

## New Species of *Apolocystis* (Aseptatorina: Monocystidae) from the Coelom of *Microscolex dubius* (Oligochaeta: Acantodrillidae) in Los Talas, Buenos Aires, Argentina

Bettina Sandra GULLO<sup>1</sup> and Laura Cecilia ARMENDÁRIZ<sup>2</sup>

<sup>1</sup>Cátedra Zoología Invertebrados I, <sup>2</sup>Cátedra Zoología General, Facultad de Ciencias Naturales y Museo, Paseo del Bosque s/n 1900- La Plata, Argentina

**Summary.** *Apolocystis janovyi*, a new species of acephaline monocystid gregarine is described from the coelomic cavity of the oligochaete *Microscolex dubius* Fletcher, 1887; *A. janovyi* is the first monocystid species described from this host. These parasites differ from previously described species of *Apolocystis* in having a larger size range, especially smaller forms in all life cycle stages, and many more sporocysts within the gametocysts.

**Key words:** *Apolocystis janovyi* sp. n., Monocystidae, coelom, *Microscolex dubius*, Argentina.

### INTRODUCTION

Monocystid eugregarines are parasitic protozoa which are frequently found in seminal vesicles and coelomic cavities of terrestrial oligochaetes. Many papers have been published on these parasites, but almost all of them are taxonomic studies and lists of species and their hosts (Ruston 1959; Marek 1967; Segun 1971 a, b; Levine 1988; Pizl 1989 a, b). Rees (1961, 1962 and 1963) described new species and some phases of monocystid life cycle. The genus *Apolocystis* Cognetti

de Martiis, 1923 was proposed to include those gregarines belonging to the genus *Monocystis* von Stein, 1848, but which had spherical trophozoites lacking polarity. *Apolocystis* was described as having spherical trophozoites without the principal axis marked by the presence of any special peripheral organ. Only three species of *Apolocystis* complete their life cycle in the host coelomic cavity: *A. catenata* Muslow, 1911, *A. michaelseni* Hesse, 1909 and *A. stammeri* Meier, 1956. The present study reports on a new species of *Apolocystis* found within the coelomic cavity of the terrestrial oligochaete *Microscolex dubius* Fletcher, 1887. This finding is also the first report of gregarines in this host. The aim of the present study is to introduce the new species and to describe the phases of its life cycle.

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Address for correspondence: Bettina Sandra Gullo, Cátedra Zoología Invertebrados I. Facultad de Ciencias Naturales y Museo. Paseo del Bosque s/n 1900- La Plata, Argentina; E-mails: bgullo@museo.fcnym.unlp.edu.ar; lauraarm@netverk.com.ar

## MATERIALS AND METHODS

The terrestrial oligochaete *Microscolex dubius* was obtained on August 27, 1997, from a vegetal substrate, mainly formed by *Azolla filiculoides* Lam. in Los Talas, Buenos Aires, Argentina (34°53'S, 57°50'W). Examination of fresh material using stereoscopic microscope revealed white, opaque, oval structures immersed in the coelomic fluid in all somites behind the clitellum. This material was fixed in Bouin's fluid, dehydrated, and embedded in paraffin for further study. Histological sections of 10 µm thickness from the host anterior half were made with a Minnot like micrometer and stained with either Meyer's or Erlich's hematoxylin and eosin. Life cycle stages found (intercellular and coelomic trophozoites, gametocysts, cysts containing zygotes and sporocysts, and sporocysts) were measured using optical microscope with micrometric ocular (n = 170) and then photographed.

## RESULTS

### *Apolocystis janovyi* sp. n.

**Host:** *Microscolex dubius* Fletcher, 1887

**Localization:** coelom.

**Description:** sub-spherical, oval, kidney-shaped intercellular trophozoites (9-36 µm in major diameter, 6-26 in minor diameter). Sub-spherical or oval coelomic trophozoites without polar differentiation and lacking of ectoplasmic processes (17-283 µm in major diameter,

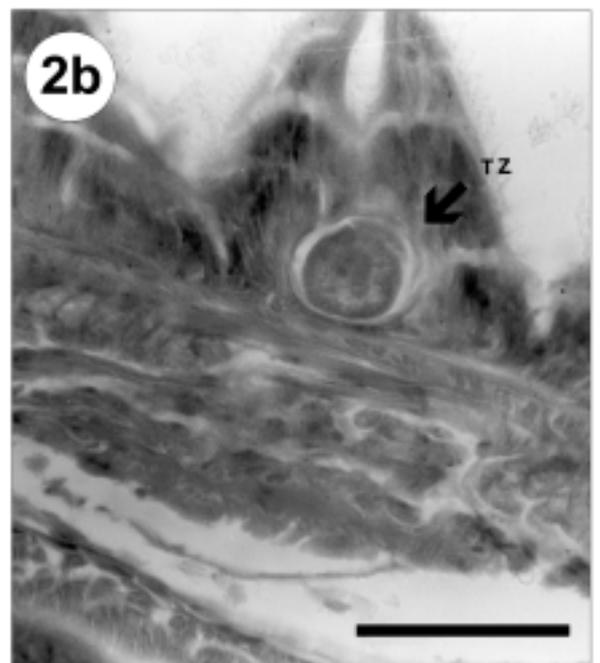
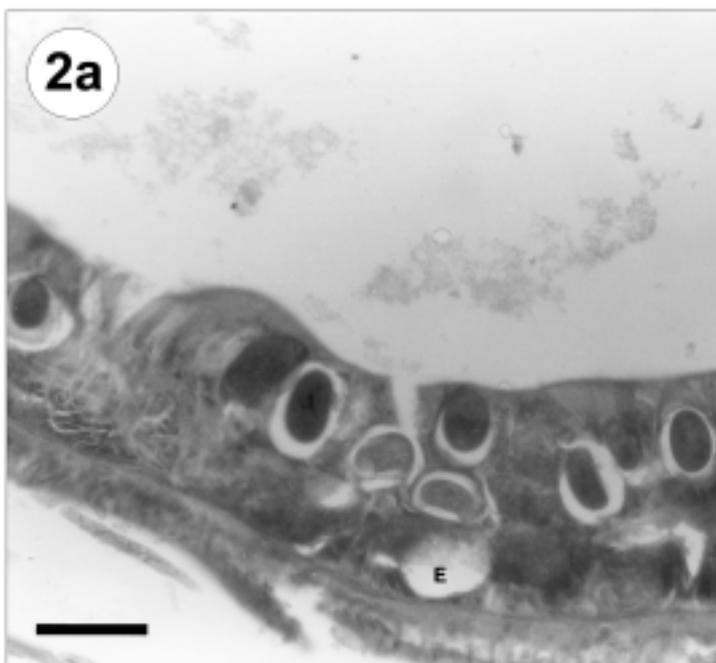
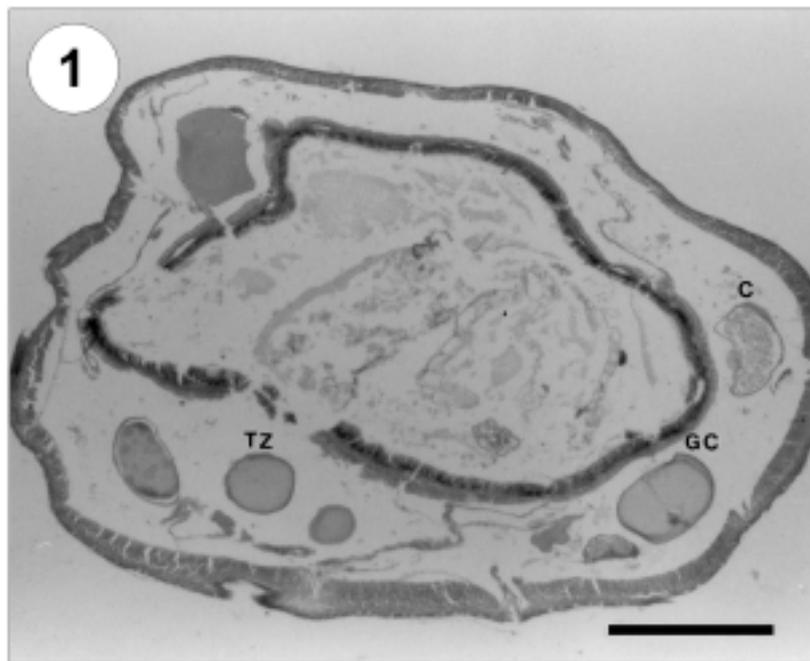
17-250 µm in minor diameter). Gametocysts 116-206 µm in minor diameter, 193-369 in major diameter. Navicular sporocysts 2.7-6.8 µm in width and 5-15 µm in length.

**Specimens deposited:** intercellular trophozoites, trophozoites, gametocysts, cysts containing sporocysts, and sporocysts. Hapantotypes (7 slides, N 11) have been deposited in Colección División Zoología Invertebrados (Protozoa), Museo de La Plata, Argentina. Other five slides were deposited in the University of Nebraska State Museum Parasite Collection, School of Biological Sciences, University of Nebraska, USA.

**Remarks:** the different developmental stages were found in the following locations: esophageal epithelium (intercellular trophozoites), coelomic cavity (trophozoites, gametocysts, cysts containing zygotes and sporocysts, and sporocysts) and only sporocysts in seminal vesicle (Fig. 1). Intercellular trophozoites were located near the basal membrane as well as slightly distal to it. They showed spherical, oval, kidney-shaped forms (Figs 2 a, b) and their sizes varied from 9-36 µm in major diameter to 6-26 µm in minor diameter (Table 1). Coelomic trophozoites were spherical and oval without polar differentiation and lacked ectoplasmic processes. They were surrounded by a mesothelial layer and, in some cases, were attached to the parietal as well as to the visceral peritoneum. Once released to the coelomic

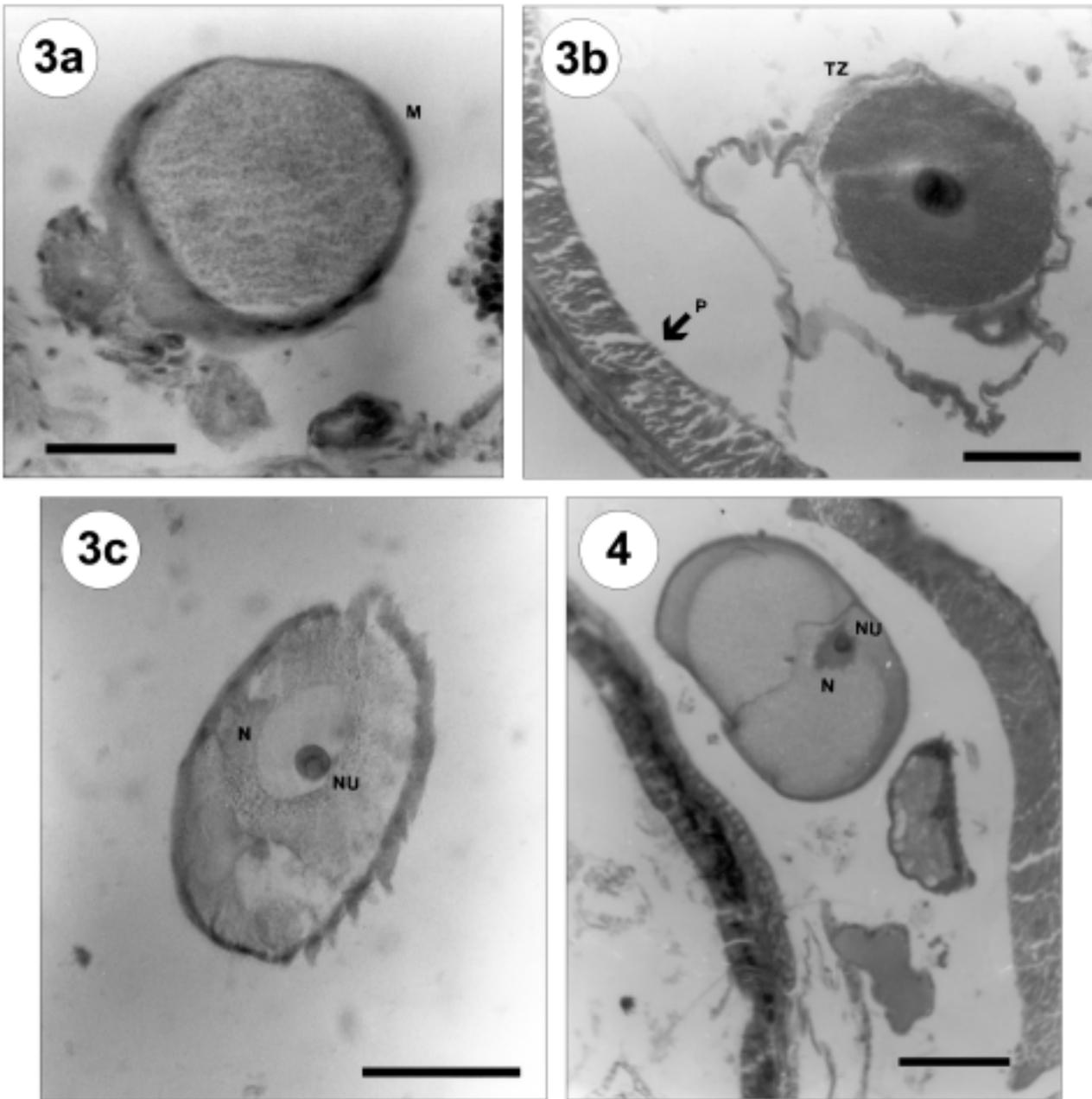
**Table 1.** Average size (µm) of the observed *Apolocystis janovyi* phases in esophageal epithelium and coelom of *Microscolex dubius*

Phase		Average	Standard deviation	n	
Intercellular trophozoite	Major diameter	26.319	5.729	26	
	Minor diameter	18.827	4.934	26	
Trophozoite within coelomic cavity	Major diameter	158.222	63.578	37	
	Minor diameter	128.484	51.778	37	
	Nucleus	Major diameter	37.462	11.503	16
		Minor diameter	32.883	8.413	16
Endosome	17.065	7.715	12		
Gametocyst	Major diameter	252.854	45.666	18	
	Minor diameter	183.01	24.89	18	
	Nucleus	Major diameter	41.624	11.332	7
		Minor diameter	30.445	4.045	5
Endosome	13.984	2.524	5		
Cyst containing sporocysts	Major diameter	262.122	44.301	25	
	Minor diameter	148.875	41.222	25	
Sporocyst	Length	9.996	2.201	55	
	Width	4.187	0.886	55	



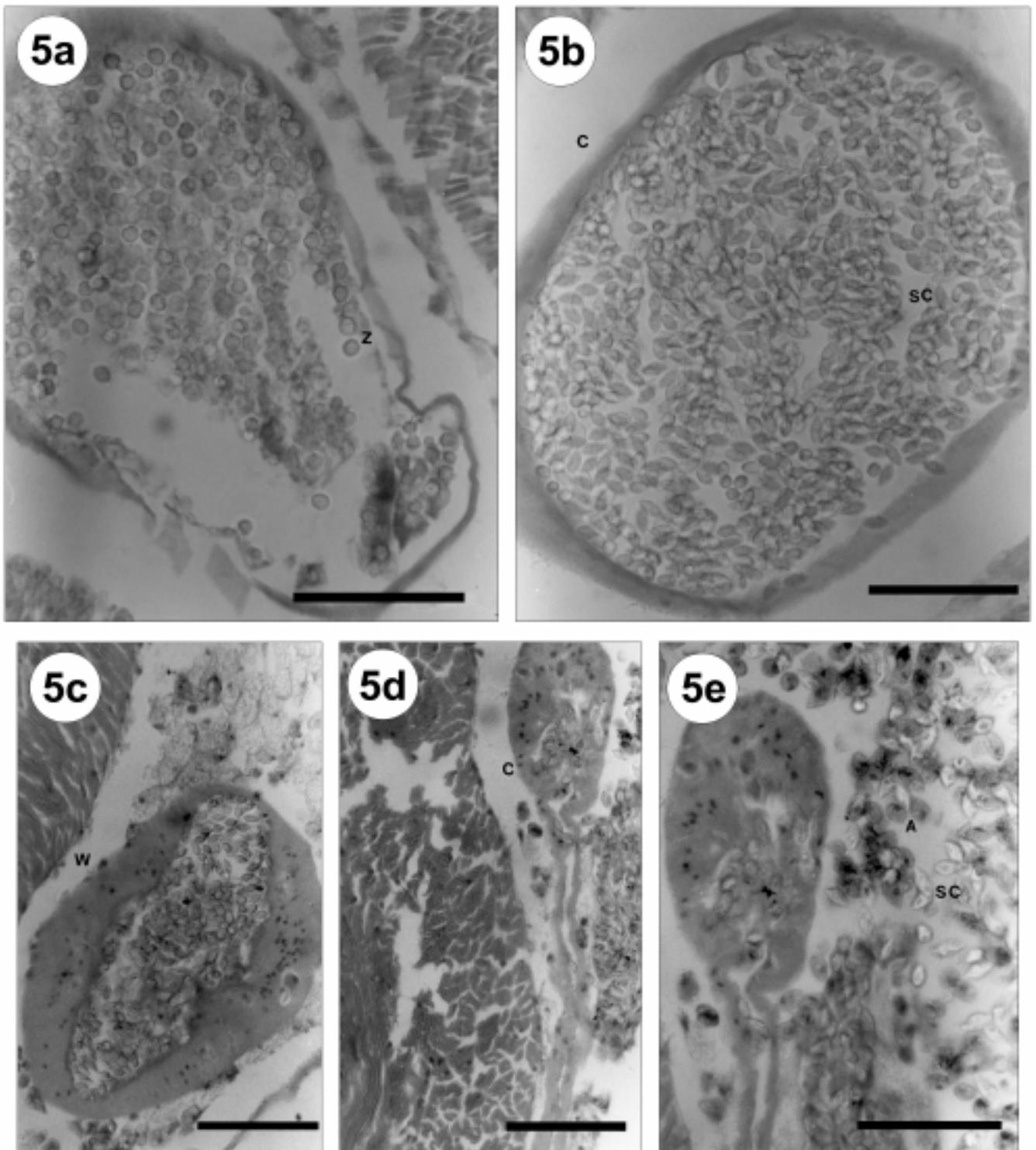
**Fig. 1.** Transverse section of *Microscolex dubius* in postclitellar segments; note trophozoites (TZ), gametocysts (GC) and cysts (C) in the coelom. Scale bar - 500 $\mu$ m

**Figs 2 a, b.** Intercellular trophozoites of *Apolocystis janovyi* in esophageal epithelium (E); **a** - different shapes and localizations can be seen; **b** - spherical trophozoite (TZ) in the basal membrane proximity. Scale bar - 50  $\mu$ m



**Figs 3 a-c.** *Apolocystis janovyi* coelomic trophozoites; **a** - note the mesothelial cell cover (M); **b** - trophozoite (TZ) attached to the parietal peritoneum (P); **c** - free trophozoite within the coelomic cavity; note the irregular cover, the nucleus (N) and endosome (NU). Scale bar - 50  $\mu$ m

**Fig. 4.** *Apolocystis janovyi* gametocyst inside coelomic cavity; note the star-shape nucleus (N) and endosome (NU). Scale bar - 100  $\mu$ m



**Figs 5 a-e.** *Apolocystis janovyi* cysts inside coelom cavity; **a** - note the zygote (Z) inside; **b** - navicular sporocysts (SC) inside the cyst (C); **c** - note the multinuclear wall (W) of cyst; **d** - collapsed cyst (C); **e** - free sporocysts (SC) within the coelomic cavity surrounded by numerous amoebocytes (A). Scale bar - 50 μm

cavity, this layer adopted an irregular aspect, showing cytoplasmic prolongations (Figs 3 a, b, c). An increase in the size of trophozoites to 17-283  $\mu\text{m}$  in major diameter and 17-250  $\mu\text{m}$  in minor diameter was observed (Table 1). The nucleus was spherical, occasionally star-shaped and showed an eccentric position, with a spherical, vesicular endosome. Basophilic granules were observed in cytoplasm, of about 0.62 to 1.87  $\mu\text{m}$ , increasing in size towards the nucleus, where they appeared to be more dispersed. Likewise, gametocysts were observed in the coelomic cavity. They also showed a mesothelial layer and a star-shaped nucleus (Fig. 4). Their sizes varied between 116-206  $\mu\text{m}$  in minor diameter and 193-396  $\mu\text{m}$  in major diameter (Table 1). Both coelomic trophozoites and gametocysts were found within the coelomic cavity, which corresponds to the postclitellar region.

The morphology of the cysts having sporocysts varied (Figs 5 a, b). Sizes ranged from 76-239  $\mu\text{m}$  in minor diameter, and 167-328  $\mu\text{m}$  in major diameter (Table 1). These stages were found within the coelomic cavity of the preclitellar, clitellar and postclitellar region, and they showed a layer formed by a syncytial wall with spherical nuclei. The thickness of this wall varied according to the degree of cyst turgidity, being thinner in turgid cysts and turning into more noticeable multinuclear nature in the collapsed ones (Figs 5 c, d). Cysts that released sporocysts to the coelom showed the presence of abundant amebocytes (Fig. 5 e).

Sporocysts were navicular, and were 2.7-6.8  $\mu\text{m}$  in width and 5-15  $\mu\text{m}$  in length (Fig. 5 b). They were found within the coelom of preclitellar and clitellar segments, and in the seminal vesicle. Abundant amebocytes were observed surrounding the sporocysts in those segments as a probable host response to the presence of the parasite.

Microanatomic observation of the host gonads suggested an incipient maturation of the ovary and the seminal vesicle, showing different stages of the spermatogenesis with little presence of spermatozooids.

**Etymology:** dedicated to the eminent gregarinologist, Prof. John Janovy, Jr., Verner Professor of Biological Sciences, University of Nebraska, Lincoln, U.S.A.

## DISCUSSION

Until this description, gregarines have never been described before from *M. dubius*, although this host has a cosmopolitan distribution (Righi 1979). There are only

three species of *Apolocystis*, which complete their life cycle within the host coelomic cavity. The different phases of the life cycle of *A. michaelsoni* Hesse, 1909 show a morphology, which can be compared to *Apolocystis janovyi* sp. n.

*A. michaelsoni* has spherical or ovoid trophozoites, granular endoplasm, and a nucleus with a large, central endosome. Gametocysts and cysts are ellipsoidal, and sporocysts are navicular. Although the morphology of the mentioned stages have a correspondence with those described for the new species, the sizes measured for *A. janovyi* are more variable than the sizes observed by Hesse (1909) for *A. michaelsoni* (225-295  $\mu\text{m}$  trophozoites, gametocysts of 235-300  $\mu\text{m}$  in major diameter and 170-220  $\mu\text{m}$  in minor diameter, and sporocysts of 9  $\mu\text{m}$  in width and 15  $\mu\text{m}$  in length).

In opposition to *A. michaelsoni*, in which trophozoites are particularly abundant in the clitellum proximity, the species here described has its trophozoites distributed along the worm. Both parasite species are frequently surrounded by host conjunctive cells, which constitute a pedicel that attaches them to the body wall. Occasionally, the trophozoites are found free in the coelom. Hesse (1909) observed in *A. michaelsoni* gametocysts with more than two individuals, not knowing their destiny. An important difference between these two species must be highlighted in relation to the number of sporocysts contained inside a cyst. Hesse (1909) found up to a maximum of 16 ellipsoidal, very voluminous sporocysts inside the cyst, while in *A. janovyi* there are over than 100 ones in a cyst.

Despite the methodology used we could not find basophilic granules, their aspect in the *A. janovyi* trophozoite cytoplasm suggest its glycogenic nature (Hesse 1909; Rees 1963; Pizl 1989 a, b). As observed in other monocystid gregarine species, (*Cephalocystis singularis* and *Dendrocystis piriformis*), the two phases in the life cycle of *A. janovyi* are: a period of trophozoite growth inside the esophageal epithelium and a second phase when the trophozoite is free within the coelomic cavity of the worm (Rees 1962). The coelomic trophozoite is an important growth phase inside the host body cavity. Nevertheless, the existence of trophozoite growth in the digestive epithelium could not be denied. As Rees (1962) observed in *C. singularis*, after the association of trophozoites, a size contraction occurred in such a way that the gametocyst had a slightly bigger size than the trophozoite.

The variety or shapes found in cysts could be attributed to its liquid content. The sporocysts seem to be the

only phase of life cycle recognized as foreign by the host. This is because in no other phase amebocytes were observed.

The possible infection mode should have been the digestive one. Different episodes of infestation could have been happened due to the presence of distinct life cycles stages in diverse localities. As Hesse (1909) suggested, we consider that the release of sporocysts could occur in different ways: by coelomic liquid exudation, host fragmentation, tissue necrosis and its death. New cycles within the same host would not happen, since the sporocysts become infective only when they reach the digestive tract. The sporocysts can remain unchanged in the environment, even after a carnivore eats the host.

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