On the occurrence of a gregarine parasite (Apicomplexa: Eugregarinorida) from the seminal vesicles of earthworms (Annelida: Oligochaeta) of Bangladesh

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Abstract In a survey of the endoparasitic acephaline gregarines in the Satkhira district of Bangladesh, seminal vesicles of earthworm, *Metaphire posthuma* were found to be infested with a new species of the genus *Enterocystis* Tsvetcov 1926, *Enterocystis elongatum* sp. nov. The new species is elongated and measures 55.3–87.8 (67.7 ± 10.3) μm in length and 33.2–37.8 (35.7 ± 1.6) μm in width. Nucleus ovoid and measures 4.4–8.8 (6.6 ± 2.2) μm in length and 4.4–6.6 (5.3 ± 1.1) μm in width. Gametocyst with two unequal sized gametocytes and measures 88.4–101.7 (97.9 ± 4.8) μm in length and 77.6–95.0 (86.9 ± 7.7) μm in width. Oocysts ellipsoidal and measure 13.7–19.9 (16.7 ± 2.6) μm in length and 6.6–8.8 (7.6 ± 1.1) μm in width. Prominent association or syzygy is present in life cycle.

Keywords Protozoa · Gregarine ·
Enterocystis elongatum sp. nov. · Earthworm ·
Seminal vesicles · Satkhira · Bangladesh

Introduction

In India researches on gregarine protozoan parasites infesting invertebrates have not been carried out with great importance. Gregarines are a group of apicomplexan protozoan parasites with two forms, septate and aseptate. Only

a few of over 350 species of Indian earthworms have been studied for the occurrence of gregarines, but this research has gained momentum since the 1980s. It has already resulted in the finding of representatives of the genera *Apolocystis* De Martis (1923); *Monocystis* Stein 1848 (Stein 1848) *Nematocystis* Hesse 1909; *Stomatophora* Drzewiecki 1907; *Zygocystis* Stein 1848; *Enterocystis* Tsvetcov (1926). Levine (1977) recognized eight species of *Enterocystis* of which *E. ensis* Tsvetcov 1926; *E. ephemerae* (Frantzius) Desportes (1963); *E. palmata* Codreanu 1940; *E. racovitza* Codreanu (1940); *E. rhithrogenae* Codreanu 1940; *E. fungoides* Codreanu 1940; *E. grassei* Desportes 1963 in *Ephemeroptera* and *E. hydrophili* (Foerster) Baudoin and Maillard 1972 in *Coleoptera*. After that *E. bengalensis* Sarkar 1983 was described from India. Since then no other species has been described from Indian sub continent. Noble (1938) described *E. bullis* from *Urechis caupo*, California. Besides these, *E. yumushii* Iit-suka 1933, *E. greefi* (Greeff) Noble 1938, were described from different hosts except earthworms. The biodiversity survey of gregarines from earthworms in the Satkhira district of Bangladesh revealed another species of the aseptate gregarine of the genus *Enterocystis*, Tsvetcov 1926 from this subcontinent which has been described here. This paper deals with the description of a new species of the genus *Enterocystis*. The morphometric comparison with closely related species and the discussion of its taxonomy and systematics are also presented.

Materials and methods

Samplings were carried out in search of earthworm hosts in the Satkhira district of Bangladesh. The host earthworms were collected during winter season (November

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2009–February 2010) and the collected earthworms were kept in soil in a tub and taken to the laboratory alive. Some of the 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 protozoan under a light microscope. After initial study of living protozoans the content of the seminal vesicles semidried and fixed in Schaudin's fluid for 20 min. The smears were stored in 70% ethanol for removal of mercuric chloride. The slides were then passed through a descending series of alcohol (5 min each) and placed in distilled water. These were transferred to a 3% Iron Alum solution (Over night) and stained with Heidenhain's Hematoxylin solution for 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 DPX. All measurements were made with a calibrated ocular micrometer (μm). In each case minimum and maximum values are given, followed in parentheses by arithmetic mean, standard deviation. For statistical analysis measurements of 20 specimens were taken into account. Photomicrographs were taken using an Olympus light microscope (model CH-2) and Olympus digital camera (model CX41). The methods of describing shapes of planes and solids have been done following Clopton 2004.

Results

Enterocystis elongatum sp. nov. (Fig. 1, a–f; Table 1).

Phylum	Apicomplexa Levine 1988
Order	Eugregarinorida Léger 1900
Suborder	Aseptatoria Chakravarty 1960
Family	Enterocystidae Codreanu 1940
Genus	<i>Enterocystis</i> Tsvetsov 1926.

With the characters of the genus *Enterocystis* Tsvetsov 1926 as given by Levine 1988; gamonts are intracellular. Early syzygy, primite enlarging at its base and the satellite remaining more or less cylindrical that the two cells have sword or dagger shape, gametocysts without sporoducts, opening by simple rupture; oocysts ellipsoidal. The shape of the mature gamont is elongated, solitary, aseptate and cleaned gated with a tunnel. Both the ends of the gamonts are blunt and there is a clear distinction between ectoplasm and endoplasm. Ectoplasm smooth. Endoplasm brown and denser with the presence of paraglycogen granules. Mucorn absent. Gamont measures $55.3\text{--}87.8 \times 33.2\text{--}37.8 \mu\text{m}$. Prominent association of mature gamonts are present. The nucleus is ovoidal with variable position within the gamont.

karyosome absent. The nucleus measures $4.4\text{--}8.8 \times 4.4\text{--}6.6 \mu\text{m}$. The gametocysts are unique shaped with two unequal sized gametocytes and measure $88.4\text{--}101.7 \times 77.6\text{--}95.0 \mu\text{m}$. Oocysts ellipsoidal without any sporozoites. Oocyst measures $13.7\text{--}19.9 \times 6.6\text{--}8.8 \mu\text{m}$.

Taxonomic summary

Species	<i>Enterocystis elongatum</i> sp. nov
Name of the host	<i>Metaphire posthuma</i>
Locality	Satkhira, Bangladesh
Habitat	Seminal Vesicles
No. of the host infested	12
No. of the specimens	20
Prevalence	12/20 (60%)
Material examined	Holotype: BD/15/09 deposited at the Parasitology laboratory of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India
Syntype	BD/EN/5/10 deposited at the Parasitology Laboratory of the Department of Zoology, University of Kalyani. Kalyani 741235, West Bengal, India
Etymology	The name of the species is given after the shape of the parasite

Discussion

The species under discussion shows great morphological variability while compared with other species of the genus *Enterocystis* described so far. The species resembles to some extent with *E. bullis* (Noble 1938) and *E. bengalensis* (Sarkar 1983). The gamonts of *E. bullis* are solitary, bulb like distention of the middle of the body, while the posterior end becomes pointed and dagger like. But here the mature gamonts are aseptate, elongated, solitary and clean gated with a tunnel. The size of the gamont is significantly larger in *E. bullis* than the species described here. Both the ends of the gamont of the present form is blunt and there is a clear distinction between ectoplasm and endoplasm. In *E. bullis* a cluster of fibrils was extended from the anterior end of the parasite which appeared to be a modification of epicyte for attachment. Nucleus of *E. bullis* was spherical with granular nucleoplasm, a large karyosome and additional chromatin bodies were also present. But here it is small, almost ovoid, contained with a group of granules and occupies variable positions within the gamont. Ectoplasm both in *E. bullis* and here is smooth. Endoplasm in both the cases are highly granular with large number of

Fig. 1 Photomicrographs of different stages of the life cycle of *Enterocystis elongatum* sp. nov. obtained from the seminal vesicles of the earthworm *Metaphire posthuma*. *a*. A mature gamont; *b–c*.

Gamonts in clusters; *d*. Gamonts in syzygy; *e*. Gametocyst; *f*. Oocysts. [Scale bars: 50 μ m (*a–d*), 100 μ m (*e*), 20 μ m (*f*)]

paraglycogen granules. The gametocyst of *E. bullis* was pyriform, one end being sharply pointed. But in the species under discussion, the shape of the gametocyst is unique having two unequal gametocytes. In *E. bullis* the oocysts are spherical and without any gelatinous wall. The oocysts are ellipsoidal and without any sporozoites in the present form. In *E. bengalensis*, the primitive is cylindrical with a

bulb-head anterior end and a round or truncated posterior end with slightly concave sides. In the present form the bulb-like anterior end is absent without any distinct primitive and the gamonts are elongated, solitary and with blunt ends. Late syzygy is observed in *E. bengalensis* whereas life history of present study encounters early syzygy. Nucleus in *E. bengalensis* is spherical but here it is ovoidal.

Table 1 Morphometric comparison of *Enterocystis elongatum* sp. nov. with *E. bullis* Noble, 1938 and *E. bengalensis* Sarkar 1983

Species→ Characters↓	<i>E. bullis</i> Noble 1938	<i>E. bengalensis</i> Sarkar 1983	<i>E. elongatum</i> sp. nov. (n = 20)
Host	<i>Urechis caupo</i>	<i>Psocatropos</i> sp. (Psocoptera)	<i>Metaphire posthuma</i>
Locality	Drakes Bay, California	Naihati, West Bengal, India	Satkhira, Bangladesh
Site of Infestation	Posterior end of the midgut of <i>Urechis caupo</i>	Midgut and hindgut	Seminal vesicles
Gamonts	Solitary, characterized by a bulb-like distention of the middle part of the body while the posterior end become pointed. Adult gamont range 400 (274–500) microns in length and 150 (110–200) microns in width	The primite cylindrical with a bulb-head anterior end and a round or truncated posterior end with slightly concave sides. The satellite was cylindroconical or lanceolate slightly longer than the primite. Syzygy present in this stage	Elongated, solitary, aseptate and clean gated with a tunnel. Gamont with blunt ends and measures 55.3–87.8 × 33.2–37.8 μm
Mucorn	No mucorn but a cluster of fibrils is present which extended from anterior end of the parasite	No mucorn	No mucorn
Ectoplasm	Smooth	Smooth	Smooth
Endoplasm	Highly granular and with a large number of paraglycogen granules	Highly granular and with a large number of paraglycogen granules	Granular with numerous paraglycogen granules
Nucleus	Spherical with granular nucleoplasm and 10 μ in diameter which was situated at about the center of the body. One large karyosome is characteristic, additional chromatic bodies were present	Spherical with nucleoplasm which situated at near about the posterior end	Nucleus ovoidal, measures 4.4–8.8 × 4.4–6.6 μm, which was not situated at about the center of the body
Gametocyst	Pyriform, one end being sharply pointed and measured 5/6 μ	Thin-walled gametocysts, 63.5 in diameter.	The gametocysts are unique shaped with two unequal sized gametocytes, measuring 88.4–101.7 × 77.6–95.0 μm
Oocyst	Spherical and measured 185/200 μ without the gelatinous wall. Spores unknown	Ellipsoidal with truncated and thickened end, measures 6 × 4 μm and contained filiform sporozoites	Oocysts ellipsoidal and measure 13.7–19.9 × 6.6–8.8 μm, without any sporozoites
Syzygy	Lateral syzygy is present	Caudo-frontal syzygy is present	Syzygy of mature gamonts are present
Reference	Noble (1938)	Sarkar (1983)	Present study

The gametocyst in *E. bengalensis* is rounded which is ovoid in the present form. Gametocyst of present species contains two unequal sized gametocytes which is lacking in *E. bengalensis*. Size of the gametocyst of *E. bengalensis* is smaller in comparison to the present form. Oocyst of *E. bengalensis* is ellipsoidal with truncated and thickened ends without any sporozoites. The size of the oocysts are significantly larger than *E. bengalensis*. Moreover, the hosts are also different. Both *E. bullis* and *E. bengalensis* were described from insect hosts whereas the present form has been described from oligochaete hosts. It is also quite different in morphology, host preference and distribution pattern from the other two *Enterocystis* species described earlier. So it is evident that the present species which is obtained from the seminal vesicles of the earthworm host *Metaphire posthuma*, is totally different from *E. bullis* (Noble 1938) and *E. bengalensis* (Sarkar 1983) and it is also found for the first time from the hosts of Bangladesh. Considering all these aspects the present form is designated

as a new species and hence the name *Enterocystis elongatum* sp.nov is being proposed.

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