

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 existence 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, Zyrocystis, 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 Cephalocystis, 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 Drzhnevskiy (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 Chatterjee (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.

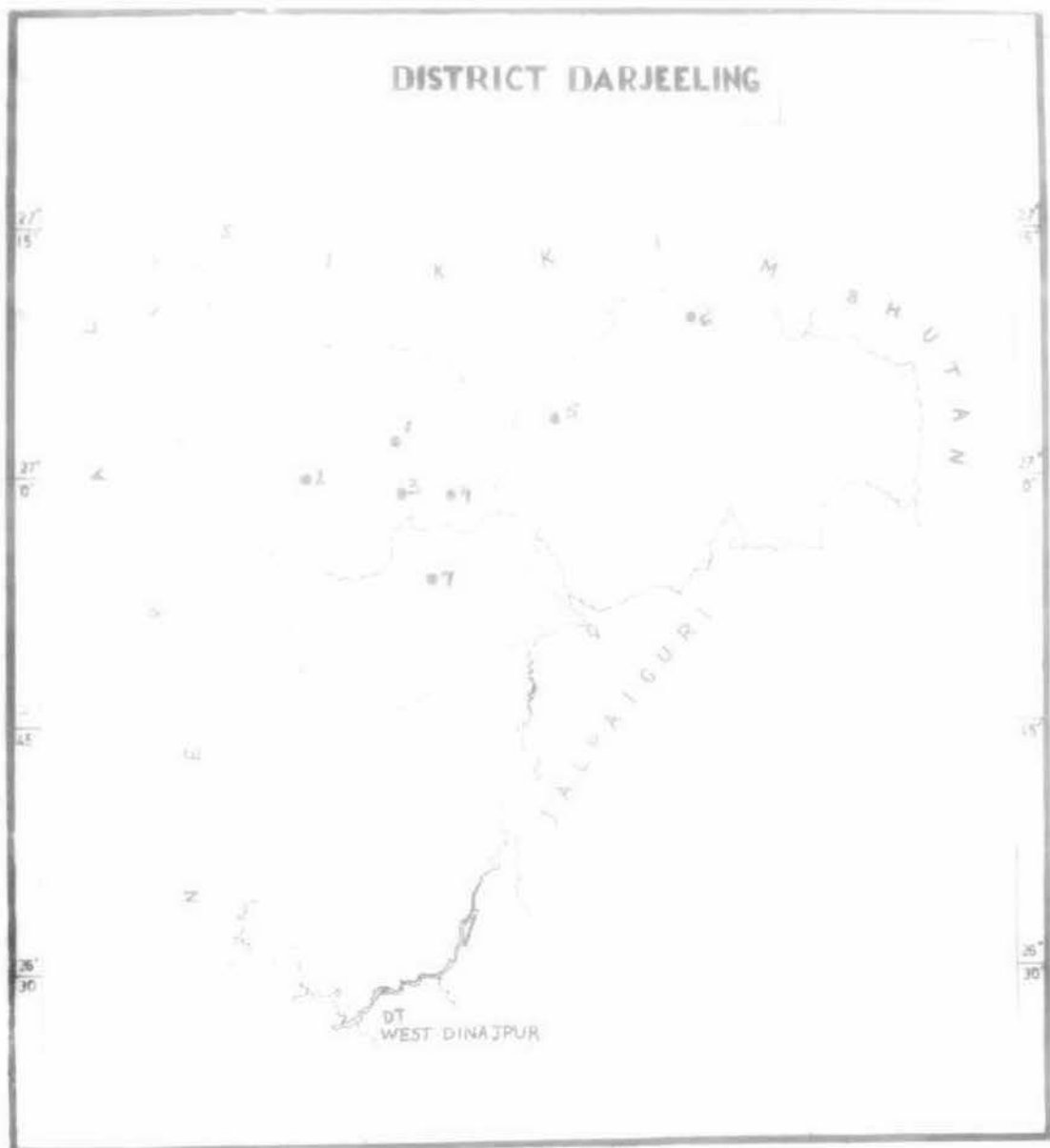
Setna (1927) created genus Grayallia.

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. Goemti (alt. 1372m).

DISTRICT DARJEELING



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)

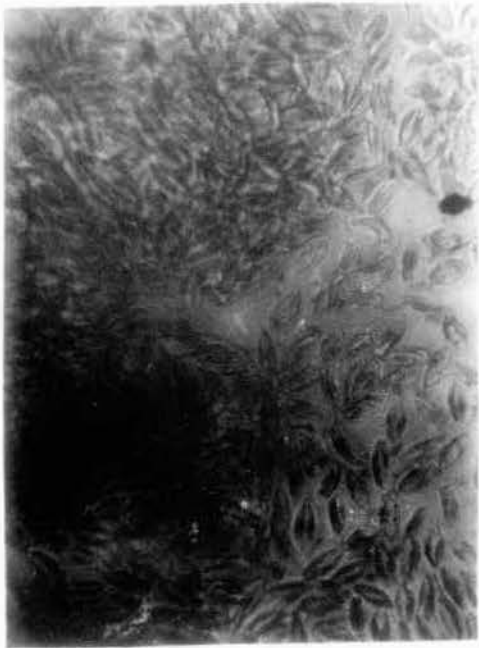
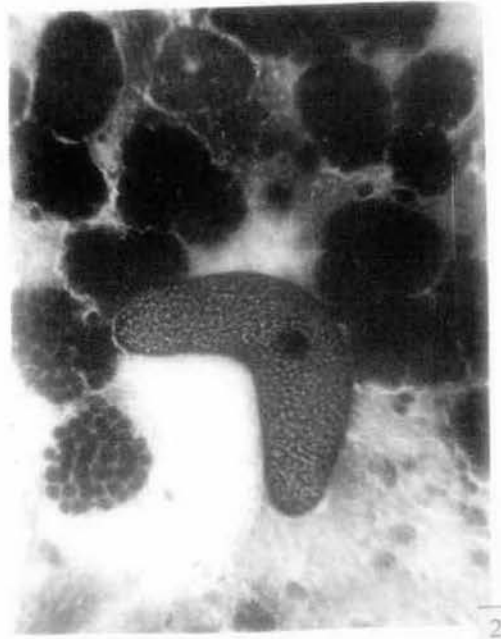
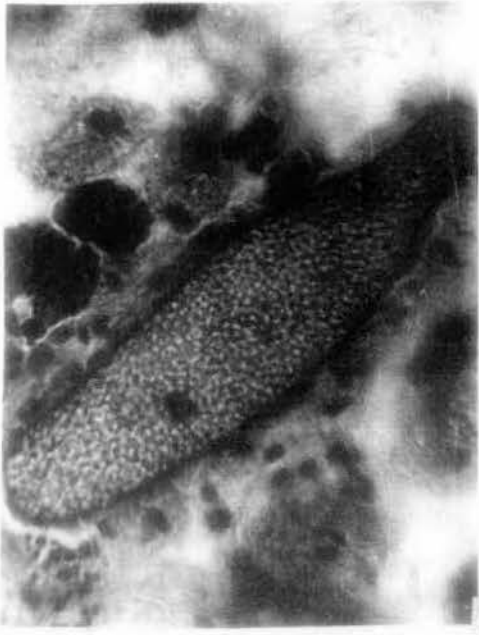
15 X 40X

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 I



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

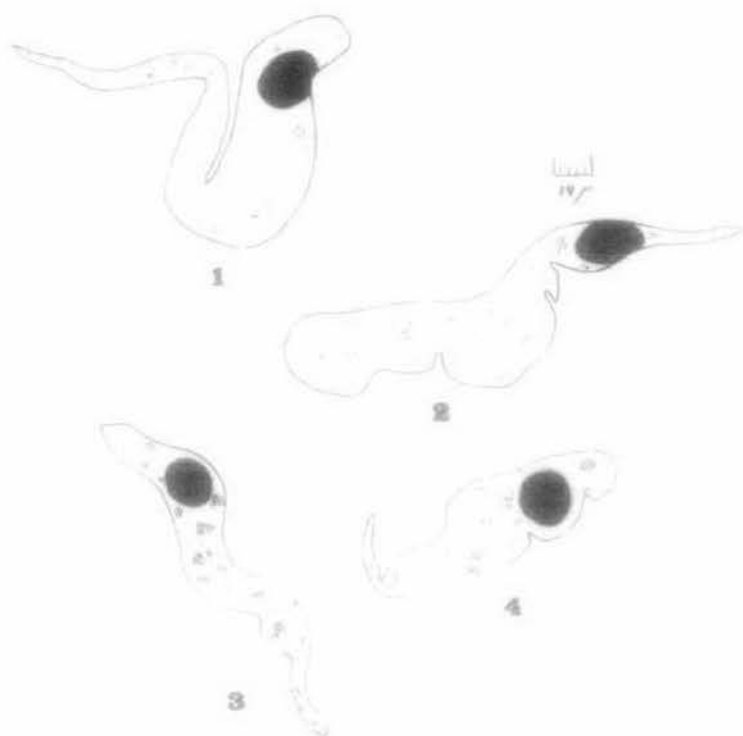
PLATE II

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).



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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	11.76 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.

(Loubatières 1955)
Nematocystis caudata 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 1906.

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; ectozoic; 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 III

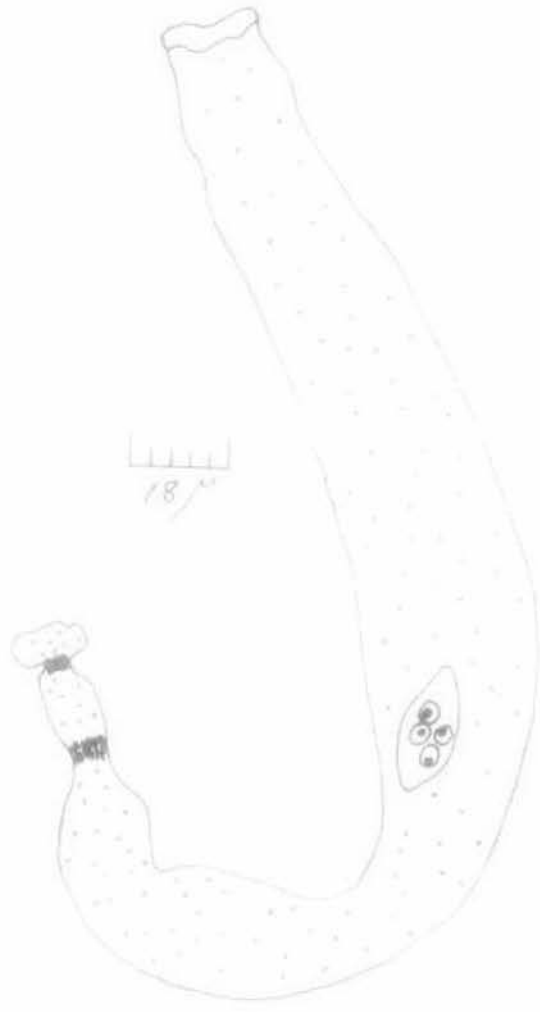


TABLE III

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: Goonti (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:

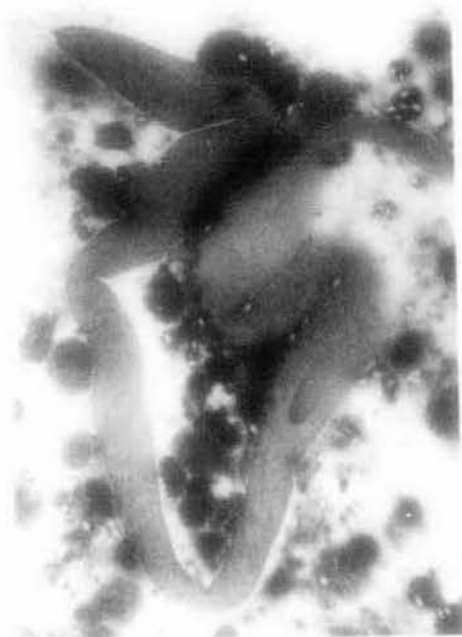
The parasite described here is identified as belonging to the genus Nematocystis on account of its elongated nematode-like body. Among all the species of Nematocystis known, few measure two millimeters or more. e.g. N.anguilla (Hesse 1909) measures 2080 μ by 40 μ , while N.tuzeti^{Loubatières 1955} measures 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^{Loubatières 1955} (2415 μ by 180 μ) and N.carensis^(Mohammed & Ramadan 1972) (2550 μ 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: Mangpoo (alt. 2164.75m), Darjeeling district
West Bengal.

T A B L E 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

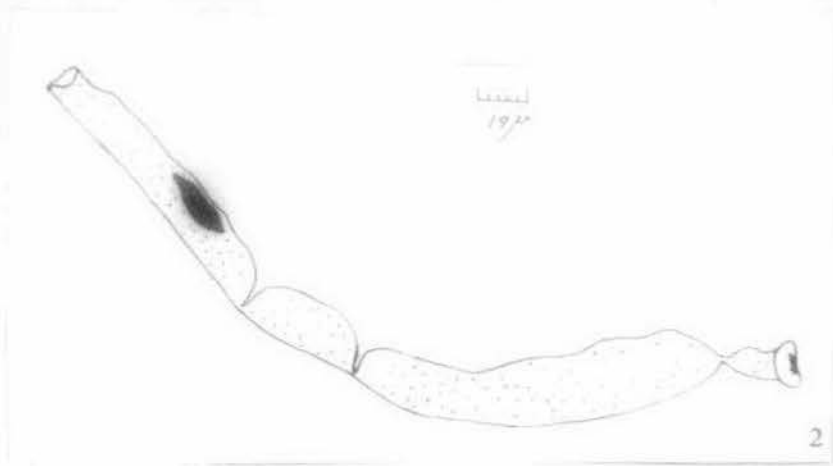
Fig. 1. Photomicrograph of Nematocystis n.sp. (d)

6 X 10X

Fig. 2. Camera lucida drawing of Nematocystis
n.sp. (a) , showing the body grooved at

intervals. 6 X 10X

PLATE V



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 - 55.10 μ	29.35 μ
Nucleus length	14.76 μ - 30.04 μ	25.82 μ
Nucleus breadth	6.84 μ - 13.30 μ	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: Goonti (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.

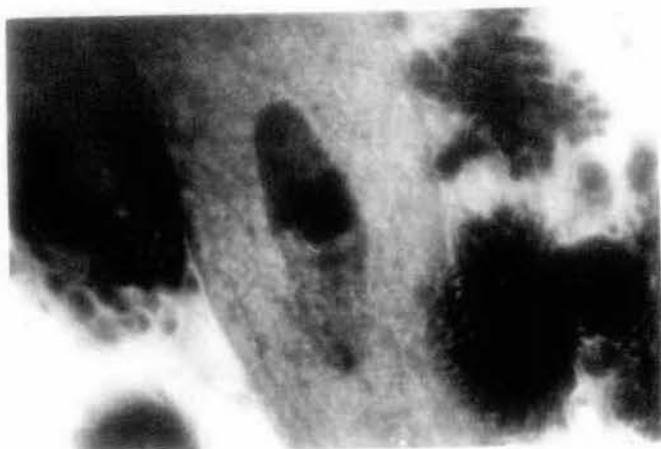
Fig.2. Nucleus of Nematocystis n.sp.(e)
showing deeply stained karyosome
surrounded by a light area.

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, and N. pilosa (Loubatieres 1955) (Tuzet & Loubatieres 1946) differs

TABLE VI

Measurements of Nematocystis n.sp. (e)

	RANGE	AVERAGE
Length	760 μ - 1339.9 μ	1172 μ
Breadth	77.52 μ - 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
Karyosome	13.5 μ	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, (Oligochaete.)

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 Zyrocystis n.sp. (a)

10 X 45X

PLATE VII

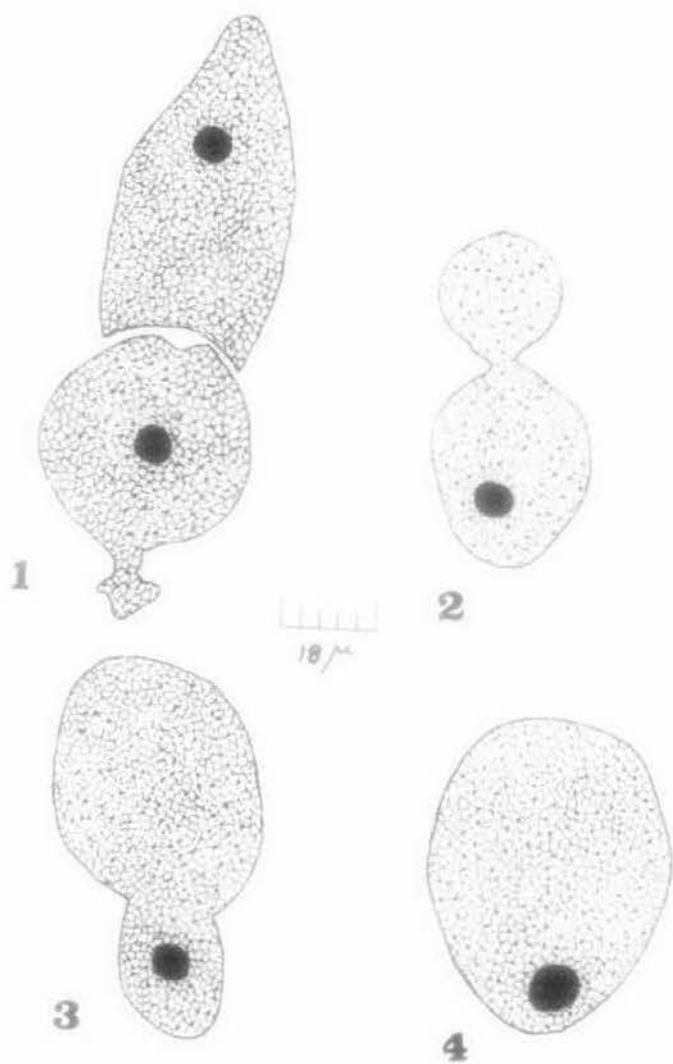
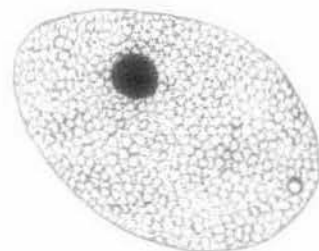


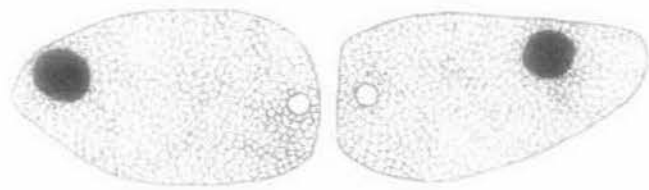
PLATE VIII

- Fig.5. Zygecystis n.sp.(a). Clear vacuole
seen at broad end of body. 10 X 45X
- Fig.6. Clear vacuoles are present during syzygy.
10 X 45X

PLATE VIII



5



6

18 μ

TABLE VII

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

	RANGES	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
Gametocyst length	75.6 μ - 144 μ	122.4 μ
Gametocyst 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

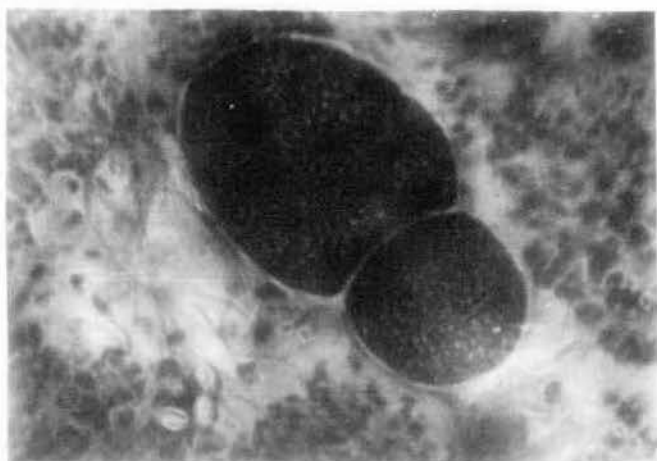
Fig. 7. Autotomy. The amputated piece does not possess a nucleus.

15 X 40X

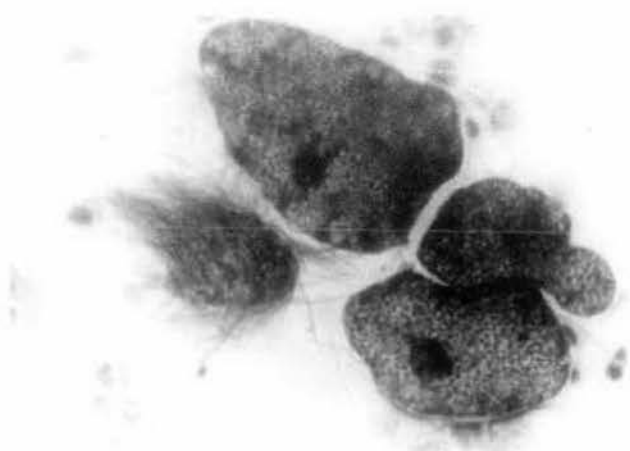
Fig. 8. The lower half of the photomicrograph shows the parasite undergoing autotomy. The amputated piece does not possess the nucleus.

15 X 40X

PLATE IX



7



8

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

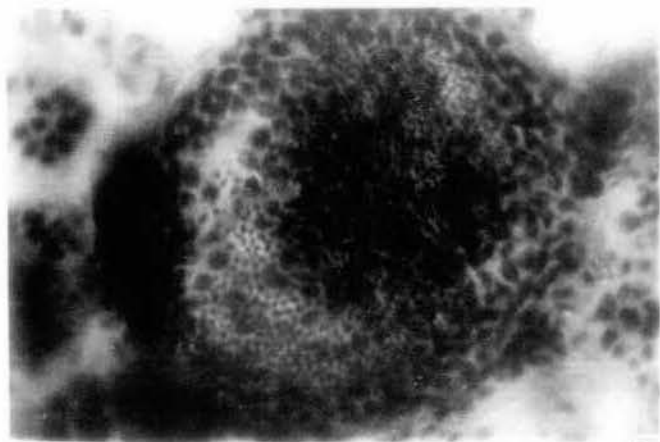
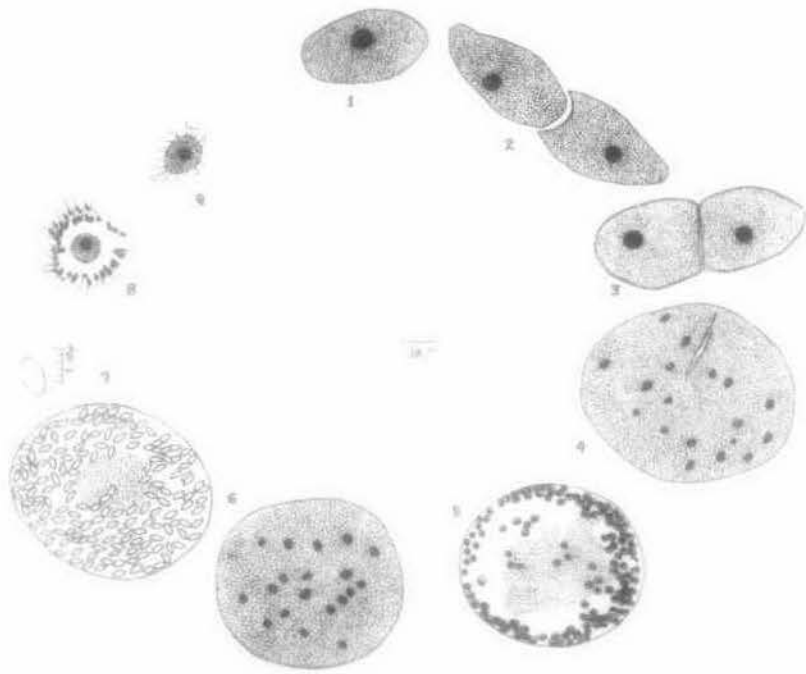
PLATE X

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. 15 X 40X

PLATE X



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 Zygozystidae, and hence the parasite has been placed under the above Family.

Previous workers have stated that Zygozystis 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 & Ramadan Mohammed⁽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 32 μ - 48 μ X 25 μ - 36 μ (two syzygites).

Zygocystis n.sp.(a) 97 μ - 129 μ X 30.4 μ - 47 μ (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 Zygozystidae Bhatia

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

Genus: Zygozystis Stein

Adult trophozoite generally pyriform; occur in pairs or groups of three; occasionally single (Troisi 1955). Spores biconical with peculiar thickenings at both poles; ectozoic; 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; Ocozoic; Coelozoic.

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

Host: Eheretima (= Metaphire) californica Kinberg
(Oligochaeta.)

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.

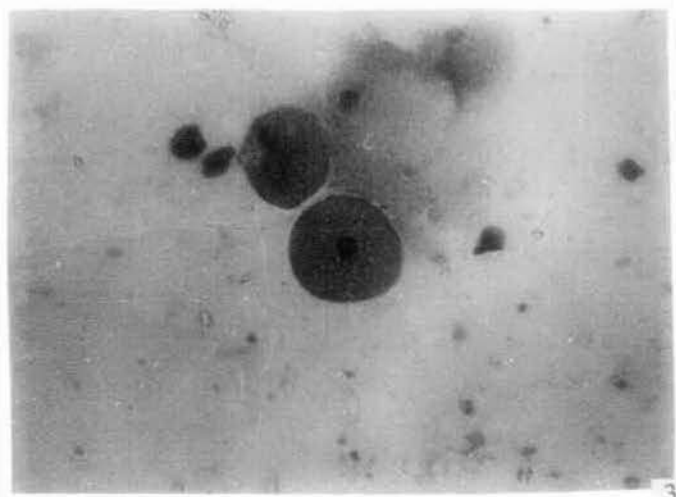
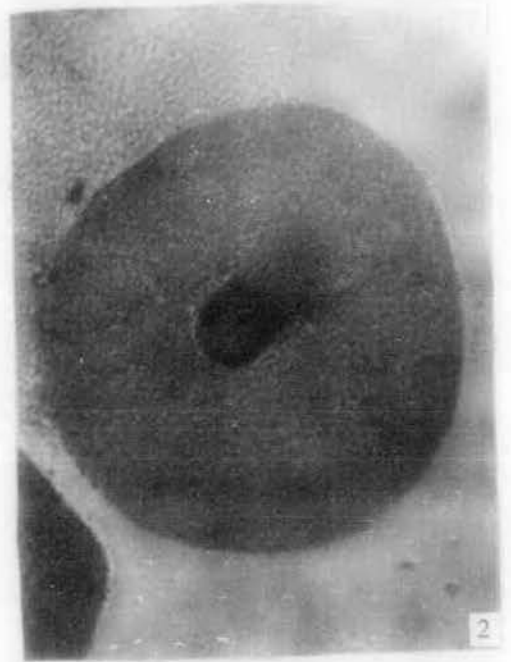
PLATE XI

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



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 ~~covered~~ with fine granules^{ulations} 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	99 μ F
Spores length	14.2 μ - 15.62 μ	14.86 μ
Spores breadth	5.9 μ - 7.10 μ	6.98 μ

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 chitinous 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 XIII

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 XIII

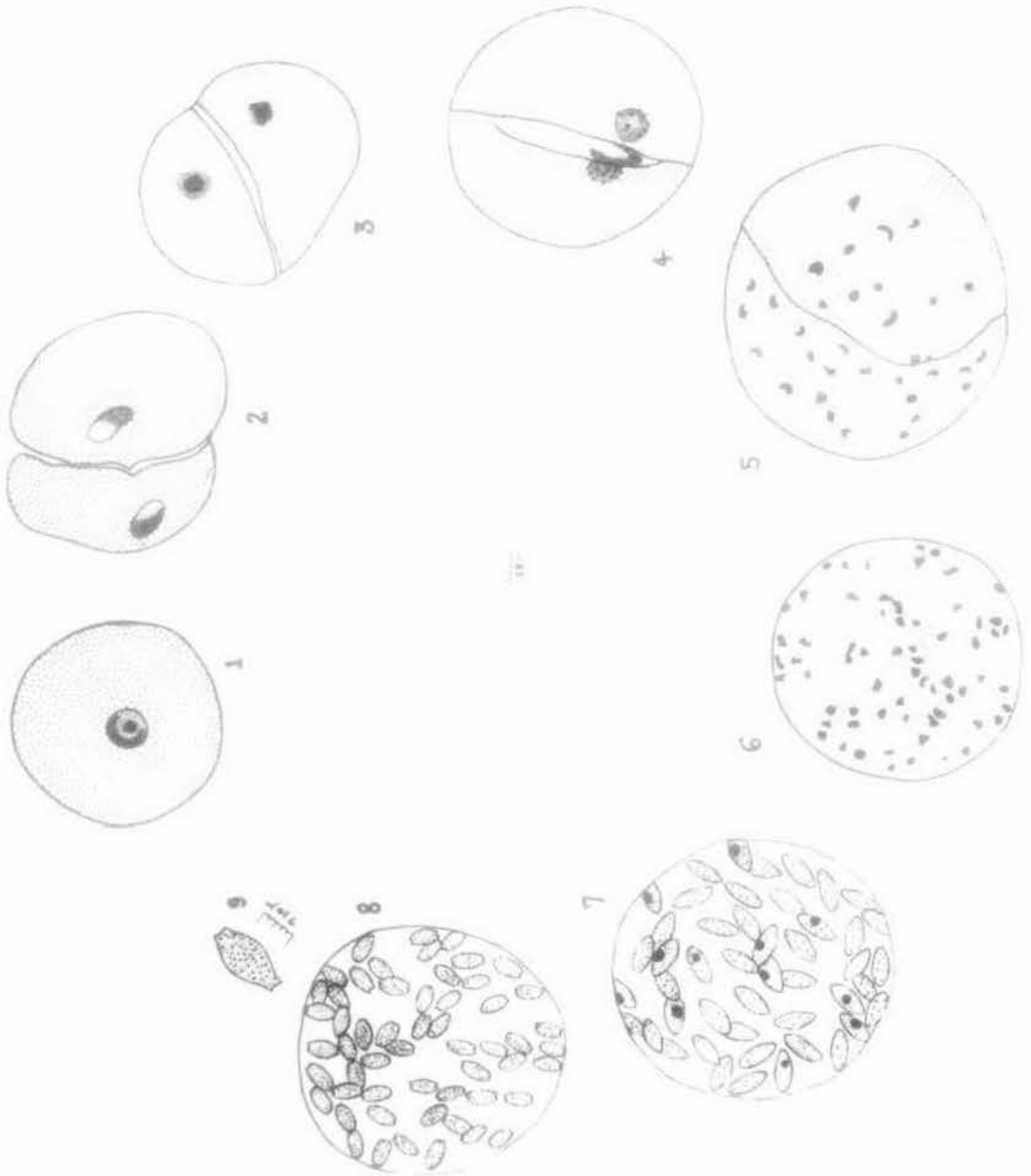


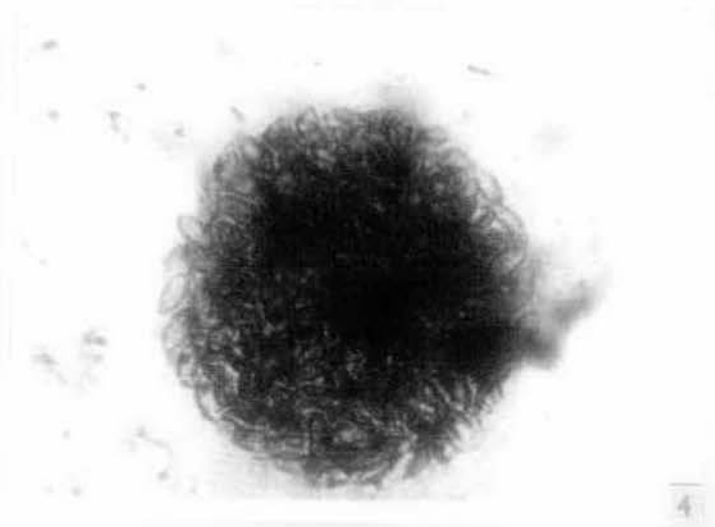
PLATE XII B

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

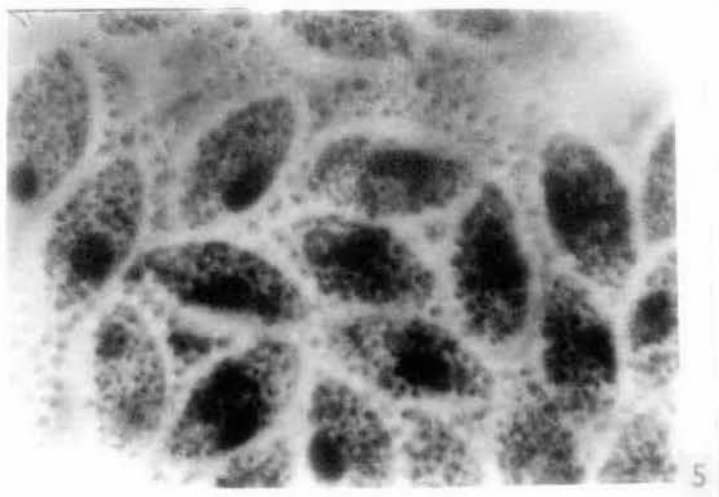
15 X 40X.

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

PLATE XIII



4



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 i.e. 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,^(Roisi 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 Bitschli

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.

TABLE IX

Measurements of Apolocystis n.sp.(b)

	RANGE	AVERAGE
Trophozoite diameter	61.2 μ - 115.2 μ	93.2 μ
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	126.2 μ
Spores length	14.2 μ	14.2 μ
Spores breadth	7.10 μ	7.10 μ

PLATE XIII

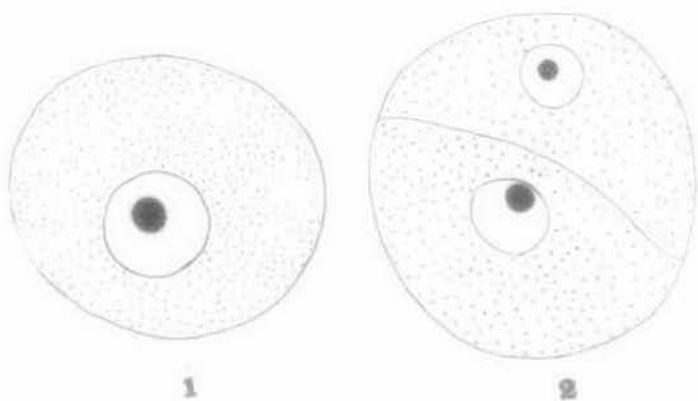
Fig. 1. Apolocystis n.sp.(b) 10 X 45X.

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

Fig. 4. Spores magnified 10 X 100X.

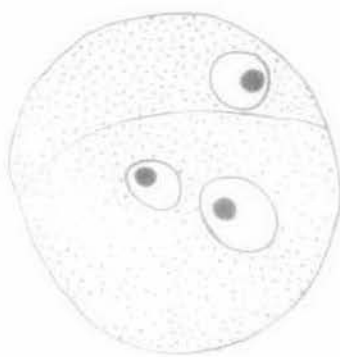
Fig. 5. Photomicrograph of two gametocytes.
15 X 10X.

PLATE XIII

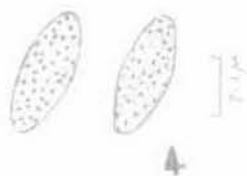


1

2



3



4



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 (= Amyntas) 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.

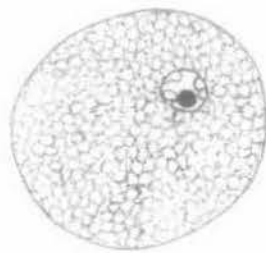
Morphology:

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.

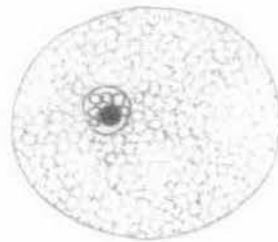
PLATE XIV

- 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



2



3



4



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 proportions of the body from all other species of the genus recorded so far.

Certain specimens of A. gigantea (Troisi 1953) have been found to show in addition to the karyosome a small spherical body, which probably could correspond to the small spherical 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 (= Amyntas) 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

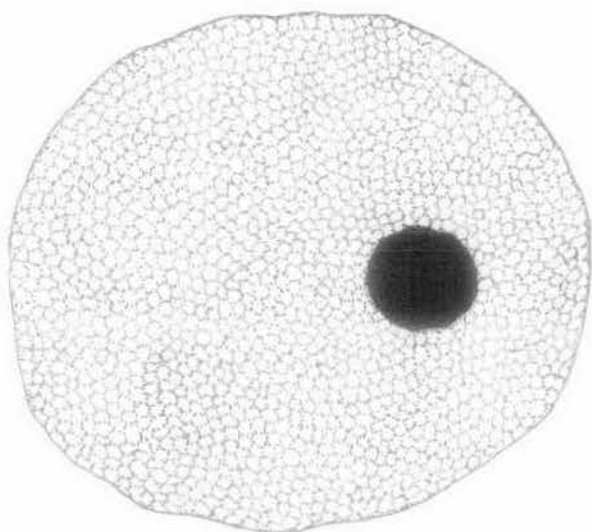
The trophozoite is more or less spherical. The body is bounded by a distinct pellicle. The endoplasm is packed with paraglycogen granules. In between these granules there occur a large number of deeply stained bodies. The nucleus is round to ellipsoidal in shape and it does not occupy a definite position 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



18μ

T A B L E 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

T A B L E 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 processes 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 occurring 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

	<u>Apolocystis</u> n.sp.(a)	<u>Apolocystis</u> n.sp.(b)	<u>Apolocystis</u> n.sp.(c)	<u>Apolocystis</u> n.sp.(d)
Shape & diameter	Spherical; 84.6 μ	Spherical; 93.2 μ	Spherical; 230.34 μ	Spherical; 98.62 μ
Endoplasm	Granular	Granular	Alveolated	Granular
Nucleus	Spherical; 15.19 μ	Spherical; 21.07 μ	Spherical & vacuolated 42.116 μ	Spherical to ellipsoidal 19.17 μ
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	<u>Pheretima robusta</u> (Perrier)	<u>Pheretima robusta</u> (Perrier)	<u>Pheretima alexandri</u> (Beddard)	<u>Pheretima diffringens</u> (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 peculiarities are sufficient to separate the

PLATE XVI

Figs. 1 - 3.

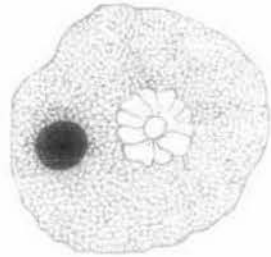
Various forms of Stomatophora n.sp.(a)

10 X 45X

PLATE XVI

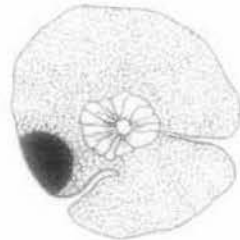


1



2

187



3

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 necessitates 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. Coelozoic; 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

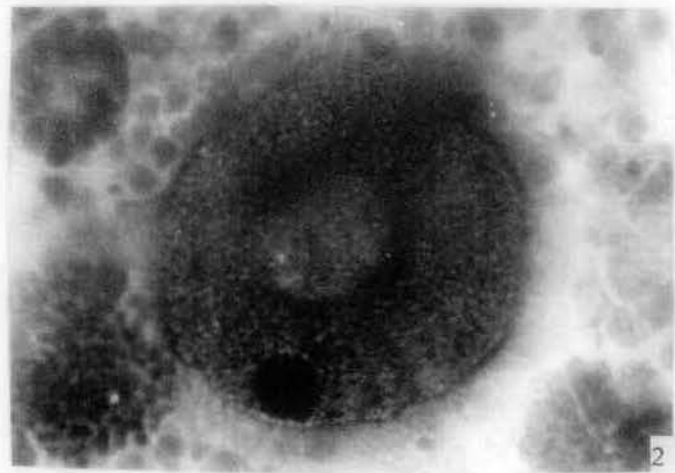
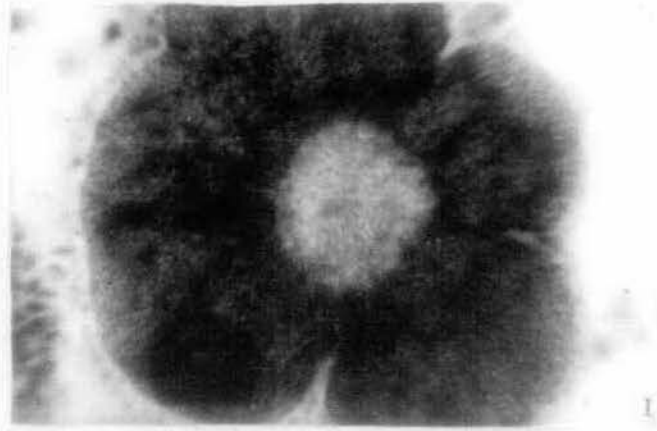
PLATE XVII

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

1. 10 X 100X.

2. 10 X 40X.

PLATE XVII



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 disappearing 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 occasionally 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

TABLE XIII

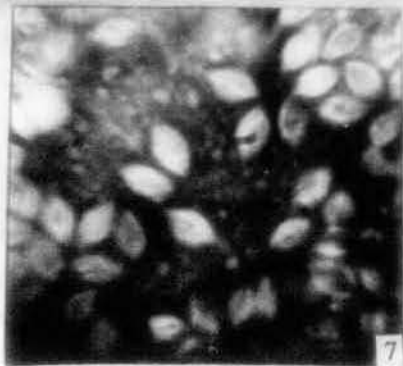
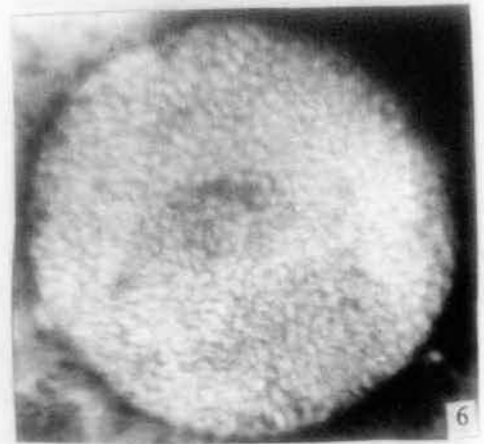
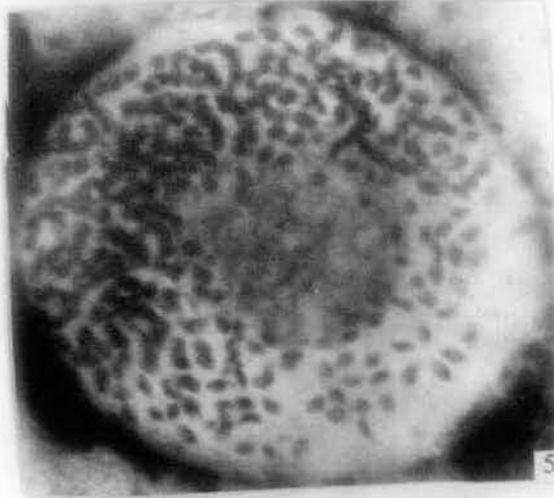
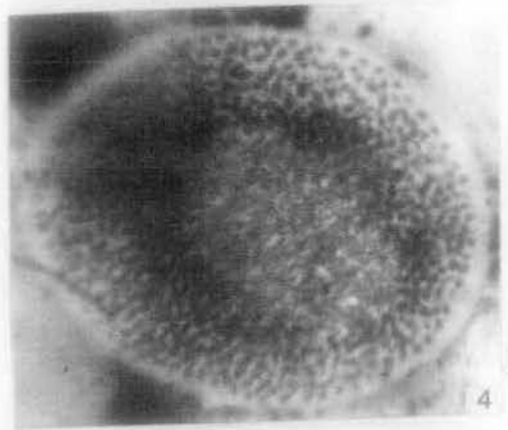
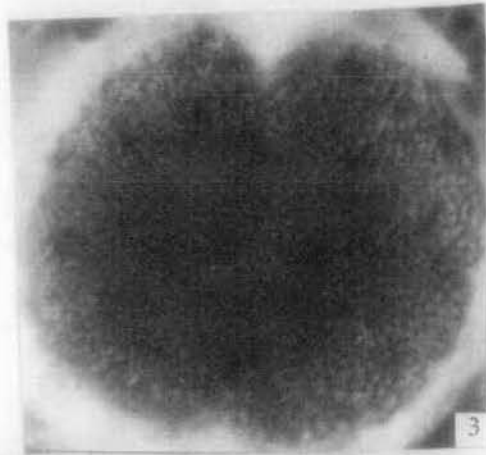
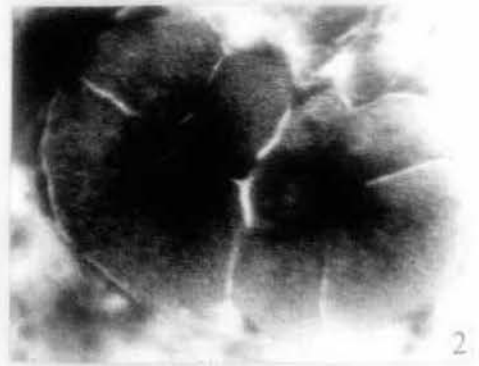
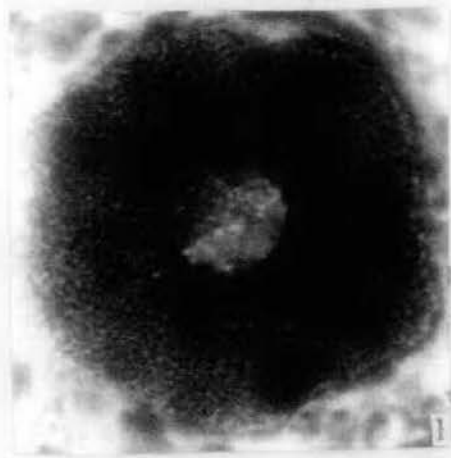
Measurements of *Stomatophora* n.sp.(b)

	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 Trophozoite Stomatopora 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



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 1209m) 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 postumi (A. Chatterjee 1971 - thesis unpublished) in that the gregarine is discoid in shape. But the sucker pattern is entirely different, whereas the micron is present in all the Stomatophoridae 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.

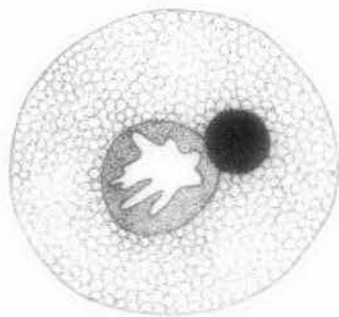
The differences are conspicuous and the parasite can safely be regarded as a new species under the genus.

PLATE XIX

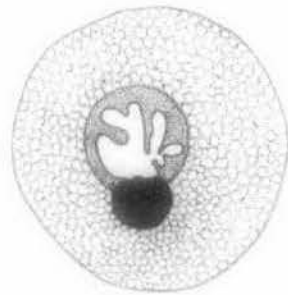
Figs. 1 - 2. Stomatophora n.sp.(c) 10 X 45X.

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

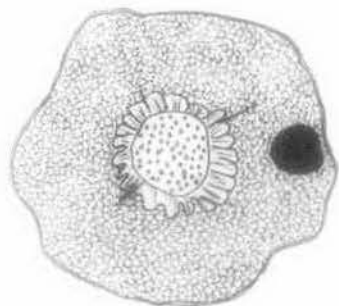
PLATE XIX



1



2



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.

T A B L E X I V

Measurements of Stomatophora n.sp.(c)

	RANGE	AVERAGE
Body diameter	30.6 μ - 97.2 μ	66.2 μ
Nucleus	10.3 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 Amyntas 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 extension 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

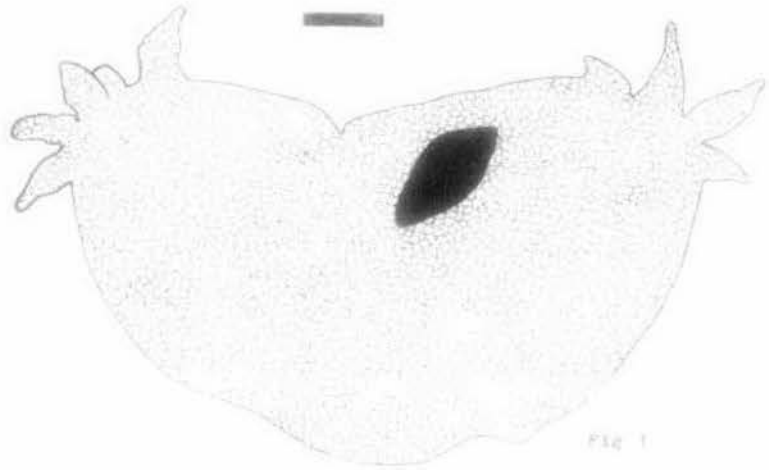


FIG 1



FIG 2

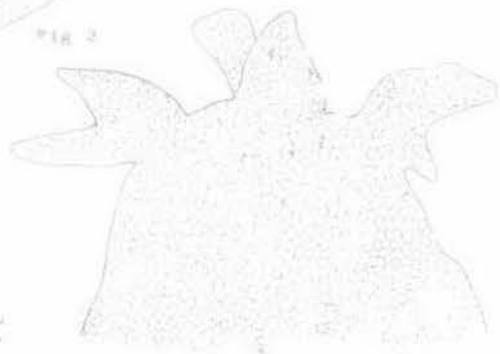


FIG 3

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.

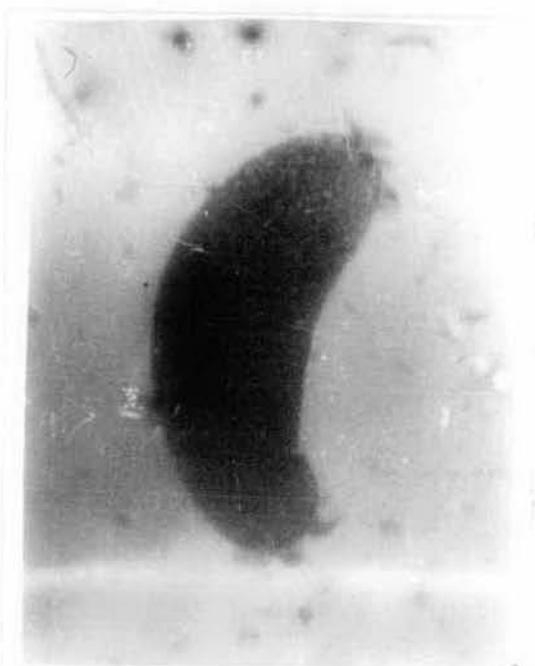
PLATE XXI

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.

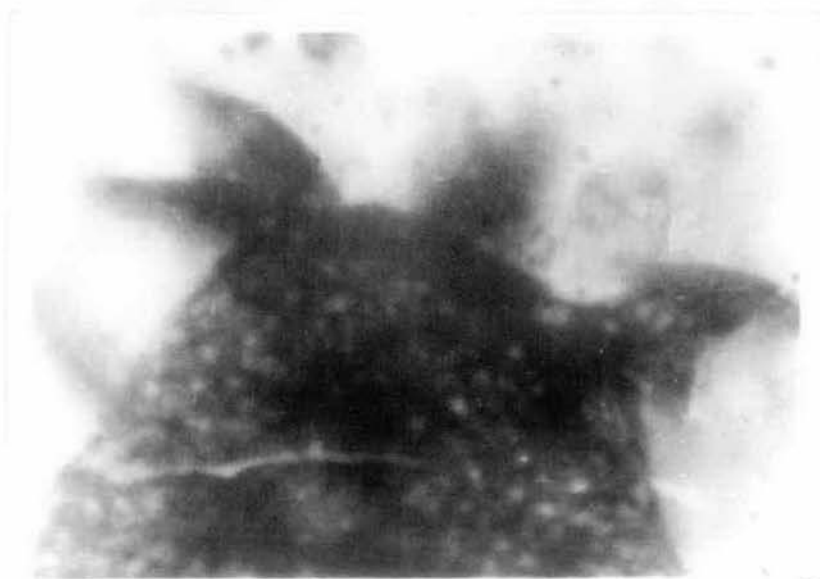
PLATE XXI



1



2

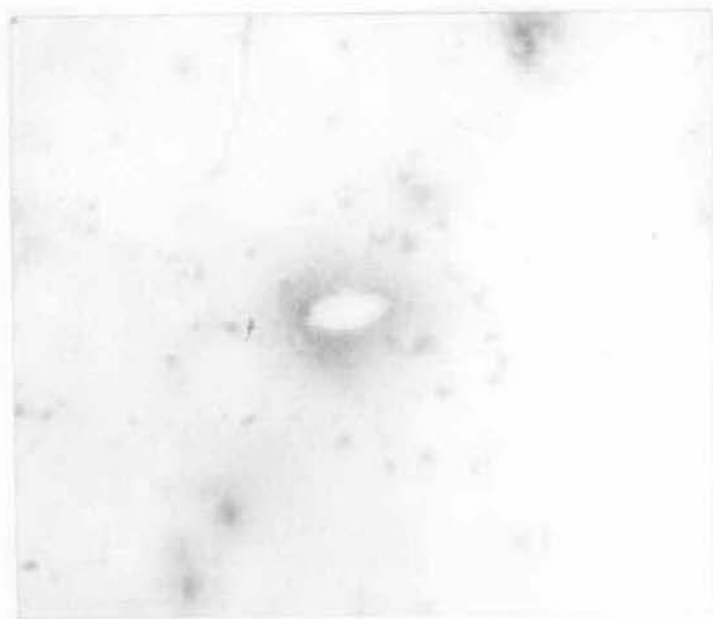


3

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(g)n.gen.,n.sp., shows certain similarities with Dendrocystis (= Arborocystis) ^(Rees 1962) piriformes 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

TABLE XVI (b)

Measurements of A(a)n.ges., 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 Bitschli .

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: Amyntas hawayanus Rosa (Oligochaeta)

Site of infection: Coelomic fluid.

Locality: Coonti (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)

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 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 membrane 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

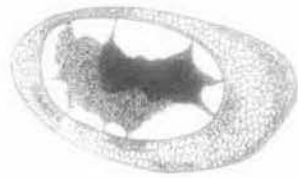


fig 3



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:

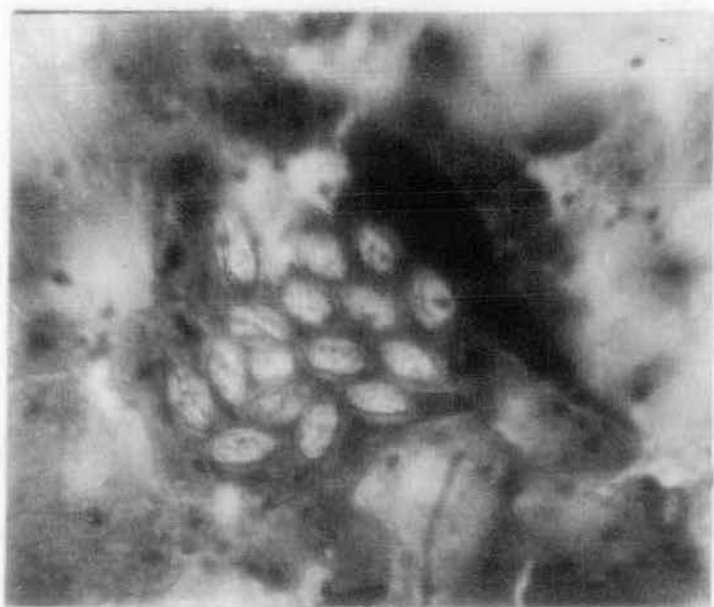
Solitary; body changes shape constantly while retaining its elongated appearance in general. One end is rounded and blunt and

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 μ
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: Aporectodea 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.

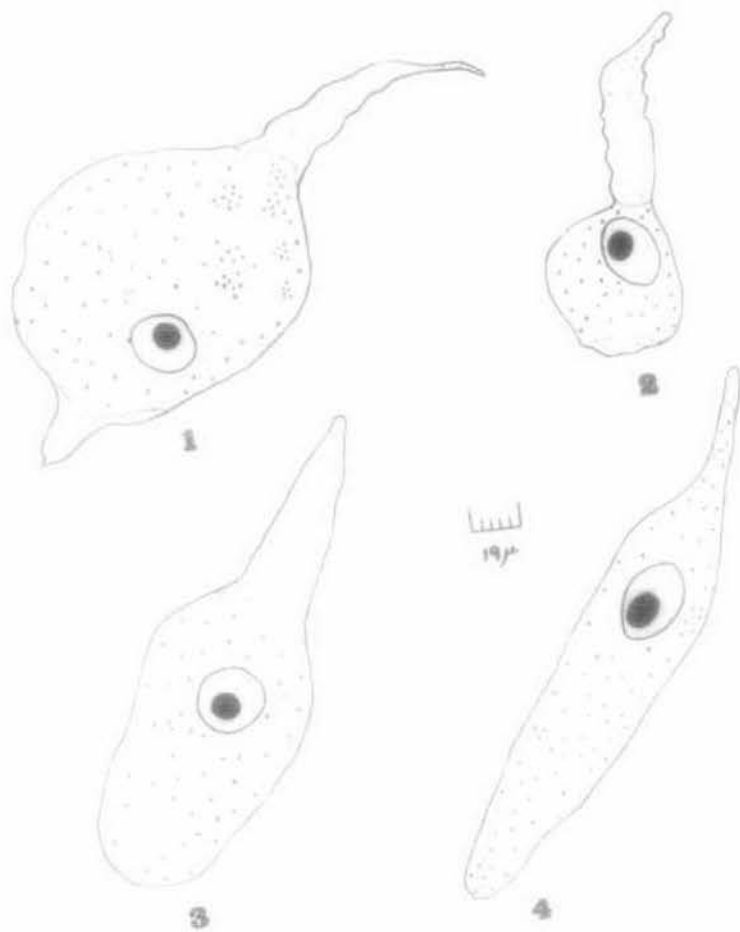
Morphology

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 extensions show some similarity to Dirhynchocystis globosa (Bhatia and Chatterjee 1925) which also exhibit extensions designated 'spine like processes' (Bhatia and Chatterjee 1925). In both the cases the ends of the extensions 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	65 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	14.98 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 (Oligochaeta.)

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 hawayan 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 separated areas outside India.

TABLE NO: XIX

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

	<u>S. coronata</u> (Hesse)	<u>S. coronata</u> 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 -7.5 μ by 3 μ	10.8 μ by 5.4 μ
Host	<u>Pheretima barbadensis</u>	<u>Pheretima californica</u>
Indian Locality	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 Chatterjee, 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 Chatterjee, 1925.
In the seminal vesicles of Pheretima heterochaeta,
collected from Punjab.
7. N. plurikaryosomata Bhatia et Chatterjee 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 Sanchal (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 gammei, collected from Mangpoo (alt. 2164.75m), Darjeeling district.

Genus: Apolocystis Cognetti de Martiis

15. A. matthei 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 Drzhevet'skiy

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 Amyntas hawayanus,
collected from Goonti (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.