

First record of a parasitic septate gregarines (Apicomplexa: Sporozoa) in the shrimp *Peneaus monodon* in Sundarbans of West Bengal

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Abstract Investigations on the incidence of septate gregarines in shrimp have immense importance because of severe pathogenicity of the parasite. The septate gregarines infect the midgut of shrimp *Peneaus monodon* and severe infection disturbs the intestinal tissues. Mostly gregarines of the genus *Nematopsis* have been identified from cultured penaeid shrimp. It has worldwide in distribution. In India, gregarine parasites have so far been reported from penaeid shrimps of Bombay and Kerala. The species which was isolated from the midgut of shrimp *Peneaus monodon* collected from Kharibari area of Sunderbans. 9 out of 20 i.e. 45% of the randomly sampled hosts were found to be infected with a species of the genus *Nematopsis*. Different developmental stages including trophozoites, sporadins, and gametocysts of the *Nematopsis* sp. infecting the shrimp have been isolated. No correlations have been established between incidence of infection and environmental parameters.

Keywords *Nematopsis sundarbanensis* · *Peneaus monodon* · Septate gregarines · Gut parasite · Sundarbans

Abbreviations

SD	Standard deviation
SE	Standard error
CV (%)	Coefficient of curvature
TL	Total length of the protomerite
LP	Length of Protomerite
WP	Width of Protomerite
WD	Width of Dueteromerite

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Introduction

Gregarines are a diverse and successful group of protozoan parasites belonging to the phylum Apicomplexa, order Eugregarinorida Leger, 1900. The Eugregarinida are all parasitic and are restricted to invertebrates (Clopton 2002). *Cephalobolus* Kruse, 1959 and *Nematopsis* Schneider, 1892, are two common genera found in cultured penaeids (Lotz and Overstreet 1990; Lightner 1996) and other decapod crustaceans. Different stages of life cycle are isolated from gut of the host but oocysts of *Nematopsis* were also isolated from gills of mussels and oysters (Padovan et al. 2003). In India the gregarine parasites have been reported from penaeid shrimps of Bombay (Bower et al. 1994) and Kerala (Prasadan and Janardanan 2001). The paper deals with the incidence of septate gregarines from *Peneaus monodon* of Sundarbans, West Bengal.

Materials and methods

Host specimens were randomly sampled from the farms of Kharibari and Canning stations of Sundarbans region, West Bengal and brought alive to the laboratory or in moribund condition. In the laboratory the gut of the shrimps were removed aseptically with fine forceps, gut materials were scrapped out on glass slides in 0.8% saline and examined under phase contrast microscope for detection of fresh gamonts. The smeared slides are air-dried, fixed in methanol and stained in Giemsa for permanent preparation. Measurements of the trophozoites were taken with the aid of a calibrated ocular micrometer. All measurements are presented in μm as mean (\pm) SD followed in parentheses

Table 1 Measurement and statistical analysis of Trophozoites, Association and gametocyst of Septate Gregarine *Nematopsis sundarbanensis* n.sp

Character	Range	Mean	SD	SE	CV%
Trophozoite: TL	5–12.5	7.4	2.15	0.48	29.05
PL	1–3	1.74	0.563	0.126	32.35
DL	4–9.5	5.93	1.71	0.38	28.84
Association length	11.5–50.5	32.66	11.95	3.98	36.65
Primites: TL	6–21	15.19	5.85	1.95	38.51
PL	1.5–5	3.22	0.94	0.314	29.19
DL	4.5–18.5	11.97	5.75	1.92	48.04
DW	7.65–13.6	8.97	1.812	0.405	20.2
PW	4.25–8.5	5.95	1.202	0.268	20.2
Satelite: TL	5.5–24.5	15.13	6.71	2.33	44.35
Gametocyst	6.8–10.2	8.33	1.061	0.237	12.74

by the range. Drawings were made on stained material with the aid of a mirror type camera lucida. Photographs were taken with Olympus phase contrast microscope fitted with digital camera.

Results *Nematopsis sundarbanensis* n. sp. (Fig. 1 A–D, E; Tables 1 and 2)

Sporadins: Multi associative, association caudofrontal.

Primites: These septate gregarines contain two segments i.e. protomerite and dueteromite, epimerite is absent. The protomerite is hemispherical in shape. It stains light with Giemsa. It is separated from the dueteromite by a straight septum which is constricted at the sides. The dueteromite is elongated with a slight constriction at the middle or it appears more or less dumbbell shaped and stains deep with Giemsa. Nucleus is ovoid in shape situated at the middle of the dueteromite.

Satellite: Protomerite absent. Only dueteromite is present. Shape of the satellite dueteromite is similar to that of primate.

Trophozoite: The mature and immature trophozoites contain only two segments i.e. protomerite and dueteromite, epimerite is absent in the septate gregarines which have been collected from the gut of shrimp. The protomerite is wide at the anterior end and narrow at the posterior end. It stains light with Giemsa. The dueteromite is elongated with slight constriction at the middle or it appears more or less dumbbell shaped. It stains deep with Giemsa. A rounded nucleus is present at the middle of dueteromerite just in the region of constriction. The anterior end of the dueteromite is straight while the posterior end is blunt and rounded.

Gametocyst: Narrow at one end and broad at the other. Both the ends are rounded almost resembling the shape of a water drop. Epicyst thin. Gametocysts are surrounded by a single epicyst.

Table 2 Morphometric comparison of the new species with previously described species of the genus *Nematopsis* n.sp

Measurement	<i>N. messor</i>	<i>N. quadratum</i>	<i>N. annulipes</i>	<i>Nematopsis sundarbanensis</i> n. sp.
Association length	114–178 (140.9)	404–678 (492.7)	213–317 (259.9)	11.5–50.5 (32.66)
Primites: Total length	59–94 (72.7)	177–370 (285.3)	86–185 (138.4)	6–21 (15.19)
Protomerite length	13–23 (18.6)	53–69 (59.7)	17–36 (28.5)	1.5–5 (3.22)
Deuteromerite Width	30–58 (40.3)	89–162 (128.1)	38–94 (59)	7.65–13.6 (8.97)
Protomerite width	23–43 (34)	54–121 (90.8)	33–56 (39.9)	4.25–8.5 (5.95)
Ratio PL: TL	1:3.9	1: 4.8	1: 4.9	1:4.7
PW:DW	1:1.1	1:1.1	1:1.3	1: 1.5
Satellites Total length	51–86 (68)	152–308 (207.3)	101–147 (121.4)	5.5–24.5 (15.13)
Deuteromerite width	25–46 (33.9)	50–117 (83.1)	26 -79 (49.2)	6–14.5 (7.78)
Trophozoites: Total length	31.3	56.1	37.1	5–12.5 (7.4)
Protomerite length	9.9	13.2	7.4	1-3 (1.74)
Deuteromerite length	21.4	42.9	29.7	4–9.5 (5.93)
Ratio PL: TL	1:3.16	1: 4.25	1:5.01	1: 4.25
PL: DL	1:2.16	1: 3.25	1: 4.01	1: 4.04
Gametocyst	54–86 (66.7)	139–246 (206.4)	112–119 (115.5)	6.8–10.2 (8.33)
References	Prasadan and Janardanan (2001)	Prasadan and Janardanan (2001)	Prasadan and Janardanan (2001)	Present study

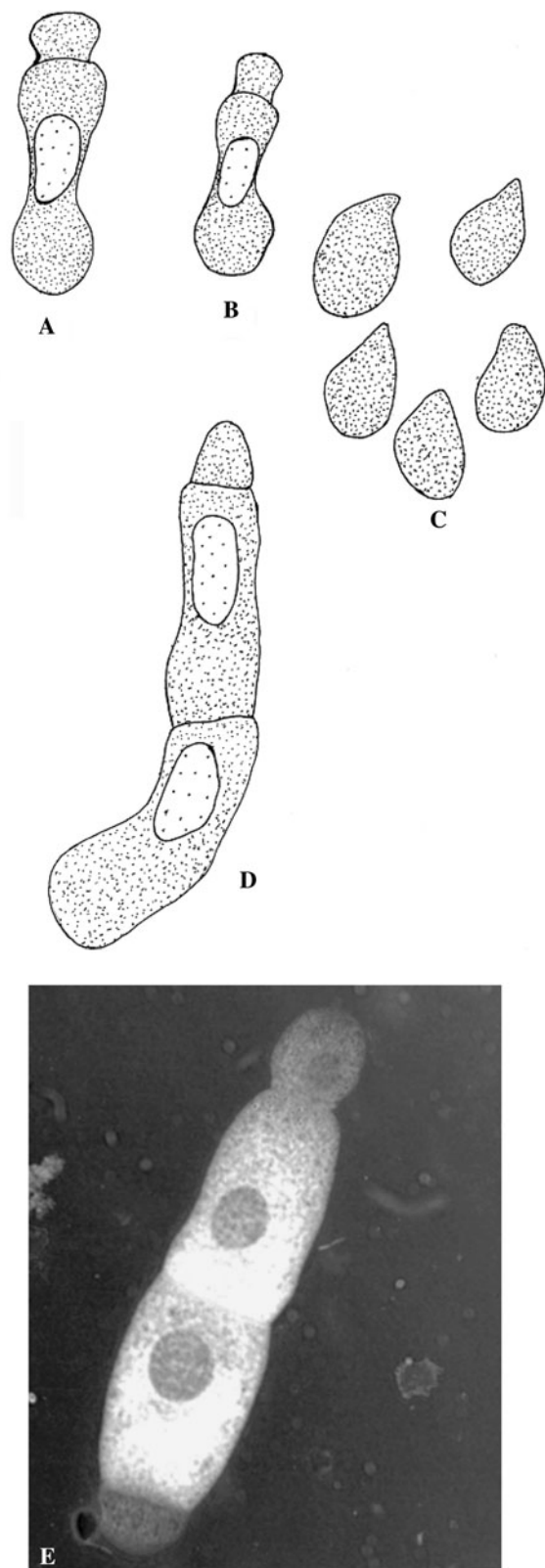


Fig. 1 A–D: Camera lucida drawings of different stages of life cycle of *Nematopsis sundarbanensis* n. sp. E. Photomicrograph of a trophozoite of *Nematopsis sundarbanensis* n. sp

Taxonomic summary

- Type specimen:** Syntypes, No. Z/Par/G/6408/09; deposited in the Parasitology laboratory, Department of Zoology, University of Kalyani, West Bengal, India.
- Type host:** *Peneaus monodon*
- Type locality:** Canning, Matla estuary, 24 Parganas (S) and Kharibari, 24 Parganas (N) West Bengal, India
- Collection time:** July, August' 2008
- Site of infection:** Intestine
- Prevalence:** 09 out of 20 hosts sampled and examined randomly. i.e. 40% of the sampled hosts are infected.

Discussion

The present species differs from other three described species namely *Nematopsis messor*, *N. quadratum*, and *N. annulipes* (Prasadan and Janardanan 2001) in shape and size in following significant ways:

(i) A prominent lens shaped structure is present at the anterior end of the protomerite of *N. messor* but it is absent in the species under description, (ii) the shape of the nucleus of the species also differs. It is ovoid in shape in the present species while it is spherical in the other species, (iii) Protomerite absent in the satellite, the described species but it is present in the other two species, (iv) Each gametocyst of the species under study resembles the shape of a water drop whereas, the gametocysts are rounded to spherical in shape in *N. messor*, *N. quadratum* and *N. annulipes*, (v) A single epicyst encloses the gametocyst of the present species as it is found in other two species.

In India, the gregarine parasites have so far been reported from penaeid shrimps of Bombay (Bower et al. 1994). It is the first record of septate gregarines from the Black Tiger shrimp of Sundarbans region. On the basis of above taxonomic and comparative study based on the morphology of trophozoites, sporadins, gametocysts the species described in this paper is designated as a new species under the genus *Nematopsis* and hence *Nematopsis sundarbanensis* n.sp. is designated.

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