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A New Species of *Monocystis* Stein, 1848 (Protista: Apicomplexa: Eugregarinida) from the Indian Earthworm, *Amynthas hawayanus* Rosa, 1891 (Annelida: Oligochaeta)

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SUMMARY: As a part of an ongoing biodiversity survey of aseptate gregarine fauna of oligochaete hosts of West Bengal, an expedition was carried out in the Darjeeling district of West Bengal and most of the earthworms collected were found to be infested with a species of *Monocystis* Stein, 1848. The monocystid species was collected from the seminal vesicles of the earthworm and was identified as a new species, *Monocystis amynthae* sp. nov. The gamont of the new species is characterized by having an elongated body with broad anterior end, separated from the narrow posterior end by a prominent constriction measuring 49.0-77.0 (66.0±1.3) µm x 32.0-41.0 (37.0±2.8) µm. The gametocysts are oval-shaped, measuring 40.0-65.0 (58.0±2.1) µm. The oocysts are navicular, measuring 8.0-12.0 (10.5±1.1) µm x 4.0-6.0 (5.5±1.1) µm.

Key Words: *Monocystis amynthae* sp. nov., endoparasite, earthworm, seminal vesicle, India

Hindistan Toprak Solucanı *Amynthas hawayanus* Rosa, 1891'dan Yeni Bir *Monocystis* Stein, 1848 (Protista: Apicomplexa: Eugregarinida)

ÖZET: Batı Bengal'in oligoket konaklarının aseptat gregarin biyoçeşitliliği ile ilgili araştırma esnasında, Darjeeling Bölgesi'den toplanan *Amynthas hawayanus* türüne dahil toprak solucanlarının çoğunun bir *Monocystis* türü ile enfekte oldukları tespit edilmiştir. Bu monocystid türü toprak solucanlarının seminal vesiküllerinde saptanmış ve yeni bir tür, *Monocystis amynthae* sp. nov. şeklinde tanımlanmıştır. Bu yeni türün gamontu 49.0-77.0 (66.0±1.3) µm x 32.0-41.0 (37.0±2.8) µm boyutlarında olup, bariz bir yapı ile daha dar posterior uçtan ayrılan, genişçe anterior uca sahip olacak şekilde uzamış bir vücuda sahip olmasıyla karakterize olur. 40.0-65.0 (58.0±2.1) µm boyularında ölçülen gametokistleri oval şekillidir. Ookistleri navikular şekilli olup uzunlukları 8.0-12.0 (10.5±1.1) µm ve genişlikleri 4.0-6.0 (5.5±1.1) µm arasında değişir.

Anahtar Sözcükler: *Monocystis amynthae* sp. nov., endoparazit, toprak solucanı, seminal vesikül, Hindistan

INTRODUCTION

In India, only 13 species of the genus *Monocystis* Stein, 1848 have been reported so far (1-5, 7-9, 11, 12). The present paper deals with a new species of the genus *Monocystis* obtained from a biodiversity survey of aseptate gregarines in the earthworm hosts of the Darjeeling district.

MATERIAL AND METHODS

Thirty one 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 mins. 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 and stained with Heidenhains haematoxylin solution (20 min). Differentiation (over night) was done with 1% iron-alum solution. The slides were then washed thoroughly,

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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. Method of describing shapes of planes and solids is mainly according to Clopton (6).

RESULTS

Phylum : Apicomplexa Levine, 1988

Order : Eugregarinida Leger, 1900

Family : Monocystidae Bütschli, 1882

Subfamily: Monocystinae Bhatia, 1930

Genus : *Monocystis* Stein, 1848

***Monocystis amynthae* sp.nov (Figs. 1-3)**

Gamont Length (GL): 49.0- 77.0 (66.0±1.3); Gamont Width (GW): 32.0-41.0 (37.0±2.8); Nucleus Diameter (ND): 6.0-12.0 (9.5±1.2); Gametocyst Diameter (GD): 40.0-65.0 (58.0±2.1); Oocyst Length (OL): 8.0-12.0 (10.5±1.1); Oocyst Width (OW): 4.0-6.0 (5.5±1.1).

The members of the genus *Monocystis* Stein, 1848 are characterized by having no distinct mucron, ovoid and solitary gamonts, bi-conical, symmetrical oocysts (Levine, 1988). The present monocystid gregarine obtained from the seminal vesicles of the earthworm *Amynthas hawayanus*, the gamont is elongated with irregular outline. The anterior end is broad and the posterior end is narrow. There is a prominent constriction between the anterior and posterior end. The broad anterior end of the gamont has a cup like depression. The posterior tip of the posterior end of the gamont is rounded. The mucron is not distinct and present at the anterior end of the body. No syzygy stage has been observed in the life cycle. Ectoplasm is thick. Distinction between the ectoplasm and endoplasm is not significant. A large number of small vacuoles are present in the endoplasm. Irregular shaped paraglycogen granules are uniformly arranged in the endoplasm. Nucleus shape is oval and is situated in the middle portion of the anterior half of the gamont. Each rounded gametocyst with almost two equal gametocytes. Oocysts are biconical.

Taxonomic summary

Type Host: *Amynthas hawayanus* Rosa, 1891

Type Locality: Darjeeling (Lat. 27°N, Lon. 88 °E)

Symbiotype: Host AH-17 deposited in the museum of the Department of Zoology, University of Kalyani, Kalyani, 741235, West Bengal, India

Type material

Holotype: Slide MA-11/2006 is deposited in the Museum of the Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India. Registration number Z/KU/2006/14.

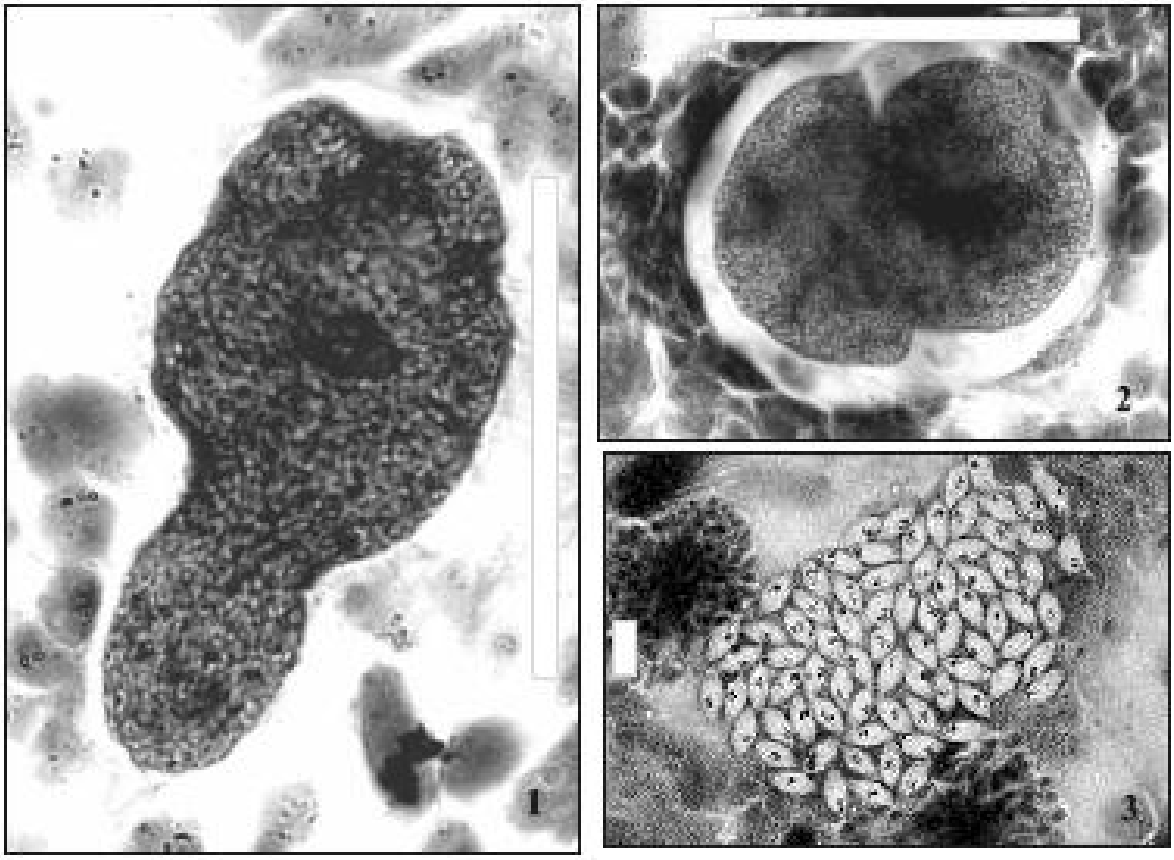
Paratype: Slides AH-04, AH-07, AH-14 are in the collection of the Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India.

Prevalence: 19/31 (61.2%) earthworms were infected with *Monocystis amynthae* sp. nov.

Etymology: The specific epithet " *amynthae*" has been given after the genus name of the host earthworm *Amynthas hawayanus* Rosa.

DISCUSSION

Cylindroid, solitary, with mucron, late syzygy 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 70 species belonging to the genus *Monocystis* Stein, 1848, only 13 have been described from India. The present monocystid gregarine obtained from the seminal vesicles of the earthworm *Amynthas hawayanus* has been compared with other species of the genus *Monocystis* and found that it resembles only two Indian species, *Monocystis clubae* Bandyopadhyay et al. 2006a and *M. metaphirae* Bandyopadhyay et al. 2006b. The species under discussion resembles *M. clubae* in having anterior broad posterior narrow end only. But the measurements are significantly different. The new species under discussion is smaller in size (66 x 77 versus 160 x 64 in *M. clubae*). The anterior end of *M. clubae* is rounded while that of the new species contains a cup-like depression at the anterior end. The anterior end of the gamont of *M. clubae* gradually tapers to a narrow tail, but the gamont of the new species does not end to any tail-like structure. In *M. clubae* the nucleus is elongated, that is oval in the proposed new species. The gametocyst of *M. clubae* with two unequal gametocytes, but in the new species the gametocyst with two almost equal gametocytes. The new species also smaller than *M. metaphirae* (66 x 77 versus 119x 66 in *M. metaphirae*). The gamont of *M. metaphirae* gradually tapers to rounded posterior end without any constriction in between the two ends. But in the new species obtained from the *Amynthas hawayanus*, a constriction is present between the two ends. The anterior tip of the anterior end of the new species contains a cup-like depression, which is rounded and without any such depression in *the M. metaphirae*. The main differences between the two species is the presence of syzygy in the life cycle of *M. metaphirae*, which is completely absent in the new species. Oocysts shape is also different in both the species.



Figures 1-3. Photomicrographs of different stages of the life cycle of *Monocystis amynthae* sp. nov.
1. Mature gamont; **2.** Gametocyst; **3.** Oocysts. Scale bars 100 µm (Figs. 1-2) and 10 µm (Fig. 3).

Considering all these differences we propose the species under discussion a new *Monocystis* species to science and designate in this paper as *Monocystis amynthae* sp. nov.

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