(From the Department of Zoology, Calcutta University.)

Studies on *Sporozoa* from Indian millipedes.

IV. Life-history of a cephaline gregarine, *Hyalosporina cambolopsisae* n. gen., n. sp.

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(With plate 7.)

Contents.

1. Introduction.

This paper contains the description of the life-history and morphology of a new cephaline gregarine found in the intestine of a millipede *Cambolopsis* sp. Specimens were collected from our college compound and from neighbourhood of Calcutta. Ray (1933), and Ray and Chakravarty (1933) described the life-histories of *Stenophora khagendrae* and *Monoductus lunatus* from the intestine of the millipedes *Zikadesmus* and *Diplopoda* sp.[1]) respectively. Chakravarty

[1]) This millipede has since been kindly identified for us as *Strongylosoma contortipes* Attems by Dr. Attems of Vienna to whom our sincere thanks are due.

(1934) described the life-history of *Stenophora ellipsoidi* from *Diplopoda* sp. The present form however, resembles *Monoductus lunatus* in having its nucleus tethered to the pellicle by means of myoneme fibres, but its other features, such as the simple nature of the epimerite and intracellular development are more near approach to the characters of the genus *Stenophora* than *Monoductus*. While cysts, though rupture by simple dehiscence spores do not resemble that of any species of *Stenophora* hetherto described. A mature spore has a hyaline coat round it which is more prominent at one pole than the other. In naming this gregarine, therefore, emphasis is laid on the hyaline coat of its spore and hence called *Hyalosporina cambolopsisae*, the species being named after its host.

Here I wish to express my indebtedness to Dr. H. N. RAY for his kind guidance and criticism and to Mr. D. MUKERJI for the partial identification of the millipede.

2. Material and methods.

Living organisms were observed under a cover glass smear sealed with vaseline. Smears were fixed in SCHAUDINN'S fluid and stained in DELAFIELD'S or HEIDENHAIN'S haematoxylin. MAYER'S Acid Haemalum was also used with great success. For detailed investigation the entire alimentary canal of infected millipedes were fixed in BRASIL'S modification of BOUIN-DUBOSCQ'S fluid for twenty four hours and ultimately cut 6 μ thick. The sections were stained in HEIDENHAIN'S haematoxylin. Small pieces of infected alimentary canal were also fixed in CARNOY'S fluid and after being cut 6 μ thick were tested for glycogen by means Iodine an BEST'S carmine methods. Methods of MANN and KOPSCH and DA FANO were also employed for studying the cytoplasmic inclusions of this gregarine. Gametocysts which were collected from the faecal matter of the host were fixed for twenty four hours (one hour on the top of the bath) in DOBELL'S modification of BOUIN-DUBOSCQ fluid.

3. Observations on *Hyalosporina cambolopsisae* n. gen., n. sp.

The youngest forms encountered with in my preparations measured 4 μ long and 1 μ broad. These forms are seen to penetrate the epithelial cells, pass beyond the nucleus and develop intracellularly while some were found to be attached to the epithelial cells of the host and grew extracellularly. The intracellular forms after having travelled beyond the nucleus of the epithelial cell

remain attached to the basement membrane of the intestine in the same way as in *Stenophora lactaria* WATSON (1917) an *S. khagendrae* RAY (1933). In the intracellular forms measuring 11 $\mu \times 6\ \mu$ one could easily differentiate a protomerite, deutomerite and a darkly staining spot, the epimerite. With the growth however, this epimerite assumes a rounded appearance with an increased darkly staining area (Pl. 7 Fig. 3). Among the intracellular stages some were found to lie with their long axis parallel to the long axis of the intestine. While those which developed extracellularly the differentiation of the epimerite, protomerite and deutomerite was found to take place at a very early stage too (see Pl. 7 Fig. 2). Forms measuring 43—150 $\mu \times$ 14—30 μ were often seen hanging from the epithelial cells by means of an epimerite. Epimerite in this gregarine is a simple structure and consists af a darkly staining collar or ring which grasps the host cell and a tongue-like elevated cytoplasmic area situated in front of it inserted into the cell. In older form very fine root-hair-like processes are seen to arise from the ring and project over the tongue-like elevation (see Pl. 7 Fig. 4).

In a full grown individual, which is found free in the lumen of the gut the epimerite is nearly always absent because it remains attached to the epithelial cell as the gregarine leaves it. It is quite probable that the body weight of a mature sporont aided by the peristaltic movement of the intestine is responsible for snapping the organism at its weak spot — the junction between epimerite and protomerite. Moreover, the function of the epimerite is over as soon as the gregarine has attained maturity and is ready to step into another phase of its life-cycle, that is, sporogony. In some, however, a darkly staining dot is seen at the orifice of the protomerite. This dot being different in its staining reaction from the granules of the protomerite is found to serve as a plug and stop the latter from collapsing. Pl. 7 Fig. 4 shows a sporont in the process of detaching from the host cell in which the darkly staining granules has appeared at the orifice. At a later stage however this dot disappears and the pellicle in this region becomes slightly thickened and protrudes into the cytoplasm of the protomerite (see Pl. 7 Fig. 6). These forms without epimerite are sporonts and measure 800 μ in length and 80 μ in breadth. Observation on living organism shows that they attain even a greater size and measure 1111 μ in length and 111 μ in breadth.

The protomerite which is more or less spherical in young forms becomes conical in shape in the adult and measures 48.5 $\mu \times$ 61.5 μ.

From the study of the sections it is evident that the protomerite is capable of elongating slightly.

The deutomerite is the longest segment of the body and is circular in transverse section. It grows more rapidly than the protomerite as is shown in the accompanying table.

Total length of Protomerite in microns	Total length in microns	Ratio of length of Protomerite to total length
15	55	1 : 3.6
15	60	1 : 4
15	65	1 : 4.3
17	85	1 : 5
15	90	1 : 6
20	150	1 : 7.5
35	407	1 : 11.3
24	247	1 : 12.1
48	877	1 : 18.2
48	1035	1 : 20.1
48	975	1 : 20.3
39	799	1 : 20.4
48	1111	1 : 23.1

Its cytoplasm gives a greenish white appearance with the reflected light. The cytoplasmic inclusions are described below.

Longitudinal epicyteal striation are more prominent on the deutomerite than protomerite and in a transverse section appear as thin ridges about 1.5 μ high. Just below this ridged layer there is a very thin homogenous layer of sarcocyte which stains pink with MALLORY'S triple stain. Next to this lies a layer of circular myonemes or myocyte to which the nuclear myonemes are tethered (see Pl. 7 Figs. 5 and 6).

The nucleus is spherical and has a central karyosome in the young forms, but it soon becomes irregularly oval in shape as the gregarine attains maturity. A normal full grown nucleus measures 55 $\mu \times 35\ \mu$ and the karyosome which is always spherical is 15 μ in diameter. There is a well defined nuclear membrane at the sides of which there are two sets of myoneme fibres which stain dark with DELAFIELD'S or HEIDENHAIN'S haematoxylin. These fibres run backwards and are tethered to the myocyte as stated above. Tethering of the nucleus to the pellicle in this gregarine is slightly different from that of *Monoductus lunatus* in having the nuclear myonemes more concentrated at the sides of the nuclear membrane and then attaching more to the side walls instead of at the posterior extremity of the deutomerite as in the latter (see RAY and CHAKRAVARTY Text-Figs. 3 and 4 a and b).

Mitochondria in this gregarine as demonstrated after DA FANO's technique being followed, consists of fine powdery mass of granules distributed all over the cytoplasm. GOLGI-bodies are small irregular osmicated patches in the deutomerite as shown in the Pl. 7 Fig. 15. Gregarines are known to contain glycogen in the form of paraglycogen in their protoplasm but these granules in this gregarine were not so characteristic as they are in certain species of monocystis (*Acephalina*). Pl. 7 Fig. 16 is a photomicrograph of a section of this gregarine on which Iodine test for glycogen was applied. It shows the irregular Iodine positive granules in the cytoplasm. Microchemical tests for iron and copper have failed to reveal the presence of any one of those chemical in this gregarine.

4. Sporogony.

The mature sporonts that are found free in the lumen associate in pairs to form gametocysts which come out with the faecal matter of its host. Large number of cysts were found in the months of August and September. These are oval in shape and measure 292—390 μ in length and 263—375 μ in breadth. The actual process of association has, unfortunately not been observed but it is certain that it is quite unlike that of *Monoductus lunatus* because sporonts did not exhibit any pseudopodic prolongation of their deutomerite, — a character which is unique of *Monoductus lunatus* (see RAY and CHAKRAVARTY Pl. 7 Figs. 19, 20). Further development of the gametocysts takes place out side the host as has already been pointed out by RAY, and CHAKRAVARTY in the cases of *S. khagendrae* and *S. ellipsoidi* respectively. If kept in moist chamber they are found to mature within five or six days. There is an ectocyst which however disappears when spores are formed (see Pl. 7 Fig. 13).

There is a distinction between male and female gametes: a male gamete has one of its ends more pointed than the other with its nucleus occupying the pointed end; while a female is spherical and possesses an oval nucleus (see Pl. 7 Figs. 7, 8). Gametes of both the types have a transverse diameter of 6—6.5 μ. A very peculiar phenomenon is observed in the process of fertilization. The male gamete, as I have called it, does not seem to fuse with the female bodily but its nucleus only appears to enter the female gamete to fertilise it; the cytoplasm of the male gamete ultimately degenerating. It is quite evident from the study of the sections that the number of chromosomes is two and the first division of the zygote nucleus is the meiotic division. Cyst wall ruptures by

simple dehiscence and liberates large number of oval spores. These spores have a hyaline coat round them which is more prominent at one pole than the other and measure 8 μ in length and 6 μ in breadth (see Pl. 7 Figs. 12, 18). The sporozoites which are eight in number are arranged superficially along the long axis of the spore. After the gametes are formed spore formation takes place all over the gametocyst and like *Stylorhynchus oblongatus* SCHMIDT (see LEGER, 1904) is not confined to the bay at the junction of two gametocyst.

Almost all the millipedes that I examined were infected with this gregarine. The millipedes swarm in dark, moist places, and are gregarious in habit and that is why the infection was found to be so rich.

5. Systematic position.

This gregarine resembles *Monoductus lunatus* in having its nucleus tethered to the pellicle by means of myonemes. But, the characters such as intracellular development, the simple nature of its epimerite, solitary sporonts, and dehiscence of cysts by simple rupture, indicate its relationship with Stenophoridae. Its spore on the other hand has a hyaline membrane round it which is quite unlike that of either *Monoductus* or *Stenophora*. At a glance therefore, it appears to occupy an intermediate position between the *Stenophoridae* on one hand and *Monoductidae* on the other. So it is proposed that a new family of cephaline gregarines *Hyalosporinidae* be created to receive the genus *Hyalosporina*. This name is given to emphasize the nature of the hyaline coat round the spore. The specific name of the type species *Hyalosporina cambolopsisae* refers to its host *Cambolopsis* sp.

Hyalosporinidae fam. nov.

Sporonts solitary; Epimerite a small tongue-like elevation bordered with a collar at its base; Gametes dissimilar, during fertilization only the nucleus of the male gamete transfered to the female gamete; Gametocysts dehisce by simple rupture; Spores oval, with a surrounding hyaline membrane.

Hyalosporina n. gen., with the characters of the family.

Type species: *Hyalosporina cambolopsisae*.

Trophozoites elongate (247—1111 $\mu \times$ 37—111 μ) with two sides of deutomerite being parallel to each

other except at the posterior end which is tapering to a blunt point. Epimerite with a collar which holds the host cell and a tongue-like elevation which is thrust into the cell. Protomerite small and conical in shape, with its orifice plugged by a special type of granule. Oval gametocysts (292—390 μ × 263—375 μ) are expelled with host's excreta and complete development in five to six days if kept in moist chamber. Gametocysts rupture by simple dehiscence and release spores measuring 8 μ × 6 μ. A hyaline membrane, more prominent at one pole than the other, surrounds each spore.

Habitat: Alimentary canal of a millipede, *Cambolopsis* sp. from Calcutta, India.

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Explanation of plate.

All figures were drawn with the aid of a camera lucida and the magnification is × 3500 unless otherwise stated. All drawings (Except Figs. 12—19) were made from smears or sections of *Hyalosporina cambolopsisae* n. gen., n. sp. in a millipede. The material was fixed in Bouin-Duboscv-Brasil's fluid and stained in iron alum haematoxylin. (Figs. 12 and 13 are made from a fresh smear, while Figs. 14—19 are photomicrographs.)

Hyalosporina cambolopsisae n. gen., n. sp.

Fig. 1. Sporozoite attacking an epithelial cell. × 1666.

Fig. 2. Same, in which the body has become differentiated into segments.

Fig. 3. Trophozoite growing intracellularly — note the darkly staining spot in the epimerite. × 1666.

Fig. 4. Attachment of the epimerite by means of the collar — note the tongue-like elevation thrust into the host cell which is hypertrophied and contains the degenerating nucleus. Protomerite is at the point of breaking and hence shows the special granule at the orifice.

Fig. 5. A portion of the pellicle in transverse section — E — ridged epicyte, S. homogeneous sarcocyte, M. myocyte.

Fig. 6. Average sized adult (epicytial striations omitted). Note the myoneme fibres tethering the nucleus to the side-wall and the plug in the protomerite. × 180. From a smear.

Fig. 7. Fertilization of a female gamete by a male — note the pointed nucleus of the male.

Fig. 8. A male gamete.

Fig. 9. A zygote — preparing for the first meiotic division.

Fig. 10. Sporocyst with six nuclei.

Fig. 11. Sporocyst with eight nuclei.

Fig. 12. A mature spore — from a fresh smear. × 1666.

Fig. 13. Living gametocyst twenty four hours after evacuation from the millipede. Note the ectocyst. Gametes are developing on the surface of the gametocyst. × 180.

Fig. 14. Photomicrograph of the section of gametocyst showing developing spores all over the surface of the cyst. × 275.

Fig. 15. Oblique section of an adult showing irregular osmicated patches. Photomicrograph. × 275.

Fig. 16. Longitudinal section of an adult showing Iodine positive granules. Photomicrograph. × 275.

Fig. 17. Male gamete with the pointed nucleus (left) and two female gametes, from a section. Photomicrograph. × 2800.

Fig. 18. Photomicrograph of spores taken from a fresh smear treated with LUGOL's solution. Note the prominence of the hyaline coat at one pole. × 1200.

Fig. 19. Longitudinal section, showing the myoneme fibres tethering the nucleus to the side-walls. Photomicrograph. × 275.

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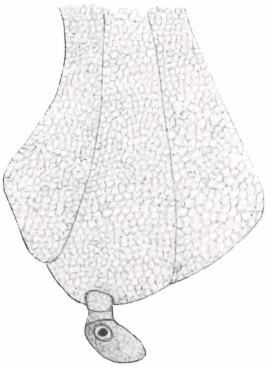

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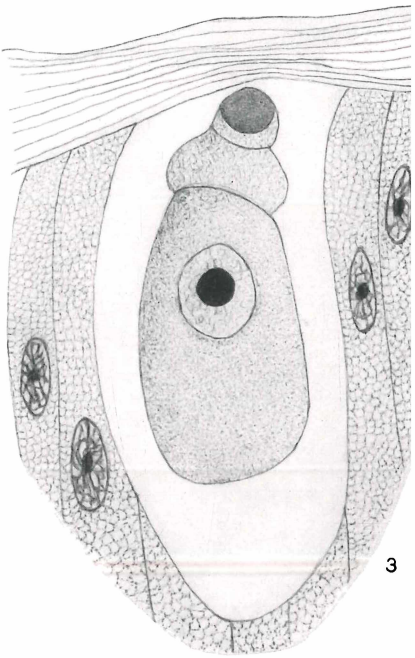

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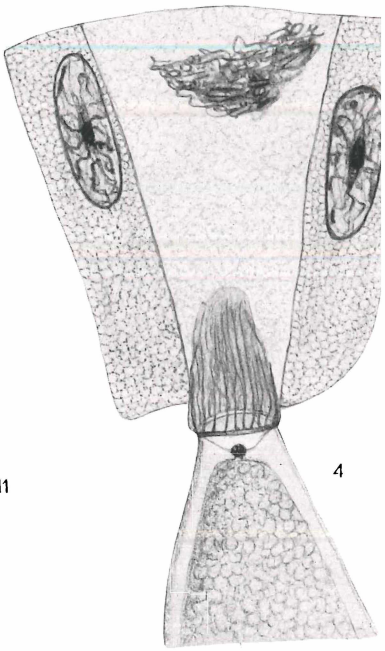

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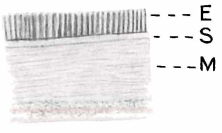

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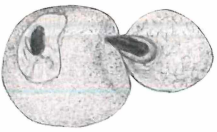

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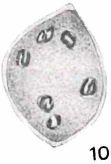

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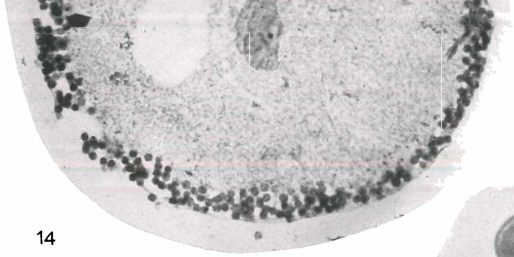

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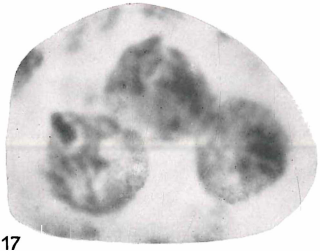

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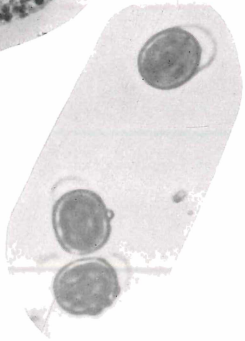

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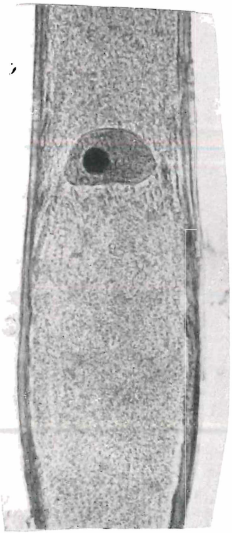

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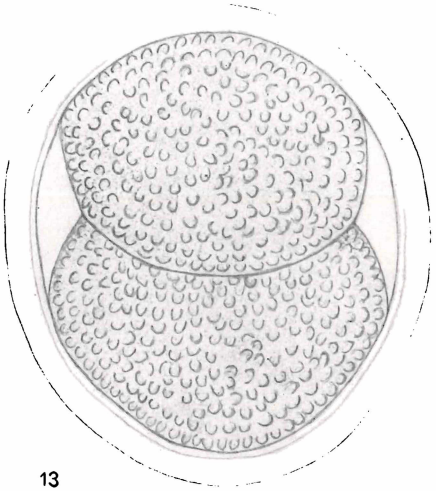

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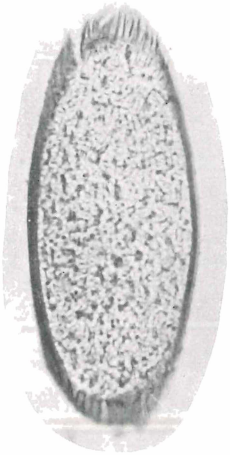

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