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Seasonal Modifications in the Life Cycle of *Parastasia fennica* (Michajłow, 1966)

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Synopsis. Annual life cycle of endoparasitic euglenid flagellate *Parastasia fennica* (Michajłow, 1966) has been studied with the use of morphometric, morphological and histochemical methods. Spontaneously infected copepods were collected from small ponds in the environs of Leningrad. Some modifications in the life cycle of *P. fennica* in various seasons of the year were observed as well as morphological variation and storage of great amount of paramylon in cell cytoplasma during late autumn. It has been also found that low temperature of water in natural reservoirs during late autumn and winter inhibits palintomic division and formation of infective flagellate cells. In spring the reproduction of *P. fennica* is the most intensive, leading to increase of parasite populations. Seasonal modifications in *P. fennica* life cycle are considered as adaptative features, they ought to be taken into account when other species of the genus *Parastasia* Michajłow, 1972 are determined and described.

The life cycle of *Parastasia fennica* (Michajłow, 1966, 1972), an endoparasitic euglenid flagellate inhabiting alimentary tract of fresh water *Copepoda*, has been already studied in detail in natural as well as in laboratory conditions (Michajłow 1972, 1978, Wita and Sukhanova 1983). It has been found that the complex life cycle of this flagellate includes three obligatory alternating phases: (1) parasitic phase — trophozoite living in host intestine, (2) reproductive phase — mature trophozoites undergo palintomic division in outer environment after leaving host intestine, (3) infective phase — flagellate cells formed as a result of palintomic division, live in water up to ingestion by copepode host. The life cycle, from the moment of infection of a copepode by flagellate cells up to the death of flagellates in the outer environment, when contact with copepodes is failing, lasts 12–15 days in spring, summer and early autumn, i.e., in warmer seasons of the year. In reservoirs in the north-west of USSR the life cycle of *P. fennica* may be repeated several times during the year.

Modifications of *P. fennica* life cycle in late autumn, winter and early spring

have not been known till now and no information about seasonality of this species were recorded. So the aim of the present study was to investigate the life cycle of *P. fennica* during the whole year with special attention paid to the stages occurring in nature in cool seasons of the year.

Material and Methods

Copepode hosts of *P. fennica* — *Cyclopidae*, were collected from small ponds in the environs of Leningrad. Samples of the material were collected in all seasons during 1980–1984. The material was used for investigation on morphology and trophozoite physiology as well as for observation of palintomic division and formation of infective flagellate individuals.

In laboratory conditions the cultures of copepods infected with *Parastasia* were maintained the whole year round according to the method described by Michajłow (1966, 1972). They were kept at room temperature and at 1–4°C.

Except living *Parastasia* the investigation was carried on the material fixed in 4% and 10% neutral formaldehyde, Carnoy's fluid and 100% methanol. Flagellate morphology was described on the base of preparations stained with Mayer's acid hemalaun, hematoxylin after Carazzi and azur-eosin after Giemsa–Romanovsky. Lysosomes were revealed due to vital staining with 0.1% neutral red in aqueous solution (Bulychev et al. 1978).

Aggregations of paramylon in trophozoite cytoplasm were observed in living *Parastasia* as well as in those fixed in 4% formaldehyde without further staining, because paramylon does not stain with any dyes for polysaccharides. Glycogen type polysaccharides were revealed with Lugol's iodine and PAS reaction. Acid mucopolysaccharides were stained after Steedman (Pearse 1968), also 0.1% toluidine blue in 30% and 1% ethanol was used. Mucopolysaccharides on cell surface were intravitally stained with 0.01% aqueous solution of accian blue (Krylenkov et al. 1979).

Neutral lipids were revealed due to staining with saturated solution of Sudan red III in 70% ethanol and in fettrot (Pearse 1968, Beyer et al. 1977), a dye specific for these biopolymeres. For phospholipids detection saturated solution of Sudan black B in 70% ethanol was used.

All drawings were made with the aid of camera lucida PA-4 with objectives 40× and 90×.

Results

In the North-West of USSR the following copepods are hosts of *P. fennica*: *Eucyclops serrulatus* (Fisch.), *Acanthocyclops viridis* (Jur.), *A. bicuspidatus* (Claus), *A. bisetosus* (Rehb.), *A. vernalis* (Fisch.), *Macrocyclops fuscus* (Jur.). Crustaceans of the genus *Cyclops* are only rarely infected by *Parastasia*, although they commonly live in small ponds. During spring, summer and early autumn the populations of *P. fennica* are morphologically not uniform — in the host gut trophozoites of various size, i.e., various age, may be found. Their localization in the host gut is also variable. The smallest young trophozoites (30–40 μm long) inhabit the anterior part of the gut, while mature ones (180–220 μm long) occur in the further part of the intestine. Thus, during growth the trophozoites pass along the gut towards the end and the largest, mature ones leave the gut together with faeces.

This diversity of body dimensions in trophozoites living in copepode gut proves that infection of hosts with flagellate forms takes place at various times (asynchronically). During the whole warm period of the year *Parastasia*, in the course of palintomic division, and flagellate cells — infective stage of the cycle (Fig. 2) are constantly present in the bottom layer of water in coastal parts of ponds. In the warm season of the year the life cycle stages succeed each other and copepode hosts become infected swallowing flagellate cells together with other food organisms.

Our continuous investigations have shown that from the beginning of late autumn (second half of October, November) the regularity of succession of the life cycle stages is disturbed. Low temperature inhibits palintomic division and flagellate cell formation. Thus, during late autumn, winter and early spring, when water temperature is not higher than 4°C, infection of hosts does not occur. This phenomenon occurs also in laboratory conditions when the aquarium with copepods is kept in low temperature (1–4°C).

In late autumn and winter trophozoites occur in host intestine. Out of copepods active in winter, *P. fennica* occurs most frequently in *Acanthocyclops bisetosus* and *A. bicuspidatus*. The trophozoites are localized in the anterior portion of the gut. They do not leave the gut spontaneously. Their populations are not homogenous as to body dimensions, being composed of individuals of various size. However, the smallest protozoans, originating from fresh infection, are absent (Fig. 1).

By the morphological characters the smallest trophozoites observed in late autumn and winter do not differ from those found in summer. But fully mature ones undergo some morphological changes and differ from summer forms (Fig. 3) in some respects. They attain great body dimensions, up to 900 μm in length and 60–80 μm in width. The posterior body end becomes thicker and the protozoan attains sausage-like shape (Fig. 3). The increase of body dimensions and the change of shape is due to a great amount of paramylon grains stored in the cell, producing the most characteristic feature of its morphology and metabolism. Except paramylon, in the endoplasm of mature trophozoites in autumn glycogen-type polysaccharides accumulate in the form of compact granules 0.5–0.8 μm in diameter (Fig. 5). There are also more acid mucopolysaccharides than in summer; they produce β and γ metachromasia when stained with toluidine blue (Fig. 6). The glycocalyx becomes slightly thicker — it is visible due to staining with alcian blue, which reveals hyaluronid acid (Fig. 7).

At the beginning of the cool season, in the endoplasm of mature trophozoites a great amount of neutral lipids accumulates. This substance is stored in the form of small droplets situated among paramylon granules (Fig. 8). Autumn is a period of intensive feeding of *P. fennica*, resulting in an accumulation of glycogen, paramylon and neutral lipids. This is proved by a great quantity of lysosomes (primary and secondary) present in the trophozoite cytoplasm (Fig. 4).

Mature trophozoites, fulfilled with paramylon granules, are almost motionless. After the removal from the host gut and placing in water they do not move inten-

Fig. 1. Size structure of *Parastasia fennica* population in the intestine of *Eucyclops serrulatus* (mass infection). Living specimens

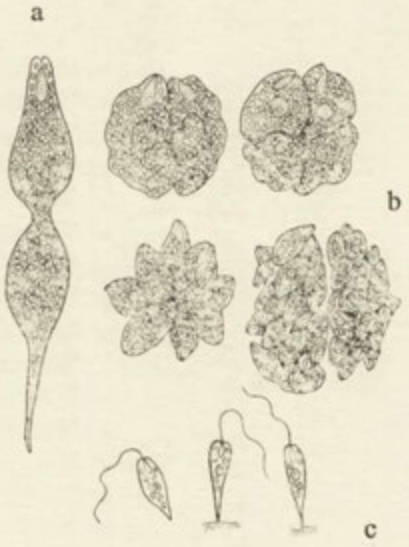

Fig. 2. *Parastasia fennica*. a – mature trophozoite, b – stages of palintomic division, c – flagellate forms (infective phase). Living specimens

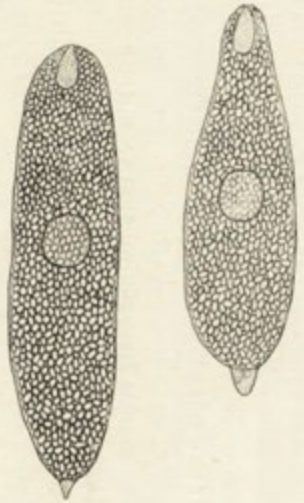

Fig. 3. Mature trophozoites of *Parastasia fennica* in late autumn and winter. Living specimens

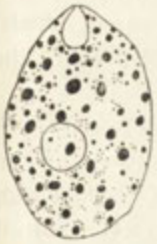

Fig. 4. Lysosomes of *Parastasia fennica*, stained with 0.1% neutral red

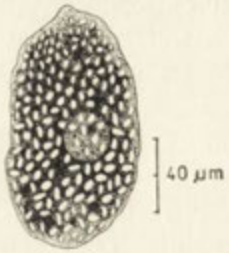

Fig. 5. Glycogen type polysaccharide in the endoplasm of winter trophozoites of *Parastasia fennica*. PAS reaction

Fig. 6. Acid mucopolysaccharides in a winter trophozoite cell. Stained with toluidine blue

Fig. 7. Surface of winter trophozoite of *Parastasia fennica* (glycocalyx). Stained with alcian blue

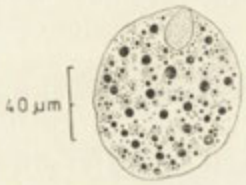

Fig. 8. Neutral lipids inclusions in the cytoplasm of winter trophozoite of *Parastasia fennica*. Fettrot staining

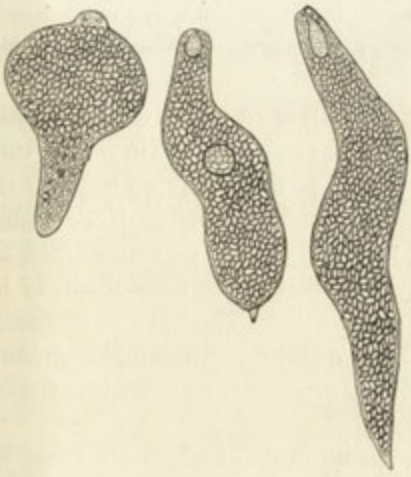

Fig. 9. Character of metabolic movements of winter trophozoite of *Parastasia fennica*. Living specimens

Fig. 10. Palintomic division of winter trophozoites of *Parastasia fennica* maintained in room temperature. Living specimens

sively (Fig. 9), in contrast to mature trophozoites in spring and summer, not overloaded with paramylon granules. Observations made on various individuals have shown that the more paramylon granules in the cytoplasm are present, the less mobile is the trophozoite.

Observations on the development of mature trophozoites, removed from the gut of wintering hosts, were carried in the laboratory from November to February. This study aimed in recognition of palintomic division of *Parastasia* during the winter season. Under natural conditions, low winter temperature (1–4°C) inhibits palintomic division during the whole cool season. In cultures kept at room temperature the trophozoites removed from the host gut become rounded and form a mucous envelope around themselves. However, only few specimens begin palintomic division, irrespective of the optimum temperature maintained in the laboratory. Moreover, many abnormalities occur during this process, e.g., many winter trophozoites with great paramylon burden live 5–6 days or more in culture, do not undergo division and die. Many specimens undergo division induced by increasing temperature, but much more slowly than in spring and summer. Besides, in many cases the process of division is not accomplished and the protozoon die. In cases when the process of palintomic division runs its course to the end, most infective flagellate forms do not produce flagella. Sometimes a short, reduced flagellum is formed, which cannot be used for swimming. Such cells can move only on the substrate due to metabolic contractions, and most of them soon die (Fig. 10).

In natural reservoirs, already in the second half of March and in April, the large trophozoites overloaded with paramylon granules, occur rarely and usually as single individuals. Simultaneously with them small trophozoites originating from new infection may be observed in copepode gut.

During winter, when the feeding of copepods is rather limited, the *Parastasia* live at the cost of paramylon, reducing its stock many times in comparison to that in late autumn.

In spring, when ponds become free of ice, the period of intensive reproduction of the *Parastasia* begins and its natural populations quickly increase in size. A study on palintomic division in laboratory conditions showed that in spring not only mature trophozoites divide, but also small, not yet fully mature individuals. Palintomic division begins within a few hours of leaving the host gut and all trophozoites undergo division (100%). The number of infective flagellate cells, descendants of immature trophozoites is smaller than of large, mature ones. The number of descendants depends on the size of trophozoite and the quantity of paramylon granules (Fig. 11a). Most frequently 2–32 flagellate cells are produced, while mature trophozoites give rise to 32–128 cells.

It has been also observed that young trophozoites (about 30–35 μm long), not yet ready for division, produce flagellum when placed in water and renew the infective stage (Fig. 11b).

Intensive reproduction, induced in nature by a change of temperature, sunlight

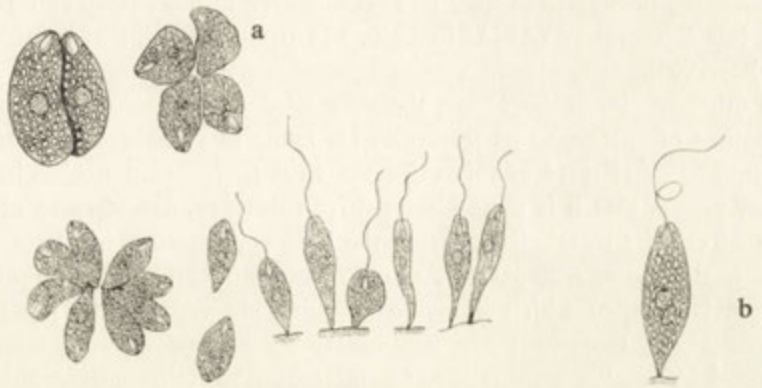

Fig. 11. Palintomic division of young trophozoites of *Parastasia fennica* in spring (a), formation of flagellum by young trophozoite taken out of the host gut (b). Living specimens

and increasing host activity, represents a characteristic peculiarity of spring season in the life cycle of *P. fennica*. In summer the life cycle of this species runs regularly and may be repeated many times (Michajłow 1972, 1978, Wita and Sukhanova 1983).

Discussion

In climatic conditions of the North-West of USSR, with clearly cut seasons of the year, the life cycle of *Parastasia fennica* shows seasonal modifications concerning its morphology and physiology as well. Also the longevity of particular life cycle stages varies depending on the season.

Low winter temperatures *P. fennica* spends in the trophozoite stage in its host organism and this phase of the life cycle is strongly elongated (up to 5 months) in comparison with spring and summer trophozoites (Michajłow 1966, 1972, 1978, Wita and Sukhanova 1983). Besides, during late autumn, winter and early spring, due to the inhibitory influence of low temperature, palintomic division is completely arrested, as well as the formation of infective flagellate cells.

All these peculiarities in the life cycle of *P. fennica* are considered as adaptative modifications of the parasites for survival in spite of unfavourable conditions in their habitat. Morphological variation observed in trophozoites is also adaptative in character, being connected with cool seasons of the year. This variation is manifested by an increase of body dimensions of mature trophozoites (Fig. 3) due to accumulation of reserve substances, especially of paramylon. Such accumulation of reserve substances is a well known phenomenon in many free-living and parasitic protozoans. It is known that glycogen and neutral lipids are accumulated by opalinids and ciliates in the autumn when the temperature of outer environment falls

down. The same phenomenon may be also observed in laboratory cultures maintained in low temperature (Zhinkin 1930, Manusova 1939, Sukhanova 1953, Poljanskij 1963).

As in other species of *Euglenida*, the paramylon in *P. fennica* is the most important source of energy, so its reserve in the cytoplasm of these flagellates is always high. The great store of this substance in *P. fennica* trophozoites in autumn and winter proves that it is the main nutritive substance, necessary to uphold all processes of cellular metabolism during the long cool season, when many of its copepode hosts undergo diapausa. Autumnal and winter mature trophozoites of large dimensions, filled with paramylon granules, show only little possibility to perform metabolic movements, in contrast to spring and summer trophozoites characterized by smaller dimensions. Characteristic "wave" constrictions are not produced by large trophozoites kept in water, outside the host body. So the number of "waves" observed during metabolic movement cannot be regarded as a taxonomic character of *Parastasia* (Palienko 1980).

Trophozoites taken out of the host gut and placed in the culture at 4°C do not undergo division and die. It is barely possible to evoke palintomic division by placing the trophozoites, taken out of a wintering copepode, in room temperature. Most of them die without any attempt to divide, in others the division is disturbed. The offspring produced as a result of such aberrant palintomy is also abnormal (Figs 2, 10). So, such characters as a short, reduced flagellum cannot be regarded as a specific taxonomic character for the discrimination of a new species, especially if the material is collected in autumn and winter (Palienko 1980).

The most intensive and quick reproduction of *P. fennica* in nature takes place in spring. In this season palintomic division is performed not only by mature trophozoites, but also by young ones leaving the host gut. There are also some other peculiarities in the life cycle of *P. fennica*, e.g., secondary transformation of young trophozoite into infective flagellate cell. Thus, spring is the period of quick increase of *Parastasia* population, remaining later, in other seasons of the year, at a defined level.

Particularities of the annual life cycle of *P. fennica* ought to be taken into account when this or other new species of endoparasitic *Euglenida* are to be described (Fize 1969, Michel and Fize 1972, Wita 1978, Palienko 1980).

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Thécamoebiens des plages de la Mer du Nord en Angleterre

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Synopsis. Les résultats concernant les Rhizopodes Testacés de quelques plages des côtes anglaises, ont donné une liste de 17 espèces.

Depuis la découverte des premières espèces de Thécamoebiens psammobiontes et la création de la Famille des *Psammonobiontidae* (Golemansky 1974), leur nombre n'a cessé d'augmenter au fur et à mesure des recherches dans ce biotope particulier.

Toutes les espèces strictes vivant dans ce milieu, possèdent plusieurs particularités spécifiques qui les caractérisent et les distinguent des autres Thecamoebiens d'eau douce, des Mousses ou du sol (Golemansky 1978).

Les recherches sur les Thécamoebiens interstitiels supralittorals des Mers et Océans entreprises jusqu'à présent, semblent démontrer que la majorité des espèces connues ont une répartition cosmopolite.

Matériel et méthode

Les espèces reprises dans cette étude, proviennent d'une série de prélèvements exécutés sur les plages de la région de Douvre (Angleterre).

L'eau interstitielle des plages de la côte anglaise, possède des caractères physico-chimiques proches de ceux des côtes belges (Chardez 1977).

Les prélèvements ont été Fait à marée haute; distance variant de 2 à 4 m du bord de mer, profondeur dans le sable de 20 à 40 cm pH = 7.50 à 7.60, salinité 31.600 NaCl/l. Température de l'eau 17°C (aout 1984).

Au laboratoire, les prélèvements ont été placés en cristallisoires, l'évaporation compensée régulièrement par apport d'eau stérile.

Liste faunistique

Centropyxiella arenaria Valkanov
Centropyxiella gibbula Valkanov
Centropyxiella oopyxiformis Chardez
Centropyxiella platystoma Golemansky
Ogdeniella maxima Golemansky Pl. I 1–3
Ogdeniella elegans Golemansky
Ogdeniella lucida Golemansky
Ogdeniella septentrionalis (Chardez) Golemansky
Ogdeniella taschevi Golemansky Pl. I 4
Psammonobiotus communis Golemansky
Psammonobiotus plana Chardez
Pseudocorythion acutum (Wailes) Valkanov
Pseudocorythion undulacollis Chardez et Thomas
Centropyxis constricta (Penard) Deflandre Pl. I 5

Incertea sedis

Lagenidiopsis elegans (Grubber) Golemansky
Lagenidiopsis valkanovi Golemansky
Volutella hemispiralis Chardez

Ces trois espèces, n'ont peut-être pas leur place parmi les Thécamoebiens, elles appartiennent actuellement à un groupe mal défini, probablement proche des Foraminifères.

L'étude des pseudopodes granuleux et la profonde invagination tubulaire du pseudostome chez les *Lagenidiopsis* cont comparables à certains Foraminifères *Sacamminidae*.

Note sur *Ogdeniella maxima* Golemansky, 1982

Les spécimens observés, présentaient quelques variantes morphologiques, la thèque est plus fortement comprimée que chez le type décrit par Golemansky et le col est plus largement évasé.

Ces variations peuvent être considérées comme intra-spécifiques (Pl. I 1) à 39.

Commentaires

L'ensemble des taxons inventoriés appartient à la faune caractéristique du mésopsammon.

Ogdeniella taschevi et *Pseudocorythion acutum* représentent ici les espèces les plus abondantes.

Il est bien connu que plusieurs espèces venues des eaux douces ou des Mousses

du continent, ont déjà été observées dans le psammon, mais le plus souvent sous forme de thèques vides, ces espèces peuvent être considérées comme erratiques.

Quelques espèces toutefois, ont été observées vivantes et semblent pouvoir s'adapter en milieux salés ou saumâtres.

Cela semble notamment le cas pour les espèces suivantes: *Centropyxis conxtricta* signalée par Valkanov (1936), Golemansky (1970) et Chardez (1972). *Hyalosphenia cuneata* signalée par Wailes (1927), Golemansky (1970, 1973, 1983). *Hyalosphenia minuta* signalée par Gourvitch (1934), Chardez (1972). *Antarcella atava* signalée par Decloitre (1972). *Cochliopodium granulatum* signalée par Valkanov (1970), Decloitre (1972). *Irinema lineare* signalée par Golemansky (1971), Sudzuki (1979) et Chardez (1972).

Bien que nous n'ayons pas observé *Centropyxis constricta* actif dans ces prélèvements, le grand nombre de thèques présentes nous incite à considérer cette espèce comme psammophile.

Un des caractères important qui semble le démontrer réside dans la nature des xénosomes entrant dans la structure de la thèque, en effet, on y reconnaît souvent des fragments de Diatomées marines mélangés à des micromorpholites toujours abondants dans les sables marins, or la construction d'une thèque représente incontestablement une manifestation vitale.

SUMMARY

The results about the Testate Amoebae from English coast beaches, produced the list containing seventeen species.

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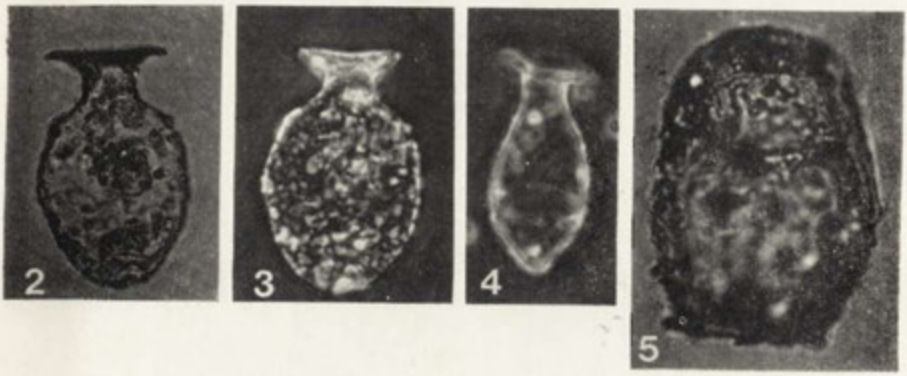
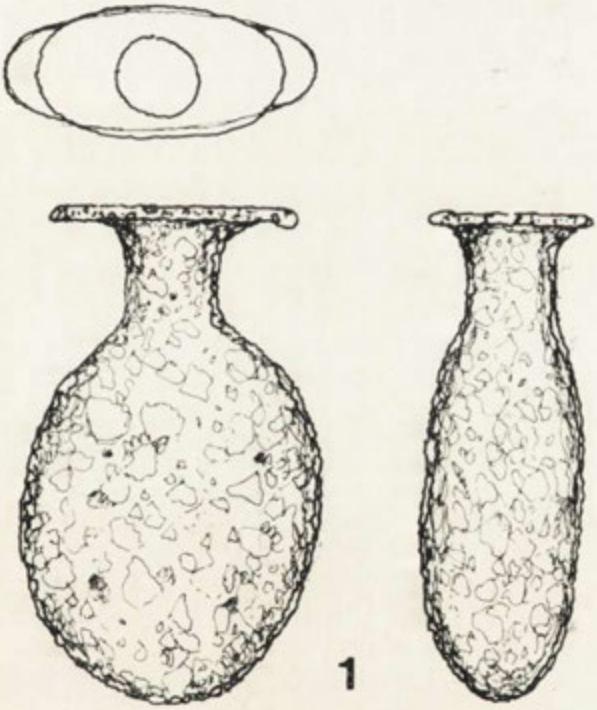
EXPLICATIONS DE PLANCHE I

1: *Ogdeniella maxima* Golemansky (Variante face, profil et plan)
2: *O. maxima* (×400)
3: *O. maxima* (×400 contraste de phase)
4: *Ogdeniella teschevi* (×400 contraste de phase)
5: *Centropyxis constricta* (×400)





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Ciliaten aus dem Interstitial des Ontario Sees

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Synopsis. Die Ciliaten im Interstitial des Ontario Sees bei Burlington, Ontario (Kanada) wurden nach der Methode von Uhlig (1964) qualitativ erfaßt. Insgesamt wurden 12 verschiedene Arten nachgewiesen. Es sind einesteils Ciliaten, deren Habitat das Kapillarwasser ist: *Cyclidium plouneouri, Loxodes magnus, Loxodes rostrum, Sathrophilus muscorum* sowie sapropelische, biotopunspezifische Arten: *Loxocephalus luridus, Dexiotricha plagia, Cristigera media, Histiobalantium marinum.* Die Infraciliatur von 10 Arten wurde genauer untersucht und abgebildet. Drei neue Arten wurden gefunden: *Cyclidium setiger, Cristigera hammeri, Histiobalantium minor.*

Die von Remane (1933) entdeckte Mikrofauna im Lückensystem des Meeressandes ist seitdem Gegenstand zahlreicher, besonders zoologischer Untersuchungen gewesen, die zur Entdeckung speziell an diesen Lebensraum angepaßter Metazoen und Protozoen geführt haben.

Im marinen Interstitial sind Ciliaten unter allen in Frage kommenden Besiedlern die dominierende Gruppe. Nach Bock (1953), Fauré-Fremiet (1948), Dragesco (1960) und Hartwig (1974) leben allein im Sandlückensystem der europäischen Meere über 250 verschiedene Arten.

Im Vergleich mit der marinen Sandlückenfauna ist die Interstialfauna des Süßwassers deutlich artenärmer. Dies trifft sowohl für Metazoen als auch für Protozoen zu, wie Untersuchungen von Pennak (1940, 1951), Goulder (1971) und Reinnarth (1979) zeigen. Nach Ruttner-Kolisko (1956) stehen auch im Limnopsammon die Ciliaten vor den Nematoden, den Rotatorien und Oligochaeten an erster Stelle. Dragesco (1960) traf im Interstitial des Genfer Sees "nur" 50 verschiedene Ciliaten-Arten an.

Verantwortlich für die relative Artenarmut des Limnopsammons ist die Besiedlungsfähigkeit des Porenwassers, die unter der Voraussetzung, daß Sauerstoff ausreichend vorhanden ist, in erster Linie von seinem Nährstoffgehalt bestimmt wird.

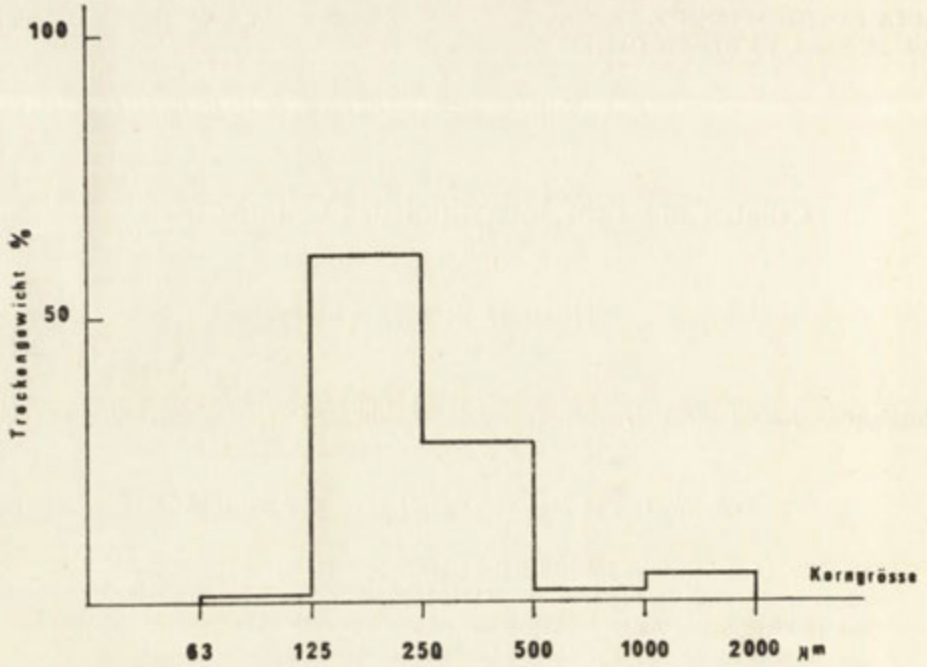

Abb. 1. Korngrößenverteilung. Trockengewicht in % gegen Korngröße. Probestelle Burlington, Ontario See

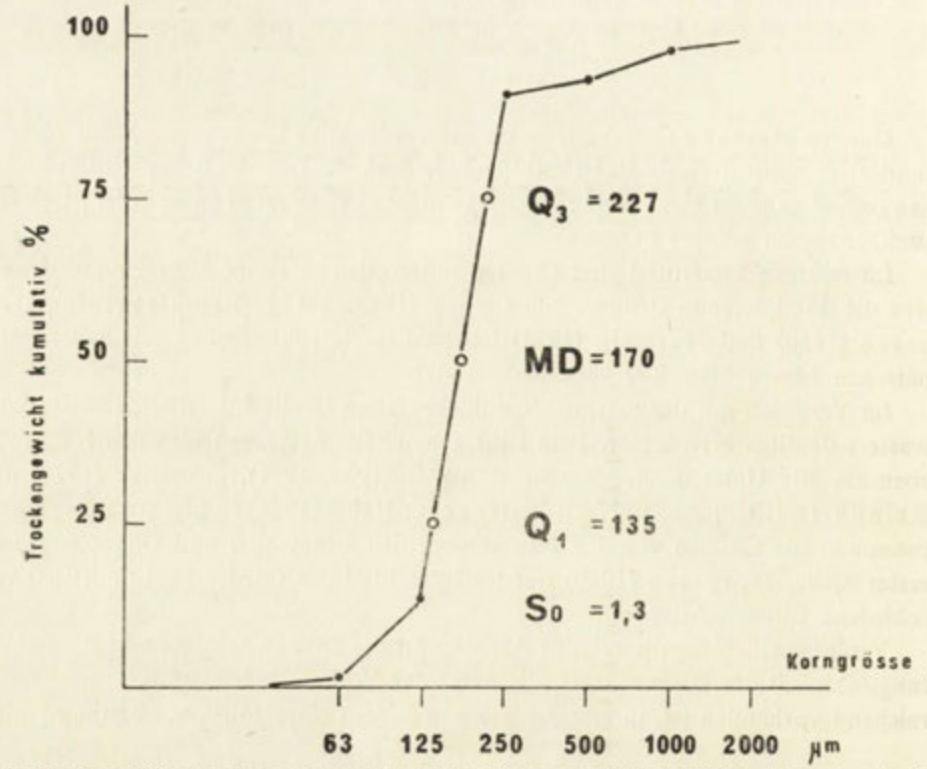

Abb. 2. Korngrößenverteilung. Summenkurve der Daten aus Abb. 1. MD — Zentralwert oder Median, Q_1, Q_3 — Quartile 1 und 3, S_0 — Sortierungskoeffizient — $\sqrt{Q_3/Q_1}$

In den Kapillarräumen können Tiere nur dann existieren, wenn deren Zufuhr und Erneuerung ständig gewährleistet ist. Die hierzu erforderliche Wasserventilation hängt vom Wellenschlag ab. Sie wird außerdem beeinflußt von der Korngrößenverteilung im Sand, da diese über das Porenvolumen auf den Wasserdurchsatz einwirkt. In den marinen Sandbiotopen ist allein schon durch den ständigen Gezeitenwechsel die Wassererneuerung regelmäßiger und intensiver. Aus diesem Grunde sind hier die Lebensbedingungen günstiger und ist die Artenvielfalt größer als im Limnopsammal.

Die nordamerikanischen Großen Seen sind von ihren Dimensionen her Meere. Die Küsten mit ihren Sandstränden, die Brandungszonen und der Wellengang sind denen der Ozeane durchaus vergleichbar. Besonders an der Wasserlinie trifft man die Bedingungen an, die im marinen Bereich erfahrungsgemäß auf eine artenreiche Interstitialfauna schließen lassen. Hier, an der Westseite des Ontario Sees, habe ich im September 1981 eine Bestandsaufnahme der Ciliaten im Interstitial durchgeführt.

Material und Methode

Untersucht wurde das Interstitial vor dem Canada Centre of Inlandwaters, Burlington, Ontario. Das litorale Benthal gliedert sich hier in einen mäßig steil zum Wasser hin abfallenden Sandhang, an dem sich die Brandungswellen brechen, und in einen ständig mit Wasser bedeckten Teil. In diesem wurde ungefähr 1 m von der Wasserlinie entfernt die Ciliatenfauna untersucht. Die Sedimentproben habe ich mit Plexiglasröhren (Länge 50 cm, Durchmesser 3 cm), die an den Enden zu verschließen waren, entnommen, indem 10 cm lange Sandzylinder ausgestochen wurden. Um Vergleiche mit marinen Sandbiotopen anstellen zu können, wurde die Korngröße des Materials analysiert. Zur Methode siehe Schmidt (1968). Die Analyse (Abb. 1, 2) zeigt, daß die Proben vorwiegend aus Feinsand und nur zu einem geringen Teil aus grobkörnigen Material bestehen. Nach Fauré-Fremiet (1950), Dragesco (1960) und Hartwig (1973) beherbergen vergleichbare marine Sande eine artenreiche Ciliatenfauna.

Die Extraktion der Ciliaten erfolgte nach der Methode von Uhlig (1964) mit dem Unterschied, daß die Ciliaten nicht mit Seewasser- sondern mit Süßwassereis des Standortes ausgetrieben wurden. Da dieses Verfahren im limnischen Bereich noch nicht angewandt wurde, habe ich seine Leistungsfähigkeit überprüft, indem ich es mit dem Extraktionsverfahren der "Klimaverschlechterung" Schlieper (1968) verglichen habe. Dieser Vergleich gab aber bezüglich der qualitativen Zusammensetzung der Infauna keinen Unterschied. Aus praktischen Gründen habe ich dann ausschließlich nach der Uhlig-Methode extrahiert.

Die Ciliaten habe ich nach Kahl (1930–35) bestimmt. In zweifelhaften Fällen wurde die Infraciliatur durch Imprägnation mit Silbernitrat nach Chatton-Lwoff (1930) und mit Protargol nach Wilbert (1975) untersucht. Neue und wenig bekannte Arten sind abgebildet. Die systematische Einordnung der angetroffenen Arten basiert auf Corliss (1979).

Der Maßstab in den Zeichnungen entspricht 10 μm.

Folgende Abkürzungen gelten für die Biometrie:
M — Median, n — Anzahl der untersuchten Individuen, Sx — Standardabweichung, $S\bar{x}$ — mittlerer Fehler des Mittelwertes, $\bar{x}$ — Mittelwert.

Ergebnisse

Unterklasse: *Hypostomata* Schewiakoff
Ordnung: *Cyrtophorida* Fauré-Fremiet
Familie: *Lynchellidae* Jankowski

Gastronauta clatratus Deroux, 1976 (Abb. 3)

Länge 45–65 µm. Die Art gleicht der Beschreibung von Jutrczenki (1978). Mit der von Deroux (1976) gefundenen Population stimmt sie in Gestalt, Kernverhältnissen und Lage der kontraktilen Vakuolen überein. Sie unterscheidet sich aber in der Gliederung der von Deroux so bezeichneten "cinetie droite externe". Diese bezeichne ich hier als "periphere Kinete". Zu ihr gehören ein ventrales (vpK) und mehrere dorsale Fragmente (dpK 1–3). Deroux gibt für seine Art 3 präorale periphere Kineten an. Meine Population hat aber präoral nur 2. Eine dritte (dpK 3) steht postoral, caudal. Diese hat Deroux nach eigener Bekundung übersehen.

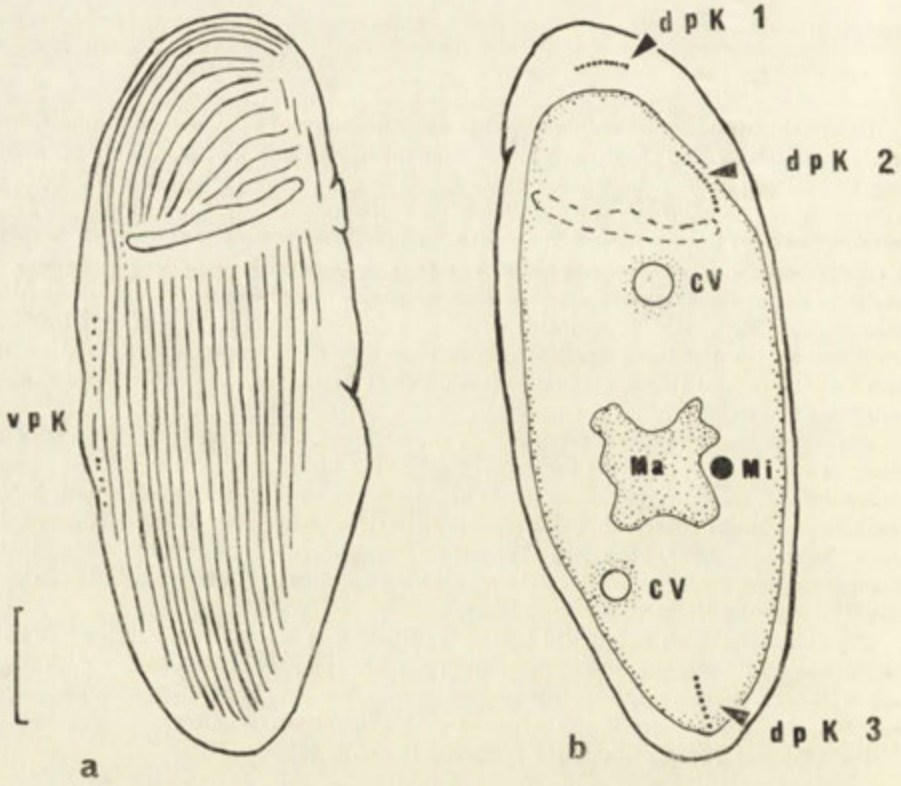

Abb. 3. *Gastronauta clatratus* Deroux, 1976. (a) Ventrales Kinetom. (b) Dorsalansicht. Nach Lebendbeobachtung und Protargolimprägnation. CV — kontraktile Vakuole, vpK — ventraler und dpk 1–3 — dorsale Anteile der peripheren Kinete. Ma — Makronucleus, Mi — Mikronucleus

Faßt man die Merkmale meiner Population, der von Deroux und von Jutrczenki zusammen dann lautet die Artdiagnose: gattungstypische ventrale Bewimperung, dorsale peripherc Kinete (dpK) 3–4 teilig, davon eine postoral, caudal gelegen.

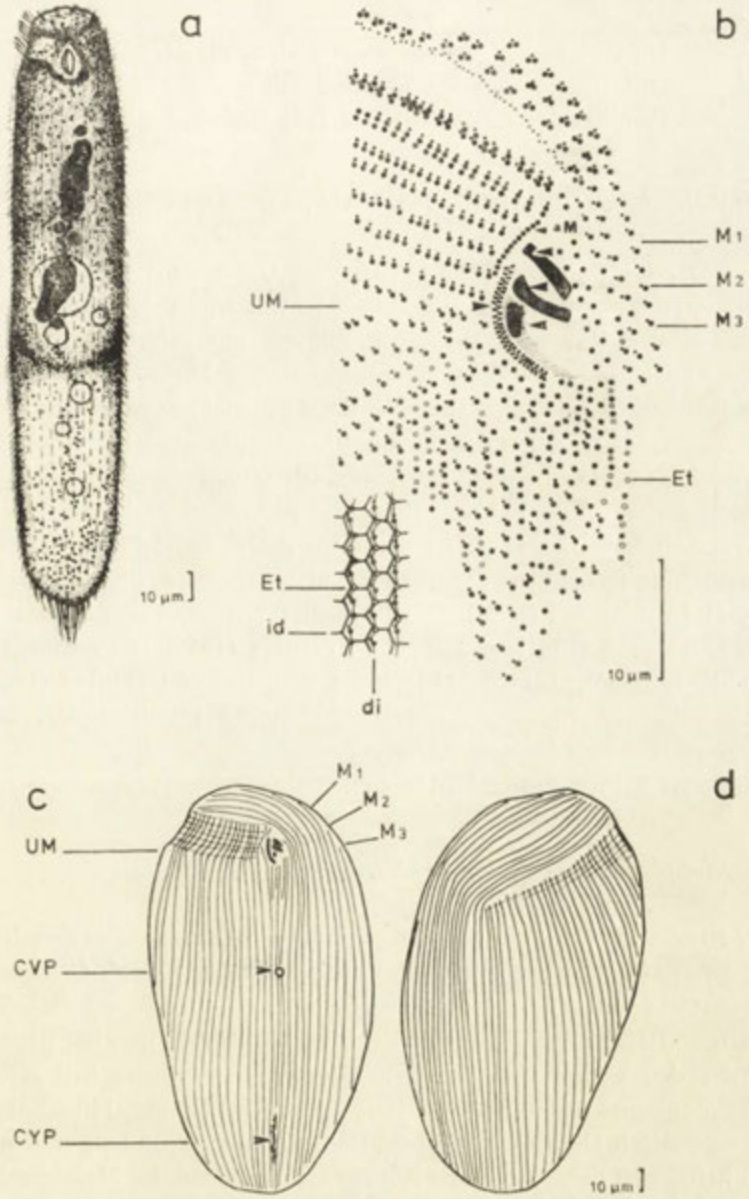

Abb. 4. *Loxocephalus luridus* Eberhard, 1862. Nach Lebendbeobachtung (a) und Chatton-Lwoff Präparationen gezeichnet. (b) Infraciliatur des Mundes und Teilaspekt des Silberliniensystems. (c, d) ventrales und dorsales Kinetom. (Tier durch die Präparation kontrahiert). aM – additional membrane, CVP – Exkretionsporus, CYP – Cytopyge, Et – Extrusome (Trichocysten), di – direkt verbindende Silberlinien, id – indirekt verbindende Silberlinien, M_{1-3} – Membranellen, UM – undulierende Membran

G. clatratus wurde nur vereinzelt angetroffen. Die Art lebt als Weidegänger von Bakterien und Algen.

Vorkommen: marin (Deroux 1976), im Aufwuchs von Bächen (Jutrczenki 1978), Limnopsammal.

Unterklasse: *Hymenostomata* Delage et Hérouard
Ordnung: *Scuticociliatida* Small
Familie: *Loxocephalidae* Jankowski

Loxocephalus luridus Eberhard, 1862 (Abb. 4)

Länge 200–250 µm. Wurmförmig. Vorne im Querschnitt rund, mit schwarzer Granulierung. Hinten durchsichtig und abgeflacht. Bewimperte Stirnplatte, deutlich von einer adoralen Rinne abgesetzt. Ektoplasma mit zahlreichen Trichocysten. In der vorderen Hälfte ein ovaler bis hantelförmiger Makronucleus, 3–4 Mikronuclei. In Körpermitte eine kontraktile Vakuole mit einem ventralen Exkretionsporus. Körper metabol und wenig kontraktil. Cilien in über 100 Kineten.

Der Mund am Ende einer spiral verlaufenden Wimperrinne ist mit 8 µm Länge im Vergleich zur Körpergröße des Tieres ausgesprochen klein. Der Oralapparat ist tetrahymenal. Bemerkenswert ist ein zusätzliches Strudelorganell, eine membranartige Bildung rechts über der undulierenden Membran, die "additional membrane" (aM) Jankowski (1964). Die Infraciliatur dieser Population stimmt mit der von Jankowski (1964) und Fauré-Fremiet (1968) untersuchten überein. Nach Kahl (1930–35), Jankowski (1964) und Fauré-Fremiet (1968) ist der Körper aber nicht wurmförmig, sondern gedrungen spindelförmig. Der Ciliat frißt Bakterien und kam regelmäßig vor.

Vorkommen: Sapropel, Limnopsammal.

Dexiotricha plagia Stokes, 1885 (Abb. 5)

Nach Jankowski (1964) ist die Art synonym mit *Loxocephalus annulatus* Kahl, *Loxocephalus luridus* Smith, *Loxocephalus simplex* (Penard) Kahl, *Uronema simplex* Penard und *Colpidium pannonicum* Gelei.

Größe 65–80×25–35 µm. Schlank oval, mit flacher, unbewimperter Frontalplatte. Unter den Cilienreihen liegen in Streifen angeordnete hell erscheinende Schollen. Entoplasma vorne meist schwarz granuliert. Kontraktile Vakuole und Kernapparat zentral. Die Cilien in 30–35 Reihen, etwa 10 µm lang und spreitzbar. Mit ihnen heftet sich das Tier in den Ruhephasen während der Nahrungsaufnahme Substratpartikeln an. Die "additional membrane" ist deutlich über dem Peristom zu erkennen, als eine Verdichtung von Kinetosomen der 1. Kinete. *D. plagia* ist ein Bakterienfresser und wurde regelmäßig aber nicht zahlreich angetroffen.

Vorkommen: Sapropel (Jankowski 1964), Limnopsammal.

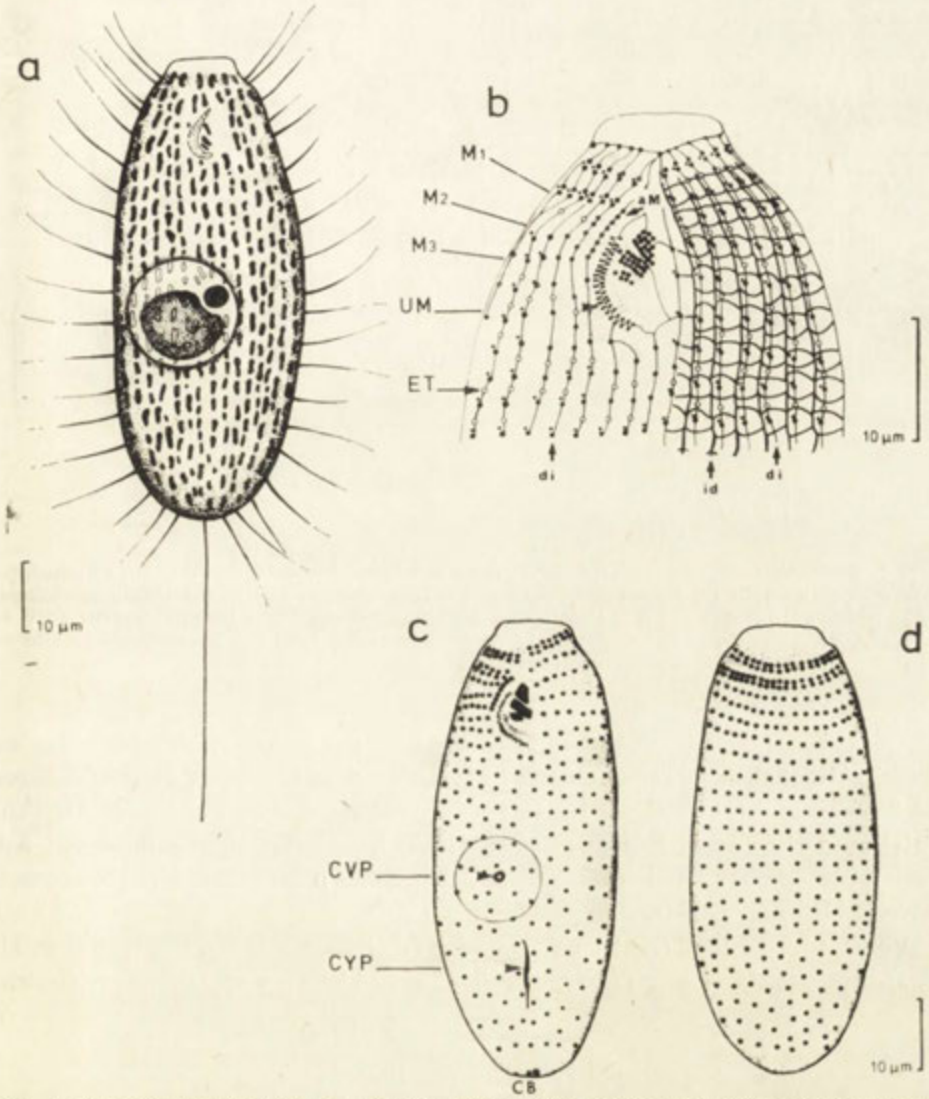

Abb. 5. *Dexiotricha plagia* Stokes, 1885. (a) Nach Lebendbeobachtung, (b) Infraciliatur ventral im Bereich des Mundes. Indirektes Silberliniensystem (id) rechts nicht eingezeichnet, Chatton–Lwoff-Präparation, (c) Ventrales- und (d) Dorsales Kinetom, Protargolimprägnation. aM – additional membrane, CB – Basalkörper der Schwanzcilie, CVP – Exkretionsporus, CYP – Cytopyge, Et – Extrusome, di – direkt und id – indirekt verbindende Silberlinien. M_{1-3} – Membranellen, UM – undulierende Membran

Familie: *Cinetochilidae* Perty

Sathrophilus muscorum Kahl, 1931 (Abb. 6)

Länge 25–35 µm, Breite um 20 µm, dorsoventral abgeflacht. Mund vor der Mitte, dem rechten Körperrand genähert. Vierzehntens seiten 16 Kineten, in denen

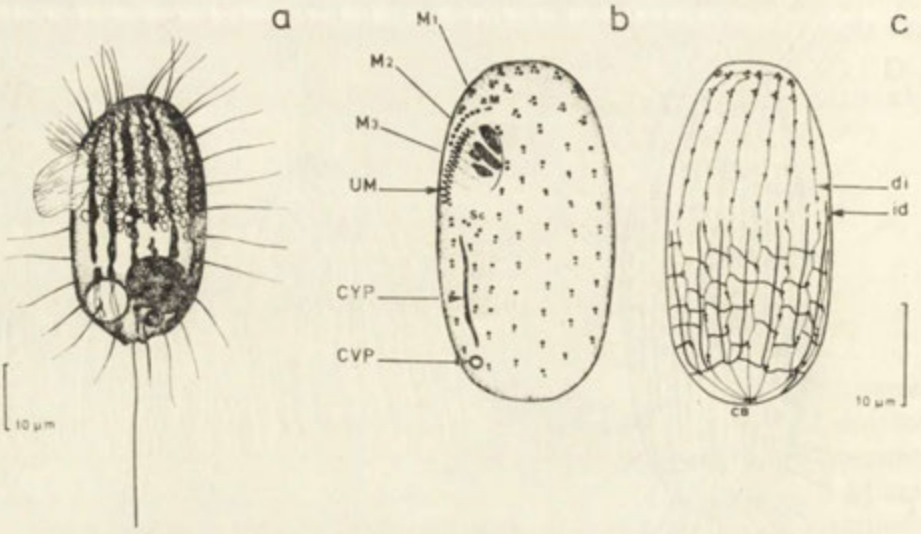

Abb. 6. *Sathrophilus muscorum* Kahl, 1931. (a) Nach Lebendbeobachtung und (b, c) Chatton-Lwoff-Präparation. In (c) Silberliniensystem nur in der hinteren Hälfte vollständig gezeichnet. aM — additional membrane, CB — Basalkörper der Schwanzcilie, CVP — Exkretionsporus, CYP — Cytopyge, di — direkt-, id — indirekt verbindende Silberlinien, M_{1-3} — Membranellen, UM — undulierende Membran

die Cilien vorne paarweise stehen. (1) Kinete über der undulierenden Membran zu einer "additional membrane" differenziert. Unter dem Cytostom die Basalkörper des Scuticus und (2) postorale Kineten. Unter den Kineten lichtbrechende Streifen. Buitkamp (1977) und Foissner et al. (1982) haben die Infraciliatur dieser Art beschrieben. Die Merkmale meiner Population stimmen mit jenen im großen ganzen überein. Nahrung Bakterien.

Vorkommen: Moos (Kahl 1931), Boden (Buitkamp 1977), (Foissner et al. 1982). Im Interstitial war die Art regelmäßig in kleinen Populationen vertreten.

Familie: *Cyclidiidae* Ehrenberg

Cyclidium setiger sp. n. (Abb. 7, Tab. 1)

Länge 15–20 µm, Breite 9–12 µm. Körperumriß eiförmig, breite Frontalplatte. Cilien über 10 µm lang, in Ruhestellung abgespreitzt. In der vorderen Hälfte stehen die Cilien in den einzelnen Kineten in 5–6 dicht beieinander liegenden Paaren, hinten dagegen einzeln und weiter entfernt. Die Caudalcilie ist über körperlang. Makro- und Mikronucleus liegen in der Vorderhälfte. Die kontraktile Vakuole caudal rechts gelegen. Bewegung typisch cyclidienartig: hin und herhüpfend mit langen Pausen bevorzugt an Substratpartikeln.

Die Zahl der Kineten variert von 11–13. Sie sind polar. Nach Behandlung mit

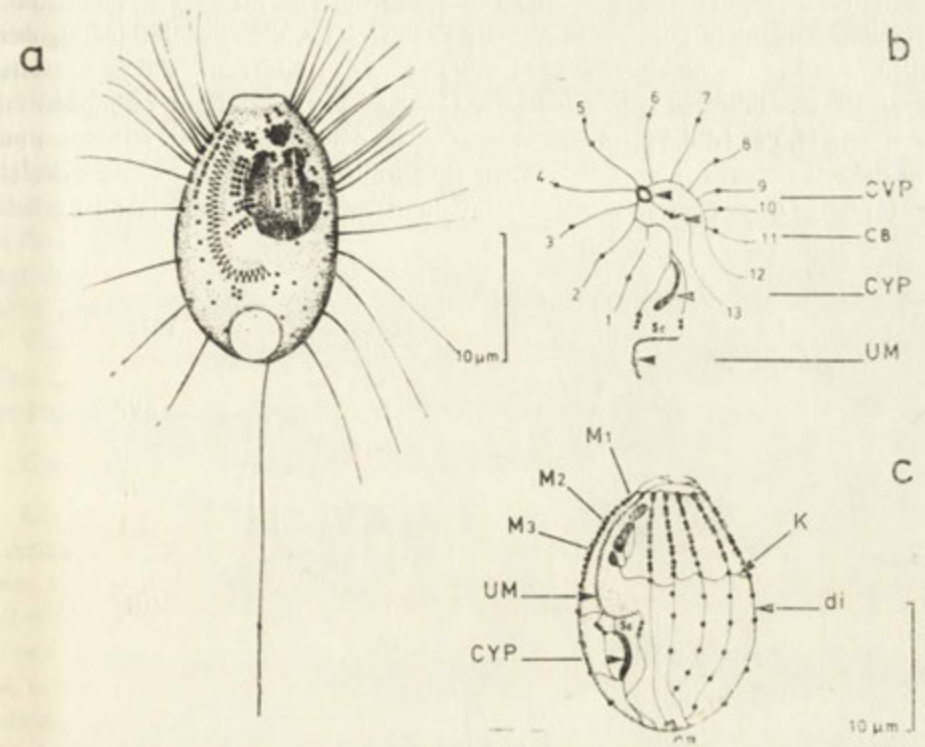

Abb. 7. *Cyclidium setiger* sp. n. (a) Nach Lebendbeobachtung und Protargolpräparaten, (b) Caudalpol, (c) Ventralansicht Chatton–Lwoff-Präparation. CB – Basalkörper der Schwanzwimper, CVP – Exkretionsporus, CYP – Cytopyge, di – direkt verbindende Silberlinie, K – kommissurale Silberlinie, M_{1-3} – Membranellen, UM – undulierende Membran

Tabelle 1

Biometrische Charakteristik von *Cyclidium setiger* sp. n.

Merkmal	$\bar{x}$	M	Sx	$S\bar{x}$	Extremwerte	n
Länge	17.6	17	1.6	0.3	15–20	19
Breite	10.7	11	1.7	0.4	9–12	19
Länge UM in der Längsachse	9.7	9	1.5	0.3	9–11	7
Länge UM zur Körperlänge	0.55					
Zahl der Kineten	12	12	0.7	0.3	11–13	10
Kinetosomen in der 1. Kinete	19.3	21	1.0	0.7	17–23	8
Kinetosomen in der N. Kinete	10.6	10	0.9	0.4	10–12	8
Kinetosomen in der N-1 Kinete	17.3	18	0.8	0.4	15–19	8
Kinetosomen einer dorsalen Kinete	13	13	1.2	0.3	12–14	8
Kinetosomenpaare der UM	31.2	32	1.9	0.9	28–34	7

Maße in µm von $AgNO_3$-imprägnierten Tieren (Chatton-Lwoff-Technik)

Silbernitrat erscheint frontal und caudal eine Silberlinie, die die Kineten miteinander verbindet. Außerdem Silberlinien auf der Frontalplatte, die eine Verbindung herstellen zwischen der undulierenden Membran und den Kineten 4 und 6. Am Hinterpol der Basalkörperkomplex (CB) der Caudalcilie. Er ist direkt verbunden mit der Kinete N, das ist die erste Kinete links neben dem Oralapparat, außerdem mit dem Exkretionsporus (CVP), der auf der ringförmigen Silberlinie zwischen der (4) und (5) Reihe liegt. Eine weitere Querverbindung der Kineten unterreinander schafft

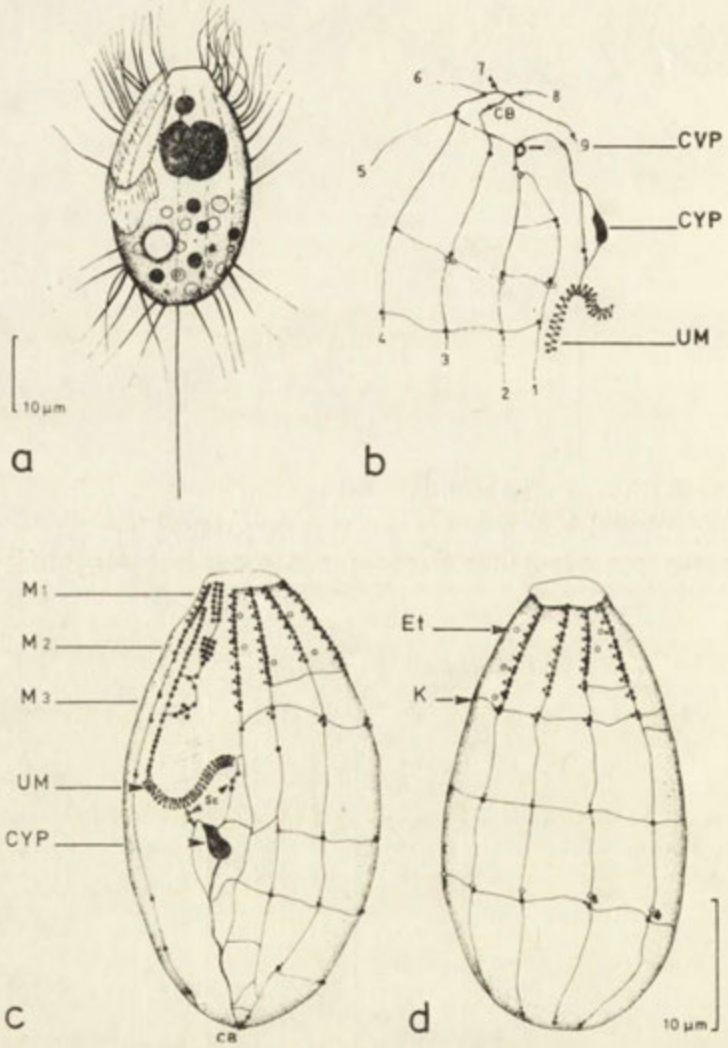

Abb. 8. *Cyclidium plouneouri* Dragesco, 1963. (a) Nach Lebendbeobachtung, (b) Caudalpol, (c) Ventral- (d) Dorsal-ansicht. CB – Basalkörper der Schwanzcilie, CVP – Exkretionsporus, CYP – Cytopyge, Et – Extrusome, K – kommissurale Silberlinie, M_{1-3} – Membranellen, UM – undulierende Membran

eine kommissurale Fibrille (K). Das Peristom ist über halbkörperlang. Scuticus (Sc) unter der undulierenden Membran in zwei Portionen aufgeteilt. Als ein besonderes Artmerkmal kann die Kinete N angesehen werden. Sie ist sehr kurz und endet in Höhe der 3. Membranelle (M 3). Arttypisch ist auch die Lage des Exkretionsporus am Ende der (4) und (5) Kinete. Bei allen mir bekannten Cyclidium-Arten liegt er auf der caudalen Ringfibrille zwischen (1) und (2), meistens aber vor der 2. Kinete. Nach Grolière (1973) liegt der Exkretionsporus von *C. sphagnetorum* im Peristom. Das beruht auf einer Fehlbeobachtung. Nach eigenen Untersuchungen liegt der Exkretionsporus dieser Art am Ende einer stark verkürzten 2. Kinete in Höhe des Peristoms.

Vorkommen: ich habe diese Art außer im Ontario See auch im Interstitial des Biwa-Lake (Japan) und im Preßwasser aus Sphagnum eines Tümpel der Wahner-Heide bei Bonn gefunden.

Cyclidium plouneouri Dragesco, 1963 (Abb. 8)

Länge 25–37 µm, ovoid. Mit 11, in seltenen Fällen auch 12 polaren Kineten. Kinetosomen praeoral paarweise und dichter gestellt als postoral. Den Paaren aber auch den einzelnen Kinetosomen sind ein Parabasalsack zugeordnet. Das Silberliniensystem kennzeichnet 3 komissurale Fibrillen (K), die das die Kinetosomen direkt verbindende System verknüpfen. Für diese Art charakteristisch ist die orale Infraciliatur, hier besonders das eigenartig geschwungene Ende der undulierenden Membran. Der Kernapparat aus Makro-und Mikronucleus liegt vorne. Der Makronucleus meiner Population bestand meist aus zwei Teilen.

Neben der Originalbeschreibung und dieser gibt es noch eine weitere von Borror (1965). Seine Population kennzeichnet ein linksspiraliger Verlauf der Kineten 1 bis 4. Dies ist der Hauptunterschied zu den anderen Populationen mit ausnahmslos meridionalen Kineten. Weitere Unterschiede zwischen den drei Populationen habe ich unten gegenübergestellt.

Vorkommen: Sapropel, Brackwasser (Dragesco 1963), marin (Borror 1965), Limnopsammon.

Vergleich von *C. plouneouri* — Populationen

Merkmal	Dragesco (1963)	Borror (1965)	Ontario See
Länge in µm	30–40	25–30	25–37
Anzahl der Kineten	14–16	12–13	11–12
Kinetosomen in der (1). Kinete	25	17	20
Kinetosomen in der N. Kinete	? (nur präoral)	15	21 (nur präoral)
Zahl der Makronuclei	1	1	1–2
Zahl der Mikronuclei	1	1	1
Länge UM zur Körperlänge	0.79	0.54	0.63

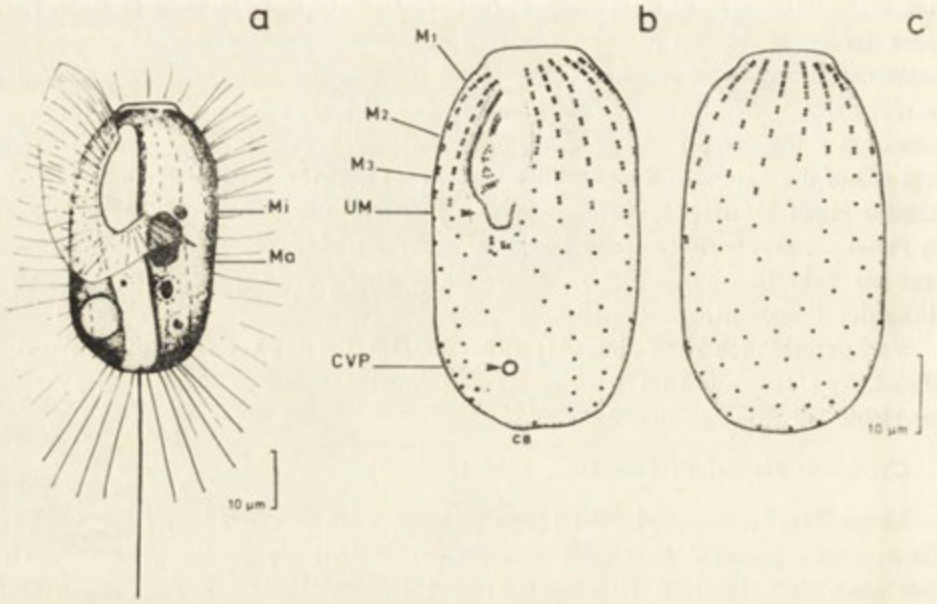

Abb. 9. *Cristigera media* Kahl, 1928. (a) Nach Lebendbeobachtung, (b) Ventral- und (c) Dorsalansicht nach Protargolimprägnation. CB – Basalkörper der Schwanzcilie, CVP – Exkretionsporus, M_{1-3} – Membranellen, UM – undulierende Membran

Cristigera media Kahl, 1928 (Abb. 9, Tab. 2)

Länge 40–50 µm, von ovaler Gestalt (2:1), hinten breit gerundet, der Vorderpol gerade abgestutzt und als Frontalplatte ausgebildet. Gattungstypisch sind die dorsoventrale Abflachung und die postorale rinnenartige Vertiefung. In den Kineten sind die sonst einzeln stehenden Kinetosomen nach vorne hin in bis zu 9 Kinetosomenpaaren verdichtet. Aus diesem Grunde ist auch die Bewimperung in der hinteren Hälfte wesentlich lockerer als vorne. Die kontraktile Vakuole liegt hinten rechts. Sie entleert über einen Exkretionsporus in der postoralen Rinne. Kernapparat zentral gelegen. Abweichend von Kahl's Beschreibung hat der linke Peristomrand meiner Population keine zahnartigen Höcker.

Vorkommen: Sapropel, halobiont (Kahl 1935), Limnopsammal.

Cristigera hammeri sp. n. (Abb. 10)

Länge 45–51 µm, Breite 23–29 µm. Körper schwach abgeflacht, im Umriß rechtsseitig fast gerade, linke Seite konvex. Der Vorderpol wird von einer breiten, deutlich abgesetzten Frontalplatte gebildet. Die rechte und linke Konturlinie stoßen caudal versetzt aufeinander. Dadurch entsteht ein Vorsprung, an dessen Basis die körperlange Caudalcilie steht. Ein wenig rechts der Mitte die gattungstypische Depression. Sie teilt sich in die vorne liegende etwa 25 µm lange Mundhöhlung und in die postorale Rinne, in der der Exkretionsporus (CVP) und die Cytopyge

Tabelle 2

Biometrische Charakteristik von *Cristigera media* Kahl

Merkmal	$\bar{x}$	M	Sx	$S\bar{x}$	Extremwerte	n
Länge	47.8	47	3.8	1.5	43–54	10
Breite	23.8	23	1.7	0.8	21–25	10
Länge UM in der Längsachse	25.5	26	1.4	0.6	24–28	10
Länge UM zur Körperlänge	0.53					
Distanz Vorderpol Makronucleus	21.7	23	3.5	1.5	16–25	10
Länge Makronucleus	10.4	10	1.2	0.6	9.8–11.2	7
Breite Makronucleus	7.2	8	1.3	0.6	7–9	7
Durchmesser Mikronucleus	2.2	2.2	0.3	0.1	2.0–2.8	6
Zahl der Kineten	16.0	15	1.8	0.8	14–18	10
Kinetosomen in der 1. Kinete	29.2	–	–	–	27–32	5
Kinetosomen einer dorsalen Kinete	25	–	–	–	24–28	4
Kinetosomenpaare der UM	61.8	–	–	–	60–65	4

Maße in µm von Protargol-imprägnierten Tieren

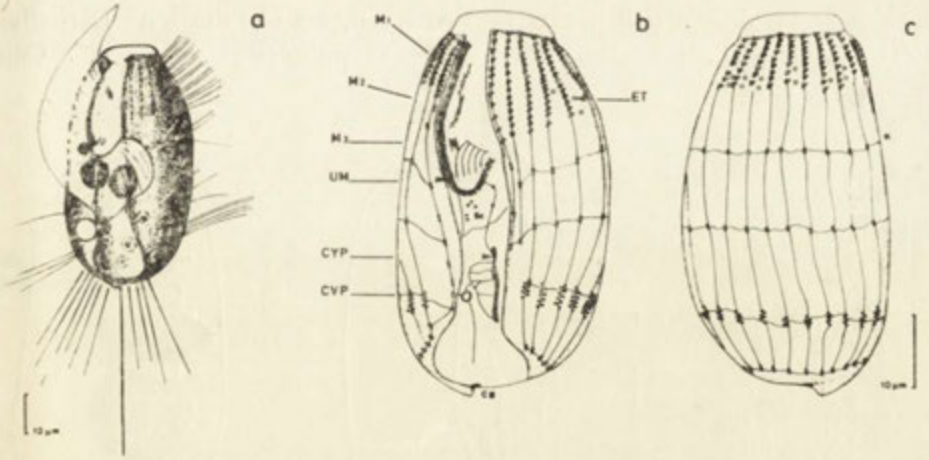

Abb. 10. *Cristigera hammeri* sp. n. Nach Lebendbeobachtung (a) und Chatton–Lwoff- Präparation (c, b), (b) ventrale, (c) dorsale Ansicht. CB – Basalkörper der Schwanzcilie, CVP – Exkretionsporus, CYP – Cytopyge, Et – Extrusome, K – kommissurale Silberlinie, M_{1-3} – Membranellen, Sc – Scuticus, UM – undulierende Membran

(CYP) liegen. Ein ovaler Makronucleus (9×6 µm) liegt zentral, in geringer Entfernung von ihm ein Mikronucleus, 2 µm im Durchmesser. Wie in Abb. 10a dargestellt, kommen mitunter auch Individuen mit zwei Makronucleusteilen vor.

Die Bewimperung und das Kinetom zeigen die Abbildungen. Bemerkenswert ist die Gliederung der Kineten: eine erste, apikale Verdichtung von Kinetosomen,

die sukzessiv von ventral nach dorsal abnimmt. Dann caudal ein zweiter Kinetosomengürtel. Im Unterschied zum ersten, mit paarweise gestellten Kinetosomen, stehen sie hier zig zag. Dazwischen nur zwei Kinetosomenpaare.

Das Silberliniensystem gehört zum streifenförmigen Typ. Das direkt verbindende System wird durch 5 kommissurale Fibrillen (K) quer verbunden. Ein indirektes Silberliniensystem konnte ich nicht feststellen. Es is möglich, daß es der Gattung fehlt. Untersuchungen an *Cr. minor* von Foissner et al. (1982) lassen das vermuten. *Cristigera hammeri* bewegt sich hüpfend. In den Ruhepausen werden die Cilien gespreizt. Nahrung Bakterien.

Ich widme diese Art meinem Freund Herrn Prof. Dr. U. T. Hammer, Limnologe an der University of Saskatchewan, Saskatoon, Kanada.

Familie: *Histiobalantiidae* de Puytorac et Corliss

Histiobalantium marinum Kahl, 1933 (Abb. 11)

Länge 60–102 µm, $\bar{x} = 75.3$, $M = 70$, $Sx = 15.0$, $S\bar{x} \times 5.0$, $n = 10$. Breite 30–42 µm, $\bar{x} = 36.0$, $M = 35$, $Sx = 4.7$, $S\bar{x} = 1.7$, $n = 10$. Maße von Protargol – imprägnierten Tieren. Kahl (1933) hat diese Art ausführlich beschrieben. Meine Beobachtungen decken sich mit seinen Angaben, die hier durch die Darstellung der Infraciliatur des Buccalapparates weiter vervollständigt werden. Die Cilien

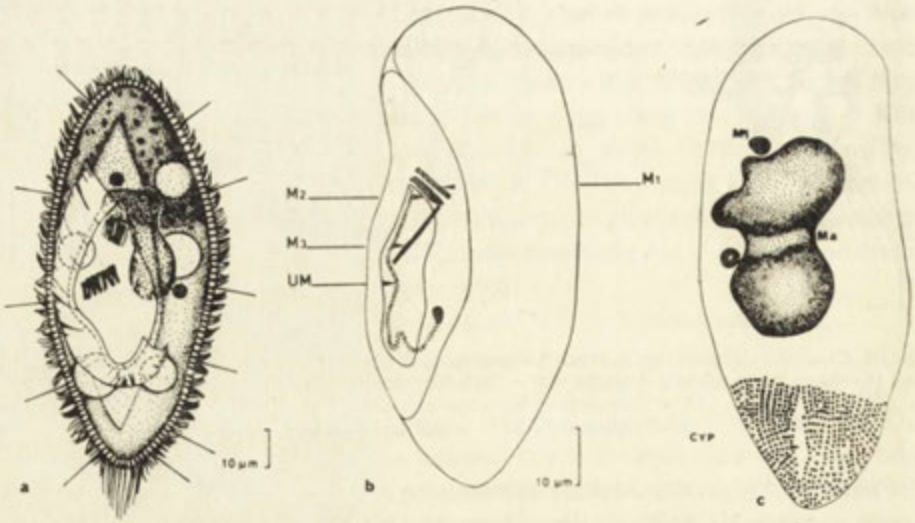

Abb. 11. *Histiobalantium marinum* Kahl, 1933. (a) Nach Lebendbeobachtung und Protargolimprägnation, (b) Oralapparat, (c) Kernapparat und Infraciliatur des dorsalen Hinterendes. CYP – Cytopyge, Ma – Makronucleus, Mi – Mikronucleus, M_{1-3} – Membranellen, UM – undulierende Membran

stehen in bis zu 120 Kineten. Erwähnenswert zwischen den kurzen Körpercilien zahlreiche verlängerte Tastcilien, im Ektoplasma darunter dicht an dicht stehende Trichocysten und immer findet sich frontal eine Anhäufung schwarzer Granula. Abweichend von Kahl ist der Makronucleus nicht zwei- sondern einteilig.

Vorkommen: Sandgrund der Kieler Bucht (Kahl 1933), Limnopsammal.

Histiobalantium minor sp. n. (Abb. 12, Tab. 3)

Länge 40–55 µm. Bewegung: ein lang anhaltendes Schwimmen unterbrochen von Ruhepausen, die immer in Kontakt mit Substratpartikeln gehalten werden. Dies und die Gestalt erinnern sehr an *Pleuronema*-Arten. Das Plasma ist farblos und frontal meist mit schwarzen Granula und kristallin aussehenden Körnern angefüllt. Drei bis vier kontraktile Vakuolen. Die Cytopyge (CYP) dorsal im hinteren Drittel.

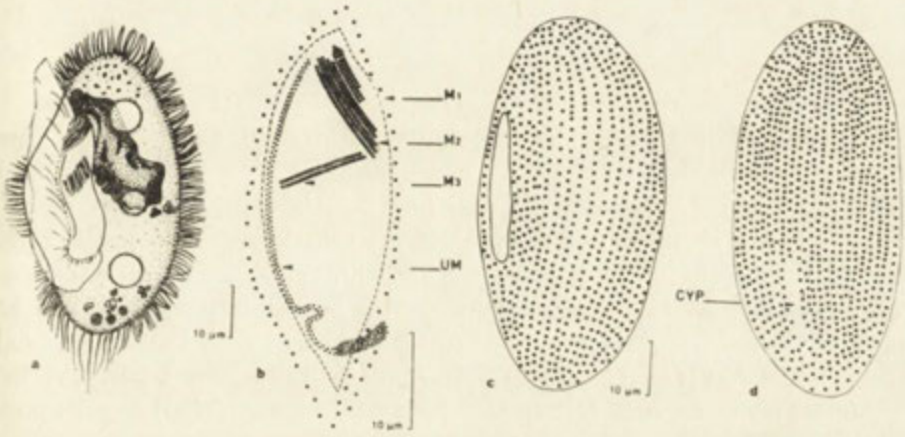

Abb. 12. *Histiobalantium minor* sp. n. (a) Nach Lebendbeobachtung, (b) Intraciliatur des Oralapparates, der Ventral- und Dorsalseite (c,d). CYP – Cytopyge, M_{1-3} – Membranellen, UM – undulierende Membran

Tabelle 3

Biometrische Charakteristik von *Histiobalantium minor* sp. n.

Merkmal	$\bar{x}$	M	Sx	$S\bar{x}$	Extremwerte	n
Länge	48.4	45	4.6	1.3	42–57	12
Breite	22.9	22	3.3	0.95	19–26	12
Länge UM in der Längsachse	26.8	25	4.4	1.5	23–36	10
Länge von M 1	6.00	6	0.85	0.28	4.5–7.0	10
Länge von M 2	9.8	9.6	1.52	0.44	8.5–13	10
Länge von M 3	8.3	8.4	1.00	0.27	8.0–9.0	10
Länge Makronucleus	23.7	21.6	3.5	1.7	19.5–25.5	10
Zahl der Kineten	42.4	43	2.2	0.84	41–45	12

Maße in µm von Protargol-imprägnierten Tieren

Im Ektoplasma etwa 3 µm lange Trichocysten. Die Mundhöhle ist einhalb körperlang. An ihrer rechten Seite die undulierende Membran, die außerdem die Öffnung hinten weit umgreift. Diagonal über die Mitte des Munddaches zieht die Membranelle 3, zu ihr fast im rechten Winkel die anderen.

Der Makronucleus, immer vorne gelegen, hat keine konstante Form. Meist ist er tief gebuchtet. Zwei runde Mikronuclei (2 µm Durchmesser) liegen ihm an. Die Wimpern, um 8 µm lang, stehen in 41–45 meridionalen Reihen. Verlängerte Tastcilien, die eigentlich für die Gattung typisch sind, habe ich nicht gesehen. Nahrung Bakterien. *H. minor* kam in allen Proben regelmäßig und zahlreich vor.

Beschreibungen der oralen Infraciliatur liegen von den Arten *H. natans* Dragesco et Iftode (1972) und *H. majus* Dragesco (1968), Grolière (1973) vor. Im Vergleich zeigt sich, daß die Lage der Membranellen zueinander und ihre Größenverhältnisse arttypisch sind.

Diskussion

Ziel meiner Untersuchungen war festzustellen, ob das Interstitial von Süßwasserseen der Größe der Nordamerikanischen Seen eine spezielle Ciliatenfauna beherbergt. Diese Frage war bislang offen, da bei Untersuchungen, die Pennak (1940, 1951) hier im Interstitial durchführte, die Ciliaten nicht berücksichtigt wurden. Ich habe 12 Arten im Interstitial festgestellt, von denen ich 10 taxonomisch genauer untersuchte. Mit den Arten *Loxodes magnus* und *Loxodes rostrum* habe ich mich nicht weiter auseinandergesetzt. Sie kamen in allen Proben regelmäßig vor und sind nach Dragesco (1960) und eigenen Beobachtungen typische Vertreter des Limnopsammons. Ihre Infraciliatur haben Dragesco (1960) und Foissner (1983) erarbeitet.

Ein Teil der angetroffenen Ciliaten ist zu charakterisieren als euryök und stenoplastisch in bezug auf wenige Faktoren. Das sind die Arten *Cyclidium plouneouri*, *Cyclidium setiger*, *Loxodes magnus*, *Loxodes rostrum*, *Sathrophilus muscorum*. Ihr Lebensraum sind die verschiedenartigsten Kapillarwasser z.B. im Moos und Boden. Auch *Gastronauta clatratus* gehört in diese Gruppe. Die Art ist haptisch und saugt sich bei ankommenden Wasserturbulenzen fest. So vor dem Verdriften geschützt, ist sie in besonderer Weise an diesen Lebensraum angepaßt. Die restlichen Arten sind nicht biotopgebunden und unspezifisch für diesen Lebensraum. Es sind dies ausschließlich sapropelische Tiere wie *Loxocephalus luridus*, die *Cristigera-* und *Histiobalantium-* Arten, die auf eine geeignete Bakteriennahrung angewiesen sind, die sich hier vorfindet.

Die Artenarmut und die verhältnismäßig hohe Präsens sapropelischer Arten lassen den Verdacht aufkommen, daß unbestimmbare Gifte oder auch bakterielle Abbauprozesse die Milieubedingungen so verändert haben, daß viele Arten von einer Besiedlung des Kapillarwassers ausgeschlossen sind. Die Sandproben, die

ich genommen habe, waren immer oxydiert, was an ihrer grauen bis weißen Färbung zu erkennen war. Demnach war Sauerstoff ausreichend vorhanden und fällt damit als ein limitierender Faktor aus. Nach den Untersuchungen von Weiler (1973) sind die abiotischen Bedingungen des untersuchten Limnopsammals von keinem Parameter her gesehen auffällig. Dies trifft insbesondere auf den Sauerstoff zu, der ausreichend vorhanden ist, und auf das Fehlen von Zellgiften z.B. Ammonium. Obwohl alle Bedingungen scheinbar optimal sind, ist hier eine Infauna, die man eine Biozönose nennen könnte, nicht existent.

DANKSAGUNG

Meine Untersuchungen wurden durch ein Stipendium der Heinrich Hertz-Stiftung des Lan, des Nordrhein-Westfalen ermöglicht. Für diese Unterstützung danke ich herzlich. Herrn Dr. J. Barica, Chief der Aquatic Ecology Division am Canada Centre for Inlandwaters, Burlington, Ontario-danke ich für einen Arbeitsplatz in seinem Institut.

SUMMARY

Qualitative measurements of ciliates in the interstitial of Lake Ontario near Burlington, Ontario (Canada) were made by the method of Uhlig (1964). During this study 12 species were detected. The infraciliature of 10 of these was studied by means of the silver nitrate or protargol methods. Three new species are described: *Cyclidium setiger*, *Cristigera hammeri*, *Histiobalantium minor*.

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Inhibition of Macromolecule Syntheses in a Ciliate Protozoan, *Tetrahymena pyriformis* by Hexachlorocyclohexane (HCH) Isomers

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Synopsis. The syntheses of macromolecules in *Tetrahymena* treated with various isomers of HCH was studied. The peak incorporation of ^{3}H-thymidine in *Tetrahymena* cultures treated with δ, β and α-HCH occurred on the 4th day of treatment as compared with the control and lindane treated cells where maximum incorporation was observed on the 3rd day. The range of inhibition for DNA synthesis was 1–89%. δ and β-HCH shifted the peak incorporation of ^{3}H-uridine to the 4th day while in the case of α and γ-HCH its maximum incorporation was observed on the 3rd day of treatment. The range of inhibition for RNA synthesis was 1–86%. The maximum incorporation of ^{3}H-lysine in treated and control cells occurred on the 3rd day, the percentage inhibition, however, ranged from 1–81.

HCH is one of the most extensively used insecticides for agriculture and public health in India (Krishnamurti et al. 1982). Although only the γ-isomer of HCH is the isomer with insecticidal properties, nevertheless, other isomers of HCH viz., α, β, δ and ε do occur in different proportions in the technical grade of HCH marketed and used (Brooks 1974). Besides, there have been reports of the isomerization of γ-HCH to α-HCH (Benezet and Matsumura 1973, Matsumura et al. 1976 and Vonk and Quirjns 1979) and thus along with lindane, other isomers of HCH also would be present in the environment and can affect the organisms. γ-HCH and its isomers have been known to lower the convulsive threshold for pentylenetetrazol in mice (Hulth et al. 1976), to cause extensive myofilament degradation in rats (Publicover et al 1979), to cause severe hypoplastic anemia (Morgan et al. 1980), to increase the values of hepatic microsomal enzyme, O-demethylase in albino rats (Chand and Ramachandran 1980), to inhibit somatic and

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meiotic divisions in male swiss mice (Babu et al. 1981) and to affect some enzymes of liver and kidney in *Heteropneustus fossilis* (Sengupta et al. 1984).

The target site of HCH has been very clearly established in higher organisms but it is not so in microorganisms where one or more sites are affected. BHC/lindane has been reported to interfere with many basic biochemical processes like photosynthesis (Kopecek et al. 1976), enzyme activity (Gray 1954) and oxidation of ammonium to nitrite and conversion of nitrite to nitrate (Ray 1983).

Besides the target organisms, HCH isomers affect the non-target organisms as well. Among these, ciliates are important as they constitute important link in the food chain and food webs.

Our earlier studies have shown that HCH isomers inhibit the cell population growth of a ciliate protozoan, *Tetrahymena pyriformis* (Mathur et al. 1984). In the present paper, the effect of sublethal concentrations of HCH isomers on the syntheses of macromolecules viz., DNA, RNA and protein in *T. pyriformis* are described.

Materials and Methods

Stock cultures of *T. pyriformis* were grown in 15 ml tubes containing 5 ml of 1% proteose peptone supplemented with 0.5% NaCl and 0.3% yeast extract at 27±1°C in a B.O.D. incubator.

Lindane, the gamma isomer of 1, 2, 3, 4, 5, 6 Hexachlorocyclohexane (HCH) was obtained from ICN Pharmaceuticals Inc., Life Sciences Group, Cleveland, Ohio. The other isomers of HCH viz., α, β and δ were obtained through the courtesy of Dr. Gunter Zweig, Environmental Protection Agency, U.S.A.

The stock solutions of the insecticide isomers were prepared in acetone and then diluted with the culture medium to obtain the desired concentration of the insecticide. The acetone concentration of the insecticide treated cultures was kept at 0.1% as our earlier studies showed that concentrations of acetone upto 0.5% did not have any adverse effect on the organisms.

Our earlier studies (Mathur et al. 1984) also showed that 2.5 and 5.0 ppm of α and γ-HCH and 1.0 and 2.5 ppm of β and δ-HCH were the sublethal concentrations for the growth of *T. pyriformis*. Hence, these concentrations of HCH isomers were used to study their effects on the syntheses of DNA, RNA and proteins.

A volume of 2 ml of 48 h old culture of *Tetrahymena* was transferred to 48 ml of sterile medium in 100 ml conical flasks and allowed to grow for 24 h. Insecticide solutions were added directly to the culture medium so as to get the required concentration of the insecticide and the cultures were allowed to grow for another 24 h. The incorporation of radioactive thymidine (Sp. Act 18.8 Ci/mM), uridine (Sp. Act 13.8 Ci/mM) and lysine (Sp. Act 5.6 Ci/mM) in the presence of sublethal concentrations of HCH isomers has been used as an index of DNA, RNA and protein synthesis respectively in *T. pyriformis* treated with these insecticides. The method adopted was the same as described by Freeman and Moner (1976). After every 24 h a volume of 2 ml was pipetted out aseptically from both the treated and the control flasks. Out of these, three aliquots of 0.5 ml each were incubated with the respective radioisotope i.e., ^{3}H-thymidine (for DNA synthesis), ^{3}H-uridine (for RNA synthesis) and ^{3}H-lysine (for protein synthesis) at 27±1°C, at a specific activity of 5 μCi/ml. After 30 min of incubation, 0.5 ml of ice cold trichloroacetic acid (TCA) was added to each of these. The precipitated material was pipetted onto Whatman GF/C glass fiber discs, washed twice with 10 per cent ice cold TCA and finally dried. The dried discs were counted for radioactivity in the Packard tricarb liquid scintillation spectrophotometer. The scintillation fluid consisted

of 5 gms PPO and 50 mgs POPOP dissolved in 1 l of Toluene. The remaining 0.5 ml of the culture medium from both the control and the treated flasks was fixed with 0.5 ml of 10 per cent neutral formalin and cell number was determined with the help of a haemocytometer. A minimum of six replicates were taken for each treatment and the experiments were repeated twice.

Results

The incorporation of radioactive precursors in controls followed a pattern similar to the normal cell cycle of *Tetrahymena*. During the log phase of growth of *Tetrahymena* the cell number increases till 72 h and then the cells enter into stationary phase during which the cell number remains more or less constant. The incorporation of radioactive precursors (^{3}H-thymidine, ^{3}H-uridine, ^{3}H-lysine) increased gradually from the 1st to the 3rd day and thereafter declined on the 4th and the 5th day.

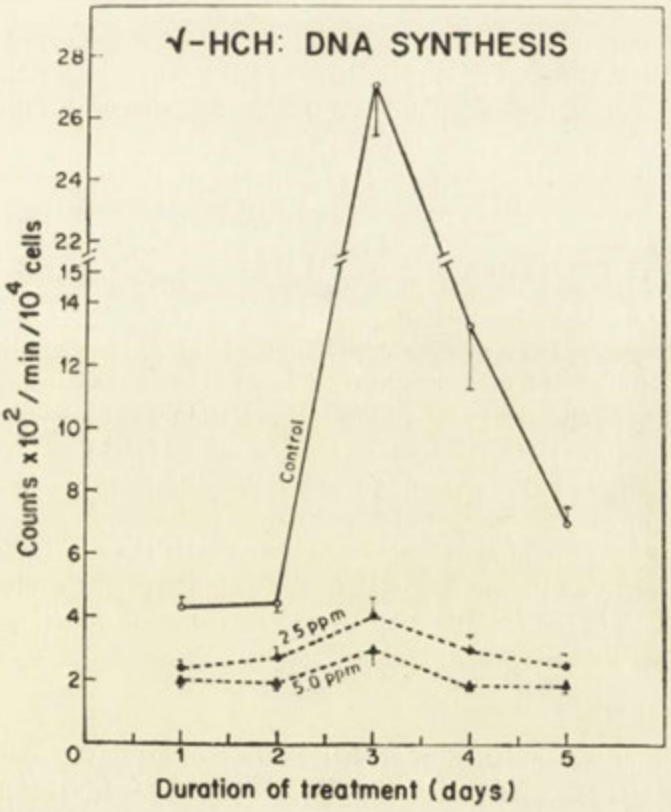

Fig. 1. ^{3}H-thymidine incorporation into DNA of *Tetrahymena pyriformis* treated with 2.5 and 5.0 ppm γ-HCH

DNA Synthesis

γ-HCH: The peak incorporation of ^{3}H-thymidine in cultures of *T. pyriformis* treated with 2.5 and 5.0 ppm lindane was obtained on the 3rd day of treatment as in the case of control (Fig. 1). In the cultures treated with 2.5 ppm lindane about 44% reduction in ^{3}H-thymidine incorporation was observed within 24 h with respect to the control. Maximum reduction (upto 85%) occurred on the 3rd day of treatment. With 5.0 ppm lindane treatment the reduction at the end of 24 h was 55% which increased to 89% on the 3rd day of treatment and at the end of 5th day of treatment it decreased to 73%.

The peak incorporation of ^{3}H-thymidine in *Tetrahymena* cultures treated with sublethal concentrations of δ, β and α-HCH was observed on the 4th day of treatment as against the control where maximum incorporation was observed on the 3rd day. The percentage inhibition ranged from 2–74 for δ-HCH (Fig. 2), from 10–60 for β-HCH (Fig. 3) and from 1–84 for α-HCH (Fig. 4).

RNA Synthesis

The incorporation pattern of ^{3}H-uridine into RNA of the control cultures of *T. pyriformis* is shown in Fig. 5. ^{3}H-uridine incorporation into RNA increased initially reaching a peak incorporation on the 3rd day after which it declined on the 4th and the 5th day.

The peak incorporation of ^{3}H-uridine into RNA of *T. pyriformis* treated with 1.0 and 2.5 ppm concentrations each of δ and β-HCH was observed on the 4th day of treatment unlike the case in control where the peak incorporation was observed on the 3rd day. Within 24 h of treatment, 16 and 22% inhibition occurred in RNA synthesis in 1 and 2.5 ppm δ-HCH treated cultures respectively with respect to control which increased to a maximum value of 69 and 72% respectively with 1 and 2.5 ppm δ-HCH after 72 h of treatment (Fig. 5). In the cultures treated with 1 ppm β-HCH about 8% reduction in ^{3}H-uridine incorporation was observed within 24 h. Maximum reduction (25%) occurred on the 3rd day of treatment. With 2.5 ppm β-HCH the reduction at the end of 24 h was 13% which increased to 29% on the 3rd day (Fig. 6).

The peak incorporation of ^{3}H-uridine in α and γ-HCH treated cultures of *Tetrahymena* occurred on the 3rd day of treatment as in the case of control. The percentage reduction in incorporation for α-HCH ranged from 6–69 (Fig. 7) and for γ-HCH it ranged from 39–86 (Fig. 8).

Protein Synthesis

The pattern of incorporation of ^{3}H-lysine into proteins in the control cultures of *T. pyriformis* is shown in Fig. 9. The ciliates showed a gradual increase in the incorporation of the precursor reaching its maximum after 72 h. Subsequently, the rate of incorporation declined on the 4th and 5th day.

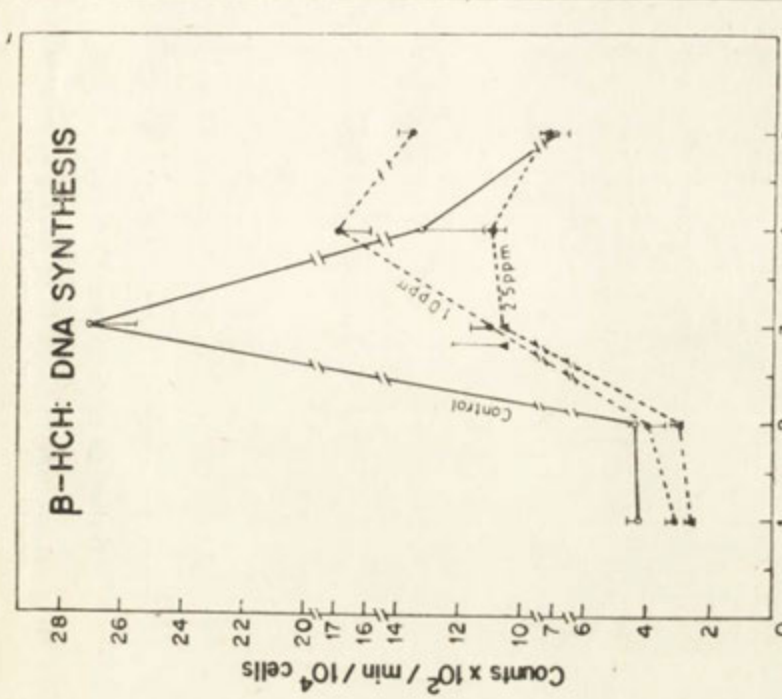

Fig. 3. ^{3}H-thymidine incorporation into DNA of *Tetrahymena pyriformis* treated with 1.0 and 2.5 ppm β-HCH

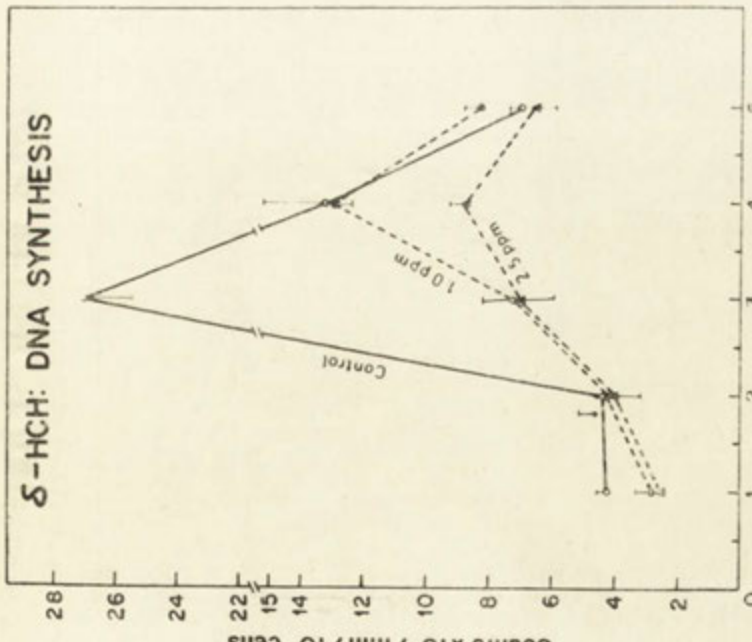

Fig. 2. ^{3}H-thymidine incorporation into DNA of *Tetrahymena pyriformis* treated with 1.0 and 2.5 ppm δ-HCH

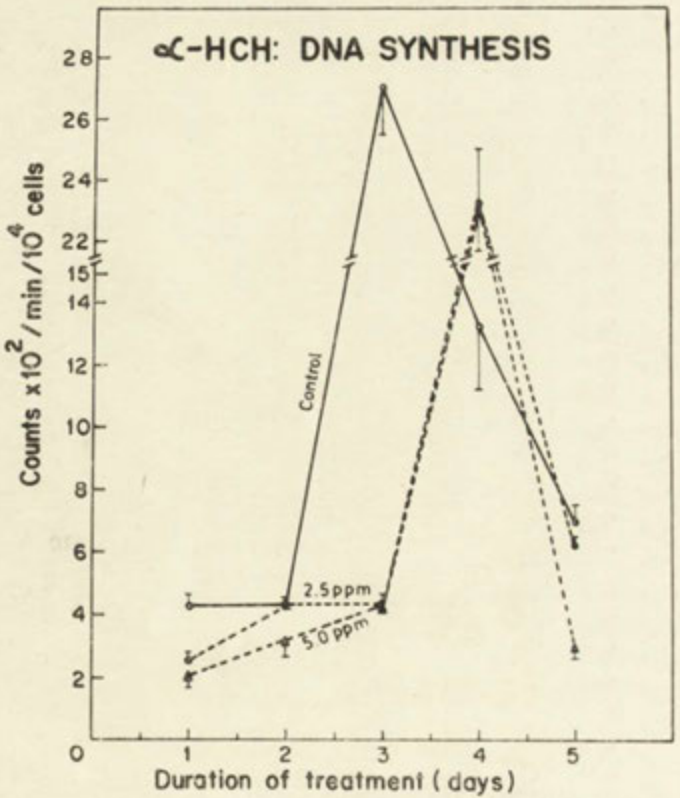

Fig. 4. ^{3}H-thymidine incorporation into DNA of *Tetrahymena pyriformis* treated with 2.5 and 5.0 ppm α-HCH

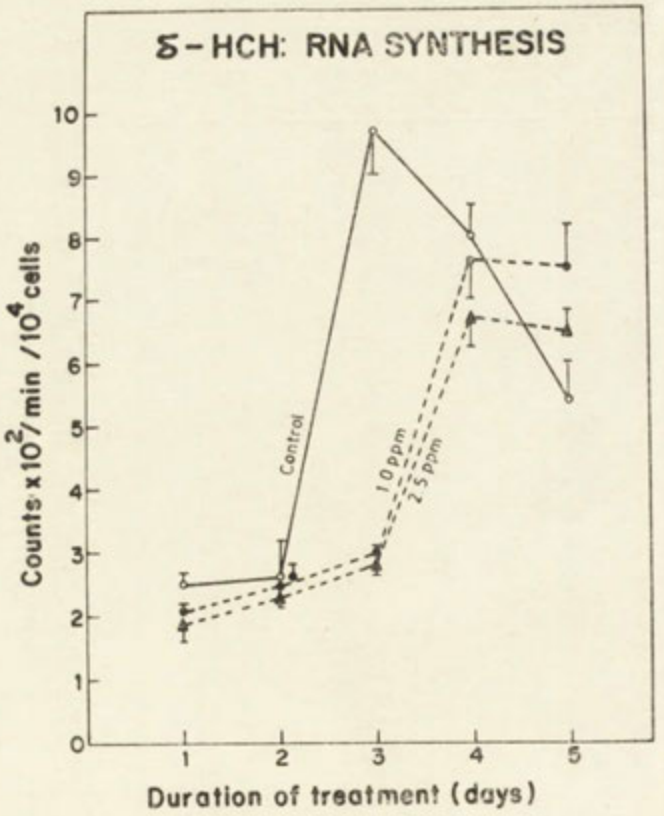

Fig. 5. ^{3}H-uridine incorporation into RNA of *Tetrahymena pyriformis* treated with 1.0 and 2.5 ppm δ-HCH

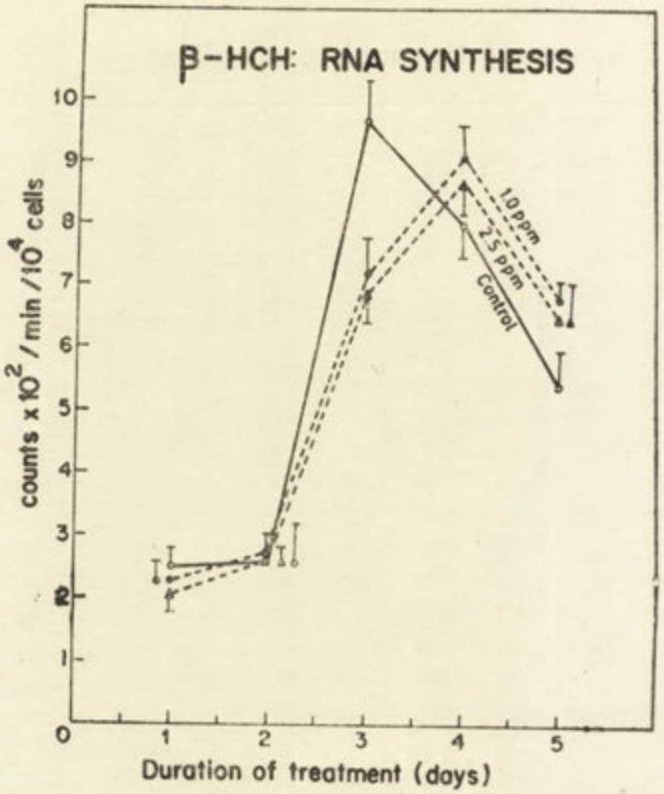

Fig. 6. ^{3}H-uridine incorporation into RNA of *Tetrahymena pyriformis* treated with 1.0 and 2.5 ppm β-HCH

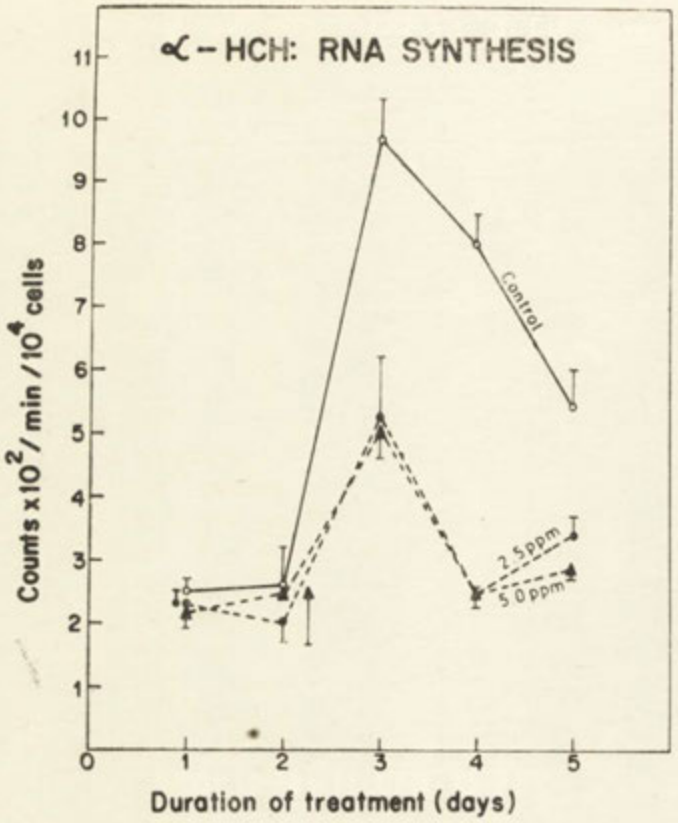

Fig. 7. ^{3}H-uridine incorporation into RNA of *Tetrahymena pyriformis* treated with 2.5 and 5.0 ppm α-HCH

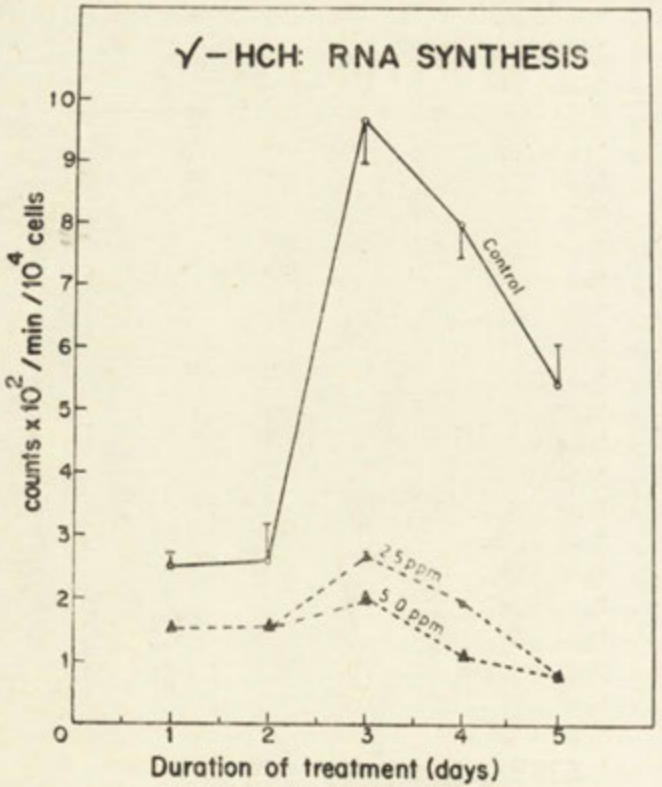

Fig. 8. ^{3}H-uridine incorporation into RNA of *Tetrahymena pyriformis* treated with 2.5 and 5.0 ppm γ-HCH

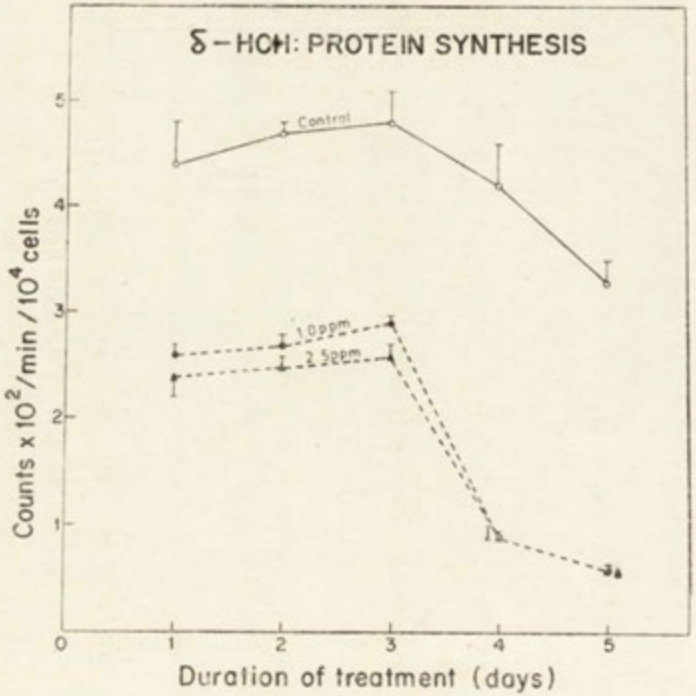

Fig. 9. ^{3}H-lysine incorporation into proteins of *Tetrahymena pyriformis* treated with 1.0 and 2.5 ppm δ-HCH

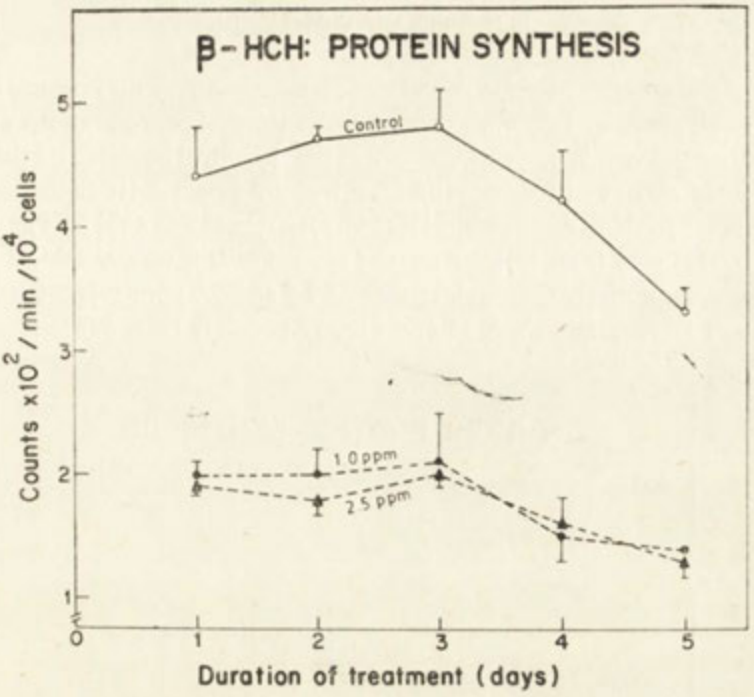

Fig. 10. ^{3}H-lysine incorporation into proteins of *Tetrahymena pyriformis* treated with 1.0 and 2.5 ppm β-HCH

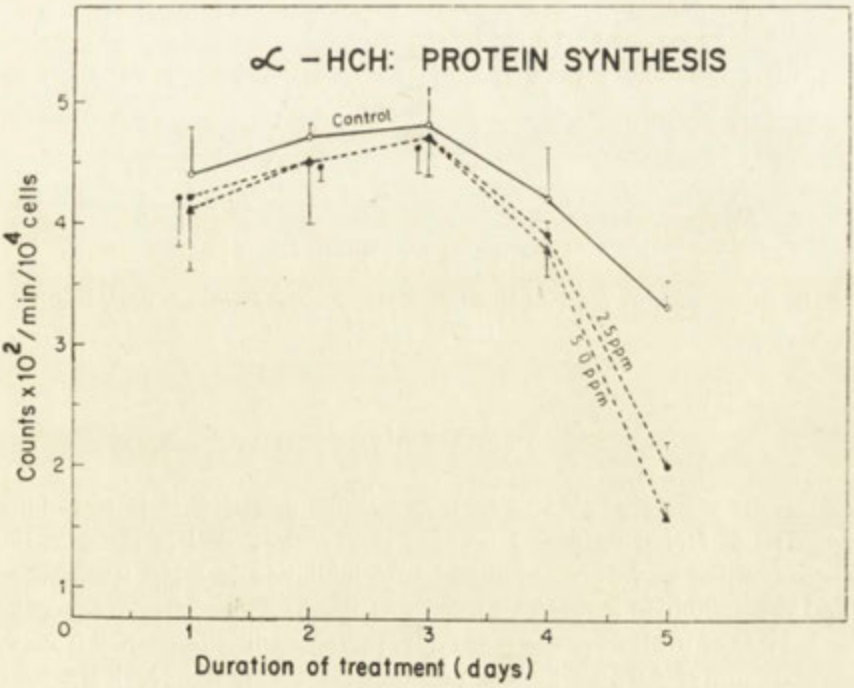

Fig. 11. ^{3}H-lysine incorporation into proteins of *Tetrahymena pyriformis* treated with 2.5 and 5.0 ppm α-HCH

The maximum incorporation of the radioactive precursor into proteins of asynchronous culture of *T. pyriformis* treated with sublethal concentrations of each of the HCH isomers viz., δ, β, α and γ as well as in the control occurred on the 3rd day. The extent of maximum incorporation for 1 and 2.5 ppm δ-HCH treated cells as compared to the control was 61 and 54% (Fig. 9); it was 43 and 42% for 1 and 2.5 ppm β-HCH treated cells (Fig. 10); for α-HCH treated cells it was 99 and 98% for 2.5 and 5.0 ppm respectively (Fig. 11). In case of 2.5 and 5.0 ppm γ-HCH treated cells the range of incorporation was 83 and 42% respectively (Fig. 12).

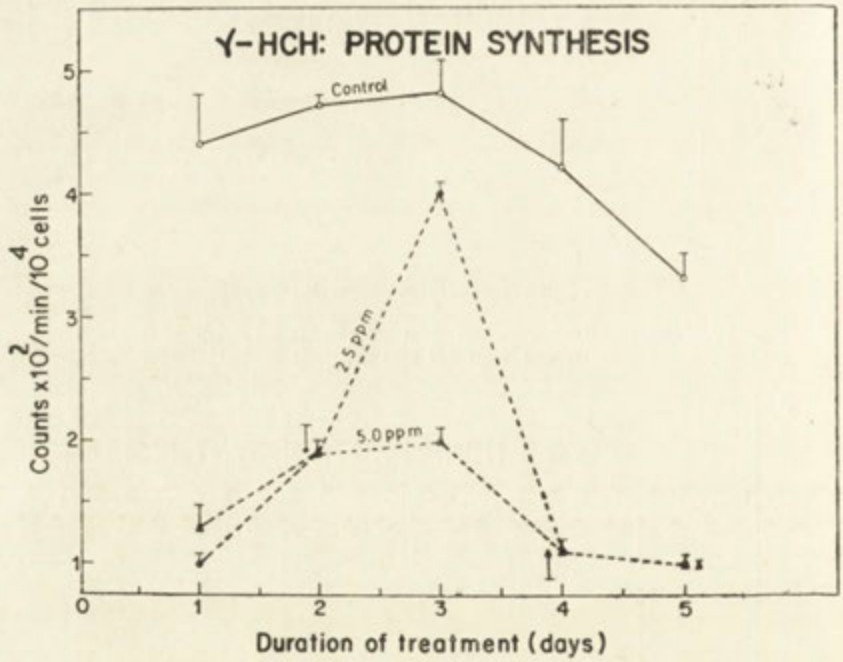

Fig. 12. ^{3}H-lysine incorporation into proteins of *Tetrahymena pyriformis* treated with 2.5 and 5.0 ppm γ-HCH

Discussion

Our results show that sublethal concentrations of all HCH isomers inhibited the synthesis of DNA, RNA and proteins in *Tetrahymena*. For instance, lindane at a concentration of 2.5 ppm produced 40% inhibition in DNA synthesis within 48 h and 89% inhibition in DNA synthesis was observed with 5.0 ppm concentration on the 3rd day of treatment. The range of percentage inhibition varied from 40–89. A concentration of 5.0 ppm lindane produced 39% inhibition in RNA synthesis within 24 h of treatment and 86% inhibition occurred on the 4th day. γ-HCH at

2.5 ppm concentration caused 78% inhibition in protein synthesis within 24 h. However, on the 3rd day only 17% inhibition in protein synthesis was noticed.

Similarly other isomers of HCH also inhibit macromolecule syntheses to different extents. The inhibition of macromolecule syntheses in this sensitive ciliate by HCH isomers could explain the observed inhibitory effects of HCH isomers on its growth (Mathur et al. 1984).

Although reports on the effects of lindane on macromolecule syntheses in microorganisms are scanty, lindane and other isomers of HCH have been reported to inhibit DNA, RNA and protein synthesis in higher organisms.

Borghi et al. (1973) reported that lindane inhibited DNA, RNA and protein synthesis in the alga, *Acetabularia mediterranea*. Jeanne (1979) found that in *Dunaliella bioculata* DNA synthesis was strongly inhibited by 10 ppm lindane and depressed by 5 ppm in the first cell cycle. The synthesis of DNA was not resumed during the second cell cycle. The RNA and the protein content increased in the treated cells during the first cell cycle but the increase was slow. However, in the second cell cycle the RNA and protein contents increased rapidly.

Miller et al. (1980) while studying the effect of 250 ppm concentration of each of the HCH isomers on the nuclear and mitochondrial DNA synthesis in the mouse liver, however, reported that the treated animals showed increased nuclear DNA specific activities as well as an increase in the radioactivity incorporated into mitochondrial DNA. Roux et al. (1980) studied the effects of lindane *in vitro* on the mouse peritoneal macrophages and reported a pronounced inhibition of uridine incorporation correlated with the reduction of intracellular uridine pool. Roux et al. (1978) studied the effects of lindane on mouse peritoneal macrophages and their results revealed that the rate of leucine incorporation associated with protein synthesis rate remained uniform during the exposure of macrophages to lindane suggesting that leucine transport was not affected.

On comparison of the concentrations of lindane which have been reported to inhibit macromolecule synthesis in other systems like *Dunaliella* (Jeanne 1979) and mouse liver (Miller et al. 1980) with those we have found effective in *Tetrahymena* it is evident that this ciliate is sensitive to lower concentration of lindane.

The exact mechanism of interaction of HCH isomers with macromolecule syntheses is not known. However, the work of Puiseux-Dao et al. (1977) revealed that plasma membrane could be the possible target site of γ-HCH in *Acetabularia mediterranea*. Such interactions besides interfering with the essential transport mechanisms like Na^+, K^+ and Mg^{2+} ATPase systems which are associated with the membranes, may also affect various other biochemical processes within the cell. Srinivasan and Radhakrishnamurty (1980) found that albino rats fed 800 ppm of β and γ-HCH each for two weeks showed decreased levels of hepatic mitochondrial DNA/Mg^{2+}/Ca^{2+} activated ATPase and microsomal Na^+, K^+ activated ATPase. The levels of SDH were elevated while there was a reduction in the activities of hepatic MDH and LDH. Such interferences of HCH isomers with the normal

activities of important enzymes might lead to uncoupling of oxidative phosphorylation and since protein synthesis is an energy consuming process, inactivation or inhibition of oxidative enzymes by HCH isomers could, therefore, alter the rate of oxidative phosphorylation and this in turn could inhibit protein synthesis.

Sengupta et al. (1984) studied the effect of 1.0 and 5.0 ppm concentrations of BHC on some enzymes of liver and kidney of the fish, *Heteropneustus fossilis*. The γ-amylase activity increased in the treated fishes and glucose was released directly from glycogen to meet the energy demand of BHC stressed fish. The GOT and GPT activities were found to increase and were responsible for the process of transamination to gluconeogenesis. The alkaline phosphatase activity decreased while that of acid phosphatase increased indicating hepatotoxicity produced by BHC.

Organochlorine insecticides other than HCH isomers such as DDT, aldrin and dieldrin have been reported to inhibit/stimulate DNA, RNA and protein synthesis in other systems such as HeLa cells (Chung et al. 1967 and Litterst et al. 1969), *Dictyostelium discoideum* (Bushway and Hanks 1976), *Euglena gracilis* (Ewald et al. 1976), *Crithidia fasciculata* (French 1976), *Stylonychia notophora* (Lal and Saxena 1980 and Saxena and Lal 1981), *Blepharisma intermedium* (Lal et al. 1981) and *Tetrahymena pyriformis* (Saxena et al. 1981).

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Biochemical Changes of *Acanthamoeba* Following Attenuation and the Role of Cysts in Retaining the Characteristics of Strains[1]

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Synopsis. We compared the virulence of the environmental *Acanthamoeba* strains, maintained paralelly in prolonged axenic culture at 37°C and in cyst form stored at 4°C beginning at the original isolation. The changes in the lytic enzymes activity and isoenzyme patterns, concomitant with the strain attenuation, were also studied. After $3^1/_2$ to 4 years the strains remained virulent and lost their primary virulence only after numerous passages (8 years). Still, the amoebae retained the virulence when stored in cyst form for 9 years. The attenuation of the strains is related to the decrease in trypsin activity. However, the isoenzyme patterns of non-specific esterases and acid phosphatase remained stable throughout the time.

The amphizoic *Acanthamoeba* species, widespread in nature, are the etiological agents of granulomatous amoebic encephalitis and other diseases in humans. The cysts of *Acanthamoeba* stored at 4°C are able to survive moths (De Jonckheere and Van DeVoorde 1976, Biddick et al. 1984) or even years (Mazur 1984). However, the main method for laboratory continuous maintenance of *Acanthamoeba* strains in axenic culture without passage through host tissue affects the amoeba behavioural characteristics, e.g., the loss of encystation potential, alteration in drug sensitivity and, most important, the loss of pathogenicity (Stevens and O'Dell 1974, Wong et al. 1977). The changes in virulence concern mainly the population of amoeba isolated from environment. Cloud (1969) explained the attenuation by the loss of enzyme systeme which enabled the amoebae to invade the host tissue.

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The aim of our observation was to compare the virulence of *Acanthamoeba* strains maintained paralelly in long-term axenic culture in fluid medium at 37°C, and in cyst form stored at 4°C since early passage level close to original isolation. We also tested the changes in lytic enzymes activity and isoenzyme patterns, which were concomitant with the strain attenuation.

Materials and Methods

Cultivation of amoeba strains and storage of cysts. Two strains of *Acanthamoeba castellanii* (309 and 319) and one strain of *A. astronyxis* (190), primarily mouse-pathogenic, were isolated in 1972 from the environment (Kasprzak and Mazur 1972). The isolates were maintained on Bacto-Casitone medium (Červa 1966, 1969) supplemented with horse serum in axenic culture without animal passage through the total time of over nine years. The strains were grown at 37°C in 50 ml Erlenmeyer flasks containing approximately 30 ml of medium and transferred weekly by inoculation of three drops of vigorously shaken amoeba cultures. The cultures were periodically tested on thioglycollate broth for inapparent contamination.

The sterile tap-water-suspension of cysts of these strains, obtained at early passage level close to the original isolation from the environment, were stored at 4°C in 2 ml screw-capped tubes. To establish fresh cultures the amoebic cyst suspension was plated on 1.5% non-nutrient agar previously spread with *Enterobacter aerogens*; after 48 to 72 h the trophozoites were washed off the agar plates and, after Červa, inoculated with antibiotics (penicillin 100 units and streptomycin 50 μg per one ml of medium) on fluid medium. After axenization, the cultures were tested for inapparent contamination on thioglycollate broth.

As control for biochemical study, non-pathogenic strains were employed: *A. rhysodes* (Chang strain), *A. castellanii* (Neff strain), and a strain 23Ś isolated from a molusc (Mazur 1975), initially recognized as *A. castellanii*.

Virulence assays. Weanling Swiss mice, lightly anaesthetized with ether, were instilled intranasally by administering one drop (about 0.03 ml) of amoebae (2×10^4) into the nostrils. Authopsis were performed immediately after death and pieces of forebrain and lung were placed on non-nutrient agar spread with living *E. aerogenes* for culturing amoebae. Mice which survived over 2 months were etherized, dissected, and their brains and lungs were also examined.

Preparation of cell extracts. Amoebae were harvested from 4-day-old culture (late logarithmical phase) by centrifugation for 5 min at 900 × g, and the pellet was washed thrice in PBS. The amoeba pellet was resuspended in extraction buffer (PBS pH 7.4) 1:4 v/v and homogenized at 4°C. The homogenates were centrifuged for 30 min at 15 000 × g and the resulting supernatant fraction was decanted; both fractions were stored in ice until investigation. Protein was determined by the method of Lowry et al. (1951).

Enzyme activity assays. The activity of lytic enzymes in homogenate and supernatant was estimated colorimetrically (520 nm) by using Azocoll (Calbiochem) as substrate; the activity of pepsin-like enzymes was determined at pH 2.5 (50 nM glycin buffer), and the activity of trypsin-like enzymes at pH 8.2 (50 nM Tris-HCl buffer). The activity was defined in international units (IU) as specific activity per 1 mg of protein.

Polyacrylamide gel electrophoresis. The disc electrophoresis was performed on 7.5% polyacrylamide gel in a Quickfit apparatus according to the method of Davis and Ornstein (1959) as modified by Hadaś et al. (1977) at 2.5 mA for each gel, using bromophenol blue as a marker. The gel and electrode buffer was a Tris-glycin pH 8.5 (3 g Tris-hydroxymethyl-amino-methane and 14.4 g glycin in 1000 ml distilled water). The electrophoresis was stopped when the marker was at the end of the gel. The isoenzyme patterns of non-specific esterases were visualized by se-

Table 1

Results of intranasal inoculations in mice with *Acanthamoeba astronyxis* (strain 190) and *Acanthamoeba castellanii* (strain 309 and 319) after continuous subculturing of trophozoites in Bacto-Casitone Medium at 37°C or cysts storage at 4°C

Strain	Number of subculture	Period of cyst storage	Time of deaths (days)	Mean survival time (days)
190	2		3+, 7+, 9+, 12+, 12+, 14+	10
	52		13+, 14+, 15+, 16+, 18+, 20+, 22+, 24+, 26+, 26+, 29+, 34+, 43+, 45+, S+, S+, S+, S−, S−, S−, S−, S−, S−, S−	25
	83		13+, 13+, 17+, 18+, 18+, 18+, 20+, 20+, 69+, S−	22
	102		5+, 6+, 7+, 9+, 9+, 10+, 12+, S−, S−, S−	9
	208		8+, 9+, 9+, 10+, 15+, 23+, 25+, 25+, 30+	17
	416		S+, S+, S−, S−, S−, S−, S−, S− S−, S−	
	(8 years)		S−, S−, S−	
		9 years	8+, 14+, 19+, 19+, 47+, 55+, S−, S−, S−, S−	27
309	2		15+, 15+, 16+, 17+, 20+	17
	52		6+, 8+, 9+, 10+, 10+, 12+, 12+, 12+, 12+, 13+, 16+, 16+, 17+, 17+, 17+, 21+, 21+, 22+, 22+, 29+, 33+, 43+, S+, S+, S−	17
	83		21+, 25+, 28+, 29+, 29+, 30+, 67+, 75+, S−	38
	102		12+, 14+, 19+, 19+, 33+, 56+, 61+, S+, S+, S−	31
	180		18+, 19+, 23+, S+, S+, S+, S−, S−, S−, S−	20
	416		S−, S−, S−, S−, S−, S−, S−, S−, S−, S− S−, S−	
	(8 years)			
		9 years	4+, 4+, 5+, 5+, 7+, 7+, 14+, 14+, S+, S−,	8
319	2		4+, 4+, 7+, 9+, 12+,	6
	52		3+, 3+, 6+, 8+, 12+, 13+, 13+, 15+, 15+, 18+, 19+, 20+, 21+, 21+, 22+, 27+, 43+, 47+, S+, S−, S−, S−	18
	83		22+, 24+, 25+, 28+, 36+, 40+, 44+, S−, S−, S−	31
	180		34+, 35+, 35+, 39+, S+, S+, S+, S+, S−, S−	36
	416		S+, S−, S−, S−, S−, S−	
	(8 years)			
		9 years	5+, 13+, 14+, 18+, 20+, 34+, 41+, S+, S−, S−	21

S — survivor, + — brain culture positive for amoebae, − — brain culture negative for amoebae

lective staining of the enzymes after their electrophoresis in a mixture which in 50 ml contained 200 mM phosphate buffer (pH 7.9), 50 mg Fast Red Violet, and 15 mg naphtol-acetate as a substrate. The bands of acid phosphatase were visualized using a mixture whose 50 ml contained 200 mM acetate buffer (pH 4.0), 10 mg Fast Blue TR, and 50 mg naphtol-phosphate as a substrate. After incubation the gels were fixed with 7% acetic acid and 5% ethanol.

Results

The virulence of strains tested in mice is summarized in Table 1. In 1972, at the time close to the original isolation, the examined *Acanthamoeba* strains showed high virulence with many deaths when instilled intranasally to mice. After $3^1/_2$ to 4 years the strains remained virulent but the survival time and the number

Table 2

Activity of lytic enzymes from cultures of virulent, attenuated and non-pathogenic *Acanthamoeba* spp. Results are presented in international units (IU) as means ± SD of seven separate experiments

Specification	Trypsin-like enzymes activity (pH 8.2)		Pepsin-like enzymes activity (pH 2.5)	
	homogenate	supernatant	homogenate	supernatant
A. astronyxis strain 190				
virulent*	25.5±1.8	8.5±0.4	61.0±1.0	48.6±1.6
attenuated**	18.6±1.0	7.4±0.4	50.5±1.5	41.7±1.4
A. castellanii strain 309				
virulent	22.6±1.7	8.8±0.8	58.4±2.2	55.8±2.3
attenuated	18.2±0.7	7.4±1.1	56.1±1.9	52.3±2.1
A. castellanii strain 319				
virulent	30.1±2.3	9.8±0.9	68.4±2.6	58.5±2.8
attenuated	17.4±0.8	5.3±0.3	60.2±1.9	51.1±2.8
A. rhysodes strain Chang, non-pathogenic	14.9±1.8	2.0±0.2	55.1±2.6	23.3±2.9
A. castellanii strain 23Ś, non-pathogenic	14.4±1.8	2.0±0.4	68.5±2.1	37.7±1.8
A. castellanii strain Neff, non-pathogenic	4.0±0.3	0.2±0.2	60.1±2.6	32.5±2.3

* – isolated recently from cysts stored at 4°C beginning at the original isolation (1972), ** – isolated in 1972 from the environment and maintained in long-term axenic culture in Bacto-Casitone Medium at 37°C.

of dead mice varied. All the strains were attenuated after eight years of serial passages in axenic cultures at 37°C. On the other hand, the strains isolated recently from cysts kept at 4°C retained, after nine years, their virulence, though lower than that initially observed, with similar survival time.

Table 2 quantitates the lytic enzyme activity associated with strains isolated primarily in 1972 from the environment and recently from cysts. Maximal activities were found in homogenate at pH 2.5, however, the activity of pepsin-like enzyme of all the strains tested (virulent, attenuated, and primarily non-pathogenic) seemed to be similar. On the other hand, the activity of trypsin-like enzymes in homogenate at pH 8.2, differentiated the virulent and attenuated strains. The virulent strains obtained recently from cysts kept at 4°C since the early passage level contained 24–73% more activity than did the attenuated strains. The control strains, primarily non-pathogenic, particularly the *A. castellanii* Neff strain isolated nearly thirty years ago, showed the lowest trypsin-like activity.

Figures 1 and 2 are diagrammatic presentation of the isoenzyme bands position of all the amoebae tested. The species of *Acanthamoeba* were not clearly distinguished by the isoenzyme patterns of non-specific esterases and acid phosphatase, nor the

Fig. 1. Diagrammatic presentation of the isoenzyme bands positions in non-specific esterases of *Acanthamoeba strains*. *1* — *A. astronyxis* strain 190, *2* — *A. castellanii* strain 309, *3* — *A. castellanii* strain 319, *4* — *A. rhysodes* strain Chang, *5* — *A. castellanii* strain 23Ś, *6* — *A. castellanii* strain Neff. *A* — attenuated strains, *B* — virulent strains, *1–11* — band numbers

Fig. 2. Diagrammatic presentation of the isoenzyme bands positions in acid phosphates of *Acanthamoeba* strains. *1* — *A. astronyxis* strain 190, *2* — *A. castellanii* strain 309, *3* — *A. castellanii* strain 319, *4* — *A. rhysodes* strain Chang, *5* — *A. castellanii* strain 23Ś, *6* — *A. castellanii* strain Neff. *A* — attenuated strains, *B* — virulent strains, *1–5* — band numbers

virulent and attenuated strains could be differentiated. All primarily pathogenic strains, irrespective of the amoeba species and actual virulence, had common bands (1), (3), (9) and (11) in non-specific esterases, whereas the non-pathogenic strains had a common, diffuse, slow-running (2) band. However, pathogenic *A. castellanii* had a faint (5) band of non-specific esterases, which was not seen in *A. astronyxis*, and did not have band (7) which appeared in *A. astronyxis*. In acid phosphatase the pathogenic strains, irrespective of their taxonomic position and actual virulence, had two common bands (2) and (4), while the non-pathogenic strains had a common, diffuse cathodic band in the (1) position.

Discussion

The decrease in virulence of *N. fowleri*, which occurs after prolonged axenic culture, was observed by several authors (Visvesvara and Callaway 1974, Wong et al. 1977, De Jonckheere 1979, Dempe et al. 1982). The gradual decrease in virulence with the subculture number was described by De Jonckheere (1978) and the author found differences in the virulence decrease between *N. fowleri* isolated from humans and from the environment. All the environmental strains were attenuated in axenic serum-casein-glucose-yeast extract medium after many transfers (53–106) with high survival range. Dempe et al. (1982) found that the attenuated strains of *N. fowleri* might have produced a chronic disease in mice.

On the other hand, there is little information on the decrease in virulence of *Acanthamoeba* strains grown in axenic culture. Stevens and O'Dell (1974) did not observe any gradual decrease in virulence of *A. culbertsoni* strains A-1. However, the virulence was far lower than that observed initially by Culbertson et al. (1965) and varied considerably. Our study indicated that the environmental strains of *Acanthamoeba* lost their primary virulence in axenic suspension culture only after much greater number of transfers (180–208) than that defined by De Jonckheere (1978) in *N. fowleri*. Instead, like in Stevens and O'Dell study (1974), the survival time and survival rate of mice were not stable. It is interesting to observe that the environmental strains of *Acanthamoeba* retained the virulence when stored in cyst form. Therefore we may assume that the completing of the whole life cycle, i.e., the cyclical processes of encystation and excystation, and thereby undergoing alternately the vegetative stage and resting cyst stage in the natural environment, conditioned the potential of amoeba pathogenicity.

Cloud (1969) suggested that one possible explanation of the virulence attenuation of *A. castellanii* is the loss of the enzyme systems which enable the penetration of the host tissues. The results of the studies conducted by Mattern and Keister (1977), Bos (1979), and recently presented by Gadasi and Kobiler (1983), demonstrated a correlation between the virulence of *E. histolytica* strains and the activity of proteolytic enzymes, and raised the possibility of the involvement of

these enzymes in the penetration of amoebae into the tissues of the host. Likewise, our study shows that the attenuation of *Acanthamoeba* strains — as a consequence of numerous subcultures — is related to the decrease in trypsin activity.

The isoenzyme patterns of non-specific esterases and acid phosphatase, which characterized the potentially pathogenic *Acanthamoeba* strains, did not change in the course of the prolonged axenic cultivation. The band position were very similar in *Acanthamoeba* strains, irrespective of culture age and their actual degree of virulence, showing only minor changes. Similarly, Sargeaunt et al. (1980) reported a stability of isoenzymes having single band in some strains of *E. histolytica* which lost their virulence to laboratory animals. These results disagree with those of Godfrey (1975), Kilgour (1976), and of Romanha et al. (1979), which found, after frequent serial passages of *Trypanosoma* cruzi strains, a partial change in their isoenzymes with multiple bands. The authors presumed that if the multiple isoenzyme bands were related to the alternative metabolic pathway, the changes in the bands might be related to the lengthy dissociation from the host concomitant with frequent sub-passing. The stability in the isoenzyme having multiple bands, observed in our experiments after numerous subcultures, indicated that the *Acanthamoeba* strains, being amphizoic organisms, may not be submitted to such strong differentiation as true parasites.

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The Nutritive Factors Affecting the Growth of the Rumen Ciliate *Diploplastron affine in vitro*

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Synopsis. The rumen ciliate *Diploplastron affine* has been cultured in a phosphate medium for over 2 years with or without rumen fluid. The supplementation of the medium consisting of phosphates, hay and wheat gluten with cellulose supported the growth of protozoa, while the starch and pectins diminished their number and glucose caused a disappearance of ciliates. There were no changes in population density in relation to the proportion of hay and barley flour in the diet. The elimination of wheat gluten from the diet caused a decrease or disappearance of ciliates. A negative correlation between protozoa number and food protein solubility was observed, while there was no correlation between bacteria and protozoa number.

There is a lot of papers about the cultivation of rumen ciliates, but only few of them are concerned with the protozoa from the genus *Diplodinium* (Coleman et al. 1972, 1976, Sugden 1953). The results presented here concern the cultivation of *Diploplastron affine*. Our own observations showed that the concentration of these protozoa in the rumen often reached 3×10^4 cells/ml. Since their cells are over 100 μm long (Dogiel 1927), they form a large part of the ciliate biomass in the rumen and hence they can play an important role in the rumen metabolism.

Material and Methods

The protozoa were taken from the rumen of sheep. The single-species population was obtained by picking up the cells that showed the typical features of *Diploplastron affine* (Dogiel 1927) and introducing them into flasks containing the culture medium and bacterial flora. Between 50 and 100 cells were put in a flask containing 40 ml of culture medium and cultured as described elsewhere (Michałowski 1975). When the population density reached about 1000 cells/ml, the protozoa were transferred into continuous culture system (Michałowski 1980). The samples taken from this system were used as inocula for particular experiments. Four different salt solutions were used for the cultivation of ciliates (Table 1). The sheep rumen fluid was temporarily used as

Table 1

The chemical composition of salt solutions used for cultivation of *Diploplastron affine* (g/l)

Ingredient	A	B	C	D
K_2HPO_4	6.3	4.9	0.0	0.0
KH_2PO_4	5.0	3.8	1.0	0.0
$NaH_2PO_4 \times 12H_2O$	0.0	0.0	0.0	9.3
$NaHCO_3$	0.0	6.6	5.0	9.8
KCl	0.0	0.0	0.0	0.57
NaCl	0.65	0.49	6.0	0.47
$CaCl_2 \times 6H_2O$	0.09	0.07	0.2	0.08
$MgSO_4 \times 7H_2O$	0.09	0.07	0.2	0.0
$MgCl_2$	0.0	0.0	0.0	0.06
CH_3COONa	0.75	0.0	0.0	0.0
pH	6.84	7.53	7.76	8.52

A – "caudatum type" salt solution, B – "simplex type" salt solution (both according to Coleman et al. 1972), C – "Hungate type" salt solution (according to Hungate 1942), D – artificial saliva (after McDougal 1948)

the supplementation of culture medium. It was taken 4 h after feeding, filtered through filter paper and kept frozen. The rumen fluid was added to salt solution on the days of transferring the ciliates into fresh medium (see below) in the amount of 10% of the culture medium volume. Ground hay, barley flour, barley starch, cellulose powder (Koch Light Lab.), pectins, glucose (P.O.Ch. Gliwice), wheat gluten, glycynin, phaseolin, legumin and casein (BDH) were used for preparing a food. Pure barley starch was obtained according to Whelan (1955). After purification it contained about 0.5% protein. Wheat gluten was obtained from commercial wheat flour, while glycynin, phaseolin and legumin from soya, beans and peas respectively. They were purified according to Klein (1933) and Pace (1955). The solubility of proteins in the "caudatum type" salt solution was 15, 37, 57, 78 and 100% for wheat gluten, glycynin, phaseolin, legumin and casein respectively.

The cultures were initiated by introducing 20 ml of *Diploplastron affine* suspension into Erlenmeyer flasks containing 20 ml of culture medium with appropriate food. Three cultures were run simultaneously in relation to any factor studied. The ciliates were fed every day and the content of flasks was gassed with CO_2 according to Michałowski (1975). Every fourth day a half of the culture volume was transferred after thorough mixing into another flask containing fresh medium.

Samples for ciliate and bacteria counts were taken every fourth day from the material remaining after ciliate transfer (see above) and were fixed with 4% formalin solution. Occasionally the population density was also determined on consecutive days after transferring the protozoa to the fresh medium. The ciliate number was estimated by counting all the cells present in 0.1 ml of the sample, and the number of bacteria – in Thoma counting chamber. Each sample was analysed three times. All analyses were made using light microscope. The concentration obtained immediately after transferring the ciliates to fresh medium and that one observed before the next transfer were used for growth rate calculation. Standard deviations of the mean values were also calculated. The significance of differences between the mean values was determined by Student-t test.

Results

The changes in the number of ciliates cultured in four culture media are shown in Table 2. All the cultures received daily 40 mg of food consisting of 30% ground hay, 47% barley flour and 23% wheat gluten. The experiment lasted until the death

of ciliates in the particular media. In the "caudatum type" salt solution they survived up to date, i.e., over 2 years. The mean number of these ciliates in continuous culture system was about 8.5×10^3/ml. The culture volume was there about 1 l, the daily ration — 2 g and the turnover rate about 0.6/24 h.

The elimination of wheat gluten from the diet caused a decrease in ciliate num-

Table 2

The concentration of *Diploplastron affine* in relation to the chemical composition of the culture medium ($\times 10^2$/ml)

Days	A	B	C	D
1		12.6		
4	30.6±4.47	25.7±3.39	19.1±6.91	11.0±2.48
8	26.0±4.87	22.3±0.91	14.8±1.13	8.8±1.36
12	21.0±3.76	14.6±2.49	9.5±1.12	2.0±1.74
16	22.6±9.40	4.8±0.61	6.6±1.62	0.2±0.1
20	22.6±7.96	0.5±0.30	2.5±0.77	0.0
24	17.7±1.84	0.0	0.0	0.0

A, B, C, D — the culture media (see Table 1)

ber by about two times ($P < 0.01$), whereas the rumen fluid addition did not affect the population density (Table 3).

It was impossible to maintain the ciliates on wheat gluten alone, on a mixture of pure barley starch with wheat gluten and on pectins with wheat gluten, whereas

Table 3

The concentration of *Diploplastron affine* in relation to diet and the presence of rumen fluid in the medium ($\times 10^2$/ml)

Diet	Concentration	
	A	B
(1) Hay (12 mg), barley flour (19 mg), wheat gluten (9 mg)	10.6±2.44	10.7±2.71
(2) Hay (32 mg), wheat gluten (8 mg)	10.9±3.47	10.9±3.45
(3) Hay (40 mg)	3.8±1.71	4.4±1.74

A — control cultures without rumen fluid, B — experimental cultures with 10% of rumen fluid in the medium

they grew about 2 months on a mixture of cellulose with wheat gluten (Fig. 1).

The number of *Diploplastron affine* in the cultures receiving hay and wheat gluten decreased after an addition of pure barley starch and pectins. The addition of glucose or elimination of wheat gluten caused a disappearance of ciliates, while supplementation of the medium with cellulose increased the population den-

sity (Fig. 2). Significant differences in ciliate number were found only between the cultures fed on pectins and other cultures ($P < 0.01$). The bacteria number varied from 10.9×10^7/ml to 13.0×10^7/ml and from 3.0×10^5/ml to 14.0×10^5/ml for single-cells counts and thread-like colony counts respectively. However, there were

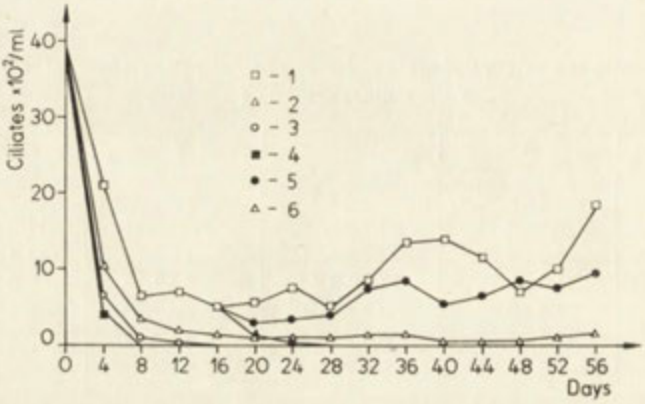

Fig. 1. The changes in number of *Diploplastron affine* in the cultures receiving daily 8 mg of wheat gluten supplemented with 32 mg of: ground hay (1), cellulose powder (2), barley starch (3), and pectins (4). (5) — the cultures receiving daily 32 mg of hay; (6) — the cultures receiving daily 8 mg of wheat gluten

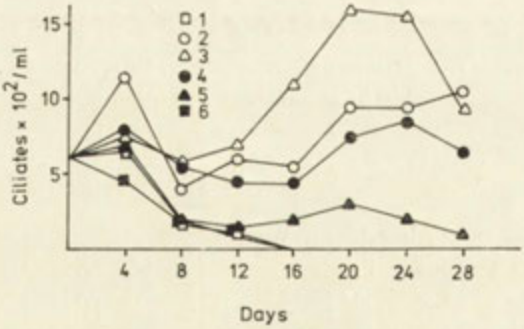

Fig. 2. The changes in concentration of *Diploplastron affine* in the cultures receiving daily 24 mg of ground hay (1) or 24 mg of hay and 8 mg of wheat gluten (2) supplemented with 8 mg of cellulose (3), 8 mg of barley starch (4), 8 mg of pectins (5), and 8 mg of glucose (6)

no significant differences in bacteria number due to large day-to-day variations occurring in the number of thread-like colonies. The pH values in this experiment varied between 6.8 and 7.2.

The population density and the growth rate of protozoa were not affected by the proportion of hay and barley flour in the medium (Table 4). The population

Table 4

The concentration of *Diploplastron affine* ($\times 10^2$/ml) and its growth rate ($\times 10^2$ml//day) in the cultures receiving different proportions of hay and barley flour in the diet. The wheat gluten content of the diets used, was always 10% of daily ration

Diet	A	B
(1) Hay (36 mg)	9.0±2.57	1.1±0.37
(2) Hay (28 mg), barley flour (8 mg)	12.0±3.83	1.5±0.44
(3) Hay (20 mg), barley flour (16 mg)	8.9±2.87	1.7±0.56
(4) Hay (12 mg), barley flour (24 mg)	11.8±6.48	1.4±0.55
(5) Hay (4 mg), barley flour (32 mg)	9.7±3.44	1.2±0.66

A – concentration, B – growth rate

Table 5

The concentration of *Diploplastron affine* ($\times 10^2$/ml), single-cells of bacteria ($\times 10^7$/ml), and thread-like colonies of bacteria ($\times 10^5$/ml) in the cultures receiving daily 36 mg of ground hay and 4 mg of proteins of different solubility. Solubility of proteins in %

Proteins	Solubility	Ciliates	Singlecells	Colonies
Gluten	15	16.7±5.49	10.2±1.93	5.0±3.02
Glycinin	35	9.3±1.79	9.7±2.08	4.0±3.21
Phaseolin	57	8.1±2.57	10.0±1.63	5.0±2.13
Legumin	78	7.1±2.30	9.6±1.83	7.0±5.61
Casein	100	6.7±1.89	8.9±2.34	4.0±3.12

usually showed a permanent increase on the days succeeding the culture transfer. It was often the highest within the first 24 h.

The ciliate number visibly tended to decrease with the increase of protein solubility (Table 5), but only the cultures fed on wheat gluten differed significantly from the others ($P < 0.01$). There was no significant differences in bacteria counts ($P > 0.05$).

Discussion

Among the four culture media studied, only "caudatum type" salt solution (Coleman et al. 1972) provided appropriate conditions for the development of *Diploplastron affine*. This solution contains a high amount of phosphates and its pH is about 6.8. Such conditions are preferred by many species of rumen ciliates (Coleman et al. 1972, 1976, Michałowski et al. 1985).

The addition of rumen fluid to culture medium is considered as a factor stimulating the growth of protozoa *in vitro* (Coleman 1969). In the experiments described here the positive influence of rumen fluid addition was not observed, which

might be connected with the fact that the diet consisted largely of unpurified plant material.

An addition of pure barley starch and especially of pectins decreased the number of protozoa, and glucose caused the disappearance of ciliates. The reasons of this reaction are unknown. It was certainly not caused by the acidifying of the environment, since the fall in pH values was not observed. The similar reaction was noted, however, in the case of *Entodinium exiguum* (Michałowski et al. 1985). Perhaps such substances may cause some unfavourable changes in the culture medium.

In contrast to *Entodinium exiguum* (Michałowski et al. 1985), *Diploplastron affine* did not react by increasing its number to the increase of the amount of starch-rich component in the diet. This fact may be connected with the ability of these protozoa to utilize the carbohydrates from the plant cell walls. This supposition can be supported by microscopic observations showing that *Diploplastron affine* readily ingested plant fragments and by the fact that they survived for two months on the mixture of pure cellulose and wheat gluten (Fig. 1). The literature data also reported the occurrence of α-D-glucosidase and β-D-glucosidase in the cells of these protozoa (Williams et al. 1984).

The tendency to a negative correlation between the food protein solubility and population density of *Diploplastron affine* was observed. A similar correlation was found also in *Entodinium exiguum* (Michałowski et al. 1985) and *Entodinium caudatum* (Muszyński et al. 1985). We suppose that reason for that is perhaps a small ability of these ciliates to uptake high-molecular compounds soluble in the culture medium. From the other hand the bacteria number was too low to cover the nutritive requirements of the numerous ciliate population if there was no insoluble (i.e., readily ingestible by ciliates) protein in the diet.

In the present experiment wheat gluten allowed the population of *Diploplastron affine* to maintain a higher density that the other proteins did. Our other experiments showed that wheat gluten enabled the maintenance of high population density of other rumen ciliates, although it is relatively poorly degradable by both protozoa and bacteria (unpublished). Perhaps, apart from the fact that the solubility of wheat gluten is much lower than of other proteins used, the presence of components like lipids and carbohydrates in the gluten molecule (Miflin et al. 1983) had a positive effect on ciliate growth. This problem needs further investigations.

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Sur une nouvelle espèce de cilié *Paranophrys carnivora* sp. n. (*Scuticociliatida*)

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Synopsis. Une nouvelle espèce du genre *Paranophrys* a été trouve dans l'eau de mer. Elle se distingue des autres représentants de ce genre par l'ensemble des caractères suivants: la taille moyenne mesure 40×20 μm, le nombre des cinéties somatiques est 21–22, la parorale en descendant s'incurve vis-à-vis M_3, en formant un petit sinus. Ce cilié se nourrit des tissus d'animaux. Au cours de la division ce sont la parorale et le scuticus qui forment l'appareil buccal de l'opisthe.

Depuis quand Thompson et Berger (1965) ont créé le nouveau genre *Paranophrys* et décrit la première espèce *Paranophrys marina* vivant dans la cavité gastrale de *Plumularia*, trois autres espèces ont été trouvées: deux dans l'eau marine et une dans l'eau douce. Ici nous présentons une nouvelle espèce différente des précedantes.

Matériel et techniques

Ce cilié a été trouvé par Wilbert dans un prélévement provenant de la côte d'Israel et presque en même temps par Czapik dans une mare rocheuse sur la côte de l'ile Malta. C'est une espèce histophage, qui se nourrit des tissus frais des animaux. On la cultivait dans l'eau marine, en lui donnant comme nourriture les vers coupés en morceaux (*Enchytraeidae*).

La forme du corps est variable, suivant les stades trophiques. Si la nourriture est abondante les ciliés se multiplient très rapidement. Privés de la nourriture pendant deux ou trois semaines les ciliés deviennent très minces et petits et la plupart d'eux disparaissent. On n'a pas observé la formation des kystes.

La morphologie et la stomatogenèse ont été étudié sur les préparations imprégnées au protéinate d'argent d'après la méthode de Wilbert et aussi au nitrate d'argent d'aprés Chatton afin de mettre en evidence les fibres argentophiles.

Morphologie générale de *Paranophrys carnivora* sp. n.

Le cilié est fusiforme, sa partie antérieure un peu amincie, le bout postérieur arrondi (Pl. I). Le corps du théronte mesure 36–40 μm de long et 18–20 de large, celui du trophonte 38–35 μm de long et 19–28 de large. La vacuole contractile est

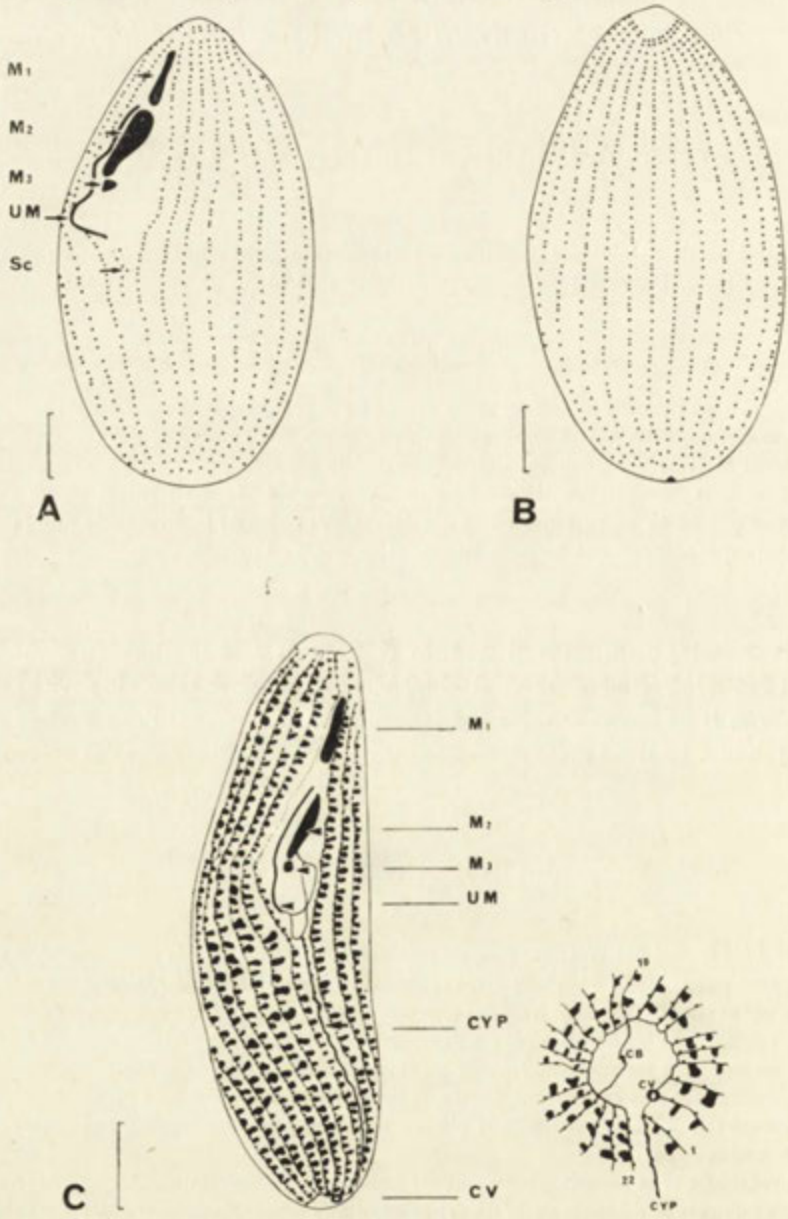

Fig. 1. Morphologie de *Paranophrys carnivora*. A, B – animal imprégné au protéinate d'argent: A – côté ventral, B – côté dorsal. C, D – animal imprégné au nitrate d'argent: C – système argentophil, D – pôle postérieur

située au bout du corps. La ciliature somatique consiste en 21–22 cinéties. Les extremités des cinéties n'atteignent pas le pôle postérieur mais se terminent sur un fibre argentophile qui l'entoure. Sur le pôle postérieur il y a un long cil caudal.

Au-dessous du péristome un petit groupe des cinétosomes (3–5) forment le scuticus. Plus bas une ligne argentophile désigne le lieu où se trouve le cytopyge.

L'appareil buccal s'étend sur la partie antérieure du corps, presque jusqu' à son équateur (Fig. 1 A). Les trois membranelles sont disposées en file méridienne. Les deux premières membranelles sont allongées, presque de la même longueur. M_1 mesure 8.2 μm de long et contient deux rangées des cinétosomes, M_2 (9.2 μm de long) est plus large — elle contient quatre rangées des cinétosomes dont une partie est disposée d'une façon irrégulière. M_3, très petite, a la forme d'un triangle irrégulier. La parorale commence au lieu, où se termine M_1, en descendant elle s'incurve vis-à-vis de la M_3 et plus bas elle entoure la partie droite de la cavité buccale.

Stomatogenèse

Le debut de la stomatogenèse chez *Paranophrys carnivora* se manifeste comme chez *Paranophrys carcini*, où elle a été décrite minutieusement par Grolière et Leglise (1977): la partie antérieure de la parorale se dédouble (Fig. 2 B) et les cinétosomes de sa partie postérieure ainsi que ceux du scuticus se multiplient et forment d'abord trois et plus tard quatre champs (Fig. 2 C). A l'étape suivant la parorale et le scuticus du proter se reconstruit (Fig. 2 B). Les membranelles restent intactes et ne participent pas à ce processus. Ensuite trois petits champs du côté droit disparaissent et à leur place la parorale de l'opisthe se forme en entourant un grand champ en forme de V renversé et le scuticus au-dessous de lui (Fig. 2 E). Le grand champ donne l'origine aux membranelles de l'opisthe. Au moment où la division commence et les cinéties somatiques s'interrompent, les structures orales chez le proter subissent une désintegration afin de se reconstituer plus tard (Fig. 2 F).

Discussion

Par sa morphologie, structures buccales et le type de stomatogenése l'espèce trouvé par nous appartient sans doute au genre *Paranophrys*. Jusqu'ici on a décrit 4 espèces de ce genre, qui se distinguent parmi eux par le nombre des cinéties, par les détails dans la structure du péristome et par le milieu, dans lequel ils vivent (Table 1), les différences dans les dimensions du corps etant trop petites pour être significatif. S'il s'agit des cinéties somatiques on voit que chez *Paranophrys magna* ainsi que chez l'espèce décrite ici elles sont plus nombreuses, les autres en possèdent beaucoup moins. Finalement c'est surtout d'après les différences dans la structure de la bouche qu'on peut distinguer les espèces. Figure 3 présente schemati-

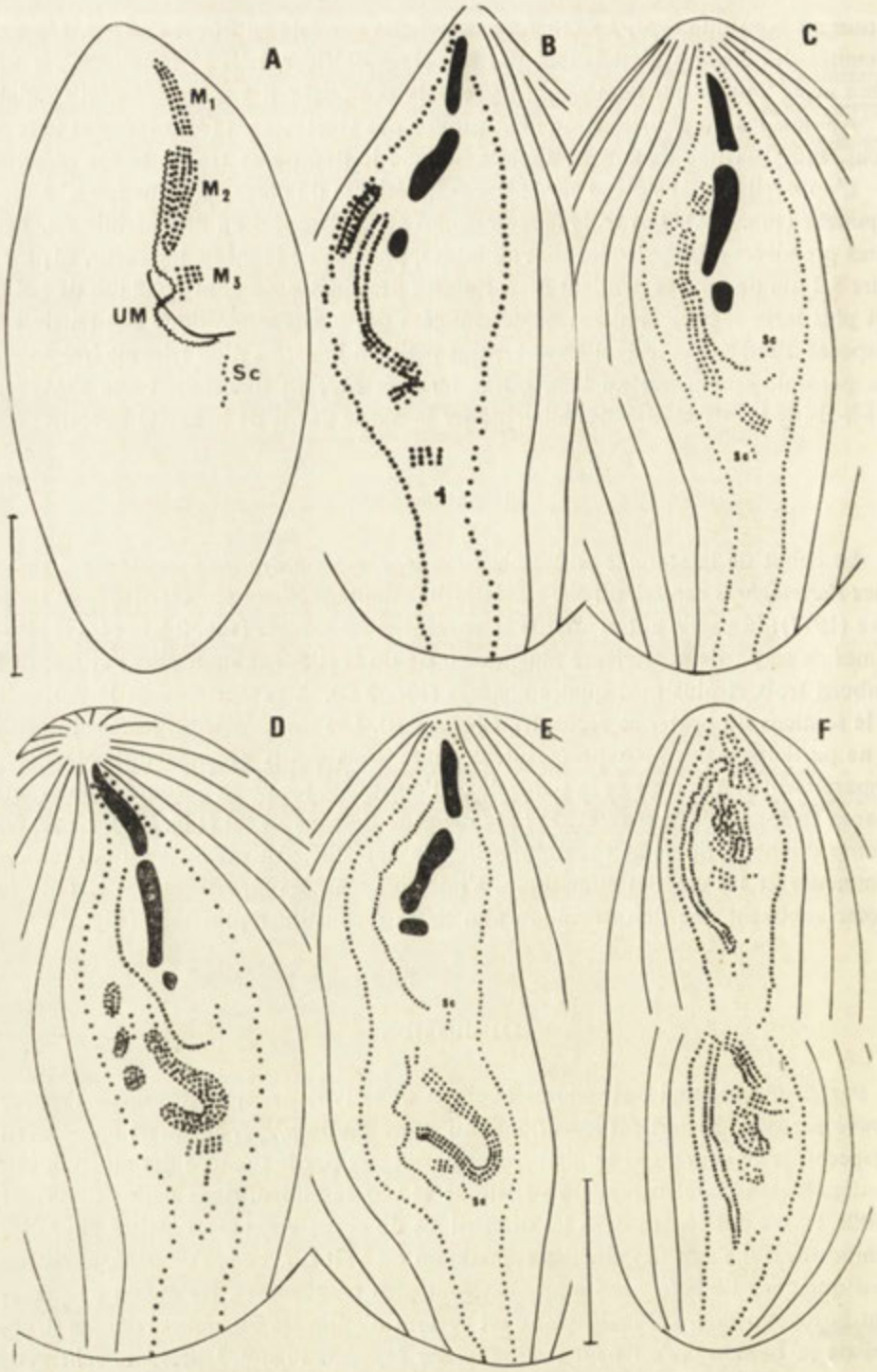

Table 1

Comparaison des espèces du genre *Paranophrys* Thompson et Berger, 1965

Espèce	Dimensions en μm	Cinéties	Nourriture	Milieu
Paranophrys marina Thompson et Berger, 1965	39–44 de long 19 de large	10	tissus	mer
Paranophrys magna Borror, 1972	45–75 de long 17–25 de large	21	bacteries?	mer
Paranophrys thompsoni Didier et Wilbert, 1976	40–45 de long 15–17 de large	13	bacteries	eau douce
Paranophrys carcini Grolière et Leglise	35–55 de long 12–14 de large	11	tissus	mer
Paranophrys carnivora sp. n.	36–56 de long 18–35 de large	21–22	tissus	mer

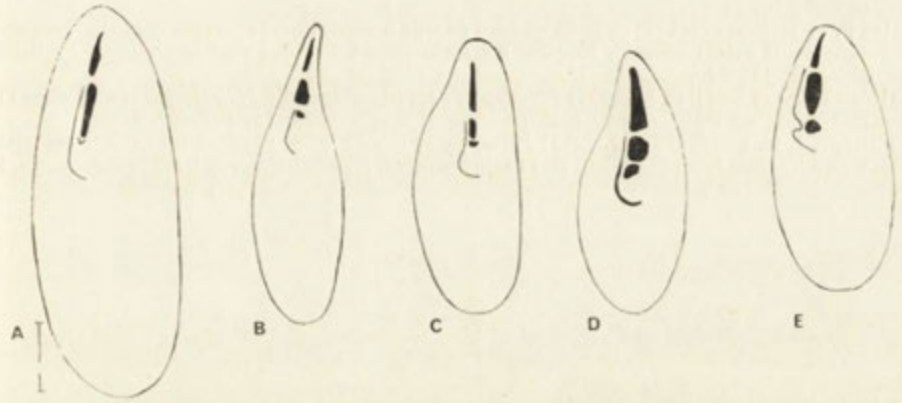

Fig. 3. Appareil buccal chez différentes espèces de *Paranophrys* (schematiquement): A — *P. magna* Borror, B — *P. carcini* Grolière et Leglise, C — *P. marina* Thompson et Berger, D — *P. thompsoni* Didier et Wilbert, E — *P. carnivora*

quement les péristomes de toutes les espèces de *Paranophrys*. M_1 est toujours allongée et mince, M_3 toujours petite. C'est M_2 qui est la plus variable — chez *P. magna* allongée mais plutot mince, chez *P. carnivora* sp. n. allongée et large, chez trois autres espèces courte et large. Un autre caractère, qui distingue bien *P. carnivora* c'est la forme de sa parorale. Chez toutes les espèces connues jusqu'ici la parorale en descendant vers le cytostome suit la ligne droite, seulment chez *P. marina* elle est legèrement courbée. Chez l'espèce que nous avons trouvée la parorale s'incurve vers la M_3, en formant un petit sinus.

Fig. 2. Morphogenèse chez *Paranophrys carnivora*: A — appareil buccal de l'animal, B — dédoublement de la parorale, multiplication des cinétosomes de sa partie postérieure et ceux du scuticus, C — formation des champs, D — reconstitution de la parorale du proter, E — formation de la parorale de l'opisthe, F — formation des membranelles de l'opisthe

On a constaté, que trois espèces appartenant au genre *Paranophrys* se nourissent des tissus des animaux (*P. carcini*, *P. marina* et *P. carnivora*). En ce qui concerne la *Paranophrya magna*, Borror ne definit pas la nourriture de cet animal. Il dit seulement que "the animal feeds by rubbing the buccal apparatus against the surface scum". Ce cilié absorbe probablement les bactéries présentes dans l'écume. *Paranophrys thompsoni* trouvée dans les pontes des *Chironomides* se nourrit, d'après les auteurs, des bactéries et flagellés qui se developpent dans la gelée, mais mise en culture dans les conditions pareilles elle ne se developpe pas bien. Il est possible, que ces dernières espèces sont histophages facultatifs et comme les autres espèces de ce genre ont besoin des tissus des animaux.

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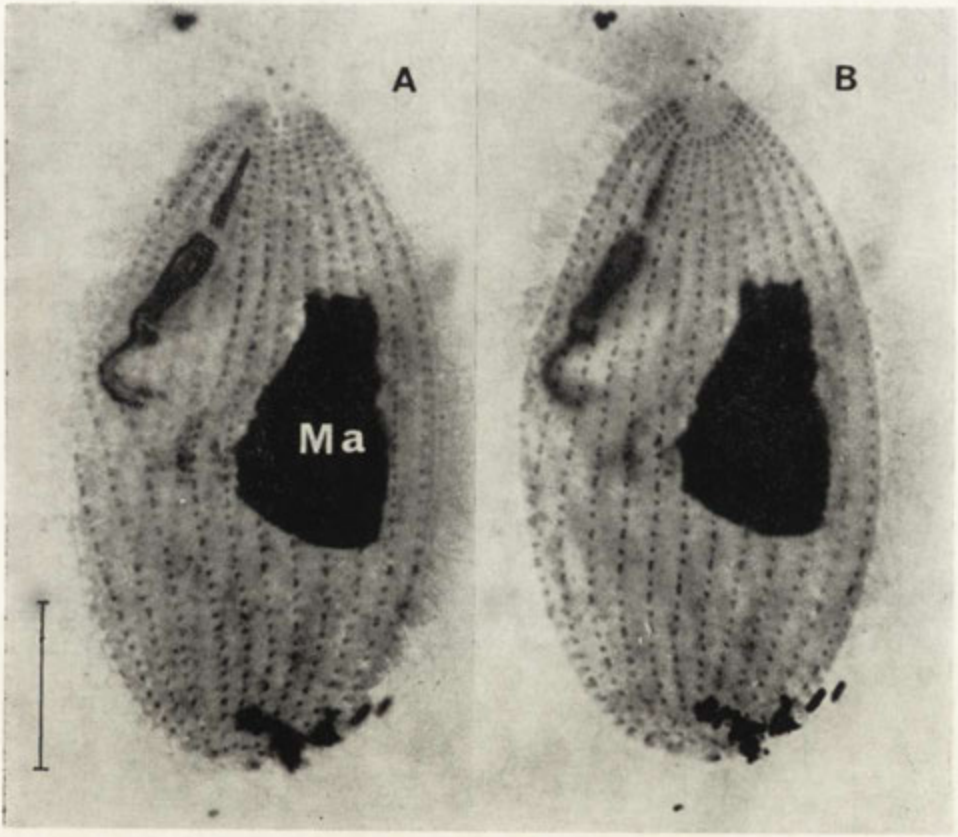
EXPLICATION DE PLANCHE I

1: *Paranophrys carnivora*: côté ventral et dorsal



A. Czapik et N. Wilbert auctores phot.

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The Ciliate *Paratrichodina africana* sp. n. (*Peritricha, Trichodinidae*) from *Tilapia* fish (*Cichlidae*) from Africa

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Synopsis. A new species of trichodina, *Paratrichodina africana* sp. n. is described from fishes of the genus *Tilapia* from Lake Victoria (Kenya) and the Nile Delta (Egypt). The new species is characterized by an elongate outer fragment of the central part of the denticle forming a prominent spine-like processus. Metric and meristic data concerning the new species are given jointly and separately for both localities. Affinities of the new species with other representatives of the genus *Paratrichodina* Lom, 1963 are discussed.

In the material collected by the senior author from Lake Victoria in Kenya (Kazubski 1985, 1986) and by the junior author in the Nile Delta (El-Tantawy and Kazubski 1985, 1986) a new species of trichodina, parasitizing fish of the genus *Tilapia* Smith, was found. A precise analysis showed that the trichodinas from both localities were identical. By the body structure and the adhesive disc and denticle shape these trichodinas appeared to be the most closely related with the genus *Paratrichodina* Lom, 1963, but they represented the new species. Due to origin of the new species from the African continent, the name *Paratrichodina africana* sp. n. is proposed for it.

Material and Methods

The trichodinas were collected in the following localities:

(1) Lake Victoria in Kenya. The ciliates were found on gills of all 5 examined fishes *Tilapia* sp., caught by anglers near the molo in Homa Bay, South Nyanza Distr., on 3rd August 1983. The parasites were not numerous.

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Table 1

Paratrichodina africana sp. n., metric and meristic data (dimensions are given in μm)

Characters	Trichodinas from the Nile Delta				Trichodinas from Lake Victoria				Whole material			
	range	M	SD	*n*	range	M	SD	*n*	range	M	SD	*n*
Body diameter	24–45	32.7	2.9	228	26–42	33.5	4.7	21	24–45	32.8	3.9	249
Adhesive disc diameter with border membrane	18–29	23.8	2.4	108	19–27	22.9	2.6	8	18–29	23.7	2.4	116
Adhesive disc diameter without border membrane	15–25	20.5	1.3	106	15–23	19.1	2.7	9	15–25	20.4	1.4	115
Denticulate ring diameter	8.5–16.5	12.2	1.0	81	9–16	12.0	2.2	8	8.5–16.5	12.2	1.1	89
Number of denticles	19–27	23.9	1.5	66	20–27	22.5	2.4	6	19–27	23.8	1.7	72
Height of denticle	4–7	6*	–	53	5–7	6.5*	–	6	4–7	6*	–	59

* mode

(2) The Nile Delta in Egypt. The ciliates were found in February 1984 on 9 out of 17 examined fishes *Tilapia nilotica*, originating from a small branch of the river near El-Mansoura. The ciliates were localized mainly on gills, in small amount also on body surface. In 3 fishes the ciliates were abundant.

Smears were made from freshly caught fish. They were dried and silver impregnated with $AgNO_3$ after the method of Klein. Examination of preparations and photographs were made under the light microscope Amplival C. Zeiss Jena.

Results

Description of *Paratrichodina africana* sp. n.

Fairly small trichodinas with the adoral spiral forming a circle of about 270°. Height of the body, evaluated on the base of dried and silver impregnated preparations, is equal to 3/4 of the body diameter. Dimensions of the body and of the adhesive disc and the number of denticles in the ciliates from both localities are given in Table 1.

The denticles have a pronounced centrifugal blade, well formed central part with characteristic spine and straight centripetal ray. The shape of the blade is similar to equilateral triangle with the top directed towards the adhesive disc centre. The right edge of the blade, when seen from the adhesive disc centre, is slightly concave and the left edge is convex. The outer edge of the blade, opposite to the top of the triangle, is also convex. The blades of the denticles have usually rounded tips, which give them a slightly irregular, semilunar shape. The central part of the den-

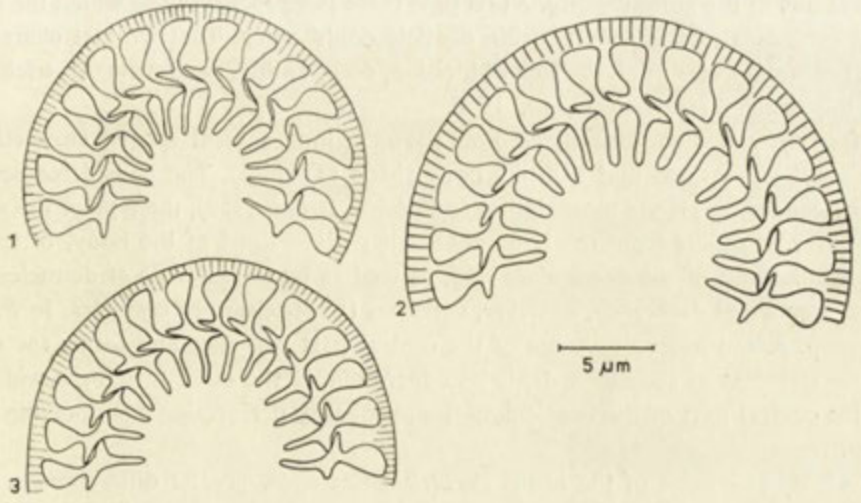

Fig. 1. *Paratrichodina africana* sp. n., 1–3 fragments of adhesive disc and denticulate ring; all specimens from *Tilapia nilotica* from the Nile Delta

ticle has an elongate outer fragment with the blade settled near its middle and the final part forming a characteristic spine-like processus. This element lies parallel to the corresponding central part of the neighbouring denticle, forming thus a ring 2–3 μm wide in the central part of the denticulate ring. This is probably a supporting structure. Centripetal rays are straight, finger shaped, fairly short and narrow. The bases of the blade and the ray are in a visibly shifted position. For each denticle there are five to six radial pins (Fig. 1, Pl. I).

The border membrane surrounding the adhesive disc is fairly wide; it measures about 2.5 μm in width.

Horse-shoe shaped macronucleus measures 20–25 μm in diameter and 7–9 μm in width. Micronucleus was not visible in preparations.

Hosts: *Tilapia nilotica* in the Nile Delta and *Tilapia* sp. in Lake Victoria.
Localization: gills, rarely body surface of fish.
Distribution: The Nile Delta, environs of El-Mansoura, Egypt and Lake Victoria, Homa Bay, South Nyanza Distr., Kenya.

As the type host and type locality, the fish *Tilapia nilotica* from the branch of the Nile near El-Mansoura are indicated.

Discussion

Taking into account structure of the cell, length of the adoral spiral and shape of the denticles, the described species is the most closely related to the genus *Paratrichodina* Lom, 1963. The denticles are composed of three elements: outer centrifugal blade, central part and centripatal ray. The blade is straight, in line with the radius of the adhesive disc. At its base there is no incision into which the spine-like processus of the neighbouring denticle could enter. The two last characters: the position of blade and the lack of incision, do not allow to allocate this trichodina to the genus *Tripartiella* Lom, 1959.

To the genus *Paratrichodina* Lom, 1963 belong several species characterized by small dimensions and bell-like body shape (Table 2). The considered species, *P. africana* sp. n., is the most similar to *P. incisa* (Lom, 1959), the type of the genus. However, it differs from the latter by smaller dimensions of the body, of the adhesive disc and of the denticulate ring, as well as by the number of denticles. The most important difference, however, concerns the structure of denticles. In *P. africana* sp. n. the outer processus of the central part is longer and forms the spine. These elements of successive denticles adhere one to the other forming a wide ring in the central part of the denticulate ring, probably performing a supporting function.

All other species of the genus *Paratrichodina* show greater differences in comparison with the described new one, so it is not necessary to consider them in this place.

Table 2

Main morphometric data of the species allocated to the genus *Paratrichodina* Lom, 1963 (dimensions are given in μm)

Species	Body diameter		Adhesive disc diameter		Denticulate ring diameter		Number of denticles		Distribution	References
	range	M	range	M	range	M	range	M		
Paratrichodina africana sp. n.	24.0–45.0	32.8	15.0–25.0	20.4	8.5–16.5	12.2	19–27	23.8	Africa	present paper
P. incisa (Lom, 1959)	14.0–54.5		11.0–36.0		7.0–20.0		17–31		Europe, Asia	Stein in Shulman (1984)
P. uralensis Kaschkowsky et Lom, 1979	30.0–44.0		20.0–26.0		12.0–16.0		18–25		Asia (river Ob)	,,
P. phoxini Lom, 1963	30.0–44.0		30.0–43.0		19.0–30.0		30–39		Europie, Asia	,,
P. corlissi Lom et Haldar, 1977	27.0–48.0		17.4–26.0		9.0–16.0		17–25		Europe, Asia	,,
P. alburni (Voitek, 1957)	32.2–63.2		25.3–44.2		18.1–32.6		23–29		Europe	,,

The described species is the first representative of the genus on the African continent and in fishes of the family *Cichlidae*. All arguments presented in this paper show that the described species is new. Due to its occurrence in Africa, the name *P. africana* sp. n. is proposed for it.

P. africana sp. n. has been primarily described under the name *Tripartiela* sp. in short communications at the VIIth International Congress of Protozoology in Nairobi (Kazubski 1985, El-Tantawy and Kazubski 1985). As it has been discussed above, this species shows closer affnities with the genus *Paratrichodina* Lom, 1963. This taxon was originally regarded as the subgenus within the genus *Tripartiella* (Lom 1963) and mentioned under this name by Ergens and Lom (1970). It was recently established as an independent taxon (Corliss 1979, Stein in Shulman 1984).

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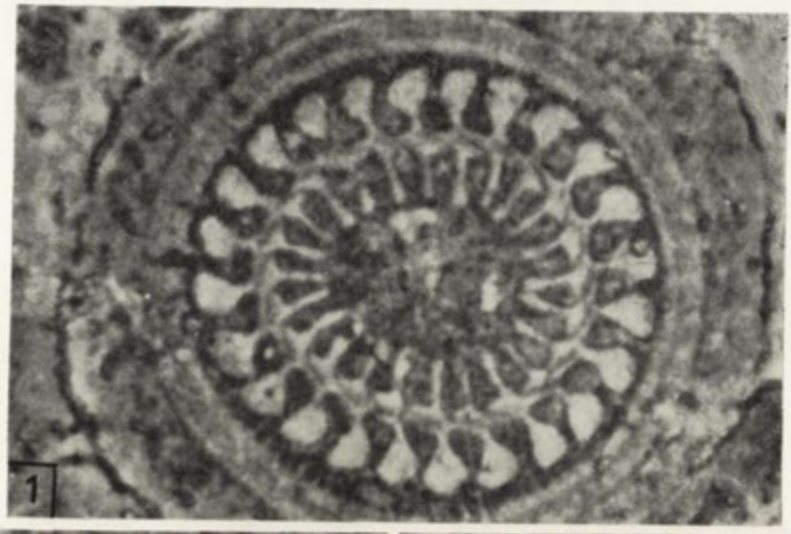
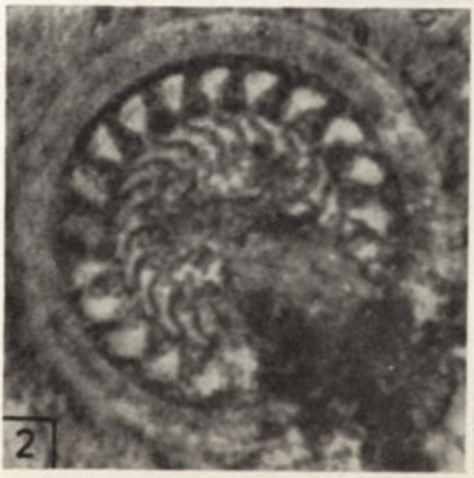
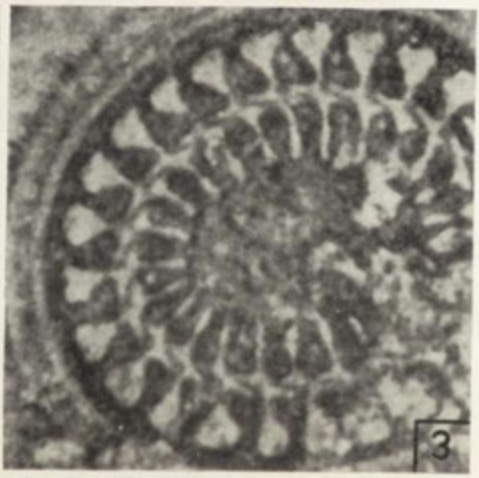
EXPLANATION OF PLATE I

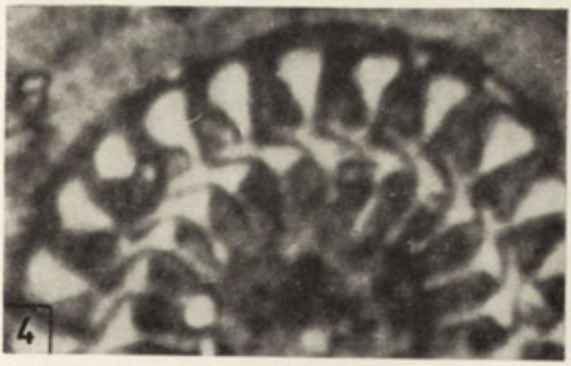
Paratrichodina africana sp. n.

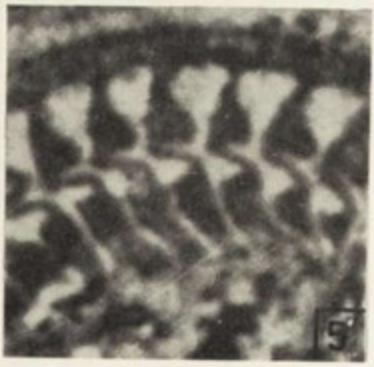
1–3 – adhesive disc (3000×)
4–5 – fragments of denticulate ring (5000×)
All specimens from *Tilapia nilotica* from the Nile Delta











S. L. Kazubski et S. A. M. El-Tantawy auctores phot.

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The Trichodinid Ciliates from Fish, *Tilapia nilotica* from the Nile Delta (Egypt)

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Synopsis. Four species of trichodinas were found on the fish *Tilapia nilotica* from the Nile Delta (near El-Mansoura), namely *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983 *Paratrichodina africana* Kazubski et El-Tantawy, 1986 and two species of the genus *Trichodina*, whose specific determination was not possible. *Trichodina* sp. I seems to be identical with the species determined by Basson et al. (1983) as *T. nigra* Lom, 1961 occurring in Europe. *Trichodina* sp. II is probably a new species, not recorded till now in fish from Africa. Descriptions of collected trichodina species are given and their systematic status discussed. Some problems connected with parasitism and geographic distribution of the species are also discussed.

Trichodinas parasitizing fish in Africa are rather poorly known. The only serious publication on this subject concerns the parasites of cichlid and cyprinid fish is South Africa (Basson et al. 1983). In this paper 8 species representing the genus *Trichodina*, one species of *Trichodinella* and one *Tripartiella*, are recorded. A small amount of material from the eastern shore of Lake Victoria was also collected (Kazubski 1985, 1986). The trichodinas from the Nile have not been investigated till now, although in Isreal the parasites of fish were thoroughly studied. The aim of the present paper was to pay attention to this group of fish parasites on the African continent.

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Material and Methods

The material was collected by the first author in the Nile Delta in February 1984. The fish, *Tilapia nilotica* (fam. *Cichlidae*) were caught in a small branch of the Nile near El-Mansoura. The fishes measured 9–17 cm (*longitudo totalis*). Smears were made from the gills and skin of freshly caught fishes. Dried smears were impregnated with $AgNo_3$ after Klein. The preparations, mounted in Canada balsam, were examined under the light microscope Amplival C. Zeiss, Jena. In order to analyze the ciliates photographs were made.

Results

The trichodinas were found in all 17 examined fishes *Tilapia nilotica*, in numbers ranging from several specimens to several dozens, depending on the species of trichodina and the fish specimen. The following species were noted:

(1) *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983. The body structure of ciliates from *T. nilotica* was typical of the species and the adhesive disc revealed characteristic ridges in the centre (Fig. 1). Dimensions of this trichodina were as follows: body diameter of silver impregnated specimens ranged from 48 to 66 μm, arithmetical mean 56.8 μm, standard deviation 5.1 μm (number of measurements n = 35); adhesive disc diameter with border membrane measured respectively 36–52 μm, 43.9 μm and 4.9 μm (n = 22); adhesive disc diameter without border membrane 32–49 μm, 38.1 μm and 4.3 μm (n = 26); diameter of the denticulate ring 20–29 μm, 24.2 μm and 2.2 μm (n = 36); number of denticles 24–29, 27.0 and 1.3 (n = 25). The height of completely formed denticle was 11–14 μm,

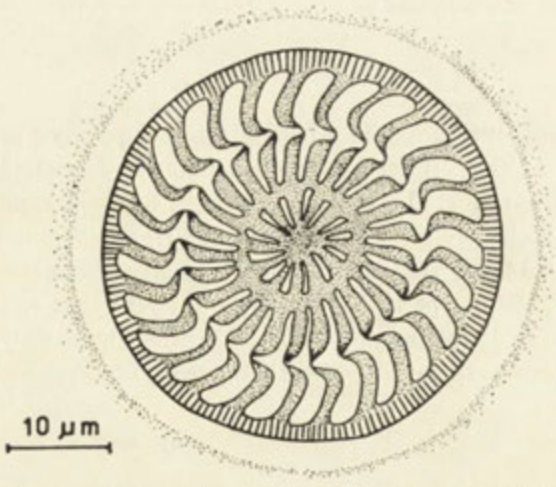

Fig. 1. *Trichodina centrostrigeata* Basson, Van As et Paperna, 1983, adhesive disc

most frequently (mode) 12 μm (n = 12). The border membrane was fairly wide, measuring up to 4.5 μm, usually about 3 μm.

T. centrostrigeata was found on the gills of 4 specimens of *Tilapia nilotica*. Sometimes, the ciliates occurred numerously.

(2) *Paratrichodina africana* Kazubski et El-Tantawy, 1986. Small-sized ciliates with adoral spiral of about 270°. Body dimensions: diameter of silver impregnated specimens ranged from 24 to 45 μm, arithmetical mean 32.7 μm, standard deviation 2.9 μm (number of specimens measured n = 228); diameter of adhesive disc with border membrane respectively 18–29 μm, 23.8 μm and 2.4 μm (n = 108); diameter of adhesive disc without border membrane 15–25 μm, 20.5 μm and 1.3 μm (n = 106); diameter of denticulate ring 8.5–16.5 μm, 12.2 μm and 1.0 μm (n = 81), number of denticles 19–27, 23.9 and 1.5 (n = 66). Height of denticles 4–7 μm, most frequently (mode) 6 μm. The structure of denticles was typical of the genus having a characteristic spine-like processus of the outer fragment of the central part. Detailed description of the species is given by Kazubski and El-Tantawy (1986).

P. africana was found in 9 out of 17 fishes originating from the Nile. The ciliates occurred mainly on gills, rarely on the body surface. In 3 fishes the ciliates were abundant — dozens of specimens were found on preparations.

Besides, two other species of *Trichodina* were found on gills and skin of examined fish. The condition of these ciliates in the preparations was poor, which made species identification impossible. However, some elements of their structure, e.g. adhesive discs, fairly well preserved in preparations, allowed us to made the following description:

Trichodina sp. I. Ciliates somewhat smaller then *T. centrostrigeata*. Silver impregnated specimens measured 47–52 μm in diameter, mean 50.5 μm, standard deviation 2.4 μm (n = 4); diameter of adhesive disc with border membrane measured respectively 40–45 μm, 41.7 μm and 1.7 μm (n = 6); diameter of adhesive disc without border membrane 31–41 μm, 36.5 μm and 3.0 μm (n = 13); diameter of denticulate ring 19–30 μm, 24.3 μm and 2.5 μm (n = 32); number of denticles 17–21, 18.9 and 0.9 (n = 32). Height of denticles 9–12 μm, most freqently (mode) 9 and 10 μm. The denticles had strongly curved, sickle-like blades, massive central part and fairly short, thick rays, slightly narrowing towards the adhesive disc centre (Fig. 2). The border membrane was 2.0–2.5 μm wide; there were 8–9 radial pins to one denticle.

Trichodina sp. I was found on the skin, rarely on the gills of 11 examined fishes. Infections was rather mild.

Trichodina sp. II. Body dimensions larger than in all other trichodinas mentioned in this paper. Body diameter of silver impregnated specimens ranged from 70 to 118 μm, mean 94.1 μm, standard deviation 18.6 μm (n = 9); diameter of adhesive disc with border membrane measured respectively 55–80 μm, 65.5 μm and 8.3 μm

(n = 11); diameter of adhesive disc without border membrane 46–73 μm, 60.7 μm and 9.6 μm (n = 13); diameter of denticulate ring 28–49 μm, 39.8 μm and 6.5 μm (n = 19); number of denticles 25–29, 27.1 and 1.3 (n = 19). Height of the denticle 16–25 μm, most frequently (mode) 17 μm. Denticle large, slender. Centrifugal blade sickle-shaped, slender, slightly curved, with central part fairly narrow. Centripetal ray clearly longer than the blade and slightly curved, being almost of the same width at the whole length (Fig. 3). Width of the border membrane about 3 μm. Number of radial pins corresponding to each denticle ranges from 9 to 11.

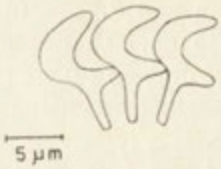

Fig. 2. *Trichodina* sp. I, fragment of denticulate ring

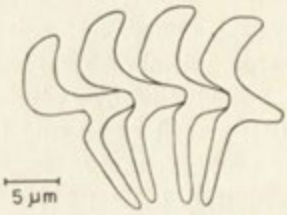

Fig. 3. *Trichodina* sp. II, fragment of denticulate ring

Trichodina sp. II was found on the gills, rarely on the skin of 8 fishes. Infection mild.

Discussion

The present investigation has shown that trichodinas are frequent parasites of fish in Africa. All examined fish specimens were infected, some of them even intensively. Four species of these ciliates were found, in many cases one fish harboured two, three or even four species.

What concerns particular species of trichodina, the following remarks may be added:

Trichodina centrostrigeata Basson, Van As et Paperna 1983 has been recorded so far only in South Africa from: *Oreochromis mossambicus*, *Pseudocrenilabrus philander*, *Tilapia rendalli*, *T. sparrmanii* and *Cyprinus carpio*. The present investigation extended the distribution area of this species to the Nile Delta in North Africa and added one fish species, *Tilapia nilotica*, to the list of hosts. This shows that *T. centrostrigeata* is probably widely distributed in Africa, occurring in various habitats. Recognition of intraspecific variation of this trichodina species would be interesting.

The remaining two species of the genus *Trichodina* are probably fairly frequent,

but less numerous in comparison with the former one. Unfortunately, the collected material did not make their specific identification possible.

The species described in the present paper as *Trichodina* sp. I is probably identical with that described by Basson et al. (1983) under the name *T. nigra*. Dimensions of the body, of the adhesive disc and of the denticulate ring of this trichodina are very close to those of *Trichodina* sp. I in the present material, although the latter has slightly smaller number of denticles. However, the ranges of this character overlap in both groups of ciliates. The shape of denticles is also very similar. Unfortunately, the lack of well-preserved specimens in the present material does not allow to identify these trichodinas. An additional difficulty is connected with the fact that *Trichodina* sp. I and probably the species described by Basson et al. (1983) are not identical with *Trichodina nigra* Lom, 1961, widely distributed in Europe. The differences concern body and adhesive disc dimensions, number of denticles and, above all, the shape of denticles. However, the final decision in this matter requires further comparative studies of specimens from Europe, Egypt and South Africa, as well as the recognition of morphological variation of the considered species.

The trichodina described in the present paper as *Trichodina* sp. II is probably a new species, although it shows affinities with the species described by Basson et al. (1983) as population A of *Trichodina mutabilis* from *Carassius carassius*. Differences concern body dimensions (our specimens are larger and have larger adhesive disc) and the shape of centripetal rays. As in the case of the former species, further studies are needed, including an evaluation of morphological variation, in order to resolve the problem of species identity.

Paratrichodina africana Kazubski et El-Tantawy, 1986 is a new species, found for the first time in Lake Victoria and in the Nile Delta. It is described in a separate paper (Kazubski and El-Tantawy 1986). Taking into account its prevalence — 64.7% of *Tilapia nilotica* from the Nile Delta and 100% of *Tilapia* sp. from Lake Victoria (Kazubski 1986), it is a common parasite of fish in Africa. This species was recorded in a preliminary note for the International Congress of Protozoology in Nairobi (El-Tantawy and Kazubski 1985, Kazubski 1985) under the name *Tripartiella* sp. More precise investigation on its morphology showed that it represents the genus *Paratrichodina* Lom, 1963 (Kazubski et El-Tantawy 1986).

Summing up, it can be stated that trichodinas play an important role as parasites of cichlid fish in Africa, but further investigations are necessary to recognize better this group of parasitic ciliates in African continent. Such studies are also necessary to solve some taxonomic problems concerning the species discussed above.

ACKNOWLEDGEMENTS

The authors wish to thank their Egyptian colleagues for helpful assistance in this investigations.

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The Trichodinid Ciliates from Fish, *Tilapia* sp. from Lake Victoria (Kenya) and Description of *Trichodina equatorialis* nom. nov.

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Synopsis. Two species of trichodina from fish, *Tilapia* sp. from Lake Victoria (Kenya) are described. The new name, *Trichodina equatorialis* nom. nov. is given to ciliates already described by Basson et al. (1983) from cichlid and cyprinid fish from South Africa and Israel under the name *T. heterodentata* Duncan, 1977. The differences between trichodinas from Africa and the Philippines are discussed and the appropriateness of creating a separate species for African trichodinas is justified. The second species, *Paratrichodina africana* Kazubski et El-Tantawy, 1986 was described partly from the material for this paper. Attention is paid to the distinctness of the fauna of trichodinas in Africa and in Europe.

Trichodinas parasitizing on fish in equatorial Africa have never been the object of investigation. Some information about this group of parasites originate from the peripheries of the African continent — from South Africa (Basson et al. 1983) and from Egypt (El-Tantawy and Kazubski 1985, 1986). The task of the present paper is to fill this gap.

Material and Methods

The trichodinas were collected from five fishes, *Tilapia* sp. originating from the eastern part of Lake Victoria. The fishes were caught on the 3rd August 1983 by anglers near the molo in Homa Bay, South Nyanza Distr., Kenya. The fishes measured several centimeters in length. Smears made from gills, just after taking the fish out of the water, were dried and impregnated with silver nitrate after Klein. The preparations were examined under a light microscope Amplival C. Zeiss, Jena. In order to morphological analysis of the ciliates numerous photographs were made.

Results

In all five examined specimens of *Tilapia* sp. two species of trichodinas were present.

(1) *Trichodina equatorialis* nom. nov. Dimensions of silver impregnated specimens from *Tilapia* sp. from Lake Victoria were as follows: diameter of the body ranged from 72 to 84 μm, diameter of adhesive disc with border membrane 50–61 μm, diameter of adhesive disc without border membrane 45–52 μm, diameter of denticulate ring 27–39 μm, number od denticles 24–27, height of denticles 15–18 μm, width of the border membrane 2–5 μm. As the number of this trichodina was small, only the ranges of dimensions are given, mean values have not been calculated. The blades of the denticles were sickle-shaped and the centripetal rays straight.

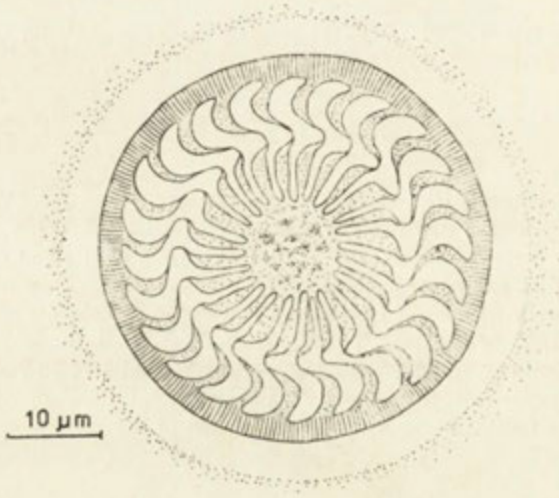

Fig. 1. *Trichodina equatorialis* nom. nov., adhesive disc

The central part of the adhesive disc was not uniformly dark, but gave an appearance of marble pattern (Fig. 1).

This species of trichodina were found on 3 out of 5 examined fishes.

The collected trichodinas, determined here as *T. equatorialis* nom. nov., are identical with ciliates described by Basson et al. (1983) from cichlid and cyprinid fish in South Africa and Israel, and especially with the specimen represented in Fig. 5 of the quoted paper (p. 247), and classified to the species *T. heterodentata* Duncan, 1977. The present finding confirms the differences observed by the mentioned authors between trichodinas from Africa and Israel and those described by Duncan (1977) from the Philippines. These differences concern the structure and shape of denticles, especially of their centripetal rays. The rays of trichodinas from Africa are much longer than the blades, slender and gradually narrowing, while the rays in *T. hete-*

rodentata from the Philippines are short and wide, abruptly tapering at the end. These differences prove that the process of formation of the centripetal rays differs between the both species of trichodinas. The described differences enable the author to recognize these trichodinas as distinct species: *T. heterodentata* described by Duncan (1977) from the Philippines and the trichodinas from Africa and Israel recorded by Basson et al. (1983) and in the present paper. For the latter the name *T. equatorialis* nom. nov. is proposed (syn. *T. heterodentata sensu* Basson, Van As et Paperna, 1983).

(2) *Paratrichodina africana* Kazubski et El-Tantawy, 1986. Small trichodinas with adoral spiral of about 270°. Specimens from *Tilapia* sp. from Lake Victoria had the following dimensions: diameter of the body of silver impregnated specimens — mean 33.5 μm, standard deviation 4.7 μm, diameter of adhesive disc with border membrane respectively 22.9 μm and 2.6 μm, diameter of adhesive disc without border membrane 19.1 μm and 2.7 μm, diameter of denticulate ring 12.0 μm and 2.2 μm, number of denticles 22.5 and 2.4, height of denticle 5–7 μm, most frequently (mode) 6.5 μm. The structure of denticles corresponded with that in other representatives of the genus *Paratrichodina* Lom, 1963, with the characteristic outer processus of the central part of the denticle. A detailed description of the species is given by Kazubski and El-Tantawy (1986). This species has been mentioned as *Tripartiella* sp. in a communication for the VIIth International Congress of Protozoology in Nairobi (Kazubski 1985).

P. africana was found on the gills of all 5 examined fishes. In some cases the intensity of infection was high. Finding of the same species on gills and skin of *T. nilotica* from the Nile Delta (El-Tantawy and Kazubski 1986) suggests that the species is a common parasite of cichlid fish.

Discussion

Investigations on trichodinas suggest that these ciliates are common parasites of fish in Africa. In publications by Basson et al. (1983), El-Tantawy and Kazubski (1985, 1986) and Kazubski (1985) as many as 11 species have been recorded, including some new species. This number is likely to increase after new investigations are performed. However, great care must be taken in identifying the African species of trichodina with the species occurring in other regions of the world, living in different climatic conditions and on other host species. As an example, the differences in specific characters observed between trichodinas formerly identified as *T. heterodentata* from the Philippines and those from Africa and Israel may be indicated. A detailed analysis of the material resulted in creating a new species, *T. equatorialis*, for trichodinas from Africa and Israel. Another example may be the species identified by Basson et al. (1983) as *Trichodina pediculus* (specimen represented in Fig. 14 of the quoted paper). By the shape of denticles, especially of

their centripetal rays, this species greatly differs from *T. pediculus*, a common parasite of hydras in Europe, occurring also on tadpodes and small fish. These example underline the necessity of serious studies on morphological variation in this group of parasitic ciliates.

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The author is greatly indebted to his colleagues in Kenya — professor Thomas R. Odhiambo and dr Leonard H. Otieno, of ICIPE in Nairobi, for organization of the trip to Lake Victoria, during which the material for this study was collected.

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Occurrence of *Trypanosoma mukasai* Hoare, 1932 in *Tilapia mossambica* (Peters) from India

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Synopsis. Trypanosomes (*Trypanosomatidae*) are described from the peripheral blood of *Tilapia mossambica* (Peters) collected from Bongaon, 24-Parganas, West Bengal, India and identified as *Trypanosoma mukasai* Hoare, 1932 as redescribed by Baker (1960). *Trypanosoma choudhuryi* Mandal, 1977 is compared with the present parasite and considered a synonym of *Trypanosoma mukasai* Hoare, 1932.

This is the first instalment of the series dealing with the trypanosomes of fresh water fishes in West Bengal, India. This communication records the occurrence of *Trypanosoma mukasai* Hoare, 1932 as redescribed by Baker (1960) from the different species of *Tilapia* of East Africa, found in the peripheral blood of American Koi, *Tilapia mossambica* (Peters) collected from the ponds of Bongaon, 24-Parganas, West Bengal, India. This host fish popularly known as "American Koi" belongs to family *Cichlidae* (*Perciformes*).

Material and Methods

In a survey (Sinha 1984) 25 specimens of this host fish were examined and two were found positive for *Trypanosoma* infection. Blood was obtained from branchial blood vessels or caudal veins and stained with Giemsa's or Leishman's stains. Measurements of parasites were made from fixed and stained materials and Camera lucida drawings were made.

Results

Trypanosoma mukasai Hoare

Trypanosoma mukasai Hoare, 1932: Parasitology, 24: 210, Baker (1960), Parasitology, 50: 515.

Trypanosoma choudhuryi Mandal, 1977: Acta Protozool., 16, 1–4

Description

Trypomastigotes are monomorphic, small, slender with bluntly pointed extremities (Fig. 1 *1–12*). These measure 21.4 μm (18.9–24.1 μm) in length including free flagellum and 1.5 μm (1.2–2.2 μm) in width. The cytoplasm is light purple in colour and contains a few volutin granules and vacuoles. The nucleus is oval, subcentral and stains deep purple measuring 1.9×1.0 μm. It lies towards the anterior part. A karyosome is not found. The nuclear index is 1.3 μm (1.1–1.35 μm). The kinetoplast is small, sub-terminal, dot-like and deep purple in colour, measuring 0.9×0.3 μm. The kinetoplastic index measures 1.04 μm (1.02–1.12 μm). The fla-

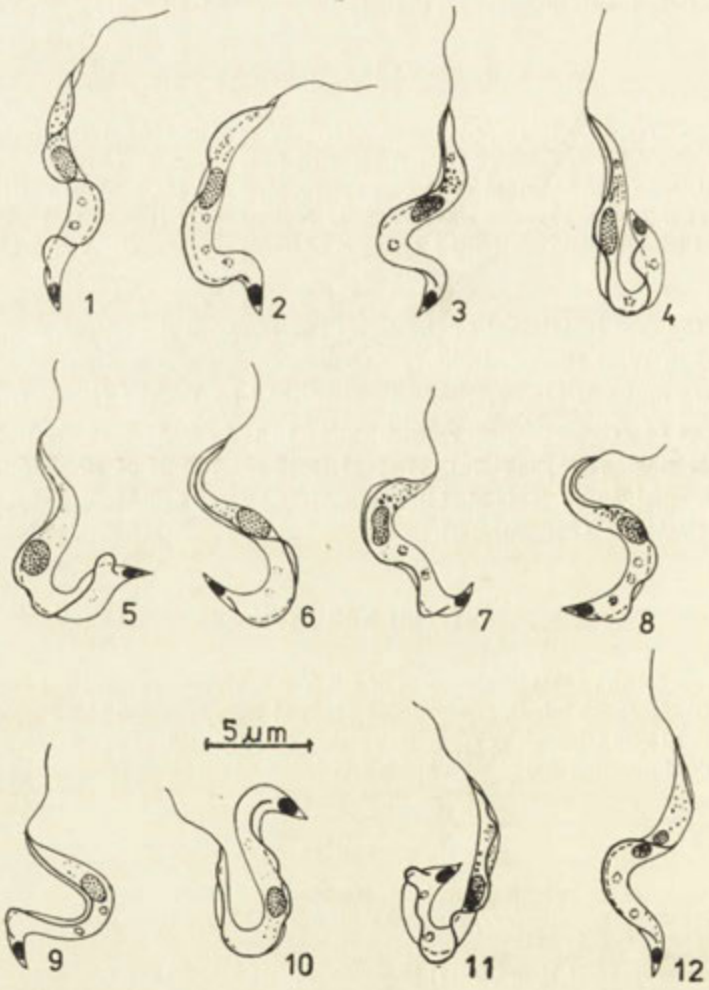

Fig. 1. *1–12.* Camera lucida drawings of *Trypanosoma mukasai* Hoare (1932). *1–12* — Trypomastigote forms, *12* — Dividing form of a trypomastigote showing two nuclei

gellum arises from the base of kinetoplast forming an undulating membrane with 2–4 folds. The free part of the flagellum is short at the anterior tip and measures 5.1 μm (4.4–5.8 μm).

Dividing forms of these trypomastigotes are not seen except for one individual (Fig. 1 *12*), where the cell body contains two nuclei but the kinetoplast remains undivided.

Host: *Tilapia mossambica* (Peters)
Locality: Bongaon, 24-Parganas, West Bengal, Bagmari 24-Parganas, West Bengal, India
Site of infection: Blood plasma

Discussion

Trypanosomes have been recorded in different species of *Tilapia* from Africa and India. Wenyon (1908) described an unnamed species of *Trypanosoma* in *Tilapia zilli* of the Nile river in Sudan. Léger and Léger (1914) found trypanosomes in *Tilapia lata* from the river Niger in the then French West Africa, but assigned no specific name to the parasite. These authors believed that the trypanosomes were identical with those described by Wenyon (1908) from *T. zilli*. After a lapse of many years, Dias (1955) described three species of trypanosomes *viz.*, *T. napolesi*, *T. rebeloi* (in which were included the trypanosomes described by Wenyon, 1908 in *T. zilli*) and *T. serranoi* (in which were included the trypanosomes which Neave, 1906 reported from *Synodontis schall*) from *T. mossambica* in Mozambique.

Baker (1960) reviewed the trypanosomes of African fresh water fishes and considered Dias's three species as variants of a single species and regarded them as synonyms of *T. mukasai* Hoare, 1932. He also reported *T. mukasai* from the different species of *Tilapia* (*T. nilotica*, *T. esculenta*, *T. variabilis* and *T. leucosticta*) of George and Victoria lakes of East Africa. Mandal (1977) described *T. choudhuryi* in *T. mossambica* collected from Bagmari, 24-Parganas, West Bengal, India.

The present parasite under discussion is identified as *T. mukasai* Hoare, 1932 as redescribed by Baker (1960) from different species of *Tilapia* on the basis of the morphological characters described above.

T. choudhuryi Mandal, 1977 resembles very much the present parasite as well as the small forms of *T. mukasai* in its morphology and dimensions. Therefore, *T. choudhuryi* and the present parasite are both identified as *T. mukasai* Hoare, 1932. The only difference is that the large forms of *T. mukasai* were not found in the present study, nor were these reported in *T. choudhuryi*.

A comparative study on the morphometric measurements of these trypanosomes is given in Table 1, which reveals that differences in dimensions, which almost overlap each other, are minor in nature and not sufficient for considering *T. choudhuryi* and the present parasite as valid species distinct from *T. mukasai* Hoare, 1932.

Table 1

Comparative measurements (in μm) of *T. mukasai* Hoare, 1932 as described by Baker (1960), *T. choudhuryi* Mandal, 1977 and the present parasite

Specification	*T. mukasai*		*T. choudhuryi*	The present parasite
	Range	Range		Range
	Small forms	Large forms	Small forms	Small forms
Total length (including free flagellum)	22–35	45–62	23–37.8	18.9–24.1
Body length (excluding free flagellum)	16–27	33–54	16.5–25.3	14.3–18.8
Body width	1–2.7	2–6	1.5–1.8	1.2–2.2
Free flagellum	5–12	7–17	6.5–12.5	4.4–8.2
Posterior end to kinetoplast	0–2	0.5–2	0.8–2.5	0.01–0.5
Nucleus length	1.7–3.5	3–5.5	3.5–5.2	1.7–2.4
Nucleus width	0.75–1.7	1.5–5.7	1–2.5	0.6–1.2
Kinetoplast to nucleus	5–17	14–29	5.5–9.5	5.6–7.9
Nucleus to anterior end	4.5–11.5	13–19	4.5–8.5	4.4–8.2
Kinetoplast length	0.25–1.5	0.5–1.5	1–1.5	0.7–1.1
Kinetoplast width	0.25–1	0.5–1	1–1.05	0.2–0.6

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Neohirmocystidae fam. n., a New Family of Septate Gregarines (*Apicomplexa*: *Sporozoea*) from Insects

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Synopsis. A new family, *Neohirmocystidae* fam. n., is proposed in this communication to accommodate the new genus *Neohirmocystis* gen. n. for the two septate gregarines from insects. These are *Neohirmocystis grassei* and *N. dercetini* from *Tribolium castaneum* and *Dercetina* sp. respectively. The affinity of the new family with the closely related families is discussed.

In the course of our study on septate gregarines (*Apicomplexa*: *Sporozoea*) in insects we obtained some parasites lacking an epimerite, having solitary as well as biassociative sporadins, spherical gametocyst dehiscing by simple rupture and double-walled spherical spores. The combination of such characters is unique among the group necessitating the proposition of a new genus to accommodate them.

In 1953 Grassé separated from the family *Gregarinidae* Labbé the genera *Hirmocystis*, *Hyalospora*, *Tettigonospora*, *Euspora*, *Caulocephalus*, *Sphaerocytis* and *Didymophyes* and placed them in a new family *Hirmocystidae*. Chakravarty (1959), however, regarded the family *Hirmocystidae* Grassé as a synonym of *Didymophyidae* Léger as in his opinion, "yet according to the laws of priority in nomenclature the first family name shall stand as it is formed from a valid generic name. The name *Didymophyidae*, therefore, should stand". Kudo (1971) accepted the family *Didymophyidae* to include the genus *Didymophyes* Stein only and regarded features like two to three sporadins in association and satellite without septum to be the characters of the family as did Kamm (1922). In a recent revision of the septate gregarines Levine (1979) accepted the family *Hirmocystidae* Grassé for those having epimerite ordinarily papilla-like or simple knob-like; gametocysts dehisce by simple rupture; oocyst ellipsoidal, prismatic, fusiform, ovoid or even spherical. It is apparent from the above discussion that the gregarines obtained by us do not fit

Table 1

Comparative characters of the four closely related families of septate gregarines

Characters	*Didymophyidae* Léger, 1892	*Gregarinidae* Labbé, 1899	*Hirmocystidae* Grassé, 1953	*Neohirmocystidae* fam. n.
Epimerite	—	Simple, symmetrical	Papilla-like or simple knob-like	Epimerite lacking
Sporadin	Sporonts in associations of two or three	Sporadins in association	—	Solitary as well as biassociative
Association	No septum in satellite	—	—	Caudo-frontal; satellite with septum
Gametocyst	—	Cysts with or without ducts	Dehisces by simple rupture	Spherical gametocyst; dehiscence by simple rupture
Spore	—	Symmetrical	Ellipsoidal, prismatic, fusiform, ovoid or even spherical	Double-walled spherical
Development	—	—	—	Intracellular or extracellular

properly either in the family *Didymophyidae* or *Hirmocystidae*. These can also be separated from the family *Gregarinidae* since these lack an epimerite and their cysts do not have any sporoducts. We are, therefore, proposing a new family *Neohirmocystidae* fam. n. for these gregarine parasites as these have characters more closer to those of the family *Hirmocystidae*. The characters of the family *Didymophyidae*, *Gregarinidae*, *Hirmocystidae* and *Neohirmocystidae* fam. n. are given in Table 1.

The genus *Neohirmocystis* gen. n. is being erected here under the family *Neohirmocystidae* fam. n. The new genus has the characters of the family. The structures and life histories of two new species under this genus are given. These are *Neohirmocystis grassei*, the type species of the genus and *Neohirmocystis dercetini* sp. n. from *Tribolium castaneum* (Herbst) and *Dercetina* sp. respectively.

Materials and Methods

The host insects *Tribolium castaneum* (Herbst) and *Dercetina* sp. were collected from a stock of the dry fruits of *Trachyspermum amni* at Chinsurah and from the potato plants, *Solanum tuberosum* at Kalyani in West Bengal respectively. The methods employed in the present study have been elaborated in our previous communication (Ghose et al. 1985).

The following abbreviations have been used: TL — Total length; LE — Length of epimerite; LP — Length of protomerite; LD — Length of deutomerite; LN — Length of nucleus; WE — width of epimerite; WP — Width of protomerite; WD — Width of deutomerite; WN — Width of nucleus. The ratios used in this paper are those of length of protomerite to total length (LP:TL) and the width of protomerite to width of deutomerite (WP: WD). The holotype and the paratype materials are deposited at the Department of Zoology, University of Kalyani.

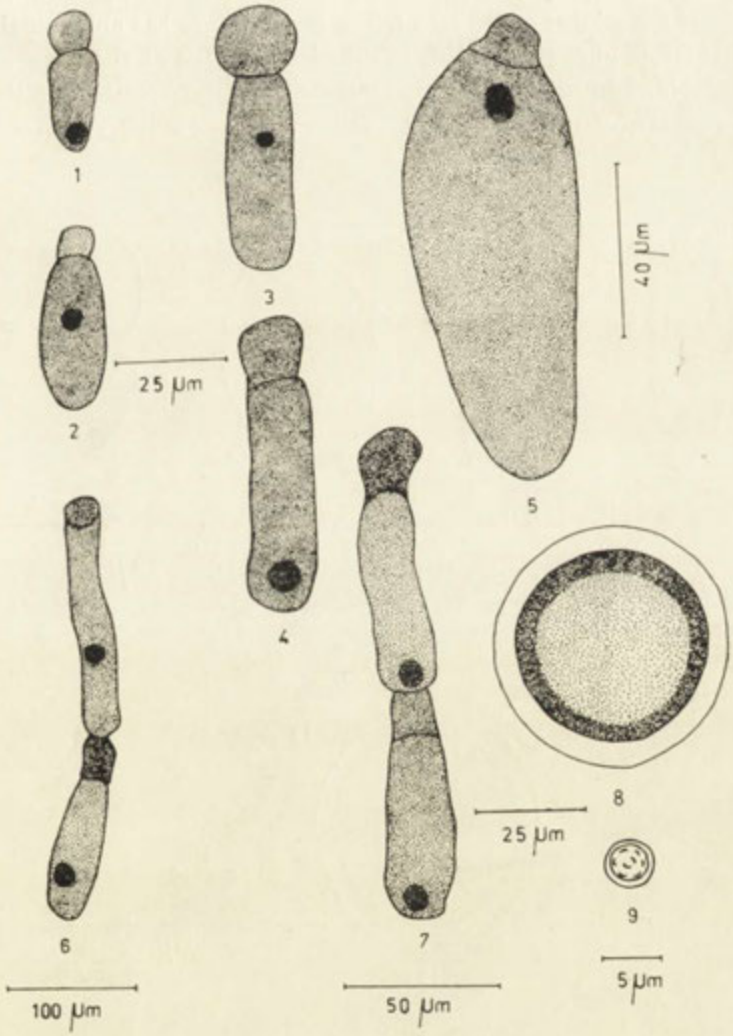

Fig. 1. *1–9* — Camera lucida drawings of *Neohirmocystis grassei* gen. n., sp. n. *1*, *2* — Young sporadins, *3–5* — Mature sporadins with various shapes and sizes, *6*, *7* — Sporadins in syzygy, *8* — Gametocyst with prominent ectocyst, *9* — Double-walled spherical spore with eight sporozoites

Observations

Type species: *Neohirmocystis grassei* gen. n., sp. n.

Host: *Tribolium castaneum* from the dry fruits of *Trachyspermum amni.*
Incidence: 91 out of 284 hosts (32%) examined are infected with this gregarine, with the peak of infection in the month of August (54.5%).

Development: Early development of this gregarine takes place inside the epithelial cells of the midgut of the insect. The earliest stage has an ovoidal body measuring 6.52×8.15 μm. The nucleus is spherical and lies at the posterior portion of the developing parasite. The infected cell shows a clear area around the parasite.
Sporadin: Biassociative as well as solitary. Young solitary sporadins (Fig. 1

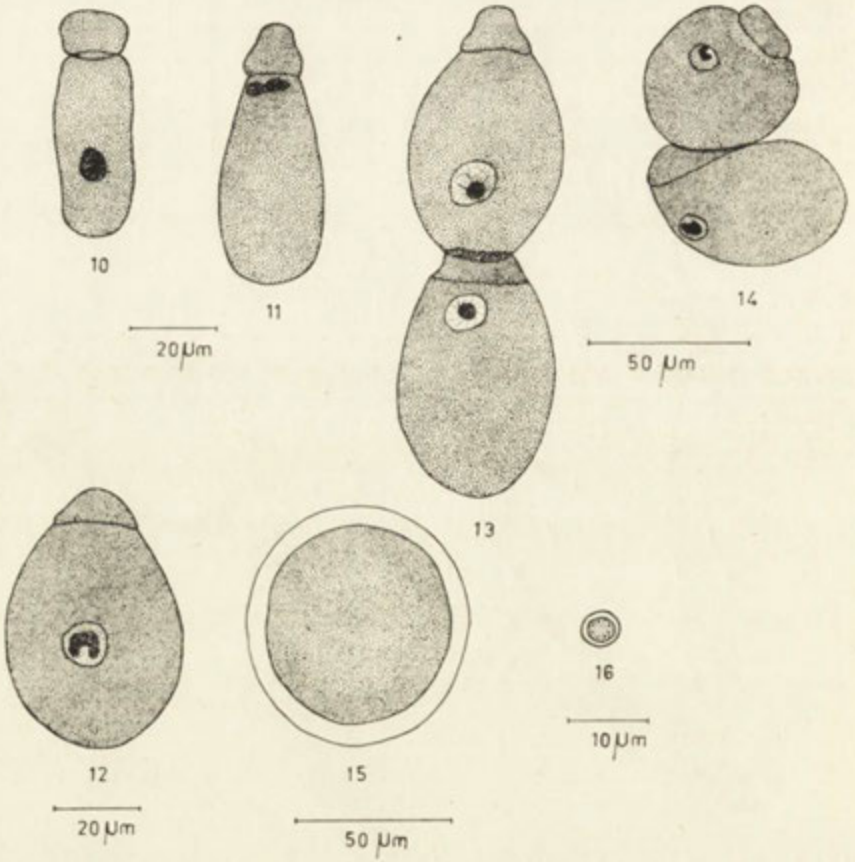

Fig. 2. *10–16* — Camera lucida drawings of *Neohirmocystis dercetini* sp. n. *10–12* — Sporadins of various shapes and sizes, *13, 14* — Sporadins in syzygy, *15* — Gametocyst with prominent ectocyst, *16* — Double-walled spherical spores

1, *2*) are elongated with globular protomerite while mature sporadins (Fig. 1 *3–5*) are elongated cylindrical in shape with a hat-like, tongue-like or globular protomerite. A distinct septum separates protomerite from deutomerite, the latter being elongated with rounded posterior end. Cytoplasm is uniformly granulated. Nucleus is rounded and variously located.

Association: This is always caudo-frontal in nature (Fig. 1 *6*). Primite and satellite are morphologically different. Protomerite of primite is fan-shaped containing high amount of cytoplasm while protomerite of satellite is dome-shaped and its cytoplasm is hyaline in nature (Fig. 1 *7*).

Gametocyst and spore: Gametocysts are collected from the midgut of the infected hosts. These are blackish white in colour and rounded in shape with prominent ectocyst (Fig. 1 *8*) and measure 58.05 μm in dimension. After 35 h of development in the moist chamber cysts dehisce by simple rupture and release the spores.

Double-walled spherical spores (Fig. 2 *9*) measure 10.8 μm in diameter. Formation of eight small rod-like sporozoites is completed at 90 h of development.

Measurements (in microns): The summary of measurements of 20 specimens with the mean within parentheses is given below:

Sporadin
TL = 25.35–199.8 (74.1);
LP = 6.75–24.3 (13.3); WP = 8.1–27.0 (14.8);
LD = 14.85–175.5 (60.8); WD = 10.8–40.5 (19.44);
LN = 4.05–13.5 (7.3); WN = 4.05–10.8 (6.65);
LP:TL = 1:3.07–12.3 (5.9);
WP: WD = 1:0.8–2.7 (1.4)

Details of measurements of individual specimens are given in Table 2.

Material:

(a) Holotype: on slide No. $M_{1/3}$ prepared from the contents of the midgut of *Tribolium castaneum* (Herbst) collected from the fruits of *Trachyspermum amni* at Chinsurah, Hooghly, West Bengal, India by S. Ghose on August 12, 1983.

(b) Paratype: Many, on the above-numbered slide and on other slides; other particulars are the same as for holotype material.

Neohirmocystis dercetini sp. n.

Host: *Dercetina* sp. from the potato plants, *Solanum tuberosum*.
Incidence: fourteen out of 76 hosts (18%) examined are infected with this gregarine during the months of November and December.
Development: Extracellular.

Sporadin: Biassociative as well as solitary sporadins are commonly encountered. Solitary sporadins are obese in shape (Fig. 2 *10–12*). Protomerite is conical and distinctly greater in length than breadth. Deutomerite is elongated, sometimes ovoidal to elliptical, broadest near the posterior third and with rounded posterior

Table 2

Showing details of measurements (in microns) of different parts of 20 individuals of *Neohirmocystis grassei* gen. n., sp. n.

Sl. No.	TL	LP	LD	LN	WP	WD	WN	LP:TL	WP: WD
(1)	29.7	8.1	21.6	5.4	8.1	10.8	5.4	1:3.6	1:1.3
(2)	25.35	10.5	14.85	4.05	8.1	10.8	5.4	1:3.6	1:1.3
(3)	45.9	6.75	39.15	8.1	9.45	12.15	5.94	1:6.8	1:1.2
(4)	97.2	8.1	89.1	9.45	21.6	35.1	8.1	1:12.0	1:1.6
(5)	99.9	8.1	91.8	8.1	14.85	40.5	6.75	1:12.3	1:2.7
(6)	91.8	16.2	75.6	5.4	8.1	21.6	5.4	1:5.6	1:2.6
(7)	72.9	9.45	63.45	6.75	8.1	13.5	5.4	1:7.7	1:1.6
(8)	62.1	16.2	45.9	5.94	20.25	20.26	5.94	1:3.8	1:1.0
(9)	81.0	13.5	67.5	6.75	12.15	29.7	6.75	1:6.0	1:2.4
(10)	49.95	8.1	41.85	5.94	8.1	10.8	5.94	1:6.16	1:1.3
(11)	199.8	24.3	175.5	10.8	27.0	24.3	10.8	1:8.2	1:0.9
(12)	64.8	16.2	48.6	6.75	18.9	16.2	6.75	1:4.0	1:0.85
(13)	67.5	18.9	48.6	6.75	16.2	18.9	8.1	1:3.6	1:1.2
(14)	54.0	13.5	40.5	6.75	18.9	16.2	6.75	1:4.0	1:0.85
(15)	91.8	10.8	81.0	7.29	13.5	27.0	8.1	1:8.5	1:2.0
(16)	59.4	18.9	40.5	8.1	18.9	16.2	6.75	1:3.14	1:0.85
(17)	54.0	17.55	36.45	6.75	20.25	21.6	6.75	1:3.07	1:1.06
(18)	40.5	8.1	32.4	5.4	9.45	14.85	5.94	1:5.0	1:1.57
(19)	132.3	18.9	113.4	13.5	21.6	17.55	8.1	1:7.0	1:0.81
(20)	62.1	13.5	48.6	8.1	13.5	10.8	5.4	1:4.6	1:0.8

extremity. Cytoplasm is almost uniformly granulated throughout proto- and deutomerite. Nucleus is more or less spherical in shape with a distinct nuclear membrane and a single endosome inside it. Pellicle is moderately thick and epicyteal striations are not observable.

Association: Always caudo-frontal in nature (Fig. 2 *13, 14*) and of the associated partners, primite and satellite are almost equal in size. Association appears to be very firm as it is observed that deutomerite of primite pushes into protomerite of satellite.

Gametocyst and spore: Cysts are collected from the hindgut of the hosts and are almost spherical in shape (Fig. 2 *15*) measuring 79.0 μm × 77.0 μm in the average. When freshly viewed these appear white in colour. Cysts dehisce by simple rupture at about 48 h. The double-walled spherical spores (Fig. 2 *16*) measure 4.1 μm in diameter. Formation of eight sporozoites is completed at 72 h. The small, ovoidal sporozoites are arranged in a circular fashion inside the spore.

Measurements (in microns): The summary of measurements of 20 specimens with the mean within parentheses is given below:

Sporadin
TL = 44.8–106.4 (66.0);

LP = 8.4–15.4 (10.9): WP = 12.6–28.0 (17.6);
LD = 33.6–92.4 (55.2); WD = 14.0–56.0 (30.0);
LN = 2.8–16.8 (8.6); WN = 2.8–16.8 (7.6);
LP:TL = 1:4.2–10.2 (6.1);
WP: WD = 1:1.0–2.9 (1.7).

The details of measurements of different parts of 20 specimens have been shown in Table 3.

Table 3

Showing details of measurements (in microns) of the different parts of 20 specimens of *Neohirmocystis dercetini* sp. n.

Sl. No.	TL	LP	LD	LN	WP	WD	WN	LP:TL	WP: WD
(1)	56.0	9.8	46.2	8.4	14.0	16.8	6.3	1:5.7	1:1.2
(2)	44.8	8.4	36.4	11.2	14.0	14.0	2.8	1:5.3	1:1.0
(3)	53.2	12.6	40.6	7.0	12.6	23.8	5.6	1:4.2	1:1.8
(4)	64.4	14.0	50.4	2.8	16.8	28.0	11.2	1:4.6	1:1.6
(5)	58.8	11.2	47.6	8.4	16.8	36.4	8.4	1:5.3	1:2.2
(6)	50.4	8.4	42.0	5.6	14.0	16.8	7.0	1:6.0	1:1.2
(7)	61.6	11.2	50.4	5.6	16.8	19.6	5.6	1:5.5	1:1.2
(8)	95.2	11.2	84.0	11.2	21.0	61.6	8.4	1:8.5	1:2.9
(9)	64.4	11.2	53.2	7.0	16.8	33.6	5.6	1:5.8	1:2.0
(10)	85.4	8.4	77.0	14.0	22.4	42.0	14.0	1:10.2	1:1.9
(11)	89.6	15.4	74.2	11.2	14.0	37.8	9.8	1:5.8	1:2.7
(12)	75.5	14.0	61.6	9.8	16.8	25.2	5.6	1:5.4	1:1.5
(13)	42.0	8.4	33.6	8.4	14.0	22.4	5.6	1:5.0	1:1.6
(14)	67.2	12.6	54.6	7.0	16.8	25.2	5.6	1:5.3	1:1.5
(15)	106.4	14.0	92.4	16.8	28.0	56.0	16.8	1:7.6	1:2.0
(16)	72.8	9.8	63.0	5.6	22.4	30.8	4.2	1:7.4	1:1.4
(17)	68.6	12.6	56.0	7.0	16.8	30.8	5.6	1:5.4	1:1.8
(18)	47.6	8.4	39.2	7.0	16.8	22.8	9.8	1:5.6	1:1.4
(19)	67.2	8.4	58.8	10.5	23.8	33.6	9.1	1:8.0	1:1.4
(20)	50.4	8.4	42.0	8.4	16.8	22.8	5.6	1:6.0	1:1.4

Material:

(a) Holotype: On slide No. $T_{1/1}$, prepared from the contents of the midgut of the insect, *Dercetina* sp. collected from the horticulture garden of Bidhan Chandra Agricultural University, Kalyani, West Bengal by S. K. Ray on November 2, 1982.

(b) Paratype: Many, on the above-numbered slide and on other slides; other particulars are the same as for holotype material.

Remarks: This gregarine differs from the type species of the genus in the shape of the sporadin, colour of the gametocyst, development, measurements as well as ratios of different body parts (Table 4). As such it has been assigned a new species status and named *Neohirmocystis dercetini* sp. n., after the generic name of its host.

Table 4

Comparative characters of *N. grassei* and *N. dercetini*

Characters	*Neohirmocystis grassei* Type species	*Neohirmocystis dercetini* sp. n.
Epimerite	lacking	lacking
Sporadin	Solitary as well as biassociative: young sporadins are elongated with globular protomerite while mature sporadins with hat-like, tongue-like or globular protomerite	Solitary as well as biassociative; solitary sporadins are obese in shape with conical protomerite
Association	Caudo-frontal syzygy; primite with fan-shaped protomerite while it is dome-shaped in satellite	Caudo-frontal syzygy; associated partners are equal in size
Gametocyst	Blackish white in colour and rounded in shape with prominent ectocyst	White in colour and spherical in shape
Spore	Double-walled, spherical	Double-walled, spherical
Development	Intracellular	Extracellular
LP:TL	1:3.07–12.3 (5.9)	1:4.2–10.2 (6.1)
WP: WD	1:0.8–2.7 (1.4)	1:1.0–2.9 (1.7)
Host	*Tribolium castaneum* (Herbst)	*Dercetina* sp.

Diagnosis

Family *Neohirmocystidae* fam. n.

Epimerite lacking; sporadins solitary and biassociative; satellite with septum during association; gametocyst dehisces by simple rupture releasing spherical spores; development intracellular or extracellular.

Genus *Neohirmocystis* gen. n.

Characters same as in the family.

Type species *Neohirmocystis grassei*

Mature sporadins with hat-like, tongue-like or globular protomerite; cyst blackish white in colour; spores spherical; development intracellular; LP:TL = 1:5.9; WP: WD = 1:1.4; from the gut of *Tribolium castaneum* (Herbst).

Neohirmocystis dercetini sp. n.

Sporadins with conical protomerite; gametocyst white in colour; development extracellular; LP:TL = 1:6.1; WP:WD = 1:1.7; from the gut of *Dercetina* sp.

Discussion

The importance of epimerite and sporoduct is immense in the taxonomy of eugregarines. New genera and families are often established on the basis of presence or absence of these two features. In course of the present investigation we examined 284 hosts of *Tribolium castaneum* and 76 hosts of *Dercetina* sp. of which 91 and 14 respectively were infected by eugregarines and these never showed any epimerite. In some gregarines the epimerite is fragile and very alimentary giving a false impression that it is altogether lacking. However, we examined more than one thousand individual gregarines from the infected hosts with utmost care under the phase contrast microscope in both living and stained conditions and could never find any trace of this structure in them. The obvious conclusion that can be drawn from this is that the epimerite is altogether lacking in the present study. Chances of its non-observance due to fragility are, therefore, remote. The gregarines, as such, cannot be fitted well in the family *Hirmocystidae* to which they have a superficial resemblance only.

Species of *Tribolium* have been found to be infected by the Gregarine, *Gregarina minuta* in different parts of the world. *G. minuta* has earlier been reported from these hosts in USSR (Wellmer 1911), Japan (Ishii 1914), France (Théodoridès 1955), Canada (Laird 1959), Poland (Lipa 1967) and Germany (Geus 1969). In a recent communication Ghose et al. (1986) have reported two other species namely *G. basiconstrictonea* and *Hirmocystis oxeata* from *T. castaneum* in India infesting ground nuts and walnuts respectively. In another communication Haldar and Sengupta (1986) have shown that species of *Tribolium* infesting different food items have their own distinctive eugregarine fauna. In the present study the hosts were collected from fruits of *Trychyspermum amni*, a common spice in India. The gregarines from this host are altogether different from *G. minuta* as will be apparent from Table 5.

Characters of *G. minuta* as given by Ishii (1914), Lipa (1967) and Geus (1969) have been compiled in Table 5 and have been compared with *Neohirmocystis grassei*, the type species of the new genus proposed under the new family. The eugregarine under report is distinctive in the structure of the sporadin, general shape of the associating partners, appearance of the gametocysts and ratios of different body parts. Information on the mode of development, structure of epimerite, nature of dehiscence and details of spore morphology of *G. minuta* are also not available. It is,

Table 5

Summarizing differences between *G. minuta* as given by various authors and *N. grassei* gen. n., sp. n. as revealed in the present study

	Gregarina minuta Ishii			*Neohirmocystis grassei* gen. n., sp. n.
	Ishii (1914)	Lipa (1967)	Geus (1969)	Present study
(1) Development	–	–	–	Intracellular
(2) Epimerite	–	–	–	–
(3) Sporadin	Larger sporonts are usually in association, the smaller ones often solitary Length of protomerite – 5–8 μm Length of deutomerite – 22–112 μm Breadth of the body – 6–28 μm Total length of the body – 27–120 μm	Maximum length 165 μm; width 50 μm	Solitary sporonts 110 μm long	Young solitary sporadins are elongated with globular protomerite while mature sporadins are elongated cylindrical in shape with a hat-like, tongue-like or globular protomerite. Deutomerite is elongated with rounded posterior end. Total length – 25.35–199.8 μm Length of Protomerite – 6.75–24.3 μm Length of Deutomerite – 14.85–175.5 μm Breadth of the body – 10.8–40.5 μm
(4) Association	Protomerite ovoid, sometimes more or less half-moon-shaped, broader than long. It is not large, especially so in the satellite, in which it is not infrequently hidden from view, being entirely imbedded in	Primite: Protomerite oval with flattened posterior end, wider than long. Deutomerite cylindrically elongated with oval end. Nucleus has one karyosome. Satellite: Protomerite twice wider than long. Deutome-	Primite: Protomerite of the primite is egg or small cap-like. Satellite: Protomerite close to the septum. Deutomerite is long with rounded end. Nucleus rounded situated at the middle or in the hinder half of the	The association is always caudofrontal in nature. Primite and satellite are morphologically different. Protomerite of primite is fan-shaped containing high amount of cytoplasm while protomerite of satellite is dome-shaped and its cyto-

	the deutomerite of the primite. Deutomerite elongate, cylindrical, rounded posteriorly. Nucleus large, spherical, usually situated near the middle of the deutomerite of both primite and satellite Larger association Total length – 188 μm Smaller association Total length – 118 μm	rite elongate. Nucleus located in the anterior end of deutomerite	deutomerite with central rounded karyosome. Maximum length of association 180 μm	plasm is hyaline in nature
(5) Gametocyst	Small, spherical, 36–48 μm	Oval upto 160 μm	Rounded 36–48 μm	Rounded blackish white in colour with prominent ectocyst and measure 58.05 μm
(6) Dehiscence	–	–	–	Simple rupture
(7) Spore	–	–	–	Double-walled spherical spores measure 10.8 μm in diameter. The eight rod-like sporozoites are arranged in circular fashion
(8) LP:TL	–	1:5–16		1:3.07–12.3 (5.9)
(9) WP: WD	–	1:1.7–2.7	–	1:0.8–2.7 (1.4)
(10) Host	*Tribolium castaneum* (= *ferrugineum*)	*Tribolium confusum*	*Tribolium castaneum* (= *ferrugineum*) and *Tribolium confusum*	*Tribolium castaneum*
(11) Locality	Japan	Poland	Germany	India

thus, difficult to identify the present form as *G. minuta* under the family *Gregarinidae*. As pointed out by Ghose et al. (1986) climatic variations and food habit of the host might have caused variations in the structures of this gregarine.

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Leidyana bimaculata a New Cephaline Gregarine Parasite of a Cricket, *Gryllus bimaculatus* De Geer

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Synopsis. A new species of Cephaline gregarine *Leidyana bimaculata* from the mid-gut of a gryllid, *Gryllus bimaculatus* De Geer is described. The gregarine has a simple, sessile globular knob-like epimerite. Adult sporonts measure up to 315 μm in length and their various ratios are: PL:TL, 1:3–7 and PW:DW, 1:1–2. Oval (330×350 μm in dimension) gametocysts after 72 h release doliform (3.5×5.0 μm) oocysts through 10 sporoducts each 50–90 μm in length.

Watson (1915), while working on polycystid gregarines, created the genus *Leidyana* under the family *Gregarinidae* Labbe (1899) and transferred some species to this genus which were previously described by Cuenot (1897) and Crawley (1903 a, b, 1907) under different genera. The generic characters of *Leidyana* are: epimerite simple sessile globular knob, sporonts solitary, cyst with ducts and sporocysts doliform. Later, Keilin (1918) described a species, *L. tinei* from a lepidopteran larva and Daviault (1929) reported *L. ephestiae* from *Ephestia kuhniella* Zeller. Kudo (1954) based on the specific characteristic nature of the solitary sporadins, created a separate family *Leidyanidae* to include the genus *Leidyana* alone.

Subsequent contributions to this genus have come from Hoshide (1957), Corbel (1967 a, b), Hoshide (1973, 1978), Haldar and Sarkar (1979), Patil and Amoji (1979) and Lipa and Martignoni (1984).

During the course of studies on cephaline gregarines of insects, a gregarine species has been obtained from the mid-gut of a gryllid, *Gryllus bimaculatus* De Geer, which possesses features agreeing with the genus *Leidyana* Watson.

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Material and Methods

The host insects were brought alive to the laboratory and their alimentary canals dissected out in physiological solution. Smears of infected mid-gut contents were made on clean microscope slides and fixed in Carnoy's fixative. The smears were stained subsequently in iron alum haematoxylin.

The development of gametocysts was observed after placing them in cavity blocks with few drops of insect Ringer's solution and incubating at 25°C–37°C. The oocysts were examined with Lugol's iodine solution. The Figures have been drawn with the help of Camera lucida.

Observations

Cephalont: The youngest cephalont (Fig. 1 A) observed measures 13–25 μm in length. The body is divided by septa into epimerite, protomerite and deutomerite. The epimerite is sessile and varies from subspherical to papilla-like structure (Fig. 1 B) measuring 3–7 μm in length and 2.5–6.0 μm in breadth. The protomerite is dome-shaped and the deutomerite is ovoidal with broad posterior end and con-

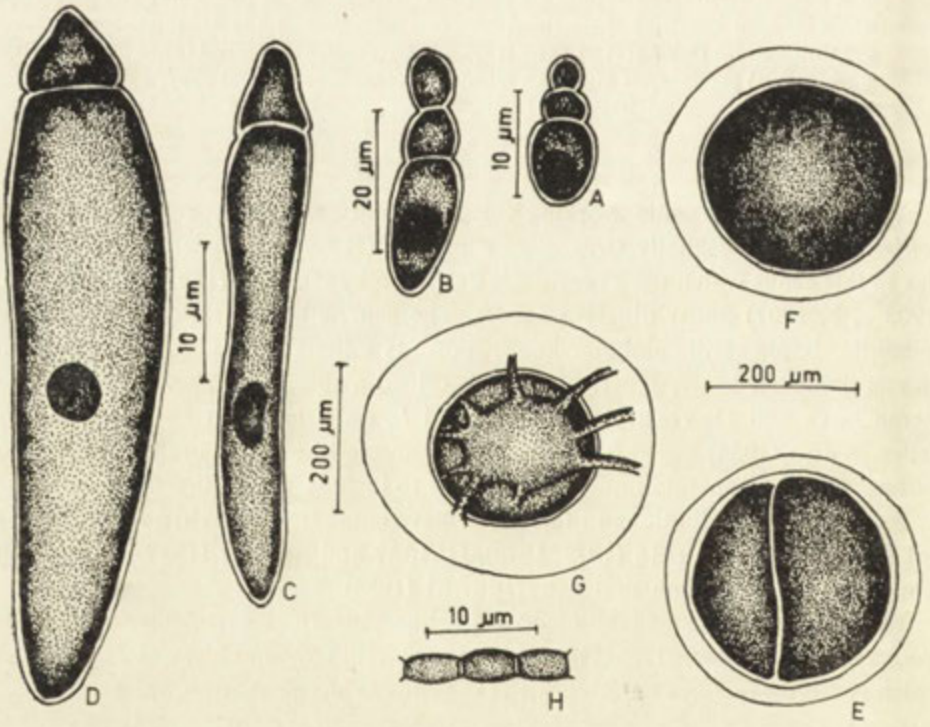

Fig. 1. *Leidyana bimaculata* sp. n., explanations see text

Table 1

Measurements (in microns) of different parts of 20 specimens of *Leidyana bimaculata* sp. n.

No.	TL	LE	LP	LD	WE	WP	WD	N	LP:TL	WP:WD
1	13.5	3.0	3.0	7.5	4.0	5.0	7.5	3.0	4.5	1.5
2	14.5	3.0	4.0	7.5	6.0	5.0	6.0	—	3.625	1.2
3	17.0	4.0	5.0	8.0	6.0	6.0	7.0	4.0	3.4	1.166
4	15.5	5.0	3.0	7.5	3.0	5.0	5.0	—	5.166	1.0
5	14.0	4.0	2.5	7.5	3.0	5.0	6.0	3.0	5.6	1.2
6	25.0	7.0	5.0	13.0	5.0	6.0	7.5	5.0	5.0	1.25
7	15.5	2.5	5.0	7.5	2.5	5.0	7.5	2.5	3.1	1.5
8	180.0	—	40.0	240.0	—	60.0	100.0	30.0	7.0	1.666
9	315.0	—	45.0	270.0	—	70.0	110.0	30.0	7.0	1.571
10	250.0	—	40.0	210.0	—	50.0	100.0	40.0	6.25	2.071
11	160.0	—	40.0	120.0	—	50.0	70.0	—	4.0	1.4
12	270.0	—	50.0	220.0	—	60.0	110.0	50.0	5.4	1.833
13	60.0	—	15.0	45.0	—	20.0	20.0	10.0	4.0	1.0
14	270.0	—	60.0	210.0	—	90.0	15.0	30.0	4.5	1.666
15	42.5	—	12.5	30.0	—	12.5	17.5	7.5	3.4	1.4
16	60.0	—	10.0	50.0	—	20.0	25.0	—	6.0	1.25
17	95.0	—	25.0	70.0	—	40.0	35.0	15.0	3.8	0.875
18	60.0	—	20.0	40.0	—	22.5	25.0	—	3.0	1.111
19	170.0	—	40.0	130.0	—	50.0	90.0	25.0	4.25	1.8
20	220.0	—	50.0	170.0	—	50.0	100.0	30.0	4.4	2.00

Abbreviations: TL — total length, LP — length of protomerite, WE — width of epimerite, WD — width of deutomerite, LE — length of epimerite, LD — length of deutomerite, WP — width of protomerite, N — diameter of the nucleus.

tains a spherical nucleus. The septum between epimerite and protomerite is distinct and convex.

Sporont: The sporadins (Fig. 1 C, D) are solitary, elongated cylindrical in shape, consisting of protomerite and deutomerite. These measure 42.5–315 μm in length and 17.5–150 μm in breadth. The protomerite is conical in shape, measuring 10–60 μm in width. The deutomerite is an elongated and cylindrical structure measuring 30–240 μm in length and 17.5–150.0 μm in width. Average width of the deutomerite is always greater than the protomerite. The breath near the anterior 1/4th is maximum and tapers gradually, terminating in a blunt posterior end. The cytoplasm is uniform and finely granulated in protomerite, whereas the deutomerite is filled up with a fine and coarse granules. The nucleus is almost in the middle of the deutomerite and stains deeply with iron-alum haematoxylin. Its shape is usually spherical, but various forms like kidney-shaped, spindle-like and oval form are also observed. The spherical nucleus measures 7.5–40 μm in diameter.

The sporadins at any stage do not show syzygy. Hence, these are solitary in nature.

The ratios PL:TL and PW:DW range between 1:3–7 and 1:1–2 respectively. The details of measurements are given in Table 1.

Gametocyst: The gametocysts (Fig. 1 E, F, G) are oval measuring 330×350 μm in dimension. The cyst wall is thin. There is a thick (40 μm) hyaline ectocyst. After 48 h of incubation the gametocyst develops 10 sporoducts, each measuring 50–90 μm in length.

Oocysts: The oocysts (Fig. 1 H) are doliform measuring 5.0×3.5 μm and are extruded in chains after 72 h of gametocyst development.

Table 2

Comparative characters of *Leidyana* spp. to show distinctiveness of *Leidyana bimaculata* sp. n.

Specification	*L. erratica* Crawley, 1903	*L. suzumushi* Hoshide, 1973	*L. saigonensis* Corbel, 1967	*L. bimaculaa* sp. n.
Body shape and size	Elongated cylindrical, 300–500 μm	Elongated cylindrical, 380 μm	Elongated cylindrical, 350 μm	Elongated cylindrical, 42.5–315 μm
Epimerite	Spherical knob like set on short cylindrical stalk	Simple sessile knob	—	Varies from subspherical to papilla like
Protomerite	Conical (broadly cone shaped)	Conical	Spherical	Conical
Nucleus	—	Spherical, 25 μm diameter	Spherical, 120 μm diameter	Spherical, 7.5 μm to 50 μm diameter
PL:TL ratio	1:5–1:7	1:6.1	—	1:3–7
PW:DW ratio	1:1.3–1:1.7	1:1.4	—	1:1–2
Gametocyst	Spherical, 350 μm with 30 μm thick cyst wall	Spherical, 200 μm	—	Oval, 330 μm×350 μm with thick (45 μm) ectocyst
Sporoducts	1–12, each 1200 μm long	4–6, each 70–75 μm long	—	10, each 50–90 μm long
Oocyst	Barrel shape 6 μm×3 μm	Barrel shape, 5.5 μm×3 μm	Doliform, 7.5 μm×3.5 μm	Doliform, 5.0 μm×3.5 μm
Host	*Gryllus abreviatus*; *G. pensilvanicus*	*Homocogryllus japonica* de Haan	*Gryllus bimaculatus* De Geer *Gryllodes sigallatus* (Walter)	*Gryllus bimaculatus* De Geer
Locality	America	Japan	Vietnam	India

Seasonal Intensity and Site of Infection

Infection commences from October, attains maximum during November and comes to an end during December. On an average 30% of the insects dissected are found to be parasitized. The site of infection is mid-gut.

Discussion

The described gregarine is assigned to the genus *Leidyana* Watson on account of its solitary sporadins, simple epimerite, cyst with sporoducts and doliform oocysts.

The epimerite of this species varies from subspherical to papilla-like structure which is not found in any of the previously described species under *Leidyana*. However, it possesses conical epimerite like *L. erratica* (Crawley 1903), and *L. suzumushi* (Hoshide 1973) and infects *Gryllus bimaculatus* like *L. saigonensis* (Corbel 1967). But, these three species differ from the gregarine in question in various features. The comparative list of characters (Table 2) indicates its distinctiveness. Therefore, it is considered a new taxon for which the name *Leidyana bimaculata* sp. n. is proposed. The specific name signifies that of the host *Gryllus bimaculatus* De Geer.

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Hepatozoon mucosus sp. n. from Indian Rat Snakes, *Ptyas mucosus* (Linnaeus)

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Synopsis. Hepatozoon mucosus sp. n. is described from the peripheral blood of rat snakes, *Ptyas mucosus* (Linnaeus) (*Squamata*: *Colubridae*). Gametocytes are intra-erythrocytic, their sex could not however, be conclusively distinguished. Erythrocytic schizonts were not found. Two types of tissue schizonts (macro and microschizonts) were found in liver and lung sections. Mature macromerozoites are also observed. The present species is compared with the allied species and is considered to be a new species described for the first time from the Indian subcontinent.

Ophidian *Hepatozoon* were reported for the first time by Garnham (1950) from an African swamp snake. Since then, many investigators like Hull and Camin (1960), Marquardt (1966), Mkrtchyan (1967), Ball et al. (1967, 1969), Clark and Bradford (1969), Booden et al. (1970), Oda et al. (1971), Pessoa and Biasi (1973) and Sinha (1980, 1981, 1984) encountered Ophidian *Hepatozoon* from various parts of the world.

The present paper records a new species of *Hepatozoon* found in the blood of rat snakes collected from Bongaon, 24-Parganas, West Bengal, India.

Material and Methods

Out of five rat snakes, two were positive for *Hepatozoon* in their peripheral blood. Blood was collected from the facial vein or by clipping the tail of the snakes. Small pieces of tissues (liver, lung, heart, spleen and kidney) were fixed in Bouin's or Carnoy's fixatives and sectioned. No ecto-parasites were found on the snakes. Blood films were stained with Giemsa's or Leishman's stains. Tissue sections were stained in haematoxylin-eosin and Heidenhain's iron-haematoxylin stains. Measurements of parasites were made from fixed and stained materials and Camera lucida drawings were made.

Results

Hepatozoon mucosus sp. n.

Type host: *Ptyas mucosus* (Linnaeus).
Type locality: Bongaon, 24-Parganas, West Bengal, India.
Site of infection: Peripheral blood, liver and lung tissues.

Morphology. In the circulating blood, parasites occurred in the form of intra-erythrocytic gametocytes. Two distinct morphological types were found, depending on their size, shape and staining properties. The sex of these gametocytes could not, however, be conclusively distinguished.

Gametocytes type I. Young individuals (Fig. 1 *1–2*) were oval or broad and measured 5.5 μm–9.2 μm × 1.6 μm–3.8 μm (average 7.2 μm × 2.5 μm). Cytoplasm was homogeneous and stained faintly. Nucleus was central or sub-central, deep pink and the average measurements were 3.3 μm × 2.3 μm. Mature forms (Fig. 1 *3–4*) were encapsulated measuring 11 μm–13.2 μm × 2.7 μm–33 μm (average 12 μm × × 3 μm). Polar caps were seen at both ends: they stained deep blue and fitted over the parasite like a cup. Polar caps were very prominent with a maximum length of 2.6 μm. Cytoplasm was basophilic and the nucleus stained deep pink measuring 3.3 μm × 2.6 μm (average).

Gametocytes type II. Young forms (Fig. 1 *5–6*) were thin and narrow, broad and tapering ends and measured 9.7 μm–11.2 μm × 1.4 μm–1.6 μm (average 10.5 μm × 1.5 μm). Cytoplasm was homogeneous and the nucleus was oval. Mature vermicular forms (Fig. 1 *7–10*) soon developed a capsule. One end of the parasite was sharply bent, forming a hook-like structure (Fig. 1 *9–10*); the other end was broad or tapered slightly. These measured 12 μm–16 μm × 2 μm–2.5 μm (average 14.2 μm × 2.2 μm). Nucleus stained deep pink measuring 4.3 μm × 2.4 μm (average).

Tissue phase. No divisional stages were encountered in peripheral blood smears. Schizogony occurred in the livers and lungs of snakes and was studied in sections.

Trophozoites (Fig. 1 *13–14*) were ovoid to sub-spherical, measuring 6.4 μm–7.7 μm × 4.8 μm–6.5 μm (average 6.8 μm × 5.5 μm). Cytoplasm was homogeneous, non-granular and light pink in colour with eosin stain. Nucleus was large, oval, deep blue measuring 3.3 μm × 1.6 μm (average). A schizont with two nuclei (Fig. 1 *15*) measured 6.8 μm × 5.6 μm. Cytoplasm took up eosin stain and nuclei were deep blue in haematoxylin and blue-black in iron-haematoxylin stains measuring 2.3 μm–2.5 μm × 1.4 μm–1.7 μm. As the development proceeded. two kinds of schizonts formed. Mature macroschizonts (Fig. 1 *21–25*) containing 4–8 merozoites were found in the sections of liver and lung tissues. They measured 10 μm–13 μm × × 8 μm–11 μm (average 11.5 μm × 9 μm). Microschizonts (Fig. 1 *16–20*) in lung tissues were sub-spherical to oval measuring 7.7 μm–11 μm × 6.5 μm–8.8 μm

(average 9.5 μm × 7.2 μm) with thirteen (Fig. 1 *18*), sixteen (Fig. 1 *17*), twenty four (Fig. 1 *19*) and thirty two (Fig. 1 *20*) nuclei. Cytoplasm contained a reticular network of fibres with dark blue nuclei scattered throughout the cytoplasm. Nuclei

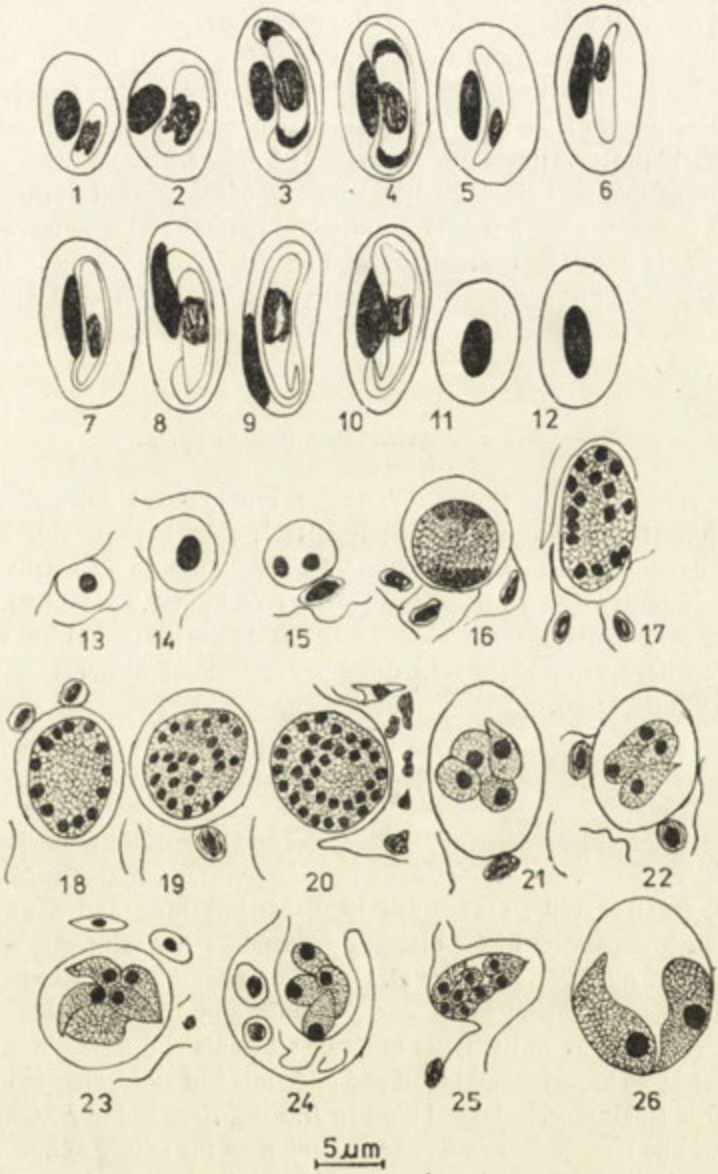

Fig. 1. *1–26.* Camera lucida drawings of *Hepatozoon mucosus* sp. n. *1–4* — Gametocytes type I, *5–10* — Gametocytes type II, *11–12* — Normal erythrocytes, *13–14* — Trophozoites in liver sections, *15* — Binucleate schizont in liver, *16–20* — Microschizonts in lung sections, *21–24* — Immature and mature macromerozoites in lung, *25* — Macromerozoites in liver, *26* — Macromerozoites in lung smear

were arranged around the peripheral margins of the cytoplasm as dense spherical masses about 1.2 μm in diameter. Mature micromerozoites were not seen. Mature macromerozoites (Fig. 1 *21–26*) had a pointed anterior end, and a broadly rounded posterior end, measuring 3.5 μm–12 μm ×1.6 μm–4 μm (average 5.7 μm×2.4 μm). Cytoplasm resembled a loose net-work with a terminal, spherical nucleus measuring 1.2 μm in diameter (average). In one individual (Fig. 1 *23*) four elongate macromerozoites were found lying beside a large residual body. In lung smears (Fig. 1 *26*), two macromerozoites lay on two sides of the periphery of macroschizont and measured 10 μm–12 μm×3.3 μm–4 μm (average 11.5 μm×3.3 μm).

Invasion of this parasite caused hypertrophy of the infected erythrocytes measuring 16.3 μm–20.9 μm×7 μm–12.2 μm while normal erythrocytes were 12 μm–16.5 μm×7.5–11 μm. The nucleus of the infected erythrocyte measured 5.2 μm–10 μm×1.6 μm–3.3 μm and normal erythrocyte nucleus measured 3.3 μm–6.6 μm × 5.2 μm–10 μm. The host cell nucleus was displaced, but not seen in a fragmented condition.

Diagnosis of *Hepatozoon mucosus* sp. n.

Gametocytes exhibiting two morphological types; type I is broad or oval measuring 5.5 μm–11 μm×1.6 μm–3.8 μm; type II is thin, narrow with a broad and tapering ends and measures 9.7 μm–16 μm×1.4 μm–2.5 μm. In tissues (liver and lung) parasite is found to develop as trophozoites (6.4 μm–7.7 μm×4.8 μm–6.5 μm), and schizonts (macroschizonts measuring 10 μm–13 μm×8 μm–11 μm with mature macromerozoites 3.5 μm–12 μm×1.6 μm–4 μm and microschizonts measure 7.7 μm–11 μm×6.5 μm–8.8 μm).

Discussion

Occurrence of schizogony in cells of the liver and lung of the snake host identifies the parasite as a member of the genus *Hepatozoon* (fam. *Hepatozoidae*, *Adeleina*, *Sporozoea*) (Miller 1908, Garnham 1950, Mohammed and Mansour 1959, Bray 1964). The parasite under discussion resembles *Hepatozoon minchini* Garnham (1950), *H. rarefaciens* Ball et al. (1967) and *H. fusifex* Ball et al. (1969) in the general pattern of schizogony in tissues of the snake, but differs in other morphological features as well as in the types of tissue schizonts and merozoites. In *H. minchini*, gametocytes in peripheral blood and schizonts and merozoites in lung sections exhibit only a single morphological type. In *H. rarefaciens* and in *H. fusifex* gametocytes are of one type and three types (depending on the nature of infected erythrocytes) respectively. Ball et al. (1969) considered that more then one species of haemogregarine occurred in *Boa constrictor*. A comparative study of these species of *Hepatozoon* with the present parasite is given in Table 1, which reveals that the present parasite does not completely resemble any other known species of the genus,

Table 1

Comparative study of *Hepatozoon minchini* Garnham, 1950, *Hepatozoon rarefaciens* Ball et al. 1967, *Hapatozoon fusifex* Ball et al. 1969 and the present parasite, *Hepatozoon mucosus* sp. n. Measurements (range) in micrometers (μm)

H. minchini	*H. rarefaciens*	*H. fusifex*	*H. mucosus* sp. n.
Gametocytes (Intra-erythrocytic)			
One type	One type	Three types as per description of gametocytes	Two types
13–14×3–4	11–18×2.5–9	9.2–17.3×2.3–8.1 15–23×4.6–11.5 15–18.4×4.6–9.2	5.5–13.2×3.3–9.2 9.7–16×1.4–2.5
Sexual dimorphism:			
Monomorphic	Monomorphic	Trimorphic, sexes were not stated	Dimporphic, sexes could not be conclu sively distinguished
Development in vertebrate host:			
In lung tissue	In liver, lung, spleen, pancreas and heart tissues	In lung, liver, spleen, brain, heart and kidney tissues	In liver and lung tissues
Type of tissue schizonts:			
One type of schizont with merozoites	Two types, macro and microschizonts with merozoites	Two types, macro- and microschizonts with merozoites	Two types, macro- and microschizonts with only mature macromerozoites
Vector:			
Unknown	*Culex tarsalis, Anopheles albimanus, Aedes sirrensis*	*Culex tarsalis, Aedes togoi, Amblyomma dissimile*	Unknown
Site of infection:			
Peripheral blood	Peripheral blood	Peripheral blood	Peripheral blood
Vertebrate host:			
Crotaphopeltis degeni	*Drymarchon corais, Constrictor constrictor*	*Boa constrictor*	*Ptyas mucosus*
Locality:			
Kavirondo Gulf of Lake Victoria, East Africa	Colima and Tepic, Mexico, North America	Colima, Mexico, North America	Bongaon, 24-Parganas, West Bengal, India

and it is therefore considered as a new species. The specific name of the parasite is given after the name of the species host. The holotype and paratypes will be deposited in the National Zoological Collection of Zoological Survey of India, Calcutta.

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A New Myxosporidan *Myxidium sciaenae* sp. n. (*Myxozoa* : *Myxididae*) from the Gall Bladder of a Marine Teleost of West Bengal, India

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Synopsis. A new myxosporidan *Myxidium sciaenae* sp. n. (*Myxozoa*: *Myxididae*) has been described from the gall bladder of a marine teleost *Sciaena bleekeri* Day, caught from the Digha coast of Bay of Bengal, West Bengal, India. The characteristic mensural data of the myxosporidan are as follows: spore — 19.36 μm × 5.32 μm, polar capsule — 5.26 μm × 2.5 μm. It is the eighth myxosporidan species reported from the coastal water of Bay of Bengal, West Bengal, India.

During the investigation about the parasitic protozoa of the marine fishes of the coastal water of Bay of Bengal, West Bengal, India, a new myxosporidan parasite has been recovered from the gall bladder of a teleost *Sciaena bleekeri* Day. It has been described as a new species for some of its distinctive characters given later.

Material and Methods

All autopsies were performed on frozen fish collected from Digha coast of Bay of Bengal, West Bengal, India. The parasite has been studied in fresh treated with Lugol's iodine solution and from dry smears stained with Giemsa after fixation in absolute Methanol. The polar filament could not be extruded even after repeated attempts with various concentrations of KOH and Urea solutions have been made. The measurements are given in micrometers (μm). The Figures have been drawn with a camera lucida (Prism type).

Observations

Myxidium sciaenae sp. n.

Description: No trophozoite or other vegetative stages were found. The spores were coelozoic, fusiform with rounded ends in valvular view (Fig. 1 *A*) while in sutural view the spores appeared more fusiform (Fig. 1 *B*) than the spores in

valvular view. The shell valves were two — each with five to six longitudinal striations arranged almost parallel to each other. The suture was distinct and finely curved — nearly S-shaped. The polar capsules were two — one on either end of the spore. These polar capsules were small, pyriform to elongately pyriform with six to seven coils of polar filament. In a few spores, one side appeared more convex

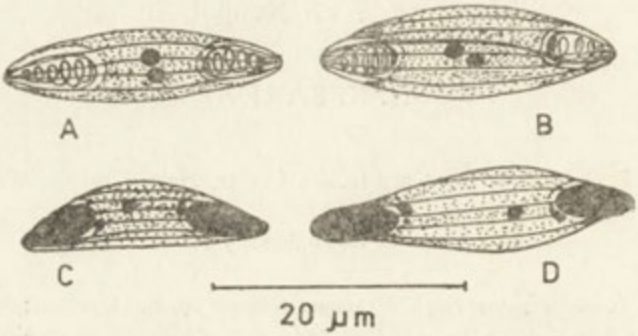

Fig. 1. *A–D.* Spores of *Myxidium sciaenae* sp. n. *A* — A spore in valvular view — Lugol's iodine treatment, *B* — A spore in sutural view — Lugol's iodine treatment, *C* — A spore having convex upper surface — Giemsa stained, *D* — A spore having its breadth shifted — Giemsa stained

than the other side (Fig. 1 *C*) and in a few others, the maximum width of the spore was shifted from the middle of the extracapsular region to the capsular region (Fig. 1 *D*). The extracapsular biconcave cavity was filled with granular sporoplasm containing two round nuclei. No iodinophilous vacuole was detected in the sporoplasm.

Measurements: The mensural data of 25 fresh spores and 44 polar capsules with sample standard deviation are given.

Length of the spore — 19.36±0.47
Breadth of the spore — 5.32±0.28
Length of the polar capsule — 5.26±0.13
Breadth of the polar capsule — 2.50±0.12
Infection locus: Gall bladder
Indidence: Two infected out of 15 examined
Pathogenicity: Not apparent
Host: *Sciaena bleekeri* Day
Locality: Digha coast of Bay of Bengal, West Bengal, India
Date of collection: 2 January, 1985

Discussion

Jayasri and Hoffman (1982) reviewed the genus *Myxidium* Butschli, 1882 and listed 116 species. In mensural data the present myxosporidan resembles closely with *Myxidium lentiformе* Fujita, 1927 reported from the kidney of *Anguila japo-*

nica and *M. melanocetum* Noble, 1966 reported from the gall bladder of *Melanocetus johnsoni*. The myxosporidan in study differs from the former species in having longitudinally striated shell valves (shell valves thin-walled and smooth in *M. lentiforme*). It also differs from *M. melanocetum* by lesser number of longitudinal striation in shell valves (shell valves with 8–9 longitudinal striations in *M. melanocetum*), distinct suture (indistinct in *M. melanocetum*) and smaller polar capsule (6.7 μm long polar capsule in *M. melanocetum*). Moreover, in general shape of the spore, the present parasite also resembles *M. bajacalifornium* Noble, 1966 reported from the gall bladder of *Bajacalifornia burragei*, but differs from the latter by its distinct mensural data (the mensural data of *M. bajacalifornium* are: spore — 22.1 μm × 4.0 μm, polar capsule — 7.4 μm long with 12–14 coils of polar filament). The present myxosporidan is, therefore, considered to be a new species and the name *Myxidium sciaenae* sp. n. is given after the name of its host.

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