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Observation on *Monocystis constricta* n. sp. (Protozoa: Apicomplexa: Monocystidae) from an Indian Earthworm, *Eutyphoeus quaripapillatus* Michelsen, 1907

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SUMMARY: A biodiversity survey of aseptate gregarines in earthworm hosts in the Calcutta district of West Bengal State revealed the existence of a new species of aseptate gregarine under the genus *Monocystis* Stein, 1848. The monocystid gregarines obtained from the earthworm host, *Eutyphoeus quaripapillatus* Michelsen, 1907 have been identified as a new species. The mucron was indistinct. The gamonts are elongated, ovoid, have a hood like structure at the anterior end and measure 150.1-212.4 (188.1± 2.1) µm x 66.1-112.1 (72.3 ± 1.1) µm. The gametocysts are ellipsoid and measure 92.3– 136.3µm (111.2 ± 2.1) × 78.3–114.4 µm (82.6 ± 3.6) µm. Prominent syzygy was apparent. Oocysts are navicular, measuring 14.1–22.3 (18.1 ± 3.2) µm × 9.1–15.2 (11.9 ± 1.1) µm.

Key words: Earthworm, seminal vesicles, new species, *Monocystis*, India.

Hindistan'daki Bir Toprak Solucanı, *Eutyphoeus quaripapillatus* Michelsen, 1907'ndan *Monocystis constricta* n. sp. (Protozoa: Apicomplexa: Monocystidae)'nın Tespiti

ÖZET: Hindistan'ın Batı Bengal eyaletinin Calcutta bölgesinde toprak solucanları üzerinde yaşayan aseptat gregarinlerin biyolojik çeşitliliği üzerine yapılan çalışmada *Monocystis* Stein, 1848 genusu altında yeni bir aseptat gregarin türü tanımlanmıştır. Yeni Monokistik gregarin türünün konağı bir toprak solucanı olan *Eutyphoeus quaripapillatus* Michelsen, 1907'tur. Mucron belirgin değildir, gamontlar elongat, ovoid ancak anterior kısmı kapişon benzeri bir yapıda olup 150.1-212.4 (188.1± 2.1) µm x 66.1-112.1 (72.3 ± 1.1) µm boyutlarındadır. Gametositler ellipsoid ve 92.3– 136.3µm (111.2 ± 2.1) × 78.3–114.4 µm (82.6 ± 3.6) µm şeklinde ölçülmüştür. Sizigi evresi belirgin. Ookistler navicular şekilli olup 14.1–22.3 (18.1 ± 3.2) µm × 9.1–15.2 (11.9 ± 1.1) µm boyutlarındadır.

Anahtar Sözcükler: Toprak solucanı, seminal vesiküller, yeni tür, *Monocystis*, Hindistan.

INTRODUCTION

Gregarines are chiefly coelozoic or lumen-dwelling protozoan parasites of invertebrates, especially arthropods and annelids under the order Eugregarinorida Léger, 1900. Of the two major groups of gregarines, septate and aseptate, insects harbor the septate and earthworms harbor the aseptate forms. Diversity of the gregarines is surpassed only by the coccidians among the phylum Apicomplexa. Eugregarinorida contains more than 1500 species under more than 250 genera. Gregarines have been reported from only about 3.124 invertebrate species, less than one third of one percent of the named invertebrate fauna (22). The majority of the gregarines reported so far from insects are septate gregarines. But less attention has been paid on the study of the biodiversity of aseptate gregarines found from annelids. 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. Research work on this group in India has gained momentum since 1980. Exploration of aseptate gregarine fauna inhabiting oligochaete hosts in India have discovered representatives of the genera *Apolocystis* Cognetti de Martiis 1923; *Monocystis* Stein, 1848; *Nematocystis* Hesse, 1909; *Stomatophora* Drzewiecki, 1907 and *Zygocystis* Bhatia, 1930 (1-17, 19-29]. As a part of the ongoing biodiversity survey one species of aseptate gregarines infesting the earthworms of West Bengal, India of the genus *Monocystis* have been obtained from the seminal vesicles of the earthworm, *Eutyphoeus quaripapillatus* Michelsen, 1907. The present paper deals with the description, taxonomy and systematic of the said aseptate gregarine, *Monocystis*

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constricta n.sp. The morphometric comparisons with closely related species have also been incorporated here.

MATERIAL AND METHOD

Samplings were carried out in the basin area of the river Ganges in the Calcutta district of West Bengal (Latitude 22° 34' N, Longitude 88° 24 E'). The earthworms were collected during the rainy season and the collected earthworms were kept in 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 microscope (Model- Olympus CX41). After 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 each) and placed in distilled water. These were transferred to a 3% iron alum solution (overnight) 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 alcohol, cleared in xyline and mounted in Canada balsam. Camera Lucida drawings of different stages of gregarines were made and photo-micrographs were taken with an Olympus phase contrast microscope (X 400 magnification) and an Olympus camera (Model-C5060). 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. The methods of describing shapes of planes and solids have been done following Clopton (18).

RESULTS

Monocystis constricta n. sp. (Figs 1–4, 5-8, Table 1)

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

Gamont Length (GL): 150.1-212.4 (188.1± 2.1) µm; Gamont Width (GW): 66.1-112.1 (72.3 ± 1.1) µm; Nucleus Length (NL): 14.3–20.5 (17.3 ± 1.9, 25) µm; Nucleus Width (NW): 13.3–21.9 (18.2 ± 2.1) µm; Gametocyst Length (LG): 92.3–136.3µm (111.2 ± 2.1); Gametocyst Width (GW): 78.3–114.4 µm (82.6 ± 3.6) µm µm; Oocyst Length (OL): 14.1–22.3 (18.1 ± 3.2) µm; Oocyst Width (WO): 9.1–15.2 (11.9 ± 1.1) µm.

The members of the genus *Monocystis* Stein, 1848 are characterized by having no distinct mucron, ovoid and solitary gamonts, bi-conical, symmetrical oocysts (22). In the present form the gamonts are solitary, ovoid and with constrictions at the anterior end forming a hood like structure. The position of the nucleus is almost in the middle of the gamonts. Cytoplasm granular. Syzygy stage present in the life cycle. Pellicle thin. Each gametocyst contains two unequal gametocytes. Oocysts navicular.

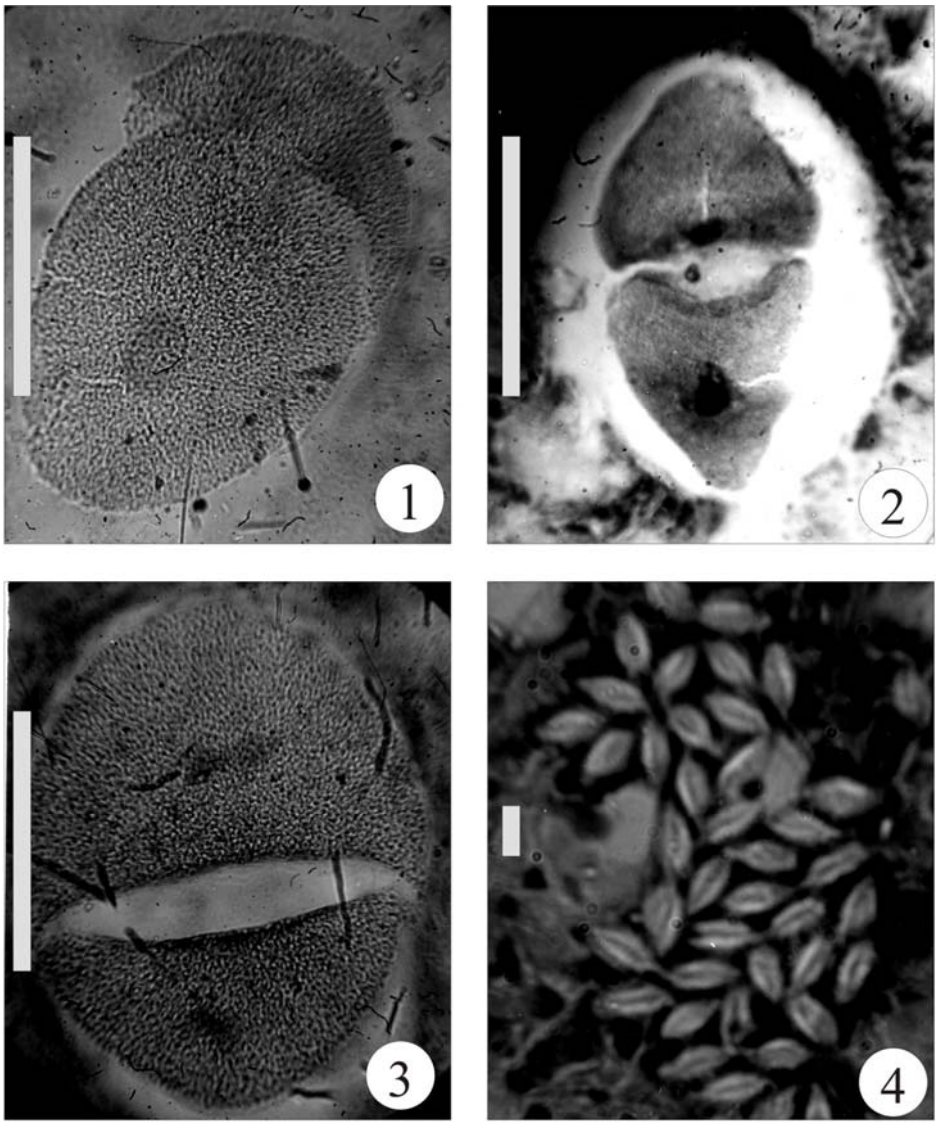
Taxonomic summary

Type material	*Monocystis constricta*
Type host	*Eutyphoeus quaripapillatus* Michelsen, 1907
Type of locality	Calcutta, West Bengal, India (Latitude 22° 34' N, Longitude 88° 24 E')
Symbiotype	EQ/08/16 deposited in the museum of the Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India.
Site of infection	Seminal vesicles
Prevalence	08/32 (25%)
Holotype	MQ/2008/12 is deposited in the museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.
Paratypes	MQ/2008/03, MQQ/2008/03 are in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.
Etymology	Since there is a constriction in the anterior side of parasite, hence the species is named after the shape of the parasite, i.e., *Eutyphoeus constricta* n.sp.

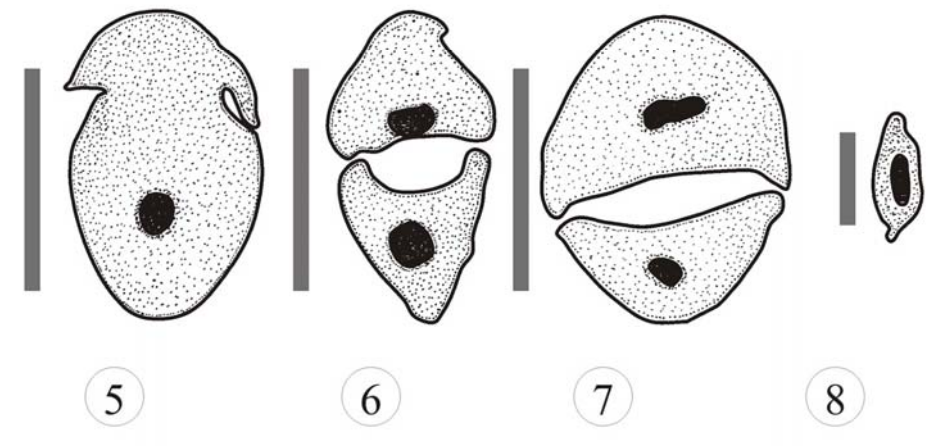
DISCUSSION

Cylindroid, solitary, with mucron with late syzygy of the parasite in an earthworm justifies its inclusion under the family Monocystidae, subfamily Monocystinae and genus *Monocystis* Stein, 1848. Of more than 70 species (22) belonging to the genus *Monocystis* Stein, 1848. Only fifteen have so far been described from India. Of these, *M. odontotermi* Kalavati 1979 (20) has been reported from the haemocoel of the termite *Odontotermes obesus* (a non oligochaete host) and *M. pontodrilus* Subbarao *et al.* 1979 (29) from the littoral oligochaete, *Pontodrilus bermudensis*. All other species of *Monocystis* have been reported from terrestrial earthworms, but none from the present host, *Eutyphoeus quaripapillatus* Michelsen, 1907. The species under discussion shows great morphological variability when it is compared with other species of the genus and only shows resemblance with *M. metaphirae* Bandyopadhyay et al. (14).

The gamont of the *Monocystis constricta* is elongated, slightly rounded, with a constriction at the anterior end of the body that forms a hood-like structure. While the gamont of *M. metaphirae* Bandyopadhyay *et al*. (14) is bean shaped with broad anterior end and comparatively narrow posterior end. The size of the gamont of the new species is comparatively larger than *M. metaphirae* (Table 1). There is a significant difference in cytoplasic structure in between the two species. In the new species the cytoplasm is granular where as is thin and non granular in *M. metaphirae*. There is no basic difference in the endoplasmic structure in both the species. The endoplasm in both the species are granular.



Figures 1-4. Photomicrographs of different stages of the life cycle of *Monocystis constricta* n. sp. obtained from the seminal vesicles of the earthworm *Eutyphoeus quaripapillatus* Michelsen, 1907 **1-2.** Mature gamonts, **3.** A gametocyst, **4.** Oocysts. Scale bars: 100 μm (5-6), 50 μm (7), 10 μm (8).



Figures 5-8. Camera lucida drawings of different stages of the life cycle of *Monocystis constricta* n. sp. obtained from the seminal vesicles of the earthworm *Eutyphoeus quaripapillatus* Michelsen, 1907 **5-6.** Mature gamonts, **7.** A gametocyst, **8.** Oocysts. Scale bars: 100 μm (5-6), 50 μm (7), 10 μm (8).

In *M. metaphirae* a large number of unequal sized paraglycogen granules are present in the cytoplasm. The number of paraglycogen granules are very few in the new species. In *M. metaphirae*, rounded nucleus is present in the broad portion of the gamont, but it is central in the gamonts of *M. constricta*. The size of the nucleus is slightly larger than *M. metaphirae* described from the seminal vesicles of *Metaphire houlleti* (Perrier). The structure and shape of the gametocysts and oocysts of both the species are almost identical but the size varies slightly. The most significant similarity in the life cycle of both the species is the presence of syzygy. While the new species is compared with all other species of the same genus obtained so far from oligochaete hosts, presence of a constriction at the anterior end giving a hood-like shape to the of the present form has not been found in any gregarines described earlier.

Table 1. Comparison of *M. constricta* n. sp. with *Monocystis metaphirae* (14) (all measurements are in µm. In each case minimum and maximum values are given, followed in parentheses the arithmetic mean).

Species	***Monocystis metaphirae***	***Monocystis constricta* n. sp.**
Characters		
Host	*Metaphire houlleti* (Perrier)	*Eutyphoeus quaripapillatus* Michelsen
Locality	North 24 Parganas, India	Kolkata, India
Gamonts	Solitary, bean shaped, measuring 94.0-151.0 µm x 53.0-81.0 µm	Solitary, ovoid, having a constriction at anterior end with hood like structure, measuring 150.1-212.4 µm x 66.1-112.1 (72.3 ± 1.1) µm
Ectoplasm	Ectoplasm thin	Ectoplasm granular
Endoplasm	Granular	Granular
Nucleus	Mostly rounded nucleus, situated at the wider portion of the gamont, measuring 4.0-16.0 µm	Mostly rounded nucleus, situated mostly at the middle of the gamont, measuring 13.3–21.9 µm
Gametocyst	Gametocyst with two unequal gametocytes,measuring 85.0-102.0 µm	Gametocyst with two unequal gametocytes, measuring 92.3–136.3µm
Oocyst	Oocysts navicular, measuring 4.0-7.5 µm	Oocysts navicular, measuring 9.1–15.2 µm
Site of Infection	Seminal vesicles	Seminal vesicles
References	Bandyopadhyay *et al.* (2006e)	Present paper

Considering all the aspects, it can be concluded that the species described here can not be compared with other species described so far from the seminal vesicles of the earthworm, *E. quaripapillatus* Michelson, 1907 and hence it is considered as a new species and therefore it is designated as *M. constricta* n.sp. in this paper.

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