(From the Department of Zoology, Calcutta University.)

Studies on Sporozoa from Indian millipedes.

I. *Stenophora khagendrae* n. sp., with a Note on its Method of Progression.

By

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(With 9 figures in the text.)

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1. Introduction.

In December 1930 I was struck by the richness with which the common millipedes of Calcutta are infected with gregarines. This has induced me to make a systematic survey and at the same time work out the life-cycle in some detail of certain of these parasites.

Out of thirty species of *Stenophora*, the cysts and spores of only six have been recorded, and *S. lacteria* Watson (1917) is the only species of which the life-history is known in some detail. The reason for this probably is that the laborious task of examining the excreta for cysts has seldom been undertaken.

In this article I have based my observations on a *Stenophora* from *Zikadesmus* sp., a millipede belonging to the family Cresptodesmidae [1]), usually found in damp places under stones and bricks or old masonry. It will be clear from what follows that my species differs from all those described hitherto under this genus. I shall therefore call it *Stenophora khagendrae* n. sp.

I have also studied the method of progression of this species under normal conditions, and have ample proof that some sort of mucus is secreted from the body, which, together with the contraction of myonemes, helps the organism to move forward.

2. Material and Methods.

Throughout the year I was supplied with these millipedes by my friend Mr. KHAGENDRA nath DAS [2]), to whom I am deeply indebted. As soon as they were received in the laboratory, they were transferred to petri dishes, which were kept moist by placing a wet blotting-pad at the bottom. Here the millipedes were kept for weeks without any mortality.

The entire alimentary canal of the animal was taken out, placed on a slide, mounted in saline and examined under slight pressure from a cover-glass. Observations were made on living gregarines taken from the infected gut and placed in drops of saline.

Smears were fixed in SCHAUDINN'S fluid and in BRASIL'S modification of BOUIN-DUBOSCQ'S fluid, and stained in DELAFIELD'S or HEIDENHAIN'S haematoxylin. Smears, after fixation in the second of these, were also stained in DOBELL'S modification of MANN'S methyl-blue-eosin. In order to see if there were any intracellular stages, the entire alimentary canals of infected specimens were fixed in BRASIL-BOUIN-DUBOSCQ for twenty-four hours and ultimately cut into sections 4 μ thick, which were stained with HEIDENHAIN'S haematoxylin. Faeces deposited by the millipedes on the blotting-pad were examined at regular intervals of twelve hours. Most of the cysts I came across were those which appeared to me to have just formed. These were picked up by means of a micro-pipette and transferred to a moist chamber. Cysts were thus kept under

[1]) I sent this millipede to Dr. ATTEMS in Vienna, and received the following reply from him: "The millipedes sent in January belong to a new species and probably to a new genus related to *Zikadesmus* CHAMB. (Fam. Cresptodesmidae, Polydesmoidea), and I will describe them when opportunity comes". So until the creation of the new genus I must refer to this myriapod as *Zikadesmus* sp.

[2]) I have named this species of *Stenophora* after him.

observation in the living condition and watched at the end of every sixth hour. This experiment was repeated a number of times, and it was found that, within three to five days after defaecation, mature spores were formed. A few which did not develop spores soon showed signs of degeneration. Camera lucida drawings of gametocysts, zygotes and mature spores were made from living material.

The medium used for studying the method of progression was normal saline, with a fine carmine suspension in it where necessary.

3. Morphology of *Stenophora khagendrae.*

The spherical gametocysts, 100—123 μ in diameter, dehisce by simple rupture and release numerous spindle-shaped spores, measuring 10.25×4 μ, each containing eight sickle-shaped sporozoites. In sections of the proventriculus, I have found this infective stage. At one end of a sporozoite, which measures 6×2 μ, is seen a darkly staining area (Fig. 1 a) which I take to be the precursor of the epimerite, because later I have found the organism attached to the epithelium by this end. The sporozoite gradually penetrates the epithelial cell and journeys past the nucleus while maintaining the original dimensions (Fig. 1 b, c, d). In this respect its behaviour agrees with that of *S. lacteria* Watson. The nucleus of the young parasite is oval and possesses a karyosome: the nucleoplasm at this stage does not show any granular structure and appears colourless in a stained preparation. The gregarine now begins to grow, and its body becomes differentiated into epimerite, protomerite and deutomerite [1]). The earliest stage which shows this differentiation into segments measures 10×6 μ (Fig. 1 d). As growth proceeds, i. e. when the trophozoites range between 10—30 μ in length and 6—16 μ in breadth, the cytoplasm of the protomerite becomes densely granular, while the epi-

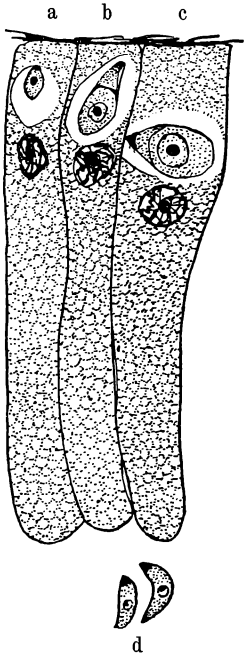

Fig. 1.

[1]) In no stage of *S. lacteria* has an epimerite been observed by Watson (1917). As a matter of fact, she has definitely declared that this is a species without epimerite (although in her earlier communication (1916), mention of an epimerite was made).

merite remains as a hyaline, conical structure. With further growth the cytoplasm of the deutomerite also begins to show dense granules (Fig. 2). The epimerite now appears as a hyaline, rounded elevation.

Usually when a trophozoite has attained dimensions of $30 \times 10\ \mu$, the outer end of the infected cell gives way: the organism appears to remain attached to the cell by its epimerite and continues to grow until it reaches sexual maturity. Having then lost its hold on the gut wall, it remains floating in the lumen. A full-grown sporont measures $225 \times 56\ \mu$, the broadest part being a short way behind the septum between protomerite and deutomerite: the epimerite has disappeared, as a rule. The protomerite is slightly broader than long, and measures $28 \times 37\ \mu$ (Fig. 3).

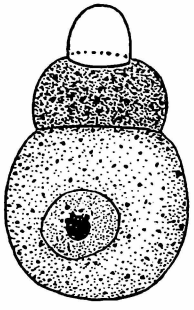

Fig. 2.

The epicyte is about $2\ \mu$ thick: the striations on it are very fine, about $2\ \mu$ high, and are discernible only on very close examination. In a tangential section of the organism, the disposition of the longitudinal and circular myonemes is seen very clearly (Fig. 4).

Fig. 3.

Fig. 4.

The longitudinal myonemes appear beaded: the circular myonemes are smooth and stain less deeply. I could see no trace of myoneme canals such as ROSKIN and LEVINSON (1929) describe for *Monocystis* and *Nematocystis*. The endocyte, which was transparent at an earlier stage, becomes very opaque and densely granular as the sporont grows. It appears white under reflected light. The nucleus is spherical. In a full-grown sporont it has a diameter of $16\ \mu$, and contains a large spherical karyosome, $8\ \mu$ in diameter. At the approach of association, the nucleus becomes compressed and spindle-shaped and the karyosome oval (Fig. 5). As stated above, the nucleus of the young parasite contains no stainable granules other than the karyosome. But I find that, as it grows, the karyosome presents a series of pictures in my preparations which suggest that karyosomic material oozes out, later appearing as granules in the nucleoplasm (Fig. 6, 7 and 8). In smears stained with MANN'S methyl-blue-eosin, the karyosome stains deep pink. What is its ultimate fate I do not know.

How the sporonts attach themselves in pairs, I have, unfortunately, not been able to observe. The earliest stage in the sexual phase that I have come across is a gametocyst shown in Fig. 5. If such a cyst is kept in a moist chamber, small protuberances appear on the periphery of each gametocyte at the end of the third day, and at the end of the fifth day mature spores can be obtained by bursting the cyst. I did not have enough material to enable me to cut sections for detailed study of the cyst contents prior to spore-formation, but I hope to be able to do so in course of time.

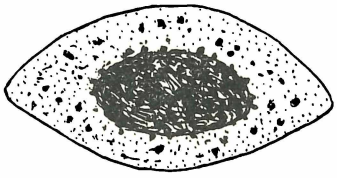

Fig. 6.

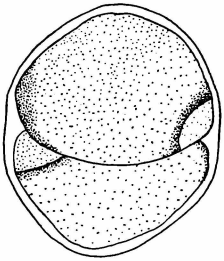

Fig. 5.

The ripe spores are spindle-shaped and measure about $10 \times 4\ \mu$. I could not observe the equatorial line upon them such as has been described for the spores of Stenophoridae. They are released by rupture of the cyst, and are found lying in the debris, where they are swallowed by the gregarious millipedes. It was a great relief to find that there was no other protozoan parasite to deal with in this case: out of the fifty millipedes examined, thirty were found to be infected with one and the same form.

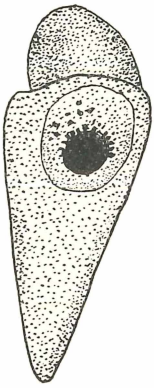

Fig. 8.

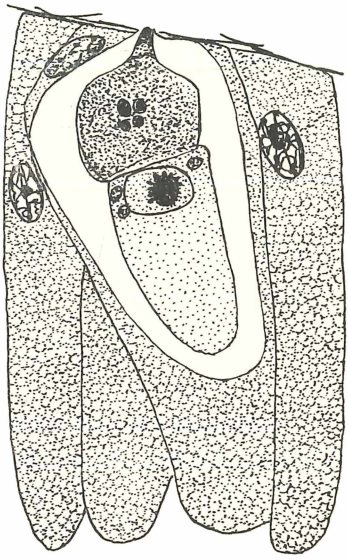

Fig. 7.

The features characterising *Stenophora khagendrae*, then, may be summed up as follows:

Sporonts solitary, "parrot-shaped". Length $225 \times 56\ \mu$. Epimerite hyaline and dome-shaped. Protomerite rounded at the anterior end and flattened at the septum; slightly

broader than long. Deutomerite (eight times as long as the protomerite and half as broad again) broadest slightly behind the septum and gracefully tapering toward the posterior end. Nucleus spherical. Gametocyst sherical 100—123 μ in diameter, dehiscing by simple rupture to release spindle-shaped spores measuring $10.25 \times 4\,\mu$. An intracellular stage is present. Habitat: intestine of a millipede, *Zikadesmus* sp., from Calcutta, India.

4. Movement.

Here I would say something about the movements of this *Stenophora*; but, before I do so, I must briefly summarise what other authors have noted, for there is much divergence of opinion as to what is responsible for movement in gregarines. I would explain, however, that I made my own observations and came to my own conclusions before referring to the literature on this subject.

SCHEWIAKOFF (1894), from his studies on *Clepsidrina munieri*, decided that a slimy substance was secreted from the body of the gregarine, which hardened in the surrounding medium and pushed the organism from behind forwards; and he held that the circular myonemes did not take any part in the progression.

SOKOLOW (1912), although he agrees in the main with SCHEWIAKOFF, apparently failed to find any hardening of the secreted slime, and says that movement is due to its forceful expulsion from the body.

PORTER (1897 p. 13), from his studies on a gregarine from *Rhyncobolus americans*, gave quite a different explanation of the movement of translation. He describes it as follows: "It is a very slow movement of translation in a straight line' without any apparent contraction of the walls of the body. It is probably caused by a very slight undulatory motion of the under surface of the animal".

CRAWLEY (1902, 1905), unaware of PORTER's work, came to the same conclusion from his observations on *Stenophora juli* and *Echinomera hispida*. He denies that slime has anything to do with the forward movement and thinks that the progression is due to continuous contraction of the circular myonemes.

ROSKIN and LEVINSON (1929) in their study of Nematocystis sp., *Monocystis agilis*, *Selenidium mesnili* [1]), and *Polycystis* sp., say that in

[1]) I have studied *Selenidium mesnili* at Plymouth, and I feel reasonably certain (RAY, 1930) that ROSKIN and LEVINSON were not dealing with that schizogregarine

these gregarines no slime is extruded, and that contraction of the myonemes is responsible for the progression. Other authors such as LEIDY (1853), LÜHE (1904), PAEHLER (1904), SCHELLACK (1907), DOGIEL (1907), VOSS (1922), BERLIN (1924) COGNETTI DI MARTIIS (1927) and others, belong to the Porter school and consider that myonemes are responsible for the movements of translation.

WATSON (1911 p. 24), from detailed study of *Leidyana erratica*, has made a compromise between the two antagonistic views, by suggesting that "the gregarine moves forward by imperceptible vertical movements in the myonemes on that side of the body which happens to be ventral at the time, friction being produced with the under surface by exudation of mucus from the body". She compares this movement with the locomotion in Limax. She also describes the mucus thread as continuous and says that the gregarines sometimes get entangled there and ultimately perish in trying to escape.

The movements in my *Stenophora* are of two kinds (1) "active" movements, where pocket-like outgrowths of the body are formed just behind the septum, and (2) "passive" movements of translation. These two sorts of movements may take place singly or together.

A fresh cover-glass preparation in saline shows a sporont either to glide slowly on the substratum or else try to bend its head from side to side. This bowing movement is brought about by a pocket-like extension of the epicyte of the deutomerite just below the septum (as has already been noted by WATSON for *Leidyana erratica* and *Stenophora lacteria*, and by SOKOLOW for *Stenophora juli*, — see his figs. 1 and 2). Cytoplasmic granules are seen to travel towards this pocket, gradually fill it up, and then, by a sudden jerk, the cytoplasm pushes the protomerite up which was temporarily sunk in this pocket. The pocket-like outgrowth of the deutomerite may originate from any part round the septum, but, under normal conditions, never simultaneously at two or three places.

But it is the "passive" movement which is of greater interest, and it is round this point that controversy has chiefly centred. I do not profess to have cleared the matter up — far from it — and must content myself with setting down my unbiassed observations.

About half-a-dozen specimens of *Stenophora* were put in a drop of saline and examined under dark-ground illumination with the aid of an oil-immersion lens. No slimy exudation was observed. Next time, instead of pure saline a carmine suspension was used. (The carmine should not be powdered too fine, as it then exhibits Brownian movement in the aqueous medium). Now, as the organism moves

forwards, the carmine particles collect round it, especially on that surface which happens to touch the slide (Fig. 9). Watching a carmine granule carefully, one see that it passes backwards along the epicyteal grooves, rolling with sudden jerks, until it comes to lie at the extreme posterior end of the gregarine. My first impression was that some of the carmine accidentally got in the grooves and was just washed backwards with the fluid around the organism as it moved forward in the saline. But I soon noticed that the particles carried to the posterior end did not fall off there, but stuck together and were dragged along by means of some invisible sticky substances which must have been secreted from the body of the moving creature. This sticky substance, or mucus, certainly did not dissolve at once in the saline, for one could see the particles of carmine trailing behind a gregarine for some time. This mucus tail apparently often snapped, for frequently carmine particles, when gathered into a mas, were seen to drop off the body and knock about erratically for a while, in a way suggesting that the mucus tail was drawn out by the weight of the dragging carmine mass until it could bear the strain no longer. The result was that the mucus trailed off here and there, scattering the carmine particles irregularly. These were always found to be in groups of two or more, which indicated clearly that there was a mucus coating round them, which bound them together even after they were severed from the main thread. The impression, then, that I have so far obtained concerning the method if translation of this *Stenophora*, agrees closely with the account of *Stenophora juli* given by SOKOLOW (1912) and with PORTER'S views (1897). Had the method of progression been such as SCHEWIAKOFF described, the carmine particles would never been dragged along the organism, but would have been left in succession on the hardened portion of the mucus tail. Moreover, I noticed at times that even when the gregarine was stationary, the carmine particles kept sliding down the grooves just the same. I have no doubt that secretion of some kind of mucus or slime goes on from all over the body of this *Stenophora* [1]). But while this secretion is continuing, I could also see a wave of

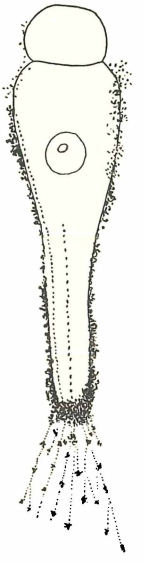

Fig. 9.

[1]) WATSON (1916) failed to discover any gelatinous stalk in *S. lacteria*.

contraction running down the body from before backwards: this contraction becomes more conspicuous when the movement is very rapid.

From what has been said it must be clear that my observations are in strict conformity with WATSON's (1916), except that I have not been able to demonstrate the continuity of the mucus tail, as she has done in *Leidyana erratica* (see her Fig. 236). Later on, I hope to be in a position to say more concerning the actual process of exudation of the mucus; but before doing so, I wish to make an exhaustive study of the various species of *Stenophora* at my disposal.

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