

# *Monocystis appoectodae* sp. nov. (Protozoa: Apicomplexa: Eugregarinida), from an Indian earthworm *Appoectodea trapezoides* Duges

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**SUMMARY:** A survey aimed at exploring the endoparasitic acephaline gregarine diversity in South-western Bengal, detected a new species of the genus *Monocystis* Stein, 1848, that resides in the seminal vesicles of the earthworm, *Appoectodea trapezoides* Duges collected in the district of Bankura from alluvial soil. *Monocystis appoectodae* sp. nov. is a ribbon-like organism with one or more prominent constrictions especially in some mature forms and measures 178.0-224.0 (203.0±5.0) µm × 37.0-58.0 (46.0±1.5) µm. The extreme ends are pointed. Its gametocysts are ovoid and measure 108.0-118.0µm (113.0±1.1) × 79.0-89.0 (83.0±1.1) µm. Oocysts are navicular in shape. The length of the oocysts ranges from 10.0-14.6 and the width, from 5.5-8.1 µm.

**Key Words:** *Monocystis appoectodae* sp. nov., gregarine, earthworm, seminal vesicle, India

## Hindistan'da Yaşayan Toprak Solucanı *Appoectodea trapezoides* Duges'den *Monocystis appoectodae* sp. nov.

**ÖZET:** Güneybatı Bengal'deki enoparazitik asefalin (aseptat) gregarin çeşitliliğini tespit etme amaçlı araştırma esnasında Bankura Bölgesi alüvyon toprağından toplanan toprak solucanı *Appoectodea trapezoides* Duges'in seminal vesiküllerinde yaşayan *Monocystis* Stein, 1884 cinsine dahil yeni bir tür ile karşılaşmıştır. *Monocystis appoectodae* sp. nov. bir veya daha fazla bariz yapısal farklılığa sahip çubuk benzeri bir organizmadır; bilhassa bazı olgun formları 178.0-224.0 (203.0±5.0) µm × 37.0-58.0 (46.0±1.5) µm boyutlarında olup, uç kısımlarda sivridir. Gametokistleri ovoid şekilli ve 108.0-118.0µm (113.0±1.1) × 79.0-89.0 (83.0±1.1) µm boyutlarındadır. Ookistleri navikular (kayık) şekilli olup uzunlukları 10.0-14.6 µm ve genişlikleri 5.5-8.1 µm arasında değişir.

**Anahtar Sözcükler:** *Monocystis appoectodae* sp. nov., gregarin, toprak solucanı, seminal vesikül, Hindistan

## INTRODUCTION

One well known endoparasitic group of protozoans (Apicomplexa) of invertebrates, especially arthropods and annelids, the "Gregarines" are chiefly coelozoic or lumen dwelling. Gregarines are distinguished into two major groups, acephaline and cephaline, of which acephaline forms are harboured by the earthworms. The acephaline or aseptate forms are characterized by the presence of a cell body lacking a septum near the anterior end (6).

Aseptate gregarine fauna have been reported from various parts of the world including India. But especially in India the search is far from complete. Investigations in search of

acephaline gregarines infesting earthworms of West Bengal, India revealed the occurrence of a new species of the genus *Monocystis* Stein, 1848. Until now, there are only records of ten species of *Monocystis* from India (1-3, 5, 7, 9, 10).

## MATERIAL AND METHODS

Earthworms were collected from alluvial soil of Bankura district. 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 Schaudin's fluid (66 ml HgCl<sub>2</sub>, 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 ethanol (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 micrometre ( $\mu\text{m}$ ). 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 (4).

## RESULTS

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

### *Monocystis apporectodae* sp.nov (Figs. 1-4)

Gamont Length (GL): 178.0-224.0 (203.0 $\pm$ 5.0, 11); Gamont width (GW): 37.0-58.0 (46.0 $\pm$ 1.5, 11); Nucleus Diameter (ND): 12.0-16.0 (15.0 $\pm$ 0.59); Gametocyst Length (GL): 108.0-118.0 (113.0 $\pm$ 1.1, 11); Gametocyst Width (GW): 79.0-89.0 (83.0 $\pm$ 1.1); Oocyst Length (OL): 10.0-14.6 (13.4 $\pm$ 1.1); Oocyst width (OW): 5.5-8.1 (7.7 $\pm$ 2.3).

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 obtained from the seminal vesicles of the earthworm *Apporectodea trapezoides* Duges from the alluvial soil of Bankura district of West Bengal the gamonts are solitary, elongated and flattened, ribbon like, having parallel sides but not uniform due to presence of constriction especially in mature form. Both the end of the body is with pointed tips. Ectosarc thin, (1-3 $\mu\text{m}$ ). Epicyte smooth i. e. free from external process. In some places distinction between ectoplasm and endoplasm is not clear. Endoplasm with evenly distributed vacuoles and with some dotted appearance, which are not uniform in distribution and their size also unequal. Almost rounded to ovoid nucleus at some distance from the periphery but not in exact middle portion of the gamont in almost all cases.

A number of vacuoles are present throughout the nucleoplasm. Hold fast organ not visible. Each ovoid gametocyst with two gametocytes, which are in close proximity with each other. Oocysts are navicular in shape. Sexual reproduction takes place in this organism by means of lateral syzygy.

## Taxonomic summary

**Type material:** *Monocystis apporectodae* sp. nov.

**Type host:** *Apporectodea trapezoides* Duges

**Symbiotype:** AT1/2005, AT6/2005 and some other slides are deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India

**Site of infection:** Seminal vesicles

**Type locality:** South-western Bengal

**Prevalence:** 11/44 (25%)

### Type material

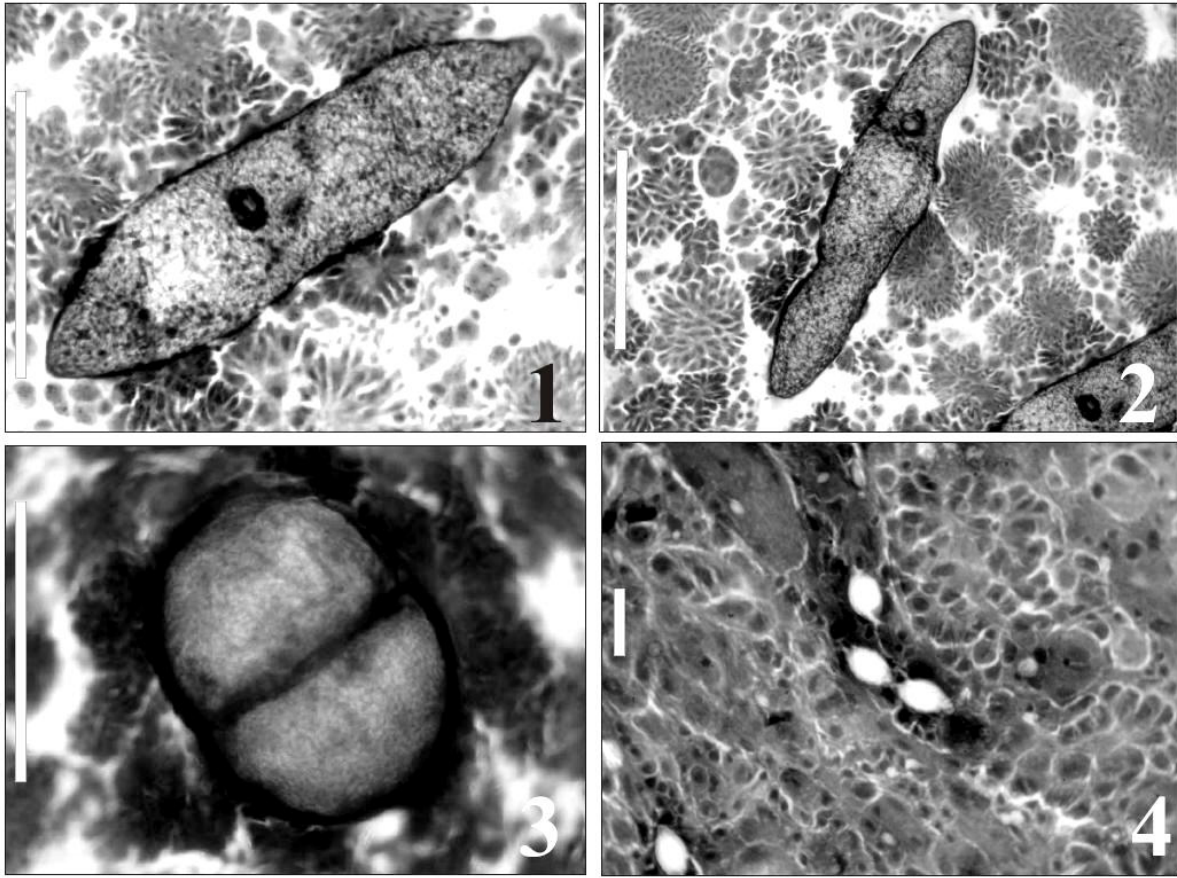
**Holotype:** Slide MA/2/2005 is deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India. Registration number ZD/KU/0144

**Paratypes:** Slides MA/03/2005, MA/06/2005, MA/07/2005 are in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

**Etymology:** The species is named after the generic name of the type host *Apporectodea trapezoides* Duges

## DISCUSSION

Till date, there are only records of 11 species of *Monocystis* from India (1-3, 5, 9, 10). The present monocystid gregarine obtained from the seminal vesicle of the earthworm *Apporectodea trapezoides* Duges has been compared with other species of the genus *Monocystis* Stein, 1848 and found that it resembles only two indian species, i.e., *Monocystis senchalensis* Pradhan *et* Dasgupta, 1982 (9) and *M. darjeelingensis* Bandyopadhyay *et* Mitra, 2005 (1) by having a few similarities. The new species obtained from the present investigation resembles *M. senchalensis* by having elongated gamont shape, but differs significantly in gamont diameter. *M. senchalensis* is comparatively a smaller sized (43.02-185.4 x 10.8-48.6) parasite than the new species (178.0-224.0 x 37.0-58.0). Endoplasm of *M. senchalensis* is packed with numerous paraglycogen granules, but endoplasm of the new species does not have paraglycogen granules. The shape and size of the oocysts also differs. The oocysts of the monocystid gregarine reported by Pradhan and Dasgupta (9) are bi-conical, which is navicular in the new species. The new species also resembles *M. darjeelingensis* in having the elongated gamont shape and size. The gamonts of *M. darjeelingensis* are cylindroid with round extremities and one end is slightly wider than the other. But in the new species we obtained from Bankura, the gamonts are elongated, ribbon like and with gradually tapering pointed ends. Ectoplasm of the gamonts of *M. darjeelingensis* is with fine, longitudinal striations, which is smooth in the new species. The size of the nucleus of *M. darjeelingensis* is significantly larger (26.0-40.0 x 20.0-32.0) than the new species (12.0-16.0). Nucleus of the gregarine reported by Bandyopadhyay *et al.* (2) is ellipsoidal or ovoidal with a little



**Figures 1-4.** Photomicrographs of different stages of the life cycle of *Monocystis apporectodae* sp. nov. obtained from the seminal vesicles of the earthworm *Apporectodea trapezoides* Duges. **Figs. 1-2.** Mature gamonts; **Fig. 3.** Gametocyst; **Fig. 4.** Oocysts. Scale bars 100  $\mu$ m (Figs. 1-3) and 10  $\mu$ m (Fig. 4).

amount of karyosome scattered throughout. The nucleus of the new species is ovoid with densely packed karyosome. Moreover, the hosts and localities are different. *M. darjeelingensis* has been reported from an elevation of 2310 meters from an earthworm *Amyntas robusta* Perrier. The new species has been reported from an earthworm *Apporectodea trapezoides* Duges from alluvial soil in the Gangetic plane.

Considering all the differences we propose the *Monocystis* species we have obtained from the seminal vesicle of the earthworm *Apporectodea trapezoides* Duges as new and designate as *Monocystis apporectodae* sp. nov.

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