

***Nematocystis bayrami* sp. n. (Protozoa, Apicomplexa, Eugregarinida)
monocystid gregarine from *Eutyphoeus orientalis* Beddard
(Annelida, Oligochaeta)**

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Abstract. A survey was conducted to explore the diversity of gregarine parasites of the earthworms of West Bengal, India. During this survey a new species under the genus *Nematocystis* Hesse, 1909 has been obtained from the seminal vesicles of the earthworm, *Eutyphoeus orientalis* Beddard. The species are solitary and bears a perfect nematoid like appearance with pointed terminals, especially in mature forms. It measures 938.79-1061.91 (998.81±36.32) µm in length and 26.58-32.72 (29.24±1.64) µm in width. Nucleus rounded, measures 12.27-16.36 (14.52±1.13) µm in diameter. Extreme ends conical and pointed. Gametocysts rounded to ovoidal, measuring 94.07-106.34 (99.79±3.48) µm in diameter. Oocysts biconical, measuring 12.70-13.86 (13.24±0.32) µm × 6.54-7.31 (7.04±0.31) µm.

Key words: gregarine, parasite, *Nematocystis bayrami*. sp. n., seminal vesicle, earthworm, *Eutyphoeus orientalis*, India.

Introduction

Nematocystis Hesse, 1909 is parasitic to oligochaetes especially to earthworms. Levine (1977) listed 27 species of *Nematocystis* Hesse, 1909. Later on Segun (1978) added one new species, followed by Pradhan and Dasgupta (1980) who reported five new species. Roychoudhury and Haldar (1984) described two new species. Bandyopadhyay and Mitra (2005) identified a new species. Again Bandyopadhyay et al. (2006 and 2007) recorded two new species. Presently 39 species of *Nematocystis* have been reported from oligochaete hosts, of which 14 species have described from India.

The present paper deals with a new species of *Nematocystis* obtained from the

seminal vesicles of the earthworm *Eutyphoeus orientalis* Beddard.

Material and Methods

A survey was conducted to explore diversity of gregarine parasites in oligochaetes of West Bengal, India. Many earthworms were collected and taken to the laboratory, of which thirty-nine earthworms *Eutyphoeus orientalis*, Beddard were examined for *Nematocystis* infection. They were dissected alive and their seminal vesicles were carefully removed. These seminal vesicles were placed on clean watch glass with few drops of 0.65% NaCl solution. A thin film of seminal fluid was drawn out on a slide and examined under a phase contrast microscope for live gregarine parasites. 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 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 the help of an Olympus phase contrast microscope and Olympus camera. All measurements are in micrometers (μm). Description of plane shapes follows the guidelines of Clopton (2004).

Results and Discussion

Nematocystis bayrami sp. n. (Fig: 1-6)

Phylum: Apicomplexa Levine, 1977

Order: Eugregarinida Leger, 1900

Family: Monocystidae Bztschli, 1882

Subfamily: Monocystinae Bhatia, 1930

Length of body (LB): 938.79-1061.91 (998.81 ± 36.32); width of body (WB): 26.58-32.72 (29.24 ± 1.64); diameter of nucleus (DN): 12.27-16.36 (14.52 ± 1.13); diameter of gametocyst (DG): 94.07-106.34 (99.79 ± 3.48); length of oocyst (LO): 12.70-13.86 (13.24 ± 0.32); width of oocyst (WO): 6.54-7.31 (7.04 ± 0.31); LB : WB = 34 : 1; LO : WO = 1.88 : 1 (Statistics based on 20 observations for all cases).

The specimen showed the characters of the genus *Nematocystis* Hesse, 1909 as given by Levine (1977) "Gamonts large, cylindroid, nematoid, often with mucron at anterior end, solitary, oocysts biconical". In the present form, gamonts are solitary, elongated, and ribbon-like. But the edges are not perfectly parallel, since constrictions and slight bulging region observed throughout the length of the body in mature forms

especially in the posterior end. Both ends of the gamont rounded. Ectoplasm thin. Endoplasm granulated. It harbors granules of various sizes and vacuoles are also present. Granules are not uniformly distributed but all exhibit deep staining property. Nucleus rounded, not present in the middle of the gamont but at one half which is presumed to be designated as anterior half. When stained, the entire nucleus exhibit deep staining property. Gamonts do not possess any kind of anchoring device. Each rounded to slightly ovoid gametocyst bears two gametocytes of unequal size. Oocysts are biconical and with sharp pointed end. Large and long solitary nematoid gamont and biconical oocysts justify the inclusion of the present species under the genus *Nematocystis* Hesse, 1909.

The species under discussion has some unique characters, elongated, cylindrical body with roughly parallel sides, ending in a V-shaped tapering at both ends, but the posterior end is more pointed. Slightly narrow zone followed by slightly bulging zone observed in the body. Nucleus is rounded and compact. Cytoplasm granulated and vacuoles are also present. Besides, this present form shows some similarities with other species, such as *N. bunmii* Segun, 1978; *N. levinei* Pradhan and Dasgupta, 1980, *N. gardenica* Bandyopadhyay and Mitra 2005, *N. majumdari* Bandyopadhyay et al. 2007. The gamont of *N. majumdari* measures only 129.10-151.70 μm in length and 8.2-22.5 μm in width. So, the present form is about seven times longer and more than two times wider than *N. majumdari*. The new species also differs from *N. gardenica* in their gamonts' shape. The gamonts of *N. gardenica* are almost straight with sharply pointed tips and possess no constrictions. But the gamonts of

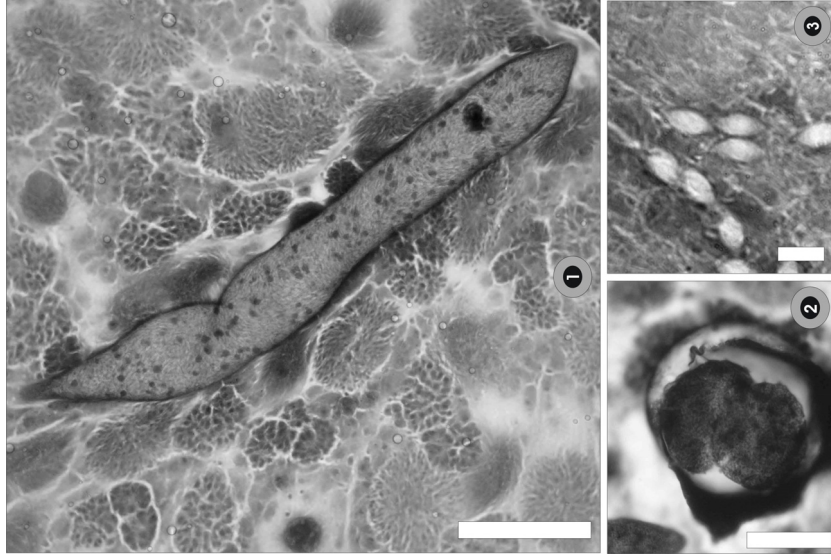


Figure 1-3. Photomicrographs of different stages of the life history of *Nematocystis bayrami* n. sp. (1) Trophozoites; (2) Gametocyst; (3) Oocysts. Scale bars: (1) 200 μ m, (2) 100 μ m and (3) 10 μ m.

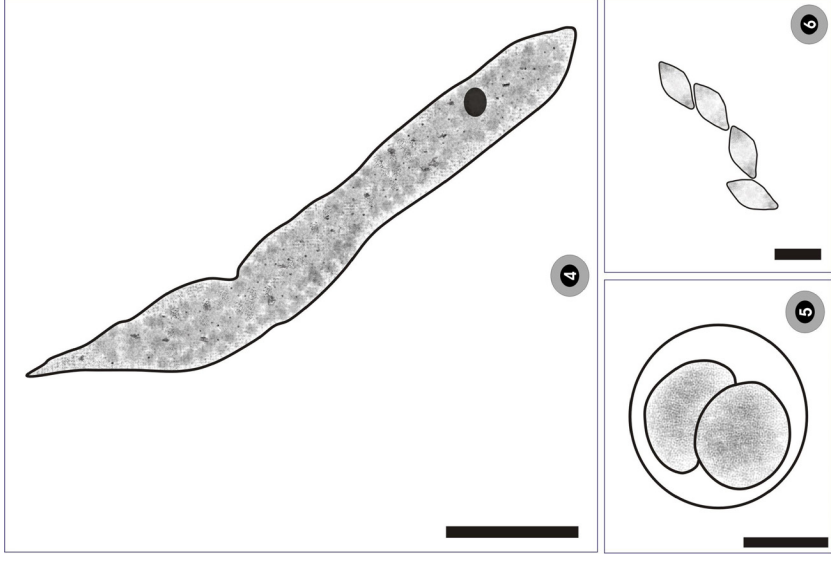


Figure 4-6. Camera lucida drawings of different stages of the life history of *Nematocystis bayrami* n. sp. (4) Trophozoites; (5) Gametocyst; (6) Oocyst. Scale bars: (4) 200 μ m, (5) 100 μ m and (6) 10 μ m.

the present form are cylindrical, much longer and with several constrictions along the body. Nucleus of *N. gardenica* is oval or elongated but that of present form is rounded. Present species exhibits some similarities in gamont shape with *N. burnii*, but they differ in size. In both cases gamonts bear pointed anterior terminal and gradually tapering pointed posterior end (Segun, 1978). *N. burnii* differ in size with the present species. The oocysts of *N. burnii* contain bipolar plugs, but no plugs have been observed in the present form. The ends of the gamonts of *N. levinei* are blunt, while in the species under discussion, it is pointed. In *N. levinei* the body has fine longitudinal striations converging at both ends. But no such striations observed in the present form. Considering all these differences, we propose that the gregarine studied here is a new species and designated it as *Nematocystis bayrami* sp. n.

Taxonomic summary

Type host: *Eutyphoeus orientalis* Beddard.

Symbiotype: EO/08/08 deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India.

Infection site: Seminal vesicles.

Type locality: Bankura, West Bengal, India.

Prevalance: 40% (13/32)

Holotype: NB/08/08 is deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Paratypes: NB/08/2006/P-1, NB/08/2006/P-2 and NB/08/2006/P-3 in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Etymology: This species was named after the name of Prof. Dr. Bayram Göcmen from Turkey for his outstanding contribution in the field of Protozoology.

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Submitted: 05 April 2009

/ Accepted: 21 November 2009

Published Online: 22 November 2009