwanenfeldii* (Bleeker) (6), *Cyclocheilichthys apogon* (Cuvier & Valenciennes) (2), *Mystacoelescens marginatus* (C. & V.) (1), *Hampala macrolepidota* van Hasselt (2), *Channa micropeltes* (C. & V.) (2) and *Mystus nemurus* (C. & V.) (1)] were caught in the National Park Area of Tasik Kenyir Reservoir.

Some of the fishes were seined with small nets, carried to the laboratory of the university and maintained in aerated aquaria for some days until coarse faecal particles were excreted. Fishes from the Tasik Kenyir Reservoir were caught by gill-nets and examined immediately. Fishes were killed by cutting off their heads with scissors, and the intestinal tract and other visceral organs were examined for infection. Pieces of visceral organs were squashed under a coverslip on a glass slide, and mucus and epithelial scrapings from the intestine were examined. Oöcysts found were studied using the method described by Molnár (1977). When an infection with unsporulated coccidia was found, oöcysts in mucus were placed in a small Petri dish containing clean water, to which a drop of penicillin and streptomycin were added. After 24 h sporulation at least 25 oöcysts were studied. Measurements were taken under a compound microscope and freehand drawings were made immediately after the oöcysts had been detected. Micrographs from semisporulated and sporulated oöcysts were made using a Leica compound microscope attached to computer via a CCD camera and pictures were digitised via an Image Analysis Video Master Program. For histological examination, segments from the intestine were fixed in 10% neutral buffered formalin and embedded in syntetic resin (Durcupan), 1 μm thick semithin sections were cut using a Reichert OM-U2 ultramicrotome and stained with 0.5% toluidine blue.

All measurements are in micrometres (μm), with then mean $\pm$ standard deviation in parentheses.

Results

Six of the 14 examined fish species were infected by coccidians. All coccidians found belonged to the genus *Goussia*. Oöcysts were found only in the gut. Of the fishes infected by coccidia, *Apocheilus panchax* and *Trichogaster pectoralis* harboured large semisporulated oöcysts in the intestinal mucus, while *Betta splendens, Hemirhamphodon pogonognatus, Rasbora sumatrana* and *Mystacolescens marginatus* had small *Goussia carpelli*-type unsporulated or semisporulated oöcysts in the gut. In most of the fishes, oöcysts were found in the transparent intestinal mucus, but less often in the epithelial scrapings and in the gut contents. Oöcysts from clean mucus placed in tap water sporulated within 24 hours. Of these, oöcysts from *Apocheilus panchax, Betta splendens* and *Hemirhamphodon pogonognatus* finished sporulation, while the sporulation of oöcysts from *Trichogaster pectoralis* stopped at an early phase. Due to the lack of clean mucus and the contaminating faces particula, sporulation of oöcysts failed in samples from *Rasbora sumatrana* and *Mystacolescens marginatus*. Three of the species having sporulated oöcysts proved to be new species and semisporulated oöcysts from the gut of *Trichogaster pectoralis* were identified as *Goussia trichogasteri* Székely & Molnár, 1992.

Goussia malayensis n. sp.

Type-host: Apocheilus panchax (Hamilton); Cyprinidae.
Type-locality: Small streams around Kuala Terengganu, Malaysia.
Site: Epithelium of the foregut.
Prevalence of infection: Only one infected specimen, 6.6%.
Intensity of infection: Moderate.
Type-material: Phototypes have been deposited in the parasitological collection of the Hungarian Natural History Museum, Budapest. Coll. No. HNHM-67439.

Description (based upon 25 sporulated oöcysts) (Figures 1, 2)

Oöcysts (Figures 1, 2) spherical, 33-38 (35.3 $\pm$ 2.1). Wall of oöcyst smooth, colourless and thin. Micropyle, polar granule and oöcyst residuum absent. Oöcysts contain 4 elongate-ellipsoidal sporocysts which loosely fill oöcyst space and are arranged in most case in one direction but less frequently in unregulated positions inside oöcyst. Size of sporocysts 21-26 $\times$ 5.0-6.5 (23.9 $\pm$ 1.9 $\times$ 5.6 $\pm$ 0.63). Sporocyst wall very thin, single layered. Two sporocyst valves are connected by indistinct, longitudinal suture. Stieda-body or Stieda-body-like formation is not seen. Sporozoites stout, pointed only at one end, located at both ends of sporocyst, measure 12.0-16.0 $\times$ 5.0-6.0 (14.0 $\pm$ 1.3 $\times$ 5.4 $\pm$ 0.5). Sporocyst residuum finely

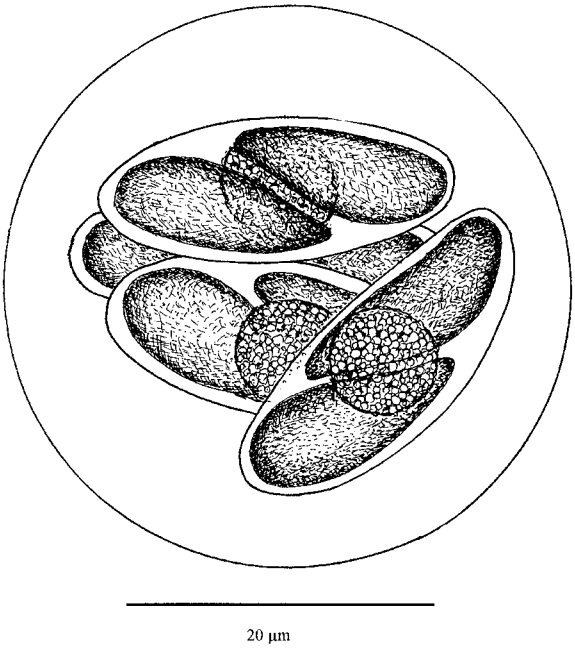

Figure 1. Schematic drawing of the oöcyst of *Goussia malayensis* n. sp.

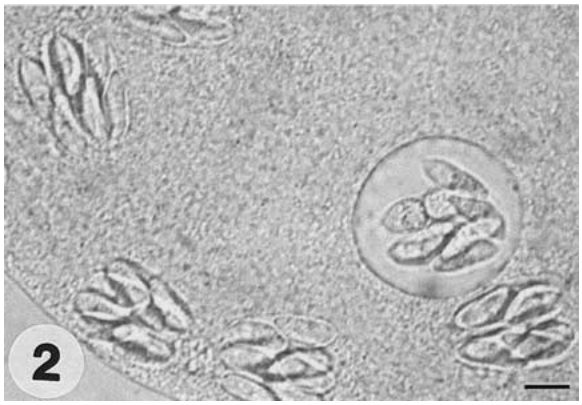

Figure 2. Oöcyst of *Goussia malayensis* n. sp. Only one of the oöcysts is intact; in most of the oöcysts the oöcyst wall collapsed and tightly covers the sporocysts. The relatively short and thick sporozoites are located at the two opposite ends of the sporocyst. *Scale-bar*: 10 μm.

granulated, fills available space within sporocyst in young oöcysts, globular or amorphous and *c.* 8-10 in diameter after 24 hours sporulation. During sporulation only minority of oöcysts preserve their shape; wall of most of oöcysts collapses and makes contact with wall of sporocysts (Figure 2).

Oöcysts pass from gut semisporulated. No endogeneous stages were found.

Remarks

In its morphology and size *Goussia malayensis* n. sp. is rather unique among intestinal species. By its large oöcyst size *G. malayensis* resembles oöcysts of *G. scardinii* Pellérdy & Molnár, 1968, a renal parasite of cyprinid fishes, but it differs from them by the lack of polar granules. It also resembles *G. trichogasteri* Székely & Molnár, 1992, but it has larger oöcysts and has no oöcyst residuum in the oöcyst.

Goussia bettae n. sp.

Type-host: Betta splendens Regan; Belontiidae.
Type-locality: Small streams around Kuala Terengganu, Malaysia.
Site: Epithelium of the foregut.
Prevalence of infection: Five infected specimens, 100%.
Intensity of infection: Moderate to heavy.
Type-material: Phototypes and a semithin section have been deposited in the parasitological collection of the Hungarian Natural History Museum, Budapest. Coll. No. HNHM-67438
Etymology: The species was named after the host.

Description (based upon 25 sporulated oöcysts) (Figures 3-5)

Oöcysts (Figures 3, 4) spherical, 7.5–8.5 (8.0 ± 0.4) in diameter. Wall of oöcyst smooth, colourless and thin. Micropyle, oöcyst residuum and polar granule absent. Oöcysts contain 4 sporocysts, which compactly fill available oöcyst space and are arranged in oöcysts in one direction. Sporocysts elongatedly ellipsoidal. Length of sporocysts 6.5–7 (6.7 ± 2.11), width and thickness 2–3 (2.5 ± 0.28); sporocyst wall very thin, single layered. Two sporocyst valves are connected by indistinct, longitudinal suture. Stieda-body or Stieda-body-like formation not seen. Sporozoites vermiform, with reflexed end located head to tail in sporocyst, $6.0–6.5 \times 1.0$ ($6.3 \pm 0.21 \times 1.0$). Sporocyst residuum globular, finely granulated, *c.* 1.5 in diamter.

Oöcysts pass from gut semisporulated.

In semithin sections unsporulated oöcysts were found in epithelium located basally to nuclei of epithelial cells. In same location some old, short, elliptical microgamonts containing only remainder of microgametes were observed (Figure 5a). In some sections macrogamonts were also found in goblet cells (Figure 5b). Less frequently sporulation progressed within

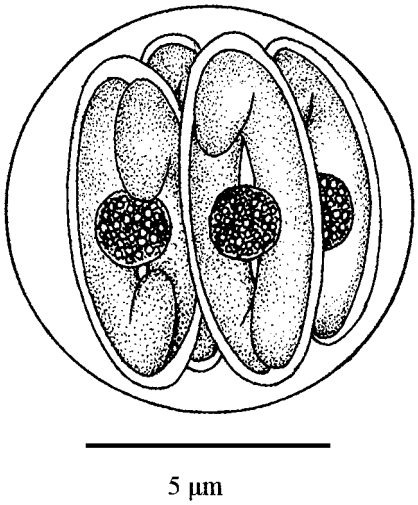

Figure 3. Schematic drawing of the oöcyst of *Goussia bettae* n. sp.

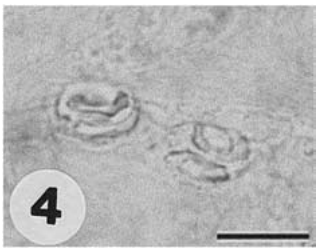

Figure 4. Sporulated oöcyst of *Goussia bettae* n. sp. *Scale-bar*: 10 μm.

fishes and semisporulated oöysts were observed in epithelium (Figure 5c).

Remarks

With its elongate sporocysts, the oöcyst of *Goussia bettae* n. sp. resembles *G. sinensis* (Chen, 1956), a parasite of the silver carp *Hypophthalmichthys molitrix* C. & V., but it is smaller in size and the taxonomic position of the hosts is different.

Goussia pogonognathi n. sp.

Type-host: Hemirhamphodon pogonognathus (Bleeker); Hemirhamphidae.
Type-locality: Small streams around Kuala Terengganu, Malaysia.
Site: Epithelium of the foregut.
Prevalence of infection: Twelve infected specimens, 52%. *Intensity of infection*: Moderate.
Type-material: Phototypes have been deposited in the

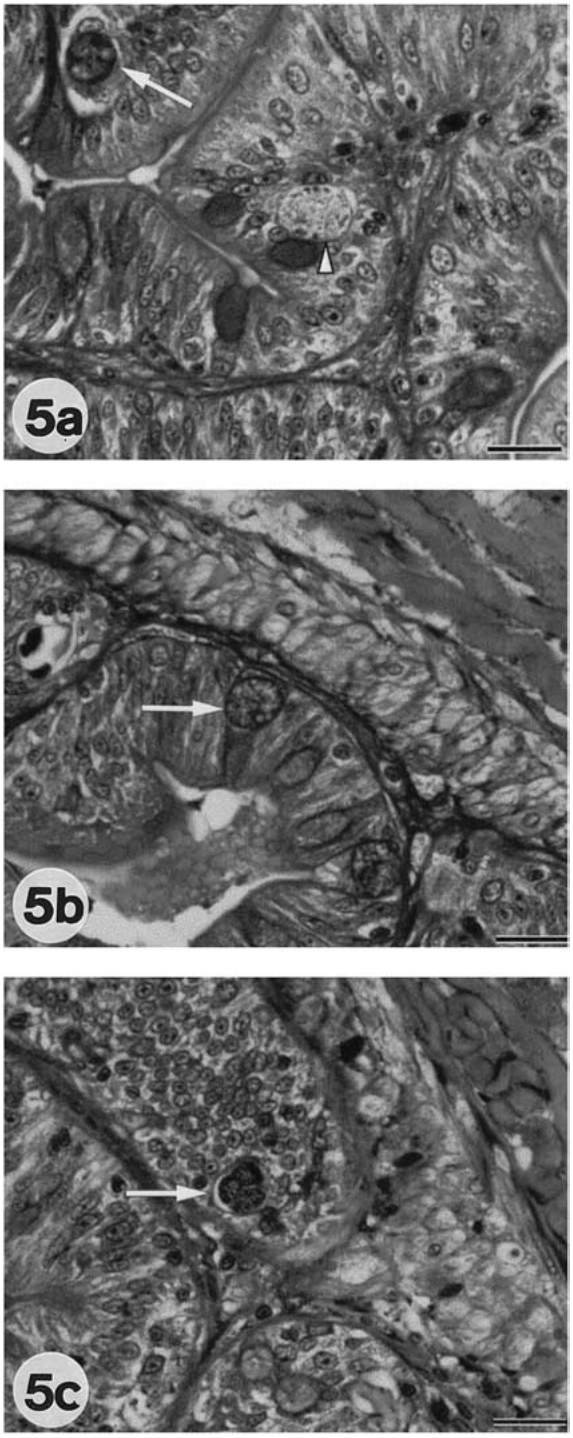

Figure 5. Semithin sections of the gut of *Betta splendens*: a. An unsporulated oöcyst (arrow) and a microgamont (arrow head) of *Goussia bettae* n. sp. in the basal region of the epithelium; b. Oöcyst developing at the base of a dark staining goblet cell (arrow); c. Semisporulated oöcyst (arrow) in the epithelium. *Scale-bar*: 10 μm.

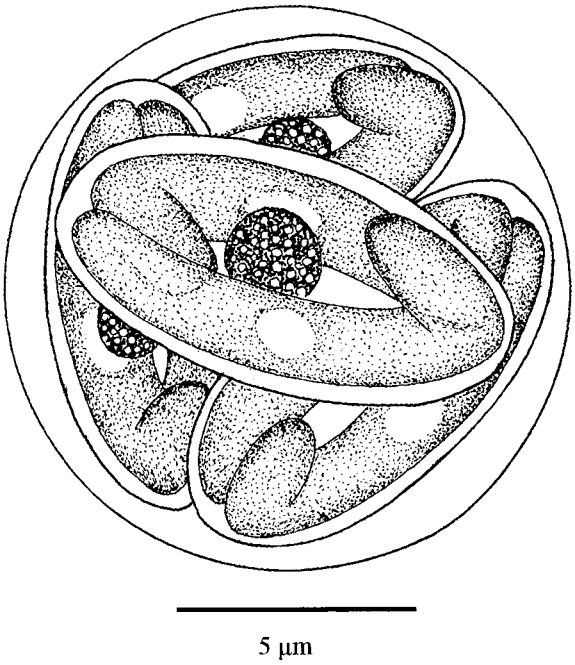

Figure 6. Schematic drawing of the oöcyst of *Goussia pogonognathi* n. sp.

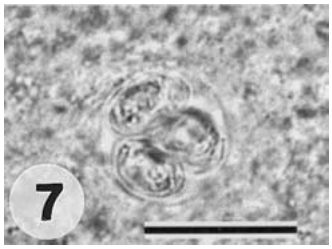

Figure 7. Sporulated oöcyst of *Goussia pogonognathi* n. sp. from the gut of *H. pogonognathus. Scale-bar*: 10 μm.

parasitological collection of the Hungarian Natural History Museum, Budapest. Coll. No. HNHM-67440
Etymology: The nomen triviale derives from the name of the host.

Description (based upon 25 sporulated oöcysts)
(Figures 6-8)

Oöcysts (Figures 6, 7) spherical, 10–13 (11.1 $\pm$ 1.16). Wall of oöcyst smooth, colourless and thin. Micropyle, polar granule and oöcyst residuum absent. Oöcysts contain 4 ellipsoidal sporocysts (Figure 8), which compactly fill available oöcyst space and are arranged in oöcysts so that 3 sporocysts are seen in 1 plane. Size of sporocysts 8-11 $\times$ 4–5 (9.0 $\pm$ 1.0 $\times$ 4.5 $\pm$ 0.50). Sporocyst wall very thin, single layered. Two sporocyst valves are connected by indistinct, longitudinal

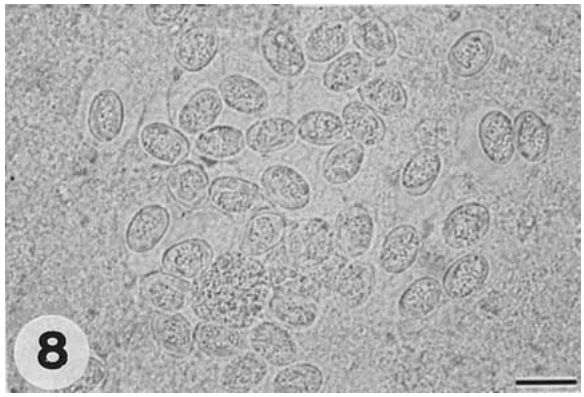

Figure 8. Sporocysts of *Goussia pogonognathi* freed from the compressed oöcysts. *Scale-bar*: 10 μm.

suture. Stieda-body or Stieda-body-like formation not seen. Sporozoites vermiform, with reflexed end, located head to tail in sporocyst, 8–9 $\times$ 1.5–2 (8.4 $\pm$ 0.5 $\times$ 1.8 $\pm$ 0.25). Sporocyst residuum globular or short-ellipsoidal, finely granulated, 2 $\times$ 2 or 2 $\times$ 1.

Oöcysts passed from gut semisporulated.

Remarks

In its sporocyst morphology this species resembles the 'carpelli' type coccidia [*G. carpelli* Leger & Stankovitch, 1921, *G. legeri* Stankovitch, 1920 and *G. iroquoina* (Molnár & Fernando, 1974)], but its sporocysts are less slender and less compact.

Goussia trichogasteri Székely & Molnár, 1992

Host: Trichogaster pectoralis Regan; Anabatidae.
Locality: Streams close to Kuala Terengganu, Malaysia.
Site: Epithelium of the foregut.
Prevalence of infection: Three infected specimens, 20%.
Intensity of infection: Moderate.

Description (based upon 25 sporulated oöcysts)
(Figure 9)

Round, unsporulated oöcysts of 12.5-14 (13.2 $\pm$ 0.51) and semisporulated oöcysts of 21-22.5 (20.94 $\pm$ 1.01) in diameter; latter contained 4 roundish sporoblasts of 5 in diameter and compact oöcyst residuum of 1.7. Semisporulated oöcysts (Figure 9) began to sporulate in tap-water within 24 h, reached size of 20-23

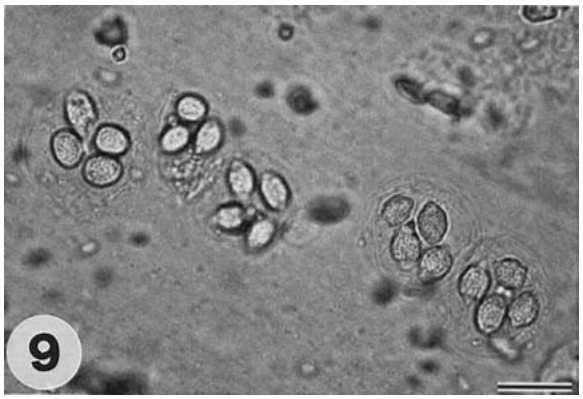

Figure 9. Semisporulated oöcysts of *Goussia trichogasteri* Székely & Molnár, 1992. Note the oöcyst residuum in the centre of the oöcyst among sporocysts. *Scale-bar*: 10 μm.

(21.4 $\pm$ 1.0) and contained 4 ellipsoidal sporocysts of 5.5-6.5 $\times$ 3.5-4.5 (6.0$\pm$0.35 $\times$ 4.0$\pm$0.14). Sporocysts loosely fill available space in oöcyst. Electrolucent oöcyst residuum is located centre of oöcyst measure 2 $\times$ 2. Banana-shaped sporozoites are aligned in sporocysts head to tail. Available space in sporocyst filled by scattered, roughly granulated sporocyst residua, but compact residuum was also observed centrally.

Remarks

Although, during this survey, only semisporulated oöcysts were found in *Trichogaster pectoralis*, the great resemblance of the oöcysts and the close relationship between the fish hosts enabled us to identify the species as *G. trichogasteri*, a species originally described from aquarium-cultured *Trichogaster trichopterus* by Székely & Molnár (1992).

Goussia sp. I

Host: Rasbora sumatrana (Bleeker); Cyprinidae.
Locality: Tasik Kenyir Reservoir, Malaysia.
Site: Epithelium of the foregut.
Prevalence of infection: One infected specimen, 20%.
Intensity of infection: Moderate.

Only unsporulated oöcysts measuring 9-10 in diameter and oöcysts sporulated to 4 sporoblasts were recorded.

Goussia sp. II

Host: Cyclocheilichthys apogon (Cuvier & Valenciennes); Cyprinidae.
Locality: Tasik Kenyir Reservoir, Malaysia.
Site: Epithelium of the foregut.
Prevalence of infection: Two infected specimens, 100%.
Intensity of infection: Moderate.

Only unsporulated oöcysts measuring 9-11 in diameter were recorded.

Discussion

Although this survey was restricted to a limited number of the known 266 species of Malaysian freshwater fishes, the results suggest that the coccidian fauna of this region shows a similar infection pattern with coccidian species as that of other parts of Eurasia. Of the 14 fish species examined six species proved to be infected by coccidians. Sporulated oöcysts of the new species found in this study exhibited features typical of *Goussia*. Although the unsporulated oöcysts of *Goussia* spp. I and II do not permit a definitive diagnosis, the results obtained on unsporulated oöcysts by Molnár (1986) suggest that in adequate media (in aerated tap-water with antibiotics) they could also develop *Goussia* oöcysts.

Fish coccidia belong to the genera *Eimeria* Schneider, 1875, *Goussia* Labbé, 1896, *Crystallospora* Labbé, 1896, *Calyptospora* Overstreet, Hawkins & Fournie, 1984 and *Cryptosporidium* Tyzzer, 1907. Of these, species of *Eimeria* and *Goussia* are the most common. *Goussia* was regarded for a long time as a synonym of *Eimeria*, but it was resurrected by Dyková & Lom (1981), who assigned *Goussia* to the Eimeriidae.

The new *Goussia* species found in this survey have some resemblance to known species but differ from them in minor but important morphological features, such as the shape and size of the sporocysts, and the presence or absence of residua or polar granules in the oöcysts. The small *G. bettae* n. sp. and *G. pogonognathi* n. sp. are typical gut coccidia most commonly known to cause dispersed coccidiosis in the gut and represented by *G. carpelli, G. iroquoina* and *G. sinensis* (Chen, 1956) (see Musselius et al., 1963; Paterson & Desser, 1982; Molnár, 1976). These coccidia are characterised by small compact oöcysts and produce a more or less permanent infection in host fishes. Due to the small size and compact nature of the oöcysts, this type of *Goussia* spp. exhibit relatively small morphological variations, but a better knowledge of their

host-specificity could effectively help in species determination. Host-specificity of fish coccidia is not well studied. There is no question that a certain level of specificity exists; however, Paterson & Desser (1982) proved that *Goussia* [as *Eimeria*] *iroquoina* can be transmitted to another closely related fish host. This means that only oöcysts differing morphologically should be described as new species.

The large-sized oöcysts of *G. malayensis* n. sp. and *G. trichogasteri* differ significantly from other intestinal species and better resemble species inhabiting the urinary ducts or oöcysts developing in nodules or in epicellular sites in the gut (Lom & Dyková, 1981; Molnár, 1986, 1989; Lukes & Dyková, 1990). Although no histological study was performed on the latter species, because of the large size of the oöcyst, the authors believe that *G. malayensis* does not develop in the gut epithelium but follows the pattern of *G. trichogasteri* and forms oöcysts epicellularly on the surface of the intestinal epithelium.

The great majority of the known fish coccidia (Dyková & Lom, 1983) were recorded from fishes in the sporulated stage. Recent data (Landsberg & Paperna, 1987; Kent et al., 1988; Molnár, 1996; Baska, 1997) have, however, revealed that the number of species shed unsporulated from the fish is much higher than previously believed. The finding of only unsporulated and semisporulated oöcysts in the Malaysian material might suggest that, due to the increased metabolism in tropical conditions, matured oöcysts remain only a short time in the gut and are released prior to sporulation.

No method has yet been elaborated for preserving fish coccidia and depositing them in collections. Bandoni & Duszynski (1988) and Duszynski & Wilber (1997) suggest that photomicrographs of the sporulated oöcysts should be used for this purpose. In addition to histological material, we have also documented our results using photomicrographs. Unfortunately, in fish coccidia micrographs of an acceptable quality can only made in the case of species with relatively large oöcysts, such as *G. malayensis* and *G. trichogasteri*. All the authors' attempts to use this technique for oöcysts of the new species with a size of *c.* 10 μm failed, as the images proved to be of poor quality.

Little is known of the pathological importance of coccidia found in this study, but knowing the rapidly growing importance of fish culture in the South-East Asian region (Ang, 1990; Lorensen et al., 1998) and assuming that some of the surveyed fish have been cultured for the aquarist market in the intensive systems of Malaysian fish farms (Mohsin & Ambak, 1983), an outbreak of an intensive infection of coccidiosis cannot be excluded. This supposition is supported by the fact that *G. trichogasteri* was originally described by Székely & Molnár (1992) from aquarium-cultured gouramis affected by a heavy coccidian infection.

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