***Monocystis julkae* sp.nov., (Protista: Apicomplexa: Monocystidae) a new aseptate gregarine species of the genus *Monocystis* Stein, 1848 obtain from an Indian Earthworm, *Eutyphoeus kherai*, Julka, 1978**

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Received: 16 January 2015 / Accepted: 8 March 2016 / Published online: 29 March 2016
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Abstract During the course of a biodiversity survey of the endoparasitic aseptate gregarines in the Malda district of West Bengal, India, seminal vesicles of the earthworm, *Eutyphoeus kherai*, Julka 1978 were found to be infested with a new species, *Monocystis julkae* sp.nov., of the genus, *Monocystis* Stein (Arch Anat Phys Med 181–223, 1848). The trophozoite is elongated but slightly constricted posteriorly. A tail like protrusion appears in the posterior end. Anterior end is rather wider than the posterior one. The whole body size of the trophozoite measures 102.2–184.0 (126.7 ± 19.9) $\mu\text{m} \times 40.9$ –81.8 (58.2 ± 10.0) μm . Size of the nucleus ranges from 10.2 to 16.3 (11.9 ± 1.9) $\mu\text{m} \times 8.1$ –12.2 (8.8 ± 1.2) μm . The gametocysts are ovoidal containing two unequal gametocytes. Diameter of it measures 61.3–98.1 (75.8 ± 8.6) μm . Oocysts are navicular and measures 6.9–10.0 (8.7 ± 0.9) $\mu\text{m} \times 3.0$ –4.6 (4.3 ± 0.4) μm .

Keywords Aseptate gregarine · Endoparasite · *Monocystis julkae* sp.nov · Seminal vesicle · Earthworm · West Bengal

Introduction

Gregarines are a group of apicomplexan which are chiefly coelozoic or lumen dwelling protozoan parasites of invertebrates especially arthropods, molluscs and annelids. Gregarine parasites are of two types, septate or cephaline

and aseptate or acephaline. The acephaline or aseptate gregarines are characterised by the presence of aseptate body. Aseptate gregarine fauna has been reported from various part of the world including India. However, especially in India research on gregarine protozoan parasites infesting invertebrates has not been carried out with great importance. Only a few of over 350 species of Indian earthworms have been studied for the occurrence of gregarines, but this research has gained momentum since the 1980s. It has already resulted in the finding of representatives of the genera, *Apolocystis* (Cognetti de Martiis 1923; *Monocystis* Stein 1848; *Nematocystis* Hesse 1909; *Zygocystis* Bhatia 1930; Hesse 1909; Ghosh 1923 cited by Levine 1988; Bhatia and Chatterjee 1925; Bhatia and Setna 1926; Kar 1946; Kalavati 1979; Subbarao et al. 1979; Pradhan and Dasgupta 1980a, b, 1982, 1983a, b; Roychoudhury and Haldar 1984; Bandyopadhyay et al. 2001, 2004, 2005, 2006a, b, c, d, e, 2007a, b, 2008; Bandyopadhyay and Mitra 2004, 2005a, b, c, d, 2006a, 2009a, b).

During the course of a biodiversity survey of the endoparasitic aseptate gregarines in the Malda district of West Bengal, India, seminal vesicle of the earthworm, *Eutyphoeus kherai*, Julka 1978 were found to be infested with a new species of the genus, *Monocystis*, (Stein 1848). The present paper dealt with the description of *Monocystis julkae* sp.nov., the morphometric comparison with closely related species and the discussion of its taxonomy and systematic (Table 1).

Materials and methods

Earthworms were collected and taken to the laboratory. The collected individuals were put in soil-filled plastic buckets and taken to the laboratory alive. Some of them

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Table 1 Morphometric comparison of *Monocystis julcae* sp.nov., with *Monocystis pheretimi*, (Bhatia and Chatterjee 1925), *Monocystis megascolexae* (Kar 1946)

Characters	Species		
	<i>Monocystis pheretimi</i> , (Bhatia and Chatterjee 1925)	<i>Monocystis megascolexae</i> (Kar 1946)	<i>Monocystis julcae</i> sp.nov.
Host	<i>Pheretima posthuma</i>	<i>Megascolex</i> sp.	<i>Eutyphoeus kherai</i>
Locality	Lahore (Pakistan and Bombay)	Calcutta	Malda, West Bengal
Site of infection	Seminal vesicles	Seminal vesicles	Seminal vesicles
Gamont	Variable, spherical, ovoid, ellipsoidal	Variable, elongated narrow with one end rounded and other end pointed. At maturity spherical with a short tail like process	Elongated but slightly constricted. Tail like protrusion present at posterior end
Size	50–200 μ m	135.8–429.0 $\times$ 58.2–97.5 μ m	102.2–184.0 $\times$ 40.9–81.8 μ m
Ectosarc	Smooth	Smooth	Wrinkled
Endosarc	Vacuolar	Highly granular	Containing deeply stained granules
Nucleus shape	Spherical or ovoidal. Karyosome placed centrally	Spherical with a central karyosome	Ovoidal
Size of Nucleus	Not stated	25 μ m in diameter	10.2–16.3 $\times$ 8.1–12.2 μ m
Gametocyst shape	Spherical	Spherical or oval	Ovoidal
Gametocyst size	80 μ m	135 μ m in diameter	61.3–98.1 μ m
Oocyst shape	Not stated	Spindle shaped with blunt end	Navicular
Oocyst size	Not stated	15.43–8.24 μ m	6.9–10.0 $\times$ 3.0–4.6 μ m

were dissected while alive and their seminal vesicles were carefully removed. These were placed on clean glass with a drop of 0.6 % NaCl solution. A thin film of seminal fluid was drawn out on a slide covered with a cover slip for examination of living protozoans under a phase contrast microscope (Olympus CX41). After the initial study of living protozoans, 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 in each) and placed in distilled water. Then they were transferred to a 3 % iron alum solution (over night) 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 then washed thoroughly, dehydrated in an ascending series of alcohols, cleared in xylene and mounted in Canada balsam. Drawings of the different stages of gregarines were made using a camera lucida attached to the microscope; photomicrographs were taken under an Olympus phase contrast microscope ($\times 400$ magnification) with an Olympus camera (Model C5060). All measurements are in micrometres; in each case minimum and maximum values are given, followed in parentheses by arithmetic mean, standard deviation and sample size. Plane shapes are described mainly according to Clopton (2004).

Results

Phylum: Apicomplexa Levine 1988; Order: Eugregarinida Léger, 1900; Family: Monocystidae Bütschli, 1882; Subfamily: Monocystinae (Bhatia 1930); Genus: *Monocystis* (Stein 1848).

The trophozoite is elongated but slightly constricted posteriorly. A tail like protrusion appears in the posterior end. Anterior side is rather wider than the posterior one. Nucleus is ovoidal and resides in the middle of the body. Mucron is very distinct and rounded. Cytoplasm is granular. Endocytal striations are not found. Endosarc is granular and deeply stained with Haematoxyline. Episarc is 2–3 μm in wide. The whole body size of the trophozoite measures 102.2–184.0 (126.7 ± 19.9) $\mu\text{m} \times 40.9$ –81.8 (58.2 ± 10.0) μm . Size of the nucleus ranges from 10.2 to 16.3 (11.9 ± 1.9) $\mu\text{m} \times 8.1$ –12.2 (8.8 ± 1.2) μm . Gametocysts are ovoidal containing two unequal gametocytes. Diameter of it measures 61.3–98.1 (75.8 ± 8.6) μm . Large gametocyte is semi ovoidal and the small one is semicircular. Large and small gametocyte measures 57.2–81.8 (64.9 ± 6.5) $\mu\text{m} \times 32.7$ –44.9 (39.7 ± 3.4) μm and 53.1–61.3 (57.8 ± 2.9) $\times 24.5$ –36.8 (29.8 ± 3.7) μm , respectively. Both gametocytes containing a round nucleus.

Oocysts are navicular and measures 6.9–10.0 (8.7 ± 0.9) $\mu\text{m} \times 3.0$ –4.6 (4.3 ± 0.4) μm . Both ends of the oocysts are blunt and tapering at each side. Some deeply stained granules are seen inside the oocysts (Fig. 1).

Taxonomic Summary

Type material: *Monocystis julkae* sp.nov.

Type host: *Eutyphoeus kherai*

Type of locality: Malda district of West Bengal, India.

Type locality: Malda (Lat. 25°6'N–Lon. 88°6' E), West Bengal.

Symbiotype: EKW/02 deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani-741235, West Bengal, India.

Site of infection: seminal vesicles

Prevalence: 12/32 (37.5 %)

Holotype: MSIII/11 is deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Paratype: MSIII/05, MSIII/09 and other slides are in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Discussion

Cylindroid, solitary, with mucron, and coelomic habitat of the parasite in an earthworm justify the inclusion of the present form under the family Monocystidae, subfamily Monocystinae and genus *Monocystis* (Stein 1848). Of more than eighty species belonging to genus *Monocystis* (Stein 1848) only 20 have been described from India. Of these 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 eleven species of *Monocystis* have been reported from terrestrial earthworms. The present species of monocystid gregarine obtained from the seminal vesicles of the oligochaete host, *E. kherai*. The species which is considered to be a new, shows a little degree of affinity with *Monocystis megascloxae* (Kar 1946) and *Monocystis pheretimae* (Bhatia and Chatterjee 1925). The present species and *M. megascloxae* possesses a tail like structure at the posterior end. Middle portion of the present species is slightly constricted and having a mucron at the anterior end. Nucleus with karyosome and ovoidal in shape but in *M. megascloxae* and *M. pheretimae* it is spherical. Cytoplasm of the present form is granular and endosarc with deeply stained granules. Oocyst of *M. megascloxae* is spindle

Fig. 1 Photomicrograph of different stages of the life history of *Monocystis julkae* sp. nov. obtained from seminal vesicles of the earthworm *Eutyphoeus kherai*, (Julka 1978). 1–2 Trophozoites, 3. Gametocyst, 4. Oocysts. Scale bars 100µm (1–3) 10µm (4)

shaped and in present form it is navicular with pointed tips. Gametocyst of the present species is ovoidal whereas it is spherical in both the comparable species. The tail of the early stage trophozoite is short but in mature form it is very much long. So comparing all the morphological features, we consider this species of the genus *Monocystis*, obtained from *E. kherai* is new to science and therefore, we propose the species as. *Monocystis julkae* sp.nov.

Acknowledgments Photomicrograph of different stages of life history of. *Monocystis julkae* sp.nov. 1–2 Trophozoite, 3. Gametocyst; 4. Oocysts Scale bar; 100 µm (1–3) 4 (10 µm).

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