

**Observations on the morphology and life history of a cephaline gregarine *Hirmocystis lepropi* n. sp. from the gut of the beetle, *Lepropus* sp.**

D P HALDAR and N CHAKRABORTY

Department of Zoology, Kalyani University, Kalyani 741 235, India

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**Abstract.** The morphology and life history of a cephaline gregarine *Hirmocystis lepropi* n. sp. from the gut of the beetle, *Lepropus* sp. are described. The parasite passes through an intracellular phase of development before reaching the trophozoite stage. The trophozoite has a length of 77.5 to 580.0  $\mu\text{m}$ , and has an epimerite, a protomerite and a deutomerite in its body. The gametocysts are ovoidal bodies and give rise to spores. Each spore measures 6.0  $\times$  4.0  $\mu\text{m}$ .

**Keywords.** Cephaline gregarine ; *Hirmocystis lepropi* ; beetle ; *Lepropus* sp. ; morphology ; life history.

## 1. Introduction

The genus *Hirmocystis* (Family Didymophyidae Léger, 1892) was created by Labbé (1899) for a cephaline gregarine obtained from the gut of *Tipula* sp. (Diptera : Insecta). *Hirmocystis ventricosa* was described as the type species of the genus. Literature available reveals that as many as eighteen species belonging to this genus have so far been reported from different arthropod hosts. Of these, eight are from Indian insects. Uttangi and Desai (1961, 1962) described five species under this genus, all from Indian termites. Haldar and Chakraborty (1979) described *Hirmocystis bengalensis*, *Hirmocystis pitcharis* and *Hirmocystis pseudoductis* from *Myloccerus* sp. 1, *Xanthoprochilus* sp. and *Myloccerus* sp. 2 respectively, all belonging to the order Coleoptera.

The present gregarine from the beetle, *Lepropus* sp. does not resemble any species of *Hirmocystis* described so far from arthropods in all its features and, therefore, is described as a new species in this communication and the name *Hirmocystis lepropi* n. sp. is proposed for it. The type slides of *Hirmocystis lepropi* n. sp. are presently deposited in the Zoology Department, Kalyani University and will later be submitted to the National Collection of the Zoological Survey of India, Calcutta.

## 2. Materials and methods

The materials for the present study consisted of the beetles, *Lepropus* sp. collected from various localities in the University campus at Kalyani. These were brought alive to the laboratory, decapitated, dissected and smears of their midgut contents were fixed in Schaudinn's fluid and subsequently stained with Heidenhain's iron alum-haematoxylin. Sections of the guts fixed in alcoholic Bouin's fluid were also stained as above. Gametocysts were collected from hind guts of infected hosts, placed in cavity slides with 0.5% NaCl solution in petri dishes, at the bottom of which moistened filter-papers were kept. These were examined at regular intervals to observe sporulation.

The ratios used in this paper are the ratio of length of protomerite to total length (LP : TL) and width of protomerite to width of deutomerite (WP : WD). Drawings were made with the aid of a camera lucida.

## 3. Observations

The gregarine passes through an intracellular phase of development before becoming free in the lumen of the host gut. This is, therefore, described first.

### 3.1. Development of the trophozoite

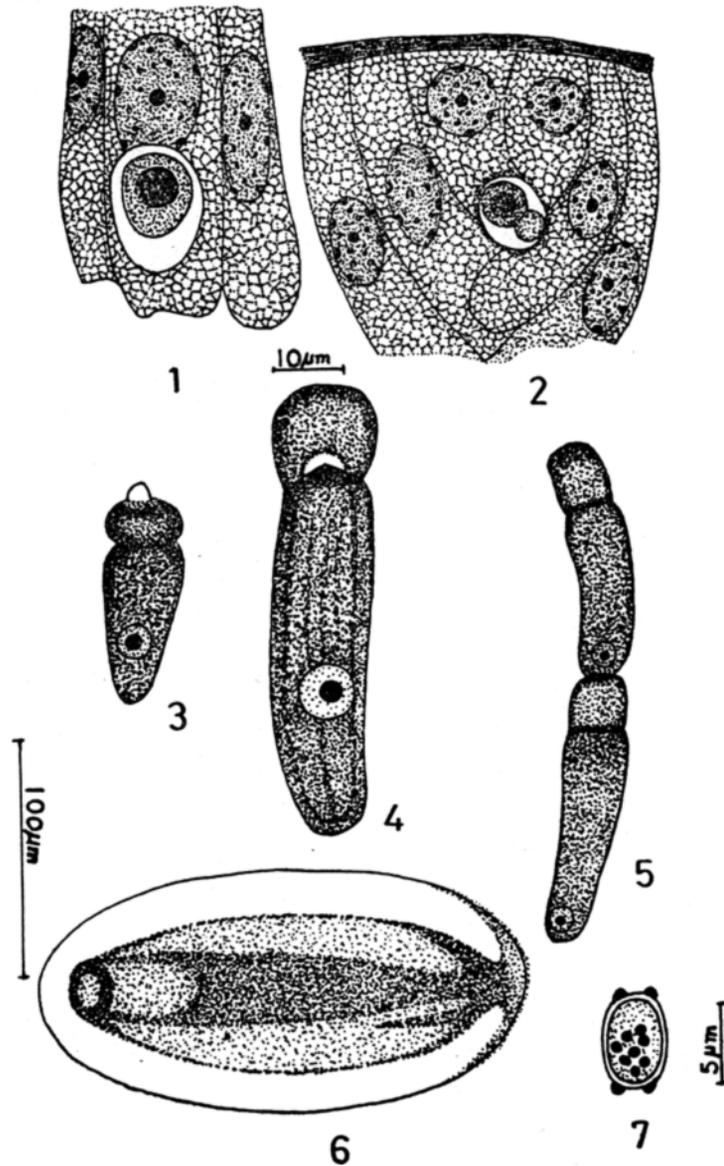
The earliest stage of the gregarine obtained in sections is an ovoidal body,  $8.0\ \mu\text{m}$  in size and has a deeply stained spherical nucleus (figure 1). With the appearance of the septum, the parasite becomes a two-segmented body in which the protomerite measures  $3.0\ \mu\text{m} \times 3.0\ \mu\text{m}$  and the deutomerite measures  $5.0\ \mu\text{m} \times 5.0\ \mu\text{m}$  (figure 2). The spherical nucleus is situated in the deutomerite. Later, an epimerite develops and the young trophozoite leaves its intracellular abode to become free in the gut lumen of its host.

### 3.2. Trophozoite

The trophozoites have a cylindrical body (figure 3). These are found to live freely within the lumen of the midgut of the host. The epimerite is a subspherical hyaline body and measures 5 to  $10\ \mu\text{m}$  in length. The protomerite is ovoidal with a flat anterior end, where the cytoplasmic granules are densely accumulated giving it a banded appearance. The deutomerite is conical and is widest behind the septum. In cross-section it appears circular in outline. The pellicle is uniformly thin and epicyteal striations are wanting. The cytoplasm is finely granulated. The nucleus is situated anywhere within the deutomerite and is spherical in outline. There is a distinct nuclear membrane. The nucleus measures  $12.5\ \mu\text{m}$  in diameter in the average. There is a large endosome, slightly eccentric in position, and the nucleoplasm contains many fine chromatin granules.

### 3.3. Sporadin

These are characterised by their large size, cylindrical outline (figure 4) and a thick pellicle. The cytoplasm is densely granulated. The characters of the nucleus are the same as in the trophozoites. An association of two sporadins is



Figures 1-7. Camera lucida drawings of *Hirmocystis lepropi* n.sp. 1. First intracellular stage ; from a section. 2. Second intra-cellular stage ; from a section. 3. A trophozoite ; from a smear. 4. A mature sporadin ; from a smear. 5. Sporadins in syzygy ; from a smear. 6. A freshly collected gametocyst. 7. A fully developed spore with four knobs and eight sporozoites inside.

regularly observed in smear preparations (figure 5). The attachment between the primite and the satellite is very superficial as these can be easily detached with the help of a needle. Both the primite and the satellite are more or less identical in their body shapes.

### 3.4. Gametocyst and spore

The gametocysts are obtained from the hind gut of the infected hosts. These are white bodies measuring  $360 \times 200 \mu\text{m}$  to  $250 \times 150 \mu\text{m}$  in dimensions. The enclosed gametocytes are of unequal size. Within 12 hr of formation the partition wall between the gametocytes disappears and the cytoplasm within the cyst retracts considerably towards the centre leaving a clear area beneath the cyst wall (figure 6). At 24 hr the cyst bursts by a simple rupture at one end and the spores are liberated in chains. The spores are ovoidal with two coats and have four knobs, two at each pole (figure 7). The knobs are probably made of a mucoid substance (Sprague 1941) and perhaps help the spores attach endwise and come out in chains after the dehiscence of the cyst. The spores measure  $6 \times 4 \mu\text{m}$ . Formation of sporozoites is completed at 90 hr of development. Their arrangement within the spores is irregular.

### 3.5. Measurements

Figures within the parentheses indicate the average of 20 specimens. Length of epimerite—5 to  $10 \mu\text{m}$  ( $8.3 \mu\text{m}$ ); Length of protomerite—17.5 to  $110 \mu\text{m}$  ( $47.3 \mu\text{m}$ ); Length of deutomerite—50 to  $480 \mu\text{m}$  ( $280 \mu\text{m}$ ); Total length—77.5 to  $580 \mu\text{m}$  ( $256 \mu\text{m}$ ); Width of protomerite—22.5 to  $200 \mu\text{m}$  ( $64.2 \mu\text{m}$ ); Width of deutomerite—25 to  $270 \mu\text{m}$  ( $72.8 \mu\text{m}$ ); Diameter of the nucleus—10 to  $40 \mu\text{m}$  ( $22.3 \mu\text{m}$ ).

$$\text{LP} : \text{TL} = 1 : 5.4; \text{WP} : \text{WD} = 1 : 1.3.$$

### 3.6. Seasonal intensity

The insects are commonly found during the months of June to September of the year. 20.4% carry the infection during this period.

### 3.7. Material

Holotype, trophozoite on slide No. F/3/2 from contents of the midgut of the beetle, *Lepropus* sp. collected at Kalyani, West Bengal, India, by N Chakraborty on 20 July 1974. Paratypes, many, on the above-numbered slide as well as other slides; other particulars are the same as for the holotype.

## 4. Discussion

According to Setna and Bhatia (1934), the genus *Hirmocystis* Labbé has features like (i) Sporadins in association of 2 to 12 (or more); (ii) Epimerite conical or cylindrical knob, caducous; (iii) Cysts spherical, dehiscence by simple rupture; and (iv) Spores ovoidal with two coats. The gregarine from the beetle, *Lepropus* sp. described in this paper, has similar features and undoubtedly belongs to the genus *Hirmocystis*. It has close resemblance with *Hirmocystis pitcharis* Haldar and Chakraborty 1979 in the ratio of WP and WD time of dehiscence and size of the spores. However, it differs from the latter in the ratio of LP and TL, shape of the epimerite and protomerite as well as development of the gametocyst. The characters definitely warrant the creation of a new species for it and the name *Hirmocystis lepropi* n. sp. is proposed after the generic name of the host insect.

Table 1. Comparative characters of the four species of *Hirmocystis* Labbé 1899 from Indian beetles.

Characters	<i>Hirmocystis bengalensis</i> Halder and Chakraborty 1979	<i>Hirmocystis pitcharis</i> Halder and Chakraborty 1979	<i>Hirmocystis pseudoductis</i> Halder and Chakraborty 1979	<i>Hirmocystis lepropi</i> n. sp.
Total length	35 to 222.5 $\mu$ m	62.5 to 480 $\mu$ m	65 to 460 $\mu$ m	77.5 to 580 $\mu$ m
Epimerite	Spherical or subspherical papilla; 7.5 to 10 $\mu$ m in length	Broadly papillated with a pointed anterior extremity; 6.2 $\mu$ m in length	Subconical papilla; 10 to 12.5 $\mu$ m in length	Subspherical papilla; 5 to 10 $\mu$ m in length
Protomerite	Hemispherical in trophozoite, subspherical in sporadin	Pitcher-like in trophozoite, hemispherical in sporadin	Ovoidal in trophozoite and globular in sporadin	Ovoidal in trophozoite as well as in sporadin
Sporadin	Biassociative or triassociative	Biassociative	Biassociative	Biassociative
Gametocyst	Egg-shaped with unequal gametocytes; 240 $\times$ 170 to 300 $\times$ 190 $\mu$ m; dehiscence at about 48 hr	Egg-shaped; gametocytes of unequal size; 320 $\times$ 190 $\mu$ m; dehiscence at 24 hr	Egg-shaped with unequal gametocytes; 400 $\times$ 250 $\mu$ m; dehiscence at 48 hr	Oval; 250 $\times$ 150 to 360 $\times$ 200 $\mu$ m; dehiscence at about 24 hr.
Spore	7 $\times$ 5 $\mu$ m; formation of sporozoites 24 hr after dehiscence	6 $\times$ 4 $\mu$ m; formation of sporozoites 48 hr after dehiscence	7 $\times$ 6 $\mu$ m; formation of sporozoites 26 hr after dehiscence	6 $\times$ 4 $\mu$ m; formation of sporozoites 66 hr after dehiscence
LP:TL	1:4.3	1:3.5	1:4.6	1:5.4
WP:WD	1:1.07	1:1.3	1:1.2	1:1.3
Host	<i>Myllocerus</i> sp. 1	<i>Xanthoprochilus</i> sp.	<i>Myllocerus</i> sp. 2	<i>Lepropus</i> sp.

The comparative characters of the four species of *Hirmocystis* described so far from Indian beetles are presented in table 1 to establish the distinctiveness of *Hirmocystis lepropi* n. sp.

Opinions differ as regards the family placement of the genus *Hirmocystis*. Watson (1916) and Kudo (1971) placed it under the family Gregarinidae, while Grassé (1953) created a new family Hirmocystidae for it. Chakravarty (1959), however, regarded the family Hirmocystidae as a synonym of the family Didymophyidae Léger, as "according to the laws of priority in nomenclature the first family name shall stand, as it is formed from a valid generic name". Sarkar and Haldar (1979) have accepted the opinion of Chakravarty (1959) and the same system of classification has been followed here.

### 5. Diagnosis

*Hirmocystis lepropi* n. sp. described from the beetle, *Lepropus* sp., collected at Kalyani, West Bengal, India, has intracellular development. Trophozoite with a total length of 77.5 to 580  $\mu\text{m}$ . Sporadins are in association. Gametocysts ovoidal. Dehiscence by simple rupture. Spores measure  $6 \times 4 \mu\text{m}$ , with two coats and four knobs.

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