Observation on *Monocystis kuidongae* sp. nov. (Apicomplexa: Eugregarinida) from an Indian earthworm *Perionyx excavatus* Perrier (Annelida: Oligochaeta)

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Abstract A survey to know the diversity of endoparasitic protozoan parasites of the earthworms of West Bengal, revealed a new species of the genus *Monocystis* Stein, 1848, that resides in the seminal vesicle of the earthworm *Perionyx excavatus* Perrier. *Monocystis kuidongae* sp. nov. is characterized by ovoidal gamonts (trophozoites) with inconspicuous mucron and measure $69.53\text{--}102.25$ (86.29 ± 11.48) $\mu\text{m} \times 24.54\text{--}61.35$ (35.17 ± 12.82) μm . Shape of the gametocysts are almost rounded and measure $81.80\text{--}110.43$ (96.11 ± 8.85) μm in diameter. Oocysts are navicular with pointed ends and measure $11.55\text{--}12.32$ (11.78 ± 0.36) $\mu\text{m} \times 5.00\text{--}5.77$ (5.39 ± 0.25) μm .

Keywords *Monocystis kuidongae* sp. nov. · Gregarine · Earthworm · Seminal vesicle

Introduction

During survey of acephaline gregarines in the oligochaete hosts in the soil of ‘Kangsabati’ river sides of Midnapur town, West Bengal, India a new species belonging to the genus *Monocystis* Stein, 1848 was obtained from the seminal vesicles of the earthworm, *Perionyx excavatus*. Till now, there are only 24 species of *Monocystis* published from India (Ghosh 1923; Bhatia and Chatterjee 1925; Kar 1946; Kalavati 1979; Subbarao et al. 1979; Pradhan and Dasgupta 1982; Bandyopadhyay et al. 2005; Bandyopadhyay and Mitra 2005; Bandyopadhyay et al. 2006a, b, c, 2007;

Bandyopadhyay and Sarkar 2013). The present paper deals with morphology, life history and systematics of a new species of the genus *Monocystis* Stein, 1848 found in the seminal vesicles of the earthworm *P. excavatus*.

Materials and methods

Forty four 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 made on clean dry slides, semidried and fixed in Schaudins fluid (66 ml HgCl_2 , 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 5 min each, and stored in distilled water. Slides were transferred to a 3 % iron-alum solution (mordant) for 12 h (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 and 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. Method of describing shapes of planes and solids is mainly according to Clopton (2004).

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Results

Phylum: Apicomplexa Levine, 1988

Order: Eugregarinida Leger, 1900

Family: Monocystidae Bütschli, 1882

Subfamily: Monocystinae Bhatia, 1930

Genus: *Monocystis* Stein, 1848

Monocystis sp. nov. (Figure 1a–h)

Gamont Length (GL): 69.53–102.25 (86.29 ± 11.48 , 20); Gamont Width (GW): 24.54–61.35 (35.17 ± 12.82 , 20); Nucleus Diameter (ND): 10.22–12.27 (11.04 ± 1.03 , 20); Gametocyst Diameter (GD): 81.80–110.43 (96.11 ± 8.85 , 20); Large Gametocytes Length (LGL): 40.90–57.26 (46.62 ± 5.03 , 20); Large Gametocytes Width (LGW): 32.72–49.08 (39.47 ± 5.39 , 20); Small Gametocytes Length (SGL): 36.81–53.17 (42.54 ± 5.03 , 20); Small Gametocytes Width (SGW): 28.63–44.99 (34.76 ± 4.69); Oocyst Length (OL) : 11.55–12.32 (11.78 ± 0.36 , 20); Oocyst Width (OW): 5.00–5.77 (5.39 ± 0.25 , 20); (20 = sample size).

Members of the genus *Monocystis* Stein, 1848 are characterized by having no distinct mucron, ovoid and solitary gamonts, biconical, symmetrical oocysts (Levine 1988). In case of acephaline gegarine forms obtained from the seminal vesicles of the earthworm *Perionyx excavatus* from Paschim Medinipur district of West Bengal, gamonts appear rounded with inconspicuous mucron. Ectoplasm is smooth and thin, 2.0–4.0 μm in thickness. Epicyte is very fine and is free from surface striation. Endoplasm neither vacuolated nor granulated, only age old forms exhibit few evenly distributed granules. Nucleus with regular rounded, spherical and not in the middle of mature gamont. Gametocyst almost rounded. There are two unequal gametes in the gametocyst. The oocysts are navicular in shape with pointed tip.

Taxonomic summary

Type material: *Monocystis kuidongae* sp. nov.

Type host: *Perionyx excavatus* Perrier

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

Site of infection: Seminal vesicles.

Type locality: Midnapur town (22°15'N, 87°39'E), Paschim Medinipur, India

Prevalance: 17 out of 44 (38.63 %).

Type material: **Holotype:** A single slide bearing number M/MS(VIII)/01 containing holotype and slides bearing number M/MS(VIII)/02, M/MS(VIII)/14 containing paratypes are deposited in the Parasitology

Fig. 1 Photomicrographs of different stages of life cycle of *Monocystis kuidongae* sp. nov. **a** Mature gamonts; **b** Syzygy **c** Gametocyst; **d** Oocyst. Scale bars 100 µm (**a–c**); 10 µm (**d**). Camera lucida drawing of different stages of life cycle of *Monocystis kuidongae* sp. nov. **e** Mature gamonts; **f** Syzygy **g** Gametocyst; **h** Oocyst. Scale bars 100 µm (**e–g**); 10 µm (**h**)

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Paratype: Slide No. 8 bearing some paratype materials has been deposited in the national collection of Zoological Survey of India, Calcutta- 53 (Reg. No. Protozool-ogy-2483)

Etymology: The species is named *M. kuidongae* after the name of Prof. Kuidong XU, Director, Taxonomy, Systematics and Marine Biology, Chinese Academy of Science, PR China; for his outstanding contribution in the field of protozoology.

Discussion

Spherical to cylindrical gamonts with inconspicuous mucron and little differentiated anterior end if at all, seminal vesicles or coelom dwelling, solitary and are parasitic to earthworms, navicular and symmetrical oocysts justify its inclusion under the family Monocystidae, sub-family Monocystinae and genus *Monocystis* Stein, 1848. Of more than 95 species belonging to the genus *Monocystis* Stein, 1848 (Levine 1988) only 24 have been described from India. Of these 18 species, *M. odontotermi* Kalavati 1979 has been reported from the haemocoel of the termite *Odontotermes obesus* (a non oligochaete host) and *M. pontodrilus* Subbarao et al. 1979 from the littoral oligochaete, *Pontodrilus bermudensis*. All other species of *Monocystis* have been reported from terrestrial earthworms, but none from the present host *Perionyx excavatus* Perrier. The species under discussion closely resembles *M. pheretimi* Bhatia and Chatterjee 1925 and *M. sanchalensis* Pradhan and Dasgupta 1982, but predominating dissimilarities promote the present form as separate entity. The trophozoites of *M. pheretimi* are variable in shape. They may be spherical, ovoidal or ellipsoidal where as the present form is ovoidal. Its one end is broader and rounded than the other. Trophozoite of *M. sanchalensis* is elongated, cylindrical with both ends blunt and its middle portion is widest. The endosarc of the present form is neither vacuolated nor granulated, only old forms exhibit few granules. On the other hand, endosarc of *M. pheretimi* is vacuolated and that of *M. sanchalensis* is alveolate, packed with paraglycogen granules. The present form obtained from a new host, *P. excavatus* where as the earlier two were reported from *Pheretima posthuma* and *Aporrectodea*

trapezoids respectively. Based on the above, morphological and morphometric observations, no other species belonging to the genus *Monocystis* Stein, 1848 resembles the species under discussion. After a thorough comparison, the present species is being considered as a new and is designated as *Monocystis kuidongae* sp. nov. in this paper.

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