UTTAR PRADESH JOURNAL OF ZOOLOGY

42(20): 128-145, 2021
ISSN: 0256-971X (P)

A NOVEL TAXONOMICAL DESCRIPTION OF FIVE NEW SPECIES OF ACEPHALINE GREGARINES (PROTOZOA: APICOMPLEXA: GREGARINOMORPHEA) OF THE GENUS *Monocystis* STEIN, 1848 FROM EARTHWORMS OF WEST BENGAL, INDIA

BIPLAB BHOWMIK[1*], BEAUTY KUNDU[2] AND PROBIR KUMAR BANDYOPADHYAY[2,3]

[1]Department of Zoology, Diamond Harbour Women's University, Diamond Harbour – 743368, West Bengal, India.

[2]Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India.

[3]Our supervisor Prof. Probir Kumar Bandyopadhyay died prior to manuscript submission; we therefore dedicate this paper to him.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Pınar Oguzhan Yildiz, Ataturk University, Turkey.
Reviewers:
(1) Biswaranjan Ray, Gayatri College of Pharmacy, India.
(2) Mahmoud Mohammed Elalfy Elhefnawy, Mansoura university, Egypt.

Received: 20 July 2021
Accepted: 30 September 2021
Published: 08 October 2021

Original Research Article

ABSTRACT

The present communication is devoted to describe five new species of acephaline gregarines (Apicomplexa: Gregarinomorphea) of the genus *Monocystis* namely *Monocystis elliptoidum* sp. nov., *Monocystis pontoscolexae* sp. nov., *Monocystis dolium* sp. nov., *Monocystis lomentum* sp. nov., and *Monocystis asmati* sp. nov. have been found from the seminal vesicles of earthworms *Metaphire posthuma, Pontoscolex corethrurus, Eutyphoeus waltoni, Eutyphoeus orientalis,* and *Perionyx excavatus* respectively. *Monocystis elliptoidum* sp. nov. is characterized by elliptoid trophozoites, rounded nucleus, orbicular gametocysts and rhomboid oocysts. *Monocystis pontoscolexae* sp. nov. have elongated, obpyriform-shaped trophozoites, rounded nucleus, fusiform shaped gametocysts and fusiform oocysts. *Monocystis dolium* sp. nov. is characterized by doliform trophozoites, rounded nucleus, orbicular gametocysts and fusiform oocysts. *Monocystis lomentum* sp. nov. have lomentiform shaped trophozoites, rounded nucleus and fusiform oocysts. Trophozoites of *Monocystis asmati* sp. nov. are obpanduriform, nucleus rounded and oocysts fusiform. The relationship of these *Monocystis* sp. nov. with their closely related species have been presented and their diagnoses were also discussed using morphometrics.

**Corresponding author: Email: panchakotbb@gmail.com;*

Keywords: *Monocystis elliptoidum; Monocystis pontoscolexae; Monocystis dolium; Monocystis lomentium*; *Monocystis asmati.*

1. INTRODUCTION

Earthworms are one of the most significant soil fauna, capable of increasing soil fertility and playing an important role in agriculture. But these earthworms are facing lots of problem due to gregarine parasitic infestation.

Gregarines belong to Phylum Apicomplexa, under the subkingdom protozoa are a diversified group of organisms infecting invertebrates. Gregarine apicomplexans are obligate endoparasites that infect the intestines, coelomic cavities and reproductive organs of terrestrial, freshwater and marine invertebrates. Adl et al. [1] proposed that gregarine groups recognized as three major groups i.e., archigregarines, eugregarines and neogregarines. Eugregarines are of two types' the aseptate or acephaline gregarines and septate or cephaline gregarines. Aseptate form harbours mainly in oligochaete host. Monocystidae is a common acephaline endoparasite of earthworms and required only single host to complete their life cycle.

Till date only 96 species of acephaline gregarines under the genus *Monocystis* have so far been recorded from different parts of the world [2-53] (Bandyopadhyay et al. 2005).

The ongoing survey revealed five new species of genus *Monocystis* Stein [2] from the seminal vesicles of earthworms. The aim of the present study is to describe these new species and the stages of their life cycle by improving our knowledge of acephaline gregarine fauna of earthworms of West Bengal, India.

2. MATERIALS AND METHODS

Earthworms collected from the districts of Murshidabad, Paschim Medinipur, Bardhaman and Nadia of West Bengal, India. They were transported alive in a plastic bucket filled with soil to the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani, West Bengal, India.

After, dissection of the earthworm seminal vesicles was carefully removed and immersed in 0.62 % NaCl solution. A thin film of seminal fluid made on a slide covered with a coverslip. The slides examined under an Olympus CX41 phase-contrast microscope. Then the content of the seminal vesicles was semi-dried and fixed in Schaudin's fluid for 20 minutes. The smears stored in 70% alcohol for removal of mercuric chloride. The slides were then passed through descending grade of alcohol and placed in distilled water. These were kept in 3% iron alum solution (throughout the night) and stained with Heidenhain's hematoxylin solution for 20 minutes. 1% iron alum solution was used for differentiation. Then the slides washed in distilled water, dehydrated in an ascending series of alcohol, cleared in xylene and mounted in Canada balsam. The photomicrographs have taken using an Olympus CX41 phase-contrast microscope. The photographs substantiated by the camera lucida drawings of different stages of gregarine parasites. The shape of the parasite described following Clopton [53].

All measurements are in µm.

3. RESULTS AND DISCUSSION

Taxonomy

Phylum Apicomplexa Levine, [55]
Class Gregarinomorphea Cavalier-Smith, [56]
Order Arthrogregarida Cavalier-Smith, [56]
Family Monocystidae Bütschli, 1882
Subfamily Monocystinae Bhatia, 1930
Genus *Monocystis* Stein, [2]

***Monocystis elliptoidum* sp. nov.**

Fig. 1 (a-c), 2 (a-c); Table 1

Type host: *Metaphire posthuma* Vaillant, 1868

Site of Infection: Seminal vesicles

locality: Bharatpur (23.88736º N, 88.08235º E) Murshidabad, West Bengal, India.

Prevalence: 35 out of 74 host earthworms (47.29%) were infected with the parasite.

Material deposited:

Holotype: MSIX/07 has been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal,

Paratype: MSIX/01 and MSV/29 have been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

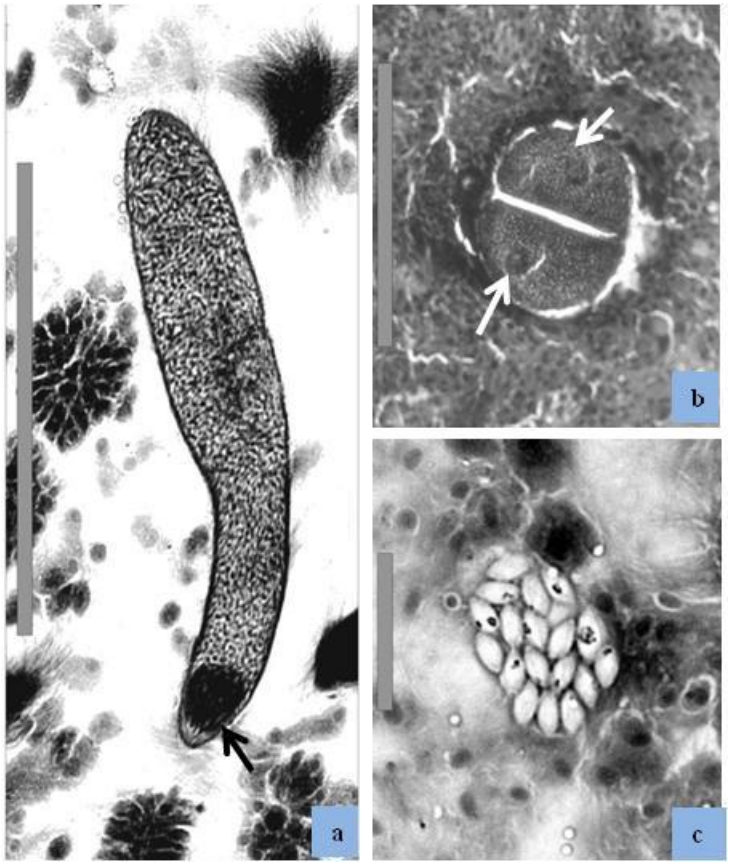

Fig. 1. *Monocystis elliptoidum* sp. nov. (Photomicrographs of different stages of the life cycle) a. Trophozoite (black arrow- nucleus); b. Gametocyst (two white arrows – two nuclei); c. Oocyst. Scale-bars. a, b - 100 µm; c - 10 µm

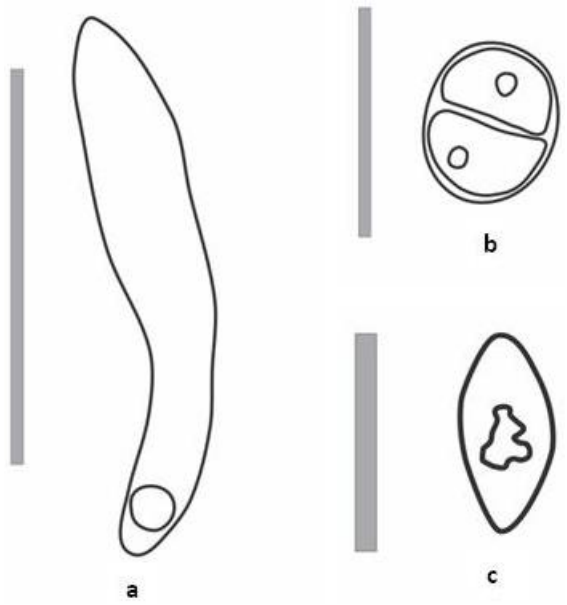

Fig. 2. *Monocystis elliptoidum* sp. nov. (camera lucida drawings of different stages of the life cycle) a. Trophozoite; b. Gametocyst; c. Oocyst. Scale-bars. a, b - 100 µm; c - 10 µm

Table 1. The morphometric comparisons of *Monocystis elliptoidum* sp. nov. with *M. senchalensis* and *M. darjeelingensis*

Species characters	*Monocystis senchalensis*	*Monocystis darjeelingensis*	*Monocystis elliptoidum* sp. nov.
Host	*Apporectodea trapezoids*	*Amynthus robusta*	*Metaphire posthuma*
Locality	Senchal, Darjeeling, West Bengal, India	Senchal, Darjeeling, West Bengal, India	Bharatpur, Murshidabad, West Bengal, India
Site of infection	Seminal vesicles	Coelomic fluid	Seminal vesicles
Trophozoite shape	Elongated and cylindrical; anterior and posterior ends blunt, middle part wide.	Cylindroid contains rounded extremities. One end slightly wider than the other. Conical or elliptical body in the early stage.	Elliptoid, anterior and posterior part blunt and the middle part slightly swollen.
Trophozoite size	43.0 – 185.4 µm × 10.8 – 48.6 µm	210.0 – 273.0 (231.0 ± 2.0) µm × 84.0 –140.0 (98.0 ± 1.8) µm	69.5 – 204.5 (135.5 ± 29.4) µm × 20.4 – 51.1 (36.4 ± 10.4) µm
Ectoplasm	Smooth	Thin, fine longitudinal striations	Poorly vacuolated
Endoplasm	Alveolated, packed with paraglycogen granules	Granular size increase with maturation	Densely vacuolated
Nucleus shape	Spherical or ovoidal	Spheroid or ovoid contains little karyosome.	Almost rounded.
Nucleus size	6.4 – 12.8µm	26.0 – 40.0 (31.0 ± 0.5) µm × 20.0 – 32.0 (27.0 ± 0.5) µm	12.2 – 20.4 (13.9 ± 3.7) µm × 8.1 – 16.3 (12.6 ± 2.7) µm
Gametocyst shape	Not stated	Spherical	Orbicular
Gametocyst size	Not stated	147.0 – 163.0 (156.0 ± 0.4) µm	57.2 – 98.1 (71.8 ± 12.0) µm
Oocyst shape	Biconical	Navicular	Rhomboid
Oocyst size	10.5 – 4.2 µm	15.4 – 18.0 (17.0 ± 1.0) µm × 8.0 – 9.0 (8.2 ± 0.5) µm	5.3 – 10.7 (8.4 ± 1.8) µm × 3.0 – 5.3 (4.2 ± 0.9) µm
References	Pradhan and Dasgupta, [33]	Bandyopadhyay and Mitra, 2005	Present study

MSIX/22 bearing paratype materials have been deposited in the national collection of the Zoological Survey of India, Calcutta-53 (Accession No. Pt/ 2434/ ZSI).

Etymology: The species name "*elliptoidum*" is given after the shape of the parasite under discussion.

Description

Trophozoites are elliptoid (Clopton B3) and slightly swollen in the middle portion. The nucleus rounded present at the anterior end of the body. Cytoplasm vacuolated. Endoplasm densely vacuolated and ectoplasm poorly vacuolated. Pellicle smooth and uniform. Episarc is thin. Gametocysts are orbicular (Clopton D3) having two almost equal-sized gametocytes. Both the gametocytes bear two distinct nuclei. Oocysts are rhomboid (Clopton C5).

Trophozoites measure 69.5 – 204.5 (135.5 ± 29.4) µm in length, the distance along the vertical axis of symmetry and 20.4 – 51.1 (36.4 ± 10.4) µm in width, the distance along the horizontal axis of symmetry. Nucleus measures 12.2 – 20.4 (13.9 ± 3.7) µm in length and 8.1 – 16.3 (12.6 ± 2.7) µm in width. The diameter of the gametocysts is 57.2 – 98.1 (71.8 ± 12.0) µm. The oocysts measure 5.3 – 10.7 (8.4 ± 1.8) µm in length and 3.0 – 5.3 (4.2 ± 0.9) µm in width.

Remarks

The species under discussion showed some sorts of similarities and dissimilarities with *Monocystis senchalensis* and *Monocystis darjeelingensis*. The present species having elliptoid gamonts while in *M. darjeelingensis* gamonts are cylindrical contain rounded extremities, one end slightly wider than the other and the young gamont has a conical body with one wide end and other end pointed. In *M. senchalensis* gamonts are elongated and cylindrical, the middle portion is wide and both ends are blunt like that of present form. The ectoplasm of *M.*

senchalensis smooth and endoplasm alveolated with paraglycogen granules. Whereas ectoplasm poorly vacuolated and endoplasm densely vacuolated in current species. Ectoplasm thin possesses very fine granular striations and endoplasm granular in *M. darjeelingensis.* In *M. senchalensis* and *M. darjeelingensis* nucleus spherical or ovoid whereas it is almost rounded in present species. Gametocysts spherical in *M. darjeelingensis* but it is ovoid in current species. In present species, oocysts rhomboid but in *M. senchalensis* and *M. darjeelingensis* oocysts biconical and navicular. Size of the oocysts is also different among compared species. Moreover, *M. senchalensis* and *M. darjeelingensis* have been reported from Senchal, West Bengal, India from *Apporectodea trapezoids* and *Amynthus robusta* respectively but the present species reported from *Metaphire posthuma* collected from Murshidabad district of West Bengal, India (Table 1).

After a thorough comparison, this discovery considered to be a new one and designated as *Monocystis elliptoidum* sp. nov. in this communication.

***Monocystis pontoscolexae* sp. nov.**

Fig. 3 (a-d), 4 (a-d) Table 2

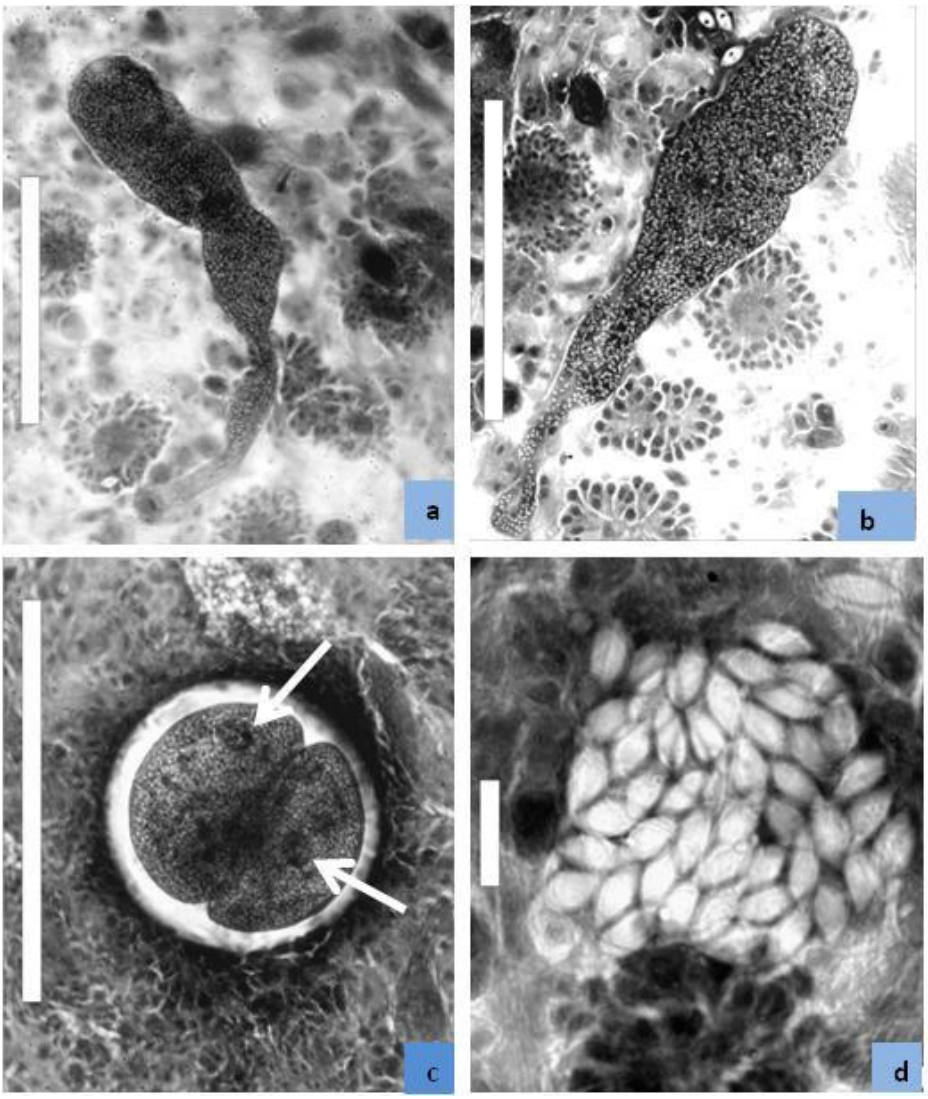

Fig. 3. *Monocystis pontoscolexae* sp. nov. (photomicrographs of different stages of life cycle) a-b. Trophozoite; c. Gametocyst (white arrows – nucleus); d. Oocyst. Scale- bars. a-c, 100 µm; d - 10 µm

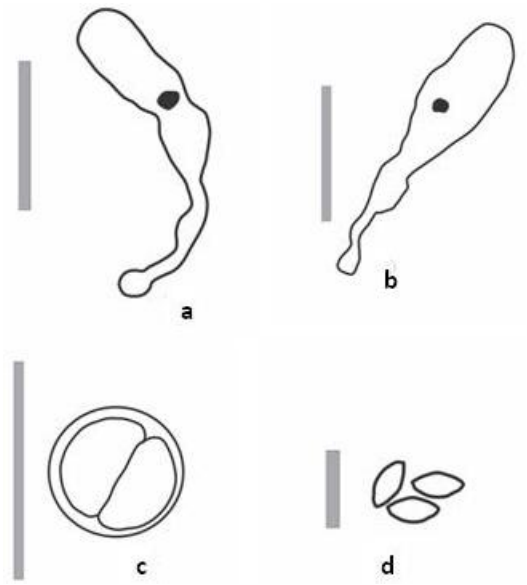

Fig. 4. *Monocystis pontoscolexae* sp. nov. (camera lucida drawings of different stages of the life cycle) a, b. Trophozoite; c. Gametocyst; d. Oocyst. Scale-bars. a-c, 100 µm; d. 10 µm

Table 2. The morphometric comparisons of *Monocystis pontoscolexae* sp. nov. with *M elongatum* and *M. bengalensis*

Species characters	***Monocystis elongatum***	***Monocystis bengalensis***	***Monocystis pontoscolexae* sp. nov.**
Host	*Perionyx excavatus*	*Pheritima posthuma*	*Pontoscolex corethrurus*
locality	Murshidabad, West Bengal, India	Calcutta, West Bengal, India	Belda, Paschim Medinipur, West Bengal, India
Site of infection	Seminal vesicles	Seminal vesicles	Seminal vesicles
Trophozoite shape	Elongated and curved at the anterior end. Body tapering towards its posterior end.	Oval shaped in the early stage, elongated or club-shaped in the mature stage. Rounded Anterior end is wider than the pointed posterior end.	Elongated, obpyriform pellicle has three constrictions.
Trophozoite size	265.0 – 346.0 (310.0 ± 2.6) µm × 46.0 – 64.3 (52.0 ± 3.1) µm	40 – 80 µm × 12 – 30 µm.	122.7 – 249.0 (205.5 ± 38.6) µm × 36.8 – 61.3 (53.3 ± 7.7) µm
Mucron	Distinct at the anterior part of the body	Not stated	Rounded
Ectoplasm	Smooth	Smooth	Not stated
Endoplasm	Densely granular and vacuolated contains a large number of paraglycogen granules.	Highly granular contains large irregular shaped paramylon grains.	Densely granulated
Nucleus shape	Round to ovoid	Rounded, contains large irregular karyosome with several compact masses	Rounded
Nucleus size	12.3 – 20.5 (19.4 ± 1.7) µm × 11.4 – 18.7 (16.3 ± 1.8) µm	Not stated	8.1 – 18.4 (13.0 ± 2.3) µm

Species characters	*Monocystis elongatum*	*Monocystis bengalensis*	*Monocystis pontoscolexae* sp. nov.
Gametocyst shape	Ovoid with two equal-sized gametocytes	Rounded or oval-shaped.	Fusiform
Gametocyst size	74.0 – 98.3 (82.0 ± 1.9) µm × 56.0 – 82.0 (72.5 ± 2.7) µm	70 – 80 µm	65.4 – 102.2 (80.2 ± 12.4) µm.
Oocyst shape	Biconical	Not stated	Fusiform
Oocyst size	9.7 – 15.3 (12.3 ± 3.1) µm × 6.8 –10.3(9.4 ± 1.1) µm	Not stated	8.1 – 10.7 (9.5 ± 0.6) µm × 3.8 – 5.3 (5.0 ± 0.5) µm
References	Bandyopadhyay et al. [43]	Ghosh, [12]	Present study

Type host: *Pontoscolex corethrurus* Müller, 1857

Site of Infection: Seminal vesicles

Type locality: Belda (22.070992ºN, 87.336639ºE), Paschim Medinipur, West Bengal, India.

Prevalence: 10 out of 17 host earthworms (58 2%) were infested with the parasite.

Material deposited:

Hololype: MSVI/VI has been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Paratype: MSVI /1 and MSVI /02 have been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

MSVI/09 bearing paratype materials have been deposited in the national collection of the Zoological Survey of India, Calcutta-53 (Accession No. Pt/ 2431/ ZSI).

Etymology: The species name "*pontoscolexae"* is given after the generic name of the host earthworm *Pontoscolex corethrurus.*

Description

Trophozoites are elongated, obpyriform (Clopton B17). Pellicle constricted throughout the body. In the body of the trophozoites have three constrictions. Posterior end of the gamonts is narrower than the anterior end. Nucleus rounded and located at the anterior end. Gametocysts are fusiform (Clopton E4) containing two equal-sized gametocytes. Each gametocyte bear nucleus. Oocysts are fusiform (Clopton C4).

Trophozoites measure 122.7 – 249.4 (205.5 ± 38.6) µm in length, distance along the vertical axis of the symmetry and 36.8 - 61.3 (53.5 ± 7.7) µm in width, distance along the horizontal axis of the symmetry. The nucleus of the trophozoites measuring 8.1 – 18.4 (13.0 ± 2.3) µm in diameter. Gametocysts measures 65.4 – 102.2 (80.2 ± 12.4) µm in diameter. Oocysts measure 8.1 – 10.7 (9.5 ± 0.6) µm in length and 3.8 – 5.3 (5.0 ± 0.5) µm in width.

Remarks

The species under discussion compared with *Monocystis elongatum* and *Monocystis bengalensis.* Gamonts of present species are elongated, obpyriform and pellicle has three constrictions. It is elongated, curved at the anterior end and tapering towards its posterior end in *M. elongatum.* In *M. bengalensis* it is oval-shaped in early-stage, elongated or club-shaped in the adult stage and the rounded anterior end is wider than the posterior end. The nucleus rounded in the present form like that of *M. bengalensis.* While in *M. elongatum* nucleus rounded to ovoid. In *M. elongatum* endoplasm is densely granulated like that present species and vacuolated with a large number of paraglycogen granules. Endoplasm is highly granular with large irregular-shaped paramylon grains in *M. bengalensis.* However, the shape and size of the gametocysts of present species are also different from previously reported species. Oocysts are fusiform in present species whereas it is biconical in the earlier described species. Furthermore, *M. elongatum* and *M. bengalensis* have been reported from *Perionyx excavatus* of Murshidabad district of West Bengal and *Pheritima posthuma* of Calcutta, West Bengal, India respectively but current species have been described from *Pontoscolex corethrurus* of Paschim Medinipur, West Bengal, India (Table 2).

After, a thorough comparison it seems that the present form cannot accommodate under any existing species of the genus *Monocystis*. Therefore, it has designated as *Monocystis pontoscolexae* sp. nov.

***Monocystis dolium* sp. nov.**

Fig. 5 (a-c), 6 (a-c) Table 3

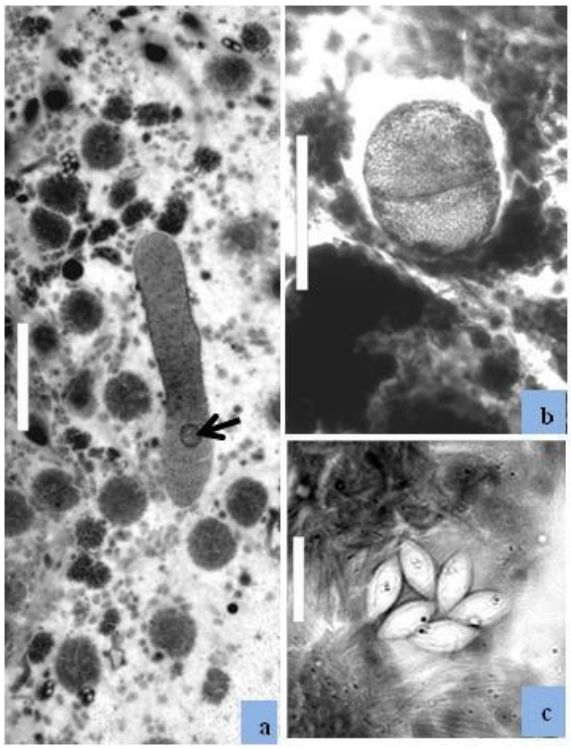

Fig. 5. *Monocystis dolium sp*. nov. (photomicrographs of different stages of life cycle) a. Trophozoite (black arrow – nucleus); b. Gametocyst; c. Oocyst. Scale-bars. a, b. 100 µm; c. 10 µm

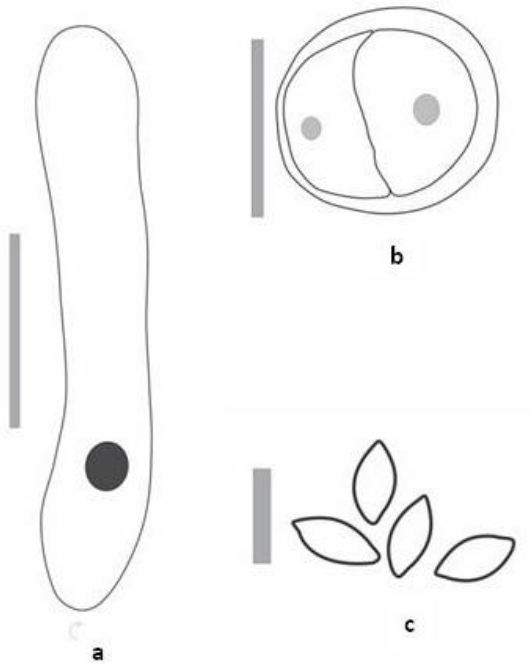

Fig. 6. *Monocystis dolium* sp. nov. (camera lucida drawings of different stages of the life cycle) a. Trophozoite; b. Gametocyst; c. Oocyst. Scale-bars. a, b. 100 µm; c. 10 µm

Table 3. The morphometric comparisons of *Monocystis dolium* sp. nov. with *Monocystis beddardi* and *M. darjeelingensis*

Species characters	*Monocystis beddardi*	*Monocystis darjeelingensis*	*Monocystis dolium* sp. nov.
Host	*Eutyphoeus nicholsoni*	*Amynthus robusta*	*Eutyphoeus waltoni*
Locality	Calcutta, West Bengal, India	Senchal, Darjeeling, West Bengal, India	Rasulpur, Bardhaman, West Bengal, India
Site of infection	Seminal vesicles	Coelomic fluid	Seminal vesicles
Trophozoite shape	Elongate fusiform when young, fusiform or variable when mature often a clear cylindrical process at one end.	Cylindroid contains rounded extremities. One end slightly wider than the other. Conical or elliptical when young.	Doliform
Trophozoite size	100 – 150 µm × 30 – 40 µm	210.0 – 273.0 (231.0 ± 2.0) µm × 84.0 – 140.0 (98.0 ± 1.8) µm	280.3 – 347.6 (293.2 ± 16.2) × 42.9 – 81.8 (52.5 ± 10.4) µm
Ectoplasm	Smooth	Fine longitudinal striations	Thin
Endoplasm	Highly granular with paraglycogen granules	Granular, size increases with matuaration	Densely vacuolated
Nucleus shape	Spherical with large central karyosome	Spheroid to ovoid-shaped contains little karyosome	Rounded
Nucleus size	Not stated	26.0 – 40.0 (31.0 ± 0.5) µm × 20.0 – 32.0 (27.0 ± 0.5) µm	20.4 – 30.6 (24.4 ± 2.9) µm
Gametocyst shape	Spherical	Spherical	Orbicular
Gametocyst size	80 - 100 µm	147.0 – 163.0 (156.0 ± 0.4) µm	69.5 – 122.2 (102.6 ± 16.2) µm
Oocyst shape	Navicular	Navicular	Fusiform
Oocyst size	12.5µm	15.4 – 18.0 (17.0 ± 1.0) µm × 8.0 – 9.0 (8.2 ± 0.5) µm	8.1 – 10.2 (9.5 ± 0.6) µm × 5.7 – 8.1 (6.6 ± 0.8) µm
References	Ghosh, [12]	Bandyopadhyay and Mitra, 2005	Present study

Type host: *Eutyphous waltoni* Michaelsen, 1907

Site of Infection: Seminal vesicles

Type locality: Rasulpur 23.184437ºN, 88.029237ºE, Bardhaman, West Bengal, India

Prevalence: 25 out of 54 host earthworms (46.2 %) were infested with the parasite.

Material deposited:

Holotype: MSII/03 has been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Paratype: MSII/04 and MSII/08 have been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

MSII/ 09 bearing paratype materials have been deposited in the national collection of the Zoological Survey of India, Calcutta-53 (Accession No. Pt/2427/ ZSI).

Etymology: The species name "*dolium*" given after the structure of the gamont is doliform.

Description

Monocystis dolium sp nov**.** have doliform (Clopton B2) shaped gamonts and smooth pellicle. Nucleus rounded present at the anterior end, cytoplasm vacuolated and endoplasm are densely vacuolated. The cytoplasm has no epicyteal striations. Mucron is not prominent. Gametocysts are orbicular (Clopton E3). Oocysts are fusiform (Clopton C4).

Trophozoites length, distance along the vertical axis of the symmetry 280.3 – 347.6 (293.2 ± 16.2) µm & width, distance along the horizontal axis of the

symmetry 42.9 – 81.8 (52.5 ± 10.4) µm. The diameter of the nucleus 20.4 – 30.6 (24.4 ± 2.9) µm. The diameter of the gametocysts measures 69.5 – 122 .2 (102.6 ± 16.2) µm. Oocysts length 8.1 – 10.2 (9.5 ± 0.6) µm and width 5.7 – 8.1 (6.6 ± 0.8) µm.

Remarks

In this communication, the present species compared with *Monocystis beddardi* and *Monocystis darjeelingensis*. *M. beddardi* have elongated and fusiform trophozoites containing prominent cylindrical process at one end in the mature stage. *M. darjeelingensis* has cylindroid trophozoite, rounded extremities, one end slightly wider than the other and young gamont has a conical body with one wide end and another pointed end. Whereas, it is doliform in the present species. *M. beddardi* has smooth ectoplasm while the current form has thin ectoplasm. *M. darjeelingensis* contains very fine longitudinal striations in ectoplasm. Endoplasm densely vacuolated in present species while it is highly granular with paraglycogen granules in *M. beddardi* and granular in *M. darjeelingensis*. In present species, nucleus rounded but in *M. darjeelingensis* it is spheroid to ovoid contains little karyosome. In *M. beddardi* it is spherical with a large central karyosome. Gametocyst spherical in both the previously described species and it is orbicular in present form. The shape and size of the oocysts of present species are also different from earlier reported species. Besides these the current species described from *Eutyphoeus waltoni* of Bardhaman district of West Bengal, India while *M. darjeelingensis* and *M. beddardi* recorded from *Amynthus robusta* of Senchal, Darjeeling and *Eutyphoeus nicholsoni* of Calcutta, West Bengal, India respectively (Table 3).

Therefore, taking into account all the differences with its closely related species, the present species is designated as new species, *Monocystis dolium* sp. nov.

***Monocystis lomentum* sp. nov.**

Fig. 7 (a-d), 8 (a-c) Table 4

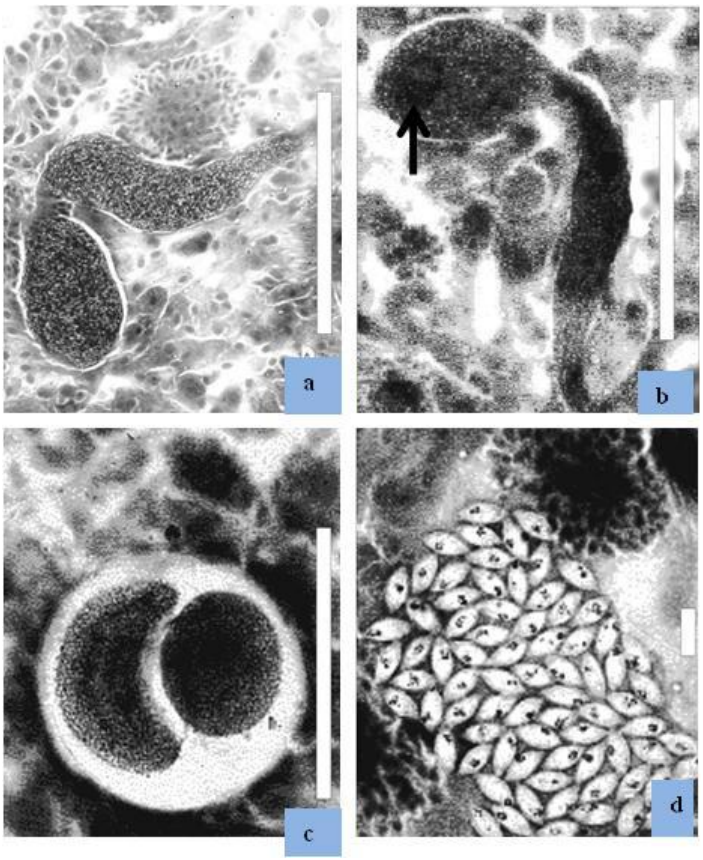

Fig. 7. *Monocystis lomentum* sp. nov. (photomicrographs of different stages of life cycle) a, b. Trophozoite (black arrow - nucleus); c. Gametocyst; d. Oocyst. Scale-bars. a-c, 100 µm; d. 10µm

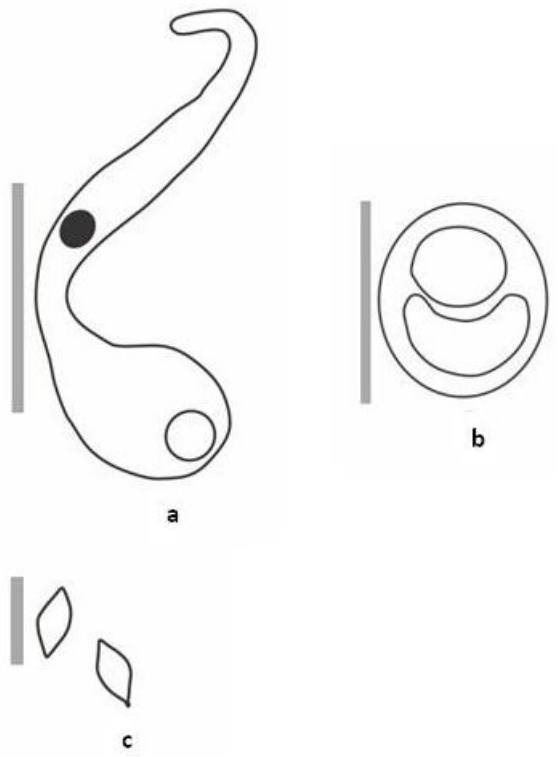

Fig. 8. *Monocystis lomentum* sp. nov. (camera lucida drawings of different stages of the life cycle) a. Trophozoite; b. Gametocyst; c. Oocyst. Scale-bars. a,b. 100µm; c. 10µm

Table 4. The morphometric comparisons of *Monocystis lonemtum* sp. nov. with *Monocystis elongatum* and *M. bengalensis*

Species characters	***Monocystis elongatum***	***Monocystis bengalensis***	***Monocystis lonemtum* sp. nov.**
Host	*Perionyx excavatus*	*Pheritima posthuma*	*Eutyphoeus orientalis*
Locality	Murshidabad, West Bengal, India	Calcutta, West Bengal, India	Ranaghat, Nadia, West Bengal, India
Site of infection	Seminal vesicles	Seminal vesicles	Seminal vesicles
Trophozoite shape	Elongated and curved tapering towards its sharply pointed posterior end.	Oval when young, elongate or club- shaped when mature. The anterior end is broad and rounded, posterior end narrow and pointed.	Motile sperm like, cylindrical, bulb like structure present at the anterior part,
Trophozoite size	265.0 – 346.0 (310.0 ± 2.6) µm × 46.0 – 64.3 (52.0 ± 3.1) µm	40 – 80 µm × 12 – 30 µm	151.3 – 306.7 (203.2 ± 48.3) µm × 14.3 – 28.6 (22.1 ± 3.9) µm
Mucron	Distinct at the anterior end of the body	Not stated	Round
Ectoplasm	Smooth	Smooth	Thin finely granulated
Endoplasm	Highly granular and vacuolated with a large number of paraglycogen granules.	Highly granular with large irregular shaped paramylon grains	Densely granulated
Nucleus shape	Round to ovoid nucleus	Rounded with a large irregular karyosome consisting of several	Rounded

Species characters	*Monocystis elongatum*	*Monocystis bengalensis*	*Monocystis lonemtum* sp. nov.
		compact masses	
Nucleus size	12.3 – 20.5 (19.4 ± 1.7) µm × 11.4 – 18.7 (16.3 ± 1.8) µm	Not stated	7.7 – 8.1 (8.0 ± 0.1) µm
Gametocyst shape	Ovoid with two equal gametocytes	Rounded or oval	Large gametocytes are luniform. Small gametocytes are orbicular
Gametocyst size	74.0 – 98.3 (82.0 ± 1.9) µm × 56.0 – 82.0 (72.5 ± 2.7) µm	70 - 80µm	85.8 – 110.4 (93.6 ± 8 .2) µm in diameter
Oocyst shape	Biconical	Not stated	Fusiform
Oocyst size	9.7 – 15.3 (12.3 ± 3.1) µm × 6.8 – 10.3 (9.4 ± 1.1)µm	Not stated	measures 7.3 – 12.0 (8.5 ± 1.5) µm × 4.0 – 8.1 (5.2 ± 1.3) µm.
References	Bandyopadhyay et al. [43]	Ghosh, [12]	Present study

Type host: *Eutyphoeus orientalis* Beddard, 1883

Site of Infection: Seminal vesicles.

Type locality: Ranaghat (23.173197ºN, 88.563523º E), Nadia, West Bengal, India.

Prevalence: 24 out of 54 host earthworms (44.4 %) were infested with the parasite.

Material deposited:

Holotype: MSI/01 has been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Paratype: MSI/04 and MSI/09 have been deposited at the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

MSI/ 03 bearing paratype materials have been deposited in the national collection of Zoological Survey of India, Calcutta-53 (Accession No Pt/ 2426/ ZSI).

Etymology: The species name "*lomentum*" has been given after the shape of the gamont is lomentiform.

Description

Trophozoites are lomentiform (Clopton B15) and motile, sperm-like, cylindroid body. The posterior end of the trophozoites pointed. Nucleus rounded present at the middle portion of the body. Ectoplasm is thin and finely granulated while endoplasm is thick and densely granulated. Gametocysts contain two unequal–sized gametocytes. Large and small gametocytes are luniform (Clopton F19) and orbicular (Clopton E3). Oocysts are fusiform (Clopton C4).

Trophozoites length, distance along the vertical axis of the symmetry 151.3 – 306.7 (203.2 ± 48.3) µm and width, distance along the horizontal axis of the symmetry 14.3 – 28.6 (22.1 ± 3.9) µm. Nucleus measures 7.7 – 8.1 (8.0 ± 0.1) µm in diameter. The diameter of the gametocysts ranges from 85.8 – 110.4 (93.6 ± 8.2) µm. The large gametocytes measure 65.4 – 102.2 (77.7 ± 9.7) µm x 35.7 – 81.8 (46.4 ± 7.7) µm. The small gametocytes measure 55.2 – 67.4 (57.4 ± 3.6) µm in diameter. Oocysts measure 7.3 – 12.0 (8.5 ± 1.5) µm in length and 4.0 – 8.1 (5.2 ± 1.3) µm in width.

Remarks

While comparing the present species with *Monocystis bengalensis* and *Monocystis elongatum* it appears that the trophozoites of present species are cylindrical, motile sperm-like and bulb-like structure found at the anterior end. Whereas in *M. elongatum* trophozoite is elongated and curved, tapering towards its posterior end and it is oval-shaped in the early stage and elongated or club-shaped in the mature stage of *M. bengalensis*. Ectoplasm is thin and finely granulated in present form. But it is smooth in previously described species. Endoplasm is highly granular and vacuolated contains numerous paraglycogen granules in *M. elongatum.* While it is densely granulated in present species and it is highly granular in *M. bengalensis* containing large paramylon granules. Nucleus rounded in present species like that of *M. bengalensis* whereas it is round to ovoid in *M.*

elongatum. Furthermore, the shape and size of the gametocyst and oocysts of the present species are also different from earlier described species. Moreover, the present species described from *Eutyphoeus orientalis* of Ranaghat, Nadia, West Bengal, India while, *M. elongatum* and *M. bengalensis* reported from *Perionyx excavatus* and *Pheritima posthuma* of Murshidabad district of West Bengal and Calcutta of West Bengal, India respectively (Table 4).

Therefore, taking into consideration of all the differences with its closely related species the present species designated as a new species, hence it is named *Monocystis lomentum* sp. nov.

***Monocystis asmati* sp. nov.**

Fig. 9 (a-c), 10 (a-c) Table 5

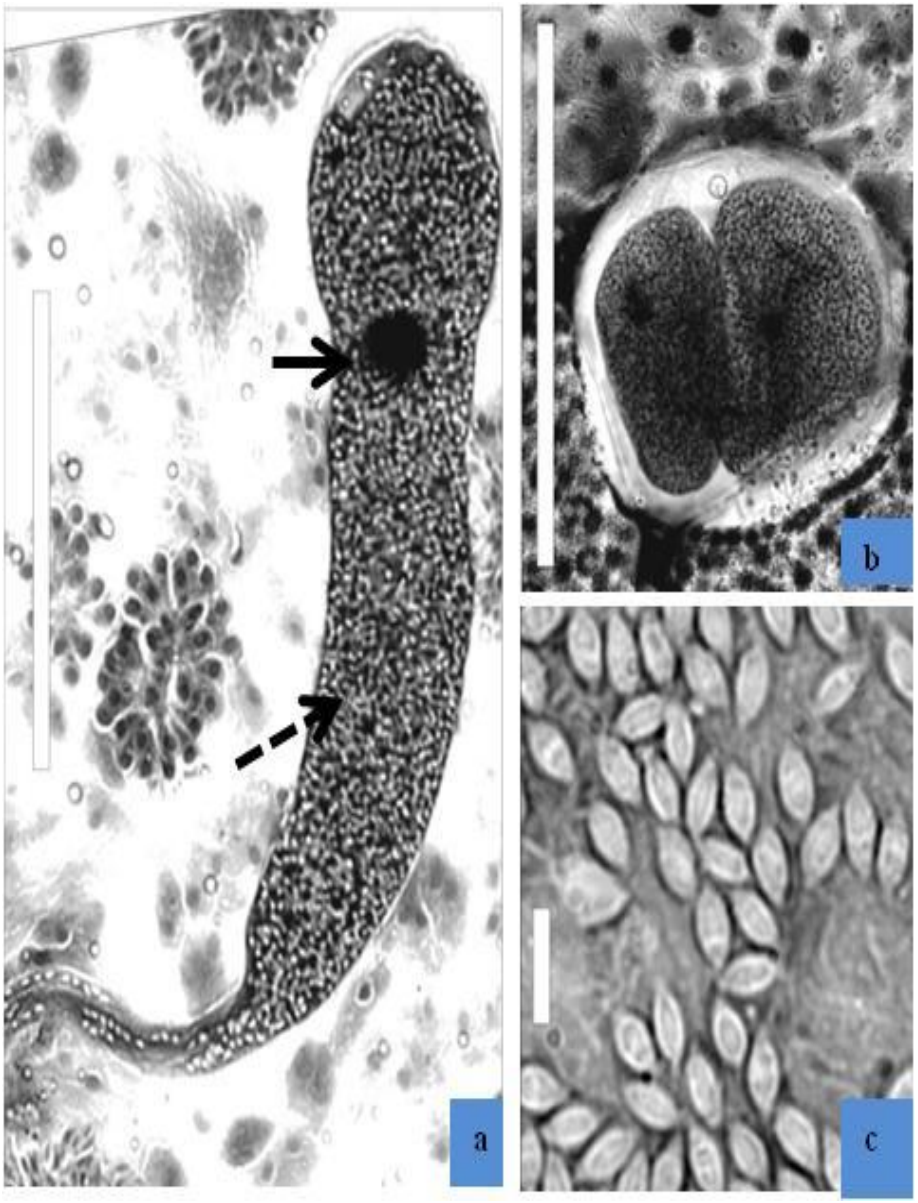

Fig. 9. *Monocystis asmati* sp. nov. (Photomicrographs of different stages of the life cycle) a. Trophozoite (black arrow – nucleus, black dotted arrow – granulated endoplasm); b. Gametocyst; c. Oocyst. Scale-bars. a, b. 100 µm; c. 10 µm

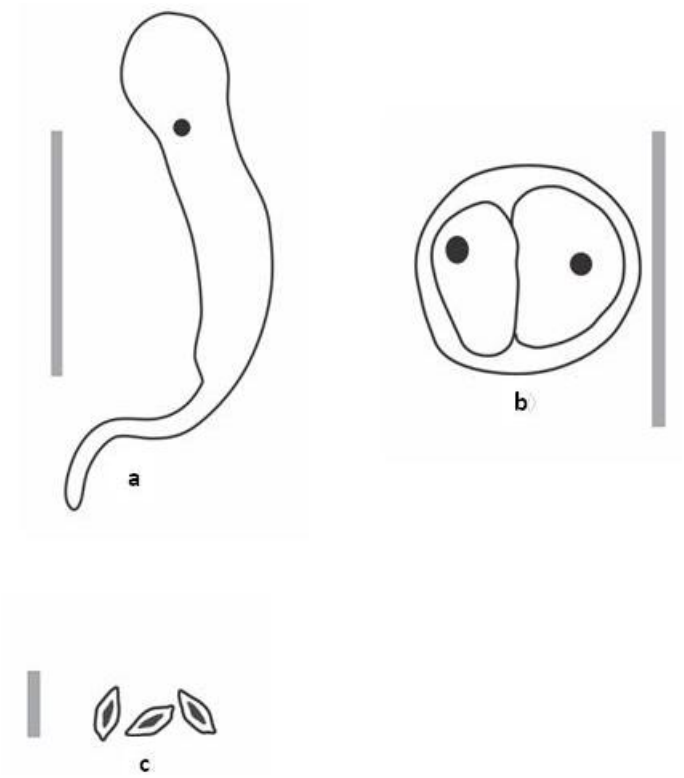

Fig. 10. *Monocystis asmati* sp. nov. (Camera lucida drawings of different stages of the life cycle) a. Trophozoite; b. Gametocyst; c. Oocyst. Scale-bars. a, b. 100 µm; c. 10 µm

Table 5. The morphometric comparisons of *Monocystis asmati* sp. nov. with *Monocystis levinei* and *M. bengalensis*

Species characters	***Monocystis levinei***	***Monocystis bengalensis***	***Monocystis asmati* sp. nov.**
Host	*Eutyphoeus incommodus*	*Pheritima posthuma*	*Perionyx excavatus*
Locality	Nadia, West Bengal, India	Calcutta,West Bengal, India India	Krishnanagar, Nadia, West Bengal, India
Site of infection	Seminal vesicles	Seminal vesicles	Seminal vesicles
Trophozoite shape	Elongated, anterior end rounded, lobe like and contains a short tail-like structure at the posterior end.	Oval when young, elongated or club-shaped when mature. The anterior end is broad and rounded, posterior end narrow and pointed.	Obpanduriform rounded anterior end and a tail like structure at the posterior end of the body.
Trophozoite size	145 – 175 (163 ± 1.5) µm x 18 – 43 (29.0 ± 1.1) µm	40 – 80 µm × 12 – 30 µm	163.6 – 245.4 (206.7 ± 31.0) µm × 28.6 – 53.1(39.4 ± 6.6)µm
Ectoplasm	Thin, 2 - 4µm	Smooth	Poorly granulated
Endoplasm	Paraglycogen granules are not prominent	Highly granular contains large irregular shaped paramylon grains	Densely granulated
Nucleus shape	Rounded to ovoidal.	Rounded contains large irregular karyosome with several compact masses	Ovoidal
Nucleus size	14.2 – 26.3 (22.3 ± 7.3) µm × 18.4 3 – 0.5 (26.4 ± 2.6) µm	Not stated	12.2 – 16.3 (13.9 ± 3.7) µm

Species characters	*Monocystis levinei*	*Monocystis bengalensis*	*Monocystis asmati* **sp. nov.**
Gametocyst shape	Ovoidal	Rounded or oval in shape.	The large gametocytes are obovoid and small gametocytes are doliform
Gametocyst size	130 – 163 (152 ± 0.4) µm	70 - 80µm	61.3 – 89.9 (70.7 ± 7.5) µm
Oocyst shape	Navicular	Not stated	Fusiform
Oocyst size	8.9 – 12.0 (10.4 ±1.0) µm × 5.2 – 7.3 (6.9 ± 0.5) µm	Not stated	9.2 – 11.5 (10.3 ± 0.9) µm × 3.0 – 5.3 (4.6 ± 0.6) µm
References	Bandyopadhyay et al, 2005	Ghosh, [12]	Present study

Type host: *Perionyx excavatus* Perrier, 1872

Site of Infection: Seminal vesicles

Type locality: Krishnanagar (23.39915ºN, 88.49249ºE), Nadia, West Bengal, India.

Prevalence: 31out of 94 host earthworms (32.9 %) were infested with the parasite.

Material deposited:

Holotype: MSV/02 has been deposited in Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Paratype: MSV/07 and MSV/29 have been deposited at the Parasitology Laboratoiy, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

MSV/ 21 bearing some paratype materials has been deposited in the national collection of the Zoological Survey of India, Calcutta (Accession No Pt/ 2430/ ZSI).

Etymology: The species name "*asmati*" has been designated after the name of the Prof. Ghazi SM Asmat, Department of Zoology, University of Chittagong, Chittagong, Bangladesh for his outstanding contribution in the field of parasitology**.**

Description

Monocystis asmati sp. nov. have obpanduriform (Clopton B16) gamonts with rounded anterior end and narrower posterior end with tail-like protrusion. Nucleus rounded present at the anterior end of gamonts. The entire pellicular lining enveloped an outer circular line. It has densely granulated endoplasm and poorly granulated ectoplasm. Gametocysts having two unequal–sized gametocytes. The large gametocytes are obovoid (Clopton F8) and small gametocytes are doliform (Clopton F2) in shape. Both gametocytes having rounded nucleus. Oocysts are fusiform (Clopton C4).

Trophozoites length, distance along the vertical axis of the symmetry 163.6 – 245.4 (206.7 ± 31.0) µm and width distance along the horizontal axis of the symmetry 28.6 – 53.1 (39.4 ± 6.6) µm. Nucleus measures 12.2 – 16.3(13.9 ± 3.7) µm in diameter. Gametocysts measure 61.3 – 89.9 (70.7 ± 7.5) µm in diameter. The large gametocytes measure 59.3 – 81.8 (65.5 ± 7.3) µm in length and 53.1 – 71.5 (58.5 ± 6.2) µm in width. The small gametocytes measure 40.9 – 73.6 (61.3 ± 8.4) × 28.6 – 40.9 (36.1 ± 3.5) µm. Length of the oocysts measures 9.2 – 11.5 (10.3 ± 0.9) µm and width of the oocysts measures 3.0 – 5.3 (4.6 ± 0.6) µm.

Remarks

While comparing the present species with *Monocystis levinei* and *Monocystis bengalensis*, the present species shows some sorts of resemblance. The species under discussion closely resembles with *M. levinei* in having rounded anterior end and a tail like protrusion at the posterior end of trophozoites. However, the anterior end of the trophozoite of the species under discussion does not have any anterior bulb-like structure. In the early stage of *M. bengalensis* trophozoites oval-shaped and in the mature stage, it is elongated or club-shaped. Ectoplasm is poorly granulated in present form whereas it is smooth in *M. bengalensis* and it is thin in *M. levinei*. Endoplasm is densely granulated in current species. It is highly granular in *M. bengalensis* contains large irregular shaped paramylon grains while in *M. levinei* paraglycogen granules are not prominent in the endoplasm. The nucleus is ovoidal in species under discussion whereas it is rounded in *M. bengalensis* and rounded to ovoidal in *M. levinei.* Shape and size of the gametocysts and oocysts are also different from earlier described

species. Moreover, present species reported from *Perionyx excavatus*, Krishnanagar, Nadia, India while, *M. levinei* described from *Eutyphoeus incommodus* Nadia, West Bengal, India and *M. bengalensis* described from *Pheritima posthuma* of Calcutta, West Bengal, India (Table 5).

Based on morphology and morpho-metrics, no other species belonging to the genus *Monocystis* resembles the new species, Therefore, the species under discussion designated as *Monocystis asmati* sp. nov.

4. CONCLUSION

There is a significant issue that a severe infection with these gregarines may cause agriculture losses due to infertility and significantly decreased the number of earthworms in agriculture. Keeping in its mind, we worked on acephaline gregarine parasites of earthworms and identified five new species. *Monocystis elliptoidum* sp. nov., *Monocystis pontoscolexae* sp. nov., *Monocystis dolium* sp. nov., *Monocystis lomentium* sp. nov., *Monocystis asmati* sp. nov. are described and illustrated in detail based on the specimens collected from the seminal vesicles of *Metaphire posthuma, Pontoscolex corethrurus, Eutyphoeus waltoni, Eutyphoeus orientalis* and *Perionyx excavatus* respectively.

ETHICAL APPROVAL

It has been informed that as per CPCSEA protocol that there is no need for taking permission from the ethical committee to carry out the research work on this animal.

ACKNOWLEDGEMENT

Authors (BB and BK) are thankful to the authorities of the University of Kalyani, Kalyani, West Bengal, India for extending permission to work at the Parasitology Laboratory of the Department of Zoology, University of Kalyani, Kalyani. One of the authors (BK) is sincerely acknowledge the University of Kalyani, Kalyani for providing fellowship to carry out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Adl SM, Bass D, Lane CE, Lukes J, Schoch CL, Smirnov A, et al. Revisions to the classification, nomenclature, and diversity of eukaryotes. Journal of Eukaryotic Microbiology. 2019;66:4–119.
2. Stein F. Über die natur der gregarinen. Archiv fur Anatomie, Physiologie und wissenschaftliche Medicin. 1848;182-223.
3. Labbé A. Sporozoa, in tierreich. eine zusammenstellung und kennzeichnung der rezenten tierformen Schulze FE, Bütschli O, Eds. Friedländer & Sohn, Berlin. 1899;5:1-180.
4. Cuènot L. Recherches sur l'evolution et la conjugaison des grègarines. Archiv Biology. 1901;17:581-652.
5. Drzhevetskiy VF. O resnichyatikh' gregarinakh'. C.R. St. Petersburg Trav Soc Nat. 1907;38:203-205.
6. Hesse E. Contribution á l'ètude des monocystidèes des oligochètes. Archives de zoologie experimentale et generale. 1909;3:27-301
7. Boldt M. Zwei neue Gregarinenarten aus octolasium complanatum ant. Dunès. Zoologischer Anzeiger. 1910;36:289-293.
8. Mulsow K. Über Fortpflanzungserscheinungen bei Monocystis rostrata n. sp. Archiv fur Protistenkunde. 1911;22:20-55.
9. Cognetti De Martiis L. Contributo alla conoscenza delle Monocistidee e dei lorofenomeni riproduttivi. Archiv fur Protistenkunde. 1911;23:205-246.
10. Pinto CDM. Contribuicao ao estudo des gregarines. Mem. Inst. Oswaldo. Cruz. 1918;15:84-108.
11. Voss H. Monocystis naidis n. sp., eine neue colomgregarine der oligochaeten. Archiv fur Protistenkunde. 1921;42:176-178.
12. Ghosh E. Monocystis from the earthworm of Calcutta. Journal Of Royal Microscopic Society. 1923;423.
13. Berlin H. Üeber drei neue Monocystiden. Archiv Zoologie. 1923;15(8):1-13.
14. Berlin H. Untersuchngen über Monocystideen in den Vesiculae seminales der schwedischen Oligochaeten. Archiv fur Protistenkunde. 1924;48:1-124.
15. Bhatia BL, Chatterjee GB. On some gregarine parasites of Indian earthworms. Archiv fur Protistenkunde. 1925;52:189-206.
16. Hahn J. Monocystis mrazeki, une nouvelle grègarine parasites de Rhynchelmis limosella et de Rh. komareki Hrabè. I. Dissèmination. Archiv fur Protistenkunde. 1928;62:1- 12.
17. Sciacchitano I. Su alcune Gregarine parassite del Criodrilus lacuum Hoffm. Bollettino di Zoologia Napoli. 1931;2(1):175-195.
18. Tuzet O, Loubatières R. Notes sur les Monocystidèes. Archives Zoologie Experementale Gènerale. 1946;84:132-149.

19. Tuzet O, Loubatières R. Phagocytose et presence d'une bouche et d'un rudiment de tube digestif chez quelques Grègarines monocystidèes. Comptes rendus de Academie des Sciences Paris. 1948;226:616-618.
20. Loubatières R. Contribution á l'ètude des gregarinomorphes Monocystidae parasites des oligochètes du Languedoc -Roussillon. Annales des Sciences Naturelles Zoologie Biologie Animale. 1955;17:73-201.
21. Tuzet O, Zuber-Vogeli M. Grègarines et Ciliès parasites des vèsicules sèminales de Dichogaster inermis Michaelsen Oligochète de Man (A.O.F.). Bulletin de I'Institut fundamental d'Afrique noire. 1955;17:369-376.
22. Tuzet O, Zuber-Vogeli M. Deuxième contribution á 1'ètude des Monocystidae des Oligochètes africains Millsonia anomala Omodeo (1956) et Dichogaster baeri Sciacchitano (1) Oligochètes de Gagnoa (A.O.F.). Bulletin de I'Institut fundamental d'Afrique noire. 1956;18:410-417.
23. Meier M. Die Monocystideenfauna der Oligochäten von Erlangen und Umgebung. Archiv fur Protistenkunde. 1956;101:335-400.
24. Boisson C. Monocystidae parasites d'oligochètes d'Indochine. Annales des Science Naturelles Zoologie. 1957;19:71-90.
25. Rees B. Studies on monocystids gregarines. Three Monocystis species including two new specics, M. cambrensis and M. lanceata. Parasitology. 1961;51:523-532.
26. Bereczky MC. Taxonomische Untersuchungen über die parasitierenden Monocystideen (Protozoa) bei Regen würmern (Lumbricidae). Opuscula Zoologica Budapest. 1967;7(1):55-69.
27. Segun AO. Studies on acephaline gregarines in British earthworms, their systematic, occurrence and possible mode of transmission. Ph.D. thesis. University of London; 1968.
28. Segun AO. Monocystid gregarine parasites of Nigerian earthworms. Journal of Protozoology. 1978;25(2):157-162.
29. Giere O. Monocystis lumbricilli n. sp. (Gregarinida, Sporozoa) aus Lumbricillus lineatus, Neubeschreibung und Literaturkritik. Archiv fur Protistenkunde. 1971;113:13-33.
30. Righi G. Alguns Sporozoa (Gregarinida e Haplosporida) de oligochaeta (Naididae e Enchytracidae) brasileiros. Papies Avulsos de Zoologia (Sao Paulo). 1974;28(11):185- 195.
31. Levine ND. Revision and check-list of the species of the aseptate gregarine family Monocystidae. Folia Parasitology. 1977;24:1-24.
32. Subbarao BVSSR, Kalavati C, Narasimhamurti CC. A new aseptate gregarine, Monocystis pontrodrili sp. n. from the litoral oligocheate annelids Pontrodilus bermudensis Beddard. Acta Protozoology. 1979;18(2):259-264.
33. Pradhan D, Dasgupta B. New gregarines in earthworms from Senchal and Goomti in Darjeeling district, West Bengal, India. Journal of Bengal Natural History Society (New Series). 1982;1(2):4-16.
34. Bandyopadhyay PK, Roychoudhuri US, Biswas G. Observation on an endoparasite (Apicomplexa: Sporozoa) from the earthworm of West Bengal, India. Uttar Pradesh Journal of Zoology. 2001;21(1):65-67.
35. Bandyopadhyay PK, Biswas G. Studies on an endoparasite Monocystis nadiensis sp. n. from the earthworm of Nadia, West Bengal, India. Transaction Zoological Society East India. 2002;6(1):12-15.
36. Bandyopadhyay PK, Mitra AK. Observation of two new species of monocystis Stein, 1848 (Protozoa: apicomplexa: monocystidae) monocystis darjeelingensis sp. n. and monocystis ranaghatensis sp. n. from earthworms (Annelida: Oligochaeta) of West Bengal, India. Animal Biology. 2005a;55(2):123-132.
37. Bandyopadhyay PK, Bhowmik B, Mitra AK. Observations on a new species of Monocystis Stein, 1848 (Protozoa: Apicomplexa: Monocystidae) Monocystis levinei sp. nov. from an Inidan earthworm (Annelida: Oligochaeta) Eutyphoeus incommodus. Zootaxa. 2005b;1016:15-19.
38. Bandyopadhyay PK, Göçmen B, Bhowmik B, Mitra AK. A new species of Monocystis Stein, 1848 (Protista: Apicomplexa: Eugregarinida) from the Indian earthworm, Amynthas hawayanaus Rosa, 1891 (Annelida: Oligochaeta). Acta Parasitologica Turcica. 2006a;30(2):155-157.
39. Bandyopadhyay PK, Mallik P, Göçmen B, Mitra AK. Monocystis apporectodae sp. nov. (Protozoa: Apicomplexa: Eugregarinida), from an Indian earthworm Apporectodea trapezoides Duges. Acta Parasitologica Turcica. 2006b;30(1):51-53.
40. Bandyopadhyay PK, Mallik P, Göçmen B, Mitra AK. Monocystis metaphirae sp. nov. (Protista: Apicomplexa: Monocystidae) from the earthworm Metaphire houlleti (Perrier). Acta Parasitologica Turica. 2006c;30(1):54-56.
41. Bandyopadhyay PK, Mitra AK, Mallik P. Biology of Monocystis clubae sp. nov. (Apicomplexa: Eugregarinida) from an Indian earthworm Lampito mauritii (Annelida:

Oligochaeta) of India. Zootaxa. 2006d;1120:51-55.

42. Bandyopadhyay PK, Mallik P, Mitra AK. Observations on Monocystis arabindae n. sp. and Nematocystis majumdari n. sp. (Protozoa: Apicomplexa: Monocystidae) from Seminal Vesicles of an Earthworm Eutyphoeus incommodus (Beddard) from West Bengal, India. Acta Protozoologica. 2007;46:147-155.
43. Bandyopadhyay PK, Chakraborty A, Mitra AK, Göçmen B. Monocystis elongatum n. sp. (Protozoa: Apicomplexa: Monocystidae) from seminal vesicles of the earthworm Perionyx excavatus (Perrier) (Annelida: Oligochaeta) in West Bengal, India. North-Western Journal of Zoology. 2008;4(1):91-98.
44. Bandyopadhyay PK, Chakraborty A, Mitra AK. Monocystis septum n. sp. (Protozoa: Apicomplexa: Monocystidae) from seminal vesicles of earthworms (Annelida: Oligochaeta) in West Bengal, India. Protistology. 2009a;6(1):66-71.
45. Bandyopadhyay PK, Mitra AK, Göçmen B. Observation on Monocystis constricta (Protozoa; Apicomplexa; Monocystidae) from an Indian earthworm Eutyphoeus quaripapillatus (Michelsen 1907). Türkiye Parazitolojii Derğisi. 2009b;33(3):254–258.
46. Bhowmik B, Mitra AK, Bandyopadhyay PK. Studies on the Biodiversity of Aseptate Gregarines from the Oligochaetes of West Bengal. Proceedings of National Congress Parasitolology. 2011;37-41.
47. Sarkar S, Bandyopadhyay PK. First record of a gregarine from an earthworm of Bangladesh. 22nd National Congress Parasitology. 2011;287-292.
48. Sarkar S, Bandyopadhyay PK. A new species of Monocystis Von Stein, 1848 (Apicomplexa: Conoidacida: Eugregarinida) from an earthworm host of Bangladesh. Proceedings of National Conference Challaenges Biodiversity Resource Management. 2013;326-333.
49. Sarkar S, Bandyopadhyay PK. Monocystis apareshae a new gregarine parasite (Protozoa: Apicomplexa: Monocystidae) of the Oligochaete host of Bangladesh. Modern Parasitology. 2014;83-90.
50. Mallik P, Bandyopadhyay PK. Observation on Monocystis kuidongae sp.nov.(Apicomplexa: Eugregarinida) from an Indian earthworm Perionyx excavatus perrier (Annelida: Oligochaeta). Journal of Parasitic Disease. 2016;41(2):361-363.
51. Sarkar S, Kundu B, Bandyopadhyay PK. Taxonomical studies of four new aseptate gregarine parasites belonging to the genus Monocystis Stein, 1848 (Protozoa: Apicomplexa: Sporozoa) from an oligochaete host, Eutyphoeus orientalis (Annelida: Oligochaeta) of West Bengal, India. Records of Zoological Survey of India. 2019;119(4):334-347.
52. Sarkar S, Kundu B, Bandyopadhyay PK. Morphotaxonomical description of six new species of acephaline gregarines infecting oligochaete host Journal of Parasitic Disease. 2020;44(4):754-771.
53. Sarkar S, Kundu B, Bandyopadhyay PK. Description of three new species of aseptate gregarine, Monocystis von Stein, 1848 of oligochaetes collected from Dhaka, Bangladesh. Records of Zoological Survey of India. 2020;120(3):211-219.
54. Clopton RE. Standard nomenclature and metrics of plane shapes for use in gregarine taxonomy. Comparative Parasitology. 2004;71(2):130-140.
55. Levine ND. The protozoan phylum Apicomplexa, vol I. CRC Press Inc., Boca Raton, Florida; 1988.
56. Cavalier-Smith T. Gregarine site- heterogenous 18S rDNA trees, revision of gregarine higher classification, and the evolutionary diversification of Sporozoa. European Journal of Protistology. 2014;50:472-495.