

Monocystis septum sp. n. (Protozoa: Apicomplexa: Monocystidae) from seminal vesicles of earthworms (Annelida: Oligochaeta) in West Bengal, India

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Summary

As a part of the ongoing biodiversity survey of aseptate gregarines in earthworms, samplings were carried out in the Murshidabad district of the state of West Bengal (India). The survey revealed a new species of the genus *Monocystis* Stein, 1848, *M. septum* sp. n., in the earthworm host *Eutyphoeus orientalis* (Beddard). The gamonts of *M. septum* are rectangular, slightly tapering towards the anterior and the posterior end. The unique feature of the gamonts is the presence of a constriction at the anterior body end that tends to divide the cell into two parts, similarly to septate gregarines. The gamonts measure 86.3–102.3 μm (90.4 ± 4.2 , 36) in length and 66.1–89.3 μm (78.2 ± 0.9 , 36) in width. The gametocyst consists of two gametocytes of unequal size and peculiar shape: one is axe-like, the other semicircular. The gametocysts measure 46.1–78.6 μm (64.2 ± 1.2 , 36) in length and 39.7–68.4 (62.4 ± 1.8 , 36) in width. Bi-conical oocysts measure 6.4–9.3 μm (8.2 ± 0.9 , 36) in length and 5.5–7.6 μm (6.1 ± 2.1 , 36) in width.

Key words: gregarines, earthworm, seminal vesicles, systematics, *Monocystis septum* sp. n.

Introduction

Gregarines are a group of apicomplexan protozoa parasitizing invertebrates. Of the two major groups of eugregarines, septate and aseptate, the former tend to parasitize arthropods, while the latter, other invertebrates, including earthworms. The majority of the gregarines reported so far are septate gregarines from insects.

In the course of the ongoing survey of the aseptate gregarines in India, 15 species of *Monocystis*

have been reported. Out of them, 13 species have been reported from oligochaete hosts. The prospects of finding new gregarine species from the new earthworm hosts are immense.

Only a few of over 350 species of Indian earthworms have been studied for the occurrence of gregarines, but this research has gained momentum since the 1980ies. It has already resulted in the finding of representatives of the genera *Apolocystis* Cognetti de Martiis, 1923; *Monocystis* Stein, 1848; *Nematocystis* Hesse, 1909; *Stomatophora* Drzew-

iecki, 1907 and *Zygocystis* Bhatia, 1930 (Hesse, 1909; Ghosh, 1923 – cited by Levine, 1988; Bhatia and Chatterjee, 1925; Bhatia and Setna, 1926; Kar, 1946; Kalavati, 1979; Subbarao et al., 1979; Pradhan and Dasgupta, 1980a, 1980b, 1982, 1983a, 1983b; Roychoudhury and Haldar, 1984; Bandyopadhyay et al., 2001, 2004, 2005, 2006a, 2006b, 2006c, 2006d, 2006e, 2007a, 2007b; Bandyopadhyay and Mitra, 2004, 2005a, 2005b, 2005c, 2005d, 2006a).

The biodiversity survey of gregarines from earthworms in the Murshidabad district of West Bengal revealed a new species of the genus *Monocystis* Stein, 1848 from the seminal vesicles of the earthworm *Eutyphoeus orientalis* (Beddard). This paper presents the description of *M. septum* sp. n., the morphometric comparison with closely related species and the discussion of its taxonomy and systematics.

Material and Methods

The Murshidabad district (latitude: 24°50'20''–23°43'30'' N, longitude: 88°46'00''–87°49'17'' E) is located 10 meters above the sea level. It is separated by the river Ganges into two distinct regions. Samplings were carried out in the alluvial soil on the east side of the river Ganges. Different species of earthworms were collected during February–March, 2007. The collected individuals were put in soil-filled plastic buckets and taken to the laboratory alive. Some of them 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 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 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 (×400 magnification) with an Olympus camera (Model C5060). All measurements are in micrometres; 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

DESCRIPTION OF *MONOCYSTIS SEPTUM* SP. N. (Figs 1–8, Table 1).

Phylum: Apicomplexa Levine, 1988; Order: Eugregarinorida Läger, 1900; Family: Monocystidae Břitschli, 1882; Subfamily: Monocystinae Bhatia, 1930; Genus: *Monocystis* Stein, 1848.

Gamont Length (GL): 86.3–102.3 µm (90.4 ± 4.2); Gamont Width (GW): 66.1–89.3 µm (78.2 ± 0.9); Nucleus Length (NL): 11.3–16.3 µm (14.3 ± 1.7); Width of Nucleus (WN): 8.5–10.6 µm (9.4 ± 1.1); Length of Gametocyst (LG): 46.1–78.6 µm (64.2 ± 1.2); Width of Gametocyst (WG): 39.7–68.4 µm (62.4 ± 1.8); Oocyst Length (OL): 6.4–9.3 µm (8.2 ± 0.9) µm; Oocyst Width (WO): 5.5–7.6 µm (6.1 ± 2.1) µm.

The monocystid gamonts obtained in the present study from the earthworm host *Eutyphoeus orientalis* (Beddard) are solitary and without any distinct mucron. The gamonts are almost rectangular, slightly tapering towards the anterior and the posterior body end. The significant characteristic feature of the gamont is a constriction that appears to divide the cell body into two halves, so that the gamont looks superficially like a septate gregarine. This constriction was present in almost all the mature gamonts obtained. In some immature forms constrictions were not very prominent. Pellicle thick. Nucleus rounded to ovoid, with variable position in the cytoplasm. Ectoplasm smooth. Endoplasm dense with vacuoles and paraglycogen granules. Gametocyst consists of two gametocytes of different size and peculiar shape: one is axe-like, the other semicircular. Oocysts bi-conical.

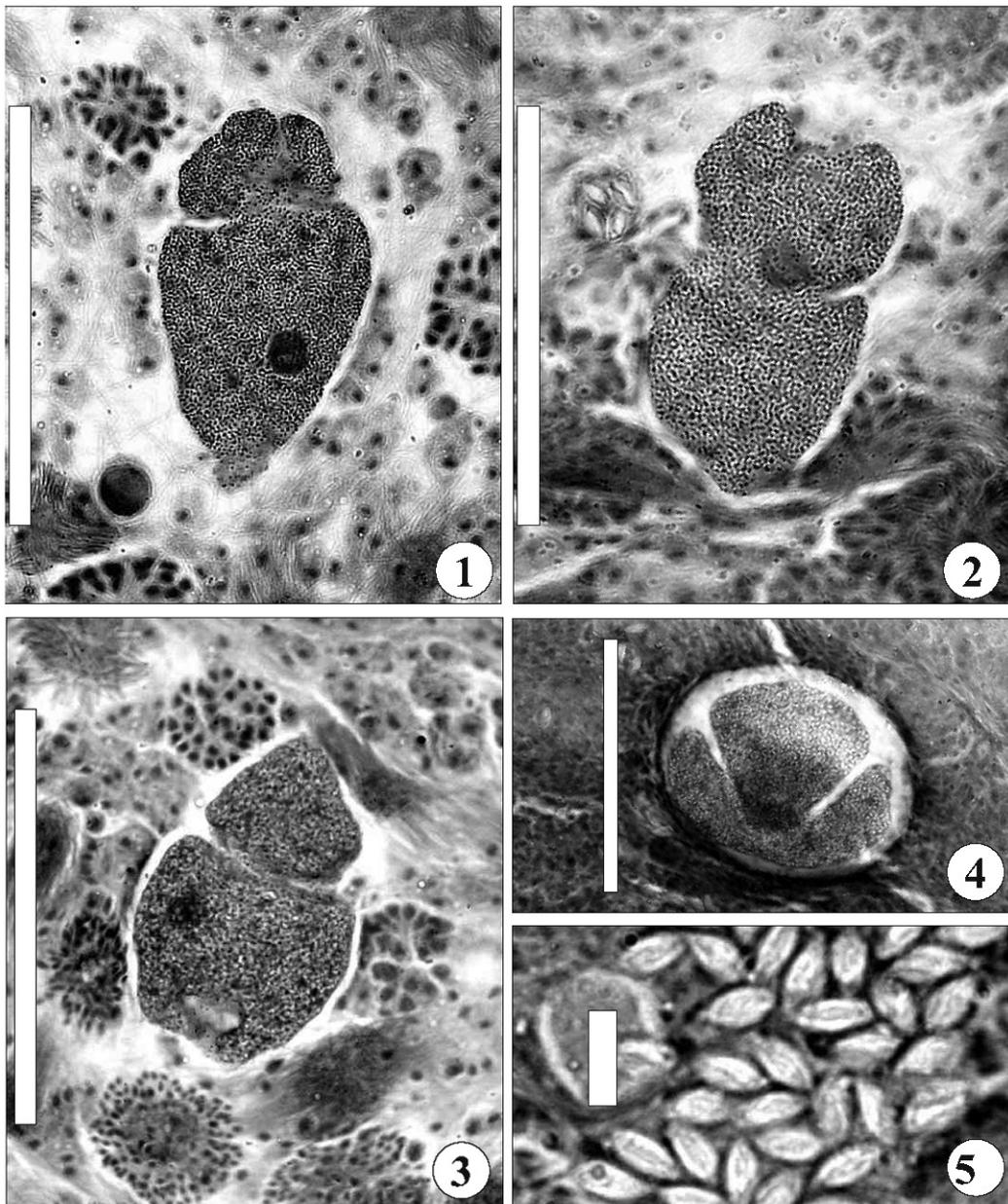
Type material: *Monocystis septum* sp. n.

Type host: *Eutyphoeus orientalis* (Beddard)

Type of locality: Murshidabad, West Bengal, India. (latitude: 24°50'20''–23°43'30'' N, longitude: 88°46'00''–87°49'17'' E).

Symbiotype: EO/03/2007 deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India

Site of infection: Seminal vesicles



Figs 1-5. Photomicrographs of different stages of the life cycle of *Monocystis septum* n. sp. obtained from the seminal vesicles of the earthworm *Eutyphoeus orientalis* (Beddard). 1-3 – Mature gamonts; 4 – a gametocyst; 5 – oocysts. Scale bars: 1-3 – 100 μ m, 4 – 50 μ m, 5 – 10 μ m.

Prevalence: 14/51 (27.4%)

Holotype: MS/010/07 is deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India

Paratypes: MS/03/07 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 name reflects the presence of the constriction on the cell body

The monocystid gregarine under discussion has cylindroid, solitary gamonts with a mucron and with late syzygy; it was found in an earthworm host. Therefore, it should be included into the genus *Monocystis* Stein, 1848. The present form was compared with the other known species of the genus *Monocystis* Stein, 1848 and was found to resemble only *M. amynthae* Bandyopadhyay, Göçmen, Bhowmik and Mitra, 2006 and *M. arabindae* Bandyopadhyay, Mallik and Mitra, 2007.

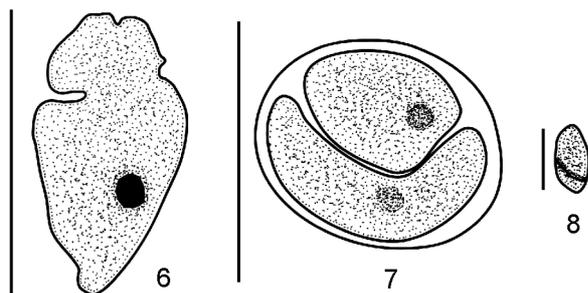
Table 1. Morphometric comparison of *Monocystis septum* sp. n. with *M. amynthae* and *M. arabindae* *.

Species Characters	<i>M. amynthae</i> Bandyopadhyay, Guzmen, Bhowmik and Mitra, 2006	<i>M. arabindae</i> Bandyopadhyay, Mallik and Mitra, 2007	<i>M. septum</i> sp. n. (n = 36)
Host	<i>Amyntas hawayanus</i> Rosa, 1891	<i>Eutyphoeus incommodus</i> (Beddard)	<i>Eutyphoeus orientalis</i> (Beddard)
Locality	Darjeeling, West Bengal	West Midnapur, West Bengal	Murshidabad, West Bengal
Gamonts	Elongated with irregular outline and a prominent constriction between the anterior and posterior end. The broad end of the gamont with a cup-like depression, measuring 49.0– 77.0 (66.0) × 32.0–41.0 (77)	Solitary, cylindrical, curved due to presence of constriction at one lateral surface, measuring 127.0– 145.5 (137.1) × 45.1–53.3 (49.3)	Solitary, rectangular, with a constriction on the body imparting resemblance with septate gregarines, measuring 86.3–102.3 (90.4) × 66.1– 89.3 (78.2)
Ectoplasm	–	Granular	Smooth
Endoplasm	Vacuolated with paraglycogen granules	Densely granular	Dense with vacuoles and paraglycogen granules
Nucleus	Oval, situated in the middle of gamont, measuring 6.0–12.0 (9.5)	Rounded, with terminal karyosome, situated in the middle of gamont, measuring 14.3–20.5 (17.3) × 12.3–18.9 (16.1)	Rounded to ovoid, measuring 11.3–16.3 (14.3) × 8.5–10.6 (9.4)
Gametocyst	Rounded with two equal gametocytes, measuring 40.0–65.0 (58.0)	Ellipsoidal, measuring 69.7–84.0 (76.2)	Ovoid with two unequal gametocytes, measuring 46.1–78.6 (64.2) × 39.7–68.4 (62.4)
Oocyst	Biconical, measuring 4.0–6.0 (5.5)	Biconical, measuring 18.4–28.6 (23.5) × 8.0–13.9 (11.9)	Biconical, measuring 6.4–9.3 (8.2) × 5.5–7.6 (6.1)
Site of Infection	Seminal vesicles	Seminal vesicles	Seminal vesicle
References	Bandyopadhyay et al., 2006e	Bandyopadhyay et al., 2007b	Present study

* All measurements are in μm . In each case minimum and maximum values are given, followed in parentheses by the arithmetic mean; n – the number of specimens measured.

The gamonts of *M. amynthae* (Bandyopadhyay et al., 2006e) are elongated, with an irregular outline; a constriction at the posterior end is present. (In *M. septum* sp. n. there is a prominent constriction at the anterior body end; as a result, the gamont looks septate. Although the gamonts of *M. amynthae* have a constriction of the cell body, it never appears to form a septum at any stage). In *M. septum* sp. n. there is a prominent constriction at the anterior body end, so that the gamont looks septate. Although the gamonts of *M. amynthae*

have a constriction of the cell body, it never looks like a septum at any stage. The size of the gamonts varies significantly between the species (Table 1): those of *M. amynthae* are much smaller than those of the new species. The nucleus size also varies significantly: it is larger in *M. septum* than in *M. amynthae*. The gametocysts of *M. amynthae* contain two gametocytes of almost the same shape and size, while the gametocysts of the new species contain two gametocytes of different shape and size. In both species, the oocysts are bi-conical, although



Figs 6-8. Camera lucida drawings of different stages of the life cycle of *Monocystis septum* n. sp. obtained from the seminal vesicles of the earthworm *Eutyphoeus orientalis* (Beddard). 6 – Mature gamont; 7 – a gametocyst; 8 – an oocyst. Scale bars: 6 – 100 μm , 7 – 50 μm , 8 – 10 μm .

those of the new species are larger (6.4–9.3 μm) than those of *M. amynthae* (4.0–6.0 μm).

The gamonts of *M. arabindae* (Bandyopadhyay et al., 2007b) resemble the gamonts of *M. septum* only in having a constriction; otherwise the overall morphology is completely different. The gamonts of *M. arabindae* are larger than those of the new species (Table 1). In the gamonts of *M. arabindae*, the constriction appears only on one side of the body, whereas in the new species the constriction appears on two sides. The mucron is distinct in *M. septum*, but is absent in *M. arabindae*. The shape and size of the nucleus are similar in these two species. The shape of the gametocytes is different in *M. arabindae* and the new species: in the former they are of the same shape, and in the latter, of different shape. Oocysts are much smaller in *M. septum* than in *M. arabindae*.

Judging by the morphology and morphometrics, no other species belonging to the genus *Monocystis* Stein, 1848 resembles the species under discussion. Hence, we designate it as *Monocystis septum* sp. n.

Discussion

The genus *Monocystis* Stein, 1848 belongs to the suborder Aseptatorina Chakravarty, 1960 with the following characters: gamonts without septa, some species with mucron and syzygy present in all the cases. Most of the *Monocystis* species that we reported from India are without a prominent mucron and do not show the syzygy stage in their life cycle. But obviously there are a few exceptions, for example, in *M. elongatum* a very prominent mucron is present. Such a prominent mucron is very rare

in other *Monocystis* species. Interestingly, we have not found any syzygy stage in the life cycle of either *M. elongatum* or *M. septum*. Only *M. metaphirae* (Bandyopadhyay et al., 2006d) has a very prominent syzygy stage in the life cycle. With the discovery of new species, new characters would probably be added to the taxonomic description of the genus.

The aseptate gregarines remain largely enigmatic due to simple neglect. Further surveys will obviously expose the taxonomic diversity of this group in the annelid hosts.

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