

## Original papers

# Observation on *Nematocystis kailashi* sp. nov. (Apicomplexa: Eugregarinida) from an Indian earthworm *Glyphidrilus tuberosus* Stephenson (Annelida: Oligochaeta)

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**ABSTRACT.** Surveys on aseptate gregarines in earthworm hosts in different districts of West Bengal state of India revealed the existence of one new species of aseptate gregarine of the genus *Nematocystis* Hesse, 1909 have been identified from the seminal vesicles of the earthworm, *Glyphidrilus tuberosus* Stephenson, 1916 in the district of Purba Midnapur, West Bengal of India. Gamonts of the organism are very much elongated, cylindrical, nematoid and without mucron. The terminal end adjacent to the nucleus rounded and the distal end pointed. The gamonts measure 846.45–1031.13 ( $931.86 \pm 70.48$ )  $\mu\text{m}$  in length and 18.40–20.45 ( $19.43 \pm 1.05$ )  $\mu\text{m}$  in width. Nucleus elongated or depressed elliptoid, measures 53.17–63.39 ( $60.33 \pm 3.28$ )  $\mu\text{m}$  in length and 13.29–16.36 ( $14.15 \pm 0.89$ )  $\mu\text{m}$  in width. The gametocysts are slightly ovoid, measuring 110.43–120.65 ( $114.31 \pm 3.44$ )  $\mu\text{m}$  in diameter. Oocysts navicular and measure 9.24–10.39 ( $9.78 \pm 0.40$ )  $\mu\text{m} \times 5.77$ –6.16 ( $6.04 \pm 0.18$ )  $\mu\text{m}$ . Based on critical analysis and comparison with earlier reported species, the species under discussion established as new one.

**Key words:** *Nematocystis kailashi* sp. nov., gregarine, earthworm

## Introduction

Gregarines belonging to the order Eugregarinorida Léger, 1900 are important endoparasitic fauna. They are chiefly coelozoic or lumen-dwelling protozoan parasites of invertebrates, especially arthropods and annelids. All known members of the order Eugregarinorida are parasitic. Of the two major groups of gregarines, septate and aseptate, arthropods harbour the septate and earthworms harbour the aseptate forms [1]. Eugregarinorida contains more than one thousand five hundred species divided among more than 250 genera, although these figures are at best a cursory survey of the group's total biodiversity. Gregarines have been reported from 3124 invertebrate species, less than one third of one percent of the named invertebrate fauna [1]. The majority of the gregarines reported so far are septate gregarines from insects although the biodiversity survey on aseptate gregarines from annelids has always been a neglected one. More than 350 species of earthworms have so far been recorded from India [2]. A very small number of

them have been studied for the occurrence of endoparasitic aseptate gregarine fauna. Studies on this group in India has got momentum since 1980. Exploration of acephaline 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. *Nematocystis* Hesse, 1909 often invade oligochaetes especially earthworms and reside as endoparasites [1]. Levine [3] listed twenty seven species of *Nematocystis* Hesse, 1909. Later, Segun [4] added one new species, followed by this; Pradhan and Dasgupta [5] reported five new species. Roychoudhury and Haldar [6] described two new species. Bandyopadhyay and Mitra [7] established one species. Again Bandyopadhyay et al. [8,9] established two new species, later on Mallik and Bandyopadhyay [10,11] established two more new species. To date, 41 species of *Nematocystis* have so far been reported from oligochaete hosts, out of which 16 species have been described from India.

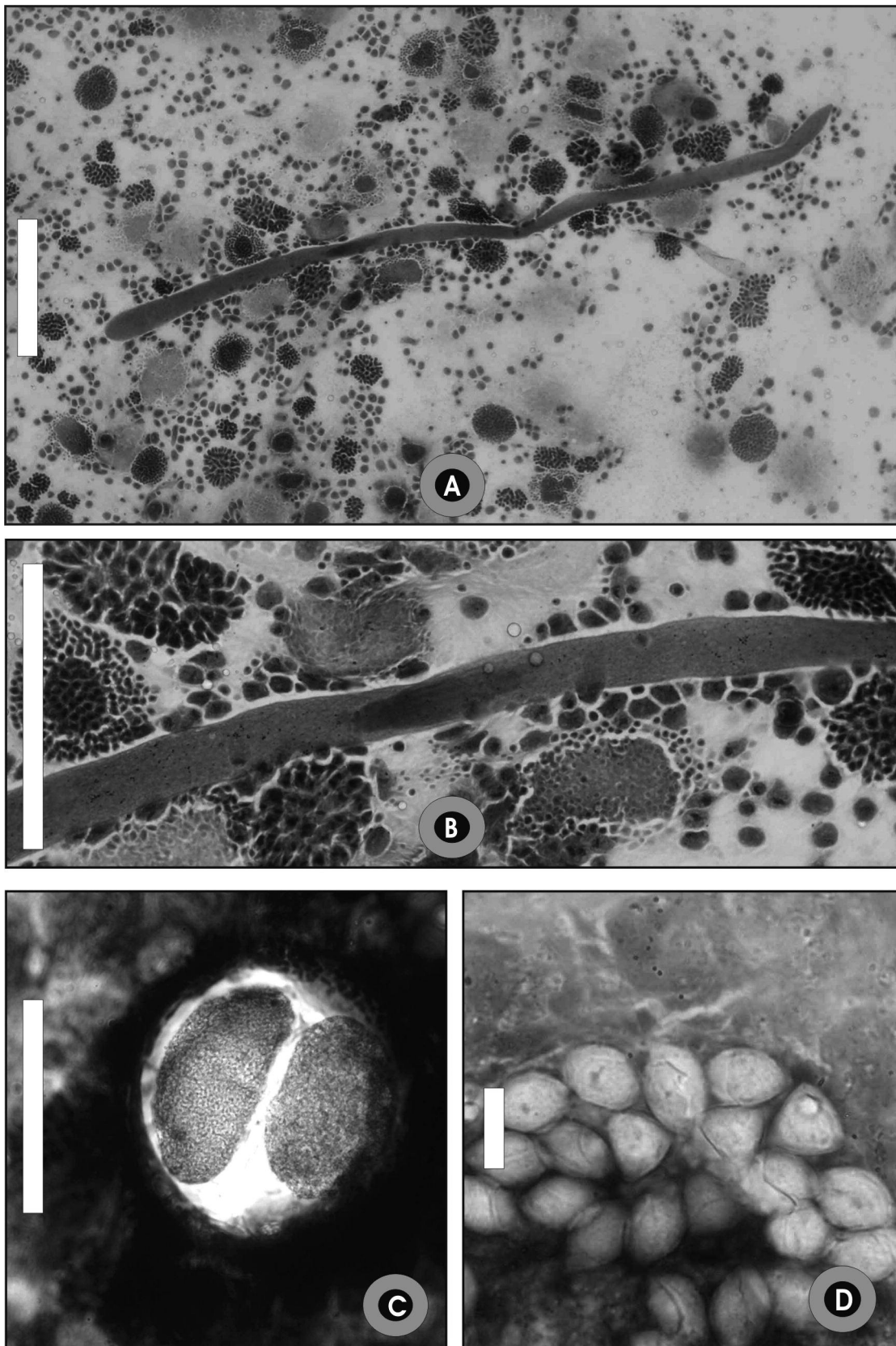


Fig. 1. Photomicrographs of different stages of life cycle of *Nematocystis kailashi* sp. nov. (A) Mature gamont; (B) Magnified view of nucleus bearing region of mature gamont; (C) Gametocyst; (D) Oocysts. Scale bars: (A-C) 100  $\mu$ m, (D) 10  $\mu$ m.

As a part of the ongoing biodiversity survey of aseptate gregarines infesting the earthworms of West Bengal, India one new species of aseptate gregarine under the genus *Nematocystis* have been obtained from the seminal vesicles of earthworm *Glyphidrilus tuberosus* Stephenson. The present paper deals with the descriptions, taxonomy and systematics of a new species of *Nematocystis* including morphometric comparisons with closely related species.

The present study is focused to explore the diversity of parasites of earthworm in West Bengal, India and also to report new aseptate gregarines belonging to the genera *Apolocystis* Cognetti de Martiis 1923; *Monocystis* Stein, 1848; *Nematocystis* Hesse, 1909; *Stomatophora* Drzewiecki, 1907 and *Zygocystis* Bhatia, 1930 from new earthworm hosts from new locality.

## Materials and Methods

Thirty mature earthworms were collected from alluvial soil. After the earthworms were identified, each was dissected in 0.65 % (w/v) NaCl solution. The perivisceral coelom, nephridia, intestine and seminal vesicles were examined immediately for monocystid gregarines. Smears of the coelomic fluid and seminal fluid were fixed in Schaudins fluid (66 ml HgCl<sub>2</sub>, 33 ml 95% ethyl-alcohol, and 1 ml glacial acetic acid) for 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 ethyl-alcohol (100% > 90% > 70% > 50%), for five minute each, and stored in distilled water. Slides were transferred to a 3% iron-alum solution (mordant) for twelve hours (over night) and stained with Heidenhains haematoxylin solution (20 min). Differentiation was done with 1% iron-alum solution. The slides were then washed thoroughly, dehydrated in an ascending series of alcohol (50%, 70%, 90%, 100%), cleared in xylene and mounted in Canada balsam. Camera lucida drawings of different stages of monocystid gregarines were made and photomicrographs were taken with the help of an Olympus phase contrast microscope and an Olympus camera. 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 method of describing shapes of planes and solids is mainly according to Clopton [12].

## Results

Phylum: Apicomplexa Levine, 1988

Order: Eugregarinida Leger, 1900

Family: Monocystidae Bütschli, 1882

Subfamily: Monocystinae Bhatia, 1930

Genus *Nematocystis* Hesse, 1909

*Nematocystis kailashi* sp. nov. (Fig. 1)

Gamont Length (GL): 846.45-1031.13 (931.86±70.48, 20); Gamont Width (GW): 18.40-20.45 (19.43±1.05, 20); Nucleus Diameter (NL): 53.17-63.39 (60.33±3.28, 20); Nucleus Width (NW): 13.29-16.36 (14.15±0.89, 20); Gametocyst Diameter (GD): 110.43-120.65 (114.31±3.44, 20); Large Gametocytes Length (LGL): 96.11-102.12 (99.79±2.26, 20); Large Gametocytes Width (LGW): 44.99-49.08(47.03±1.89, 20); Small Gametocytes Length (SGL): 79.75-85.89 (82.82± 2.85, 20); Small Gametocytes Width (SGW): 49.08-57.26 (52.56±2.97, 20); Oocyst Length (OL): 9.24-10.39 (9.78±0.40, 20); Oocyst Width (OW): 5.77-6.16 (6.04±0.18, 20); (20= sample size).

The members of the genus *Nematocystis* Hesse, 1909 are characterized by having no distinct mucron, solitary and nematode-like gamonts, bi-conical and symmetrical oocysts [1].

During the study one acephaline gregarine belonging to *Nematocystis* obtained from the seminal vesicles of the earthworm *Glyphidrilus tuberosus* Stephenson, 1916 from Purba Medinipur district of West Bengal. The species under consideration is much elongated, cylindrical and nematoid. Mucron absent. The terminal end adjacent to the nucleus is rounded and the distal end is pointed. Nucleus elongated or depressed elliptoid. Ectosarc is very thin and smooth. Endosarc mainly vacuolated, only few small fine granules observed in older organisms. Gametocysts not perfectly rounded but slightly ovoid. Each gametocyst bears two gametocytes of unequal size. The oocysts are navicular in shape with pointed tip.

## Taxonomic summary

**Type Material:** *Nematocystis kailashi* sp. nov.

**Type Host:** *Glyphidrilus tuberosus* Stephenson

**Symbiotype:** Host S/GT/07 deposited in the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India.

**Site of Infection:** Seminal vesicle

**Prevalence:** 30.00% (09/30)



Table 1. Morphometric comparison of *Nematocystis kailashi* sp. nov. with *Nematocystis gardenica* and *N. indica*

SPECIES	<i>Nematocystis gardenica</i>	<i>Nematocystis indica</i>	<i>Nematocystis kailashi</i> sp. nov.
<b>CHARACTERS</b> ↓↓	<b>Bandopadhyay and Mitra, 2005</b>	<b>Bandyopadhyay et al., 2006</b>	<b>Present study</b>
<b>Host</b>	<i>Amyantus diffirengens</i> Bairds, 1809	<i>Amyantus diffirengens</i> Bairds, 1809	<i>Glyphidrilus tuberosus</i> Stephenson, 1916
<b>Locality</b>	Nadia District, W.B.	Midnapore East district, West Bengal, India	Contai, Purba Medinipur, West Bengal
<b>Site of infection</b>	Seminal vesicles	Seminal vesicle	Seminal vesicle
<b>Trophozoite shape</b>	Elongate, nematode like, having parallel sides with pointed ends	Solitary, elongated, ribbon-like, having parallel sides and 1-4 constrictions along the length of the body in mature forms. Both ends of gamont rounded.	Elongated, cylindrical and nematoid. No constriction present on the body surface. Mucron absent. The terminal end adjacent to the nucleus is rounded and the distal end is pointed.
<b>Trophozoite Size</b>	110-1320 (505.0) × 11-18 (13.0) µm	1969.9-3139.6 (2773.3) µm × 10.4-45.8 (30.6) µm	846.45-1031.13 (931.86) µm × 18.40-20.45 (19.43) µm
<b>Ectosarc</b>	Thin, 1-2 µm	Ectoplasm very thin (1-2µm).	Very thin
<b>Endosarc</b>	Finely granulated with uniform granules	Endoplasm with very few vacuoles and with numerous granules of various size. Distribution of granules not uniform.	Mainly vacuolated, few fine granules observed in older organisms.
<b>Nucleus shape</b>	Oval or elongated	Very elongated	Elongated or depressed elliptoid
<b>Size of nucleus</b>	22.0-66.0 (32.0) µm × 6.6-13.2 (9.3) µm	37.4-58.2 (46.2) µm × 8.3-20.8 (14.8) µm	53.17-63.39 (60.33) µm × 13.29-16.36(14.15) µm
<b>Gametocyst shape</b>	Rounded	Rounded	Not perfectly rounded but slightly ovoid
<b>Gametocyst Size</b>	140.0-166.0 µm	83.2-91.5 (88.2) µm	110.43-120.65 (114.31) µm
<b>Oocyst shape</b>	Biconical	Navicular	Navicular to broadly fusiform
<b>Oocyst Size</b>	11.0-12.6 µm × 4.0 µm	16.2-19.3 (17.9) µm × 10.8-12.3 (11.7) µm	9.24-10.39 (9.78) µm × 5.77-6.16 (6.04) µm

**Type Locality:** Contai (21°50'N, 87°48'E), Purba Medinipur, West Bengal

**Type Material: Holotype:** A single slide bearing number M/NS(V)/01 containing holotype and slides bearing number M/NS(V)/02, M/NS(V)/05 containing paratypes are deposited in the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India.

**Paratype:** Slide No. 16 bearing some paratype materials has been deposited in the national collection of Zoological Survey of India, Kolkata-53 (Reg. No. Protozoology-2491)

**Etymology:** The species is named *Nematocystis kailashi* after the name of Dr. Kailash Chandra, Director, Zoological Survey of India, Kolkata for his outstanding contribution in the field of animal taxonomy.

## Conclusions

Gamonts elongated, cylindrical and nematoid with little differentiated anterior end if at all, seminal vesicles or coelome dwelling, solitary and are parasitic to earthworms. Oocysts of this species are navicular and are symmetrical. Inclusion of the present species under the family Monocystidae, sub family Monocystinae and genus *Nematocystis* Hesse, 1909 is on the basis of aforesaid characteristic features. Approximately forty species of *Nematocystis* have so far been established throughout the world, out of these, seventeen have been reported from India. The species under discussion exhibits some sorts of similarities with *N. gardenica* Bandyopadhyay and Mitra [7] and *N. indica* Bandyopadhyay et al. [8], respectively. But dissimilarities are predominating (Table 1). *N. gardenica* possess parallel sides with pointed tips. In the present form, the end nearer to the nucleus is rounded and the distal end is pointed. Present species is considerably wider than *N. gardenica*. Besides *N. gardenica* exhibits finely granulated endoplasm while endoplasm of the present species is mainly vacuolated. Nucleus of the present species differs from *N. gardenica* both in shape and size. Another species, *N. indica* reported from *Amyantus diffringens* is very long and ribbon-like, having parallel sides and few constrictions along the length of the body especially in mature organisms and its both the ends are pointed. The species under consideration is cylindrical, constriction free, and possess one rounded and one pointed end. *N. indica* and the present form possess thin, smooth

ectoplasm but differ in endoplasm. The endoplasm of *N. indica* is more granulated than the latter. *N. indica* possess comparatively small sized gametocyst and larger oocyst than the present organism. Furthermore, the present organism was observed from different host *Glyphidrilus tuberosus* Stephenson, 1916. A through comparison has been entertained, that revealed the present species is a new one and therefore, it is designated as *Nematocystis kailashi* sp. nov. in this paper.

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