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A survey of coccidian infection of freshwater fishes in South Africa, with the description of *Goussia anopli* n. sp. (Apicomplexa: Eimeriidae)

Kálmán Molnár[1], Annemarie Avenant-Oldewage[2] & Csaba Székely[1]
[1]*Veterinary Medical Research Institute, Hungarian Academy of Sciences, H-1581 Budapest, PO Box 18, Hungary*
[2]*Department of Zoology, Rand Afrikaans University, PO Box 524 Auckland Park, 2006 Johannesburg, South Africa*

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Abstract

Seventy-seven specimens of seven freshwater fish species harvested in four sites from rivers and ponds of the Gauteng, North West and Limpopo Provinces (South Africa) were surveyed for coccidian infections. Two fish species were infected with apicomplexans belonging to *Goussia* Labbé, 1896. In banded tilapia *Tilapia sparrmanii* Smith unsporulated oöcysts of *G. vanasi* (Landsberg & Paperna, 1987) were found which became sporulated in tap-water within 24 hours. Another species in the gut of chubbyhead barb *Barbus anoplus* Weber harboured sporulated oöcysts in the faeces and in the intestinal epithelium. The latter species has been described as *G. anopli* n. sp.

Introduction

Little is known of the coccidian infection of South African freshwater fishes. Adequate data concerns only the species *Eimeria* [*sensu lato*] *vanasi* Landsberg & Paperna, 1987, which was described from tilapias both from Israel and South Africa (Landsberg & Paperna, 1987). This latter species has been relatively well studied and detailed investigations have been carried out on the structure of the parasitophorous vacuoles, on epicytoplasmic stages and especially on sporozoite development of this species in Israeli material (Paperna & Landsberg, 1987; Kim & Paperna, 1992; Vilenkin & Paperna, 1997). Other data concerning the coccidian fauna of South African fishes have been presented only by Paperna (1996), who, without giving detailed information, mentioned the occurrence of *E. anguillae* Léger & Hollande 1922 in *Anguilla mosambica* (Peters) and a *Goussia*-like species from the gut of *Clarias gariepinus* (Burchell).; Other records of *Eimeria* and *Goussia* spp. are from marine fishes off Senegal (Diouf & Toguebaye, 1994, 1996).

In this paper, we describe a survey of coccidian infections of fishes from rivers and ponds in the northern part of South Africa, which resulted in the finding two species of *Goussia* Labbé, 1896, one of which is described as new to science.

Materials and methods

Altogether 77 specimens of seven fish species [*Barbus anoplus* Weber (n = 14); *B. paludinosus* Peters (n = 5); *Labeobarbus aeneus* Burchell (n = 5); *Aplocheilichthys johnstoni* Günther (n = 13); *Pseudocrenilabrus philander* Weber (n = 13); *Chetia flaviventris* Trewavas (n = 5); *Tilapia sparrmanii* A. Smith (n = 22)] were dissected. The fish were collected from four sites (Klein Nyl River above and below the Donkerpoort Dam, near Nylstroom, Limpopo Province; the Magalies River, Northwest Province; and from the Padda Dam, Johannesburg, Gauteng Province).

The fish were collected by electrofishing or seined with small nets, transported to a laboratory of the Rand Afrikaans University, maintained in aerated aquaria for some days until coarse faecal particles were voided. Fish were decapitated 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 were examined from the intestine. Oöcysts found were studied by the method described by Molnar (1977). When an infection with unsporulated coccidia was found under a coverslip on a slide, some drops of a 1% solution of azithromycinum was added to the edge of the coverslip, and the slide was

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then placed in a wet Petri dish in order to prevent evaporation. After 24 h of sporulation, a minimum of 25 oöcysts were examined. Measurements were taken with the aid of a compound microscope and freehand drawings were made immediately after the oöcysts had been detected and also after their sporulation. Digital images from semisporulated and sporulated oöcysts were made with a Zeiss Axioplan 2 compound microscope attached to a computer and the digital pictures were archived with Axiovision software. For histological examination, segments from the intestine were fixed in 10% neutral buffered formalin and embedded in paraplast. The 5 μm sections were stained with haematoxylin and eosin solutions. Photomicrographs were taken with an Olympus DH-10 digital camera mounted on an Olympus BH2 microscope.

All measurements are in micrometres, with the mean $\pm$ standard deviation in parentheses.

Results

Two of the seven examined fish species were infected by coccidians. All coccidians found belonged to *Goussia*. Oöcysts were found only in the gut. *Tilapia sparrmanii* harboured large unsporulated or semisporulated oöcysts in the intestinal mucus, while *Barbus anoplus* had small *Goussia carpelli*-like unsporulated or semisporulated oöcysts in the gut. In most of the fish, oöcysts were found in the transparent intestinal mucus and, less often, in the epithelial scrapings and in the gut contents. A smaller number of oöcysts from *T. sparrmanii* placed onto a slide in relatively clear mucus finished sporulation within 24 h, while the sporulation of the majority of oöcysts stopped at an early phase and only the division of the sporoblast into four sporonts were observed. In one preparation the sporulation progressed to 48 hours and in these oöcysts the banana-shaped sporozoites shrank to a comma-shape. Less frequently in fishes held in the laboratory for 7 days, sporulation of oöcysts also progressed in the fish gut and some sporulated oöcysts were found among unsporulated ones.

All oöcysts in the intestinal mucus of *B. anoplus* were found in a sporulated stage. Unsporulated oöcysts were recorded only from epithelial scrapings. The coccidian found in *T. sparrmanii* was identified as *Goussia vanasi* (Landsberg & Paperna, 1987), while the species from *B. anoplus* is described below as a new species.

Goussia vanasi* (Landsberg & Paperna, 1987) Lom & Dyková, 1992

Syn. *Eimeria* (*s. l.*) *vanasi* Landsberg & Paperna, 1987

Host: *Tilapia sparrmanii* A. Smith;Cichlidae).
Locality: Klein Nyl River above and below the Donkerpoort Dam, near Nylstroom, Limpopo Province; Magalies River, Northwest Province; Padda Dam, Johannesburg, Gauteng Province, South Africa.
Site: Mucus and epithelium of foregut.
Prevalence of infection: 22/22 fish infected; 100%.
Intensity of infection: Moderate to heavy.

Description (Figure 1)

[Based upon 25 sporulated oöcysts.] Oöcysts spherical, 15–17 (16.4 $\pm$ 0.4) in length and 11–13 (12.4 $\pm$ 0.6) in width. Wall of the oöcyst smooth, colourless and thin. Micropyle, oöcyst residuum and polar granule absent. Oöcysts contain 4 sporocysts, which loosely fill oöcyst space and are arranged in 1 direction. Sporocysts elongated-ellipsoidal, length 11–12 (11.6 $\pm$ 2.11), width and thickness 3–4.5 (3.6 $\pm$ 0.38); sporocyst wall very thin, single layered. Two sporocyst valves are connected by indistinct, longitudinal suture. Stieda-body or Stieda-body-like formation not seen. Banana-shaped sporozoites lie in sporocysts head to tail, 10–11 $\times$ 1.5–2 (10.3 $\pm$ 0.21 $\times$ 1.6 $\pm$ 0.31). After 24 h sporulation, sporocyst was filled with scattered, finely granulated sporocyst residua, but 48 h after sporulation, residuum had shrunk to centrally located, more compact ellipsoidal or round shape.

Oöcysts pass from gut unsporulated. In fish specimens unfed for 4–5 days, both semisporulated and sporulated oöcysts were found in gut.

Remarks

The measurements of oöcysts, sporocysts and sporozoites fell ;ithin the range given by Landsberg & Paperna (1987) for oöcysts obtained from the hybrids of *Oreochromis aureus* (Steindacher) and *O. niloticus* (L.).

*Although this species has an adequate description and reasonable illustrations (Landsberg & Paperna, 1987; Paperna & Landsberg, 1987; Kim & Paperna, 1992; Vilenkin & Paperna, 1997), it lacks a diagrammatic illustration which is necessary for completing the description. Therefore, we are adding some additional data and a schematic drawing, in order to help with the identification of this species, on the basis of oöcysts obtained in *Tilapia sparrmanii*.

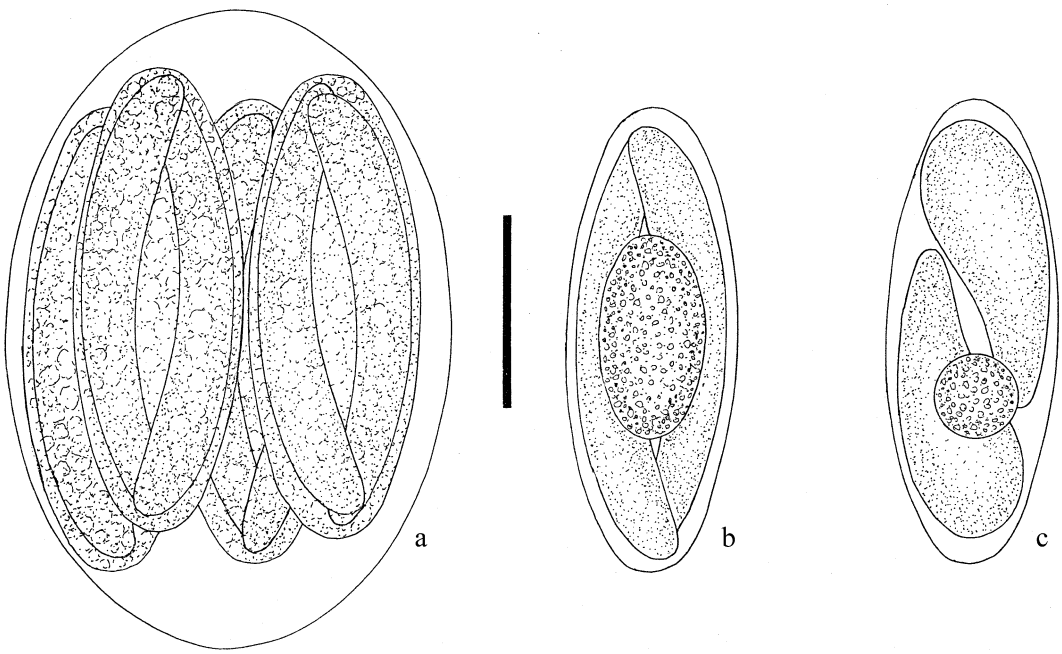

Figure 1. Diagrammatic illustration of the oöcyst and sporocysts of *Goussia vanasi* (Landsberg et Paperna, 1987): a. sporulated oöcyst; b. sporocyst after 24 h sporulation; c. sporocyst after 48 h sporulation. *Scale-bar*: 5 μm.

***Goussia anopli* n. sp.**

Type-host: *Barbus anoplus* Weber, Cyprinidae.
Type-locality: Nyl River above and below the Donkerpoort Dam, near Nylstroom, Limpopo Province, South Africa.
Other locality: Magalies River, Northwest Province, South Africa.
Site: Mucus and epithelium of the foregut.
Prevalence of infection: 11 of 14 fish infected; 78%.
Intensity of infection: Heavy.
Type-material: Phototypes and histological sections have been deposited in the parasitological collection of the Hungarian Natural History Museum, Budapest. Coll. No. HNHM-67575.
Etymology: The nomen triviale derives from the name of the fish host.

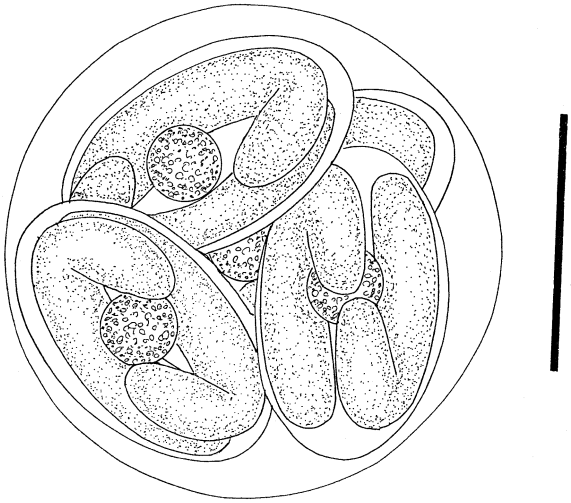

Figure 2. Diagrammatic illustration of the oöcyst of *Goussia anopli* n. sp. *Scale-bar*: 5 μm.

Description (Figures 2–4)

[Based upon 25 sporulated oöcysts.] Oöcysts spherical 8–9 (8.3 $\pm$ 2.3). Wall of oöcyst smooth, colourless and thin. Micropyle, polar granule and oöcyst residuum absent. Oöcysts contain 4 short, ellipsoidal sporocysts which fill much of oöcyst and are arranged in most cases in different directions, such that in 1 plane 3 sporocysts are seen in oöcyst. Size of sporocysts 5–5.5 $\times$ 3–4.5 (5.3 $\pm$ 1.9 $\times$ 3.9 $\pm$ 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 vermiform, with reflexed end, located head to tail in sporocyst, 7–8 $\times$ 1.5–2 (7.44 $\pm$ 0.43 $\times$ 1.7 $\pm$ 0.25). Sporocyst residuum globular or short ellipsoidal, finely granulated, 1.5 $\times$ 1.5 or 2 $\times$ 1.5.

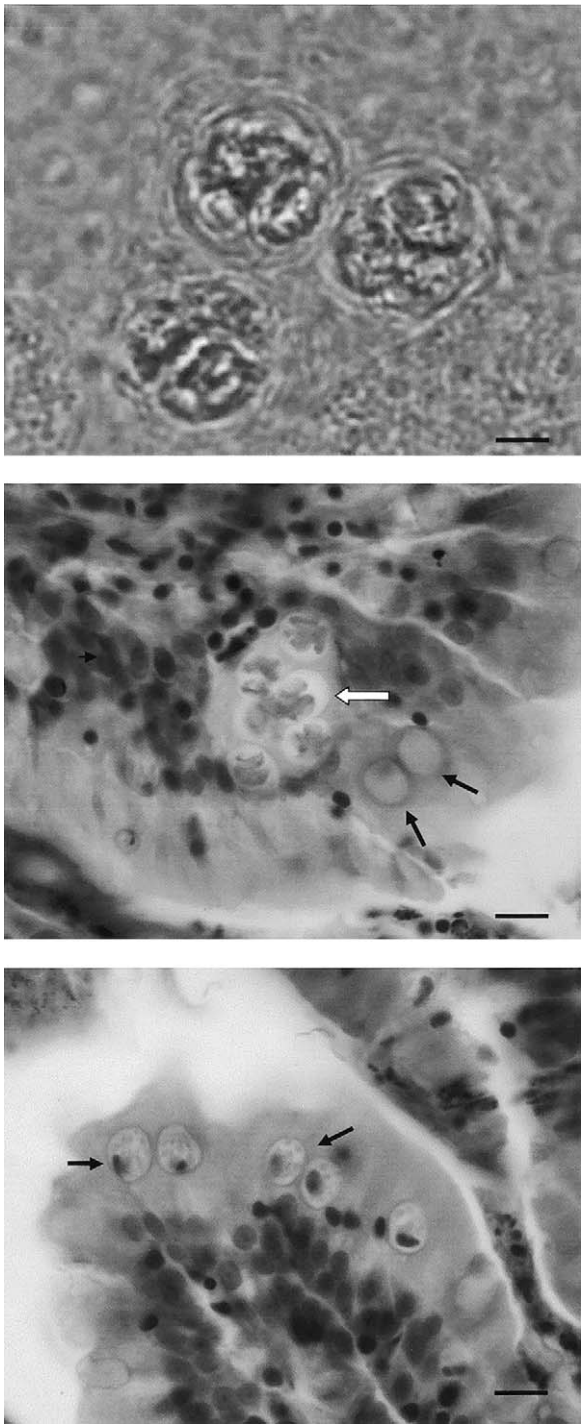

Figure 3–5. 3. Sporulated oöcysts of *Goussia anopli* n. sp. closed in a yellow body from the gut of the chubbyhead barb. Fresh smear. 4. Oöcyst batches of *G. anopli* (open arrow) under the intestinal epithelium. Histological section. H&E staining. Note that rodlet cells are located inside the epithelium (arrows). 5. Rodlet cells (arrows) in the epithelium of the chubbyhead barb's intestine. Histological section. H&E staining. *Scale-bars*: 3, 2.5 μm, 4,5, 7.5 μm.

Oöcysts pass sporulated from gut closed in yellow body. Most of yellow bodies contained 2 or 3 oöcysts.

Remarks

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

Sporulated oöcysts in histological sections were found solitary in epithelial cells or in small groups under the epithelium of the foregut (Figure 4), where they were easily recognisable by their intensive eosinophilic staining. Schizogonic or gamogonic stages were not found. No remarkable histological changes were recorded. In addition to oöcysts, however, a large number of rodlet cells were also found in the intestinal epithelium (Figure 5).

Discussion

Research on parasites of South African freshwater fishes is a fast developing area of the fish health studies. Several papers, first of all on crustacean parasites, have been published in recent years (Avenant-Oldewage, 1991, 1993; Smit et al., 2002), but other papers have also concerned protozoan parasites of fish (Van As & Basson, 1992; Basson & Van As, 1993). Little is known, however, of the coccidian infections of freshwater fishes. The only data on eimeriid coccidians of freshwater fishes were published by Landsberg & Paperna (1987), who described *Eimeria vanasi*, as a new species, from cichlid fishes, including the banded tilapia *Tilapia sparrmanii*, an endemic fish of southern Africa. During the present survey, conducted on seven species of fishes common in rivers of the northern part of the Republic of South Africa, two fish species, the banded tilapia and the chubbyhead barb *Barbus anoplus*, a cyprinid fish were infected by coccidia. The measurements and shape of the species in banded tilapia corresponded with the species description of *E. vanasi* made by Landsberg & Paperna (1987), but due to the position of the suture connecting the two equal halves of sporocyst valves, in agreement with Lom & Dyková (1992), this species should be attributed to *Goussia*, as *G. vanasi*. In histological sections, the species was found to occur in similar sites within the gut as indicated by Kim & Paperna (1992), and merogonic and gamogonic developmental

stages were found in epiplasmal and intraepithelial locations. In our survey, *G. vanasi* was typically found in the banded tilapia, but related cichlids, such as the southern mouthbrooder *Pseudocrenilabrus philander* and the canarykurper *Chetia flaviventris* were free of infection. Oöcysts from banded tilapia were shed at an unsporulated stage and completed sporulation outside the fish. A number of species from acipenserid, cyprinid and percid fishes (*G. acipenseris* Molnár, 1986, *G. danubialis* Molnár, 1986, *G. balatonensis* Molnár, 1989, *G. janae* Lukeš & Dyková, 1990, *G. desseri* Molnár, 1996 and *G. koertingi* Baska, 1997) follow this type of development in moderate climatic zones (Molnár, 1986, 1989; 1996; Lukeš & Dyková, 1990; Baska, 1997), but some from marine and tropical freshwater fish species (*G. girellae* Kent, Fournie, Snodgrass & Elston, 1988, *G. trichogasteri* Székely & Molnár, 1992 and *G. malayensis* Molnár, Shaharom-Harrison & Székely, 2003) also void unsporulated oöcysts (Kent et al., 1988; Székely & Molnár, 1992; Molnár et al., 2003). Although the temperate zone coccidians of the previous group are characterised by a year long seasonal cycle (Lukeš & Dyková, 1990; Molnár, 1996), such a seasonality has not been proved for marine and tropical species. Despite the fact that coccidians are released in an unsporulated stage they can finish their sporulation outside the fish. In the laboratory, however, due to the intense bacterial development in faeces and mucus, sporulation is successful only in a large volume clean of water (Landsberg & Paperna, 1987) or on a wet slide with antibiotic preventative measures (Molnár, 1977).

The other species, *G. anopli* n. sp., found during this survey appears to be a typical representative of the 'disperse coccidian' group, which develop in the intestinal epithelium of different fishes. These coccidia, having small (at most 14 μm in size), compact oöcysts with oval sporocysts, are frequently called 'carpelli-like' coccidia due to their resemblance to *G. carpelli*, the best studied *Goussia* species of the common carp. 'Carpelli-like' oöcysts are frequently surrounded by yellow, ceroid cell debris and are shed from their hosts at a sporulated stage. The development of the latter species takes only a few days and produces a more or less permanent infection in host fishes. Most of the cyprinids are infected by these small, almost indistinguishable oöcysts (Shulman, 1984), which, however seem to be fairly specific and infect only one host or a closely related fish species (Belova & Krylov, 2000; Molnár, unpublished). *G. anopli* n. sp. also appears to be a specific to one host, the chubbyhead barb, as it infected this latter fish with a high prevalence and relatively high intensity, but it could not be detected in two other cyprinids, *Barbus paludinosus* and *Labeobarbus aeneus*. Very little information exists on the coccidian infections of different barbel species. Patnaik & Acharya (1972) described *Eimeria ambassi* as a new species, from an Indian barbel, *B. ambassis* Day, while Shulman (1984) mentioned the occurrence of *G. carpelli* in *B. barbus* (L.). More reliable data concern *Goussia koertingi*, a parasite of *B. barbus* and *Goussia* sp. from *Hemibarbus barbus* (Temminck & Schlegel), which appear to belong to the seasonally developing group of coccidians that shed unsporulated oöcysts (Baska, 1997; Molnár & Ogawa, 2000).

The location of oöcysts in the gut of chubbyhead barb exhibited the same pattern which characterise *G. sinensis* Chen, 1956, *G. iroquoina* Molnár & Fernando, 1974 and *G. carpelli* (see Molnár, 1976; Patterson & Desser, 1981; Steinhagen et al., 1989). They seem to develop inside the epithelial cells but, after sporulation, they assume a subepithelial position. The relatively low number of tissue stages compared to the great number of free oöcysts in the faeces, in our case, is explained by the fact that during this study samples were fixed for histological purpose four or five days after the last feeding in the original biotope. Contrary to the relatively low number of oöcysts in enterocytes, the number of rodlet cells was extremely high. These enigmatic tissue cells deserve mention because their structure resembles coccidian merogonic stages and less experienced researchers, and sometimes specialists (Musselius et al., 1965), have regarded them as coccidian stages.

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