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STUDIES ON THE BIODIVERSITY OF ASEPTATE GREGARINE PARASITE FROM THE OLIGOCHAETE HOST OF BANGLADESH

Sutapa Sarkar and Probir K. Bandyopadhyay*

Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani- 741235, West Bengal, India

Abstract: Biodiversity studies in search of endoparasitic acephaline gregarines of the earthworms of Dhaka, Bangladesh, revealed a new species under the genus *Nematocystis* Hesse, 1909. The species was obtained from the seminal vesicles of the earthworm *Metaphire peguana* Rasa, 1890. It is a perfectly ribbon like organism, solitary, cylindrical, with several bulges and foldings in the middle portion of the body, especially in mature forms. Mature gamont measures 1955.85-1989.0 (1970.215 ± 13.830) µm in length and 48.62-53.04 (51.714 ± 2.135) µm in width. Nucleus small and rounded, measures 26.52-33.15 (29.835 ± 2.992) µm in length and 17.68-22.01 (19.448 ± 2.282) µm in width. Gametocysts spherical with two approximately equal sized gametocytes. It measures 110.05-121.55 (116.24 ± 4.908) µm in length and 88.04-99.45 (93.925 ± 5.823) µm in width. Oocysts biconical, measuring 22.1-30.94 (26.962 ± 3.870) µm × 11.05-15.47 (13.26 ± 2.08) µm.

Keywords: Gregarine, parasite, *Nematocystis bangladeshensis* sp.nov, seminal vesicles, earthworm, *Metaphire peguana*, Bangladesh.

INTRODUCTION

Levine (1977) listed 27 species of *Nematocystis* Hesse, 1909. Segun (1978) describe a species under the genus *Nematocystis*. Later on Pradhan and Dasgupta (1980) and Bandyopadhyay *et al.* (2005, 2006, 2007), Mallik *et al.* (2009 and 2010) worked a lot on *Nematocystis*. A total 39 species of *Nematocystis* have been reported from oligochaete hosts, of which only15 species have been from India. The present paper deals with the description of a new species of *Nematocystis* obtained from the seminal vesicles of the earthworm *Metaphire peguana* Rasa, 1890 collected from Dhaka, Bangladesh for the first time.

The present study is related with the biodiversity of the acephaline gregarine from the oligochaete host of Bangladesh. The objective of the present study is to describe a new species of aseptate gregarine from the oligochaete host, Bangladesh.

MATERIAL AND METHODS

The biotic survey of gregarine parasites in oligochaetes was performed in Dhaka, Bangladesh. Samplings were carried out in search of earthworm hosts in Dhaka, Bangladesh. The host earthworms were collected during the months of April -June 2011 from the drainage soil and the collected earthworms were kept

*Corresponding author: E-mail: prabir0432@hotmail.com

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 slide 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 the examination of living protozoan under a light microscope. After initial study of living protozoans, the content of the seminal vesicles was semidried and fixed in Schaudin's fluid for 20 minutes. The smears were stored in 70% ethanol for the removal of mercuric chloride. The slides were then passed through a descending series of alcohol (five minutes each) and placed in distilled water. These were transferred to a 3% Iron Alum solution (Over night) and stained with Heidenhain's hematoxylin for 20 minutes. 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 DPX. All measurements were made with a calibrated ocular micrometer. 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 the shapes of planes and solids have been done following Clopton (2004).

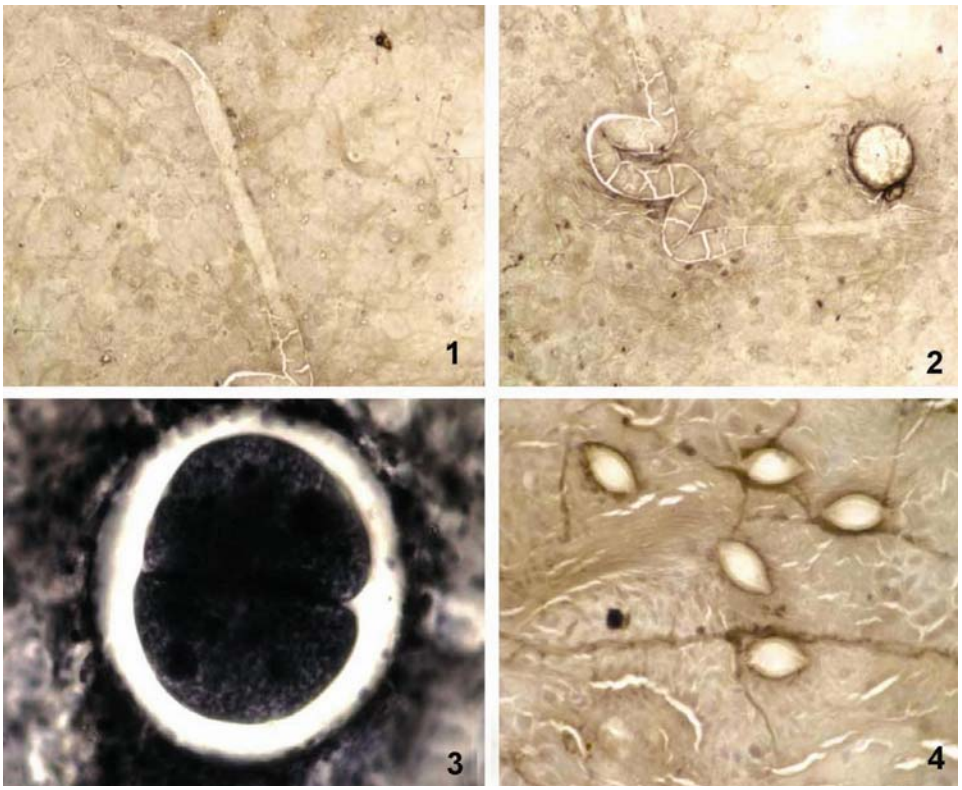
RESULTS AND DISCUSSION

Phylum : Apicomplexa Levine, 1977
Class : Sporozoa Labbe, 1899
Order : Eugregarinida Lager, 1900
Family : Monocystidae Biitschli 1882
Sub family : Monocystinae Bhatia, 1930
Genus : *Nematocystis* Hesse, 1909

***Nematocystis bangladeshensis* sp. nov (Figs 1-4, Tables 1-2)**

Mature gamont length (GL) 1955.85-1989.0 (1970.215 ± 13.830) µm and width (GW) 48.62-53.04 (51.714 ± 2.135) µm. Gamont width at the middle part 77.35-88.04 (83.759 ± 4.825) µm and gamont width at the posterior end 22.1-33.15 (28.73 ± 5.706) µm. Nucleus small and rounded. The length of nucleus (LN) 26.52-33.15 (29.835 ± 2.992) µm and width (WN) 17.68-22.01 (19.448 ± 2.282) µm. Gametocyst spherical with two approximately equal sized gametocytes. Length of gametocyst (LG) 110.05-121.55 (116.24 ± 4.908) µm and

width (WG) 88.04-99.45 (93.925 ± 5.823) µm. Oocyst biconical. Length of oocyst, 22.1-30.94 (26.962 ± 3.870) µm and width 11.05-15.47 (13.26 ± 2.08) µm.



Figs 1-4: Photomicrographs of different stages of the life cycle of *Nematocystis bangladeshensis* sp.nov. obtained from the seminal vesicles of the earthworm *Metaphire peguana.* 1. Anterior part of mature gamont. 2. Posterior part of mature gamont. 3. Gametocyst. 4. Oocysts. Magnifications : 15x40(1-3), 15x100 (4).

With the characters of the genus *Nematocystis* Hesse, 1909 as given by Levine (1977) "Gamonts large, cylindrod, nematoid, often with mucorn at anterior end solitary, oocyst biconical." In the present form the gamonts are solitary and cylindrical, with an enlarged head. The anterior end is usually swollen and rounded, but the edges are not perfectly parallel, because of some foldings. The posterior end ended gradually to a point. The mucorn well developed, made up of smooth hyalin protoplasm. Ectoplasm and epicyte thin, transparent and quite delicate. Sarcocyte is composed of dense homogeneous protoplasm. Endoplasm is composed of dense protoplasm without any paraglycogen grain and in the head region it is more transparent in comparison to other portion of the body. Nucleus elongated and present at the anterior end.

Gametocyst spherical with two approximately equal sized gametocytes, Oocyst biconical. The presence of large and long solitary nematode like gamont and biconical oocyst justifies the inclusion of the present form under genus *Nematocystis* Hesse 1909.

Table 1. Summary of Measurements (μm) of different body parts of the gamonts, gametocysts and oocysts are presented below.

Different body parts of the gamonts, gametocysts and oocyst.	Mean	Range	Standard deviation
Gamont length (GL)	1970.215	1955.85-1989.0	13.83
Gamont width (GWa) (anterior part)	51.714	48.62-53.04	2.135
Gamont width (GWm) (Middle part)	83.759	77.35-88.4	4.825
Gamont width (GWp) (Posterior part)	28.73	22.1-33.15	5.706
Protruted anterior part length	24.31	110.5-132.6	2.329
Length of nucleus (LN)	29.835	26.52-33.15	2.992
Width of nucleus (WN)	19.448	17.68-22.1	2.282
Length of gametocyst (LG)	116.24	110.5-121.55	4.908
Width of gametocyst (WG)	93.925	88.4-99.45	5.823
Length of oocyst (LO)	26.962	22.1-30.94	3.870
Width of oocyst (WO)	13.26	11.05-15.47	2.08

The species under discussion has some peculiar characters (cylindrical body with roughly parallel sides ending in a 'v' shaped pointed posterior end, several folding along the middle part of the body, anterior end is lobe like and with mucorn). While comparing the species it has some similarities with *Nematocystis levinei, N. gardenica* and *N. kalyaniensis*, but dissimilarities are predominant with *N. stephensoni* (Bhatia and Setna 1926), *N. bayrami*, *N. vinodae*. The length of the gamont of present form is greater than that of *N. bayrami*, *N. gardenica*, *N. kalyaniensis*, *N. vinodae*, *N. majumdari* and also from *N. stephensoni*. But, it does not show much difference with the length of *N. indica* and *N. Levinei.* The width of the gamont of *N. indica* and *N. levinei* has some differences with the present form. The shape of the present form also varies with the earlier described species of *Nematocystis.* The size and position of the nucleus also differs with the previously described species. In *N. indica* the nucleus is elongated. Whereas, it is spherical in *N. kalyaniensis*, rounded and compact in *N. majumdari* and *N. bayrami*, oval in *N. gardenica,* ellipsoidal in *N. stephensoni.*

The gametocyst of *N. indica* possess two almost equal gametocytes which resembles the present species. But in *N. bayrami,* the gametocyst bears two unequal sized gametocytes. The shape of the Oocyst in the present species differs from that of *N. gardenica* and *N. kalyaniensis,* but it shows some degree of similarities with the previously described species in regard to body dimensions. Moreover, the hosts are also different. *N. gardenica, N. kalyaniensis*

Table 2. Comparison of Indian species of *Nematocystis* from the seminal vesicles of the different earthworm hosts. All measurements are in microns (µm).

Parasitic sp.	*N. gardenica*	*N. kalyaniensis*	*N. indica*
Characters			
Gamonts	Elongated, nematode like, stout, parallel sides with pointed tips.	Solitary, elongated, nematode like having nearly equal bulbs like swelling at two ends.	Gamont solitary, elongated, perfect nematode organism, 1-4 constrictions with several bulges.
Size	110 to 1320 (505±12.41) µm × 11 to 18 (13.0 ± 1.8) µm.	Measuring 2462 to 1232 (565 ± 21.32) µm × 7 to 13 (10 ± 2.32) µm in diameter.	1969.9-3139.6 (2773.3 ± 404.1) µm × 10.4-45.8 (30.6 ± 2.3) µm.
Nucleus	Vesicular, oval or elongated, measuring 22.0-66.0 (32.0 ± 113;41) × 6.6-13.2 (9.3-6.6) µm in diameter, near the middle of the body.	Prominent vesicular nucleus, elliptical or ovoid, measuring 26.6 ± 1.6 × 9-18 (12.3 ± 0.6) µm in diameter and it is at one terminal bulb.	Elongated, occasionally remaining with in body constrictions,measuring 46.2x14.8 µm in diameter.
Mucorn	Absent.	Absent	Absent
Gametocysts	140 to 166 µm in diameter.	120-155 (133.0 ± 2.1) µm in diameter.	Rounded to ovoid with two almost equal gametocytes. Measuring 88.2 µm in diameter.
Oocysts	Small, biconical, 11.0-12.6 × 4.0 µm.	Biconical, measuring 8.5-11.4 × 5.0 µm in diameter.	Biconical, average size 17.9 × 11.7 µm.
Host	*Amynthas diffriengens* (Baird, 1809),	*Amynthas hawayanus*(Rasa, 1891)	*Amynthas diffriengens* (Baird, 1809).
Locality	Kalyani, Nadia, West Bengal.	Kalyani, Nadia, West Bengal.	East Midnapur, West Bengal.
Site of infection	Seminal vesicles.	Seminal vesicles.	Seminal vesicles.
Reference	Bandyopadhyay and Mitra 2005.	Bandyopadhyay and Mitra 2005.	Bandyopadhyay *et al.* 2006.

N. majumdari	*N. bayrami*	*N. vinodae*	*Nematocystis bangladeshensis sp.nov*
Solitary, elongated cylindrical worm like with terminal bulb structure.	Solitary, elongated, cylindrical body with roughly parallel sides ending in a 'V' shaped tapering at both ends.	Large, solitary, cylindrical, nematode like without any terminal bulb.	Ribbon like organism, solitary, cylindrical, with several bulges and foldings in the middle portion of the body, especially in mature forms.
129.1-151.7(143.0) × 8.2-22.5(13.9) µm.	938.79-1061.91 (998.8 ± 36.32) µm × 26.58-32.72 (29.24 ± 1.64) µm.	245.40-320.66 (270.68 ± 18.86) µm × 20.45-26.58 (24.54 ± 2.10) µm.	1955.85-1989.0 (1970.215 ± 13.830) µm × 48.62-53.04 (51.714 ± 2.135) µm.
Elongated to round, measuring 8.3-27.5(20.4 ± 6.7) µm in diameter.	Rounded and compact, present at anterior end. It measuring 12.27-16.36 (14.52 ± 1.13) µm in diameter.	Rounded to ovidal and20.45-24.54(22.69±1.74) µm.in diameter.	Elongated and measuring 26.52-33.15 (29.835 ± 2.992) µm × 17.68-22.01 (19.448 ± 2.282) µm in diameter.
Absent	Absent	Absent	Present.
Rounded, with two almost unequal size gametocytes. Measuring 73.8-96.4 (86.3) µm in diameter.	Rounded to ovoidal with two unequal sized gametocytes. Measuring 94.07-06.34(99.89±3.48) µm in diameter.	Almost rounded, measuring 77.71-85.89(82.82±2.69) µm in diameter.	Gametocysts spherical with two approximately equal sized gametocytes. It measuring 110.05-121.55 (116.24 ± 4.908) µm × 88.04-99.45 (93.925 ± 5.823) µm in diameter.
Navicular, 9.6-11.0(10.4±0.3) µm × 7.3-8.3 (7.6 ± 0.2) µm in diameter.	Biconical with sharp and pointed ends, measuring 12.70-13.86(13.24±0.32) µm × 6.54-7.31(7.04±0.31) µm in diameter.	Navicular, measuring 7.70-8.47 (8.24±0.36) µm × 4.23-4.62 (4.50±0.18) µm in diameter.	Oocysts biconical, measuring 22.1-30.94 (26.962 ± 3.870) µm × 11.05-15.47 (13.26 ± 2.08) µm.
Eutyphoeus incommodus (Beddard).	*Eutyphoeus orientalis* (Beddard).	*Eutyphoeus nicholsoni* (Beddard).	*Metaphire peguana* Rasa 1890.
West Midnapur, West Bengal.	Bankura, West Bengal.	Bankura, West Bengal.	Dhaka, Bangladesh.
Seminal vesicles.	Seminal vesicles.	Seminal vesicles.	Seminal vesicles.
Bandyopadhyay *et al.* 2007.	Mallik and Bandyo-padhyay 2009.	Mallik *et al.* 2010.	Present study.

and *N. indica* were described from *Amynthas diffriengens* and *Amynthas hawayanus* whereas *N. bayrami* and *N. vinodae* from *Eutyphoeus orientalis* and *Eutyphoeus nicholsoni,* respectively of Indian subcontinent. On the other hand, the host of the present form is obtained from *Metaphire peguana* of Bangladesh. So it is evident, that the present form obtained from the seminal vesicles of *Metaphire peguana* is totally different from the other species under the genus *Nematocystis* described earlier. It differs in shape, size and distribution pattern also and is found for the first time from different geographical region, i.e. Bangladesh. Considering all these aspects the present form is a new species and hence the name *Nematocystis bangladeshensis* sp. nov. is being proposed.

TAXONOMIC SUMMARY

Species: Nematocystis bangladeshensis sp. nov.

Name of the Host: Metaphire peguana Rasa, 1890.

Locality: Dhaka (Lat, 23° 43′ 23″ N, Long, 90° 24′ 31″ E) Bangladesh.

Habitat: Seminal Vesicles.

No. of the specimens examined: 20

No. of the host infested: 10

Prevalence: 10/20 (50%)

Material examined: **Holotype no:** 301/BD/ DH/11 deposited at the Parasitology laboratory of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India. **Paratype no:** 302/BD/DH/2/11 deposited at the Parasitology Laboratory of the Department of Zoology, University of Kalyani, Pin- 741235, West Bengal, India.

Etymology: The name of the species is given after the name of the country from where the hosts have been collected.

Concluding remarks: The biodiversity study of protozoan parasites from oligochaete hosts of Bangladesh revealed that the seminal vesicle of *Mataphire peguana* are infected with a new species of the genus *Nematocystis* and while compairing the other species described earlier, it seems to be new to science and a new name has been proposed in this communication.

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