

Monocystis metaphirae sp. nov. (Protista: Apicomplexa: Monocystidae) from the Earthworm *Metaphire houlleti* (Perrier)

Probir K. BANDYOPADHYAY¹, Partha MALLIK¹, Bayram GÖÇMEN², Amlan Kumar MITRA¹

¹Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India; ²Protozoology and Parasitology Research Laboratory, Zoology Section, Department of Biology, Faculty of Science, Ege University, Bornova, İzmir, Turkey

SUMMARY: Biodiversity studies in search of endoparasitic acephaline gregarines revealed a new species of the genus *Monocystis* Stein, 1848 in the seminal vesicles of the earthworm *Metaphire houlleti* (Perrier) residing in alluvial soil of the district of North 24 Parganas. The new species is characterized by having bean-shaped gamonts measuring 94.0-151.0 (119.0±16.0) µm × 53.0-81.0(66.0±8.0) µm. The anterior end of the gamont is always wider than the posterior end. The mucron is always present at the wider end. The occurrence of syzygy (end to end, cauda-frontal) is a very rare feature which has been observed in the life cycle of the new species. The gametocyst is ovoid consisting of two unequal gamonts, measuring 85.0-102.0 µm (93.0±6.0). Oocysts are navicular in shape, measuring 6.5-11.0 (9.0±1.1) µm × 4.0-7.5 (5.5±1.9) µm.

Key Words: *Monocystis metaphirae* sp. nov., endoparasite, earthworm, seminal vesicle, India

Toprak Solucanı *Metaphire houlleti* (Perrier)'den *Monocystis metaphirae* sp. nov. (Protista: Apicomplexa: Monocystidae)

ÖZET: Endoparazitik asefalin (aseptat) gregarin çeşitliliği ile ilgili araştırma esnasında Kuzey Parganas Bölgesi alüvyon toprağında yaşayan toprak solucanı *Metaphire houlleti* (Perrier)'in seminal vesiküllerinde *Monocystis* Stein, 1884 cinsine dahil yeni bir tür ortaya çıkarılmıştır. Bu yeni tür 94.0-151.0 (119.0±16.0) µm×53.0-81.0(66.0±8.0) boyutlarında bilhassa fasulye biçimli gamontlara sahip olması ile karakterize olur. Gamontun anterior ucu daima posterior ucundan daha geniş olup, mukron daima en geniş ucunda bulunur. Ender görülen bir özellik olan uç uca (kauda-frontal) sızıgı bu yeni türün yaşam döngüsünde gözlenmiştir. 85.0-102.0 (93.0±6.0) µm boylarında ölçülen gametokistleri ovoid şekilli olup iki eşit olmayan gamont içerir. Ookistleri navikular (kayık) şekilli olup uzunlukları 6.5-11.0 (9.0±1.1) µm ve genişlikleri 4.0-7.5 (5.5±1.9) µm arasında değişir.

Anahtar Sözcükler: *Monocystis metaphirae* sp.nov., endoparazit, toprak solucanı, seminal vesikül, Hindistan

INTRODUCTION

Till date, more than 350 species of earthworms have been recorded from India. A very small number of them have been studied so far for the occurrence of endoparasitic aseptate gregarine fauna. In India, work on this group is getting momentum since 1980. The present paper deals with a new species of the genus *Monocystis* Stein, 1848 obtained from the seminal vesicles of an earthworm *Metaphire houlleti* (Perrier). There are only records of 12 species of *Monocystis* excepting the new species reported in the present paper (1-5, 7-9, 11, 12).

MATERIAL AND METHODS

Several places of North 24 Parganas were visited for collecting earthworms. Earthworms were collected from alluvial soil. After the earthworms were identified, each was dissected in 0.65 % (w/v) NaCl solution. The perivisceral coelom, nephridia, intestine and seminal vesicles were examined immediately for monocystid gregarines. Smears of the coelomic fluid and seminal fluid were made on clean dry slides, semidried and fixed in Schaudins fluid (66 ml HgCl₂, 33 ml 95% ethyl-alcohol, and 1 ml glacial acetic acid) for 20 mins. The fixed smears were stored in 70 % ethyl-alcohol for removal of mercuric chloride. The slides were then passed through a descending series of ethyl-alcohol (100%, 90%, 70%, 50%), for 5 min each, and stored in distilled water. Slides 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 (50%, 70%, 90%, 100%), cleared in xylene and mounted in Canada balsam. Camera lucida drawings of different stages of monocystid gregarines were made and photomicrographs were taken with the help of an Olympus phase contrast microscope and an Olympus camera. All measurements are in micrometres (μm). In each case minimum and maximum values are given, followed in parentheses by arithmetic mean, standard deviation and sample size. Method of describing shapes of planes and solids is mainly according to Clopton (6).

RESULTS

Phylum	: Apicomplexa Levine, 1988
Order	: Eugregarinida Leger, 1900
Family	: Monocystidae Bütschli, 1882
Subfamily	: Monocystinae Bhatia, 1930
Genus	: <i>Monocystis</i> Stein, 1848

Monocystis metaphirae sp.nov (Figs. 1-4)

Gamont Length (GL): 94.0-151.0 (119.0 \pm 16.0, 22); Gamont Width (GW): 53.0-81.0 (66.0 \pm 8.0, 22); Nucleus Diameter (ND): 4.0-16.0 (11.0 \pm 3.0, 22); Gametocyst Diameter (GD): 85.0-102.0 (93.0 \pm 6.0, 22); Oocyst Length (OL): 6.5-11.0 (9.0 \pm 1.1, 22); Oocyst Width (OW): 4.0-7.5 (5.5 \pm 1.9, 22).

The members of the genus *Monocystis* Stein, 1848 are characterized by having no distinct mucron, ovoid and solitary gamonts, bi-conical, symmetrical oocysts (Levine, 1988). In case of the present form found from the seminal vesicles of the earthworm *Metaphire houlleti* (Perrier) the gamont is solitary, bean shaped with broad anterior end and the posterior end is comparatively narrower. Irregularly shaped and distinct mucron is present at the anterior end of the body. In syzygy, the gamonts are almost rectangular. Ectoplasm is thin (1-3 μm). Epicyte smooth, free from any external processes. Distinction between ectoplasm and endoplasm is not clear. Endoplasm with evenly distributed vacuoles. A large number of unequal sized, black paraglycogen granules are distributed in the endoplasm which are not uniform in distribution. Almost rounded nucleus, situated mostly in the wider portion of the nucleus. Nucleoplasm vacuolated. Each ovoid gametocyst with two unequal gametocytes. Oocysts are navicular in shape. Sexual reproduction takes place in this organism by means of end to end syzygy.

Taxonomic summary

Type Host:	<i>Metaphire houlleti</i> (Perrier)
Type Locality:	Madhyamgram, North 24 Parganas
Symbiotype:	Host MH07/2005 deposited in the museum of the Dept. of Zoology, Univ. of Kalyani, Kalyani, 741235, West Bengal, India.

Type material

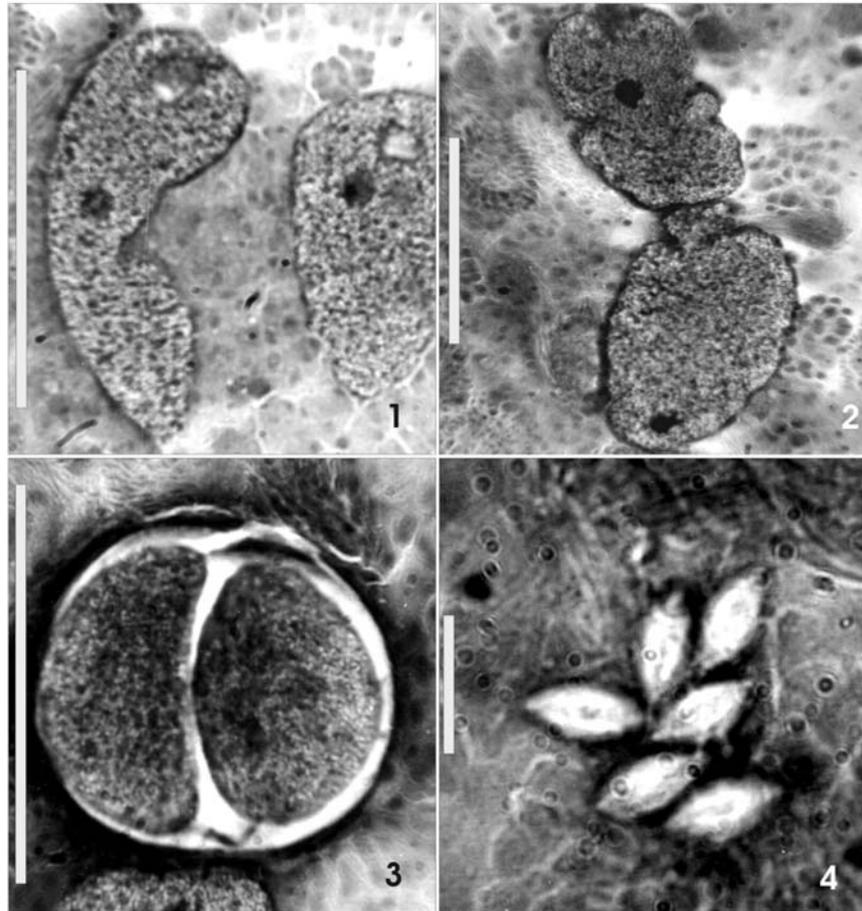
Holotype:	Slide MH-14/2005 is deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India. Registration number ZD/KU/165
Paratype:	Slides MH-03/2005, MH-11/2005, MH-21/2005 are in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India
Prevalence:	22/54 (40%) earthworms were infected with <i>Monocystis metaphirae</i> sp.nov.
Etymology:	The specific epithet " <i>metaphirae</i> " is given after the generic name of the host earthworm <i>Metaphire houlleti</i> (Perrier).

DISCUSSION

Cylindroid, solitary, with mucron, late syzygy and coelomic habitat of the parasite in an earthworm justify the inclusion of the present form under the family Monocystidae, subfamily Monocystinae and genus *Monocystis* Stein, 1848. Of more than 70 species belonging to the genus *Monocystis* Stein, 1848 (10) only 12 have been described from India. Of these species, *M. odontotermi* Kalavati, 1979 (8) has been reported from the haemocoel of the termite *Odontotermes obesus* (a non oligochaete host) and *M. pontodrilus* Subbarao, Kalavati and Narasimhamurti, 1979 (12) from the littoral oligochaete, *Pontodrilus bermudensis*. All other nine species of *Monocystis* have been reported from terrestrial earthworms, but none from the present host *Metaphire houlleti*. The species under discussion resembles to some extent only *Monocystis megascolexae* Kar, 1946 (9) in having a tail-like structure at the posterior end of the body. In *M. megascolexae* the anterior end of the gamont is rounded and the other end is sharply pointed. In the new species, the anterior end is rounded but the posterior end is not sharply pointed. Interestingly, gamonts are rectangular when in syzygy. The size of the gamonts of *M. megascolexae* is much larger (135.8–429.0 x 58.2–97.5) than the present species obtained from *Metaphire houlleti*. Endoplasm of the gamonts of *M. megascolexae* is highly granular, but that of the new species is vacuolated with few black granules which are completely absent in the former one. Oocysts of *M. megascolexae* is spindle-shaped, which is navicular in the new species.

Moreover, the life cycle of the new species represents prominent end to end syzygy. Occurrence of syzygy has not been reported by any author in the life cycle of any other Indian *Monocystis* so far.

Considering all the facts, we are sure that no other species of the genus *Monocystis* can be compared with the species we have obtained from the seminal vesicles of the earthworm *Metaphire houlleti* (Perrier) and hence we propose the species as *Monocystis metaphirae* sp.nov.



Figures 1-4. Photomicrographs of different stages of the life cycle of *Monocystis metaphirae* sp. nov. Fig. 1. Mature gamont; Fig. 2. Caudal Syzygy, Fig.3. Gametocyst; Fig. 4. Oocysts. Scale bars 100 µm (Figs. 1-3) and 10 µm (Fig. 4).

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