ON THREE NEW GREGARINES, *BHATIELLA MORPHYSAE*, n. g., n. sp., *FERRARIA CORNUCEPHALI*, n. g., n. sp., AND *EXTREMOCYSTIS DENDROSTOMI*, n. g., n. sp., FROM INDIAN POLYCHAETES.

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(Plates V, VI.)

In the following pages I have placed on record certain observations that were made by me during the past summer on some new genera of protozoon parasites. While on a short tour to Port Blair in the Andamans in connection with fishery work (1930), I also carried out observations on the intestinal protozoa of two species of polychaetes, which I found to be infected with the parasites described herein. Owing, unfortunately, to the occurrence of two gregarines in the same host, two different kinds of cysts and spores were encountered very near to each other in the same smear. I shall refer to the sporocysts and the spores under a separate heading as I am not at present able to associate satisfactorily the sporocysts with the adult stages, and so cannot definitely establish the life-cycle. From the known characters of the adult trophozoites, however, I have been able to establish two new genera. I am unaware of any previous observations regarding the occurrence of the gregarines in the hosts in question.

Bhatiella morphysae, nov. gen., nov. spec.

(Pl. V, figs. 1-2.)

Host.—*Morphysa sanguinea* Montague (=*M. furcellata* Crossland).
Habitat.—Mid-gut.
Location.—Port Blair, Andamans.

This form, together with the next species, occurred in the intestine of *Morphysa sanguinea* and is apparently an extremely common intestinal parasite.

Several specimens of this remarkable parasite were obtained and were studied both in the living condition and in permanent preparations. The total length of the trophozoite is variable, from quite young to adult specimens being found together. The full-grown trophozoite measured 200μ from base to tip and 103μ accross its greatest width. The smallest specimen observed was 100μ in length and 40μ in breadth and differed from the full grown form in size only. The trophozoites are solitary and rather small. The body is generally pear-shaped, being widest at the base and tapering towards the apex. The average ratio of length of body to total length of trophozoite is about $\frac{3}{4}$: 1. The characteristic feature of this species is the epimerite and the absence of segmentation in the trophozoite. The parasites, during the time that I was examining them on the slides, did not betray any sign of activity.

The well marked epimerite in this species is of rather unusual structure. It consists of two parts, a long slender deeply-staining style and a small bulb which is borne at the apex of the style. The style is narrowed at the apex, but broad at the base. Its total length is one-fourth the length of the animal.

There is no clear differentiation of the protoplasm. The endoplasm is highly vacuolated, the largest vacuole being 6μ in diameter. The nucleus is ellipsoidal and cannot be detected in the living specimen. It lies in the middle of the body, in the lower half. There is one large (*i.e.*, large in comparison with the size of the nucleus) karysome present, which is slightly eccentric in position. The accompanying camera lucida drawings show the general structure of the trophozoite (Plate V, fig. 1). In Plate V, fig. 2 the parasite is represented without the epimerite.

Measurements in microns were made from specimens taken at random.

Number.	Entire length.	Length of body.	Length of Epimerite.	Width of body.	Nuclear diameter.	Karysome diameter.
1	123	85	38	40	27×22½	Not visible.
2	157	126	31	67	21×18	10×10
3	160	128	32	64	19×16	12×12
4	171	130	41	67	20×15	10×10
5	180	150	30	80	17×16	12½×12½
6	220	180	40	103	22×18	10×10

Systematic position.

After a careful comparison with the accounts of all the species hitherto figured and described, I am unable to refer the present species to any known genus.

The important features of this gregarine are as follows :—Solitary ; non-septate ; pear-shaped body widest just posterior to middle. Epimerite in the form of a distinct bulb-like structure at the extreme tip of a long rigid style. Dehiscence by simple rupture, no sporoducts. Present in the digestive tract of a polychaete.

The characters enumerated above seem to be sufficient to place the gregarine in the family Lecudinidae Kamm (nov. nom. for Doliocystidae Labbe), which includes non-septate gregarines inhabiting the digestive tract of polychaetes.

The present specimens differ materially from others and are so sufficiently distinct as to warrant one in concluding that they represent a new genus. I have great pleasure in associating this genus with the name of my teacher, Prof. B. L. Bhatia, as a token of gratitude for the great help and suggestions that I have received from him.

Ferraria cornucephali, nov. gen., nov. spec.

(Pl. V, figs. 3, 4, 5 ; Pl. VI, fig. 1.)

Host.—*Morphysa sanguinea* Montagu (=*M. furcellata* Crossland).
Habitat.—Mid-gut.
Location.—Port Blair, Andamans.

This parasite, along with the one described in the preceding pages, was found abundantly in the same host. The trophozoites with epimerites are quite commonly attached to the intestinal wall or lie free in the lumen of the gut.

Trophozoites solitary, never associated, rather stout bodied. Length of the largest trophozoite 300μ and 91μ in width. Ratio length of protomerite : total length of trophozoite 1 : 4 ; width of protomerite : width of deutomerite 1 : 1·6.

The protomerite is hemispherical to sub-globular in shape ; there is no constriction at the septum, the position of which is indicated in the adult trophozoite by a clear area between the protoplasm of the protomerite and deutomerite ; the protomerite is widest in the region of the septum.

The deutomerite is very elongated, cylindrical and ovoidal ; it is widest about the middle and is well rounded posteriorly (Plate V, fig. 3).

The nucleus is large and spherical with a single large central karyosome.

The epimerite (Plate V, fig. 4) is characteristic and consists of a wide-mouthed, funnel-like structure on a long slender tubular stalk. It is transparent and measures 18μ in length.

The protoplasm of the trophozoite is dark and finely granular and not very dense in either the protomerite or the deutomerite. The epicytal striations are thin and visible under high powers only.

Measurements in microns.

Number.	Length of Trophozoite.	Breadth of Trophozoite.	Epimerite.	Protomerite.	Deutomerite.	Nucleus.	Karyosome.
1	243	81	13	54	176	23×22	9×9
2	263	90	16	67	180	27×27	9×9
3	265	90	13	54	198	29×27	11×11
4	297	90	24	58	215	31×31	11×11

Systematic position.

As the complete life-history has not been worked out, the genus is assigned tentatively to the family Polyrhabdinidae. It has several characters in common with the other genera of the family, *e.g.*, polycistid nature of the body and its occurrence in the digestive tract of a polychaete.

The form here described cannot be placed in any of the genera, of which up to the present three are reported. The cysts and spores of all three genera are still unknown.

The gregarine in question differs from those previously recorded in several points. There is a very marked difference in the size and shape of the trophozoite and in the character and structure of the epimerite. A new genus is therefore created to contain the species from *Morphysa sanguinea* Montagu.

Cysts and spores.

Two distinct types of cysts and spores were encountered in the mid-gut of the worm (*Morphysa sanguinea* Montagu). I am not able to associate satisfactorily the cysts and spores with the adult stages. One type is represented by spherical cysts, which measure between 90μ and 100μ in diameter. These cysts are full of oval spores which measure 10μ in length and 4½μ across (Plate V, fig. 5). The spores escape by rupture of the cysts.

The other type of cyst that I encountered in the same smear was single, but unfortunately it was damaged. I, however, have no doubt that this too is spherical. It contained a mass of very peculiar spores. Nothing similar to these has been recorded before. The spores are peculiarly shaped with rounded anterior and posterior ends. Each spore is bilaterally symmetrical and is covered by a membrane, the outer boundaries of which are thick and stain deeply with iron haematoxylin.

The spores are 20μ long and 4μ broad. They have a large well defined nucleus (Plate VI, fig. 1).

Extremocystis dendrostomi, nov. gen., nov. spec.

(Pl. VI, figs. 2-6.)

Host.—*Dendrostoma signifer* Sel and de Man.

Habitat.—Coelom.

Location.—Port Blair, Andamans.

This parasite was discovered inhabiting the various portions of the coelomic cavity of *Dendrostoma singnifer*. In each smear fixed in Bouin's fluid and stained with iron haematoxylin, the parasites lie coiled and contorted among the coelomic corpuscles and the hosts genital products. I have never come across any forms attached to the coelomic epithelium of either the body-wall or the gut, nor were any cysts in evidence in this situation. The parasites revealed striking uniformity, both as regards size and general structure. An important feature of the parasite is that the adult trophozoites are always associated in pairs, the association being between equal-sized individuals and attachment being end to end. This phenomenon is a constant and not an occasional characteristic (Plate VI, fig. 2).

The body of the trophozoites is cylindrical and essentially resembles that of an elongated nematode worm with more or less parallel sides and with a tendency to taper slightly towards the two extremities. There is no difference between the two ends of a free individual (such free individuals are extremely rare) : the end that is anterior at one time becomes posterior at the next. Attachment takes place by means of one tapering

end of an individual fitting into a regular concave hemispherical depression in the other (Plate VI, fig. 3). It may be mentioned that the distinct cup-like depression seems somewhat similar to the cup described in *Ganymedes anaspides* by Huxley (1910). There is, however, nothing equivalent to the ball-end and I therefore cannot regard the union as a ball-and-socket joint.

The range in length is from 100μ to 130μ and in width from 17μ to 19μ. The length of the smallest specimen observed was 50μ and there was no difference except that of size between these and the adults. The ratio of width to length in each individual is 1 : 6.

The ectoplasm is not clearly differentiated from the endoplasm. The endoplasm has a coarsely granular and light coloured structure and the stream of endoplasmic granules of the two individuals flows very swiftly and uniformly from one end of the parasite to the other, going backwards and forwards in a straight line. In the course of these movements, the body of the parasite alternately lengthens and shortens, being at the same time narrowed and broadened respectively. The ectoplasm is thin and does not show myoneme striations.

In each trophozoite there is an ellipsoidal and very greatly elongated nucleus, which lies more or less near the united ends. The nucleus measures 17μ by 6μ and lies with its long axis parallel or slightly inclined to the sides of the body. The presence of a definite nuclear membrane was demonstrated in several specimens ; in others it is indistinct and not visible, due probably to some action of the fixative. The nucleus contains one large eccentrically placed karyosome with a varying number of deeply-staining granules.

The associating pairs become short and pear-shaped. They undergo complete cytoplasmic fusion and during my observations I encountered hundreds of these pear-shaped pairs, as also pairs which had succeeded in rounding themselves of (Plate VI, fig. 5), but in no case was a cyst with spores encountered. The attachment at this stage did not seem to be very secure for I came across solitary individuals both pear-shaped and rounded, which had become detached in course of preparation of the smear. I am not sure whether the time of the year has any effect on the processes of the life of the parasite. Since the trophozoites occur associated with the genital products of the host, it seems reasonable to infer that the cysts and the spores escape with the genital products when the discharge of ova and sperms takes place ; and the formation of cysts and spores perhaps also takes place at that stage.

I came across a few spores floating in the coelomic cavity. They are spindle-shaped structures with the two ends finely pointed and the nucleus of the spore has the form of an aggregation of granules which stain deeply.

Length of the spore 29μ—35μ.

Width of the spore 6·4μ.

Nucleus of the spore 7·2μ×4·8μ.

Diagnostic characters.

Gregarine parasites found floating in the coelom of *Dendrostoma* (a polychaete). No organ for attachment to the cells of the host. Adult

trophozoites elongated, nematocystis-like, always associated in pairs; attachment being end to end, by one end of an individual fitting into a concave depression of the other, thus effecting a very close union of two individuals.

The dimensions of the trophozoites in microns are as follows :—

Pair Number.	Length of Trophozoite.	Breadth of Trophozoite.	Nuclear diameter.	Karyosome diameter.	Length of Trophozoite.	Breadth of Trophozoite.	Nuclear diameter.	Karyosome diameter.
1	70	14	16×6	4×4	80	14	16×7	4×4
2	100	15	17×6	4×4	73	12	16×6	4×4
3	102	16	19×6	4×4	103	16	19×6	4×4
4	128	19	Not visible	Not visible	118	16	20×7	4½×4½

Systematic position.

In order to determine the systematic position of this parasite, the following genera have to be considered : namely, *Nematocystis* Hesse (1909) and *Monocystis* Stein (1848) of the family Monocystidae and the genera *Zygocystis* Stein (1848) and *Pleurocystis* Hesse (1909) of the family Zygocystidae.

It will be noted that the parasite of *Dendrostoma* in point of general outline, shape of the nuclei and movement of the endoplasmic granules resembles very much a *Nematocystis*. Each individual of the pair of parasites has all the morphological characters of this genus, but the genus as described by its author has certain other well defined characters which preclude us from placing the present parasite in it. The parasites also can not be referred to either of the two genera of the family Zygocystidae. It resembles them so far as the habit of the parasite to occur in pairs is concerned. It should, however, be noted that these genera are only described from Oligochaetes and the host in the present instance is a Polychaete.

This new form is of interest as showing a remarkable combination of characters exhibited by the genus *Nematocystis* of the family Monocystidae and the genera *Zygocystis* and *Pleurocystis* of the family Zygocystidae. Under the above mentioned circumstances there are two possibilities open to me : (i) that a new genus should be established for the species or (ii) that the definition of either of the genera *Zygocystis* or *Pleurocystis* should be modified in such a way that it should include forms like the one here described. The latter alternative seems uncalled for, for in the case of the gregarines from the Oligochaetes the classification, in part at least, is based on shape or body-form. In the two genera of the family Zygocystidae, referred to above, emphasis has been laid on the mode of union, *i.e.*, the manner of adhering together in the adult condition. In one case this association is end to end and includes spherical as well as pyriform trophozoites. In the other, association is side to side and includes trophozoites which are like *Nematocystis*. The present question has regard to the classification of *Nematocystis*-like forms that are associated end to end.

Adopting the shape of the body as a distinctive criterion, Cognetti, it will be recalled, recently established a new genus *Apolocystis* by restricting the use of the term *Monocystis* to pyriform individuals only. Spherical forms, which formerly were also known as *Monocystis*, have now been relegated to the genus *Apolocystis*. Cognetti's division of these different forms into separate genera extends only to solitary forms, and it would seem desirable that a similar distinction should be drawn between the forms belonging to the family Zygocystidae. Here, as in the case of the solitary forms, on the basis of the characteristic shape of the body there is an indication of natural classification. I am of opinion that the family Zygocystidae should be classified thus :—

Zygocystis Stein.

This should include only those forms in which the adult trophozoites are permanently associated in pairs or groups of three ; spores biconical, etc. To this genus would then belong forms like *Z. cometa* and *Z. pilosa*, which otherwise agree in the main with the characters of the single forms of the genus *Apolocystis* Cognetti.

A separate genus should, I think, be established to include forms which are pyriform or oval in shape and which again in the adult condition are permanently associated in pairs. To this genus would belong forms like *Z. legeri*. This genus in the main characters of the individual would be similar to the genus *Monocystis* Stein.

Pleurocystis Hesse.

Trophozoites which in the adult condition are permanently associated in pairs ; association longitudinal and lateral, etc.

Extremocystis, nov. gen.

Lastly a new genus, for which I propose the name *Extremocystis*, should be created to include the species *dendrostomi*. This genus will include nematode-like forms associating end to end, its representatives agreeing in the main with the characters of the single individuals of Hesse's genus *Nematocystis*.

The greater part of this work was carried out in the Fisheries Laboratory, at Port Blair in the Andamans. My thanks are due to Col. M. L. Ferrar, Chief Commissioner, for facilities which I have enjoyed, without whose help the work could not have been undertaken ; to Lieut-Col. R. B. S. Sewell, Director, Zoological Survey of India for reading the manuscript of this paper and for suggestions ; and to Dr. P. Fauvel for kindly identifying the hosts.

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