

Kleinere Mitteilungen.

(From the Department of Zoology, University of Calcutta.)

Studies on Sporozoa from Indian millipedes.

V. Life-history of a cephaline gregarine, *Hyalosporina rayi* n. sp.

By

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With 5 figures in the text.

Introduction.

The family *Hyalosporinidae* was created by CHAKRAVARTY (1935) to receive the species *Hyalosporina cambolopsisae*, a cephaline gregarine found in the alimentary tract of a millipede, *Cambolopsis* sp. This paper contains the description of the life-history and morphology of a new gregarine which resembles very much the above species in having a hyaline coat around the spore and a dark staining area at the anterior extremity of the epimerite (well-marked in the intracellular forms). It also resembles *Monoductus lunatus* RAY and CHAKRAVARTY (1933) and *Hyalosporina cambolopsisae* CHAKRAVARTY (1935) in having its nucleus tethered to the pellicle by means of myoneme fibres. In addition to these and other minor features there is a lid-like structure on one side of the spore, and hence I have called it *Hyalosporina rayi*, the species being named after my teacher Dr. H. N. RAY, whose constant encouragement and guidance have made it possible for me to take up this line of work. This gregarine inhabits the intestine of the millipedes *Polydesmus* sp.¹⁾ and *Strongylosoma contortipes* ATTEMS.

¹⁾ The millipedes *Polydesmus* sp. are also infected with another species of gregarines, the life-history of which will be described when opportunity arises.

The methods employed in studying the life-history and morphology of this gregarine are the same as has already been outlined in the previous papers on the subject by RAY (1933) and CHAKRAVARTY (1935).

Here I wish to express my indebtedness to Prof. H. K. MOOKERJEE, for his kind permission to continue my studies on protozoa in the laboratory of the department of Zoology, Calcutta University, and to Mr. D. MUKERJI in helping me to identify the millipede in question.

Observations on *Hyalosporina rayi* n. sp.

The sporozoites, measuring 4.12μ long 1.03μ broad (Fig. 1 a) are seen attached to the epithelium by one of its ends which stains dark with iron-haematoxylin. The nucleus at this stage is a spherical darkly staining body. It now penetrates an epithelial cell, pass beyond the nucleus like that of *Stenophora lacteria* WATSON, *Stenophora khagendrae* RAY and *Hyalosporina cambolopsisae* CHAKRAVARTY, and develop intra-cellularly. A slight indication of epimerite

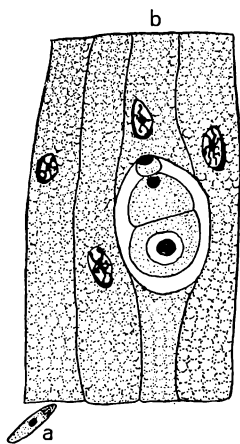


Fig. 1 a u. b. *Hyalosporina rayi* from section. $\times 1666$.

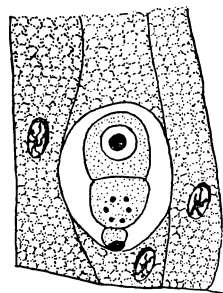


Fig. 2. *Hyalosporina rayi* from section. $\times 1666$.

can be seen in those that have entered the epithelial cells and are three times broader than the sporozoites. The nucleus too exhibits a nuclear membrane, a dark staining karyosome and a clear nucleoplasm. In forms measuring 10.3μ long and 6.18μ broad, one can easily distinguish an epimerite, protomerite and deutomerite (Fig. 1 b). The epimerite in this stage is a rounded structure with a darkly staining area at its anterior extremity. In this respect it resembles *Hyalosporina cambolopsisae* (see CHAKRAVARTY, 1935, fig. 3, Pl. 7).

The protomerite which is more or less spherical in early stages becomes triangular in shape in the adult (Fig. 4) and measures $15 \mu \times 20 \mu$. In the intracellular forms a darkly staining dot is seen at the anterior extremity of the protomerite (Fig. 1 b). In

advanced stages this dot is broken up into four to six small pieces (Fig. 2) and ultimately disappears giving the pellicle in this region a thickened appearance (Fig. 4).

The deutomerite is the longest segment of the body and is circular in transverse section. Its anterior portion is broader than the posterior which gradually becomes tapering. The cytoplasm is packed with granules which give the organism a yellowish white appearance under reflected light. The

deutomerite grows more rapidly than the protomerite as is shown in the accompanying table (see p. 119).

When an individual has grown 20 μ long and 15 μ broad, the host cell gives way and the parasite comes out of it. Forms measuring 130—173 μ \times 37—70 μ are often encountered in the lumen of the gut. These are devoid of epimerites and are apparently sporonts.

The pellicle is about 2 μ thick and there are longitudinal epicyteal striations running all over the body. Just below

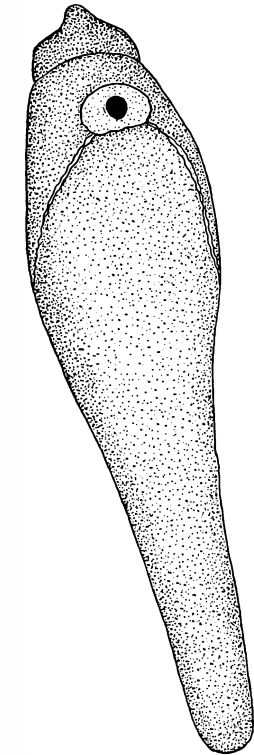


Fig. 3. *Hyalosporina rayi* from a smear. \times 730.

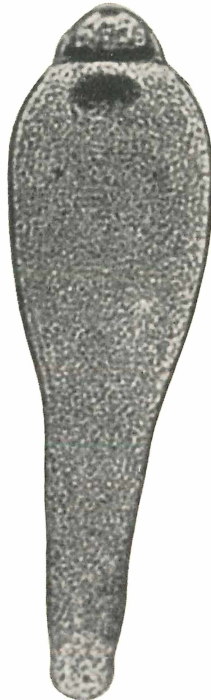


Fig. 4. *Hyalosporina rayi*. Photomicrograph from a smear. \times 730.

this is the layer of sarcocyte. Next to this lies the layer of circular myonemes or myocyte to which nuclear myonemes are tethered.

The nucleus is spherical and has a central karyosome in the young forms, but it soon becomes oval as the gregarine attains maturity. A normal full-grown nucleus measures 15 μ \times 10 μ and the karyosome which is always spherical measures 6 μ in diameter. The nuclear membrane is well defined and has at its sides two sets

Total length of Proto-merite in microns.	Total length in microns.	Ratio of the length of Proto-merite to total length.
6.18	14.42	1 : 2.3
7.5	20	1 : 2.6
10	47.5	1 : 4.75
10	50	1 : 5
10	58	1 : 6.8
12.5	92.5	1 : 7.4
10	85	1 : 8.5
10	95	1 : 9.5
12	120	1 : 10
15	158	1 : 10.53
7.5	90	1 : 12
10	130	1 : 13
10	140	1 : 14
10	160	1 : 16

of myoneme fibres (Fig. 3) disposed off in the same manner as in *Hyalosporina cambolopsisae* CHAKRAVARTY.

Spherical gametocysts measuring 77—116.5 μ in diameter are formed in the lumen of the gut and come out with faecal matter. Their further development takes place outside the host; if kept in moist chamber they are found to mature within four or five days and dehisce by simple rupture. There is an ectocyst. The spores which are elongately oval measure 6.2 μ long and 4.12 μ broad and have an outer hyaline coat round them which is prominent at both the poles. On one side of the spore there is a circular lid-like structure (Fig. 5) through which, most probably, the sporozoites escape. When fresh spores are stained with LUGOL'S solution this lid-like structure stains deep brown. It is particularly this character which distinguishes it from the type species *Hyalosporina cambolopsisae* and thus warranted me to give it a new specific name.



Fig. 5. *Hyalosporina rayi*. Spore from a fresh smear stained in Lugol's sol. $\times 2700$.

Diagnosis.

Sporonts, solitary. Trophozoites elongate, the broadest part being slightly behind the septum and measure 130—173 $\mu \times 37$ —70 μ . Epimerite, a round body with a darkly staining area. Protomerite small and triangular in shape, with special types of granules which serves as a plug. The adult nucleus is oval. Spherical gametocysts 77—116.5 μ in diameter rupture by simple dehiscence, and liberate elongately oval spores measuring 6.2 \times 4.12 μ . A hyaline membrane,

prominent at both the poles, surrounds the spore, and, at one of its sides there is a lid-like structure.

Habitat. Alimentary canal of the millipedes, *Polydesmus* sp. and *Strongylosoma contortipes* ATTEMS.

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Zeitschrift/Journal: [Archiv für Protistenkunde](#)

Jahr/Year: 1936

Band/Volume: [88_1936](#)

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