STUDIES ON SOME PARASITIC PROTOZOA IN SOME INVERTEBRATES IN DARJEELING

[GREGARINES IN EARTHWORMS]

DEVIKA PRADHAN
DARJEELING GOVERNMENT COLLEGE
DARJEELING
WEST BENGAL (INDIA)

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (SCIENCE)
OF THE UNIVERSITY OF NORTH BENGAL

1980

AN EXPLANATORY NOTE REGARDING THE TITLE OF THE WORK.

At the outset (1975) I had intended to work on the parasitic protozoa of some invertebrates in Darjeeling. As the work progressed, it became increasingly apparent that a full account of all parasites of invertebrates would result in an edition which would be too voluminous and which would involve a far greater period of time.

In view of practical considerations, the present volume has been limited to a study of one particular group of protozoa i.e. the gregarines infecting just one member of the invertebrates *i. e.* earthworms.

Devika Pradhan.

CONTENTS

A C K N O W L E D G E M E N T S

In the preparation of the thesis, I must earnestly recall the help and encouragement I received from my teachers, friends and benefactors. In particular, I would like to mention Dr. B. Dasgupta, Principal, Darjeeling Government College, to whom I am most indebted, for the meticulous and painstaking supervision of the thesis. I had the benefit of his wise criticisms, able and kind guidance. Without his active encouragement I would not have completed the thesis. I owe the most to him.

I have a deep sense of gratitude to Dr. Kanaleah Chatterjee, Reader, North Eastern Hill University, Shillong, who encouraged me to undertake research in this field.

I am indebted to Dr. S.K. Dasgupta, who is at present at Presidency College, Calcutta, for giving me timely access to the laboratory at Government College, Darjeeling, and also other facilities he so gladly made available to me.

I have sincere appreciation of the help given to me by Dr. T.D. Soota, of the Zoological Survey of India, Calcutta, who kindly identified the host earthworms.

Grateful acknowledgement is made to Dr. J. Theodorides, of Laboratoire d ' Evolution, Paris, France, for giving me useful suggestions as far as the chapter on hyperparasitisation is concerned.

I must devote some space for thanks to all my friends and well wishers from whom I have benefitted in one way or the other. Here I must mention my friend and colleague Mr.C.K. Sinha, whose keen interest and encouragement I very much appreciate.

Dr. B.C. Nandi is also remembered for the help he has offered.

Last but not the least, it is the co- ordinated effort of many others who share my commitments to higher learning.

S Y N O P S I S

The problem dealt with in the course of the present investigation is entitled ' Studies on some parasitic protozoa in some invertebrates in Darjeeling'. The work is divided into four parts.

Part I includes the description of seventeen acephaline gregarines new to science, including two proposed new genera, along with their photographs and figures. Life cycles of three of these gregarines have been worked out and Pheretima californica is recorded for the first time in India.

Part II deals with the cytochemical studies on some of the gregarines, mentioned in Part I of the thesis. It has been noticed that the nuclei of all the gregarines studied showed a negative response to the Feulgen technique, though the presence of DNA could be shown in the nuclei by Fluorescence microscopy.

In Nematocystis n.sp.(a), when stained with periodic/acid Schiff method, there was either a complete absence of paraglycogen granules or, if present, these were very few in number.

Part III includes cases of gregarines being parasitised by organisms apparently of microbial nature. It is shown that such cases of hyperparasitisation, may lead to changes in the staining characteristics of the host gregarines, finally leading to their death, in some instances. Intracellular bacteria apparently of harmless nature are also reported.

Part IV records that a heavy concentration of gregarines occurring in the coelomic fluid of Apporectodea trapezoides may lead to the death of the host.

Growth of a new species of Apolocystis on the dorsal blood vessel of Pheretima robusta, is shown to be correlated to the phenomenon of autotomy i.e., amputation of certain parts of the body of the host. Cystic bodies representing another new species of Apolocystis are found to exert physical pressure on the alimentary canal of the host, which as a consequence tend to flatten at the point of such parasitic growth.

P A R T I

MORPHOLOGY AND SYSTEMATIC POSITION OF

NEW GREGARINE PARASITES

(In this account seventeen new species and two new genera are proposed; new names will be assigned to these parasites at the time of actual publication so as to satisfy the conditions of availability, and as per ARTICLES 9, 10, and 11 of the International Code of Zoological Nomenclature.

After publication the holotypes will be deposited with the Zoological Survey of India.)

I N T R O D U C T I O N

Earthworm is one of the most common representative of the Oligochaeta found in different parts of the world. Much work has been done on various aspects of biology of this Annelid. Acephaline gregarines parasitic in Oligochaetes have received the attention of various investigators from time to time. The checklist prepared by Levine (1977) mentions about 187 species in the Family Monocystidae alone. To date, 1400 species have been named in 37 Families. It is however, noticed that parasites of earthworms in the hill areas of Darjeeling district were not studied earlier and therefore the present work was undertaken. The present study has revealed the existance of 17 acephaline gregarines new to science, present in the earthworms of the study area.

The presence of certain hyperparasitic microbes in some of these gregarines, and their effect on their respective hosts has been reported in the course of the present investigation. The present study also reveals the possible deleterious effect of heavy parasitisation, by at least two of the gregarines studied, on the host Oligochaete. Such

parasitisation has fatal effect.

An attempt has also been made to undertake fluorescent microscopic studies on some of the gregarines reported here.

New names have not been provided for the gregarines mentioned in the thesis. In lieu of this capital letters viz., A, B, C, etc. have been used to denote a new genus, and small letters viz., a, b, c. etc. to denote new species. This procedure has been followed to satisfy the Criteria of Availability (Articles 9, 10, 11) of the International Code of Zoological Nomenclature.

REVIEW OF LITERATURE

According to Levine (1977) gregarines have been known for over 150 years, and about 1400 species in 37 Families have been discovered as of date.

The literature on gregarines is widely distributed and the work of Levine (1977) in providing a checklist of the gregarines belonging to various Families has been of paramount importance to taxonomists working in the field.

In an earlier review of work on gregarines, Watson (1916) mentioned that Redi was the first person ever to describe a gregarine; but Dufour's (1828) work on Gregarina ovata parasitic in an insect, is the first authentic account of a gregarine.

Dujardin (1835) described a gregarine Proeteus tenax for the first time in an earthworm Lumbricus terrestris . Stein (1848) later transferred this parasite to genus Monocystis, and renamed it Monocystis agilis. The same author (1848) also described a gregarine Gregarina

cometa (syn. Zygocystis cometa) in Lumbricus terrestris. Subsequently, the work on gregarines was systematically extended by Butschli (1881).

Beddard (1888) described a new species and named it Gregarina perichaetae. It was later renamed Monocystis perichaetae (Labbe 1899).

Subsequently, Bosanquet (1894) detected white bodies that filled the hind part of Lumbricus herculeus, and identified these bodies as Monocystis herculea. Later on Meier (1956) transferred the parasite to genus Apolocystis and renamed the parasite Apolocystis herculea.

Drzhevetskiy (1907) created genus Stomatophora, with a description of the type species Stomatophora coronata. He also described a new species Monocystis ciliata from the coelom and seminal vesicles of Allolobophora longa.

Hesse (1909) published a systematic study of the gregarine parasites of earthworms and created three new genera viz.,Rhynchocystis, Pleurocystis, and Nematocystis.

Boldt (1910) erected genus Rhabdocystis,and further

extended the work on Monocystid gregarines. Mulsow (1911) also worked on Monocystids.

Cognetti, in a series of publications (1911, 1918, 1921, 1923, 1925, 1926) dealt with different species of Monocystis and erected six new genera viz. Apolocystis, Dirhynchocystis, Craterocystis, Astrocystella, and Beccaricystis.

Berlin (1923, 1924) surveyed the earthworms from Germany and erected a number of new species under the Family Monocystidae.

Gates (1926, 1933) described two new genera Aikinetocystis and Nellocystis from two different genera of earthworms.

Loubatieres (1955), Tuzet and Loubatieres (1946) worked on several new species belonging to genera Monocystis, Nematocystis, Rhabdocystis, Apolocystis, Zygocystis, Rhynchocystis and Echinocystis (= Dirhynchocystis).

Meier (1956) also worked on earthworms and created

several new species under the genus Rhabdocystis and Zygocystis.

Boisson (1957) described a number of new species belonging to genera Monocystis, Nematocystis, Dirhynchocystis, and Craterocystis.

Dissanaike (1953) created a new genus Zeylanocystis, with a description of the type species Zeylanocystis burti, from the seminal vesicles of Pheretima peguana.

Ruston (1959) added one more species to the genus Dirhynchocystis.

Rees (1961,-1963) who did considerable work on earthworm gregarines erected two genera Cephalocystsis, and Dendrocystis (= Arborocystis), and described several new species under the genera Apolocystis and Monocystis.

Cox (1967) described a new species Adelphocystis from the coelom of Keffia variabilis.

Segun (1971, 1971a, 1972, 1978) also made valuable contributions to the study of acephaline gregarines from Europe and Nigeria.

Mohammed and Ramadan (1971,1972) have added to our knowledge of acephaline gregarines from Egypt by describing a few new species under the genera Nematocystis and Zygocystis.

WORK DONE IN INDIA:

A review of literature shows that Ghosh (1923) carried out a systematic study of the gregarines found in the earthworms of Calcutta. Though valuable, the work has been proved to be inadequate as some of his descriptions are not accompanied by illustrations. Moreover, his descriptions are not sufficient.

Bhatia (1924, 1930) has done major work in the field in India. In 1924, he reviewed the work done by Drzhevetskiy (1907) on Stomatophora coronata, and added one more species under the genus. He also revised (1929) and gave a concise account of the distribution of gregarines in Oligochaetes .

Bhatia and Chaterjee (1925) created a number of new species under genera Nematocystis, Rhynchocystis, and

Dirhynchocystis (= syn. Echinocystis).

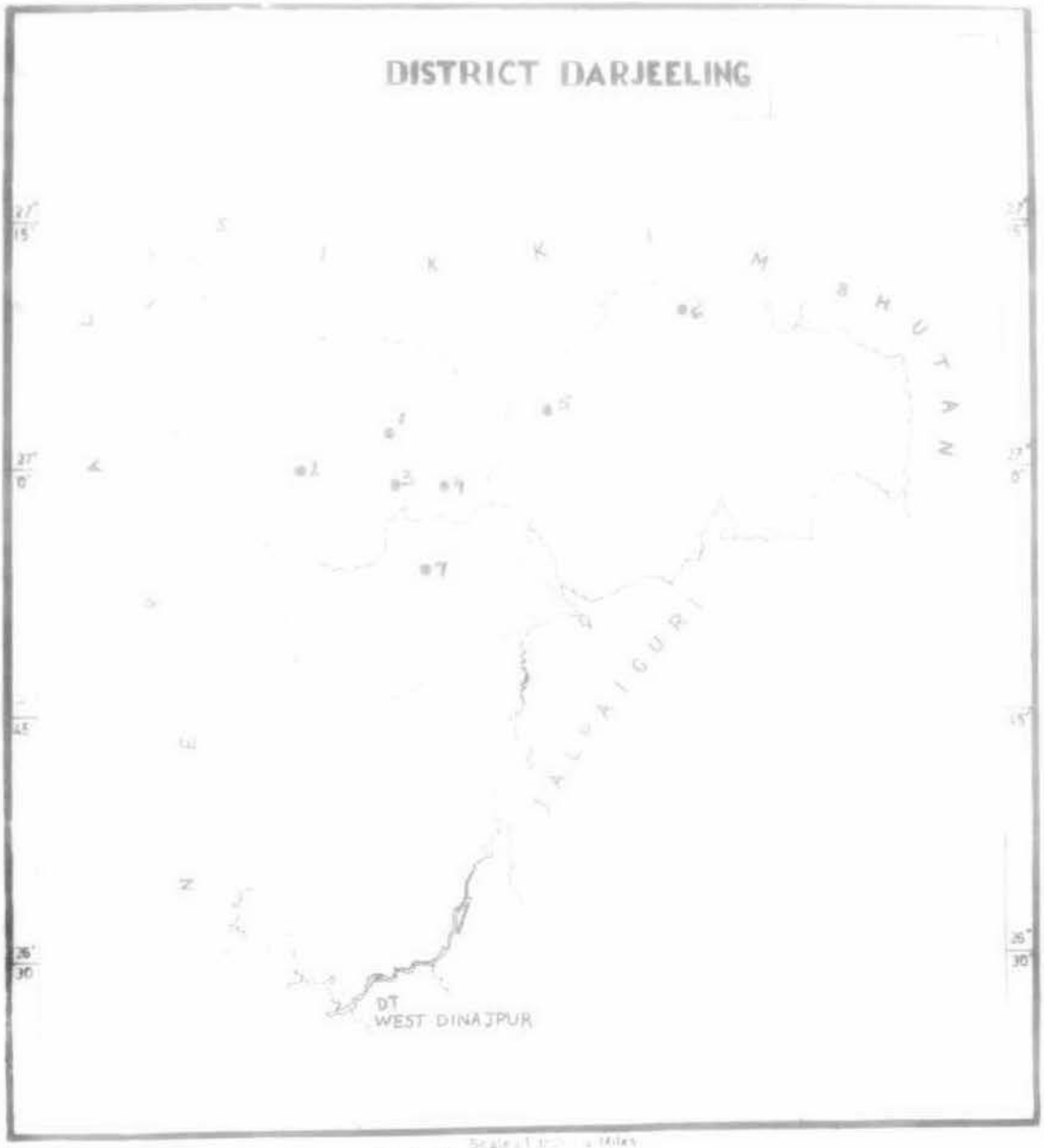
Bhatia and Setna (1926) also described some new species under genera Nematocystis, Apolocystis, and Rhynchocystis.

Setna (1927) created genus Grayallia.

Nelson (1970) has rightly mentioned in his concluding paragraph on the literature review that, 'The list of gregarines has slowly accumulated over the years. There has been no phenomenal explosion similar to that which has occurred in many other areas of science. In all likelihood, the literature will continue this slow growth. There are almost certainly many new species of gregarines yet to be discovered.'

Map of Darjeeling district showing the sites of Collection.

1. Darjeeling, North point (alt. 2001m).
2. Jor- Pokhari (alt. 1271 m).
3. Senchal (alt. 2669.69m).
4. Mangpoo(alt.2164.75m).
5. Kalimpong, Echay Bustee (alt. 1249.7m).
6. Pedong (alt. 1209m).
7. Goomti (alt. 1372m).


DISTRICT DARJEELING
BHUTAN
JALPAIGURI
DT
WEST DINAJPUR

NEW GREGARINE PARASITE (No. 1)

Monocystis n.sp. (a)

(Plate 1, Figs. 1- 4)

Occurrence

The parasite has been detected in the smear of the seminal vesicle of *Apporectodea trapezoides* (Duges), an Oligochaete collected in October and November 1977 at Senchal (altitude 2269.66 m) in Darjeeling District, West Bengal.

Morphology

The trophozoites are elongated in form and are more or less cylindrical. The two ends of the body are blunt. In some cases one end is narrower than the other end. The middle part of the body represents the widest part. Cytoplasm is alveolated in appearance, and packed with paraglycogen granules. Nucleus is eccentric in position and spherical in shape though in some cases it may be slightly ovoid. An endosome is not visible. When viewed in saline water, the trophozoite exhibits sluggish movement caused by the flow of protoplasm.

Gametocysts are slightly ellipsoidal in shape. Most of the gametocysts have an empty space between the gametocyst wall and the aggregation of spores as seen in stained preparations. At times residual cytoplasm is seen in the centre.

Spores are typically biconical in shape with their ends drawn out. Plate I. Fig.3.

For measurements see Table I.

Discussion

Due to presence of typically biconical spores the parasite under discussion is assigned to Family Monocystidae. The parasite however does not resemble any known species under this genus, though there is a slight resemblance to Monocystis mammaliae (Segun, 1971) so far as the shape of the parasite is concerned. However the posterior end of Monocystis mammaliae terminates in a V shape, unlike the species under report. Moreover a mucron which is present in Monocystis mammaliae is absent in the species under report. The most striking difference between the two is the absence of cytophilia in the present parasite.

T A B L E I

Measurements of *Monocystis* n.sp. (a)

	RANGE	AVERAGE
Length	43.02 μ - 185.4 μ	99 μ
Breadth	10. 8 μ - 48.6 μ	24.23 μ
Nucleus	6.48 μ - 12.60 μ	8.53 μ
Gametocyst length	90 μ - 111.6 μ	108.36 μ
Gametocyst breadth	72 μ - 111.6 μ	92.88 μ
Spores length	9.94 μ - 11.36 μ	10.5 μ
Spores breadth	3.97 μ - 4.26 μ	4.23 μ

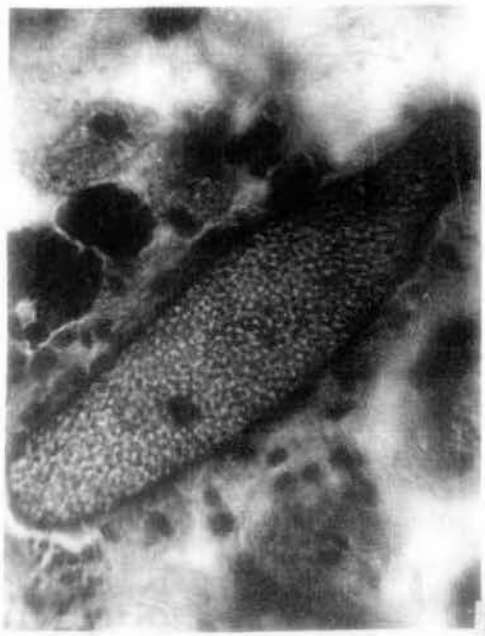
PLATE I

Figs. 1 - 2. Two forms of Monocystis n.sp.(a)
15 X 40X

Fig.3 . Spores of Monocystis n.sp.(a)
15 X 100X

Fig.4 . Mature gametocyst. Note the space between the gametocyst wall and the aggregation of spores. 15 X 40X

PLATE X



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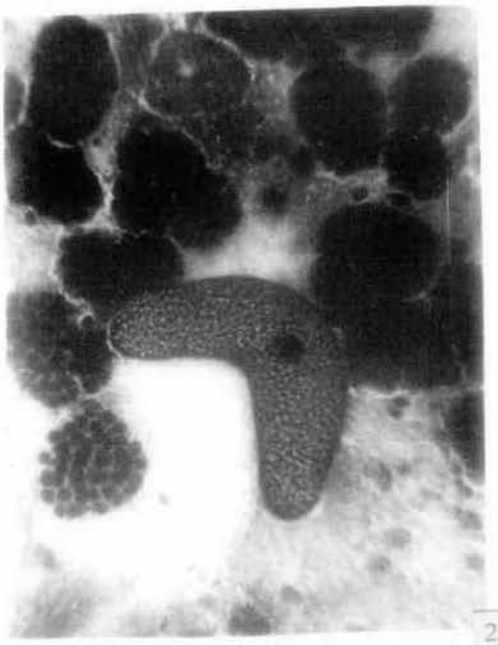

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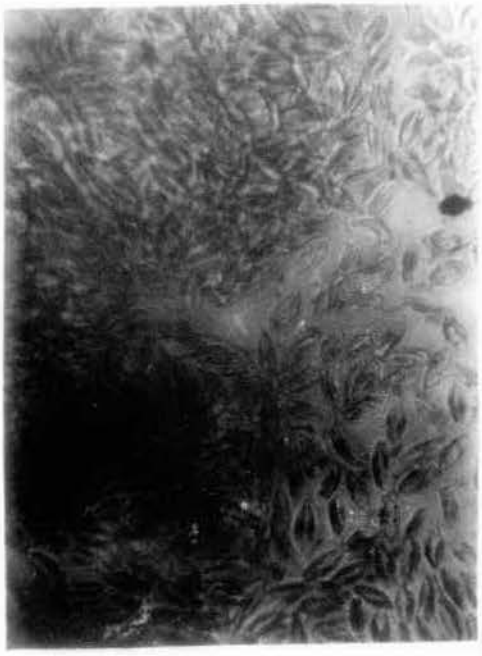

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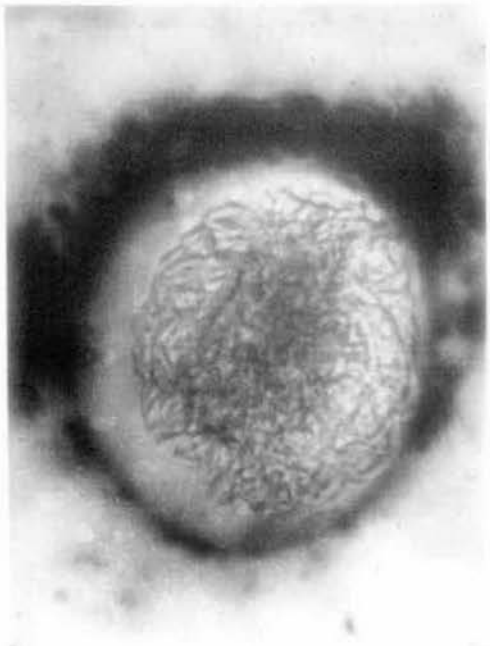


4

The parasite under report is also clearly distinguishable from M. acuta (Berlin 1924) on account of the sharply tapering end of the latter. The species under report also differs considerably in size and in other particulars from all other Monocystid species recorded from Oligochaete worms. Considering all aspects of the matter it would appear that the parasite under report may safely be regarded as a new species.

Diagnosis

Family Monocystidae Bütschli

Spores spindle-shaped.

The parasite is placed under the family on account of this.

Genus Monocystis Stein

Trophozoites variable in form; solitary, motile; incomplete sporulation in cyst; spores biconical; in coelom or seminal vesicle of Oligochaetes.

The parasite is placed under the genus.

Specific characters:

Trophozoite elongated and more or less cylindrical; Nucleus is eccentric and

spherical; endosome is not visible; cytoplasm is alveolated; spores biconical; octozoic; coelozoic.

For the present the parasite is referred to as Monocystis n.sp.(a)

Host: Apporectodea trapezoides Duges (Oligochaeta).

Site of infection: Seminal vesicles.

Locality: Senchal (altitude 2269.66 m) Darjeeling, West Bengal.

NEW GREGARINE PARASITE (No. 2)

Nematocystis n.sp.(a)

(Plate 2, Figs.1 - 5)

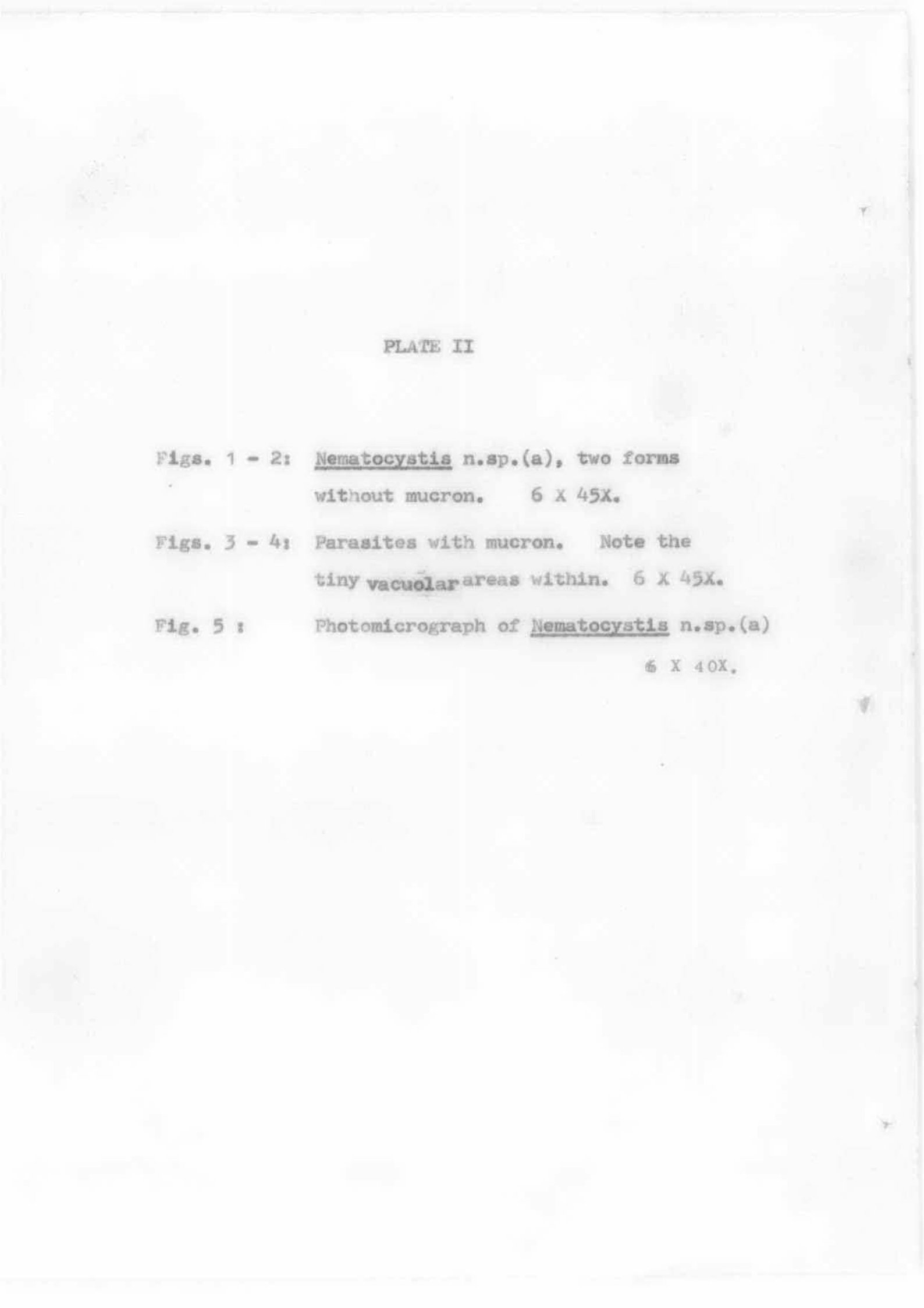
Occurrence

The parasite has been observed in the coelomic fluid of Apporectodea trapezoides (Duges) - an Oligochaete collected in the months of March and April 1978, at Senchal (alt. 2269.66 m) in Darjeeling district, West Bengal.

Morphology:-

The trophozoite is vermicular. One end is narrow and the other end is somewhat rounded and terminating in a majority of cases, in a pointed mucron. The body is surrounded by a fine pellicle. A large number of tiny vacuolar structures lie scattered in the endoplasm. The rest of the endoplasm is faintly stained with Heidenhain's haematoxylin. The endoplasm has very little storage material. Some trophozoites are practically devoid of paraglycogen granules. Faint striations which cross the

PLATE II

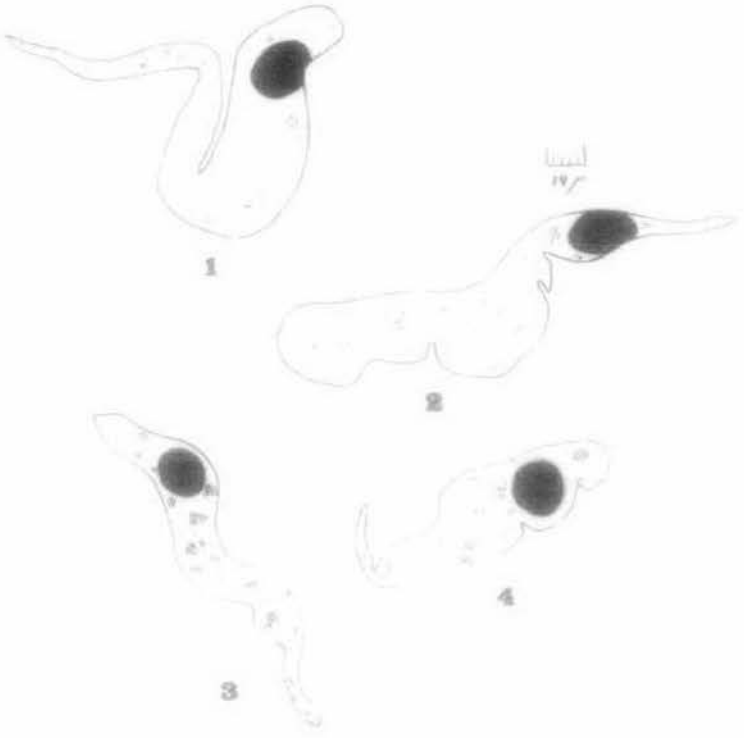


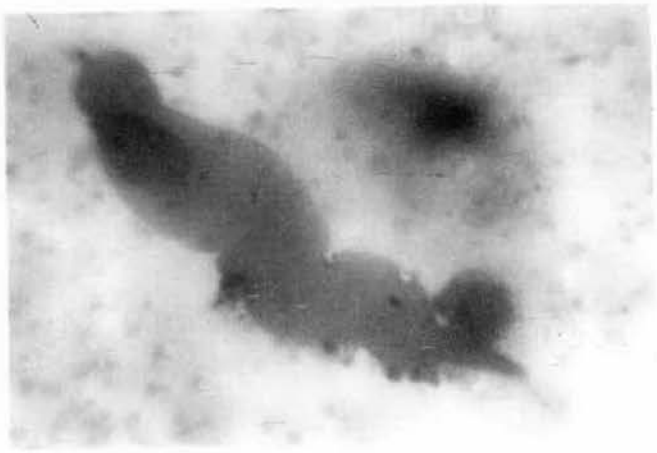
Figs. 1 - 2: Nematocystis n.sp.(a), two forms without mucron. 6 X 45X.

Figs. 3 - 4: Parasites with mucron. Note the tiny vacuolar areas within. 6 X 45X.

Fig. 5 : Photomicrograph of Nematocystis n.sp.(a) 6 X 40X.

PLATE II





body diagonally are present throughout the entire length. These striations are seen clearly under oil immersion lens. The nucleus is large, slightly ellipsoidal in shape, and occupies any position within the body. It is homogeneously, but faintly, stained. An endosome is present only in some cases. A slightly lighter area is seen within the nucleus in some cases. For measurements see Table II.

Discussion

Due to the presence of biconical spores the present parasite has been placed under the Family Monocystidae.

Close resemblance is seen to Monocystis acuta, (Berlin 1924), in its worm-like shape, and in its varying breadth at both ends. However, there are significant dissimilarities; for example, the nucleus of M. acuta, is often markedly elongated, whereas in the gregarine under report, the nucleus is ellipsoidal. Moreover the present parasite has hardly any paraglycogen granules, a substance which has been emphasised in M.acuta (Berlin 1924), and also noted by Marek (1967).

T A B L E II

Measurements of Nematocystis n.sp.(a)

	RANGE	AVERAGE
Length	91.2 μ - 286.90 μ	186.05 μ
Breadth	15.2 μ - 45.22 μ	31.49 μ
Nucleus length	16.72 μ - 32.30 μ	26.39 μ
Nucleus breadth	11.78 μ - 22.8 μ	18.13 μ

The diagonal striations and the more or less constant presence of a mucron that are present in this new parasite have not also been mentioned in the case of M.acuta.

Nematocystis caudata (Loubatieres 1955) has a globular anterior end and possesses a mucron. The posterior end is narrow and tail-like. The present parasite has the presence of a mucron and a tail-like posterior end, but the very fact that the mucron in the case of N. caudata is connected to the body by a narrow neck, differentiates the two parasites distinctly.

As no described members of this genus resemble the parasite under report, it is proposed to give this parasite a new specific status.

Diagnosis

Family Monocystidae Bütschli

Spores spindle-shaped.

The parasite is placed under this family on account of this.

Genus Nematocystis Hesse

Trophozoites large; cylindroid, nematoid, often with a mucron at the anterior end; solitary; spores biconical. The parasite has been placed under this genus.

Specific characters:

Body elongated with a blunt anterior end and a tail-like posterior end; presence of mucron at anterior end. Diagonal striations present throughout the body length; ovoidal nucleus eccentric in position; spores biconical; octozoic; coelozoic.
The parasite is referred to as Nematocystis n.sp.(a).

Host:- Apporectodea trapezoides Duges (Oligochaeta)

Site of infection:- Coelomic fluid.

Locality:- Senchal (alt. 2269.66 m) Darjeeling, West Bengal.

Additional Observations:

The parasite when viewed in fresh smears presents a transparent appearance. This could be due to the dearth of storage materials. The gregarine is however very active. The manner in which the parasite moves so vigorously and carries out its metabolic activities with hardly any food reserves as a source of energy is a problem which might require elucidation by future workers.

NEW GREGARINE PARASITE (NO. 3)

Nematocystis n.sp.(b)

(Plate 3, Fig. 1.)

Occurrence

The parasite has been detected in the coelomic fluid of *Pheretima diffringens* (Baird) - an Oligochaete collected in May 1978, at Goomti (alt. 1372m) in Darjeeling district, West Bengal.

Morphology

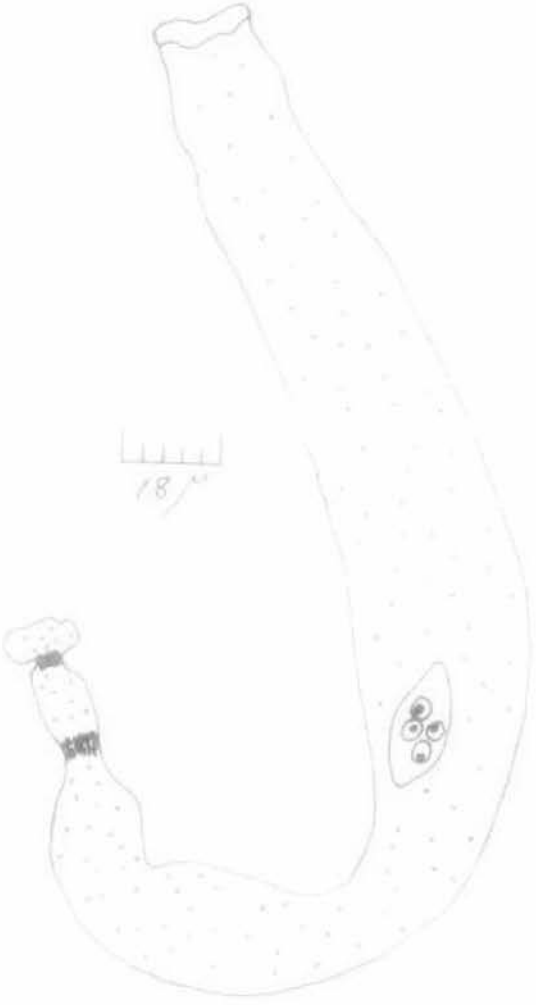
The trophozoite is long, vermiform and cylindrical but not of equal breadth throughout its length. One end is narrow and the other end is broad. The narrow end is terminated in a bulbous area followed by a constricted neck. The broad end is provided with a flattened rim. Endoplasm is granular. The body is enclosed by a fine pellicle. Nucleus is elongated and situated more towards the narrow end of the body. Four karyosomes are present within the nucleus. These are deeply stained bodies surrounded by lightly stained areas. For measurements see Table III.

PLATE III

Fig.1. *Nematocystis* n.sp. (b)

10 X 45X.

PLATE XXI



T A B L E III

Measurements of Nematocystis n.sp. (b)

(single specimen)

	RANGE	AVERAGE
Length	273.22 µ	273.22 µ
Breadth	30.78 µ	30.78 µ
Nucleus length	25.84 µ	25.84 µ
Nucleus breadth	11.4 µ	11.4 µ
Karyosomes	7.2 µ	7.2 µ

Discussion:

The parasite under report is unique in having two dissimilar poles **i.e.** one end is narrow with a constricted neck terminating in a bulbous area, while the other end is broad ending in a flattened rim. It bears no resemblance to any of the species reported so far under the genus *Nematocystis*.

A new specific status for the gregarine is justified.

Diagnosis:

Family Monocystidae Bütschli

Spores spindle-shaped

The parasite is placed under the family on account of this.

Genus *Nematocystis* Hesse

Trophozoites large; cylindroid, nematoid, often with a mucron at the anterior end; solitary; spores biconical. The parasite has been placed under this genus.

Specific characters:

Trophozoite is elongated, narrow at one pole and increases in width towards the middle; the blunt end terminating in a flattened rim. Nucleus elongated with four karyosomes, Coelozoic.

For the present the parasite is referred to as Nematocystis n.sp.(b).

Host: Pheretima diffringens Baird Oligochaeta

Site of infection: Coelomic fluid.

Locality: Goomti (alt. 1372m), Darjeeling district, West Bengal.

NEW GREGARINE PARASITE(No. 4)
Nematocystis n.sp. (c)

(Plate IV. Fig. 1)

Occurrence

The parasite has been detected in the smear of the seminal vesicle of *Eutyphoeus gammiei* (Beddard), an Oligochaete collected in August 1978, at Mangpoo (alt. 2164.75m), Darjeeling district, West Bengal.

Morphology:

The trophozoites are very large and they can be seen with the naked eye as fine white threads. In stained preparations they occur as twisted worm-like bodies often thrown into coils. In some individuals one end is club-shaped while the other is pointed, but in others both the ends are blunt. However in most cases one end is broader than the other. Pellicle is smooth and fine and the epicyte is clear. Very fine longitudinal striations run along the length of the body. These striations converge at both ends. Endoplasm has a large number of paraglycogen granules which are usually elongated and occasionally ellipsoidal in shape. These reserve

food granules are not uniformly distributed in the endoplasm. Areas within the endoplasm are encountered that are completely devoid of these granules. The nucleus is large and elongated. It is stained homogeneously with haematoxylin. Nucleus does not occupy a specific location within the body. The size of the nucleus varies in different individuals. For measurements see Table IV.

Discussion:

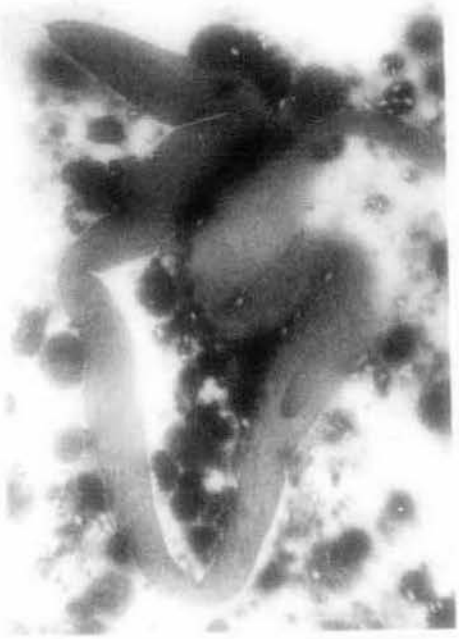
The parasite described here is identified as belonging to the genus Nematocystis on account of its elongated nematode-like body. Among all the species of Nematocystis known, few measure two millimeters or more. e.g. N.anguilla (Hesse 1909) measures 2080 µ by 40 µ, while N.tuzeti (Loubatieres 1955) measures 9000 µ by 40 µ - 360 µ. The parasite under report undoubtedly belongs to this variety of the larger types. If length is taken into consideration then the parasite comes between N.navicula (Loubatieres 1955) (2415 µ by 180 µ) and N.carensis (Mohammed & Ramadan 1972) (2550µ by 75 µ), as it measures 2523.04 µ by 107.3 µ. There is however, an important morphological difference, i.e. the apparent absence of endosome in the present parasite. The host is also different. The above evidence is enough to justify inclusion of the present parasite as a new species.

PLATE IV

Fig.1 . Photomicrograph of Nematocystis n.sp.(c)

6 X 10X

PLATE IV



Diagnosis:

Family Monocystidae Bütschli

Spores spindle-shaped

The parasite is placed under the family on account of this.

Genus Nematocystis Hesse

Trophozoites large; cylindroid, nematoid, often with a mucron at the anterior end; solitary; spores biconical. The parasite has been placed under this genus.

Specific characters:

Body long and vermiform; elongated nucleus may take up any position in the body. Longitudinal striations run along the length of the body and converge at both ends. Epicyte clear, body loosely packed with paraglycogen granules that are elongated; Coelozoic.

Host: Eutyphoeus gammiei Beddard (Oligochaete)

Site of infection: Seminal vesicles

Locality: Mungpoo (alt. 2164.75m), Darjeeling district West Bengal.

TABLE IV

Measurements of Nematocystis n.sp.(c)

	RANGE	AVERAGE
Length	839.9 μ - 2523.04 μ	1785.75 μ
Breadth	49.5 μ - 107.3 μ	79.57 μ
Nucleus length	36.3 μ - 98.8 μ	71.87 μ
Nucleus breadth	13.2 μ - 28.2 μ	19.66 μ

NEW GREGARINE PARASITE (No. 5)

Nematocystis n.sp. (d)

(Plate V. Figs. 1 & 2)

Occurrence

The parasite has been observed in the coelomic fluid of Pheretima diffringens (Baird) - an Oligochaete collected in May 1978 at Goomti (alt. 1372m), Darjeeling district, West Bengal.

Morphology:

The trophozoite is long, vermiform and ribbon-shaped. In stained smears the parasite is seen as curved, somewhat straight or as deeply grooved at intervals along its length. One of its poles shows a very distinct funnel-like structure. The region below this funnel is always constricted to form the neck. The other extremity is rather blunt. In some individuals the blunt end is club-shaped while in others the blunt end shows a distinct collar-like structure.

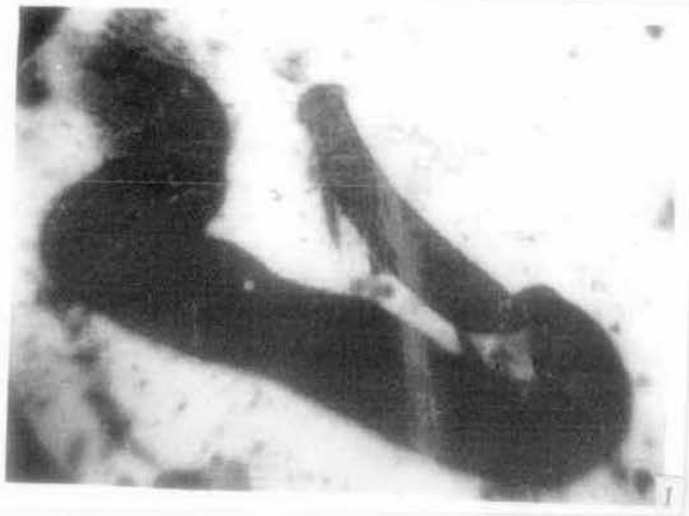
Body is enclosed by a pellicle. Endoplasm is dotted with loosely packed granules which take up the haematoxylin stain well. Nucleus is elongated and narrows

PLATE V

Fig.1. Photomicrograph of Nematocystis n.sp.(d)
6 X 10X

Fig. 2. Camera lucida drawing of Nematocystis n.sp. (d) , showing the body grooved at intervals. 6 X 10X

PLATE V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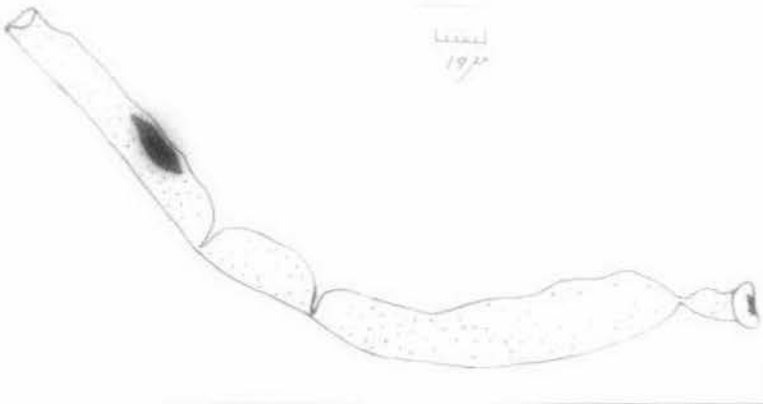

2

down towards the two ends. The position of the nucleus in the body is inconstant. The entire nucleus takes up a deep stain and an endosome is not visible. The parasite is very active as seen in fresh smears. For measurements see Table V.

Discussion:

The gregarine under report belongs to the genus Nematocystis on account of its elongated and cylindrical body.

The two ends of the parasite are different from each other. The funnel-shaped structure at one end is invariably preceded by a neck region. The other end is blunt and in most cases this end shows a structure that resembles a folded collar. In most of the Nematocystis species that have been reported so far, the two extremities are rather alike or if unlike, their poles are rather rounded at one end and tapering off towards the other. The present parasite does not resemble N. vermicularis, (Hesse 1909) in that it does not have hairs that are directed backwards, as is the case with the latter.

Most of the Nematocystis species are usually found in the seminal vesicles, except in a few like

T A B L E V

Measurements of Nematocystis n.sp. (d)

	RANGE	AVERAGE
Length	212.76 μ - 444.6 μ	348.44 μ
Breadth	14.76 μ - 55.10μ	29.35 μ
Nucleus length	14.76 μ - 30.04μ	25.82 μ
Nucleus breadth	6.84 μ - 13.30μ	9.16 μ

N. tuzeti (Loubatieres 1955), N. almae (Cognetti 1921), N. elmassiani (Hesse 1909), N. dendrobaenae (Segun 1971) and the present parasite. Apart from the fact that the parasite under consideration belongs to a different host, morphological differences noted are sufficient to justify the conclusion that the present parasite is a new addition to the genus Nematocystis.

Diagnosis:

Family Monocystidae Bütschli

Spores spindle-shaped

The parasite is placed under the family on account of this.

Genus Nematocystis Hesse

Trophozoites large; cylindroid, nematoid, often with a mucron at the anterior end; solitary; spores biconical. The parasite has been placed under this genus.

Specific characters:

The parasite is elongated and vermiform and occasionally deeply grooved. Nucleus does not occupy a definite location within the body.

Endosome is not visible. The two terminal ends are unlike. One end is funnel-shaped and the other is collar-like. Coelozoic.

For the present the parasite is referred to as Nematocystis n.sp.(d)

Host: Pheretima diffringens Baird (Oligochaeta)

Site of infection: Coelomic fluid.

Locality: Goomti (alt. 1372m), Darjeeling district, West Bengal.

NEW GREGARINE PARASITE (No. 6)

Nematocystis n.sp. (e)

(Plate 6. Fig. 1)

Occurrence

The parasite has been detected in the smear of the seminal vesicle of Eutyphoeus gammiei (Beddard) - an Oligochaete collected in August 1978 at Mangpoo (alt. 2164.75m), Darjeeling district, West Bengal.

Morphology:

The body is long and vermiform. The two ends of the body are not always similar. At times one end of the body is club-shaped whereas the other end is slender and even tapers to a tail-like structure. Sometimes both ends are slender and tapering. The entire body is enclosed by a smooth and complete pellicle. The epicyte is clear and thin. The endoplasm has large and elongated paraglycogen granules which are loosely packed. Longitudinal striations are present along the length of the parasite. These converge at both ends. Nucleus is long and spindle-shaped, tapering at both ends. The

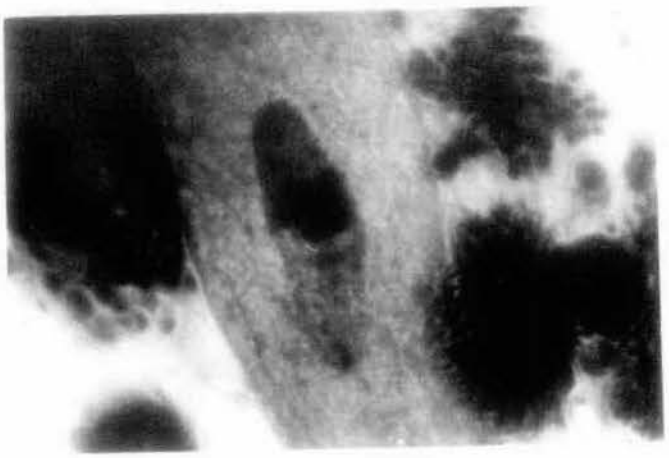
PLATE VI

Fig. 1. Nematocystis n.sp.(e). 6 X 40X.

Fig.2. Nucleus of Nematocystis n.sp.(e) showing deeply stained karyosome surrounded by a light area.
10 X 100X.

PLATE VI

1



2

nucleus does not occupy a definite position within the body. The karyosome is surrounded by a faintly staining area. For measurements see Table VI.

Discussion:

Due to its worm-like body, the parasite under discussion has been identified as Nematocystis.

The present parasite resembles N. stephensoni (Bhatia & Setna 1926) in the site of infection and in the presence of a nucleus which besides not having a fixed position has its elongated axis lying parallel to the long axis of the body. Both the parasites have a karyosome - but in the case of N. stephensoni, it is either rounded or oval. In the parasite under discussion the nucleus is distinctly elongated. A lightly stained area surrounding the karyosome is present in both the parasites. This lightly stained area has been described as a clear halo by Bhatia and Setna (1926). But apart from the rather close resemblances of the nuclear structure, major differences are present as regards the size of the trophozoites concerned and in their choice of host.

N. vermicularis, N. navicula (Loubatieres 1955) and N. pilosa (Turet & Loubatieres 1946) differs

T A B L E VI

Measurements of Nematocystis n.sp. (e)

	RANGE	AVERAGE
Length	760 µ - 1339.9 µ	1172 µ
Breadth	77.52µ - 150.48 µ	97.96 µ
Nucleus Length	64.6 µ - 83.6 µ	67.61 µ
Nucleus breadth	19 µ - 26.9 µ	24.14 µ
Karyosmme	13.5µ	13.5 µ

from the present parasite in the presence of hairs in the body.

N. carensis is cylindrical throughout the length of the body. None of the species of Nematocystis recorded so far absolutely resembles the present Nematocystis. Therefore, it appears necessary to consider this parasite as a new species.

Diagnosis:

Family Monocystidae Bütschli

Spores spindle-shaped.

The parasite is placed under the family on account of this.

Genus Nematocystis Hesse

Trophozoites large; cylindroid, nematoid, often with a mucron at the anterior end; solitary; spores biconical.

The parasite has been placed under this genus.

Specific characters:

Body long and vermiform. The two ends are dissimilar. Elongated and spindle-shaped

nucleus, with a karyosome that does not occupy a fixed position; pellicle is smooth, epicyte clear; longitudinal striations run along the length of the body and converge at both ends.

Coelozoic.

The parasite is referred to here as Nematocystis n.sp.(e)

Host: Eutyphoeus gammiei Beddard (Oligochaeta.)

Site of infection: Seminal vesicles.

Locality: Mangpoo (alt. 2164.75m), Darjeeling district, West Bengal.

NEW GREGARINE PARASITE(No. 7)

Zygocystis n.sp. (a)

(Plates VII-IX. Figs.1-9)

Occurrence

The parasite has been detected in the smear of the seminal vesicles of Pheretima (= Metaphire) californica (Kinberg) - an Oligochaete collected throughout the year-1976, at Kalimpong (alt.1249.7m), Darjeeling district, West Bengal.

Morphology:

Trophozoites are ovate in shape when at rest. When observed in saline water they are found to be very motile often assuming variable shapes. A thick pellicle surrounds the trophozoite. Cytoplasm is packed with paraglycogen granules which remain enmeshed in a tenuous matrix of cytoplasm. Vacuoles, one or two in number, may occur in the cytoplasm. These are generally clear and no inclusion or granule can be detected in them. Nucleus is round and occasionally ellipsoidal in shape. It is either centrally placed or it may occur towards the periphery. A nuclear membrane is not visible in smear preparations, but can easily be detected in sections. Endoplasm shows darkly-stained areas; under high magnification these dark areas are

PLATE VII

1 - 5. Various forms of *Zygocystis* n.sp. (a)

IO X 45X

PLATE VII

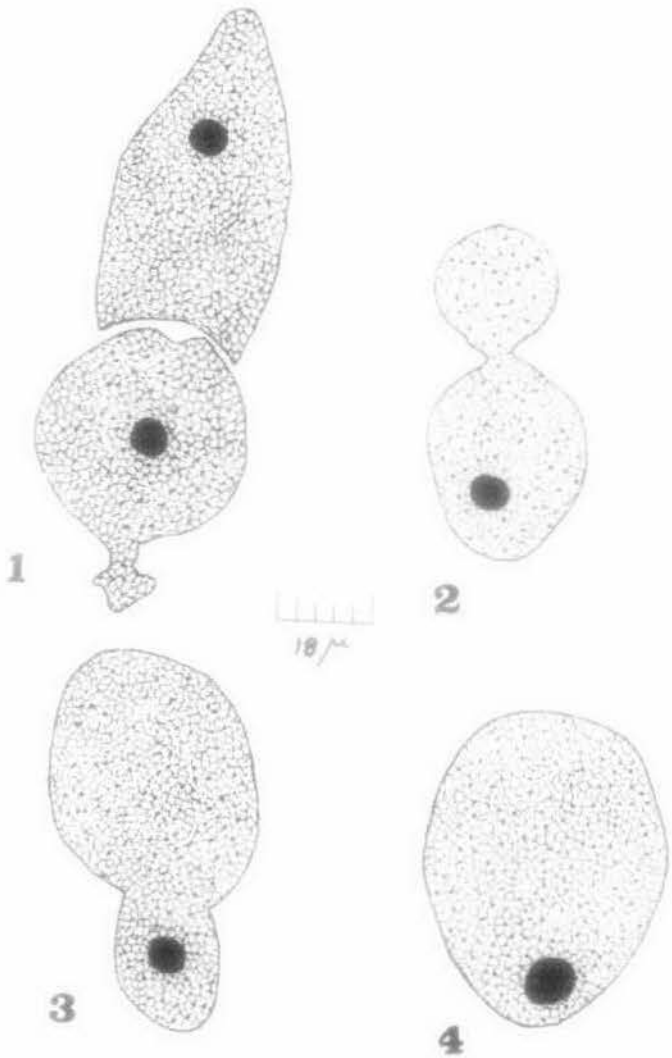
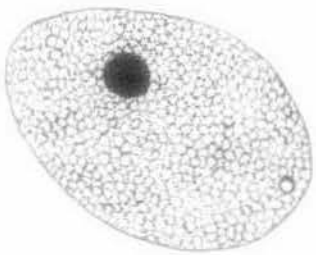


PLATE VIII

Fig.5. Zygocystis n.sp.(a). Clear vacuole seen at broad end of body. IO X 45X

Fig.6. Clear vacuoles are present during syzygy. IO X 45X

PLATE VIII



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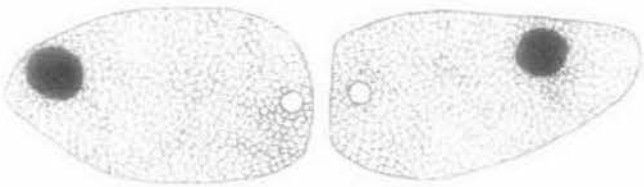

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18 μ

TABLE VII

Measurements of *Zygocystis* n.sp.(a)

	RANGE	AVERAGE
Length	43.2 μ - 86.4 μ	65.96 μ (single troph.)
Breadth	23.40 μ - 58.6 μ	41.89 μ
Nucleus	5.4 μ - 10.8 μ	8.11 μ
Cametocyst length	75.6 μ - 144 μ	122.4 μ
Cametocyst breadth	86.4 μ - 133 μ	110.2 μ
Spores length	7.65 μ	7.65 μ
Spores breadth	3.67 μ	3.67 μ

seen to consist of fine granular structures.

Trophozoites often occur in pairs, lying closely apposed to each other along their breadth, where the cup-shaped concavity of one fits into the cone- shaped projection of the other. This can be referred to as the 'cup and socket' junction as described by Huxley (1910) in Ganymedes anaspides. At a later stage the apposing ends flatten out.

Gametocysts are slightly ellipsoidal in shape.

Spores are imperfectly biconical, assuming the shape of the barrel with distinct plugs at both ends. For measurements see Table VII.

AUTOTOMY

(Plate IX., figs. 7 - 8)

In quite a number of cases, pieces of the body are thrown off. These thrown off pieces are devoid of nucleus. This peculiar phenomenon is identified as autotomy.

Cases of autotomy have been recorded by Troisi(1933) in Rhynchocystis pilosa in which the posterior portion of the body is thrown off before syzygy. The cause of autotomy

P L A T E IX

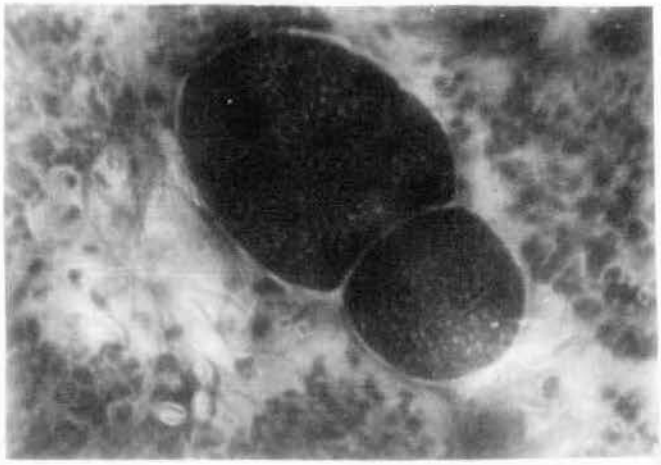
Fig. 7. **Autotomy.** The amputated piece does not possess a nucleus.

15 X 40X

Fig. 8. The lower half of the photomicrograph shows the parasite undergoing autotomy. The amputated piece does not possess the nucleus.

15 X 40X

PLATE IX



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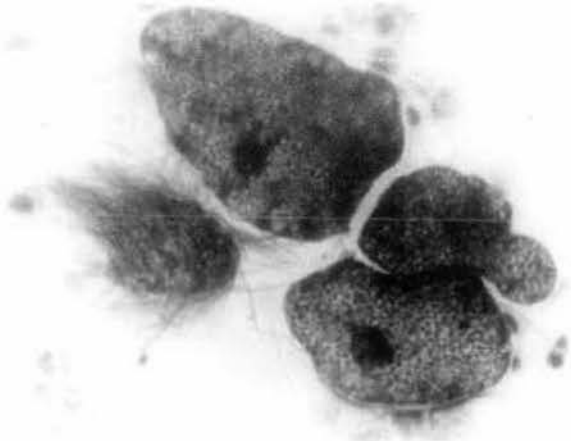

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could be the alterations in the nucleo-cytoplasmic ratio of the gregarine. The nucleus is known to be able to control the activity of just a limited quantity of cytoplasm. When the amount of cytoplasm increases beyond the controlling power of the nucleus only then, a portion of the cytoplasm will be amputated so that the original nucleo-cytoplasmic ratio may be restored, as suggested by De Robertis *et al* (1975).

LIFE HISTORY

(Plate X)

Encystation:

Mature trophozoites come together and attach themselves by their broad ends. If the trophozoites are already in a state of syzygy, then this itself acts as the beginning of the encystation phase. Association between two individuals occur irrespective of the sizes of the individuals concerned. At the beginning, two mature trophozoites associate by the typical 'ball and socket' junction described earlier. The two parasites then appear to secrete the cyst forming substance which hardens around them. Meanwhile the two individuals shorten and become more or less spherical in shape; the

PLATE X

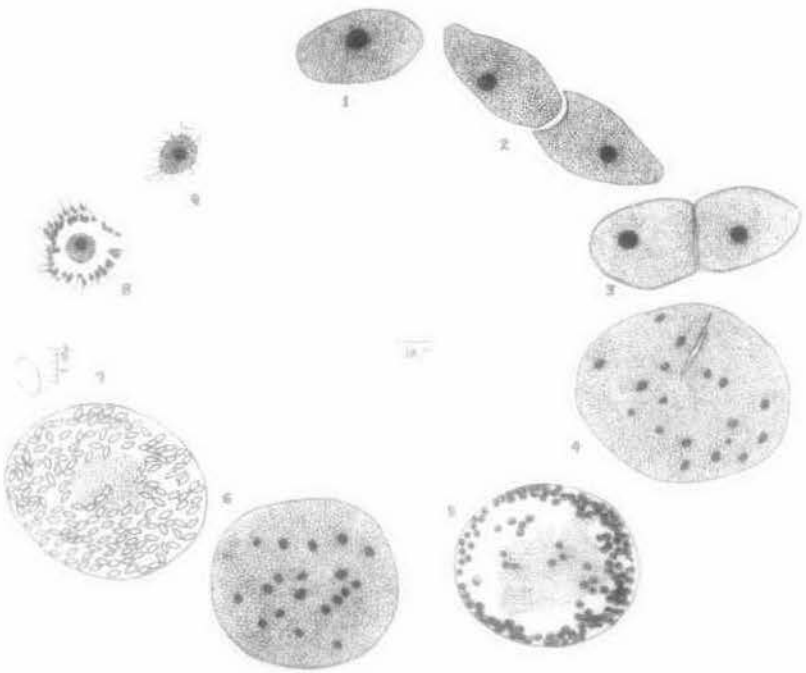
Fig.9. Life history of Zygocystis n.sp.(a)

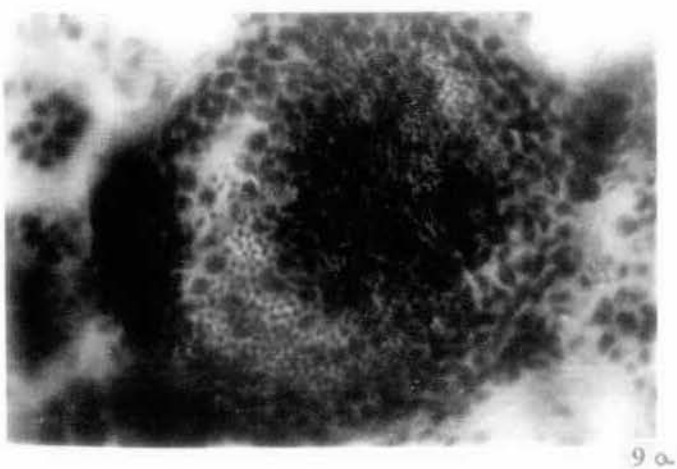
1. Trophozoite
2-3. Early syzygy and formation of gametocyst.
4. The nuclei of the two gametocytes break down to form several nuclei.
5. Gametes aligned at the periphery of the gametocyst. Residual cytoplasm seen in the centre of the gametocyst.
6. Zygote formation.
7. Formation of sporocysts. Next to it is an individual spore. Note the two plugs at either end.

8 - 9. Developing trophozoites within the sperm morula.

Fig.9a. Photomicrograph of a gametocyst with the gametes at the periphery. A few are in a state of isogamy. I5 X 40X

PLATE X





9a

mature gametocyst is ellipsoidal in shape.

Gamete formation:

Once the gametocyst is formed, the nucleus of each gametocyte starts to fragment and a large number of nuclei are formed. A little bit of cytoplasm collects around each of the nuclei and thus the gametes are formed. The gametes are somewhat spherical in shape. They arrange themselves along the periphery of the gametocyst. The dividing wall between the two gametocytes disappears. The gametes look alike in all respects, and hence can be regarded as isogametes.

In a gametocyst with immature spores, there is always a mass of residual cytoplasm in the centre.

Spore formation:

The zygotes secrete a wall around themselves and are transformed into spores. These are barrel-shaped bodies, with distinct plugs at both ends. A fully mature gametocyst is seen to be packed with such transparent spores.

The nucleus in the spore divides until a stage is reached when eight sporozoites are formed. Sporozoites

are elongated in appearance.

The trophozoites develop intracellularly within the sperm morula, in the seminal vesicles. They grow at the expense of the host germ cells which continue to develop producing the spermatogonia, spermatids and the spermatozoa on its surface. Finally the host cells are reduced to a mere membrane surrounding the gregarine. The latter has the appearance of having many cilia. The apparent cilia are actually the tails of the attached spermatozoa. Eventually the remains of the host cells rupture and a fully grown gregarine emerges to start the life cycle all over again.

Discussion:

The parasite under report has spores which are biconical in shape and possess peculiar thickenings at both poles. This character has been quoted for Family Zygocystidae, and hence the parasite has been placed under the above Family.

Previous workers have stated that Zygocystis is always associated in pairs - but Troisi (1933) has observed single individuals in Z. wenrichi. Single

individuals have likewise been observed in the present parasite, but individuals in a state of association are a more common sight.

Other recorded members of the genus possess some distinct morphological characteristics, such as the presence of a posterior tuft of fine processes in Z. cometa (Stein 1848), caudal bristles in Z. cordiformis (Loubatieres 1955), the longitudinal striations which run off the body to form a tuft of hairs in Z. wenrichi, and the elongation of the posterior end in Z. legeri (Hesse 1909). These features do not exist in the present parasite.

Another distinguishing feature of the present parasite is its small size as compared to the recorded members of the genus. However, Z. aegyptiaca Mohammed & Ramadan (1971) has some similarities with the parasite under discussion. Both the species have been found in the seminal vesicles of Pheretima (= Metaphire) californica. Both of them are much smaller in size than the other species, but differ among themselves in size, as shown below:

Z. aegyptiaca 32µ - 48µ X 25µ - 36µ (two syzygites).
Zygocystis n.sp.(a) 97 µ - 129 µ X 30·4µ - 47µ (two syzygites)

Apart from the size differences it is also noticed that the present parasite occurs singly- a factor not found in Z. aegyptiaca. According to Levine (1977), Z. coneta has been recorded from India earlier. This is the second record of the genus occurring in India.

It is also, noted here that this is the first time that the earthworm Pheretima (= Metaphire) californica is recorded from this country.

Diagnosis:

Family Zygocystidae Bhatia

Syzygy extremely early or permanent; oocysts navicular; generally in the seminal vesicles or coelom of Oligochaetes.

Genus: Zygocystis Stein

Adult trophozoite generally pyriform; occur in pairs or groups of three; occasionally single (Troisi 1933). Spores biconical with peculiar thickenings at both poles; octozoic; in the seminal vesicles or coelom of Oligochaetes. The parasite has been placed under the genus.

Specific characters:

Body ovate when at rest and often in a state of syzygy. Nucleus either at the centre or the periphery. Gametocyst slightly ellipsoidal; spores barrel shaped with plugs at both ends; Octozoic; Coelozoic.

The parasite has been referred to here as *Zygocystis* n. sp. (a)

Host: *Pheretima* (= *Metaphire*) *californica* Kinberg (Oligochaete.)

Site of infection: Seminal vesicles.

Locality: Kalimpong(alt. 1249.7m), Darjeeling district, West Bengal.

NEW GREGARINE PARASITE (No.8)

Apolocystis n.sp. (a)

(Plate XI-XII, figs.1- 5)

Occurrence

The parasites are seen as small white spheres with minute granulations on the surface. These appear closely adherent to the dorsal blood vessel of *Pheretima robusta* (Perrier) collected at North Point campus, Darjeeling. (alt.2001m). Some trophozoites and gametocysts also occur free in the coelom. In cases where infection is not very heavy, the parasites are restricted to the posterior two-thirds of the dorsal blood vessel. In other cases the parasites extend along the dorsal blood vessel upto the point where the intestinal caeca branch off from the alimentary canal. The specimens were collected throughout the year.

Morphology:

These are spherical in shape and are often in a state of syzygy. They appear immobile when placed in saline water but cytoplasmic granules within the endoplasm show active movement. Cytoplasm stains deeply with haematoxylin.

PLATE XI

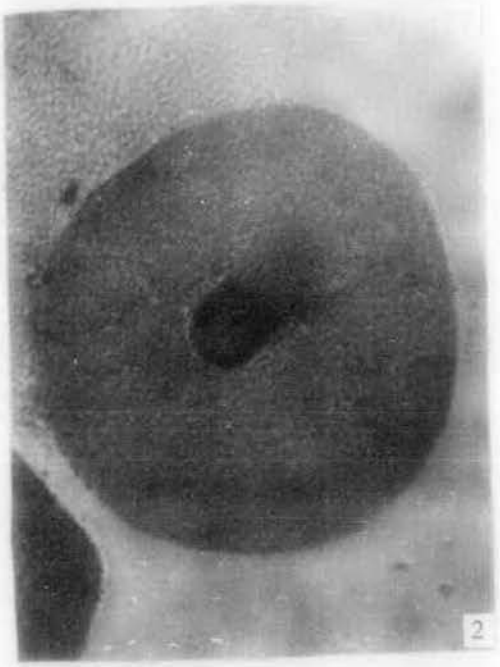
Fig.1 . Dissection of the posterior end of *Pheretima robusta* showing the growth of the cystic bodies on the dorsal blood vessel.

Fig.2. *Apolocystis* n.sp.(a), magnified 15 X 40X.

Fig. 3. *Apolocystis* n.sp.(a), magnified 15 X 10X.

PLATE XI

1



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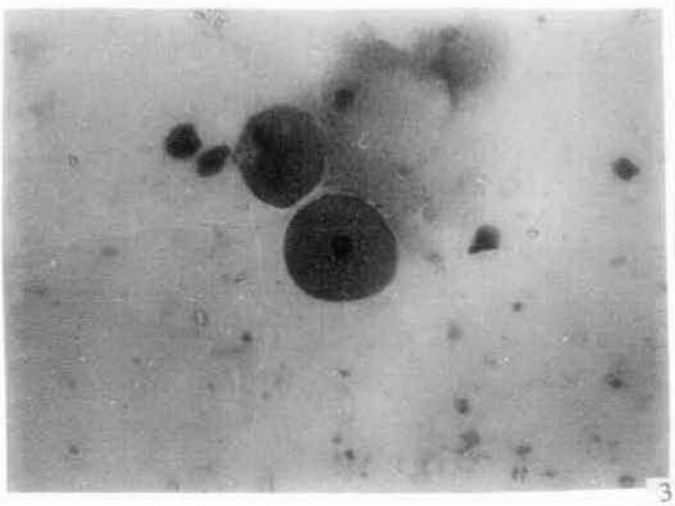


3

The outer pellicle is thin and complete and there is no ectoplasmic process. Nucleus is round and large and does not stain homogeneously with haematoxylin. There are areas in the nucleus which stain deeply in contrast to other areas which are lightly stained. Nuclear membrane and the karyosome cannot be distinguished. Nucleus is placed either in the middle of the body or is situated towards the periphery.

A gametocyst when just formed and having the gametocytes within are spherical in shape. An empty space is generally noticed at the point of contact of the two gametocytes. The space narrows down as the gametocyst gets older until only a narrow line remains. When fully mature the gametocyst is round and is slightly larger than the immature form. It is crowded with transparent spores.

The spores are biconical, but with pronounced truncated ends. The edges of the truncated ends wing out slightly. The surface of the spores are [redacted] with fine granules/lations which may be seen clearly under oil immersion lens. These granules are often accompanied by black pigmented dots. For measurements see Table VIII.

TABLE VIII

Measurements for Apolocystis n.sp.(a)

	RANGE	AVERAGE
Trophozoite diameter	54 µ - 100.8 µ	84.6 µ
Nucleus diameter	11.88 µ - 18 µ	15.1 µ
Mature gametocyst	86.4 µ - 111.6 µ	99µ
Spores length	14.2µ - 15.62µ	14.86 µ
Spores breadth	5.9µ - 7.10µ	6.98µ

LIFE HISTORY

(Plate XIII. Fig. 1)

The trophozoites come together in syzygy, irrespective of their sizes.

When the adults have reached sexual maturity, two trophozoites approach one another. At the point of contact an empty space is visible; especially so in the middle region. As the gametocytes mature the space narrows down. Next they secrete a cyst wall around themselves, forming the gametocyst. The diameter of the gametocyst is slightly less than the actual sizes of the two gametocytes.

Soon the nucleus starts to divide and a large number of gametes are formed by accumulation of a bit of cytoplasm around each nucleus. The nuclei present a large variety of forms and do not stain homogeneously.

The gametes that are formed are never aligned to the periphery as is the case in all common gregarines, but then remain scattered within the gametocyte cytoplasm. The dividing wall between the two gametocytes

disappears. The zygote that is formed by the fusion of the gametes is irregular in outline and size, and are haphazardly distributed within the gametocyst.

The zygotes produce a chitonous secretion around themselves and each of them develops into a boat-shaped spore. These are biconical with truncated ends and the edges of the truncated ends wing out slightly. Fully-formed gametocysts are packed with spores of this kind. There are eight sporozoites in a spore. Residual cytoplasm does not occur in the gametocyst.

Discussion:

The parasite bears slight resemblance to A. herculea (Bosanquet 1894) in the fact that both the parasites appear as numerous white cysts attached to some internal organs in the posterior region of the body of the earthworm. However the present parasite is found growing on the dorsal blood vessel while A. herculea was seen attached to the alimentary canal. The two parasites also differ in their choice of the host, and in their size.

PLATE XIIIA

1. Trophozoite.
2. 3. Early syzygy and formation of gametocyst.
4. Nuclei of the two gametocytes break down to form several nuclei.
5. Gametes haphazardly distributed in the gametocytes.
6. Isogamy and formation of zygotes.
7. Sporoblasts within the gametocyst.
8. Formation of sporocysts.
9. Individual spore. Note the two truncated ends.

PLATE XXIA

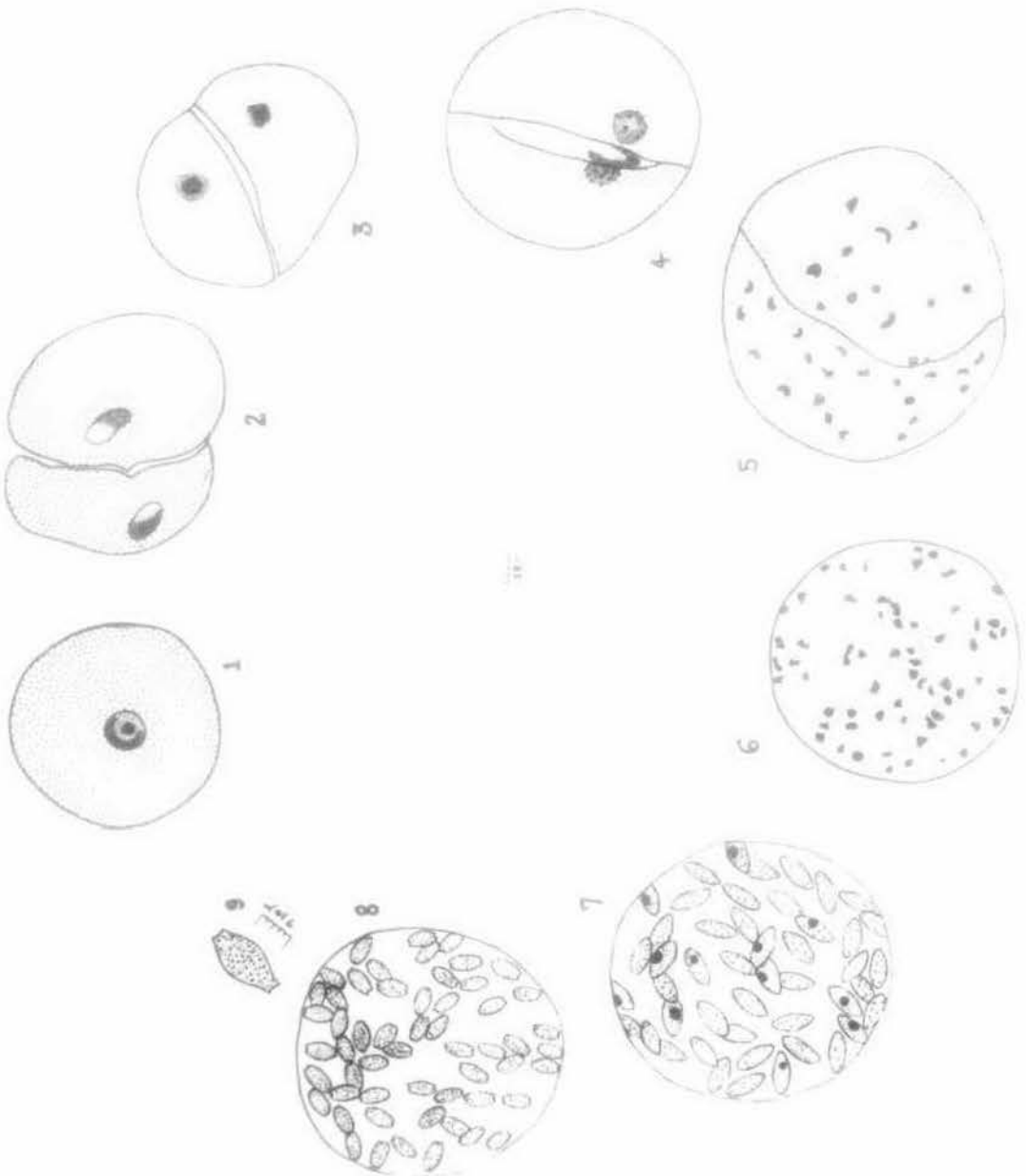
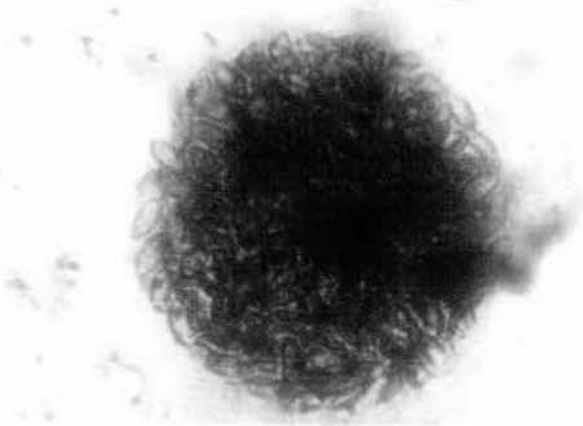


PLATE XII B

Fig.4 . Photomicrograph of a mature gametocyst packed with transparent spores.
15 X 40X.

Fig.5 . Photomicrograph of the sporoblasts magnified 7 X 100X. Black pigments are seen in the cytoplasm.

PLATE XIIB



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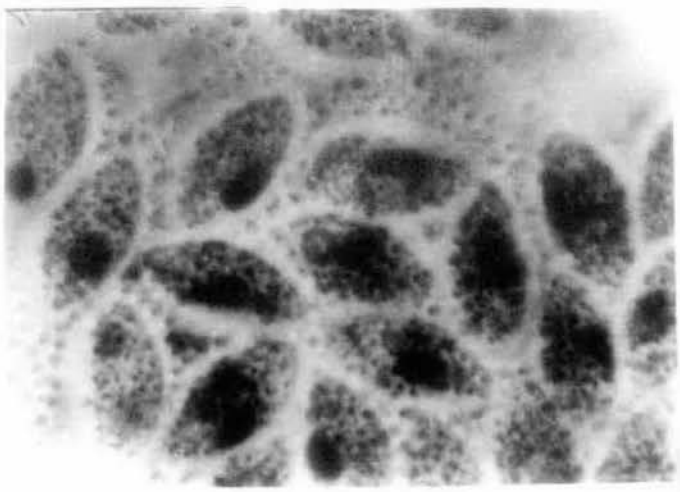


5

The present parasite resembles A. rotaria (Rees,1963) in the granular nature of the ectosarc i.e. in both the cases the ectosarc is granular not appearing as a clear region of cytoplasm as is the case in most of the species of Monocystidae. Apart from this, other major morphological differences also exist between the present parasite and A. rotaria. For example, the shape and size of the nucleus differ, and a karyosome is present in A. rotaria while it is absent in the present parasite. The site of infection also differs, A. rotaria occurring in the seminal vesicles only, while the present parasite is found attached to the dorsal blood vessel.

A. laverensis (Rees,1963), differs from the parasite under discussion in its site of infection ie. it is found in the genital segments of the host. The two parasites also differ in their respective sizes. However both possess a densely granular endoplasm and both are practically inactive individuals.

A. perfida (Rees,1963) is found in the body cavity of A. chlorotica. It has densely granular endoplasm, possesses a karyosome, and its ectoplasm is

differentiated unlike the present parasite. In contrast to the present parasite A. perfida, is found normally covered by dense amoebocytes. The body measurements also vary in the two parasites.

The presence of hair-like ectoplasmic processes which occur in the body of A. spinosa, (Rees 1963) A. gigantea, (Troisi 1933) A. villosa, (Hesse 1909) and A. lumbrici, are absent in the parasite under report. The above four species do not also occur attached to the dorsal blood vessel of the host.

Finally it may be emphasised that the spores of the parasite under discussion are unique in possessing sharply truncated ends the edges of which wing out slightly. This kind of spore has never been reported in any other member of the genus Apolocystis. Hence the name Apolocystis n.sp. (a) justifies its inclusion as a new species under the genus.

Diagnosis:

Family Monocystidae Bütschli

Spores spindle-shaped.

The parasite is placed under the Family on account of this.

Genus Apolocystis Martiis

Trophozoites are spherical; solitary; absence of any polarity. Spores biconical; in the seminal vesicles or coelom of Oligochaetes.

The parasite is placed under the genus.

Specific characters:

Spherical body; nucleus is either in the centre or eccentric; immature gametocyst slightly smaller than the mature ones. Biconical spores with truncated ends and the edges of the truncated ends wing out slightly. Coelozoic; Octozoic.

For the present the parasite is referred to as Apolocystis n.sp.(a)

Host: Pheretima robusta Perrier (Oligochaeta.)

Site of infection: It is attached to the posterior part of the dorsal blood vessel in the form of small white spheres. In heavy infection it grows all along the blood vessel, up to the point of the intestinal caeca.

Locality: North Point, Darjeeling, (alt.2001m) West Bengal.

NEW GREGARINE PARASITE (No.9)

Apolocystis n.sp.(b)

(Plate XIII.Figs.1 - 5)

Occurrence

The trophozoites are seen as small white spheres with minute granulations on their surface. They grow closely adherent to the dorsal blood vessel of an Oligochaete *Pheretima robusta* as in the case of *Apolocystis* n.sp.(a). The parasites extend along the dorsal blood vessel upto the point where the intestinal caeca branches off from the alimentary canal. When infection is not very heavy the parasites are confined to the posterior two thirds of the blood vessel. The specimens were collected throughout the year.

Morphology:

Trophozoites are spherical in outline. Solitary forms occur but many are found in a state of syzygy. These have a thin and complete pellicle, devoid of any ectoplasmic processes. Cytoplasm is finely granular. Trophozoites appear immobile when placed in saline water,

but a brisk movement of the cytoplasmic granules is noticed. Nucleus is spherical and eccentric in position. Nuclear membrane is clearly seen, and there is an unstained area around the deeply-stained karyosome. The karyosome does not occupy a central position in the nucleus.

In one case a binucleate condition of the trophozoite has been encountered. In another instance a binucleate gametocyte has been noticed within a newly formed gametocyst.

An immature gametocyst having the immature gametocytes within, is slightly smaller in size than the mature gametocyst. All the gametocysts are spherical in shape. The mature gametocysts are found to be packed with large spores.

Spores belonging to this species are biconical and with flattened ends. These spores have granulations on their surface often accompanied by black pigments which are a little more prominent here than in the case of the spores of Apolocystis n.sp.(a). For measurements see Table IX.

T A B L E IX

Measurements of Apolocystis n.sp.(b)

	RANGE	AVERAGE
Trophozoite diameter	61.2 µ - 115.2 µ	93.2u
Nucleus diameter	14.4 µ - 29.16 µ	21.07 µ
Karyosome	4.32 µ - 9 µ	5.65 µ
Mature gametocyst	108 µ - 133.2 µ	126.2µ
Spores length	14.2 µ	14.2 µ
Spores breadth	7.10 µ	7.10 µ

PLATE XIII

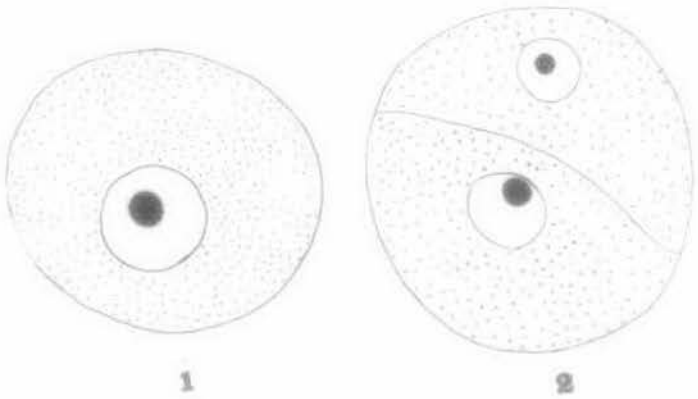
Fig. 1. *Apolocystis* n.sp.(b) 10 X 45X.

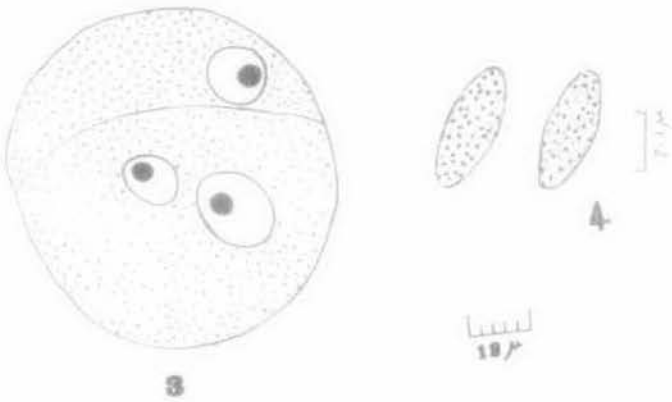
Figs. 2- 3. Trophozoites in syzygy. A binucleated condition is noticed in one of the gametocytes.

Fig. 4. Spores magnified 10 X 100X.

Fig. 5. Photomicrograph of two gametocytes. 15 X 10X.

PLATE XIII





Discussion:

The parasite under report does not have a definite polarity and is spherical in shape; it is therefore classified as genus Apolocystis. It differs distinctly from Apolocystis n.sp.(a)in the possession of a very distinct karyosome with a clear area around it and in the occurrence of occasional binucleate forms. The spores also differ in shape markedly.

In view of the differences noted above, a new specific status is considered necessary for accomodating the parasite under report.

Diagnosis:

Family Monocystidae Bütschli

Spores spindle shaped.

The parasite has been placed under the Family on account of this.

Genus Apolocystis Martiis

Trophozoites are spherical; solitary, absence of any polarity; spores biconical; in seminal vesicles or coelom of Oligochaetes.

Specific characters

Spherical bodies; nucleus eccentric in position. There is a clear area around the karyosome. Immature gametocysts are slightly smaller than the mature ones. Spores are biconical with flattened ends; Coelozoic. Octozoic. For the present the parasite has been referred to as Apolocystis n.sp.(b)

Host: Pheretima robusta (Perrier (Oligochaeta)

Site of infection: On the dorsal blood vessel.

Locality: North Point, Darjeeling (altitude 2001m), West Bengal.

NEW GREGARINE PARASITE (No.10)

Apolocystis n.sp.(c)

(Plate XIV, Figs. 1 -5)

Occurrence

The parasite has been found growing closely attached to the antero-dorsal part of the intestine of Pheretima (= Amynthas) alexandri (Beddard), an Oligochaete, as numerous white globular cystic bodies. The specimens were collected in the month of October 1978, at Echay Bustee, Kalimpong (altitude 1249.7m) Darjeeling district, West Bengal.

Morphology:

Trophozoite is spherical in outline. The pellicle is fine and smooth and is devoid of any ectoplasmic processes. When placed in saline water the trophozoites appear immobile. In stained preparations minute granules are found to occur in the cytoplasm, scattered in between tiny vacuoles. The nucleus is eccentric in position and spherical in shape. There is a well-defined nuclear membrane enclosing a deeply stained karyosome which is rounded in shape. The karyosome is situated a little away from the centre of the nucleus.

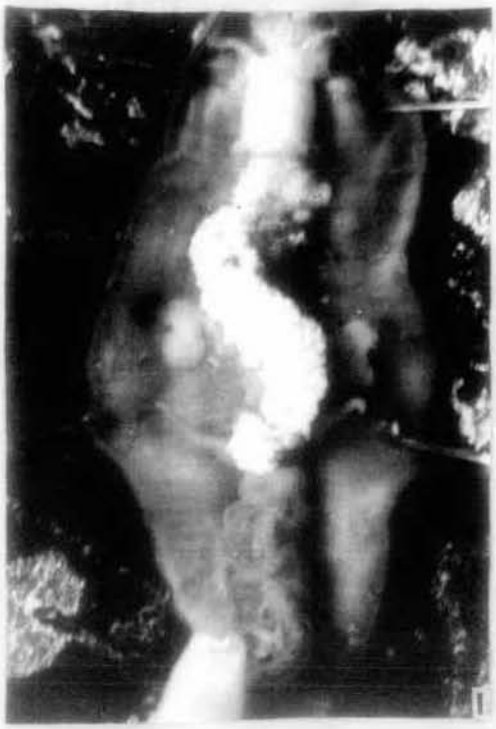
PLATE XIV

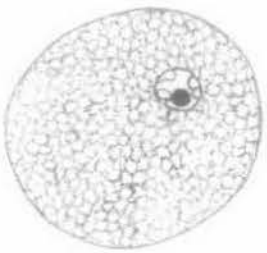
Fig. 1. Dissection of the anterior part of Pheretima alexandri showing the growth of the cystic bodies on the alimentary canal.

Fig. 2-3. Apolocystis n.sp.(c) 10 X 10X.

Figs.4-5. Nucleus of the parasite magnified 10 X 45X. Vacuoles and the spherical black bodies are seen inside.

PLATE XIV





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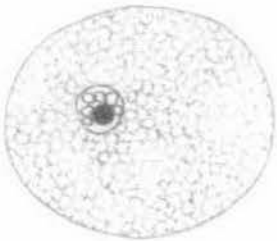

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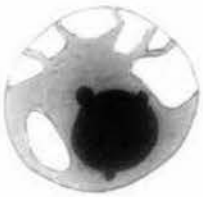
76 μ



4

18 μ

5

Vacuoles two to eight in number and of varying shape and size occur in the nucleus. Very often deeply-stained bodies varying in number are also present in the nucleus. The ground substance of the nucleus under oil immersion lens appears granulated. For measurements see Table A.

Discussion:

Apolocystis n.sp.(c), does not resemble any known species of the genus Apolocystis. However, it has got apparent similarity with Apolocystis herculea (Bosanquet 1894) in that it is found as white cystic bodies growing on top of the alimentary canal. But the present parasite differs from A. herculea, in a number of other aspects. Apolocystis n.sp.(c), has got a vacuolated nucleus, unlike A.herculea. The position of growth of the cystic bodies does not tally either. The parasite also differs in the measurements and propotions of the body from all other species of the genus recorded so far.

Certain specimens of A.gigantea (Troisi 1933) have been found to show in addition to the karyosome a small spherical body, which probably could correspond to the small spehrical bodies encountered in the parasite under discussion. Troisi has assumed their structure to be

probably ' lipoid in nature'.

The marked differences mentioned above, warrants the erection of a specific status for the gregarine under report.

Diagnosis:

Family Monocystidae Bütschli .

Spores spindle shaped.

The parasite has been placed under the Family on account of this.

Genus Apolocystis Martiis .

Trophozoites are spherical, solitary, absence of any polarity; spores biconical; in the seminal vesicles or coelom of Oligochaetes.

Specific characters:

Spherical body; cytoplasm provided with granules interspersed in between tiny vacuoles; spherical nucleus eccentric in position and possessing two to eight vacuoles. Karyosome spherical and eccentric in position; Coelozoic.

Host: Pheretima (= Amynthas) alexandri Beddard (Oligochaeta)

Site of infection: Attached to the anterior part of the intestine.

Locality: Kalimpong (altitude 1249.7m) Darjeeling district, W.B.

NEW GREGARINE PARASITE(No.11)

Apolocystis n. sp. (d)

(Plate XV, Fig. 1)

Occurrence

The parasite has been observed in the coelomic fluid of Pheretima diffringens (Baird) an Oligochaete collected in the month of May 1978, at Goomti (altitude 1372m) Darjeeling district, West Bengal.

Morphology

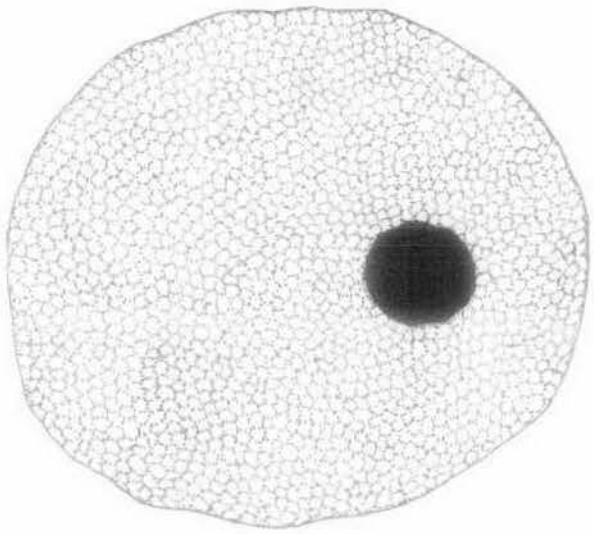
The trophozoite is more or less spherical. The body is bounded by a distinct pellicle. The endoplasm is packed with paraglycogen granules. In between these granules there occur a large number of deeply stained bodies. The nucleus is round to ellipsoidal in shape and it does not occupy a definite position in the cell body. The karyosome is not clearly visible though a faint dark body can occasionally be located in the nucleus. For measurements see Table XI

PLATE XV

Fig. 1 Apolocystis n.sp.(d)

10 X 45X

PLATE XV



18 μ

TABLE X

Measurements of Apolocystis n.sp.(c)
(5 specimens)

	RANGE	AVERAGE
Diameter	228 μ - 235.60 μ	230.34 μ
Nucleus	39.52 μ - 45.06 μ	42.116 μ
Karyosome	14.4 μ - 18 μ	16.94 μ
Small bodies	1.80 μ - 3.6 μ	2.01 μ

TABLE XI

Measurements of Apolocystis n.sp.(d)

	RANGE	AVERAGE
Diameter	57.6 μ - 154.8 μ	98.62 μ
Nucleus length	10.2 μ - 28.8 μ	19.17 μ
Nucleus breadth	14.4 μ - 25.2 μ	18.6 μ

Discussion

The present parasite differs from other members of the genus Apolocystis. A.gigantea, A. villosa , A. spinosa, A. perfida , and A. lumbrici have the presence of ectoplasmic processess present on the body surface. This feature is not found in the parasite under report.

Although A. minuta (Troisi 1933) resembles this new parasite in having the cytoplasm packed with paraglycogen granules, the nuclear structure is entirely different; the site of infection is also different. According to the checklist prepared by Levine (1977), only one species of Apolocystis has been discovered in India so far. A. mathaii (Bhatia and Setna 1926) was found occuring in the seminal vesicles of Megascolex trilobatus. It differs markedly from the present parasite in its body measurements, choice of the host and in the possession of an eccentric karyosome and also in the site of infection.

A comparative table of the four new species of Apolocystis described in the thesis is given on the following page. Due to its spherical shape the present parasite has been placed under the genus Apolocystis.

Comparative Table Of The 4 Species Of *Apolocystis*

	Apolocystis n.sp.(a)	*Apolocystis* n.sp.(b)	*Apolocystis* n.sp.(c)	*Apolocystis* n.sp.(d)
Shape & dismeter	Spherical; 84.6 μ	Spherical; 93.2 μ	Spherical; 230.34 μ	Spherical; 98.62 μ
Endoplasm	Gramular	Granular	Alveolated	Gramular
Nucleus	Spherical; 15.19 μ	Spherical; 21.07 μ	Spherical & vacuolated 42.116 μ	Spherical to ellipsoidal 19.17μ
Karyosome	Not visible	Present	Present	Not visible
Site of infection	Cystic bodies on the dorsal blood vessel.	Cystic bodies on the dorsal blood vessel.	Cysts on the dorsal aspect of alimentary canal.	Coelomic fluid
Host	*Pheretima robusta* (Perrier)	*Pheretima robusta* (Perrier)	*Pheretima alexandri* (Beddard)	*Pheretima diffringens* (Baird)
Locality	Darjeeling (alt.2001 m)	Darjeeling (alt. 2001 m)	Kalimpong (alt. 1249 m)	Goomti (alt. 1372 m)

Diagnosis

Family Monocystidae Bütschli

Spores spindle shaped.

The parasite has been placed under the Family on account of this.

Genus Apolocystis Martiis

Trophozoites are spherical; solitary; absence of any polarity. Spores biconical. In the seminal vesicles or coelom of Oligochaetes.

The parasite is placed under the genus.

Specific characters:

Body spherical; endoplasm packed with paraglycogen bodies. In between these bodies there are darkly stained granules. Nucleus spherical; karyosome not visible.

Host: Pheretima diffringens Baird (Oligochaete)

Site of infection: Coelomic fluid

Locality: Goomti (altitude 1372m) Darjeeling district, West Bengal.

NEW GREGARINE PARASITE (No.12)

Stomatophora n.sp.(a)

(PlateXVI, Figs. 1,2,3.)

Occurrence

The parasite has been detected in the smear of the seminal vesicle of Pheretima diffringens (Baird), an Oligochaete collected throughout the year at Pedong, (altitude 1209 m), in Darjeeling district, West bengal.

Morphology

Trophozoites have the form of a disc flattened between two poles; these parasites are solitary by nature and appear immobile when placed in normal saline water. The outline of the parasite is wavy, but in some cases the pellicle dips down at several places giving the parasite a petaloid appearance. The pellicle is fine. In the centre of the disc shaped body there is a sucker. The sucker has a central mucron, which is a clear ring like structure. In most cases the part of the sucker surrounding this mucron is distinctly petaloid, in other cases this feature is not so well marked. Epicytal striations are also present. These

extend from the mucron to some distance towards the periphery. Nucleus is generally round to ellipsoidal in shape. It is eccentric in position. A karyosome is not visible in material stained in iron alum Haematoxylin, but it could be detected using toluidine blue stain.
For measurements see Table XII.

Discussion

The parasite under consideration differs from other species that have been described under the genus Stomatophora. It differs from S.coronata (Hesse 1909), S.simplex (Bhatia 1924) and S. bulbifera (Bhatia 1924) in that it is spherical in shape and has a centrally placed sucker. The other species have an ovoidal to pear shaped body and the sucker is placed at the anterior end.

The present parasite resembles S. coronata, in the petaloid nature of the sucker, but the similarity ends here. The epicytal striations of the sucker of S. diadema (Bhatia 1924) gives rise to distinct lobes in the body. Such lobes are not present in the species under report. There is also a marked difference in the shape and size of the body in these two cases. These pecularities are sufficient to seperate the

PLATE XVI

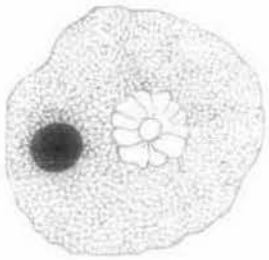
Figs. 1 - 3.

Various forms of *Stomatophora* n.sp.(a)

10 X 45X

PLATE XVI

1



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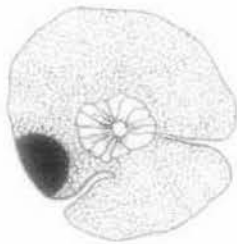

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T A B L E XII

Measurements of Stomatophora n.sp.(a)

	RANGE	AVERAGE
Body diameter	43.2 μ - 82.8 μ	63.12 μ
Nucleus	10.8 μ - 25.2 μ	15.21 μ
Sucker	9 μ - 36 μ	25.6 μ
Mucron	4.32 μ -10.8 μ	8.04 μ

present parasite from the previously described ones. This neccessiates the erection of a new species.

Diagnosis

Family Stomatophoridae Bhatia

Trophozoites are spherical to cylindrical or cup-shaped with sucker like epimerite; spores are navicular, ends truncated. Octozoic; in seminal vesicles of Pheretima.

Genus Stomatophora Drzhevetskiy

Spherical or ovoid; anterior end with a sucker like epimerite with a central mucron; spores navicular. Usually in the seminal vesicles of Oligochaetes.

Specific characters:

Body spherical; periphery of the body is often deeply indented giving the parasite a petaloid appearance. Presence of a central sucker which has a small mucron. Nucleus is eccentric in position and spherical. Cytoplasm alveolated. Spores navicular and truncated. Coelozoic.

The parasite has been referred to here as Stomatophora n.sp. (a)

Host: Pheretima diffringens Baird(Oligochaeta)

Site of infection: Seminal vesicles.

Locality: Pedong (altitude 1209 m), Darjeeling district, West Bengal.

NEW GREGARINE PARASITE (No.13)

Stomatophora n. sp. (b)

(Plate XVII, Figs. 1- 2)

Occurrence

The parasite has been detected in the smear of the seminal vesicle of Pheretima diffringens (Baird), an Oligochaete collected throughout the year at Pedong (alt.1209 m) Darjeeling district, West Bengal.

Morphology

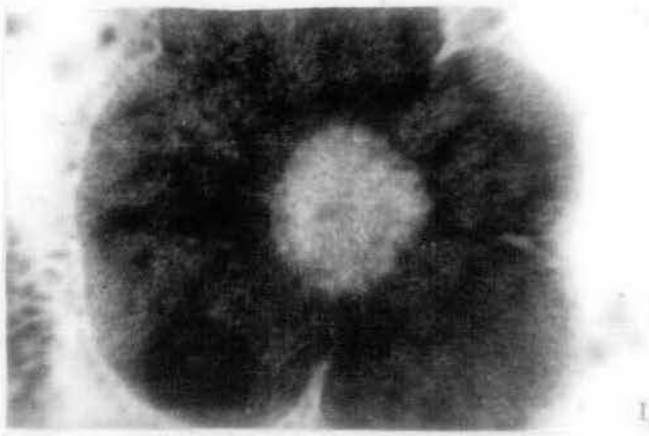
The trophozoites have the form of a disc that has been flattened between two poles. They are solitary and lie immobile in saline water. The outline of the body is wavy, but at certain points the pellicle dips down giving rise to a petaloid appearance of the body. In the centre of the disc-shaped body there is a sucker with a wide mucron which is filled with small vacuole like areas. Surrounding the mucron, clear petal like structures are observed. These structures are small and unequal in size. Nucleus is round and takes up an uniform black stain with Heidenhain iron alum Haematoxylin. In a few instances the nucleus was

PLATE XVII

Figs. 1 - 2. Photomicrographs of *Stomatophora* n.sp(b).

1. 10 X 100X.
2. 10 X 40X.

PLATE XVII



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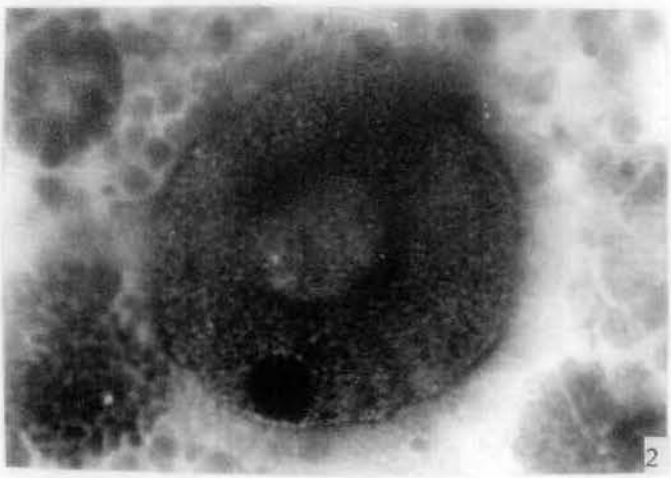

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ellipsoidal in shape. With toluidine blue stain there is an indication of the presence of a small karyosome.

Gametocysts that are observed are always spherical. The spores are irregularly crowded within the cyst.

Spores are navicular.

For measurements see Table XIII

LIFE HISTORY

(Plate XVIII)

When two trophozoites become mature, they approach each other, attach and their suckers fuse dissapearing thereafter. Both the gametocytes secrete a cyst wall around themselves. Two gametocytes enclosed by a cyst wall undergo reduction in volume. As a result the gametocyst that is formed is much smaller in size than the actual sizes of the two initial gametocytes. In fixed and stained smears, a considerable space between the cyst wall and the gametocytes is occassionally seen. This may be an artifact, caused by the action of the fixatives. The gametocyst is always spherical in shape.

Gametes are formed by the division of the nucleus and the accumulation of a bit of cytoplasm around each of

T A B L E XIII

Measurements of Stomatophora n.sp.(b)

	RANGE	AVERAGE
Body diameter	50.4 μ - 93.6 μ	71.64 μ
Nucleus	10.8 μ - 18 μ	15.08 μ
Sucker	21.6 μ - 39.6 μ	29.28 μ
Mucron	10.8 μ - 28.8 μ	19.26 μ
Spores length	7.10 μ	7.1 μ
Spores breadth	3.55 μ - 4.4 μ	4.02 μ
Gametocysts	90 μ - 118.8 μ	110.16 μ
Gametes	1.42 μ - 2.1 μ	1.86 μ

PLATE XVIII

Fig. 1 Trophozoites *Stomatophora* n.sp.(b)

Fig. 2 Syzygy.

Fig. 3 Nuclear fission in the two gametocytes.

Fig. 4 Gametes, formed by the accumulation of cytoplasm, around each nucleus are aligned in the periphery of the gametocyst. Residual cytoplasm is seen in the centre.

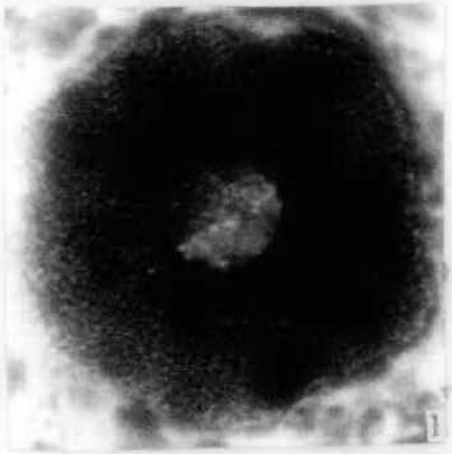
Fig. 5 Formation of sporocysts inside the gametocyst.

Fig. 6 Mature gametocyst packed with sporocysts.

Fig. 7 Individual spores 10 X 100X

Fig. 8 Development of a young trophozoite within the sperm morula.

PLATE XVIII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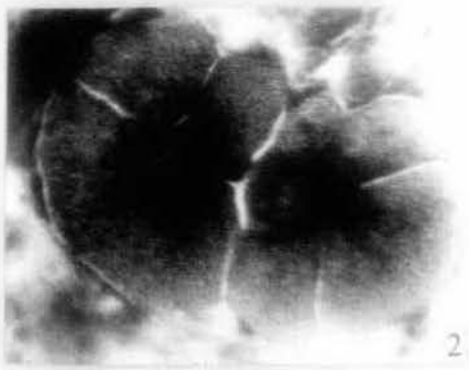
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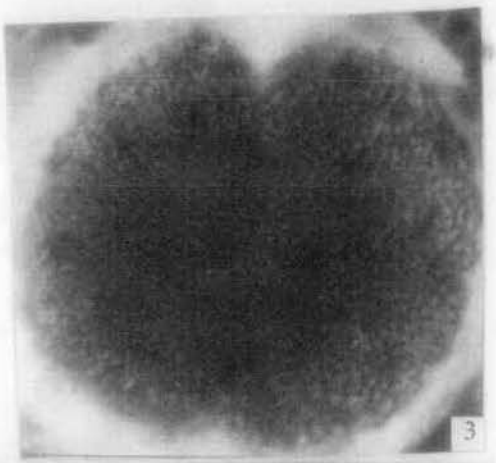
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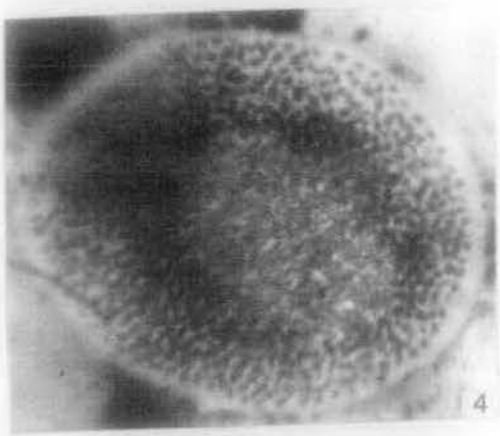
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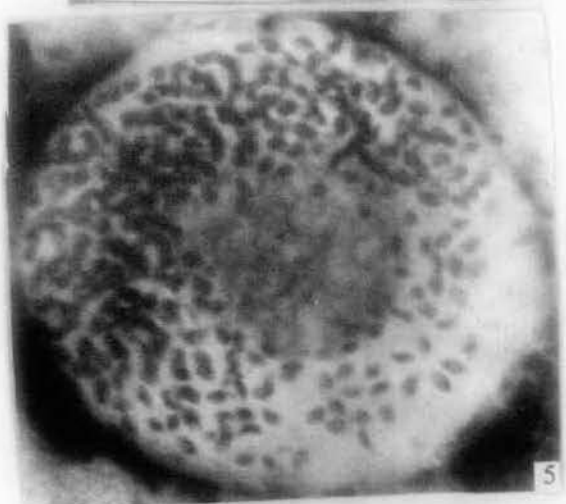
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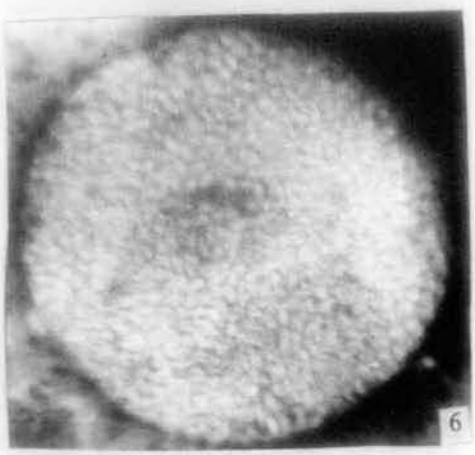
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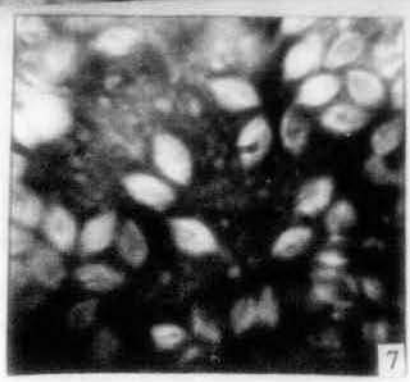
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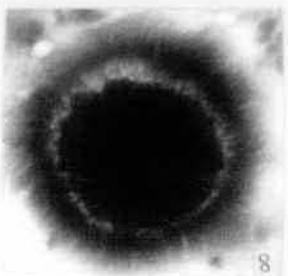
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the products of division. The line of demarcation between the two gametocysts is still discernible at this stage. Gametes arrange themselves along the periphery of the gametocysts. The gametes derived from one gametocyte then fuse with those derived from the other.

Each individual zygote secretes a hard chitinous wall and becomes navicular spores. The gametocyst always shows a bit of residual cytoplasm which soon disappears. A mature gametocyst is completely packed with spores. The spores are octozoic.

Discussion:

Stomatophora n.sp.(b) does not resemble any known species described so far under the genus. It differs from Stomatophora coronata, Stomatophora diadema, in the position of the sucker. It has close resemblance to Stomatophora n.sp.(a) except that the mucron in the parasite under report is wide and filled with small vacuolar structures. The pattern of the sucker also varies. The parasites under report also differs from Stomatophora n.sp.(a) in the measurements of the body and can safely be regarded as a distinct species.

Diagnosis

Family Stomatophoridae Bhatia

Trophozoites are spherical to cylindrical or cup- shaped with sucker-like epimerites; spores are navicular, ends truncated. Octozoic, in seminal vesicles of Pheretima.

Genus Stomatophora Drzhevetskiy

Spherical or ovoid; anterior end with a sucker-like epimerite with a central mucron; spores navicular. Usually in the seminal vesicles of Oligochaetes.

Specific characters

Body spherical; periphery of the body is often deeply indented giving a petaloid appearance to the body. Presence of a central sucker with a wide mucron filled with vacuole like areas. Nucleus is eccentric in position and spherical. Cytoplasm is alveolated. Spores navicular and truncated. Coelozoic.

Host: Pheretima diffringens Baird (Oligochaete)

Site of infection: Seminal vesicles.

Locality: Pedong(altitude 1209 m) Darjeeling district, West Bengal.

NEW GREGARINE PARASITE (No.14)

Stomatophora n.sp. (c)

(Plate XIX, Fig. 1-2)

Occurrence

The parasite has been detected in the smear of the seminal vesicle of Pheretima alexandri(Beddard), an Oligochaete collected in the month of July 1978, at Jor Pokhari (altitude 2171m), Darjeeling district, West Bengal.

Morphology

Trophozoites are spherical in shape and are surrounded by a fine pellicle. Cytoplasm is alveolated. Nucleus is situated near the periphery of the body. It is spherical in shape and takes up a dark stain uniformly. A karyosome is not visible. The sucker is the most characteristic feature of the parasite. It is more or less centrally placed. A mucron is not distinguishable. From the sucker blunt and long prolongations radiate outwards. The number of these prolongations vary from one individual to another. The entire sucker is surrounded by a dark halo. For measurements see Table XIV.

Discussion :

Stomatophora n.sp.(c) does not resemble any known species described under the genus so far. It has got apparent similarity with Stomatophora bengalensis and Stomatophora nautiyali (A. Chatterjee 1971 - thesis unpublished) in that the gregarine is discoid in shape. But the sucker pattern is entirely different, whereas the mucron is present in all the Stomatophorids that have been described by A. Chatterjee, it is absent in the gregarine under report. The blunt prolongations are not constant in number. Variations also occur in the size of the parasites.

It differs from Stomatophora n.sp.(a) and Stomatophora n.sp.(b) in the shape and structure of the sucker. In both the above cases the outline of the parasite dips down at certain intervals giving the parasite a petaloid appearance. This is not the case in the Stomatophora under report. Again the sucker pattern is an entirely new feature. No petaloid crown is present.

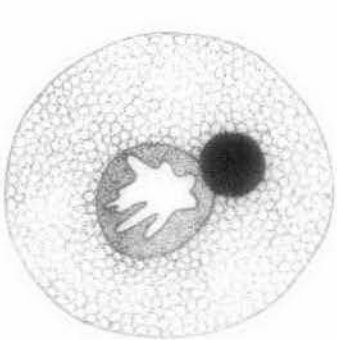
The differences are conspicuous and the parasite can safely be regarded as a new species under the genus.

PLATE XIX

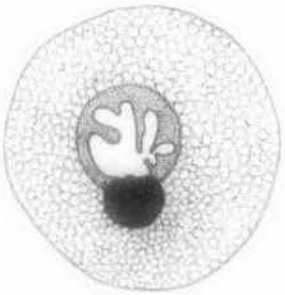
Figs.1 - 2. Stomatophora n.sp.(c) 10 X 45X.

Fig. 3. Stomatophora n.sp.(b) 10 X 45X.

PLATE XIX

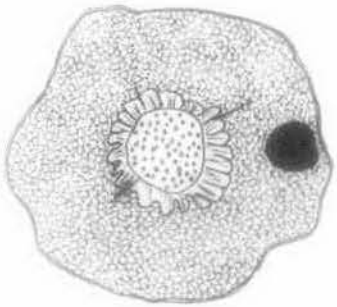


1



2

18 μ



3

Diagnosis

Family Stomatophoridae Bhatia

Trophozoites are spherical to cylindrical or cup- shaped with sucker like epimerite; spores are navicular, ends truncated. Octozoic; in seminal vesicles of Pheretima.

Genus Stomatophora Drzhevetskiy

Spherical or ovoid; anterior end with a sucker like epimerite with a central mucron; spores navicular. Usually in the seminal vesicles of Oligochaetes. The parasite has been placed under the genus.

Specific characters:

Body spherical; periphery of the body is often unbroken giving the parasite a spherical appearance. Presence of a central sucker which has no central mucron. Nucleus is eccentric in position and spherical. Cytoplasm is alveolated. Spores navicular and truncated. Coelozoic. For the present the parasite has been referred to as Stomatophora n.sp.(c).

Host: Pheretima alexandri Beddard (Oligochaeta.)

Site of infection: Seminal vesicles

Locality: Jor Pokhari (altitude 1271m), Darjeeling district, West Bengal.

TABLE XIV

Measurements of Stomatophora n.sp.(c)

	RANGE	AVERAGE
Body diameter	30.6 μ - 97.2μ	66.2 μ
Nucleus	10.8 μ -25.2 μ	14.6 μ
Sucker	12.6 μ -39.6 μ	24.3 μ

NEW GREGARINE PARASITE (No.15)

A (a) n.gen., n.sp.

(Plates XX - XXII, Figs. 1 - 3)

Occurrence

The parasite has been detected in the smear of the coelomic fluid of Amynthas hawayanus (Rosa), an Oligochaete collected in the months of October, November, May, June and July 1976, at Goomti (altitude 1372m), Darjeeling district, West Bengal.

Morphology:

Two forms of the trophozoite were encountered. A larger form and a smaller form.

Larger form:

Mostly solitary trophozoites were observed and one instance which appeared to be an early gametocyst stage. The gregarines are slightly curved bodies concave on one side and bulging out at the other. Due to active movement the parasite exhibits a large variety of shapes that range from a rotund form to an elongated one. A thin pellicle encloses the body. The cytoplasm is alveolated and packed with paraglycogen granules. At the

two terminal ends of the body two epimerites occur. These epimerites are the characteristic features of the parasite. Each epimerite consists of short, thin and contractile processes, four to seven in number, arranged in the form of a crown at each end of the trophozoite. The processes are extremely mobile and due to contractile nature they appear to change their shape frequently. The granular part of the cytoplasm, i.e. the endosarc is clearly visible within these processes. The granular part is surrounded by a clear extention of the body wall. Nucleus is more or less elongated and gently tapers at the ends. It is eccentric in position. A karyosome is not visible, though a lightly stained area occur in the centre in materials stained with iron alum Haematoxylin stain. A karyosome is however, seen in fresh material examined in saline water. The cytoplasm is heavily laden with reserve paraglycogen masses and take up deep stain.

Smaller forms:

These are very much like the bigger forms, except that there is a slight variation in the shape. The individual is sausage shaped and is occasionally bent in the middle. Epimerites consisting of flexible processes are present at the two extremities as in the case of the bigger forms.

PLATE XX

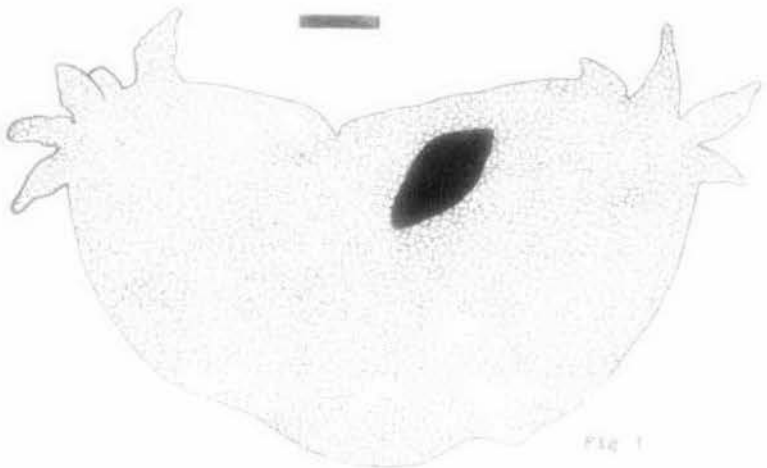


Fig 1

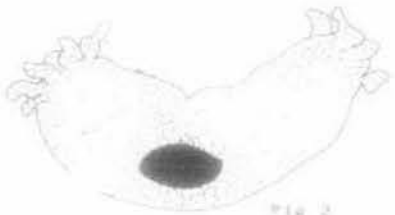


Fig 2

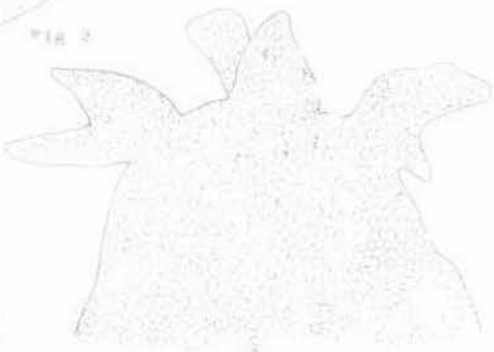


Fig 3

PLATE XX

Fig. 1. Camera lucida drawing of A (a) n.gen. n.sp. (larger form). 10 X 45X.

Fig. 2. Camera lucida drawing of the smaller form of A (a), n.gen.,n.sp. 10 X 45X.

Fig. 3. Epimerite of A (a). 10 X 100X.

PLATE XXI

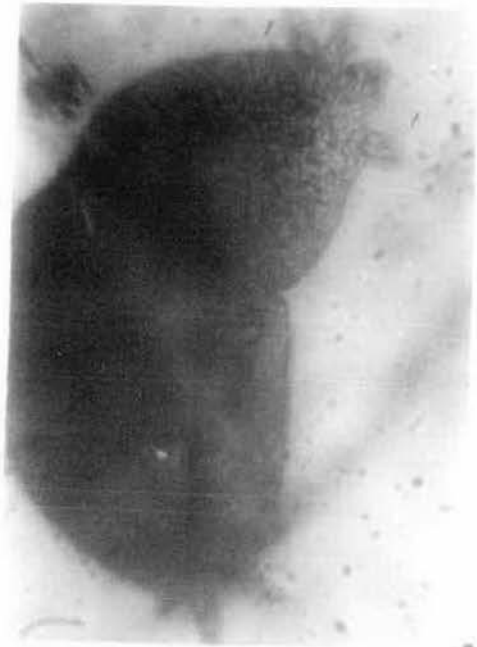
Figs. 1. Smaller form of A(a) n.gen. n.sp.
15 X 40X.

Fig. 2. Larger form of A(a) n.gen. n.sp.
15 X 40X.

Fig. 3. Photomicrograph of an enlarged view of the epimerite of A(a). n.gen.
15 X 100X.

PLATE XXI

1



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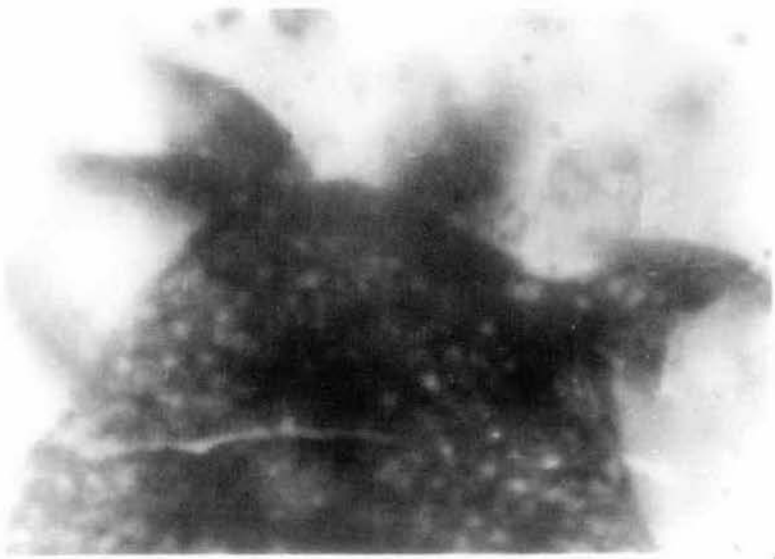
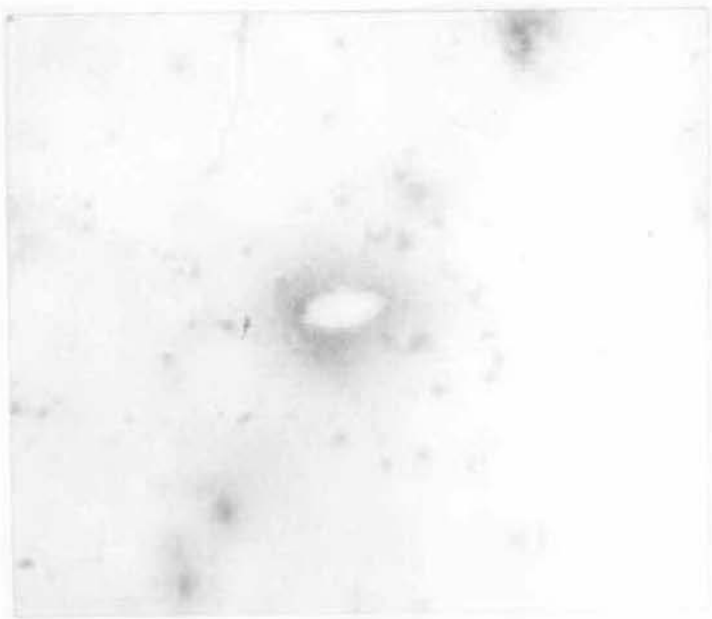


3

PLATE XXII

Fig.1. Spore of A (a) n.gen,n.sp.
10 X 100X.

PLATE XXII



The smaller form has a greater degree of flexibility and as such they present a greater variety of shapes. The nucleus is eccentric in position. As in the case of the bigger form the nucleus tapers gently at the two ends. Cytoplasm of the smaller form possesses scanty paraglycogen material, and takes up light stain.

The spores are biconical in shape.
For measurements see Table XV (a) & Table XVI (b)

Discussion:

A(a)n.gen.,n.sp., shows certain similarities with Dendrocystis (= Arborocystis)piriformes (Rees 1962) in that the epimerite is composed of blunt flexible processes consisting of internal granular endosarc surrounded by a clear ectosarc. The present parasite has two such epimerites at the two poles in contrast to a single epimerite which occurs in Dendrocystis(= Arborocystis) piriformes. Apart from this difference, the two parasites also differ in their choice of host and in their choice of the site of infection, and also in morphological characteristics.

T A B L E XV (a)

Measurements of A(a)n.gen., n.sp.

Larger forms

	RANGE	AVERAGE
Length	140 μ - 306 μ	244.68 μ
Breadth	57.96 μ - 147.06 μ	104.88 μ
Nucleus length	27.06 μ - 43.02 μ	34.16 μ
Nucleus breadth	12.60 μ - 23.40 μ	17.66 μ

T A B L E XVI (b)

Measurements of A(a)n.gen.,n.sp.

Smaller forms

	RANGE	AVERAGE
Length	54.84 μ - 172.44 μ	106.68 μ
Breadth	14.4 μ - 53 μ	29.12 μ
Nucleus length	14.76 μ - 29.52 μ	21.83 μ
Nucleus breadth	5.76 μ - 17.64 μ	9.86 μ

Earlier report of a gregarine possessing two epimerites of the described nature does not exist in the literature.

Due to the occurrence of biconical spores and also due to the fact that the gregarine possess epimerites consisting of flexible processes comparable to that in Dendrocystis(= Arborocystis) piriformis, the parasite under report has been placed under the Family Monocystidae. A new generic name is proposed to be assigned to this parasite.

Diagnosis:

Family Monocystidae Bütschli

Spores spindle-shaped.

The parasite has been placed under the Family on account of this.

Genus A n.gen.

Solitary; presence of sucker-like epimerites at both ends of the body; body elongate, generally concave on one side and convex at the other. Nucleus eccentric and elongated. Spores biconical; Coelozoic.

Specific characters:

Two epimerites consisting of variable number

of flexible processes occur one at each end of the body. Body is concave on one side and is convex on the other side. Nucleus eccentric in position. Spores biconical. Coelozoic.

Host: Amynthas hawayanus Rosa (Oligochaeta)
Site of infection: Coelomic fluid.
Locality: Coomti (altitude 1372m), Darjeeling district, West Bengal.

ADDITIONAL OBSERVATION:

From the survey undertaken in the course of the present study it is found that the parasite is seasonal, that is to say that they are available only at certain periods of the year. In the month of October, the host earthworms showed a very high incidence of infection. The collection in the month of May, showed a far lesser incidence and the few parasites that occurred were immobile. Collections of the samples made in the month of July revealed a total absence of the parasite.

NEW GREGARINE PARASITE (No.16)

B (a) n.gen., n.sp.

(Plate XXIII, Fig. 1 - 4)

Occurence

The parasite has been detected in the smear of the coelomic fluid of Apporectodea trapezoides (Duges) an Oligochaete collected in the months of March and April 1978, at Senchal (altitude 2269.66m) Darjeeling district, West Bengal.

Morphology:

Trophozoites change shape constantly though they retain an elongated appearance in general. The only constant feature as regards its shape is that one end is blunt and rounded while the other tapers to a point. The entire body is enclosed by a thin pellicle. Cytoplasm is filled with large elongated paraglycogen granules, and interspersed in between these granules there occur a large number of deeply stained bodies which are spherical in shape and variable in size. Nucleus flows along with the current of cytoplasm, and in fixed and stained materials it occupies different positions in the body. Nucleus is

ellipsoidal in shape but in occasional specimens it is slightly spherical. Nuclear memberane is very thick at certain places, thin elsewhere , and is unevenly stained with iron alum haematoxylin. Karyosome is variable in shape and size and consists of darkly stained and lightly stained areas. The whole structure of the karyosome is suspended within the nucleus by some thin connections extending towards the nuclear membrane. The empty areas in between these connections give the nucleus a vacuolated appearance.

Spores are large and typically biconical in shape. For measurements see Table XVII.

Discussion

On account of the typically biconical spores that have been found along with the trophozoites, the parasite has been placed under the Family Monocystidae. No member of the various genera that come under this Family have any similarity with the gregarine under report, which is unique in exhibiting a constant change of shape in the living condition. The only other member of the Family Monocystidae, having an elongated shape is Nematocystis.

PLATE XXIII

Figs. 1 - 2. Two forms of the parasite B(a) n.gen. n.sp. The deep stained bodies are seen interspersed in the endoplasm. 6 X 45X.

Fig. 3 - 4. Enlarged view of the nuclei of the above parasites. 10 X 100X.

Fig. 5. Photomicrograph of B (a)n.gen., showing the nucleus and the deep stained bodies. 10 X 40X.

PLATE XXIII

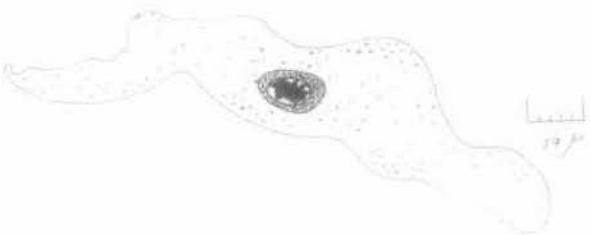


fig 1

fig 2

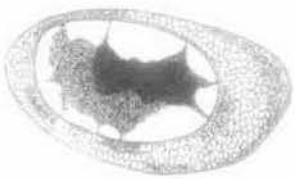
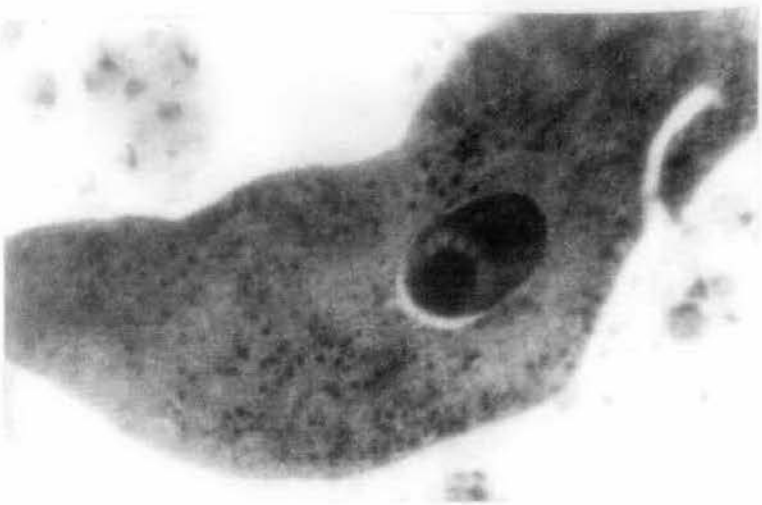


fig 3

fig 4



The parasite under report, however, changes its form constantly unlike Nematocystis , and exhibits distinct polarity in having a rounded end and a tapering end, characters not seen in Nematocystis. The nucleus of this parasite also possesses peculiar features not comparable to anything seen in the nucleus of gregarines belonging to other genera.

In view of these peculiarities it is proposed to create a new genus for receiving this parasite.

Diagnosis

Family Monocystidae Bütschli

Spores spindle shaped.

The parasite has been placed under the Family on account of this.

Genus B n.gen., n.sp.

Solitary; changes shape constantly though they retain an elongated shape in general.

Spores biconical. Coelozoic.

The parasite has been placed under this genus.

Specific characters:

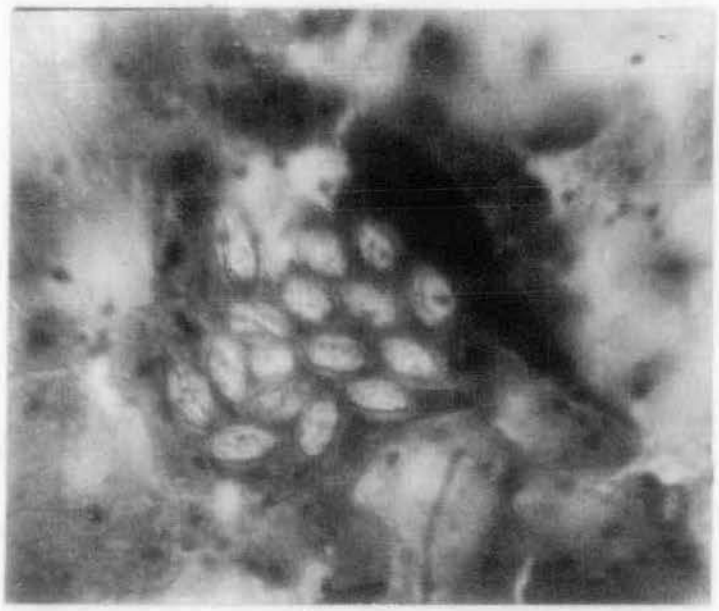
Solitary; body changes shape constantly while retaining its elongated appearance in general. One end is rounded and blunt and

PLATE XXIII A

Speres of B(a) n.gen., n.sp.

10 X 100X

PLATE XXIII A



T A B L E XVII

Measurements of B(a)n.gen.,n.sp.

	RANGE	AVERAGE
Length	254.22 μ - 448.4 μ	320.836 μ
Breadth	34.2 μ - 83.52 μ	51.796 u
Nucleus length	19 μ - 38 μ	26.95 μ
Nucleus breadth	17.10 μ - 25.2 μ	19.94 μ
Paraglycogen granules	.28 μ - 4.40 μ	2.72 μ
Deep stained bodies	.28 μ - 3.55 μ	1.715 μ
Spores	29.52 μ X 10.8 μ	29.5 μ X 10.8 μ

the other end tapers to a point. Nucleus ellipsoidal, with a thick nuclear membrane. Karyosome variable in shape and size and consisting of deeply stained and lightly stained areas... suspended within the nucleus by thin filamentous connections. Cytoplasm dotted with deeply staining bodies; Spores biconical; Coelozoic.

Host: Apporectodea trapezoides Duges (Oligochaeta)

Site of infection: Coelomic fluid.

Locality: Senchal (altitude 2269.66m) Darjeeling, West Bengal.

NEW GREGARINE PARASITE (No. 17)

B (b)n. gen., n. sp.

(Plate XXIV, Figs.1 - 4)

Occurrence

The parasite has been detected in the smear of the coelomic fluid of Apporectodea trapezoides (Duges) an Oligochaete collected in the months of March and April 1978, at Senchal (altitude 2269.66m), Darjeeling, West Bengal.

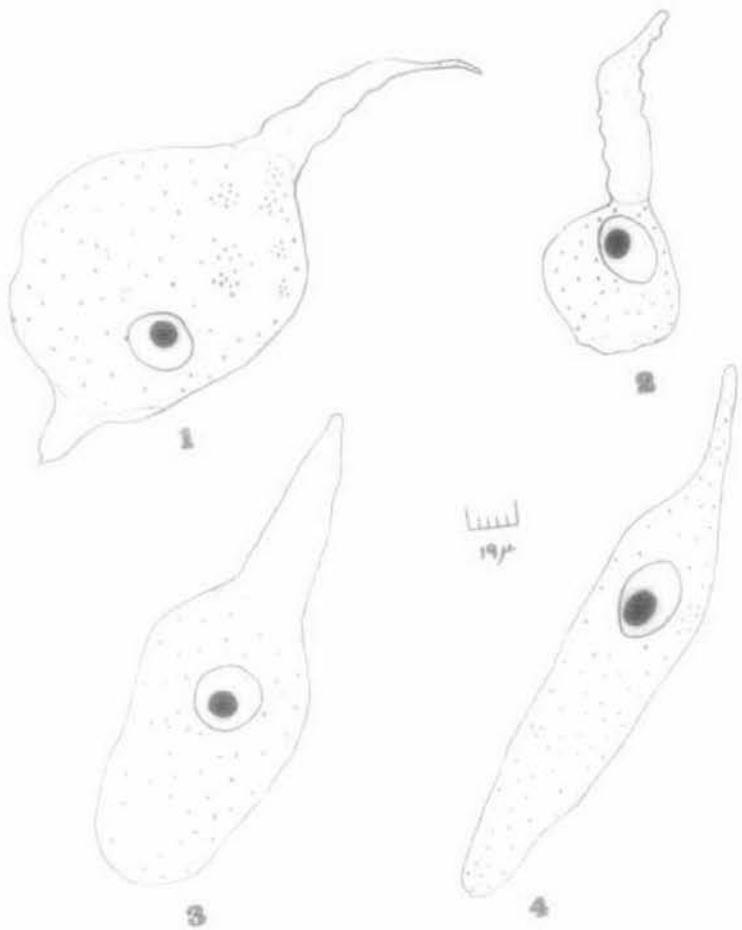
Morphology

Trophozoites change shape actively, forming in a majority of cases one or two elongated processes of the pellicle, at the terminal ends of the body, while the rest of the body, consisting mainly of endoplasm occur as an elongation or as a temporary bulge. Endoplasm is packed with large elongated paraglycogen granules, but scattered in between these there occur a number of deeply staining spherical bodies. Nucleus is ellipsoidal in shape, but spherical ones have also been encountered. In fresh smears the nucleus flows along with the endoplasm. In stained preparations the nucleus takes up various positions. A

PLATE XXIV

Figs. 1 - 4. Various forms of B(b) n.gen. n.sp. The pellicular extentions seen vary in number. 6 X 45X.

PLATE XXIV



spherical and deeply stained karyosome, eccentric in position is present in the nucleus.

Spores are large and biconical in shape. For measurements see Table XVIII.

Discussion:

The present parasite is placed under the Family Monocystidae because of the presence of biconical spores.

Forms having temporary pellicular extentions show some similarity to Dirhynchocystis globosa (Bhatia and Chaterjee 1925) which also exhibit extentions designated 'spine like processes' (Bhatia and Chaterjee 1925). In both the cases the ends of the extentions or 'spine like processes' are not always of the same length, and in both cases these are devoid of the granular endoplasm. This similarity is however, more apparent than real because in D.globosa the spine like processes, are permanent features while the processes seen in the parasite under report are temporary in nature.

D.globosa is restricted to the coelom of Pheretima posthuma and in other species of Pheretima, whereas

TABLE XVIII

Measurements of B (b) n.gen., n.sp.

	RANGE	AVERAGE
Length	63 µ - 323 µ	186.95 µ
Breadth	14.04 µ - 98.08 µ	52.43 µ
Nucleus Length	9 µ - 28.50 µ	19.97 µ
Nucleus breadth	9 µ - 20.90 µ	14.88 µ
Karyosome	6.48 µ - 11.04 µ	7.48 µ

the present parasite occurs in the coelomic fluid of Apporectodea trapezoides.

The parasite differs from B (a)n.gen., n.sp. in the structure of the nucleus and in possessing extentions of the body formed of pellicle alone.

In view of the inconstant shape of the body, it has been placed under genus B , and due to the presence of occasional temporary pellicular projections simulating permanent spine like processes of Dirhynchocystis, features found nowhere else in other gregarines, it is felt necessary to erect a new species to receive this parasite.

Diagnosis:

Family Monocystidae Bütschli

Spores spindle shaped.

The parasite has been placed under the Family on account of this.

Genus B n.gen.

Solitary; changes shape constantly though they retain an elongated appearance in general. Spores biconical; Coelozoic.

Specific characters:

Trophozoites change shape actively, and in a majority of cases produces one or two elongated pellicular projections, the rest of the body consists of endoplasm which is either elongated or appear as a temporary bulge. Nucleus spherical to ellipsoidal with a large spherical karyosome. Endoplasm dotted with several deeply staining bodies. Spores biconical. Coelozoic.

Host: Apporectodea trapezoides Duges (Oligochaete)

Site of infection: Coelomic fluid.

Locality: Senchal (altitude 2269.66m) Darjeeling, West Bengal.

NEW HOST RECORD

Stomatophora coronata has been previously recorded by Hesse (1909) from Pheretima hawayanus and Bhatia (1924) from Pheretima barbadensis.

During the present investigation it was found that this parasite occurred also in Pheretima californica, differing only in measurements.

GENERAL CHARACTERS of Stomatophora coronata. (Hesse)

Body ellipsoidal; sucker present at the anterior end. The sucker consists of a central mucron surrounded by a crown of petals, which are variable in number. The nucleus is spherical. See Table No. XIX for a comparison of Stomatophora coronata discussed earlier and the same parasite reported here.

DISCUSSION

From the above data, it is clear that the species obtained from Kalimpong are much smaller in

in size than the one described by Hesse (1909) and Bhatia (1924). Apart from the size factor, the general body outline of the parasite, spores and sucker are more or less similar as also the various stages of the life cycle of the parasite.

It is possible that the variations in the size could be due to environmental factors, particularly so as the altitude of Kalimpong is much higher than that of Punjab or Lahore.

Hence to date it can be concluded that Stomatophora coronata , has been recorded from India for the second time with its host being Pheretima californica; the specimens were collected from Kalimpong (alt. 1249m). Pheretima barbadensis was collected from Punjab and Lahore by Bhatia.

It may also be mentioned here that Pheretima californica has been recorded for the first time from India, (Soota 1977). It was recorded previously from several seperated areas outside India.

TABLE NO: XIX

COMPARISON BETWEEN *S. coronata* (HESSE 1909) & *S.coronata* UNDER REPORT.

	S. coronata (Hesse)	S.coronata under report
Length	38 μ - 80 μ (average) 147 μ - 180 μ (maximum)	11 μ - 72 μ
Breadth	60 μ - 105 μ (maximum)	21 μ - 46 μ
Gametocysts	60 μ - 70 μ (average)	45 μ - 65 μ
Spores	Macro - 9 μ-11 μ by 5 μ-6 μ Micro - 7 μ-7.5μ by 3 μ	10.8 μ by 5.4 μ
Host	*Pheretima barbadensis*	*Pheretima californica*
Locality (Indian)	Punjab & Lahore	Kalimpong

REVIEW AND CHECKLIST OF THE ACEPHALINE GREGARINES FOUND IN THE TERRESTRIAL OLIGOCHAETES (EARTHWORMS) IN INDIA

(* indicates new species described in the thesis)

Family:- Monocystidae Bütschli,

Genus:- Monocystis Stein

1. M. beddardi Ghosh 1923
   In the seminal vesicles of Eutyphoeus nicholsoni, collected from Calcutta, West Bengal.

2. M. bengalensis Ghosh 1923
   In the seminal vesicles of Pheretima posthuma, collected from Calcutta, West Bengal.

3. M. lloydi Ghosh 1923
   In the seminal vesicles of Pheretima posthuma collected from Calcutta, West Bengal.

4. M. pheretimi Bhatia et Chaterjee, 1925
   In the seminal vesicles of Pheretima posthuma, collected from Punjab and Bombay.

5. *Monocystis n.sp.(a)
   In the seminal vesicles of Apporectodea trapezoides, collected from Senchal (alt. 2269.66 m), Darjeeling.

Genus:- Nematocystis Hesse

6. N. hessei Bhatia et Chaterjee, 1925.
   In the seminal vesicles of Pheretima heterochaeta, collected from Punjab.
7. N. plurikaryosomata Bhatia et Chaterjee 1925.
   In the seminal vesicles of Eisenia foetida , collected from Punjab and Kasauli.
8. N. stephensoni Bhatia et Setna 1926.
   In the seminal vesicles of Eutyphoeus incommodus, collected from Punjab and Kasauli.
9. N. vermicularis Hesse 1909.
   In the seminal vesicles of Pheretima barbadensis, collected from Punjab.
10. * Nematocystis n. sp. (a)
    In the coelomic fluid of Apporectodea trapezoides, collected from Senchal (alt. 2269.66m), Darjeeling.
11. * Nematocystis n. sp. (b)
    In the coelomic fluid of Pheretima diffringens, collected from Goomti (alt. 1372m), Darjeeling district.
12. * Nematocystis n. sp. (c)
    In the seminal vesicles of Eutyphoeus gammiei, collected from Mangpoo (alt. 2164.75m), Darjeeling district.
13. * Nematocystis n. sp. (d)
    In the coelomic fluid of Pheretima diffringens,

collected from Goomti (alt. 1372m), Darjeeling district.

14. * Nematocystis n.sp(e)

In the seminal vesicles of Eutyphoeus gammiei, collected from Mangpoo (alt. 2164.75m), Darjeeling district.

Genus: Apolocystis Cognetti de Martiis .

15. A. mattheii Bhatia & Setna 1926.

In the seminal vesicles of Megascolex trilobatus, collected from Bombay.

16. * Apolocystis n.sp.(a)

In the coelom, growing on top of the dorsal vessel of Pheretima robusta, collected from Darjeeling (alt. 2001m)

17. * Apolocystis n.sp.(b)

In the coelom, growing on top of the dorsal vessel of Pheretima robusta, collected from Darjeeling (alt. 2001m)

18. * Apolocystis n.sp.(c)

In the coelom, growing on top of the alimentary canal (anterior region) of Pheretima alexandri, collected from Kalimpong (alt. 1249.7m) Darjeeling district.

19. * Apolocystis n.sp.(d)

In the coelomic fluid of Pheretima diffringens, collected from Goomti (alt. 1372m), Darjeeling district.

Family: Zygocystidae Bhatia

Genus: Zygocystis Stein .

20. * Zygocystis n.sp.(a)

In the seminal fluid of Pheretima californica, collected

from Kalimpong (alt. 1249.7m), Darjeeling district.

Family: Stomatophoridae Bhatia

Genus: Stomatophora Drzhevetskiy

21. S. bulbifera Bhatia and Setna 1926

In the seminal vesicles of Pheretima elongata, collected from Bombay.

22. S. coronata Bhatia 1924.

In the seminal vesicles of Pheretima barbadensis, collected from Punjab.

23. S. diadema Bhatia 1924.

In the seminal vesicles of Pheretima barbadensis collected from Punjab, and Pheretima posthuma collected from Calcutta.

24. * Stomatophora n.sp.(a)

In the seminal vesicles of Pheretima diffringens, collected from Pedong (alt. 1209m), Darjeeling district.

25. * Stomatophora n.sp.(b)

In the seminal vesicles of Pheretima diffringens collected from Pedong (alt.1209m), Darjeeling district.

26. * Stomatophora n.sp.(c)

In the seminal vesicles of Pheretima collected from Jor Pokhari (alt. 2171m), Darjeeling district.

Genus: A n.gen.

27. * A (a) n.gen. n.sp.

In the coelomic fluid of Amynthas hawayanus, collected from Goomti (alt. 1372m), Darjeeling district.

Genus: B n.gen.

28. * B (a) n.gen. n.sp.

In the coelomic fluid of Apporectodea trapezoides, collected from Senchal (alt. 2269.66m), Darjeeling.

29. * B (b) n.gen. n.sp.

In the coelomic fluid of Apporectodea trapezoides, collected from Senchal (alt. 2269.66m), Darjeeling.

UNPUBLISHED WORK ON GREGARINES OF INDIAN EARTHWORMS

While reviewing the literature on gregarines described from Indian earthworms, I have come across descriptions of twenty seven new taxa proposed by Dr. A. Chatterjee in his thesis submitted for the Ph.D. degree of the University of Calcutta, 1971.

Consideration of these taxa posed a problem, as the proposed scientific names remain unpublished. The matter was referred to Dr. R.V. Melville, Secretary, International Commission on Zoological Nomenclature, and he kindly opined that under the circumstances it will be right to ignore Dr. Chatterjee's new names, as his work does not satisfy Articles 8 - 9 of the International Code of Zoological Nomenclature.

It may be mentioned here, that none of the gregarines described by me in the thesis resemble those described by Chatterjee, 1971.

The letter from Dr. Melville is given in the Appendix.

P A R T II

CYTOCHEMICAL STUDIES OF THE GREGARINES

STUDIED IN PART I

INTRODUCTION

Gregarines have been the objects of great interest and much work has been done on their systematics. However, little information has been done on their cytochemical nature.

The polysaccharide content of the gregarines have been of interest to many workers in the field. It has been found to be present in a smaller or larger quantity in all the gregarines studied so far. The knowledge on the chemical nature of the paraglycogen has been extended by Mercier, Schrevel and Stark (1973), who have classified the reserve polysaccharide in Gregarina blattarum as an amylopectin. In the present study the presence of such reserve bodies reacting positively for polysaccharides has been traced in the different stages of the life cycles of various gregarines of earthworms. Such study on an earthworm gregarine was undertaken earlier by Sathanantnan (1977) who worked on Zeylanocystis burti.

The presence of DNA in the nucleus and RNA in the nucleolus and cytoplasm is well known. The presence of

extranuclear DNA in sporozoa has evoked the interest of many investigators in the field. (Preer 1950, Lwoff 1952, Ray and Gill 1955, Dasgupta 1959). They assumed these structures to be of bacterial or viral nature. In the present study toluidine blue and Fluorescence microscopy have been used for studying the Feulgen-positive and methyl green positive bodies in the cytoplasm of gregarines.

In this part of the investigation (Part II), a complete cytochemical study on the polysaccharide and nucleic acid contents in the different stages of the life cycles of the gregarines including the abnormal trophozoites that have been studied in Part I of the thesis is reported.

MATERIAL AND METHODS:-

Earthworms from various localities in Darjeeling district were brought to the laboratory, dissected, and smears of the coelomic fluid as well as of the seminal vesicles and cysts were prepared on clean dry glass slides.

Where sections were required seminal vesicles were taken out and fixed in freshly prepared Carnoy's fixative for about ten minutes, dehydrated and infiltrated with cedarwood oil overnight, cleared in Xylol and embedded in paraffin for one hour at 60°C. Sections were cut at 6 μ and 3 μ thickness, the latter being used for fluorescence microscopy.

The following histochemical staining methods were used.

A. Feulgen Reaction (Feulgen and Rossenbeck 1924, modified after Pearse 1968) to detect the presence of DNA.

B. Detection of DNA and RNA by

(i) Methyl green-pyronin stain after Kurnick 1955

(ii) Acridine Orange method for nucleic acids (DNA and RNA) by Fluorescence method after Armstrong, 1956.

C. Periodic Acid- Schiff method after Glick 1949, was used for the detection of Polysaccharides.

D. Carmine stain for glycogen (see Glick 1949) and Bauer Feulgen (see Glick 1949) for glycogen was used.

E. Toluidine Blue stain (see Pearse 1968) was used for studying basophilia.

PERIODIC ACID/SCHIFF REACTION IN SOME OF THE GREGARINES STUDIED IN PART I

(Plates XXV Figs 1 - 3)

Apolocystis n.sp(a)

Trophozoites:-

The reacting substance occurred as red spherical bodies. Such bodies filled up the cytoplasm. The nuclear area appeared colourless.

Gametocytes:-

Cytoplasm of the gametocytes was filled up with spherical bodies similar to those seen in the trophozoites. The wall of the gametocyst was negative to PAS technique.

Gametes:-

The cytoplasm of the gametes took up deep red colour.

Spores:-

Spore was faintly positive to PAS reaction.

Apolocystis n.sp.(b)

The colour reaction in the trophozoites, gametocytes, gametes and sporoblasts was similar to that seen in the corresponding stages of the parasite, Apolocystis n.sp.(a).

The colour reaction in the spores differed from that seen in the spore of the parasite described earlier, in that the polar ends of the spores took up deep red colour while the main part of the walls of the spores was negative to the reaction.

In both the above cases the reacting materials could be eliminated by treatment with saliva at 37°C, for an hour.

Apolocystis n.sp. (c)

Due to the paucity of materials, cytochemical work on Apolocystis n.sp.(c)could not be carried out.

Apolocystis n.sp.(d)

Normal trophzoites:

The cytoplasm was filled with minute spherical bodies positive to PAS reaction. A negative reaction was noted in the nucleus.

Abnormal trophozoites:

Black patches described in page127, seen in the abnormal forms of the parasite were negative to the reaction, but took up Delafield haematoxylin which was used as a counterstain. Certain granules in between the reacting bodies were also stained with haematoxylin. When treated with saliva at 37°C for about an hour the reacting bodies disappeared.

A(a) n.gen.,n.sp.

Trophozoites:

The two forms of the parasite (larger and the smaller form) varied in their reaction.

Larger forms:

The cytoplasm of these forms were packed with [illegible] bodies which took up deep red colour while the nucleus did not react to the stain.

The nature of the reacting substance could be confirmed with Bauer Feulgen method.

Smaller forms:

With the PAS technique the reacting substance was found to occur as red spherical granules but these granules

were fewer in number and widely scattered.

The rest of the body looked dull blue - when Delafield haematoxylin was used as a counterstain. In both the above cases the reacting substance could be removed by treatment with saliva.

Stomatophora n.sp.(a)

Trophozoites:

Cytoplasm was packed with deeply staining granules. In some cases these granules lay scattered in the cytoplasm.

Gametocyst:

The wall of the gametocyst did not take up stain. The residual cytoplasm in the gametocyst occurred as deep red mass.

Gametes and Zygotes:

Cytoplasm of the gametes and zygotes looked red.

Spores:

Spores were faintly positive to the reaction. In all the stages mentioned above, the reacting material disappeared when treated with saliva for about an hour at 37°C.

Stomatophora n.sp.(b)

The staining reaction of this parasite was the same as in the corresponding stages of Stomatophora n.sp.(a).

Zygocystis n.sp.(a)

Normal trophzoites:

The trophozoites both solitary and in a state of syzygy showed strong affinity to periodic acid/Schiff method. The intensity of staining reaction varied from one individual to another. Under high magnification, the reacting materials were found to be deeply stained on the periphery and lightly in the middle. The trophzoites stained red with Best Carmine method.

Abnormal trophozoites:

The abnormal trophozoites were distinctly negative to periodic acid/Schiff method. In cases where the infection occurred in patches, the normal cytoplasm stained red with the stain, but the infected areas remained colourless.

Gametocyst:

The wall of the gametocyst was negative to the reaction; the residual cytoplasm was however strongly positive to the reaction.

Sporozoites:-

The cytoplasm was positive to the periodic acid/Schiff reaction.

Spores:-

A weak reaction was detected in the wall of the spore. Two plug-like structures present at the two ends of the spore were negative to the reaction.

The positive reaction in all the stages mentioned above could be removed by the treatment with saliva at 37°C.

B (a) n.gen. n.sp.

Cytoplasm of the gregarine was filled with large elongated bodies that took up red colour very deeply. In between the reacting materials, haematoxylin-stained round bodies were visible. Saliva controls could not be tested due to the lack of sufficient material.

Nematocystis n.sp(a)

This parasite presents a very remarkable study as the cytoplasm hardly contained any reacting substances. Some cases were encountered where there were no signs of the reacting material, and yet others

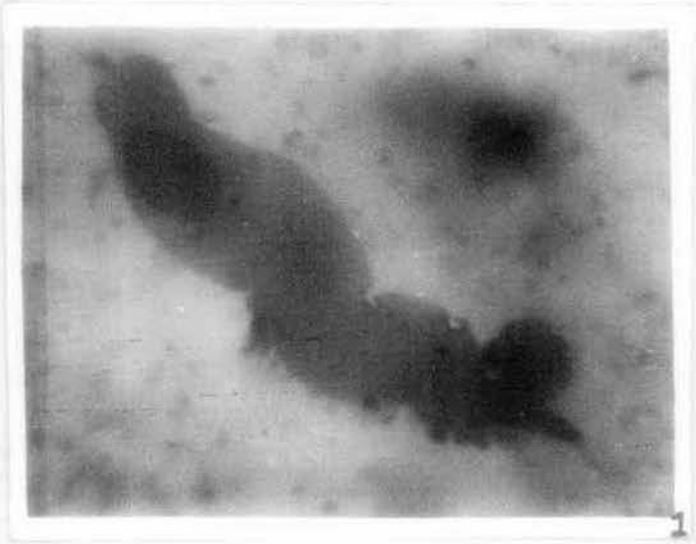
P L A T E XXV

Fig. 1. Photomicrograph of Nematocystis n.sp(a) showing the rarity of paraglycogen grains. About 6 can be seen in the cytoplasm. 6 X 40X

Fig. 2. Photomicrograph of B(a) showing the paraglycogen granules. Note the black bodies in the cytoplasm. Nucleus is masked by the paraglycogen granules 10 X 10X

Fig. 3. Photomicrograph of A (a) showing the paraglycogen granules. 10 X 10X.

PLATE XXV



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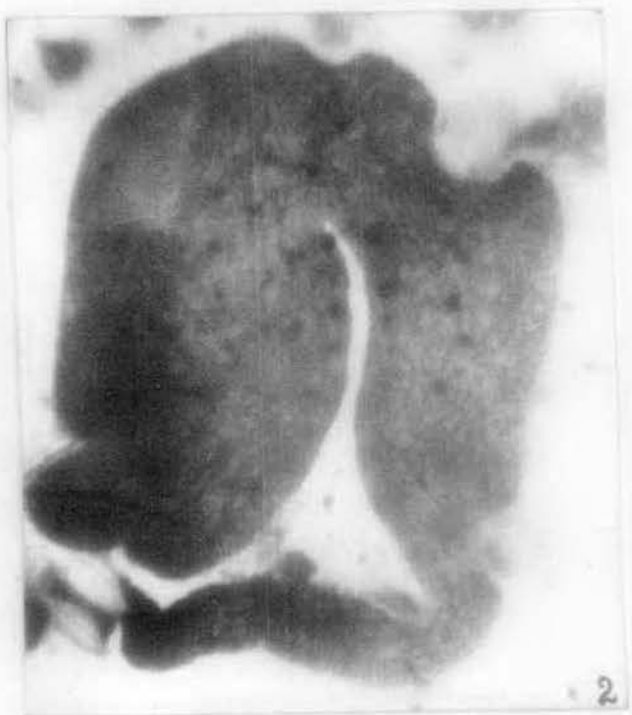

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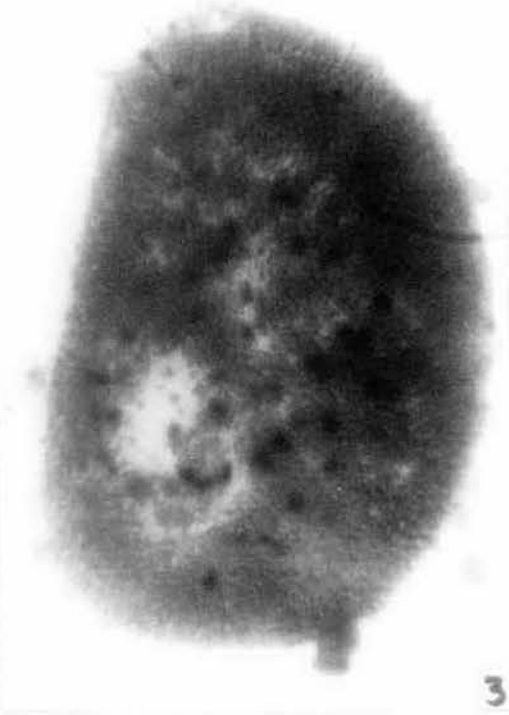


3

were present which possessed about six or seven such granules. Saliva controls could not be tested for paucity of material.

Nematocystis n.sp.(c)

Trophozoites:-

Cytoplasm was strongly positive to periodic acid/ Schiff reaction. A negative reaction was noticed in the nucleus.

B (b)n.gen.,n.sp.

Trophozoites:-

Cytoplasm was strongly positive and appeared purplish red to periodic acid/ Schiff reaction. The nucleus was negative to the reaction.

DISCUSSION

Paraglycogen is known to constitute the largest granular inclusions in the cytoplasm of gregarines in general, and the most widely distributed polysaccharides of parasites is undoubtedly glycogen (von Brandt 1966). Such material has also been considered to represent amylopectin in some of the gregarines (Mercier, Schrevel and Stark 1973). However , in most of the

cases studied here, the polysaccharide present could be identified as glycogen by using saliva digestion method. In A(a)n.gen., n.sp. and Zygocystis (a) the presence of glycogen could also be confirmed by using Bauer-Feulgen and Best Carmine methods respectively. The results recorded here are also in contrast to that reported by Dutta (1962), who stated that paraglycogen present in Stomatophora diadema consisted of neutral polysaccharides other than glycogen. In the course of the present study the reacting materials in the two Stomatophorids Stomatophora n.sp.(a) and Stomatophora n.sp.(b), could be identified as glycogen by using saliva digestion method.

In the course of the present investigation it has been noted that the granules reacting positively to the periodic acid/Schiff technique varied in shape and size from one parasite to another. For example in Apolocystis, n.sp. Stomatophora, Zygocystis and in A(a) these were spherical and granular in shape, but varied from spherical to elongated in the different species of Nematocystis, and elongated in A(a). In most instances the paraglycogen granules present in this parasite were slightly curved. All these granules lay scattered and were not localised in any

specific area in the cytoplasm of any of these parasites. The number and the concentration of the granules varied from one individual to another, of the same species.

The heavy deposition of the glycogen in the cytoplasm of the parasites studied here could mean either that these were used up as energy by the parasites or that these represented reserve food materials to be used in future. According to von Brand (1952) it is an universally accepted fact that the endogeneous glycogen masses have an exogeneous source i.e., these are derived from carbohydrates imbibed as food from the host. The same author also notes that the production of energy is not always the sole purpose of glycogen, and that glycogen can be used also for the synthesis of chitin of exoskeleton and the chitinous egg membrane. Gill and Ray (1954) assumed that in all probability the glycogen in E. tenella contributed to the formation of various structures developing within the oocysts. The same authors believed that the presence of some positively reacting material in the sporozoites could mean that these were used up as energy to enable them to penetrate the tissues of the host.

Sathananthan (1977) stated that the materials reacting positively to PAS technique, on the walls of the spores could be a precautionary measure on the part

of the parasite to tide over the unfavorable conditions outside the host, implying thereby that these stages would remain dormant and survive long periods before infecting another host.

In Zygocystis n.sp.(a), the entire area of the cytoplasm in some cases assumed a brilliant colour, while yet others presented the reacting materials as numerous granules scattered all over the cytoplasm.

In the abnormal individuals of the same parasite, the infected patches failed to respond to the periodic acid/Schiff method; the only plausible explanation that can be given is that the waste products and secretions given out by the hyperparasites represented areas which yielded negative reaction to the PAS technique.

In Apolocystis n.sp.(d), the presence of the patches of cytoplasmic inclusions, appeared to have no effect on the number and concentration of the paraglycogen granules lying elsewhere in the cytoplasm of the host.

In the studies made regarding the distribution of paraglycogen granules in the different stages of the life cycle, it has been noted that the amount is heavy

in the trophozoite and the gametocyte stages. During gametogenesis, the residual cytoplasm shows strong positive reaction to PAS technique. Later on the positively reacting material is entirely used up when the spores start developing.

The trophozoite imbibes as much food as possible and builds up a good glycogen reserve which is utilized subsequently by the parasite. As the gametes and the spores start to develop these reserve food materials are gradually used up. Finally when the spores are fully formed all the glycogen reserve materials are used up.

No differences in the amount of polysaccharides was noted when studies were made in the different seasons of the year.

Nematocystis n.sp.(a) presented a very interesting case in that the glycogen granules were few and could be counted easily. Instances where just one or two granules were present were many and in a few individuals a complete absence of the reacting material was noted. A survey of literature reveals that all gregarines should have a smaller or larger store of polysaccharides.

The rapidity with which Nematocystis n.sp.(a)moves and the manner in which it carries out its metabolic activities with hardly any reserve food materials as a source of energy is a matter which needs further investigation.

In the smaller forms of A(a) the amount of glycogen reserves were far less in amount than in the bigger forms. This could mean that these were the younger stages of the parasite, and as they grew up they accumulated more and more of the glycogen which is further stored up in the cytoplasm.

SUMMARY

In the above investigation which has been conducted to study the polysaccharide content in the different stages of the life cycles of gregarines of earthworms, the following conclusions have been reached:-

1. The polysaccharide present in the different stages of the life cycle is undoubtedly glycogen.

2. The polysaccharide content is maximum in the trophozoite and gametocyte stages and decreases gradually during development.

3. Infected areas of Zygocystis n.sp(a) failed to show any presence of polysaccharides.

4. Nematocystis n.sp(a) is unique in that it hardly posseses such reserve bodies in its cytoplasm.

THE STUDY OF NUCLEIC ACIDS IN SOME OF THE GREGARINES STUDIED IN PART I

(Plate 26, Figs.1-2)

(Plate 27, Figs.1-2)

Apolocystis n.sp.(a)

The nucleus of the trophozoite was distinctly negative to Feulgen reaction. Certain individual trophozoites were found to harbour Feulgen-positive bodies in the cytoplasm. Usually these bodies were in the form of minute granules. In two instances, however, young trophozoites showed patches of Feulgen-positive areas in the cytoplasm. The use of pyronin-methyl green stain produced comparable results in that the nucleus showed no trace of methyly green. The nuclear membrane and the area adjoining the membrane took up the pyronin stain deeply, becoming lighter towards the central region.

Violet metachromasy was noticed in the nuclear membrane and the area immediately beneath it. The central area of the nucleus was lightly stained. Several metachromatic granules were present in the ground matrix of cytoplasm as minute dots.

Gametocytes:-

The gametocytes were distinctly Feulgen-negative and metachromasy existed in the cytoplasm and periphery of the nucleus.

Gametes:-

Nuclei in a state of division in the gametocytes were very faintly Feulgen-positive and also took up faint methyl green stain. The nucleus of the fully formed gamete was strongly Feulgen-positive, and also took up strong methyl green stain. Gametes exhibited slight metachromasy within the gametocyst.

Zygotes:-

The nucleus of the zygote lying within the gametocyst was strongly Feulgen-positive, and stained strongly with methyl green.

Sporozoites:-

The nucleus of the sporozoite was Feulgen-positive.

In all the stages mentioned above, the use of

pyronin methyl green showed that the cytoplasm always stained deep red with pyronin. A faint and uneven stain with pyronin was also noticed in the nucleus of the trophozoite and the gametocytes.

Yellow fluorescence was noticed in the nucleus of all the different stages of the life cycle excluding the trophozoites and gametocytes in which a positive result with Feulgen method was not obtained.

Apolocystis n.sp.(b)

The colour reactions in the trophozoite, gametocytes, gametes and zygotes were the same as seen in the corresponding stages of the parasite described above, though with a difference i.e. the parasite Apolocystis n.sp.(b) has a very prominent nucleolus which took up a very strong pyronin stain and showed metachromasy with toluidine blue. With acridine orange, the nucleolus showed orange fluorescence and the nucleus appeared with a somewhat clear area around it.

Apolocystis n.sp.(d)

Trophozoites:

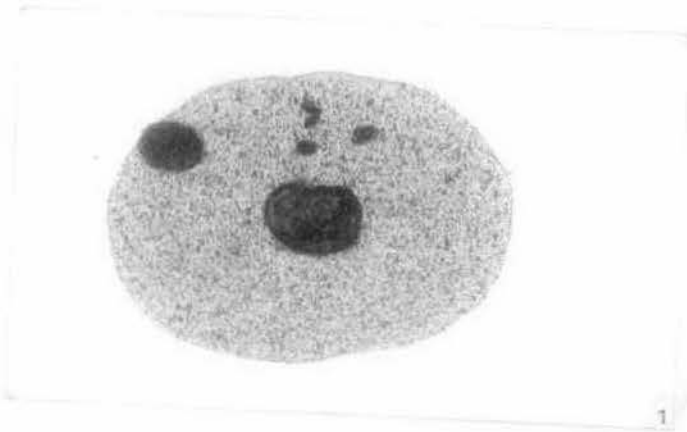
Nucleus was Feulgen- negative. Some trophozoites

P L A T E XXVI

Figs. 1 & 2.

The inclusion bodies in the cytoplasm of Apolocystis n.sp(d) show red metachromasy when stained with toluidine blue. 10 X 45

PLATE XXVI



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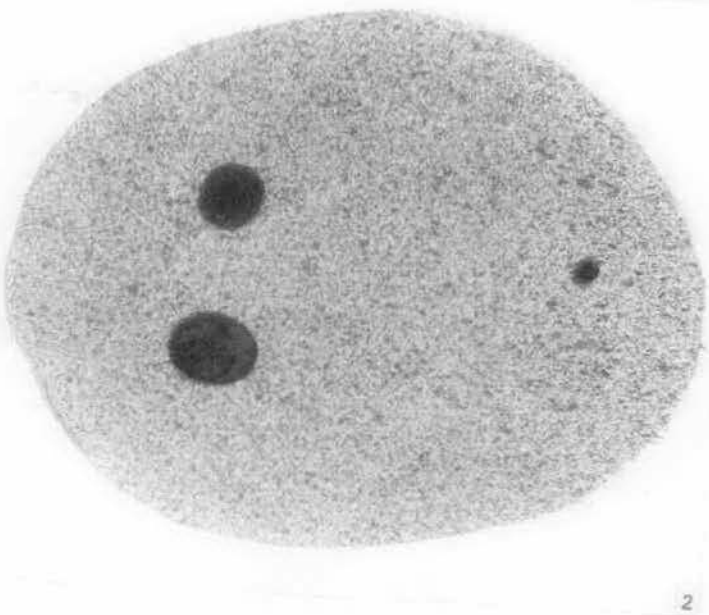

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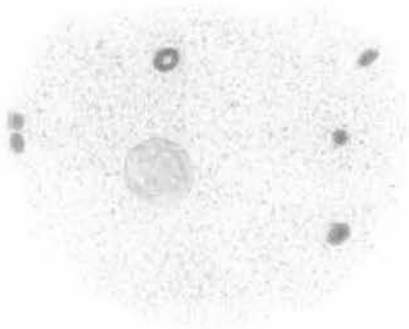
P L A T E XXVII

Figs. 1 & 2.

Feulgen positive inclusion bodies in the cytoplasm of Apolocystis n.sp(d)

10 X 45 X

PLATE XXVII



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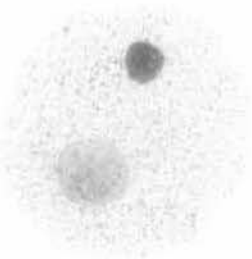

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were encountered, where Feulgen-positive bodies appeared in the cytoplasm. In one instance these Feulgen-positive bodies were so numerous that the gregarine took up a pinkish hue. The nucleus took up a faint pyronin stain and the cytoplasm took up the stain deeply.

The inclusions in the cytoplasm which presented various shapes (generally spherical) and which stained intensely with iron alum haematoxylin, corresponded to the Feulgen-positive areas. These areas occurring in patches did not appear homogeneous, as seen in iron alum haematoxylin stain. The patches appeared to harbour granular inclusions which were very strongly Feulgen-positive, and which occurred in a faintly Feulgen-positive matrix. Prominent metachromasy was observed in areas corresponding to the Feulgen-positive patches. The granular inclusions are believed to be inclusion bodies of bacterial or viral origin. These bodies did not stain with the alcian blue. In a few cases one or two ortho-chromatic dots were noticed when stained with toluidine blue. With pyronin methyl green the entire cytoplasm took up the pyronin stain while methyl green stained areas corresponded to Feulgen-positive areas noted above. The nucleolus took up pyronin stain very deeply.

Strong yellow fluorescence with acridine orange was noticed in areas in the cytoplasm which exhibited Feulgen - positive reaction.

Zygocystis n.sp.(a)

Trophozoites:

The cytoplasm as well as the nucleus of the trophozoites were Feulgen- negative. With pyronin methyl green stain, the cytoplasm took up faint red colour and the nucleus also took up a faint red colour. No sign of metachromasy in the cytoplasm of the trophozoite was noticed, but the nucleus exhibited faint violet metachromasy. With acridine orange the entire cytoplasm was orange including the nucleus which took up a slightly lighter colour compared to the surrounding cytoplasm.

Gametocytes:

The nucleus was Feulgen-negative and stained red with pyronin. Some of these showed black pigments. The residual cytoplasm showed red metachromasy (probably representing mucopolysaccharides).

Gametes:-

Nucleus of the gametes was faintly Feulgen- positive, and stained faintly with methyl green. The gametes showed faint metachromasy.

Zygotes:-

Nucleus of the zygote was strongly Feulgen - positive.

Sporozoites:-

Nucleus of the sporozoite showed positive reaction to Feulgen method and took up methyl green stain.

A (a) n.gen.,n.sp.

Trophozoites:-

In most of the cases, innumerable Feulgen - positive bodies occurring in dot like forms were found lying scattered in the cytoplasm. In one instance it was noted that these bodies were elongated in shape. Both the bigger as well as the smaller forms of the parasite appeared to have these Feulgen - positive bodies in the cytoplasm. The nucleus was Feulgen negative. These bodies also took up methyl green stain.

ytoplasm stained red with pyronin and the
i took up deeper pyronin stain. In some cases very

very faint Feulgen-positive patches were also observed in the cytoplasm.

With acridine orange the cytoplasm fluoresced bright orange. In this bright orange matrix there occurred minute granules that fluoresced bright yellow. These appeared to correspond to the Feulgen-positive bodies. The nucleoli looked pale orange with a slightly darker area in the centre. In some cases however, the nucleus fluoresced faintly.

The area of the sucker fluoresced orange only lightly and appeared transparent.

An interesting observation was made in the cytoplasmic region. Cytoplasm instead of being of uniform texture, seemed to show a blotchy picture. As such the orange colour also appeared in blotches.

Stomatophora n.sp(a)

Trophozoites:-

Nucleus was Feulgen-negative. In one or two instances there were a number of Feulgen-positive granules lying scattered in the cytoplasm. The regions of the sucker and the mucron were also Feulgen-negative. Cytoplasm and the nucleus took up pyronin stain.

With toluidine blue stain a large number of metachromatic granules were seen in the cytoplasm, and the nucleus appeared metachromatic.

Gametes:-

The nucleus of the gamete was faintly Feulgen - positive and stained faintly also with methyl- green.

Sporozoites:-

Nucleus of the sporozoite was Feulgen positive and also took up the methyl green stain.

Stomatophora n.sp.(b)

Nucleus was Feulgen - negative in the trophozoite and in the gametocytes, while the gametes, zygotes, sporoblasts and sporozoites had Feulgen- positive nucleus.

When subjected to fluorescence microscopy both Stomatophora n.sp.(a) and Stomatophora n.sp.(b) exhibited orange fluorescence in the cytoplasm and in the nucleoli. The nucleus however, showed faint fluorescence. Yellow fluorescence was also noticed in the cytoplasm in the form of granules.

In *Nematocystis* n.sp.(d) similar Feulgen-positive granules were observed, lying scattered in the cytoplasm. These granules stained with methyl green.

DISCUSSION

The nucleic acids, (DNA and RNA) have been of great interest to investigators in the field of Protozoology. Feulgen and Rossenbeck (1924) designed and utilised their well known reaction for the detection of DNA. The use of basic dye pyronin along with methyl green to distinguish between the two nucleic acids is well known (see Pearse 1968). Basing observations gained by means of Feulgen reaction it has been found that the nuclei of most of the parasitic species contain DNA. On the other hand, the ground cytoplasm of all the stages of the life cycle, especially of the gregarines, contain RNA. RNA also occurs in the nucleoli (von Brand 1966).

In the course of the cytochemical work carried out on various protozoa, metachromasy has often been noticed in the nucleoli and also in the cytoplasm. In most cases there was violet metachromasy in the nucleolus and a fainter violet stain in the rest of the

nucleus. Red metachromasy was observed in the residual cytoplasm of the gametocyst and gametes of Zygocystis n.sp.(a). While violet metachromasy could represent nucleic acids, the red metachromasy could possibly be due to the presence of mucopolysaccharides. Dasgupta (1961) conducted a series of studies on certain sporozoans wherein he encountered mucopolysaccharides.

Sirlin (1960), and von Brand (1966) have proved that the nucleolus is the chief site of RNA. The use of toluidine blue and pyronin- methyl green have confirmed this finding. With toluidine blue, metachromasy was observed in the nucleolus and also faint orthochromatic stain was seen in the nucleus. Pyronin, stained the nucleolus very deeply and the nucleus faintly. Since pyronin stains RNA and the lower polymers of DNA as well, it can be said that RNA is present in the nucleolus in heavy concentration. Metachromasy in the cytoplasm as well as pyronin staining in the same implies that the nucleic acid (RNA) is present in a lesser or greater quantity in the cytoplasm also.

In Apolocystis n.sp.(a), metachromasy existed in

the nuclear membrane and the area immediately adjoining it. Comparable results were obtained in the same species stained with methyl green pyronin. The central area of the nucleus took up the stain lightly. The nucleolus is considered to be the site for RNA, this structure being apparently absent in this species of Apolocystis, it is possible that RNA (essential for protein synthesis) is localised in the area specified for this parasite in the above description.

In the course of the present investigation on earthworm gregarines I have encountered more or less a uniform pattern of distribution of nuclear DNA in the various stages of the life cycle. For example, all the trophozoites, and the gametocytes had their nuclei negative to the Feulgen-reaction. During gametogenesis, however, the daughter nuclei showed slight positive reaction to the Feulgen's method. These gametes are also stained faintly by methyl green. The nucleus of the zygote in all the parasites was strongly Feulgen-positive. The nucleus of the sporozoites too showed similar reaction with Feulgen method. In all the above cases (gametes, zygotes, sporoblasts and sporozoites) the nuclei were stained with methyl green.

Daniels (1938) reported Feulgen-negative reaction for DNA in gregarines. Dutta (1962) also reported Feulgen-negative reaction in the nucleus of *Stomatophora diadema*. Histological literature reveals that the nucleus generally always shows a positive reaction for Feulgen's technique. But histochemical work on most of the gregarines under report, show that this is not so. Sathananthan (1977) has however, detected DNA in the nucleus of the gregarine *Zeylanocystis burti*.

A case of Feulgen-negative reaction in the nucleus was reported by Garnham (1954) in *Hepatozoon argantis* where the zygote nucleus was Feulgen-negative, though the nuclei during the sporogony were Feulgen-positive. Dasgupta and Meedeniya (1958), and Dasgupta (1959) made comparable observations on *H.sciuri*.

Survey of literature reveals that external factors may somehow influence the result of Feulgen reation in protozoa *e.g.*, starvation, oxygen deficiency and low temperature may lead to a diminution of DNA content in the macronucleus of *Paramaecium aurelia* (Gromova 1941); or death and degeneration could mean the loss of Feulgen-positive appearance in the nucleus of the crithidiae of

Trypanosoma melophagium (van Thiel 1925). But in the course of the present study all the gregarines when subjected to the Feulgen technique have failed to show any trace of Feulgen-positive material in the nuclei. This finding was confirmed by staining with methyl green.

DNA is well known to be mainly responsible for nuclear division and to be closely associated with the chromosomes that are present in the nucleus. The use of Fluorescence microscopy revealed the presence of DNA in the nuclei of the trophozoites and the gametocytes, where a positive reaction of the Feulgen technique was not obtained.

Judging from the above, the apparent absence of DNA in the nucleus of all the gregarines studied here, does not necessarily mean the complete absence of DNA. Daniels (1938), suggested that the negative Feulgen reaction could be due to the fact that the chromatin in the gregarines probably existed in a very dispersed condition which could not even be seen with the oil immersion lens. Ray and Gill's work(1955) brought forward a similar arguement regarding the gametocytes of Eimeria. Dasgupta(1959)

made similar observations on *Plasmodium* and *Eimeria*, and von Brand (1966) referred to the same.

A plausible explanation for the negative Feulgen reaction in the trophozoites and the gametocytes, and the positive reaction in the gametes, zygotes and the sporozoites can be formulated thus: The amount of DNA in the trophozoites and the gametocyte stages being in a highly dispersed condition could not even be detected by the Feulgen method. During gametogenesis, there is a considerable increase in the nucleoprotein, which probably results in the deep Feulgen staining of the material.

Of greater interest is the extranuclear occurrence of DNA especially so in the cytoplasm. Extranuclear DNA has been found to coincide with the specific cytoplasmic organelles such as the blepheroplast-kinetoplast complex of the trypanosomes. Freer(1950) demonstrated microscopic Feulgen-positive bodies in paramaecia. Lwoff(1952) also described certain Feulgen-positive bodies, the pro-virus particles, in certain lysogenic bacteria. Ray and Gill (1955) showed the presence of DNA particles in the cytoplasm of 10% of the oocysts of *E. tenella*. Dasgupta(1959) showed

a similar occurrence of DNA in the cytoplasm of the stages of E.stiedae. The present study is an added example to the text.

Many Feulgen-positive materials have been noticed in the cytoplasm of Apolocystis n.sp.(a), Apolocystis n.sp.(b) Stomatophora n.sp.(a), Stomatophora n.sp.(b), Nematocystis n.sp.(d) and A(a). These were generally in the form of minute granules and in a few cases like in Apolocystis n.sp(a) and A(a), they were present in the form of lightly stained patches. In Apolocystis n.sp.(d) however, Feulgen-positive granules were present but in an aggregated mass in the cytoplasm. It may be presumed that these Feulgen-positive materials are of viral or bacterial nature. Occurrence of such microbes were earlier reported in the oocysts of E.labbeana by Yakimoff and Timofeef (1940). Dasgupta (1959) also assumed such a case in the cytoplasm of the oocyst of Plasmodium cynomolgi. Lately, Mackenzie & Walker (1979) reported the presence of such microbes in the cytoplasm of G.garnhami. The nature of these Feulgen-positive bodies are even more convincing in the case of A(a) as some of the bodies show rod-like or coccoid shapes.

SUMMARY

In the course of the present investigation it has been found that the nuclei of all the gregarines studied showed a negative response to methyl green and Feulgen's technique. This could be dus to the fact that the chromatin in the gregarines existed in such a dispersed condition that it could not be seen even under oil immersion lens. However, the use of Fluorescence microscopy showed the presence of DNA in the nucleus of most of the gregarines.

A large number of extra- nuclear Feulgen-positive bodies were noticed in some of the gregarines studied in Part I. These were either scattered as minute spherical bodies or present as inclusion bodies in the cytoplasm of Apolocystis n.sp.(d). These bodies were Feulgen-positive, methyl green positive, showed red metachromasy and fluoresced yellow when subjected to Fluorescence microscopy. These could be of bacterial or viral nature.

PART III

HYPERPARASITES IN GREGARINES

OF EARTHWORMS

INTRODUCTION

Like metazoan organisms the protozoa are also susceptible to parasitic infection. The problem of organisms living on and in protozoa was discussed at length by Kirby (1941) From his review it would appear that various protozoa might be infected by Schizomycetes, by organisms of uncertain systematic position viz. Phycomycetes, by certain fungi viz. Sphaerita and Nucleophaga as well as by hyperparasitic protozoa.

Review of literature shows that Monocystid gregarines are no exceptions. They too have often been found infected by micro-organisms which often led to their destruction. For example, Hesse(1909) found that Monocystis lumbricilli, Monocystis striata, Monocystis agilis, Rhynchocystis pilosa, and Stomatophora coronata had their own peculiar bacterial parasites. Such bacteria, apparently belonging to Schizomycetes, varied in different species of gregarines ovoid to filamentous forms. The presence of such sitic bacteria in the majority of cases appeared ental to the gregarines. For example, Hesse(1909)

mentioned that bacterial parasites noted by him were uncommon and when present attacked host individuals of the species often leading to their death. To date, the association between a bacteria and its host (especially gregarines) have usually been cases of endoparasitism. Recently a case has been reported where bacteria have been seen lying attached to the surface of Porospora, a gregarine (Desportes & Theodorides/1977). Bacteria in this case have been found inserted between the epicytic folds of the gregarines, apparently inducing morphological changes similar to those noticed at the level of the junction between two biassociative gregarines. The author believed that in this case the gregarines constituted a substrata for the bacteria, all of them using for their food the intestinal contents of the host.

Mackenzie and Walker (1979) reported the presence of bacteria- like structures in the endoplasm of Gregarina garnhami. These structures were gram- negative.

According to Leger and Duboscq (1909), Frenzelinae conformis, a gregarine, parasitised by Nosema frenzelinae, developed normally up to a certain point only, the

formation of the gametes not taking place at all.

Caullery and Mesnil (1919) stated that Metchnikovellidae, haplosporidians, parasitic on gregarines (host-specific), had little pathogenic action on the host, particularly on the vegetative stages, though the author did not rule out mechanical injury in cases of heavy infection. According to Mackinnon and Ray (1931) the spores of Metchnikovellida escaped from their cyst into the endoplasm of the gregarines.

Kirby (1941) pointed out that according to a manuscript prepared by Stubblefield (1937), Amphiacantha, a haplosporidian, released gametocytes within the host gregarine Ophiodina elongata, by rupture of the cyst. Reduction division took place, resulting in the formation of the gametes, which underwent fertilization leading to further development; all these taking place within the remnant of the host gregarine.

According to Ganapati and Aiyar (1937), cytoplasm of Lecudina brazili, a dicystid gregarine, could be packed with cysts of a haplosporidian resulting in a mis-shapen

body of the host and a degenerating nucleus. Such parasitised gregarines were not found to associate, thus giving rise to the belief that heavily infected gregarines could not complete sexual development. (see Caullery and Mesnil 1919).

The presence of crystalline inclusions resembling virus particles have also been reported in certain parasitic protozoans including malarial parasites (Dasgupta 1968, Terzakis 1969, Davies & Howells 1971) . Malarial parasites harbouring such inclusions have been reported to undergo death and degeneration, and the significance of the presence of such inclusions remains to be clarified. Similar virus like particles have also been reported in certain gregarines and coccidia by Porchet & Richards (1969) and Porchet and Vivier (1971).

Pathogenic effects were also described in protozoa other than gregarines; for example, Bourne (1891) and Penard (1893) mentioned that Pelomyxa was generally always parasitised by endobiotic bacteria. Heavy infection led to hypertrophy, alteration in the body, and finally to the disintegration of the host (Leiner 1924).

Similar observations were made by Nagler (1910) and Epstein (1935) who worked on two different varieties of amoeba parasitised by micrococci.

Fatal effects were noticed by Wenrich (1937) on Iodamoeba butschlii, Endamoeba citelli (Becker 1926) and Nyctotherus which had macronuclear infection by microorganisms (Bassuchin 1928). Protozoa parasitised by Nucleophaga continues its activity with no change in protoplasmic structure. Lavier (1935) noticed hypertrophy of the nucleus of the parasitised Endamoeba ranarum and relegated it to a sort of defense mechanism on the part of the amoeba.

Marked physiological change was observed in paramecia infected with Dreparospira, Spirillaceae. The development in the host was rapid followed by the drying up of the nuclear contents. Bozler (1924) and Fiveiskaja (1929) noted vacuities in Paramecia infected with macronuclear infection. Fat droplets increased in amount, trichocysts were disarranged, food vacuoles stopped forming, leading to the disappearance of the mouth, gullet and the cytopyge. The host died eventually. It seemed that the above bacteria had a certain indirect

action on the host cell, the secretions and waste products of the hyperparasites seeping into the cytoplasm and resulting in the intoxication of the nucleus.

Stentor coeruleus infected with bacilli, lost their bright green colour and their capacity for motor responses (Hetherington 1932). According to Kirby (1941) many observers had reported the death of the host infected with Sphaerita. Dangeard (1889, 1895) Puymaly(1927), Mitchell (1928) and Jahn (1933) stated that Euglena when parasitised by Sphaerita lost chromatophores, suffered inhibition of flagellar movements and changes in nuclear structure. It finally burst to liberate the zoospore.

In view of all the facts stated above, it would appear that there is enough scope of work on the presence of parasitic infection in various gregarines occurring in nature. In the course of the present work an effort has been made to detect any such cases of hyperparasitisation and abnormalities in staining reactions in the gregarines studied and an effort has been made to elucidate the causes of such differences in staining characteristics.

Observations

The abnormal forms of Zygocystis n.sp.(a), were characterised by lightly stained areas in the cytoplasm, as seen in iron alum haematoxylin stain. In quite a number of cases it was observed that the entire trophozoites were lightly stained. Such lightly stained individuals were the abnormal forms of the species.

The abnormality arose as a small lightly stained patch in the cytoplasm of an otherwise normal trophozoite. The patch extended and such extended abnormal areas tended to lose compactness. As a result the trophozoites tended to lose characteristic shape. More patches developed and expanded. Such patches later on became confluent until the whole body of the trophozoite assumed a lightly-stained condition. In this final stage the trophozoite was again seen in its old characteristic shape.

These lightly-stained trophozoites appeared to harbour bacteroids, possibly of the kind reported by Hesse (1909). These imparted a faintly rosy hue to the cytoplasm. Some of these bacteroids were seen to be in a stage of binary fission. Lightly-stained

single trophozoites were occasionally found. In such cases the nucleus was either in a fragmented or in a diffuse condition. Furthermore, in some of the lightly stained individuals, black refractile pigments were found to be present. Those possessing these black refractile pigment granules had the nucleus in a disintegrating condition. The phenomenon of autotomy is also noticed in the abnormal forms of Zygocystis n.sp.(a).

When stained with Feulgen's method, the abnormal patches took up faint green stain with light green, in contrast to the surrounding areas which took up a deeper stain. No trace of Feulgen-positive body was noted in the cytoplasm of these forms.

When stained with pyronin-methyl green, the abnormal patches took up lighter colour with pyronin than the surrounding areas. With toluidine blue stain, the abnormal patches did not take up any stain as compared to the normal areas. The nucleus in the abnormal gametocyte was negative to toluidine blue but in some cases metachromasy has been observed in the nucleolus.

With acridine orange (Fluorescence microscopy),

P L A T E XXVIII

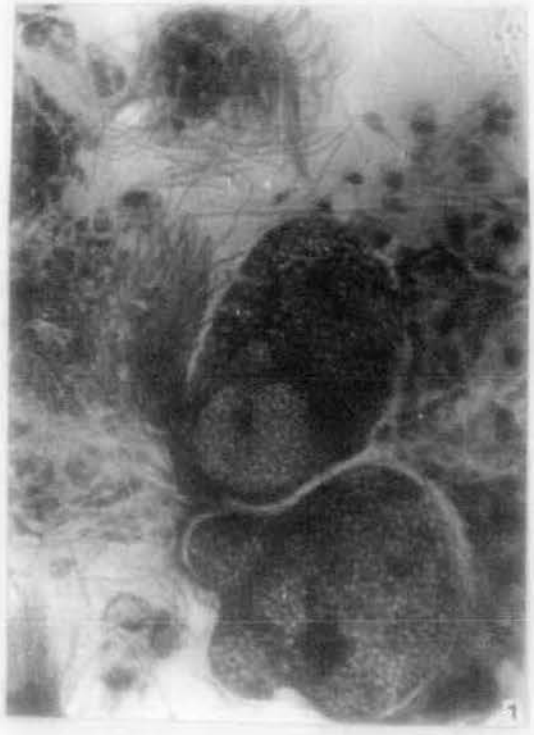
Fig.1. Patch forming in a trophozoite, which tends to lose its shape.
15 X 40X.

Fig.2. Association between a normal and an abnormal trophozoite. Note the nuclear change in the abnormal one.
15 X 40X.

Fig.3. Degenerating trophozoite. Nucleus has disintegrated. 15 X 40X.

Fig.4. Inclusion bodies in the cytoplasm of Apolocystis n.sp(d) 15 X 40X

PLATE XXVIII



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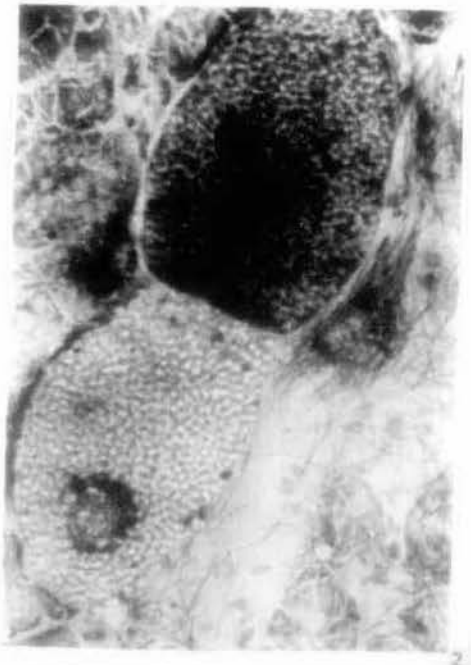

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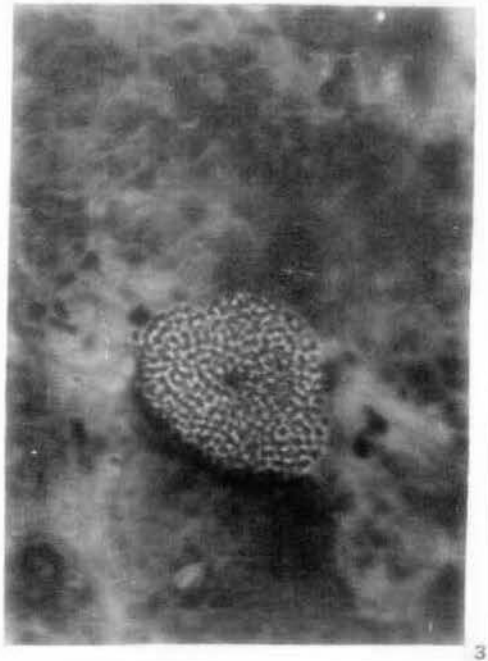

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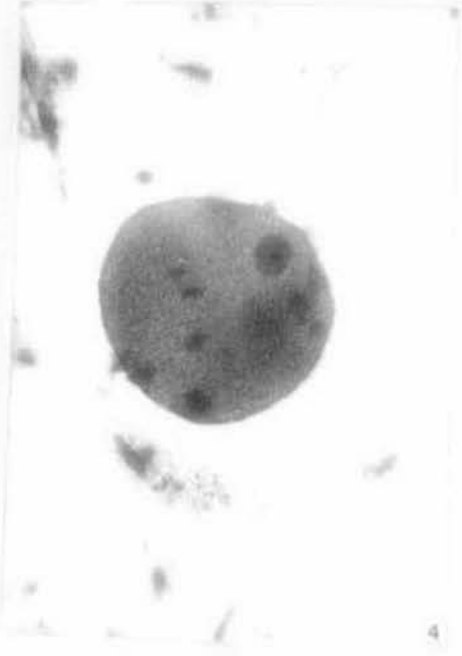

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an interesting phenomenon has been observed. The cytoplasm of these forms did not exhibit orange fluorescence as seen in the normal trophozoites. But scattered in the cytoplasm were a number of minute bodies, which fluoresced bright yellow apparently representing DNA present in the micro-organisms. The nucleus of such trophozoites showed no indication of having any trace of DNA or RNA.

Apolocystis n.sp.(d)

98% of the above gregarines possessed one or more darkly-stained patches scattered at random in the cytoplasm. These patches were circular or irregular in outline. It was noticed that a very young trophozoite (50.4 μ in diameter) might have such a patch, while an older one (104.4 μ in diameter) was without such a patch.

With Feulgen's method, the nucleus did not take up the stain. Some trophozoites were encountered in which Feulgen-positive bodies appeared in the cytoplasm. In one instance these Feulgen-positive bodies were so numerous that the gregarine took up a pinkish hue.

The patches in the cytoplasm, which represented various shapes (generally spherical) and which stained deeply with iron alum haematoxylin, corresponded to Feulgen-positive areas. These areas occurring in patches did not appear homogeneous, as seen in the iron alum stain. The patches harboured granular inclusions which were strongly Feulgen - positive, and which appeared in a faintly Feulgen-positive matrix. Prominent red metachromasy was observed in areas corresponding to Feulgen- positive patches (when stained with toluidine blue), and these areas are believed to represent inclusion bodies of bacterial or viral origin. Such patches did not stain with alcian blue. One or two orthochromatic dots were noticed in the general cytoplasm.

When pyronin-methyl green was used, the entire cytoplasm took up the pyronin stain, while areas corresponding to the Feulgen-positive patches took up methyl green. The nucleolus took up the pyronin stain very deeply.

With acridine orange (Fluorescence microscopy), strong yellow fluorescence was noticed in the Feulgen-positive patches occurring in the cytoplasm.

Besides the above two, other abnormalities were encountered in the course of the present investigation.

In A (a), innumerable Feulgen-positive bodies occurring in dot-like forms were found to lie scattered in the cytoplasm. In one instance it was noted that these bodies were elongated in shape. Both the larger as well as the smaller forms of the parasite appeared to have these Feulgen-positive bodies in the cytoplasm. In about two instances, however, a very faint patch of Feulgen positive area was seen. The nucleus was Feulgen-negative. These bodies also took up methyl green stain.

With acridine orange, the entire cytoplasm fluoresced orange. However, the bright orange cytoplasm did not appear homogeneous but was bunched up in lumps, presenting a blotchy appearance. The nucleoli looked pale orange with a slightly darker area in the centre. In some cases, however, the nucleus fluoresced faintly yellow.

Minute Feulgen-positive bodies were also found scattered in the cytoplasm of Stomatophora n.sp.(a). Likewise, a large number of similar Feulgen-positive bodies which stained with methyl green were found in the

cytoplasm of Nematocystis n.sp.(d).

DISCUSSION

The difference in staining reaction either in one or both the members of a pair of Zygocystis n.sp.(a), could possibly be due to the difference in the quantity and distribution of cytoplasmic inclusions. Similar observations were also made by Mühl(1921) and Joyet- Lavergne (1926) who used neutral red. These authors attributed the differences, to the difference in the oxidation-reduction potentials of the two gametocytes and also in the quantity and distribution of the cytoplasmic inclusions.

The finding of the fragmented or diffuse nucleus in the abnormal forms may lead to another assumption that the breaking down of the nucleus is in some way responsible for the difference noticed in the staining reaction of the cytoplasm.

We may however, rule out the above possibilities on account of the fact that all these lightly stained parasites showed extensive cytoplasmic invasion by the bacteroids,

which are definitely not normal inclusions in the species. Micro-organisms resembling these bacteroids and referred to as bacteria occurring in Monocystis species, and also in Rhynchocystis pilosa and Stomatophora coronata were reported by Hesse (1909). The bacteroids under consideration are variable in form i.e. are spherical, while others are rod-shaped. These divide by binary fission. Gradually the increase of the hyperparasites within the host, leads to the alterations in the nucleus. The changes are degenerative. The nucleus soon fragments and eventually disintegrates. Thus finally the invasion of the bacterial parasites leads to the destruction of the gregarine host.

Similar kinds of nuclear disintegration, which led to the death of the host, have been reported in Amoeba verrucosa (see Mattes 1924) and Endamoeba disparta (see Kirby 1941), which were infected by Nucleophaga. Leiner (1911) described Pelomyxa hyperparasitised by Cladothrix pelomyxa, a bacterium.

Caullery and Mesnil (1897) stated that Metchnikovella

caulleryi, a hyperparasite in a gregarine, "seemed to have little pathogenic effect on the host; particularly on the vegetative stages. What injury there is, is mechanical, when infection is heavy."

Heavy parasitisation might result in the waste products and secretions of the hyperparasite accumulating in the cytoplasm of the host gregarine, which might upset the metabolism of the gregarine leading to the lightly-stained condition. This directly or indirectly, has an effect on the nucleus which soon starts to fragment and disintegrate.

The death of the host leads to the death of the hyperparasites. Chitinization occurs around the dead micro-organisms resulting in the formation of black pigment which are refractile by nature, and the body of the host slowly disintegrates.

The association of the normal and the abnormal trophozoites, does not lead to the formation of the gametocyst. A similar observation was made by Caullery and Mesnil (1919) and Ganapati and Aiyar (1937). The latter however mentioned that the parasitised gregarines

did not associate in the case of Metchnikovellidae. In the course of the present study it has been found that association of this sort took place in Zygocystis n.sp.(a), but development ceased altogether after this. A few cases, where both the gametocytes were abnormal were also encountered. An assumption can be made that when association just occurred, only one of the members of a pair was parasitised by the bacteroids; infection spread rapidly to the other member, until both of them were heavily infected and did not take up the stain. The nuclei in such cases are not normal and are in a state of disintegration.

The above instance of hyperparasitisation proved to be fatal to the host. However in the course of the study, we have come across another case of hyper-parasitisation, which does not appear to cause any deleterious effect. For example about 98% of the trophozoites of Apolocystis n.sp.(d) harboured bacteroids, which were not spread throughout the cytoplasm, but remained in aggregates, forming some sort of inclusion bodies. No definite membrane was observed around these aggregates. The aggregates

consisted of granules. These granules were grouped in masses of irregular form.

The association between the parasites and the host is more or less a constant one, and can often be mistaken for normal structures of the host itself. Cytochemical studies have revealed no drastic changes in the polysaccharide contents of the host; this in all probability could mean that the inclusion bodies are not detrimental to the host. In Kirby's words (1941), ' They have come to occupy a normal place in the metabolism of the combination'.

Such aggregates of micro-organisms have also been found to be present in Devescovina glabea by Grassi and Foa (1911).

The micro-organisms in the other gregarines reported here also do not cause any harmful effect on the host. Their variability in number and the fact that they do not occur in all specimens is evidence enough that they are not normal inclusions of the host body. However, gregarines harbouring such inclusions appear to lead a normal life.

PART IV

A NOTE ON THE HARMFUL EFFECTS OF THE GREGARINES

ON THE HOST EARTHWORM

The invasion of the parasites into the annelid body can at times lead to detectable injuries. In some cases serious effects resulting in the death of the host has been noticed. Instances of responses of the host to parasitic invasion is also on record and many annelids have developed means to defend themselves. The coelom in most Polychaetes and Oligochaetes have coelomocytes, which phagocytose foreign materials. Leeches have botryoidal tissue which accumulates alien material by phagocytosis. The earthworms defend themselves by autotomising their posterior segments. In this way they void any unwanted material and alsc coelomic parasites, which accumulate at the posterior segments of the body.

Keilin(1925) has suggested autotomy of the host as a mode of liberation of the coelomic parasites from the body of the earthworm.

Cameron (1932) and Bang (1973) have observed the reaction of the amoebocytes to large foreign bodies in the coelom of earthworms. Bang pointed out that though

encapsulation of the experimentally introduced foreign objects took place, phagocytosis could not occur as the objects were too large. However, parasites like nematodes were found to be encapsulated. Fibrous capsules were found to form around Monocystid gregarines.

Stephenson (1930) reported that adult gregarines of Oligochaetes were not attacked, but cysts were encapsulated though the sporozoites were not affected.

In the course of the present study, three instances have been encountered, where the parasites concerned affected the host in some way.

In one instance, Amynthas alexandri had white globular cystic bodies - Apolocystis n.sp.(c) growing on the dorsal aspect of the anterior region of the intestine. (see page 60). Close scrutiny revealed the presence of a fine transparent membrane covering them.

Examination of sectioned material of the infected intestine revealed that these cysts which contained adult

trophozoites were composed of an outer wall formed from the peritoneal layer of the host intestine. This layer seperated the parasites from the underlying muscle layers and the intestinal mucosa. This could be a case of response mechanism on the part of the earthworm, caused by the presence of the parasites. The growth of the cystic bodies exerts a pressure on the alimentary canal flattening it to a certain extent.

A similar case has been noticed in the course of the present investigation in Pheretima robusta which had Apelocystis n.sp.(a) and Apelocystis n.sp.(b), growing as white bodies on its dorsal blood vessel. They were so intimately attached to the dorsal vessel that any attempt to dislodge them led to the rupture and breaking up of the latter.

In cases of heavy infection the entire dorsal blood vessels were found to be parasitised. Very often earthworms infected thus were seen to autotomise their posterior segments, the other half of the body leading a normal life. The autotomised parts, when dissected were seen to be replete with the parasites and cysts.

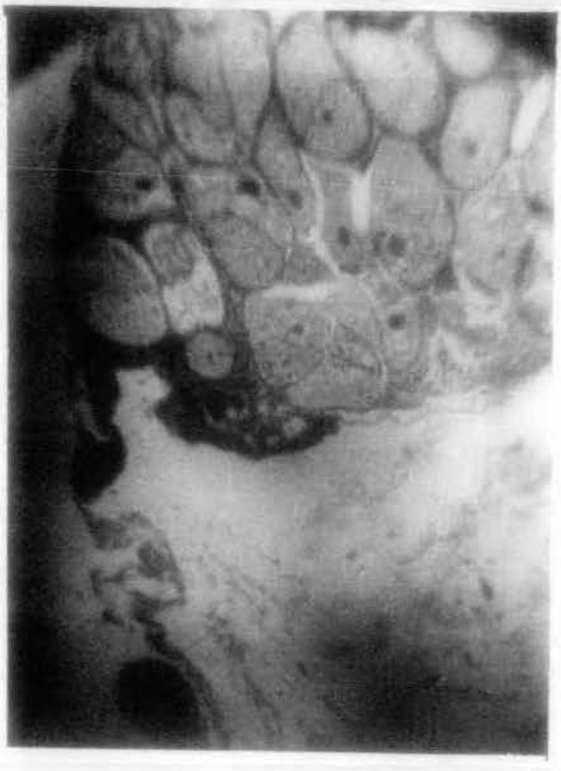
Prior to autotomisation, the blood vessels constricts,

PLATE XXIX

Examination of the sectioned material appears to indicate that the continuity of the epithelial layer is interrupted at the point where the parasitic mass presses against the intestine. Such interruption in the continuity of the epithelial layer later on extends towards the sides.

10 X 10X

PLATE XXIX



due to the weight of the parasitic bodies. The blood vessel kept constricting until cessation of blood flow resulted. This is the beginning of autotomy.

In contrast to the above two parasites, another case has been encountered, in which the presence of coelomic parasites proved fatal to the host.

Apporectodea trapezoides, has been found to be infected with a very heavy population of B(a), B(b), and Nematocystis n.sp.(a) . Earthworms infected with these three species of gregarines, invariably always died.

The infection led to the body of the earthworm accumulating excess coelomic fluid, which assumed a dirty yellow colour. The body bloated to such an extent that it lost its usual body contour. In some places the swollen part of the body had excess fluid, and the rest of the body had none. The latter region appeared wrinkled. The body became very soft to the touch. Constrictions occurred at several points of the body, followed by the ligation of the body at these points. Earthworms died soon afterwards. Putrefaction set in rapidly after death.

When dissections were made at the stage when the body was extremely bloated, it was found that parts of the internal organs viz.,stomach, and the intestine were in a state of evagination.

Smears prepared of the coelomic fluid of the autotomized portions showed a very heavy concentration of Nematocystis n.sp.(a). The other two gregarines viz. B(a), and B(b) though present in the earlier stage (before autotomy) were strangely absent. In one or two instances , one or two of these could be found.

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Copy of Dr.R.V.Melville's letter Ref.No.Z.N.(G) dated 11th March 1980.

Dear Miss Pradhan,

Thank you for your letter of 2 March asking a question about publication. I can assure you that your earlier letter of 28 January has not been received.

Certainly, if Dr.Chatterjee's thesis of 1971 has never been published, his names cannot enter into zoological nomenclature. His new names are not so much nomina nuda - a nomen nudum is normally a name that is published but without satisfying the provisions of Articles 10 to 20 - as simply non-existent names.

A thesis does not become a published work merely by being deposited in a library from which copies can be supplied on demand. On the other hand, some theses are genuinely published: for example, some German universities require 100 printed copies of a thesis to be submitted, and in both Germany and France I have seen theses published and on sale as separate works, or published as papers in standard scientific periodicals. So it is not the simple fact that

a work is a thesis that prevents it being published; it may well be published if - and when - it satisfies Articles 8 and 9 of the Code. The availability of the names in a published work is then a separate matter, to be examined in the light of Articles 10 to 20.

I hope this is a helpful answer. Certainly, on the basis of what you tell me, you are right to ignore Dr. Chatterjee's new names.

Yours sincerely,

Sd/- R.V.Melville

Secretary, International Commission
on Zoological Nomenclature