Apolocystis cognetti sp. nov. (Apicomplexa: Monocystinae) a new aseptate gregarine species from the earthworm *Amyntas hawayanus* (Annelida: Oligochaeta) from West Bengal, India

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Received: 4 January 2012 / Accepted: 22 February 2012 / Published online: 16 March 2012
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Abstract During the course of surveys of endoparasitic aseptate gregarines in Darjeeling district of West Bengal, India, seminal vesicles of *Amyntas hawayanus* (Rosa 1891) found to be infested with a new species of the genus *Apolocystis* (Cognetti de Martiis 1923). Trophozoites are spherical or rounded and without any polar differentiation. Diameter of the trophozoite ranges from 65.4 to 126.7 (91.8 ± 17.5) μm . Nucleus diameter measures 12.2–20.4 (14.4 ± 2.2) μm . Gametocyst is round and measures 73.6–110.4 (93.3 ± 14.0) μm and contains two unequal gametocytes. Oocyst measures 7.7–10.7 (9.5 ± 0.9) $\mu\text{m} \times 3.8$ –5.3 (4.6 ± 0.4) μm .

Keywords Gregarine parasite · Aseptate ·
Apolocystis cognetti sp. nov. · Seminal vesicle ·
Earthworm · *Amyntas hawayanus*

Introduction

The order Eugregarinida contains aseptate gregarines with over 400 species and septate gregarines with about 900 species (Levine 1977). In the aseptate gregarines (acephaline), the trophozoite has one compartment; in the septate

forms (cephaline) there are several compartments. Gregarines are certainly significant from an evolutionary perspective because of their suspected early diverging position within the apicomplexan and their possession of several characteristics presumed to be plesiomorphic such as an extracellular trophozoite stage, a monoxenous life cycle and prevailing presence in the marine host (Cox 1994; Théodoridés 1984; Vivier and Desportes 1990). The genus *Monocystis* (Stein 1848) under the order Eugregarinida was revised by Hesse (1909) and was reorganized to four genera, namely *Monocystis*, *Nematocystis*, *Rhyncocystis* and *Pleurocystis*. In 1923, Cognetti de Martis created the genus *Apolocystis* in order to accommodate all those species of the genus *Monocystis* which had spherical trophozoites and lack of polarity. Several species of the genus *Apolocystis* were described from various parts of the world (Bhatia and Setna 1926; Phillips and Mackinnon 1946; Lacy and Miles 1959; Levine 1977; Segun 1978; Pradhan and Dasgupta 1983; Gullo and Armendáriz 2002; Bandyopadhyay et al. 2004).

The present paper describes one new species of the genus *Apolocystis* along with a comparative account with closely related species. The new species was obtained for the first time from the seminal vesicles of the earthworm *Amyntas hawayanus* (Rosa 1891) from the podzol soil of Darjeeling district of West Bengal, India.

Materials and methods

Samplings were carried out in the podzol soil in Darjeeling district of West Bengal. Different species of earthworm were collected during June–July, 2010. The collected individuals were put in soil-filled plastic buckets and taken to the laboratory alive. Some of them were dissected while

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alive and their seminal vesicles were carefully removed. These were placed on clean grease free slide with a drop of 0.6% NaCl solution. A thin film of seminal fluid was drawn out on the slides, covered with a cover slip for examination of living protozoans under a phase contrast microscope (Olympus CX41). After the initial study of living protozoans, the content of the seminal vesicles was semidried and fixed in Schaudin's fluid (20 min). The fixed smears were stored in 70% ethyl alcohol for removal of excess of mercuric chloride. The slides were then passed through a descending series of alcohols (5 min in each) and placed in distilled water. Then they were transferred to a 3% iron alum solution (over night) and stained with Heidenhain's haematoxylin solution (20 min). Differentiation was done with 1% iron alum solution under the low power objective lens of the light microscope. The slides were then washed thoroughly, dehydrated in an ascending series of alcohols, cleared in xylene and mounted in Canada balsam. Drawings of the different stages of gregarines were made using a camera lucida attached to the microscope; photomicrographs were taken under an Olympus phase contrast microscope ($\times 400$ magnifications) with an Olympus camera (Model C5060). All measurements are in micrometers; in each case minimum and maximum values are given, followed in parentheses by arithmetic mean, standard deviation and sample size. Plane shapes are described mainly according to Clopton (2004).

Results

Apolocystis cognetti sp. nov. (Figs. 1–4; Table 1)

Phylum: Apicomplexa (Levine 1977)

Order: Eugregarinida (Leger 1900)

Family: Monocystidae (Bütschli 1882)

Subfamily: Monocystinae (Bhatia 1930)

Genus: *Apolocystis* (Cognetti de Martis 1923)

Trophozoites are spherical or rounded without any polar differentiation. Diameter of the trophozoite ranges from 65.4 to 126.7 (91.8 ± 17.5) μm . Ectosarc is finely vacuolated and measuring 4–5 μm in thickness and does not bear any surface striations. Sarcocyte and myocyte are not very distinct. Endosarc is densely vacuolated. Shape and size of the granules depend on the size of the trophozoite. Granules are not uniformly distributed. Small trophozoites contain coarse granules and the large ones have finer granules. There are clear demarcation between ecto- and endoplasm. Nucleus of the trophozoite is round and takes the position at the ectosarc region. Diameter of the nucleus measures 12.2–20.4 (14.4 ± 2.2) μm . Nuclear membrane

is very thin. A faint vacuole is seen at the centre. The gametocyst is round and measures 73.6–110.4 (93.3 ± 14.0) μm . Gametocyst contains two unequal gametocytes. Larger gametocyte is semicircular and measures 61.3–94.0 (77.9 ± 11.3) $\mu\text{m} \times 40.9$ –61.3 (50.5 ± 7.8) μm . The smaller gametocyte is ovoid and measures 57.2–89.9 (74.6 ± 9.3) $\mu\text{m} \times 34.7$ –63.3 (44.6 ± 7.4) μm . Nucleus of both the gametocytes are round. Oocysts are navicular and pointed at both ends. Oocyst contains eight sporozoites and measures 7.7–10.7 (9.5 ± 0.9) $\mu\text{m} \times 3.8$ –5.3 (4.6 ± 0.4) μm .

Taxonomic summary

Type material: *Apolocystis cognetti* sp. nov.

Type host: *A. hawayanus* (Rosa 1891)

Symbiotype: Host AH/11 is deposited in the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Site of infection: Seminal vesicles

Type locality: Darjeeling (Lat. $26^{\circ}31'N$ $87^{\circ}59'$), West Bengal.

Prevalence: 25/66 (37.8%)

Type material: Holotype on a single slide no. API/17, and paratypes on slides API/1, API/21 are deposited in the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India. Slide no. API/9 bearing some paratype materials has been deposited in the national collection of the Zoological Survey of India, Calcutta-53 (Accession no. Pt/2435/ZSI).

Etymology: The new species has been named after the scientist Cognetti de Martis who created the genus *Apolocystis*.

Discussion

Being a parasite in the seminal vesicles and having spherical gamont with lack of polarity justifies its inclusion under the family Monocystidae, subfamily Monocystinae and the genus *Apolocystis* (Cognetti de Martis 1923). Till now six species of *Apolocystis* have been reported from India. The species under discussion was obtained from an oligochaete host *A. hawayanus* (Rosa 1891) from podzol soil of Darjeeling district. When the species under discussion was compared with other related species, it was found to resemble three Indian species to some extent, viz. *Apolocystis chotonagpurensis* (Bandyopadhyay et al. 2004), *Apolocystis goomtiensis* (Pradhan and Dasgupta 1983), and *Apolocystis monokaryosomiferus* (Pradhan and Dasgupta 1983). The size and shape of present form varies

Figs. 1–4 Photomicrographs of different stages of the life history of *Apolocystis cognetti* sp. nov. obtained from seminal vesicles from earthworm *A. hawayanus* (Rosa 1891). 1 Trophozoite, 2 Syzygy, 3 Gametocyst, 4 Oocysts. Scale bars 50 μ m (1–3), 10 μ m (4)

from all other three closely related species. The present form is almost rounded or ovoid and ectosarc is finely vacuolated but in case of *A. chotonagpurensis* ectosarc is very thin. Endosarc of the new species is densely vacuolated and widely varied from that of other three closely related species. The most important feature of the present form is the occurrence of syzygy in the life history, the

presence of cytoplasmic amalgamation during this stage which was not described in the three closely related species. The shape of the gametocyst is egg-like and ellipsoidal in *A. chotonagpurensis* and *A. goomtiensis* successively, but the shape of the present form is almost rounded. Oocyst of the present form is navicular whereas it is biconical in *A. chotonagpurensis*

Table 1 Morphometric comparison of *Apolocystis cognetti* sp. nov. with *A. chotonagpurensis* (Bandyopadhyay et al. 2004), *A. goomtiensis* (Pradhan and Dasgupta 1983), and *A. monokaryosomifeus* (Pradhan and Dasgupta 1983)

Characters	Species			
	<i>A. chotonagpurensis</i>	<i>A. goomtiensis</i>	<i>A. monokaryosomifeus</i>	<i>Apolocystis cognetti</i> sp. nov.
Host	<i>Amyntas robusta</i> (Perrier 1892)	<i>Phretima diffrenses</i> (Baired)	<i>Pheretima robusta</i> (Perrier 1892)	<i>Amyntas hawayanus</i>
Locality	Chotonagpur, Bihar	Goomti, Darjeeling, West Bengal	North point Darjeeling, West Bengal	Darjeeling, West Bengal
Site of infection	Seminal vesicle	Coelomic fluid	Coelom	Seminal vesicle
Trophozoite	Gamonts spherical, rounded or differentiation	Spherical	Spherical	Spherical or rounded
Size	42.0–83.0 µm	57.6–154.8 µm	61.2–115.2 µm	65.4–126.7 (91.8 ± 17.5) µm
Ectosarc	Very thin	Devoid of any process	Not covered by hairs	Finely vacuolated
Endosarc	Homogenously granular	Granular	Not stated	Densely vacuolated
Nucleus shape	Oval or spherical	Rounded or ellipsoidal	Spherical or eccentric	Rounded
Size nucleus	12.0–17.0 µm	10.2–28.8 µm × 14.4–25.2 µm	14.4–29.1 µm	12.2–20.4 (14.4 ± 2.2) µm
Gametocyst shape	Egg shaped	Ellipsoid	Not stated	Rounded
Size	71.0–99.0 × 46.0–83.0 µm	101.2–114.4 µm	Not stated	73.6–110.4 (93.3 ± 14.0) µm
Oocyst shape	Biconical	Biconical	Not stated	Navicular
Size	6.5–7.0 × 3.5–7.1 µm	3.5–7.1 µm	Not stated	3.8–5.3 × 7.7–10.7 µm
References	Bandyopadhyay et al. (2004)	Pradhan and Dasgupta (1983)	Pradhan and Dasgupta (1983)	Present study

(Bandyopadhyay et al. 2004), and in *A. goomtiensis* (Pradhan and Dasgupta 1983) and most importantly, the present form is larger than the other three described species. Considering all these differences, we consider this species of the genus *Apolocystis* new to science and designate it as *Apolocystis cognetti* sp. nov.

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