

***Monocystis elongatum* n.sp. (Protozoa: Apicomplexa:
Monocystidae) from seminal vesicles of the
earthworm *Perionyx excavatus* (Perrier) (Annelida: Oligochaeta)
in West Bengal, India**

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Abstract: As a part of the ongoing biodiversity survey of aseptate gregarines in earthworms, samplings were carried out in the East part of the river Ganges of the Murshidabad district. The survey revealed the occurrence of a new species of *Monocystis* Stein, 1848, namely *M. elongatum* n. sp. from the earthworm host *Perionyx excavatus* Perrier, 1872. The gamonts of *M. elongatum* n. sp. are solitary, elongated and curved at the anterior end and gradually tapering towards the posterior end. The mucron is distinct. Gamonts measure 265.0-346.0 μm (310.0 ± 2.6 , $n=36$) in length and 46.0-64.3 μm (52.0 ± 3.1 , $n=36$) in width. The gametocysts are unique shaped with two equal sized gametocytes, and measure 74.0-98.3 μm (82.0 ± 1.9 , $n=36$) in length and 56.0-82.0 μm (72.5 ± 2.7 , $n=36$) in width. The oocysts are bi-conical, measuring 9.7-15.3 μm (12.3 ± 3.1 , $n=36$) in length and 6.8-10.3 μm (9.4 ± 1.1 , $n=36$) in width.

Key Words: Gregarines, earthworm, seminal vesicles, new species,
Monocystis elongatum n.sp., India.

Introduction

Gregarines are a group of api-complexan protists and essentially parasites of invertebrates. Many members of the various groups of worms (especially annelids) harbour the parasites, and certain very interesting forms are found in echinoderms. Gregarines are chiefly coelozoic or lumen-dwelling protist parasites belonging to the order Eugregarinorida Léger, 1900. All the

members of the order Eugregarinorida are symbiotic. There are two major groups of gregarines: septate and aseptate. Arthropods tend to harbour the septate ones, while a number of other invertebrate taxa, including earthworms, harbour the aseptate gregarine forms. The majority of the gregarines reported so far are septate gregarines collected from insects.

A very few of the more than 350 earthworm species reported from India have been studied so far for the

occurrence of endoparasitic aseptate gregarine fauna, but since 1980 work has gained momentum on this group in India. Exploration of acephaline gregarine fauna inhabiting oligochaete hosts in India has resulted in discovery of representatives of the genera *Apolocystis* Cognetti de Martiis, 1923; *Monocystis* Stein, 1848; *Nematocystis* Hesse, 1909; *Stomatophora* Drzewiecki, 1907 and *Zygocystis* Bhatia, 1930 (Hesse 1909, Bhatia & Chatterjee 1925, Bhatia & Setna 1926, Kar 1946, Kalavati 1979, Subbarao *et al.* 1979, Pradhan & Dasgupta 1980a, b, 1982, 1983a, b, Roychoudhury & Halder 1984, Bandyopadhyay *et al.* 2001, 2004, 2005, 2006a, b, c, d, e, f, 2007a, b, Bandyopadhyay & Mitra 2004, 2005a, b, c, d).

The biodiversity survey on earthworms of the Murshidabad district from West Bengal revealed a new species of the genus *Monocystis* Stein, 1848 from the seminal vesicles of the *Perionyx excavatus* Perrier, 1872 earthworms. In this paper the description, taxonomy and systematic of *Monocystis elongatum* n.sp. and morphometric comparisons with closely related species are presented.

Materials and Methods

The Murshidabad district is located 10 meters above sea level (24°50'20"-23°43'30"N, 88°46'00"-87°49'17"E). The district comprises two distinct regions separated by the Bhagirathi River. Samplings were carried out in the alluvial soil in the east side of the river. Earthworms of different genera were collected during February-March 2007. The collected earthworms were kept in the soil in a plastic

bucket and taken to the laboratory alive. Some of the collected earthworms were dissected while alive and their seminal vesicles were carefully removed. These were placed on clean glass with a drop of 0.6 % NaCl solution. A thin film of seminal fluid was drawn out on a slide covered with a cover slip for examination of living protozoans under a phase contrast an Olympus CX41 microscope. After initial study of living protozoans, the content of the seminal vesicles was semidried and fixed in Schaudin's fluid for 20 minutes. The smears were stored in 70% ethanol for removal of mercuric chloride. The slides were then passed through a descending series of alcohol (5 minutes each) and placed in distilled water. These were transferred to a 3% iron alum solution (overnight) and stained with Heidenhain's hematoxylin solution for 20 minutes. 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 alcohol, cleared in xylene and mounted in Canada balsam. Camera Lucida drawings of different stages of gregarines were made and photomicrographs were taken with an Olympus phase contrast microscope (x400 magnification) and a C5060 Olympus camera. All measurements are in micrometers (μm). In each case minimum and maximum values are given, followed in parentheses by arithmetic mean and standard deviation. In all cases, sample size was $n=36$. Method of describing shapes of planes and solids is mainly according to Clopton (2004).

Results

Monocystis elongatum n.sp. (Figs. 1-5, 6-8, Tab.1)

Description:

Gamont length: 265.0-346.0 (310.0 ± 2.6); gamont width: 46.0-64.3 (52.0 ± 3.1); nucleus length: 12.3-20.5 (19.4 ± 1.7); nucleus width: 11.4-18.7 (16.3 ± 1.8);

gametocyst length: 74.0-98.3 (82.0 ± 1.9); gametocyst width: 56.0-82.0 μm (72.5 ± 2.7); oocyst length: 9.7-15.3 (12.3 ± 3.1); oocyst width: 6.8-10.3 (9.4 ± 1.1).

The members of the genus *Monocystis* are characterized by having no distinct mucron, ovoid and solitary gamonts, and bi-conical, symmetrical oocysts (Levine, 1988). In case of the present forms the gamonts are solitary, elongated, curved at the anterior end and gradually tapering towards the posterior end. The mucron is significantly prominent. Nucleus rounded to ovoid with variable position within the gamont. Pellicle thin. Ectoplasm smooth. Endoplasm dense with presence of vacuoles and paraglycogen granules. Each gametocyst contains two equal sized gametocytes. The outer layer of gametocytes is constricted. Oocysts are bi-conical.

Taxonomic summary:

Phylum Apicomplexa Levine, 1988;
Order Eugregarinorida Léger, 1900;
Family Monocystidae Bütschli, 1882;
Subfamily Monocystinae Bhatia, 1930;
Genus *Monocystis* Stein, 1848

Type host: *Perionyx excavatus* Perrier, 1872

Type material: *Monocystis elongatum* n.sp.

Type locality: Murshidabad, West Bengal, India. ($24^{\circ}50'20''$ - $23^{\circ}43'30''$ N, $88^{\circ}46'00''$ - $87^{\circ}49'17''$ E)

Symbiotype: PE/12/07 deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India.

Site of infection: Seminal vesicles.

Prevalence: 19/78 (25.4 %)

Holotype: ME/09/07 deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Paratypes: ME/11/07 and other slides are in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

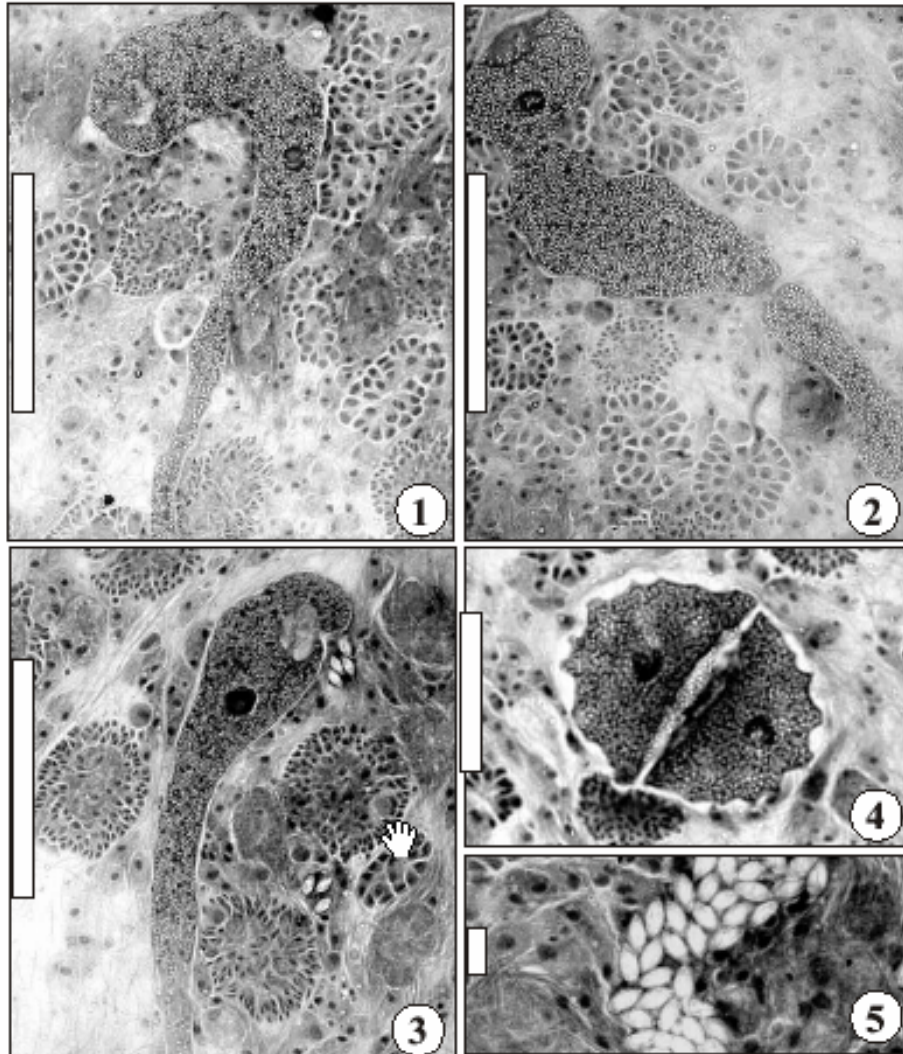
Etymology: The species has been named depending on the shape of the gamonts.

Discussion

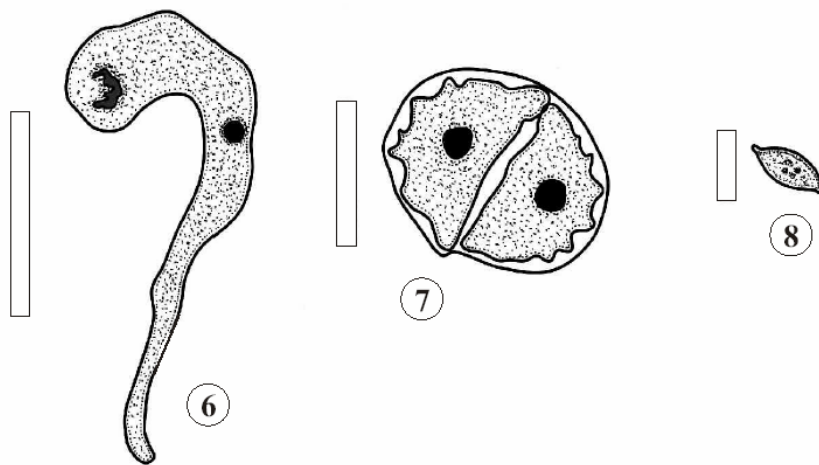
Gamonts that are cylindroid, solitary, with mucron, and exhibiting late syzygy of the parasite in an earthworm justify the inclusion of the present species under the family Monocystidae, subfamily Monocystinae, genus *Monocystis*. From more than 70 species (Levine 1988) belonging to the genus *Monocystis* only fifteen have been described from India. Among these species, *M. odontotermi* Kalavati, 1979 has been reported from the haemocoel of the termite *Odontotermes obesus* (a non oligochaete host) and *M. pontodrilus* Subbarao, Kalavati & Narasimhamurti, 1979 from the littoral oligochaete *Pontodrilus bermudensis*. All other species of *Monocystis* have been reported from terrestrial earthworms, but none from the present host *Perionyx excavatus*. *M. elongatum* n. sp. shows great morphological variability when compared with other species of the

genus *Monocystis* from India and abroad, and resembles only with *M.*

metaphirae Bandyopadhyay, Mallik, Göçmen & Mitra 2006.



Figures 1-5. Photomicrographs of different stages of the life cycle of *Monocystis elongatum* n. sp. obtained from the seminal vesicles of the earthworm *Perionyx excavatus* (Perrier)
Figs 1-3: Mature gamonts, **Fig. 4:** A Gametocyst, **Fig 5:** Oocysts. (Figs 1-3, scale bars = 100 µm; Fig.4, scale bar = 50 µm; Fig. 5, scale bar= 10 µm).



Figures 6-8. Camera lucida drawings of different stages of the life cycle of *Monocystis elongatum* n. sp. obtained from the seminal vesicles of the earthworm *Perionyx excavatus* (Perrier)

Fig. 6: Mature gamont, **Fig. 7:** A Gametocyst, **Fig. 8:** an Oocyst. (Fig. 6, Scale bar = 100 μ m; Fig. 7, scale bar = 50 μ m; Fig. 8, scale bar= 10 μ m).

The gamonts of *M. metaphirae* are bean shaped, and gradually taper towards the posterior end with blunt point (Bandyopadhyay et al. 2006e), while the gamonts of the new species are more elongated, curved and constricted at the anterior end and taper sharply to the posterior end. The gamonts of *M. metaphirae* are much smaller than those of the new species (Tab.1). The major difference is the presence of syzygy stage in the life cycle of *M. metaphirae* which is totally absent in the species described here. The shape of the nucleus of both species does not vary significantly, although the size of the nucleus of the new species is much larger than that of *M. metaphirae* (Tab.1). The gametocyst of *M. metaphirae* consist of two unequal

sized gametocytes, but that of *M. elongatum* n.sp. consists of two equal sized gametocytes. Although the size of the gametocysts does not vary significantly, the shape of the gametocytes of the new species is typical for *Monocystis*. The gametocyte membrane is constricted along the entire periphery in the gametocysts of the new species, but is smooth in *M. metaphirae*. The oocysts in both species are bi-conical, although the oocysts of the new species are much larger in size.

Based on morphology and morphometrics, no other species belonging to the genus *Monocystis* resembles the new species, hence the species under discussion is designated as *Monocystis elongatum* n.sp.

Table 1. Morphometric comparison of *M. elongatum* n.sp. with *M. metaphirae* Bandyopadhyay, Mallik, Göçmen and Mitra, 2006

Species	<i>M. metaphirae</i> Bandyopadhyay, Mallik, Göçmen and Mitra, 2006	<i>M. elongatum</i> n.sp. (n=36)
Characters		
Host	<i>Metaphire houlleti</i> Perrier, 1872	<i>Perionyx excavatus</i> Perrier, 1872
Type locality	Calcutta	Murshidabad, West Bengal
Gamonts	Bean shaped rectangular gamont, solitary. The gamonts taper towards the posterior end with blunt ends, measuring 94.0-115.0 (119.0) × 53.0-81.0 (66.0) µm, occasionally showing end to end syzygy in the life cycle	Elongated and curved body which tapers towards its posterior end with sharply pointed ends, measuring 265.0-346.0 (310.0) × 46.0-64.3 (52.0) µm. No syzygy stage has been detected in the life cycle
Mucron	Distinct, at the anterior end of the body	Distinct, at the anterior end of the body
Ectoplasm	Smooth	Smooth
Endoplasm	Granular with numerous paraglycogen granules	Highly granular and vacuolated with a large number of paraglycogen granules
Nucleus	Rounded with vacuolated nucleoplasm, 4.0-16.0 (11.0) in diameter	Rounded to ovoid nucleus, measuring 12.3-20.5 (19.4) × 11.4-18.7 (16.3)
Gametocyst	Ovoid with two unequal gametocytes, 85.0- 102.0 (93.0) in diameter	Ovoid with two equal gametocytes, measuring 74.0-98.3 (82.0) × 56.0-82.0 (72.5)
Oocyst	Navicular, measuring 6.5-11.0 (9.0) × 4.0-7.5 (5.5)	Biconical, measuring 9.7-15.3 (12.3) × 6.8-10.3 (9.4)
Site of Infection	Seminal vesicle	Seminal vesicle
References	Bandyopadhyay et al. (2006e)	Present paper

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Submitted: 11 February 2008
/ Accepted: 13 April 2008