Isolation and identification of Endoparasitic Aseptate Gregarines (Protozoa: Apicomplexa) from earthworms of West Bengal

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Abstract:

During the course of biodiversity survey of the endoparasitic aseptate gregarines of West Bengal, India, seminal vesicles of the earthworms were infested with different genus of aseptate gregarines like Stomatophora, Apolocystis, Nematocystis and Monocystis. The present paper deals with isolation, staining and identification of said genus along with their host. The genus Stomatophora was created by Drzhevetskii in 1907 with the type species S. coronata (Hesse, 1904) and the genus Apolocystis was established by Cognetti de Martiis, in 1923. The genus Nematocystis was established by Hesse (1909). Stein, in 1848 established the genus Monocystis.

Key words: Earthworms, seminal vesicles, endoparasite, aseptate gregarines, *Stomatophora, Apolocystis, Nematocystis, Monocystis.*

Introduction:

Protozoa are a diverse group of unicellular eukaryotic organisms with puzzling affinities. Originally, protozoa had been defined as unicellular protists with animallike behaviour, e.g., movement. Protozoa commonly range from 10 to 52 micrometers, but can grow as large as 1 mm, and are seen easily by microscope. According to Levine *et.al.* (1980) they are not a natural group but have been placed together for convenience. Under the subkingdom protozoa several phyla have been included. Apicomplexans are obligate intracellular parasites; these protozoa only grow and replicate within host cells and they are the causative agent of wide variety of human and agricultural diseases. Apicomplexans are a diverse group of unicellular eukaryotes harboring mainly in the alimentary canal, body cavities, and metazoan tissue. Apicomplexans are haploid during most stages of their life cycle. The rapidly proliferating haploid parasite stages cause the acute and deleterious symptoms of infection. There are approximately 4600 described apicomplexan and probably more than ten times that number is yet to be discovered. Gregarines constitute an

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apicomplexan group that is distinct from coccidians, haemosporidians. Gregarines are a group of apicomplexan which are chiefly coelozioc 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 gregarines constitute a large group of parasites limited to invertebrates. They are endoparasites, incapable of active life outside their host and their normal and primitive means of distribution is the resistant spore or sporocyst, containing the sporozoites, which are the infective stage of the life cycle.

Isolation and staining technique of aseptate gregarines from earthworms:

The host, earthworms were collected from different ecological niches of west Bengal. The collected earthworms were maintained in earthen tubs and time to time the earthworms are dissected while alive and their seminal vesicles are carefully removed. These are placed on clean grease free slide with a drop of 0.6% NaCl solution. A thin film of seminal fluid is drawn out on the slide covered with a cover slip for examination of living protozoans under a microscope, and after that the dissected earth worms were preserved 10% formalin for identification. After the initial study of living protozoans, the content of the seminal vesicles is semidried and fixed in Schaudin's fluid (20 minutes). The fixed smears are stored in 70% ethyl alcohol for removal of excess of mercuric chloride. The slides are then passed through a descending series of alcohols (5 minutes in each) and placed in distilled water. Then they are transferred to a 3% iron alum solution (over night) and stained with Heidenhain's haematoxylin solution (20 minutes). Differentiation is done with 1% iron alum solution under the low power objective lens of the light microscope. The slides are then washed thoroughly, dehydrated in an ascending series of alcohols, cleared in xylene and mounted in Canada balsam. Photomicrographs were taken under an Olympus phase contrast microscope with Dwinter camera (Model DGI-510). All measurements are in micrometres and plane shapes are described mainly according to Clopton (2004).

Identification of different endoparasitic asepatate gregarine fauna from different earthworms (Annelida: Oligochaeta)

Name of the host: Metaphire posthuma

Type locality: Bongaon, North 24 pgs, West Bengal

Nature of soil: Sandy soil

1. Name of the endoparasitic aseptate gregarines: Stomatophora sp.

The genus *Stomatophora* was created by Drzhevetskii in 1907 with the type species *S. coronata* (Hesse, 1904) Drzehevetsii, 1907 which was previously known as

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Monocystis coronata Hesse, 1904 with the characters of genus *Stomatophora* Drzhevetskii, 1907, as given by Levine (1988);trophozoite spherical or ovoid; sucker petaloid, with radiating sides. Gametocysts are ovoidal. Oocysts bioconical, a flattened button at each end attached to each other end to end in long chain inside gametocysts; usually in seminal vesicles of oligochaetes.



Fig-1: Photomicrograph of different life history stages (1-3) of *Stomatophora* sp.

Name of the host: *Eutyphoeus orientalis* Site of infection: seminal vesicle Type locality: Mogra, Hooghly, West Bengal

Nature of soil: Alluvial soil

2. Name of the endoparasitic aseptate gregarines: Apolocystis sp.

The genus *Monocystis* Stein, 1848 revised by Hesse in 1909 and recognized it under four genera namely (i) *Monocystis* (ii) *Nematocystis* (iii) *Rhynchocystis* (iv) *Pleurocystis*. In 1923 Cognetti de Martiis included the genus *Apolocystis* in order to accommodate all those species of the genus *Monocystis*, which had spherical trophozoite and lacked a polarity. Cognetii de Martiis defined the new genus as follows "Trophozoite spherical without the principal axis marked by the presence of any special peripheral organ, solitary". After that the definition of the genus *Apolocystis* remained unmodified by any subsequent author and the morphology of the species described at present agrees with the definition given by Cognetti de Martiis, 1923.



Trophozoite(10x×40x)2. Gametocyst(10x×40x)3. Oocysts (10x×100x)Fig-2: Photomicrograph of different life history stages (1-3) of Apolocystis sp.

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Name of the host: *Metaphire planata* Site of infection: seminal vesicle Type locality:Chakdah, Nadia, West Bengal Nature of soil: Sandy soil

3. Name of the endoparasitic aseptate gregarines: Nematocystis sp.

A new genus Nematocystis established by Hesse (1909) which can be distinguished from Monocystis Stein, 1848. The main characteristics of the genus are gamont large, cylindriod, nematoid. Often with mucron at the anterior end and solitary oocyst are biconical.the length of the trophozoite may be up to 5 mm and the width usually less than 50 mm. By reflected light, it is creamy white in colour.



Trophozoite(10x×40x) 2. Gametocyst(10x×40x) 3. Oocysts (10x×100x)

Fig-3: Photomicrograph of different life history stages (1-3) of Nematocystis sp.

Name of the host: Lampito mauritii Site of infection: seminal vesicle Type locality: Raghunathpur,Purulia,West Bengal Nature of soil: Rocky soil

4. Name of the endoparasitic aseptate gregarines: *Monocystis* sp.

The genus *Monocystis* was established by Stein in 1848 with the type species *Monocystis agilis* obtained from the earthworm *Lumbricus terrestris*. The generic characters were latter established by Hesse (1909) to seperate *Nematocystis*, *Rhynchocystis* and *Pleurocystis* from the genus *Monocystis*. The genus *Monocystis* is defined as : Mucron not marked, gamonts ovoid. Short or elongate, solitary, oocyst biconical, symmetrical (Levine, 1977a).



Trophozoite(10x×40x)2. Gametocyst(10x×40x)3. Oocysts (10x×100x)Fig-4: Photomicrograph of different life history stages (1-3) of *Monocystis* sp.

Discussion: The present paper deals with isolation and identification of some endoparasitic aseptate gregarines genus namely *Apolocystis, Nematocystis, Stomatophora and Monocystis.* The entire aseptate gregarines genuses were isolated from the seminal vesicles of the different earthworm's species. There are three distinct developmental stages are found in the life history of the aseptate gregarines namely trophozoite, gametocyst and oocyst. The species of the genus *Stomatophora* and the species of the genus *Apolocystis* isolated from the seminal vesicles of the earthworm, *Metaphire posthuma* and the species of the genus *Apolocystis* isolated from the seminal vesicles of the genus *Apolocystis* was found in the seminal vesicles of the genus *Nematocystis* was found in the seminal vesicles namely *Lampito mauritii.*

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