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’da Bir Toprak Solucan, *Eutyphoeus quaripapillatus* Michelsen, 1907’ndan *Monocystis constricta* n. sp. (Protozoa: Apicomplexa: Monocystidae)’nin Tespiti

ÖZET: Hindistan’ın Batı Bengal eyaletinin Calcutta bölgesinde toprak solucanları üzerinde yaşanan aseptat gregarinlerin biyolojik çeşitliliği üzerine yapılan çalışmalar *Monocystis* Stein, 1848 herausında yeni bir aseptat gregarin türü tanınmıştır. Yeni Monokistik gregarin türünü konu eden bir toprak solucan olan *Eutyphoeus quaripapillatus* Michelsen, 1907’nden. Mucron belirgin değildir, gamontlar elonat, ovoid ancak anterior kısımların benzeri bir yapıda 150.1-212.4 (188.1± 2.1) µm x 66.1-112.1 (72.3 ± 1.1) µm boyutlandırır. 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 boyutlandırır.


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 more than 1500 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*
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 xylene and mounted in Canada balsam. Camera Lucida drawings of different stages of gregarines were made and photomicrographs 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 Clupton (18).

RESULTS

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


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, MQ/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. odontotermes 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 bernudensis. 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 cytoplasmic 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 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 oocyst of the present form has not been found in any gregarines described earlier.

Consider 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* Michelsen, 1907 and hence it is considered as a new species and therefore it is designated as *M. constricta* n.sp. in this paper.

**REFERENCES**


### 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).

<table>
<thead>
<tr>
<th>Species</th>
<th><em>Monocystis metaphirae</em></th>
<th><em>Monocystis constricta</em> n.sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Characters</strong></td>
<td></td>
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<tr>
<td>Host</td>
<td><em>Metaphire houlleti</em> (Perrier)</td>
<td><em>Eutypheous quaripapillatus</em> Michelsen</td>
</tr>
<tr>
<td>Locality</td>
<td>North 24 Parganas, India</td>
<td>Kolkata, India</td>
</tr>
<tr>
<td>Gamonts</td>
<td>Solitary, bean shaped, measuring 94.0-151.0 µm x 53.0-81.0 µm</td>
<td>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</td>
</tr>
<tr>
<td>Ectoplasm</td>
<td>Ectoplasm thin</td>
<td>Ectoplasm granular</td>
</tr>
<tr>
<td>Endoplasm</td>
<td>Granular</td>
<td>Granular</td>
</tr>
<tr>
<td>Nucleus</td>
<td>Mostly rounded nucleus, situated at the wider portion of the gamont, measuring 4.0-16.0 µm</td>
<td>Mostly rounded nucleus, situated mostly at the middle of the gamont, measuring 13.3–21.9 µm</td>
</tr>
<tr>
<td>Gametocyst</td>
<td>Gametocyst with two unequal gamocytes, measuring 85.0-102.0 µm</td>
<td>Gametocyst with two unequal gamocytes, measuring 92.3–136.3 µm</td>
</tr>
<tr>
<td>Oocyst</td>
<td>Oocysts navicular, measuring 4.0-7.5 µm</td>
<td>Oocysts navicular, measuring 9.1–15.2 µm</td>
</tr>
<tr>
<td>Site of Infection</td>
<td>Seminal vesicles</td>
<td>Seminal vesicles</td>
</tr>
<tr>
<td>References</td>
<td>Bandyopadhyay <em>et al.</em> (2006e)</td>
<td>Present paper</td>
</tr>
</tbody>
</table>

257


