

Descriptions of Two New Species of Acephaline Gregarines (Protozoa: Apicomplexa: Eugregarinida), *Apolocystis chotonagpurensis* sp. n. and *Stomatophora janovyi* sp. n. from Earthworms (Annelida: Oligochaeta) of India

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Summary. During surveys of the endoparasitic acephaline gregarines in the Chotonagpur district of Bihar, seminal vesicles of earthworm *Amyntas robusta* were found to be infested with a new species of the genus *Apolocystis* Cognetti de Martiis, 1923, *A. chotonagpurensis* sp. n. Trophozoites of the new species are rounded or ovoidal in shape and measure 42-83 μm in diameter. Nucleus of the trophozoite is ovoid and measures 12-17 \times 8-16 μm . Gametocysts are ovoidal, enclose two unequal gamonts and measure 71-96 \times 46-83 μm . Oocysts are biconical measuring 6.5-7 \times 3.5-4 μm . A different species of earthworm *Amyntas hawayanus* collected from the hill region of Darjeeling district of West Bengal revealed the existence of a new species of the genus *Stomatophora* Drzhevetskii, 1907. *Stomatophora janovyi* sp. n. is petaloid shaped and measure 80-85 μm in diameter. Gametocysts are ovoid and measure 52-66 \times 33-39 μm and oocysts are navicular shaped with sharply pointed ends and measure 10-12 \times 6-7 μm .

Key words: Acephaline gregarines, *Apolocystis chotonagpurensis* sp. n., earthworm, seminal vesicles, *Stomatophora janovyi* sp. n.

INTRODUCTION

Aseptate gregarine fauna have been reported from various parts of the world including India. But especially in India the search is far from complete. While investigating acephaline gregarines in the oligochaete worms, seminal vesicles of earthworm *Amyntas robusta*

Perrier, 1872 collected from Chotonagpur district of Bihar were found to harbour an undescribed species of *Apolocystis* Cognetti de Martiis, 1923. A separate species of earthworm, *Amyntas hawayanus* Rosa, 1891 obtained from the hill region of Darjeeling district of West Bengal was infected with an undescribed species of *Stomatophora* Drzhevetskii, 1907, in their seminal vesicles. Little work has been done in India on the representatives of the genus *Apolocystis* and *Stomatophora* parasitizing the oligochaete worms. Only five species of the genus *Apolocystis* (Bhatia and Setna 1926, Pradhan and Dasgupta 1983) and six species of

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the genus *Stomatophora* (Hesse 1909, Pradhan and Dasgupta 1980, Roychowdhury and Halder 1984, Bandyopadhyay *et al.* 2001) have so far been described from India. In this paper taxonomic descriptions of two new species of acephaline gregarines of the genera *Apolocystis* and *Stomatophora*, as well as comparisons with previously species, are provided.

MATERIALS AND METHODS

Earthworms were collected and taken to the laboratory. They were dissected while alive and their seminal vesicles were carefully removed. These were placed on clean glass with a drop of 0.5 % NaCl solution. A thin film of the seminal fluid was drawn out on a slide covered with a cover slip for examination of live protozoans under a phase contrast microscope. The content of seminal vesicles was semidried and fixed in Schaudin's fluid (20 min). The fixed smears were stored in 70 % ethyl alcohol for removal of mercuric chloride. The slides were then passed through a descending series of alcohols (5 min each) and stored in distilled water. These were transferred to a 3% iron alum solution and stained with Heidenhain's haematoxylin solution (20 min). Differentiation (over night) was done with 1 % iron alum solution. The slides were then washed thoroughly, dehydrated in an ascending series of alcohol, cleared in xylene and mounted in Canada balsam.

TAXONOMY

Apolocystis chotonagpurensis sp. n. (Figs 1-4, Table 1)

Phylum: Apicomplexa Levine, 1977

Order: Eugregarinida Leger, 1900

Family: Monocystidae Bütschli, 1882

Subfamily: Monocystinae Bhatia, 1930

With the characters of genus *Apolocystis* Cognetti de Martiis, 1923, as given by Levine (1988); gamonts spherical, solitary, oocysts biconical. Trophozoite rounded or ovoidal in shape without polar differentiation. Diameter ranges from 42.0-83.0 (57.0 ± 19.0). Ectosarc very thin. Episarc fine with very fine rows of cytoplasm, which is the most characteristic feature in the species of *Apolocystis* arranged in concentric fashion. Nucleus large, elongated with deeply stained, round karyosome; lengths range from 12.0-17.0 (14.0 ± 2.0), widths from 8.0-15.0 (11.0 ± 3.0). Nucleus rather oval in young stages may also be spherical in immature stages. Gametocysts egg shaped; two unequal gametes present lengths range from 71.0-99.0 (77.0 ± 9.0), widths from 46.0-83.0 (62.0 ± 15.0). Endosarc homogeneously granu-

lar; nucleus nearly spherical but endosome not. Oocysts biconical; lengths range from 6.5-7.0 (6.0 ± 1.0) widths from 3.5-4.0 (3.0 ± 0.1).

Taxonomic summary

Type material: *Apolocystis chotonagpurensis* sp. n.

Host: *Amyntus robusta* Perrier, 1892

Type locality: India, Bihar, Chotonagpur (22° N; 84° E).

Symbiotype: Host AR-11/12/2002 deposited in the museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Site of infection: Seminal vesicles.

Prevalence: 6 of 12 (50%).

Elevation: 700 m above mean sea level.

Type material: The syntype no. AC/2-2002 deposited in the Zoological Survey of India (ZSI), Calcutta - 700016 (Catalogue No. 2407).

Etymology: The species name has been derived from the collection locality, Chotonagpur.

Remarks: Two species of *Apolocystis* have so far been described from the earthworm *Amyntus robusta*. These two species namely *A. akaryoseminiferus* and *A. monokaryoseminiferus* are known from India (Pradhan and Dasgupta 1983). Table 1 summarizes differences in morphometric characters of the three species of *Apolocystis*. Trophozoites of the present species are considerably smaller than the two previously described species. The gametocysts are ovoidal in the new species described, but gametocysts shape was not reported in the previously described species. Oocysts of *A. chotonagpurensis* differ from those of previously described species in having sharply pointed end instead of slightly flattened ends. The oocysts are biconical in the present species. The *Apolocystis* species treated in the present study is therefore considered new. The comparisons with previously described species are provided in Table 1.

Stomatophora janovyi sp. n. (Figs 5-12, Table 2)

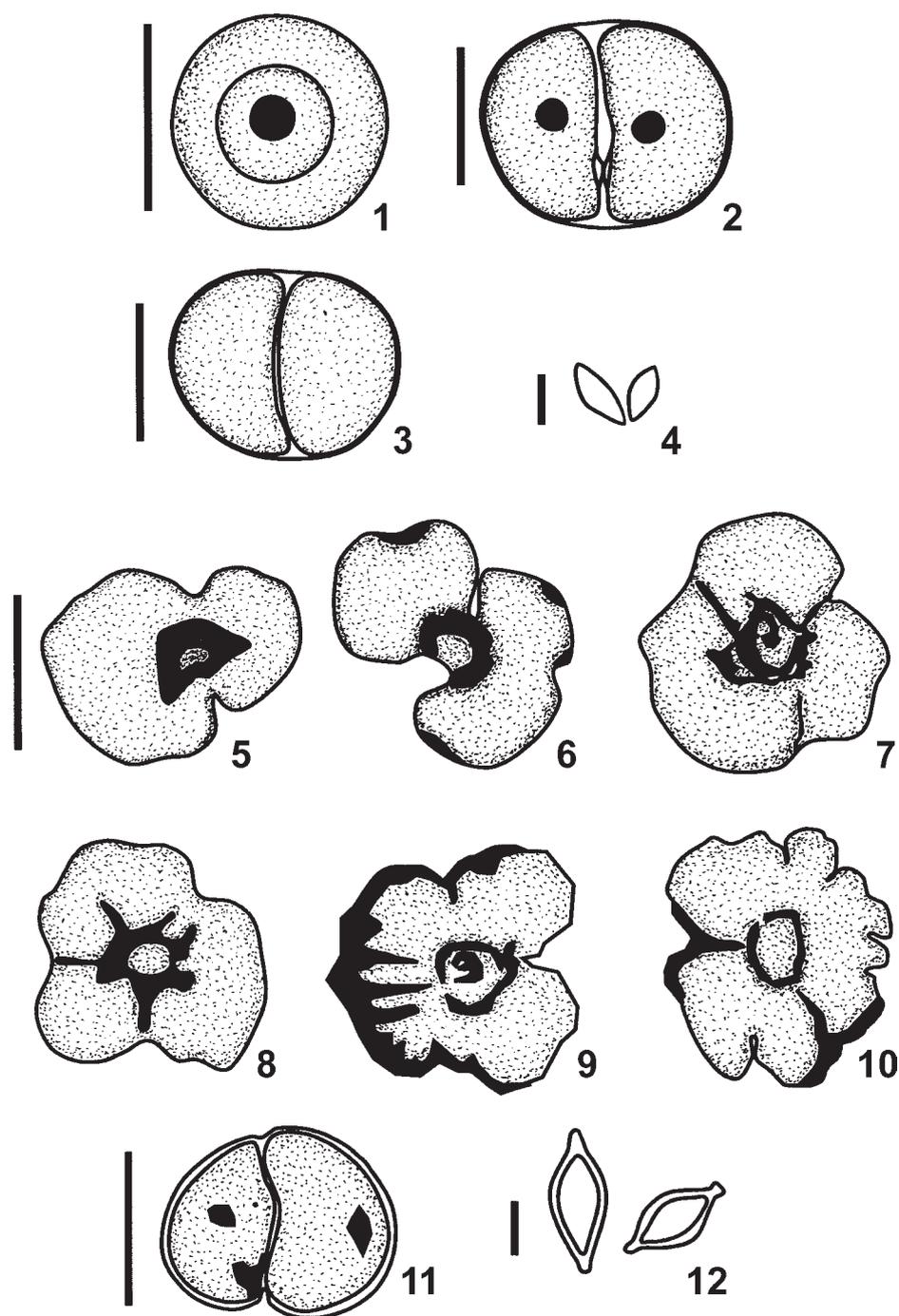
Phylum: Apicomplexa Levine, 1977

Order: Eugregarinida Leger, 1900

Family: Monocystinae Bütschli, 1882

Subfamily: Monocystinae Bhatia, 1930

With the characters of genus *Stomatophora* Drzhevetskii, 1907, as given by Levine (1988); gamonts spherical or ovoid; sucker petaloid, with radiating sides, oocysts biconical with a flattened button at each end, attached to each other end to end in long chains inside



Figs 1-4. Camera lucida drawings of different stages of *Apolocystis chotonagpurensis* sp. n. **1** - mature trophozoite; **2** - early gametocyst; **3** - late gametocyst; **4** - oocysts. Scale bars 5 μ m (4); 50 μ m (1-3).
Figs 5-12. Camera lucida drawings of different stages of *Stomatophora janovyi* sp. n. **5-8** - young trophozoites; **9-10** - mature trophozoites; **11** - gametocyst; **12** - oocyst. Scale bars 5 μ m (12); 50 μ m (5-11).

Table 1. Comparison of Indian species of *Apolocystis* from the seminal vesicles of the earthworm host *Amyntas robusta*. ni - not indicated. All measurements in microns (μm)

Parasite species/ Character	<i>A. akaryoseminiferus</i>	<i>A. monokaryoseminiferus</i>	<i>A. chotonagpurensis</i> sp. n.
Trophozoite shape	spherical, solitary	spherical, solitary	spheroidal or ovoid
Trophozoite size	54-100	61-115	42-83
Gametocyst shape	ni	ni	ovoidal
Gametocyst size	ni	ni	71-99 \times 46-83
Oocyst shape	biconical, ends slightly flattened	biconical, ends slightly flattened	biconical, ends sharply pointed
Oocyst size	ni	ni	6.5-7 \times 3.5-4
Reference	Pradhan and Dasgupta (1983)	Pradhan and Dasgupta (1983)	present paper

Table 2. Comparison of Indian species of *Stomatophora* from the seminal vesicles of the earthworm hosts. ni - not indicated. All measurements in microns (μm)

Parasite species/ Character	<i>S. bahli</i>	<i>S. globa</i>	<i>S. pedongensis</i>	<i>S. majumdari</i>	<i>S. janovyi</i> sp. n.
Trophozoite shape	discoidal; flattened between poles	spheroidal; flattened and compressed between poles	discoidal; flattened between poles	spheroidal; flattened and compressed between poles	discoidal; flattened and flower like
Trophozoite size	43-82	31-97	50-94	40-63	80-85
Gametocyst shape	ni	ni	spheroidal	ovoidal	ovoidal
Gametocyst size	ni	ni	90-119	90-130 \times 90-115	52-66 \times 33-39
Oocyst shape	ni	ni	navicular	navicular	navicular
Oocyst size	ni	ni	in	8 \times 5	10-12 \times 6-7
Host	<i>Amyntas diffringens</i>	<i>Pheretima alexandri</i>	<i>Amyntas diffringens</i>	<i>Metaphire posthuma</i>	<i>Amyntas hawayanus</i>
Soil type	podzol	podzol	podzol	alluvial	podzol
Elevation (m)	ni	ni	ni	ni	3323
References	Pradhan and Dasgupta (1980)	Pradhan and Dasgupta (1980)	Pradhan and Dasgupta (1980)	Bandyopadhyay <i>et al.</i> (2001)	present paper

gametocyst. Trophozoites solitary, flattened, flower-like and petaloid in appearance; diameter range from 80-85 (82.0 ± 2.0). Pellicle thin. Mucron is ring-like, centrally located, with some vacuolated areas range in size from 17-23 (20 ± 2.0). Nucleus round to slightly ellipsoidal; diameters from 9.0-14.0 (11.0 ± 3.0) typically located close to mucron; Cytoplasm densely granulated; epicyteal striations not distinct but in mature stages observed to extend from periphery towards mucron. Ectosarc very thin, without external processes. Gametocysts ovoidal; lengths range from 52.0-66.0 (60.0 ± 4); widths from

33.0-39 (37.0 ± 3.0). Each gametocyst encloses two gametes. Oocysts shape navicular, bluntly rounded; lengths range from 10.0-12.0 (12.0 ± 0.5); widths from 6.0-7.0 (6.0 ± 0.6).

Taxonomic summary

Type material: *Stomatophora janovyi* sp. n

Type host: *Amyntas hawayanus* Rosa, 1891

Symbiotype: Host AH - 03/22/2002 deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Site of infection: Seminal vesicles.

Type locality: Singamari, Darjeeling, W. Bengal, (Lat. 27°N, Lon. 88 °E).

Elevation: 3323 m above mean sea level.

Prevalance: 6 out of 22 (27%).

Type material: Syntypes on a single slide no. SJ/12 /02 deposited in the Zoological Survey of India (ZSI), Calcutta - 700016 (Catalogue No. 2408).

Etymology: The specific epithet “*janovyi*” is given after the name of Prof. John Janovy, Jr., of University of Nebraska, Lincoln, USA, for his outstanding contribution in the field of Apicomplexan biology.

Remarks: A new species of *Stomatophora* is described from an earthworm collected at high altitudes in podzol soil in Singamari. Pradhan and Dasgupta (1980) described three gregarine species, namely *S. bahli*, *S. pedogensis* in *Amyntas diffringens* and *S. globa* in *Metaphire alexandri* from the same locality, elevation and habitat; Singamari, Darjeeling, West Bengal, India. Bandyopadhyay and co-workers (2001) later reported *S. majumdari* from *M. posthuma* from an alluvial soil. Three of the five earthworm species belong to the genus that typically lives in podzol soil. The remaining two belong to the genus *Metaphire*, species of which live in either podzol soils (hilly areas) or alluvial soils (plains). Table 2 summarizes the characteristic features that distinguish the present form from the other mentioned species. Measurements of the trophozoites of *S. janovyi* are distinctly different from the four previously described species. The mucron of *S. janovyi* is larger in diameter in comparison with the smaller mucron in *S. bahli*. The diameter of the nucleus in *S. janovyi* is smaller than in *S. bahli*, *S. globa* and *S. pedogensis* but larger than the nucleus of *S. majumdari* and it contains some vacuolated areas. In *S. janovyi* the gametocyst is ovoid and smaller than in *S. pedogensis* and *S. majumdari*. Oocyst is similar in shape in all five

species but the measurements are distinctly longer than in both *S. pedogensis* and *S. majumdari*. Based on distinct differences in comparison with species previously reported from the Indian oligochaetes, a new species, *S. janovyi* is designated and named after the name of Prof. John Janovy Jr. of Nebraska University of U.S.A. The comparisons with previously described species are provided in Table 2.

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