

Dirhynchocystis indica (Apicomplexa, Rhynchocystinae), a new gregarine species from the earthworm *Lampito mauritii* (Annelida, Oligochaeta) in India

Probir K. Bandyopadhyay and Amlan Kumar Mitra

Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani, West Bengal, India

Summary

Dirhynchocystis indica, a new gregarine species from the earthworm *Lampito mauritii* (Annelida, Oligochaeta) from West Bengal, India, is described. Mature trophozoite has an elliptical body with two unequal elongate trunks, one at each pole. The short trunk has a rounded apex, and the long trunk is bluntly pointed. Gametocyst is spheroidal. Nucleus is spheroidal to ellipsoidal in shape, situated generally near one of the poles. Oocysts biconical.

Key words: gregarines, acephaline, earthworm, *Dirhynchocystis indica* sp. n., India

Introduction

Gregarines are chiefly coelozoic or lumen-dwelling protozoans of invertebrates, especially arthropods and annelids. Of the two major groups of gregarines, aseptate and septate, earthworms harbour the aseptate forms. The aseptate, or acephaline forms are characterized by a non-septate body. Aseptate gregarine fauna has been reported from various parts of the world including India. However, especially in India, the search is far from complete, although work on this particular group has gained momentum since 1980 (Bandyopadhyay et al.

2001, 2004, 2006a, 2006b, 2006c; Pradhan and Dasgupta, 1983a, 1983b; Roychoudhury and Haldar, 1984; Bandyopadhyay and Mitra, 2004, 2005a, 2005b, 2005c, 2005d). Further study of aseptate gregarines from Indian earthworms reveals a new species of the genus *Dirhynchocystis* Cognetti de Martiis 1921 (Martiis, 1921). Levine (1988) listed seven species under the genus *Dirhynchocystis*, only one of them described from India (Bhatia and Chatterjee, 1925). In this paper, taxonomic descriptions of a new species of genus *Dirhynchocystis*, as well as comparisons with closely related species, are given.

Material and Methods

Earthworms were collected and taken to the laboratory. They were dissected while alive and their seminal vesicles were carefully removed and 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 alcohol series (5 min each) and stored in distilled water. They were transferred to a 3% iron alum solution and stained with Heidenhain's haematoxylin solution (20 min). Differentiation (overnight) was done with 1 % iron alum solution. The slides were then washed thoroughly, dehydrated in an ascending alcohol series, cleared in xylene and mounted in Canada balsam. Photographs were taken with an Olympus camera. All measurements in this article are in micrometers. For each measurement, minimum and maximum values are given, followed in parentheses by arithmetic mean and standard deviation.

Results

Phylum: Apicomplexa Levine 1977
Order: Eugregarinida Leger 1900
Family: Monocystidae Bütschli 1882
Subfamily: Rhynchocystinae Levine 1977

Dirhynchocystis indica sp. n. (Figs 1-6; Tables 1, 2).

With the characters of the genus *Dirhynchocystis* Cognetti de Martiis 1921 (Martiis, 1921) as given by Levine (1988): gamonts metabolic, most often elongated into a conical or cylindroconical trunk, gamont with projections at both ends; oocysts biconical, in seminal vesicles and rarely coelom of Oligochaetes (Levine, 1988). The mature gamont has an elliptical body with two elongate trunks, one at each pole. The trunks are very unequal in length and parallel to the longitudinal axis of the body. The apex of the short trunk is rounded but that of the long trunk is bluntly pointed. The long trunk sometimes looks slightly angular, which might be due to distortion during smear preparation. The measurement of body length is taken as the distance between the bases of the two trunk-like appendages, and the measurement of width is taken at a right angle to this axis where the width is maximum. Ectoplasm is thin but prominent, about 1.0-1.5 µm thick all over the body except in the appendages. Endoplasm is granular and packed with homogenous granules. These granules

are restricted mostly to the trophozoite body but sometimes they are seen to invade the basal portion of the long trunk due to streaming movement of the endoplasm. The nucleus is spheroidal to ellipsoidal, situated within the body of the gamont, generally near one of the poles. Karyosome is diffused. Gametocyst is almost spheroidal. Oocysts biconical.

Measurements of gamonts and different body proportions are presented in Table 1.

Type material: *Dirhynchocystis indica* sp. n.

Type host: *Lampito mauritii*

Symbiotype: Host LM - 04/2003 deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Site of infection: Seminal vesicles.

Type locality: Dimond Harbour, South 24 Parganas, W. Bengal, (Lat. 22.11°N, Lon. 88.14°E).

Prevalence: 04 out of 19 (21.05%).

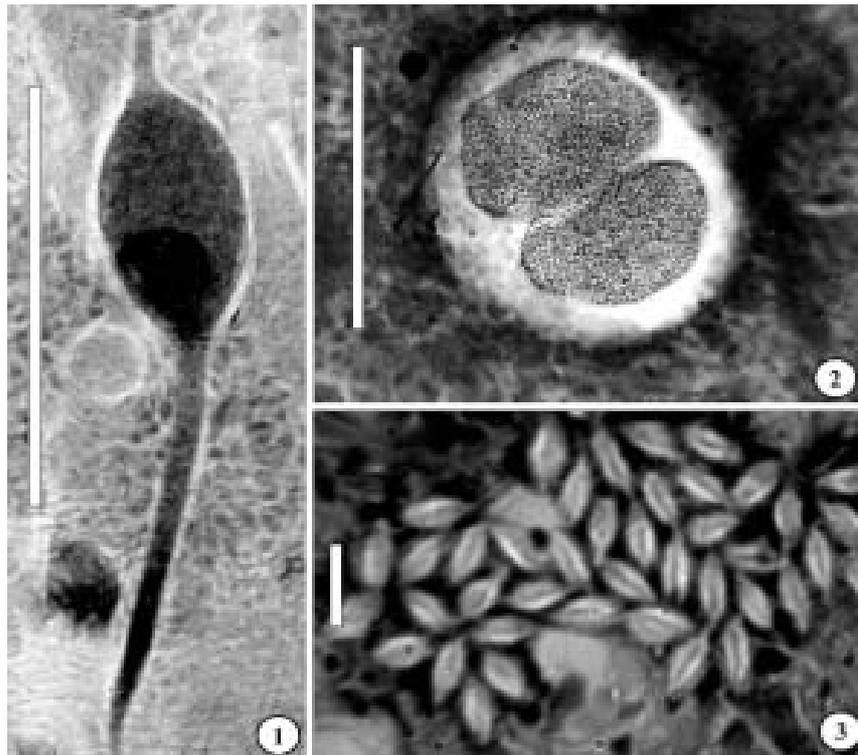
Type material: Holotype, slide DA/01/2003, and Paratypes, slides DA/02/2003, DA/03/2003 and DA/04/2003 are in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Etymology: The specific epithet '*indica*' has been derived from the name of our country.

Discussion

Being a parasite of earthworms seminal vesicle and in having ellipsoidal body with two cylindro-conical trunk-like appendages coming off the body, the parasite justifies its inclusion under the family Monocystidae, subfamily Rhynchocystinae and the genus *Dirhynchocystis* Cognetti de Martiis 1921 (Martiis, 1921). Seven *Dirhynchocystis* species have been described from all over the world. Only one of them, *Dirhynchocystis globosa*, is known from India. It was reported by Bhatia and Chatterjee (1925) from Lahore (now in Pakistan), Bombay and Lucknow. In *D. globosa* the body is globular and the long trunk is shorter than the body, whereas in the present form the body is ellipsoid and the long trunk is always longer than the body. In both species the trunks are unequal in size.

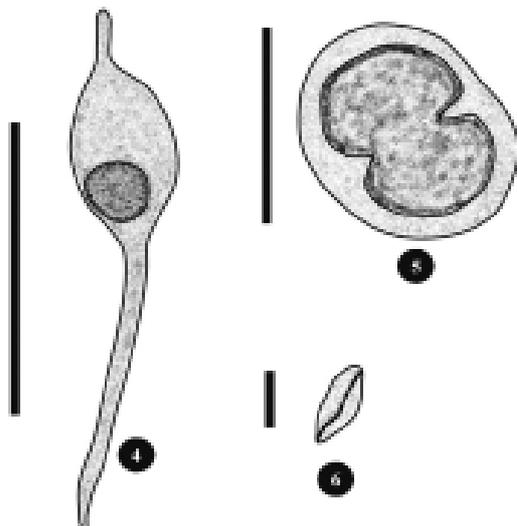
D. brasiliensis has an ovoid body, with equal trunks placed at an angle of about 130° to the body. The present form differs from this species in having a more elongate body and unequal trunks at the opposite poles. *D. oblongata* also possesses ovoid body with unequal trunks, like the present form, but differs in having a smaller nucleus and the trunks ending in rounded tips. *D. minuta* has a sub-spherical body and unequal trunks with rounded tips. But the present form has a larger body, and the ratio of large trunk and small trunk is 3:2 whereas the present form has a different type of



Figs 1-3. Photomicrographs of different stages of life cycle of *Dirhynchocystis indica* sp. n. 1 - a mature trophozoite; 2 - a gametocyst; 3 - oocysts. Scale bars: 1, 2 - 100 μ m, 3 - 10 μ m.

Table 1. Summary of measurements (μ m) of different body parts and ratios of 32 gamonts of *Dirhynchocystis indica* sp. n. obtained from *Amyntas robusta*.

Different body parts	Mean	Range	Standard deviation
Body length (BL)	62.5	48.4-88.0	1.8
Body width (BW)	33.4	22.0-48.4	2.6
Nucleus length (NL)	19.3	13.2-22.0	1.1
Nucleus width (NW)	13.6	6.6-17.6	2.8
Short trunk length (STrL)	21.0	13.2-57.2	1.9
Long trunk length (LTrL)	77.8	26.34-132.0	2.2
Short trunk Width (STrW)	5.9	3.3-8.8	1.7
Long trunk Width (LTrW)	7.8	3.3-13.2	3.1
BL/BW	1.9	1.6-2.5	2.4
NL/NW	1.5	1.0-2.5	1.9
BL/STrL	3.4	1.1-4.0	1.8
BL/LTrL	0.9	0.5-2.3	2.6
LTrL/STrL	4.2	1.5-7.3	3.5
BL/NL	3.3	2.6-4.4	1.8



Figs 4-6. Camera lucida drawings of different stages of life cycle of *Dirhynchocystis indica* sp. n. 4 - a mature trophozoite; 5 - a gametocyst; 6 - oocyst. Scale bars: 1, 2 - 100 μ m, 3 - 10 μ m.

Table 2. Comparisons between different characters of *Dirhynchocystis indica* sp. n. with closely related species (all measurements are in µm).

Characters	<i>D. brasiliensis</i> Cognetti de Martis 1921	<i>D. globosa</i> (Bhatia and Chatterjee 1925) Bhatia 1929	<i>D. minuta</i> Ruston 1959	<i>D. oblonga</i> Tuzet and Loubatiers 1946	<i>D. indica</i> sp. n.
Host(s)	<i>Fimoscolex inurus</i>	<i>Pheretima heterochaeta</i> and <i>Eutyphoeus</i> sp.	<i>Lumbricus terrestris</i>	<i>Octolasion complanatum</i>	<i>Lampito mauritii</i> (Kingberg)
Locality	Brazil	Pakistan and India	England	France	India
Trophozoite	Ovoid	Globular	Subspherical	Ovoid	Ellipsoid
Length	45-55	74.0	35.5	50.0	48.4-88.0 (62.5±1.8)
Width	55-80	65.0	31.2	75.0	22.0-48.4 (33.4±2.6)
Appendages (mucron)	Equal: round at tip	Nearly equal: short and spine-like	Unequal: round at tip	Unequal: round at end	Unequal: short arm round and long arm nearly pointed at tip
Length 1 (L 1)	25	—	43.4	—	13.2-57.2 (21.0±1.9)
Length 2 (L 2)	25	—	29.6	—	26.4-132.0 (77.8±2.2)
L1: L2	1:1	—	3:2	—	1: 4.2±1.6
Nucleus	Nearly round	Ovoid, monokaryosomic	—	—	Ovoid
Size	12 in diameter	24 in longer diameter	4.5	6-8	13.2-22.0 (19.3±1.1) by 6.6-17.6 (13.6±2.8)
ND or NL: BL	1: 4.2	1: 3.1	1:7.8	1: 7.1	1:3.3±0.5
Angle of appendages to body	130°	—	115°	—	180°
Gametocyst	—	—	—	—	Spheroidal
Size	—	—	—	—	65.0-88.0 (72.0±1.9)
Oocyst	—	—	—	—	Biconical
Size	—	—	—	—	7.8-11.3 (9.1±2.1)
Reference	Martis (1921)	Bhatia (1929)	Ruston (1959)	Tuzet and Loubatiers (1946)	Present study

attachment of the trunks to its body and the ratio of the length of long trunk: short trunk is 4.2:1.

After careful consideration the present form cannot be accommodated into any known species of the genus *Dirhynchocystis*. Therefore, a separate species has been proposed for the present form. It is designated as *Dirhynchocystis indica* sp. n. A comparison between *D. indica* and other closely related species is presented in Table 2.

References

Bandyopadhyay P.K. and Mitra A.K. 2004. Description of a new species *Zygocystis levinei* sp. n. (Eugregarinida, Zygocystidae), from the earthworm *Amyntas nicholsoni* (Oligochaeta) from West Bengal, India. Protistology. 3, 227-231.

Bandyopadhyay P.K. and Mitra A.K. 2005a. Observations on two new species of *Monocystis* Stein, 1848 (Protozoa: Apicomplexa: Monocystidae) *Monocystis darjeelingensis* sp. n. and *M. ranaghatensis* sp. n. from earthworms (Annelida: Oligochaeta) of West Bengal, India. Animal Biol. 55, 123-132.

Bandyopadhyay P.K. and Mitra A.K. 2005b. Observations on two new species of *Nematocystis* Hesse, 1909 (Protozoa: Monocystidae) from earthworms (Annelida: Oligochaeta) of West Bengal, India. Animal Biol. 55, 133-139.

Bandyopadhyay P.K. and Mitra A.K. 2005c. Description of a new species of *Zygocystis* Stein, 1848 (Protozoa: Zygocystidae) from the earthworm *Amyntas nicholsoni* (Annelida: Oligochaetasma) of West Bengal, India. Protistology. 4, 91-95.

Bandyopadhyay P.K. and Mitra A.K. 2005d. *Stomatophora cloptoni* sp. n. (Apicomplexa: Eugregarinida) from the seminal vesicles of an Indian Earthworm. Acta Protozool. 44, 385-388.

Bandyopadhyay P.K., Roychudhuri U.S. and Biswas G. 2001. *Stomatophora majumdari* sp. n. (Apicomplexa: Sporozoa) an acephaline gregarine in the seminal vesicles of earthworm of West Bengal, India. Uttarpradesh J. Zool. 21, 221-224.

Bandyopadhyay P.K., Roychudhuri U.S. and Biswas G. 2004. Descriptions of two new species of acephaline

gregarines (Protozoa: Apicomplexa: Eugregarinida), *Apolocystis chotonagpurensis* sp. n. and *Stomatophora janoyi* sp. n. from earthworms (Annelida: Oligochaeta) of India. Acta Protozool. 43, 275-279.

Bandyopadhyay P.K., Mitra A.K. and Mallik P. 2006a. Biology of *Monocystis clubae* sp. nov. (Apicomplexa: Eugregarinida) from an Indian earthworm *Lampito mauritii* (Annelida: Oligochaeta) of India. Zootaxa. 1120, 51-55.

Bandyopadhyay P.K., Mallik P. and Mitra A.K. 2006b. *Monocystis apporetodae* sp. nov. (Protozoa: Apicomplexa: Eugregarinida), from an Indian earthworm *Apporetodea trapezoides* Duges. Acta Parasitol Turcica. 51-53.

Bandyopadhyay P.K., Mallik P., Göçmen B. and Mitra A.K. 2006c. *Monocystis metaphirae* sp. nov. (Protista: Apicomplexa: Monocystidae) from the earthworm *Metaphire houlleti* (Perrier). Acta Parasitol Turcica. 54-56.

Bhatia B.L. and Chatterjee C.B. 1925. On some gregarine parasites of Indian earthworms. Arch. Protistenk. 52, 189-206.

Levine N.D. 1988. The Protozoan Phylum Apicomplexa, Vol. 1. CRC Press, Boca Raton, Florida, USA.

Martini C. 1921. Nuovo contributo alla conoscenza delle gregarines monocystidee. Monitore Zool. Ital. 31, 149-155.

Pradhan D. and Dasgupta B. 1983a. New acephaline gregarines (*Apolocystis*) in the hill areas of Darjeeling district. J. Beng. Nat. Hist. Soc. (N.S.) 2, 5-12.

Pradhan D. and Dasgupta B. 1983b. Life history and morphology of *Zygocystis indicus* n. sp. J. Beng. Nat. Hist. Soc. (N.S.) 2, 17-23.

Roychoudhuri U.S. and Haldar D.P. 1984. Studies in aseptate gregarines from earthworms of West Bengal; two new species of *Nematocystis* and one new species of *Stomatophora*. J. Beng. Nat. Hist. Soc. (N.S.) 3, 17-27.

Ruston J. 1959. *Dirhynchocystis minuta* n. sp. gregarine from the seminal vesicles of *Lumbricus terrestris* L. with a note on the association of *Rhynchocystis porrecta*. Schmidt. J. Parasit. 45, 259-261.

Tuzet O. and Loubatiers R. 1946. Notes sur les monocystidees. Arch. Zool. Exp. Gen. 84, 132-149.

Address for correspondence: Probir K. Bandyopadhyay. Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India. E-mail: prabir0432@hotmail.com

Editorial responsibility: Andrew Dobrovolskij