

Description of a new species *Zygocystis levinei* sp. n. (Eugregarinida, Zygocystidae), from the earthworm *Amyntas nicholsoni* (Oligochaeta) from West Bengal, India

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Summary

Surveys of acephaline gregarines from the Nadia district of West Bengal, India revealed a new species of *Zygocystis* Stein, 1848 in the seminal vesicles of the earthworm *Amyntas nicholsoni*. The new species is characterised by a pear-shaped body at the young stage and a conical one at maturity. Syzygy is formed early with partners of different size. Oocysts are navicular.

Key words: gregarines, acephaline, earthworm, *Zygocystis levinei* sp. n.

Introduction

Gregarines are chiefly coelozoic or lumen-dwelling parasites of invertebrates, especially arthropods and annelids. Of the two major groups of gregarines, septate and aseptate, earthworms harbour aseptate forms. Aseptate gregarine fauna has been reported from various parts of the world including India but the search for them in India is far from complete.

Research of acephaline gregarines infesting earthworms in West Bengal, India revealed a new species of *Zygocystis* Stein, 1848. This genus has pyriform gamonts, always in frontal (head to head) syzygy, and navicular oocysts. Levine (1977) listed 15 species under the genus *Zygocystis*. Two other species have been added since then (Segun, 1978; Pradhan and

Dasgupta, 1983) to this genus. Out of these 17 species only one (*Z. indicus* Pradhan and Dasgupta, 1983) has been reported from an earthworm in India. The present paper deals with description of a new species of *Zygocystis* from the earthworm *Amyntas nicholsoni* from West Bengal, India.

Material and methods

Earthworms were collected and taken to the laboratory. They were dissected while alive and their seminal vesicles were carefully removed and placed on clean glass with a drop of 0.5 % NaCl solution. A thin film of seminal fluid was drawn out on a slide covered with a cover slip for examination of live protozoans under a phase contrast microscope. The content of

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 stored in distilled water. Then they were transferred to a 3% iron alum solution and stained with Heidenhain's haematoxylin solution (20 min). Differentiation was done overnight with 1% iron alum solution. The slides were then washed thoroughly, dehydrated in an ascending series of alcohol, cleared in xylene and mounted in Canada balsam. Observations were done under an Olympus phase contrast microscope and photomicrographs were taken using an Olympus camera.

Results and discussion

Zygocystis levinei sp. n.
(Figs 1-16, Tables 1 and 2)

Early trophozoites are nearly spherical or pear-shaped, in groups of two or three. The association of trophozoites is strong and permanent, occurring early in their development. Adult trophozoites are always found in association of two or three. Mature bi-associate forms are ovoid, widest at the middle and narrowing gradually towards the ends. One partner is slightly bigger than the other. Tri-associate forms are almost triangular in shape. Bi-associate forms are more frequent than the tri-associate ones. In all the cases the length is less than the width.

Ectosarc is very thin, about 1 μm in thickness. Epicyte is smooth without any external appendages or protrusions on any part of the sporadin. Sarcocyte and myocyte are not prominent.

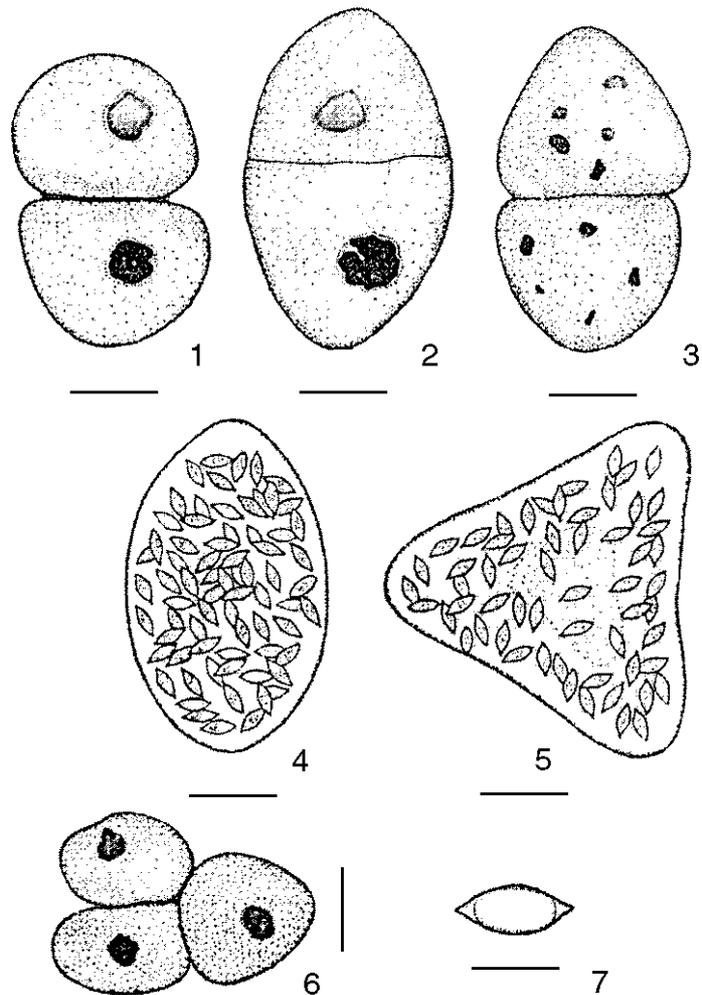
Endosarc is granular with little storage granules. Nucleus can be situated in any endosarc part. Nucleus spherical, clearly demarcated from endosarc by nuclear membrane. Karyosome dispersed within nucleoplasm. Small vacuolar spaces sometimes present within nucleus.

Syzygy very early and permanent, junction being achieved via flat margin.

Measurements of different parts of 20 bi-associate trophozoites, gametocysts, oocysts and 12 tri-associate trophozoites, as well as body proportions are given in Table 1.

Taxonomic summary:

Host: *Amyntas nicholsoni*



Figs 1-7. Camera lucida drawings of different stages in the life history of *Zygocystis levinei* sp. n. 1 - Two trophozoites in early syzygy; 2 - late syzygy; 3 - developing bi-associate gametocyst; 4 - bi-associate gametocyst; 5 - tri-associate gametocyst with oocysts; 6 - three trophozoites in early syzygy; 7 - oocyst. Scale bars: 1-6 - 30 μm , 7 - 10 μm .

Area/Locality: Gosaba, Sundarban, West Bengal, India.

Infection locus: Seminal vesicles

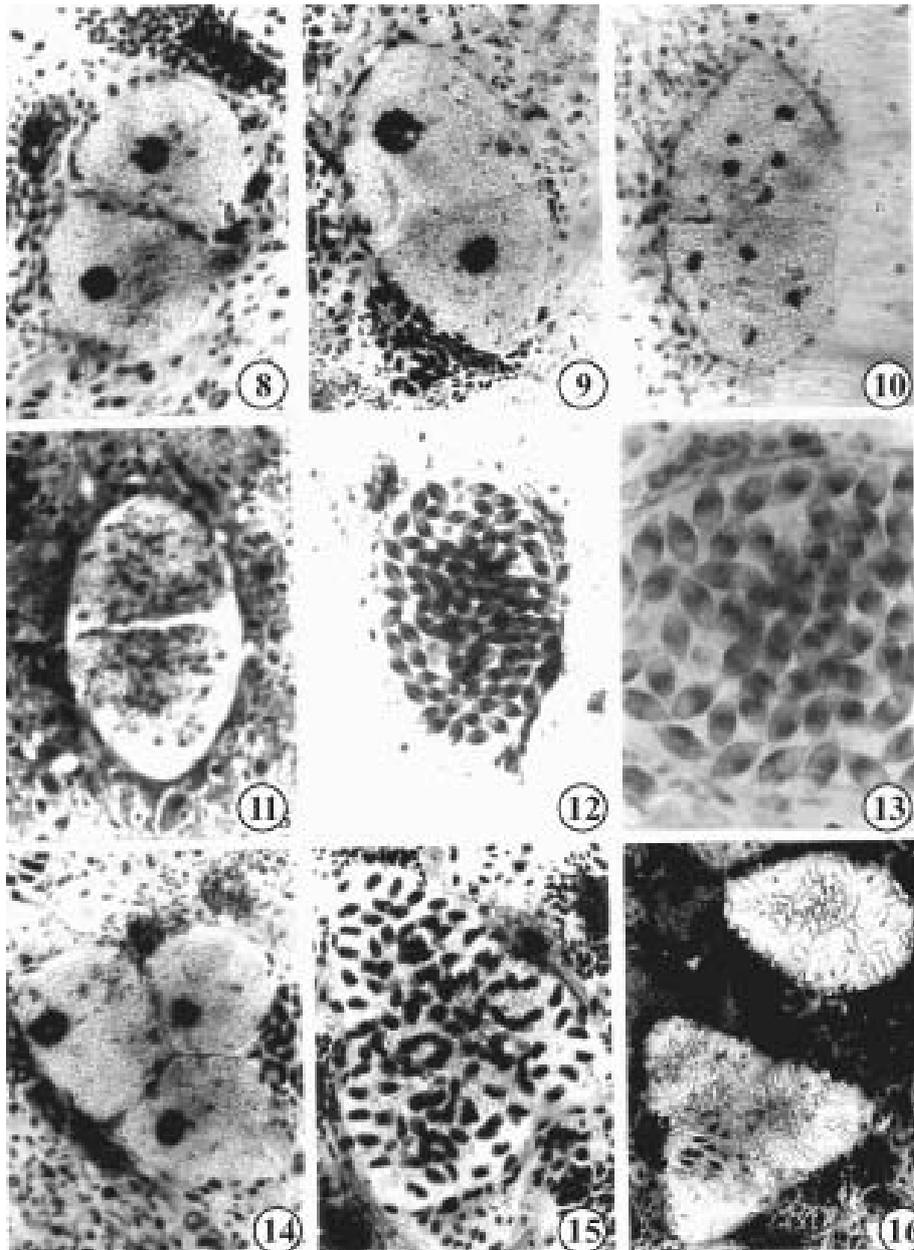
Incidence: 12 out of 20 (60%)

Materials: Holotype, slide AR-1, and paratype, slide AR-11 in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Etymology: The species name has been derived after the late Prof. N.D. Levine, to commemorate his outstanding contribution to protozoology.

Remarks

Early permanent frontal syzygy, pyriform individual gamonts in early association pairs, navicular oocysts and



Figs 8-16. Photomicrographs of different stages in the life history of *Zygocystis levinei* sp. n. (8-13 - bi-associate form and 14-16 - tri-associate form). 8 - early association; 9 - syzygy; 10-11 - developing gametocysts; 12 - mature gametocyst; 13 - oocysts; 14 - early association; 15 - gametocysts; 16 - mature bi- and tri-associate gametocysts.

occurrence in seminal vesicles of earthworms are the characters shared by members of the genus *Zygocystis*. *Z. levinei* differs from *Z. indicus*, the only known species from India, in the shape of the trophozoite and gametocyst and in the host (in *Z. indicus* the host is *Metaphire (Pheretima) californica*).

The new species can also be compared with *Z. cometa* and *Z. eiseniae*. Our species is very similar in the shape of early syzygy to *Z. eiseniae*, but differs from it in smaller size - 101,2 to 123,2 μm long in the new

species as compared to approx. 600 μm long reported in the original description of *Z. eiseniae* (Loubatieres, 1955), - and in having biconical gametocyst as compared to large spherical gametocysts in *Z. eiseniae*. *Z. eiseniae* reported by Segun (1971) from the U.K. is smaller in size but the structure of gametocyst is identical. As far as the mature syzygy is concerned, the new species bears close similarity to *Z. cometa* except for the terminal tuft of cytopilia which is absent in the present form. Moreover, in *Z. cometa* the shape of

Table 1. Details of measurements of different parts of 20 bi-associate trophozoites, gametocysts, oocysts and 12 tri-associate trophozoites as well as various body proportions of *Zygocystis levinei* sp. n. (all measurements are in μm).

Different body parts	Range	Mean	Standard deviation
Bi-associate syzygy length (BSL)	101.0-123.0	110.0	6.0
Bi-associate syzygy width (BSW)	53.0-75.0	69.0	5.0
Nucleus length (NL)	11.0-18.0	14.0	2.0
Nucleus width (NW)	11.0-18.0	13.0	1.5
Gametocyst length (GL)	114.0-123.0	121.0	3.0
Gametocyst width (GW)	66.0-75.0	71.0	0.8
Oocyst length	12.0-14.0	13.0	0.9
Oocyst width	5.0-6.0	6.0	0.3
Tri-associate syzygy length on each side	114.0-128.0	121.0	4.0
BSL/BSW	1.5-1.9	1.6	0.1
NL/NW	1.0-1.5	1.0	0.07
GL/GW	1.5-2.0	2.0	0.08

gametocyst is changed during its transformation from syzygy. In the present form there is almost no change of shape, either in the bi-associate or in the tri-associate forms during the transformation of gametocysts. Mickel (1925) reported occasional presence of solitary and tri-associate forms in *Z. cometa* obtained from the

earthworms *Helodrilus caliginosus* in the U.S.A. In the present form solitary trophozoites were not encountered and its host is also different.

The comparative characters of *Z. cometa*, *Z. eiseniae*, *Z. indicus* and *Zygocystis levinei* sp. n. are summarised in Table 2.

Table 2. Comparisons between different characters of *Zygocystis cometa*, *Z. eiseniae*, *Z. indicus* and *Zygocystis levinei* sp. n. (all measurements are in μm).

Species Characters	<i>Zygocystis cometa</i> Stein, 1848	<i>Z. eiseniae</i> Loubatieres, 1955	<i>Z. indicus</i> Pradhan et Dasgupta, 1983	<i>Zygocystis levinei</i> sp. n.
Host(s)	<i>Lumbricus terrestris</i> , <i>Eisenia foetida</i> , <i>Allolobophora longa</i> , <i>A. dubiosa</i> , <i>A. caliginosa</i> , <i>A. chlorotica</i>	<i>Eisenia foetida</i>	<i>Pheretima californica</i>	<i>Amyntas nicholsomi</i>
Locality	France, Hungary, Germany, Sweden, U.S.A.	France, U.K.	West Bengal, India	Sundarban West Bengal, India
Trophozoite	Cone shaped; ectoplasmic cytopilia prolong beyond the body as comet-like tuft of hairs	Spherical, pair through flat surfaces, partners are of different sizes	Ovate or variable in shape	Pear shaped when young, but conical at maturity, syzygy early with different sized partners, solitary forms not found
Size	41.4-98 by 20.0-75.0	# 22.0-105.0 (solitary); 44.0-200.0 (syzygy); * 325.0 (solitary) 600.0 (syzygy)	43.2-86.0 by 23.4-58.6	101.0-123.0 by 53.0-75.0 (syzygy)

Table 2. Continuation.

Species Characters	<i>Zygocystis cometa</i> Stein, 1848	<i>Z. eiseniae</i> Loubatieres, 1955	<i>Z. indicus</i> Pradhan et Dasgupta, 1983	<i>Zygocystis levinei</i> sp. n.
Ectosarc	Prominent with deep furrows, extend beyond posterior end as comet-like tuft of hairs, 12.3-60.0 µm long	No ectoplasm hairs on the body surface	Thick, without any striations	Very thin, epicyte smooth
Endosarc	With many paraglycogen granules, 3-4 µm in diameter	Granular, with large and variable shaped paraglycogen granules, 3-6 µm in diameter	Granular with many paraglycogen granules	Granular with little storage granules
Nucleus	Spherical, lying directly opposite that of the other partner with a large spherical, eccentric karyosome	Spherical, with a large, central spherical, homogenous karyosome (but upto four karyosomes have been observed)	Round or ellipsoid, karyosome diffused	Spherical, with diffused karyosome forming vacuolar space
Size	9-15 in diameter	# 8.5-18 or * 32 in diameter	5.4-10.8 in diameter	11.0-18.0 in diameter
Association	Mostly bi-associate, sometimes tri associate or even solitary forms are found	Only bi-associate forms	Only bi-associate forms	Majority bi-associate; often tri-associate forms are found, solitary forms not seen
Junction	Mostly bi-associate, sometimes tri-associate or even solitary forms are found	A flattened surface	Cup-shaped concavity fits into a cone-shaped projection	A flattened surface
Gametocyst	Spherical	Spherical	Ellipsoidal	Bi-conical (formed of two); or triangular (formed of three)
Size	108-186	228-252	75.6-144 by 86.4-133	114.0-123.0 by 66.0-75.0 (bi-associate) 114.0-128.0 on each side (tri-associate)
Oocyst	Biconical, 27-28.5 by 9.0	?	Biconical, 7.65 by 3.67	Biconical, 12.0-14.0 by 5.0-6.0
Refernce	Segun (1971); Mickel (1925)	# Segun (1971) * Loubatieres (1955)	Pradhan and Dasgupta (1983)	Present paper

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