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Dear Colleague:

Beginning with this issue we are pleased to present our journal Acta Protozoologica in a new form: bigger, with a more convenient format, with better paper, so that publication of photographs occurs with the text, as well as a shorter time for the publication of articles, which will be, we hope, our regular performance.

We want to establish the position of Acta Protozoologica as an international journal and, moreover, to join in the mainstream of European and World cooperation in developing science. We hope to accomplish these goals through a broader composition of the Editorial Board, to which we invite specialists, working in different fields of protistology in many countries. We are deeply appreciative to those colleagues, who agree to assist members of the Board, to aid editors, and take responsibility for the high scientific level of our journal. We are mainly indebted to them for taking on the heavy responsibility of critical reviews of works sent for publication in Acta Protozoologica. We thank all past members of the Board, and first of all Professor Stanisław Dryl, Editor in Chief of Acta Protozoologica for many years, for the hard work performed previously, in what were sometimes difficult periods.

We will remain, as in the past, a journal publishing original works from all fields of protistology, except papers of restricted interest or confined to limited subjects. At the same time, we also wish, as before, to be specialized in our traditional topics – morphology and morphogenesis – and experimental disciplines – physiology of movement and excitability of cells.

We will, as before, publish Acta Protozoologica as a multilanguage journal, printed in English, French and German. For technical and economical reasons we are not able to publish in Russian. We hope, however, that in the future we will publish many valuable papers from that language area.

We now have the possibility of freely modifying our journal both concerning the contents and also in form. In our opinion, the time has come for the realization of previous publishing plans, including, besides original works, also review articles and short reports. Self invited or solicited review articles sent by authors should aim to be a recapitulation of a definite stage of investigation

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as would be published to some extent in increasing authoritative journals. Short reports should intend to present information about recent results of investigation. If the short reports are well prepared and do not need author's corrections, they could be published rather quickly – between a few weeks up to three months from the time of accepting the work to its publication.

We are introducing new procedures for receiving Acta Protozoologica. Now it will be possible to order our journal directly from the Nencki Institute of Experimental Biology. For individual scientists, members of protozoological or protistological societies, we predict a low price. At the same time, one can order our journal through existing agencies as in the past.

We hope that the journal thus improved will increase the numbers of those interested, as well as among authors and current readers, and will serve still better the science.

Editors

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ACTA
PROTOZOOLOGICA

Physical Factors Effecting the Excystment of the Heterotrichous Ciliate *Fabrea salina* (Henneguy)

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Summary. Cysts of the heterotrich ciliate *Fabrea salina* were subjected to a pH range of 4–11, salinity variations of 0–66‰, and temperature changes from 16 to 44°C to determine the effect on the percentage of excystment. Two barley kernels were added to all trails to maintain a bacterial food source. One to 2 month–old cysts were used. Empty cysts were counted after 48 h as an indication of excystment. Excystment was highest at pH 7.0–9.0, 26–52‰ salinity, and 28, 34 – 38°C. Conclusions were supported by subjecting the data to statistical analysis using ANOVA. Excystment did not occur without bacteria or in the control medium.

Key words. *Fabrea salina*, excystment, physical factors.

INTRODUCTION

Fabrea salina Henneguy was first described by Stepanow (1885, 1886) and later named by Henneguy (1890), who also first described the cysts. Found in salt marshes and isolated puddles around intertidal zones (Kirby 1934, Ellis 1937, Fenchel 1968), the ciliate derives nourishment from bacteria and algae (Repak 1983, Repak 1986). The ciliate is a major food source for zooplankton and fish larvae (Fenchel 1968, Kahan 1975).

Excystment of various ciliates has been well studied (e.g. Garnjobst 1928, Strickland and Haagen–Smit 1947, van Wagtendonk 1955, Jefferies 1959, Butzel and Horowitz 1965, Beers 1966). Investigations of physical factors affecting excystment have been undertaken with other ciliates e.g. *Vorticella microstoma* (Finley and Lewis 1960) and *Blepharisma stoltei* (Repak 1968). A study of the temperature and biochemical aspects of excystment in *F. salina* was accomplished by Demark–Gervais and Genermont (1971). However, the details of the effects of temperature, pH, and salinity on excystment in this marine ciliate have not been revealed. The purpose of this study is to investigate the effect, if any, that these physical factors have on excystment in *F. salina*.

MATERIALS AND METHODS

Stock solutions consisted of 200 ml millipore sterilized, charcoal filtered sea water (CHSW) obtained from Masquamequet, RI. Initially, newly made sterile CHSW was used with little to no excystment in solutions with bacteria. After the solution was aged for 1 month excystment occurred.

The cultures were contained within finger bowls at pH 8 and a salinity of 32‰. Ten barley kernels were added to provide an infusion food source. The cultures were incubated at 26°C in the dark. Encystment usually occurred within 2–3 weeks.

Cysts were washed three times with sterile CHSW before use. Ten mililiters of CHSW was added to sterile Falcon Multiwell wells. Approximately 100–200 cysts were then introduced. Two barley kernels

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were added to each well to provide infusion as the food source. Trials were repeated twice in triplicate for 24 and 48 h at 37°C (Thirty seven degrees was chosen because of the studies of Demar–Gervais and Genermont (1971)). To avoid outside contamination aseptic technique was stringently adhered to. Each factor was studied under the condition specified for 24 and 48 h. The temperature effects were tested at 48 h only. Empty cysts were counted, averaged, and statistically analyzed. Trophozoites were not counted because their numbers would have been increased by division during the trial period.

For salinity studies, 108 g Sigma sea salt was dissolved in distilled water, filtered and adjusted to pH 8.0. The final salinity was 66‰ (The original *Fabrea* culture obtained from Andrzej Kubalski arrived in a culture at this salinity level). With the exception of a salinity of 93‰, the starter solution (66‰) was diluted by 25 % in series with glass–distilled water and millipore filtered (See Table 2).

Studies involving temperatures of 16 to 27°C were undertaken in a low temperature incubator. Temperatures from 28–44°C were studied using a high temperature bacteriological incubator. In either case sterile CHSW at pH 8.0, and a salinity of 32‰ was employed. Temperature studies were conducted at two degree increments in triplicate. Results were read after 48 h. The controls were the unaltered CHSW minus the barley kernels. Control samples were spread plated onto sea water agar (SWA) to test for sterility at 37°C, 24 h (Repak 1983). API strip–20E for gram negative rod fermentors was used to speciate the bacteria in the infusions.

In studies involving pH, CHSW was used and adjusted. The pH was tested before the experiment was undertaken, again at 24 and 48 h. A microelectrode was used to determine the pH in each well.

All data was subjected to statistical analysis using ANOVA.

RESULTS AND DISCUSSION

Escherichia coli was found to be the predominate bacterium in the barley fed cultures. After 24 h the starting pH >4 and <11 was shifted to a range from 7.0–7.8. See Table 1. The change in pH is probably caused by the buffering action of the growth of the naturally occurring bacterium with some contributions made from the metabolism of the ciliate. Excystment reached a maximum value of 9% at an initial pH 7.0 (see Table 1). This might have been expected since *Fabrea* cultures thrive at this pH range. Excystment is marginal (0.68%) at an initial pH 5.0 and did not occur at pH 4.0 or 11.0. Statistical analysis showed the results dealing with pH along with time to be significant with $p < 0.01$.

Table 1

Effect of pH Excystment of *Fabrea salina**

Initial pH	Final pH	Total No. Cysts	Number Excysted	% Excystment
4.0	4.0	150	0	0
5.0	7.0	148	6	3.1
6.0	7.1	197	6	3.1
7.0	7.1	200	18	9.0
8.0	7.2	192	13	6.8
9.0	7.4	197	12	6.1
10.0	7.5	199	13	6.5

* Studies were accomplished in cultures at 37°C and salinity of 32‰ and run 48h with barley infusion as a food source. The average % excystment ± one standard deviation per trial = 2.42 ± 1.15.

As seen in Table 2, the greatest percentage of excystment occurred at 25–53‰ salinity. The data for salinity were found also to be significant at 0.99 + confidence level using ANOVA. These results reflect the fact that the ciliate is found in estuarian salt marshes where large salinity changes (Burt and Queen 1957, Hoese 1960) occur daily. The excystment rate declines at salinities below 27‰. No excystment occurred at a salinity of or less than 11‰ while few (2%) excysted at 66‰. In nature, *F. salina* is exposed to changing salinity due to evaporation, rain fall, and the ebb and flow of the intertidal zone (Kirby 1934).

Table 2

Effect of Salinity on Excystment of *Fabrea salina**

Salinity ‰	Total No. Cysts	Number Excysted	% Excystment
93.0	101	0	0
66.0	98	2	2.04
52.8	98	11	11.2
42.2	101	12	11.9
40.0	100	7	7.0
33.7	100	10	10.0
27.0	100	11	11.0
21.6	104	3	2.9
21.0	99	5	5.1
17.3	90	6	6.7
13.8	90	6	6.7
11.0	93	0	0
10.0	100	0	0
5.0	88	0	0
2.5	101	0	0
0	103	0	0

* Studies performed in cultures at 37°C for 48h, initial pH 8 with barley infusion as a food source. Average % excystment ± one standard deviation per trial = 15.2% ± 16.1.

The % excystment peaked at 28°C, and again at 36°C. Here again the results were statistically significant at a level greater than or equal to 0.99. There was no excystment recorded below 18°C or above 40°C (Table 3). The ability of *Fabrea* to excyst at high

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Table 3

Effect of temperature on Excystment of *Fabrea salina**

Temperature °C	Total No. Cysts	Number Excysted	% Excystment
16	150	0	0
18	149	3	2.0
20	148	6	4.1
22	299	17	5.7
23	299	30	10.03
26	300	16	5.3
28	100	46	46.0
30	301	14	4.7
32	199	24	12.1
34	149	33	22.2
36	149	48	32.2
38	301	93	30.9
40	148	8	5.4
42	301	0	0
44	302	0	0

* Studies were performed in cultures at a salinity of 32% and an initial pH 8 with barley infusion as a food source. The average % excystment ± one standard deviation per trial = 15.5% ± 14.2.

temperatures has been previously noted (Demar–Gervais and Genermont 1971). However, 28°C showed a higher percentage in contrast to that reported by Demar–Gervais and Genermont without the addition of trypsin, a suspected excysting agent. The temperature peaks may also reflect the age of the cyst. One month old cysts easily excysted at lower temperatures (18–30°C) than one week old ones. On the other hand, young cysts, which were noted to not fully complete encystment, were able to reverse the process and excyst at 36–40°C. Since excystment did not occur in the controls and the controls were devoid of any bacteria, it is believed that an environment harbouring bacteria is necessary for the excystment of *F. salina*. Demar–Gervais and Genermont (1971) used *Enterobacter aerogenes* in their cultures. Without *E. coli* from the barley kernels in our trials, there was no excystment. Other ciliates also require bacteria to excyst (Beers 1946, Beers 1966, Singh 1965). Might some microbial by-product trigger excystment? Perhaps excystment follows the bacterial modification of a given medium. Barley kernels were not added to initial trials and the initial pH did not change after 48 h. There are no studies to date exploring the bacterial relationship in *F. salina* excystment. Only a suspected inducing agent is indicated thus far (Demar–Gervais and Genermont 1971).

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ACTA
PROTOZOOLOGICA

Die Strassenverkehrseinwirkung auf die im Boden lebenden Testaceenzönosen (*Rhizopoda, Testacea*) in Warschau (Polen)

The Effect of the Road Traffic Pollution on the Communities of Testate Amoebae (*Rhizopoda, Testacea*) in Warsaw (Poland)

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Zusammenfassung. Es wurde eine Analyse der zönologischen Charakteristiken von Gemeinschaftem der Bodentestaceen auf 10 Lokalitätem mit verschiedenen Grad der Strassenverkehrseinwirkung in der städtischen Agglomeration der Hauptstadt Polens (Warschau) durchgeführt. Es wurden Unterschiede zwischen den Testaceenzönosen der beschädigten und relativ unbeschädigten Lokalitäten festgestellt. Diese Unterschiede zeigten in Abundanz, Artenzahl, Artenspektrum, Artendiversitätsindex und Equitabilitätsindex. Die Auswertung auf Grund des Indexes der faunistischen Ähnlichkeit, Cluster- und Ordinationsanalysen bestädigte die Verschiedenheit der Testaceengemeinschaften aus den beschätigten und relativ unbeschädigten Lokalitäten.

Stichwörter. *Rhizopoda*, Strassenverkehreseinwirkung, städtischen Agglomeration.

Summary. The analysis of the coenological characteristics of the testacean communities from soil in 10 localities with different level of the road traffic pollution in the urban agglomeration of Warsaw (Poland) was carried out in September 1989. The differences of these characteristics between damaged and undamaged localities were found. These differences was shown in the abundance, the number of species, the species composition, the index of species diversity, and the index of equitability. These parameters on the domaged localities were lower than on the undamaged ones. Further results evaluated on the basis of Sorensen's index of faunistical similarity, cluster and ordination analyses confirmed the differences of testate amoebae communities on the domaged and undamaged localities. The effect of the road traffic on the testacean communities was therefore negative.

Key words. *Rhizopoda*, rood traffic pollution, urban agglomeration.

EINFÜHRUNG

In den letzten Jahrzehnten werden die negativen antropogenen Auswirkungen im Boden ständig stärker (sauere Regen, Erdölstoffe, schwere Metalle, Pestiziden, usw.). Der Strassenverkehr beeinflusst auch die Böden in nächster Nähe der Strassen, und zwar durch Kumulation schwerer Metalle (z. B. Blei, Kadmium, Zink), durch Einsalzung der Böden durch Reste von Winterstreumitteln, durch Einstaubung und durch Möglichkeit der Durchdringung von Erdölstoffen in die Böden nach den Verkehrsunfällen usw. Die negativen Strassenverkehrseinwirkungen auf den Zooedaphon beobachten schon einige Jahre die Mitarbeiter der Abteilung der Bodenzoologie des Zoologischen Institutes der Polnischen Akademie der Wissens-

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chaften in Warschau. Im September 1989 beschäftigte ich mich mit der einmaligen Untersuchung der Testacenzönosen im Boden auf ihren experimentellen Flächen. Die vorliegende Arbeit bringt die Ergebnisse von den Testaceenzönosen aus diesem mit dem Strassenverkehr beeinträchtigten experimentellen Fläschen.

MATERIAL UND METHODEN

Beschreibung der Lokalitäten

Im September 1989 wurden auf den langfristig studierten Flächen des Zoologischen Institutes der Polnischen Akademie der Wissenschaften in Warschau und ihrer Umgebung 10 Kollektionen von Bodenproben abgenommen. Alle studierten Lokalitäten liegen auf den linken Ufer des Wisla Flusses im Bereich einer dichten Stadtbesiedlung und eines intensiven Strassenverkehrs. Die Kontrollokalität „Chylice" liegt im landwirtschaftlichen Gebiet bei Warschau und ist ohne ausdruckvolle Strassenverkehrseinwirkung. Es geht, mit der Ausnahme der Lokalität „Naturschutzgebiet Bielany" (Laubwald), um die Natur- und Kunstgrasflächen mit dem Bestand vom Bäumen und Sträuchern. Detaillierte geologische, pedologische, klimatische und phytozönologische Charakteristiken für fast alle folgenden Lokalititäten sind in der Arbeit von Nowakowski (1981) angegeben.

Verzechnisen der Lokalitäten (Abb. 1):

1. Warschau, Plac Zbawiciela (Zbawiciela Platz), ein kleiner Platz im Stadtzentrum, grober Sandboden, eine gepflegte Grasfläche in der Mitte einer stark frekventierten Strassenkreuzung, 4. 9. 1989.

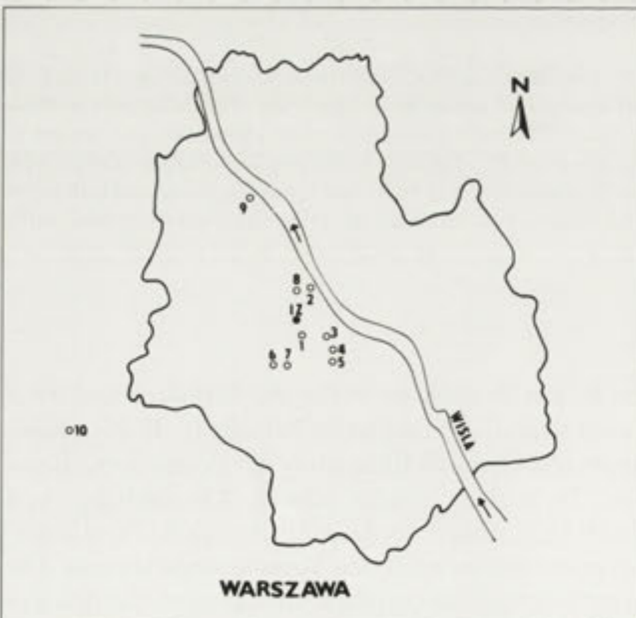

Abb. 1. Situationsschema beobachteter Lokalitäten in Warschau. IZ – Institut für die Zoologie der Polnischen Akademie der Wissenschaften, 1 – Zbawiciela Platz, 2 – Sächsischte Garten, 3 – Ujazdowskie Allee, 4 – Lazienki Park (in nächster Nähe der Strasse), 5 – Lazienki Park (Zentrum), 6 – Grabstätte der sowietischen Soldaten (Zwirki i Wigury Strasse), 7 – Grabstätte der sowietischen Soldaten (Randteil), 8 – Marszalowska Strasse, 9 – Naturschutzgebiet Bielany, 10 – Dorf Chylice.

2. Warschau, Ogród Saski (Sächsische Garten), ein Park im Ausmass von 16 ha, auf der linken Uferterrasse des Wisla Flusses, phytozönologisch Tilio–Carpinetum, sehr feiner Sandboden mit dem Schotter und Ziegelsplitt vermischt, schwer von Blei verunreinigt, eine gepflegte Grasfläche mit dem Bestand von Sträuchern und Linden, etwa 50 m von einer stark frekventierten Strasse entfernt, 5. 9. 1989.
3. Warschau, Aleje Ujazdowskie (Ujazdowskie Allee), eine sehr stark frekventierte Strasse im Stadtzentrum, sandtoniger bis toniger Boden, ein gepflegter Grasstreifen bei der Strasse unter einer Lindenallee, 5. 9. 1989.
4. Warschau, Lazienki Park, ein Park im Ausmass von 86 ha, auf der linken Uferterrasse des Wisla Flusses, phytozönologisch Arrhenatherion, toniger bis sandtoniger Boden, eine gepflegte Grasfläche mit den Sträuchern und niedrigen Koniferen, etwa 400 m von der nähsten Strasse, 5. 9. 1989.
5. Warschau, Lazienki Park, wie die Lokalität Nummer 4, ein regelmässig gemähter Rasen im Zentrum des Parks mit dem Bestand von niedrigen Koniferen und Rhododendronen, ohne direkte Strassenverkehrseinwirkung, 5. 9. 1989.
6. Warschau, Cmentarz Żołnierzy Radzieckich (Grabstätte der sowietischen Soldaten), Żwirki i Wigury Strasse, eine Strasse am Rande des Parks, feiner bis sehr feiner Sandboden, phytozönologisch Cynosurion, ein gepflegter Grasstreifen in der Mitte einer sehr frekventierten Strasse, 6. 9. 1989.
7. Warschau, Cmentarz Żołnierzy Radzieckich (Grabstätte der sowietischen Soldaten), Rand eines Parks, feiner Sandboden, ein gepflegter Rasen, etwa 20 m von Żwirki i Wigury Strasse, 6. 9. 1989.
8. Warschau, Marszałkowska Strasse, eine sehr frekventierte Strasse im Stadtzentrum, mittelgrober bis feiner Sandboden, ein gepflegter Grasstreifen in der Mitte der Strasse, 5. 9. 1989.
9. Warschau, Rezerwat Bielany (Naturschutzgebiet Bielany), ein Mischwald am Rande der Stadt im Ausmass von 130 ha, auf der linken Uferterrasse des Wisla Flusses, phytozönologisch Tilio – Carpinetum, ein Sandboden, der Boden mit dem Laubabfall aus dem Zentrum des Waldes, ohne direkte Strassenverkehrseinwirkung, 7. 9. 1989.
10. Chylice, kleines Dorf etwa 40 km südwestlich von Warschau, eine geweidete Wiese, phytozönologisch Arrhenatherion medioeuropeum, brauner Wiesenboden mild vergleyt, Wiesenboden aus der Mitte der Wiese, 11. 9. 1989.

Probenabnahmenmethode

Die Bodenproben wurden mit einer Bodensonde (1 cm^2 Arbeitsfläche) bis 5 cm Tiefe abgenommen. Auf jeder Lokalität wurden 10 Bodenstickproblem abgenommen (in 2 Reihen je 5 Proben, die Enfernung der Proben in einer Reihe war 1 m, die Entfernung zwischen den Reihen war 5 m). Die abgenommen Proben wurden in Polyethylenbeuteln aufbewahrt und bis zur Bearbeitung wurden diese Beutel mit den Bodenproben im Kühlschrank bei 4°C deponiert.

Die Isolation der Testaceen aus den Bodenproben wurde mit der Schwemmenmethode durchgeführt (Foissner et al. 1982).

Bearbeitung der Ergebnisse

Auf Grund der Angaben aus den Analysen der Bodenproben wurden die gesamte Abundanz der Testaceenzönosen und die Abundanz einzelner Testaceenarten (in ex. cm^{-2} bis 5 cm Tiefe) und Dominanz berechnet. Weiter wurden die Werte des Indexes der Artendiversität (Shanon und Weawer 1963) und Equitabilität

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(Sheldon 1969) berechnet. Die Werte der faunistischen Ähnlichkeit der Testaceenzönosen wurden nach Sorensen (1948) kalkuliert. Die zönotische Verwandschaft der Testaceenzönosen von einzelnen Flächen wurde auf Grund der Cluster- und Ordinationsanalysen (Transformation der Angaben log (x + 1)) bewertet (Computerprogramm CANOCO nach Ter Braak 1987).

ERGEBNISSE

Insgesamt wurden in allen untersuchten Lokalitäten 42 Arten, Varietäten und Formen von Testaceen aus 9 Familien gefunden (Tab. 1).

Die Lokalitäten mit der grossen anthropogenen Belastung (Lokalitäten Nr 1, 2, 3, 6, 7, 8) wurden durch eine kleine Anzahl der Testaceenarten (6-9 Arten) und durch eine niedriege Gesamtabundanz (3 600–13 800 ex. cm^{-2}) charakterisiert. In den Testaceengemeinschaften hatten die „Besiedler der beschädigten Biotopen" (d. h. kleine euryvalente Arten mit kosmopolitischer Verbreitung aus den Familien Centropyxidae, Cryptodifflugiidae, Euglyphidae und Trinematidae) eine ausdruckvolle Überlegenheit. Die Werte des Artendiversitätsindexes waren sehr niedrig (unter 2,0) und die Werte des Equitabilitätsindexes bewegten sich in einer relativ breiten Spannweite (0,72–0,95).

Auf den Lokalitäten ohne ausdruckvolle anthropogene Belastung (Lokalitäten Nr 4, 5, 9, 10) wurden die Testaceengemeinschaften durch eine höhere Artenzahl (13-28 Arten) und durch eine höhere Abundanz (13 800-55 200 ex. cm^{-2}) charakterisiert. Auch in diesen Testaceengemeinschaften hatten euryvalente Arten einen ausdruckvollen Anteil, es wurden jedoch hier auch die Bodenarten und die Arten aus ausgetrockneten Moosen vertreten. Die Werte des Artendiversitätsindexes waren hier höher als auf den Lokalitäten mit grosser anthropogener Belastung (2,1-2,5). Die Werte des Equitabilitätsindexes bewegten sich in einer engeren Spannweite als auf den gestörten Lokalitäten (0,72-0,82).

Auf allen Lokalitäten waren die Verträter der Familien *Cryptodifflugiidae* (*Difflugiella oviformis* und *D. oviformis* var. *fusca*), *Euglyphidae* (*Euglypha laevis* und *E. rotunda*), *Phryganellidae* (*Phryganella acropodia*), *Plagiopyxidae* (*Plagiopyxis callida*), *Trigonopyxidae* (*Trigonopyxis arcula*) und *Trinematidae* (*Corythion dubium*, *C. dubium* var. *terricola*, *Trinema enchelys*, *T. lineare* und *T. lineare* var. minuscula) eudominant (Tab. 2).

Die Zusammensetzung der Testaceengemeinschaften wurde wahrscheinlich auch durch die Bodentextur und die Bodenstruktur beeinflusst. Es handelte sich hier überwiegend um mittelfeine und feine Sandböden mit schwankendem Wasserregime. Deswegen wurden die Testaceengemeinschaften auf den relativ anthropogen ungestörten Lokalitäten überwiegend aus euryvalenten Testaceenarten zusammengesetzt.

Tabelle 1

Artenverzeichnis der festgestellten Testaceenarten, Varietäten und Formen auf studierten Lokalitäten.
(1-10 – Lokalitätsnummern)

Art
Arcella catinus Penard, 1890 – 9
Arcella vulgaris Ehrenberg 1832 – 2, 9
Assulina muscorum Greeff, 1888 – 4, 5, 9, 10
Assulina seminulum (Ehrenberg) Leidy, – 1879 – 5
Bulinullaria indica (Penard) Deflandre, 1953 – 2
Centropyxis aerophila Deflandre, 1929 – 4, 5, 6, 7,
Centropyxis aerophila var. *sphagnicola* Deflandre, 1929 – 4, 5
Centropyxis aerophila var. *sylvatica* Deflandre, 1929 – 3, 7, 10
Centropyxis cassis (Wallich) Deflandre, 1929 – 5, 10
Centropyxis constricta (Ehrenberg) Penard, 1902 – 10
Centropyxis minuta Deflandre, 1929 – 7
Corythion delamarei Bonnet et Thomas, 1960 – 5, 6, 10
Corythion dubim Taránek, 1881 – 5, 8, 9
Cyrythion dubim var. *aerophila* Decloitre, 1950 – 9
Corythion dubim var. *minima* Chardez, 1969 – 6
Corythion dubim var. *terricola* Schönborn, 1969 – 9
Corythion pulchellum Penard, 1890 – 4, 5, 9, 10
Cyclopyxis eurystoma Deflandre, 1929 – 5, 9, 10
Cyclopyxis eurystoma var. *parvula* Bonnet et Thomas, 1960 – 5
Cyclopyxis kahli Deflandre, 1929 – 5
Difflugiella oviformis (Penard) Bounet et Thomas, 1955 – 5, 8, 10
Difflugiella oviformis var. *fusca* (Penard) Bonnet et Thomas, 1955 – 1, 3, 5
Euglypha compressa var. *glabra* Wailes, 1915 – 5, 9
Euglypha laevis (Ehrenberg) Perty, 1849 – 3, 4, 5, 6, 7, 8, 9, 10
Euglypha polylepis Bonnet et Thomas, 1960 – 4, 5, 9, 10
Euglypha rotunda Wailes et Penard, 1911 – 1, 3, 4, 5, 6, 7, 8, 9, 10
Euglypha tuberculata Dujardin, 1841 – 4, 5, 9, 10
Heleopera picta Leidy, 1879 – 1, 9
Nebela dentistoma Penard, 1890 – 5
Phryganella acropodia (Hertwig et Lesser) Hopkinson, 1909 – 1, 2, 3, 5, 6, 8, 9
Plagiopyxis callida Penard, 1910 – 2, 5, 6, 9
Plagiopyxis declivis Thomas, 1955 – 1, 4, 5, 6, 7, 9
Plagiopyxis panardi Thomas, 1955 – 4, 5, 10
Sphenoderia quadritesta Štěpánek, 1967 –
Trigonopyxis arcula Penard, 1912 – 1, 2, 8
Trinema complanatum Penard, 1890 – 4, 5, 9, 10
Trinema complanatum var. *elongata* Decloitre, 1973 – 10
Trinema enchelys (Ehrenberg) Leidy, 1878 – 1, 4, 5, 6, 7, 8, 9, 10
Trinema enchelys var. *nasurticola* Decloitre, 1962 – 5
Trinema lineare Penard, 1890 – 1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Trinema lineare var. *minuscula* Chardez, 1971 – 8, 9
Trinema lineare var. *truncatum* Chardez, 1964 – 7, 10

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Tabelle 2

Elementare zönologische Charakteristiken studierter Testaceengemeinschaften auf 10 Lokalitäten. AZ – Artenzahl, GA – gesamte Abundanz (in ex. cm^{-2} in 5 cm Tiefe), H' – Artendiversitätsindex nach Shannon und Weawer, E – Equitabilitätsindex nach Sheldon, EA – eudominante Arten, AEA – Abundanz der eudominanten Arten, DEA – Dominantz der eudominanten Arten.

Nr. Lokalität	AZ	GA	H'	E	EA	AEA	DEA
1	8	6 300	1,565	0,753	*T. lineare*	3 000	47,9
					D. oviformis v. fusca	1 500	23,8
2	6	4 200	1,574	0,879	*Ph. acropodia*	1 800	42,9
					Pl. callida	600	14,3
					Trig. arcula	600	14,3
					T. lineare	600	14,3
3	6	3 600	1,705	0,951	*T. lineare*	900	25,0
					E. laevis	600	16,7
					E. rotunda	600	16,7
4	13	13 800	2,121	0,827	*T. lineare*	4 500	32,6
					T. enchelys	2 400	17,4
5	28	55 200	2,421	0,727	*T. lineare*	16 800	30,4
					T. enchelys	11 400	20,6
6	10	4 500	1,206	0,958	*T. lineare*	900	20,0
					T. laevis	600	13,3
					Ph. acropodia	600	13,3
					T. enchelys	600	13,3
7	9	13 200	1,652	0,752	*T. enchelys*	6 000	43,4
					E. rotunda	2 400	17,4
					T. lineare	2 400	17,4
8	9	6 600	1,937	0,882	*T. lineare*	1 800	27,3
					T. lineare v. minuscula	1 500	22,7
					D. oviformis	900	13,6
					T. enchelys	900	13,6
9	21	40 500	2,521	0,828	*T. lineare*	10 500	25,9
					T. dubium v. terricola	6 600	16,3
					C. dubium	5 700	14,1
10	18	19 200	2,337	0,808	*T. enchelys*	4 200	21,9
					T. lineare	4 200	21,9
					E. rotunda	2 400	12,5
					E. laevis	2 100	10,9

Auf Abbildung 2 ist graphisch die Abudnanz einzelner Arten auf studierten Lokalitäten demonstriert. Aus dieser Abbildung geht hervor, dass nur 11 Arten aus gesamten 42 Arten die höchste Abundanz haben. Nur bei der Art *Trinema lineare* konnten höhere Abundanzwerte auf allen Lokalitäten festgestellt werden. Alle Arten mit grosser Abundanz gehören zu den „Besiedlern der beschädigten Biotopen".

Zur Erklärung der faunistischen Identität der Testaceengemeinschaften aus einzelnen Lokalitäten wurde der Index der faunistischen Ähnlichkeit nach Sorensen angewandt (Abb. 3). Die faunistisch ähnlichsten sind die Testaceengemeinschaften aus den Lokalitäten Nr 1 und 6 (66,66 %), 5 und 9 (65,31 %), 4 und 10 (64,52%),4 und 5 (63,41%), 6 und 7 (63,16%) und 5 und 10 (60,87%). Die geringste faunistische Identität wurde unter den Testaceengemeinschaften der Lokalitäten Nr 2 und 10 (8,33%), 2 und 4 (10,53%), 2 und 7 (13,33%), 2 und 5 (17,67%) festgestellt. Dieser Vergleich drückt die Unterschiede in der Zusammenstzung

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von Testaceengemeinschaften auf durch den Strassenverkehr gestörten und ungestörten Lokalitäten deutlich aus.

Die Clusteranalyse (Abb. 4) teilte 10 studierte Testaceenzönosen in zwei Gruppen schon im ersten Niveau der dichotomischen Zweiglinie. Die erste Gruppe ist aus den Testaceengemeinschaften aus den durch Strassenverkehr gestörten Lokalitäten (Nr 1, 2, 3, 6, 7, 8) zusammengestellt. Weitere Teilung dieser Gruppe unterschied noch zwei Untergruppen. Die erste Untergruppe ist aus den Testaceengemeinschaften aus den Lokalitäten Nr 1 und 3 gebildet, was die meistbeschädigten Lokalitäten sind. Die zweite Untergruppe (Lokalitäten Nr 6 und 7) umfasst die Testaceengemeinschaften aus einem spezifischen Ort (Grabstätte der sowietischen Soldaten). Hier ist nur ein kleiner Unterschied zwischen dem Teil in nächster Nähe der Strasse (Lokalität Nr 6) und im Zentrum des Parks (Lokalitäten Nr 7). Die zweite Gruppe der Testaceengemeinschaften (Lokalitäten Nr. 4, 5, 9, 10) ist aus den Gemeinschaften aus den Lokalitäten ohne ausdruckvolle Strassenverkehrseinwirkung zusammengestellt. Innerhalb dieser Gruppe ist eine Untergruppe (Lokalitäten Nr 4 und 10) aus typischen Wiesenlokalitäten. Von dieser Untergruppe sind zwei weitere Testaceengemeinschaften deutlich zu erkennen. Lokalität Nr 5 ist praktisch ein uneingeschalteter Kulturmischwald mit zusammenhängendem Grasbestand und Lokalität Nr 9 ist ein natürlicher Laubmischwald ohne zusammenhängenden Grasbstand.

Bei der Anwendung der Ordinationsanalyse wurden die Testaceengemeinschaften der gestörten und ungestörten Lokalitäten sehr deutlich abgesondert.

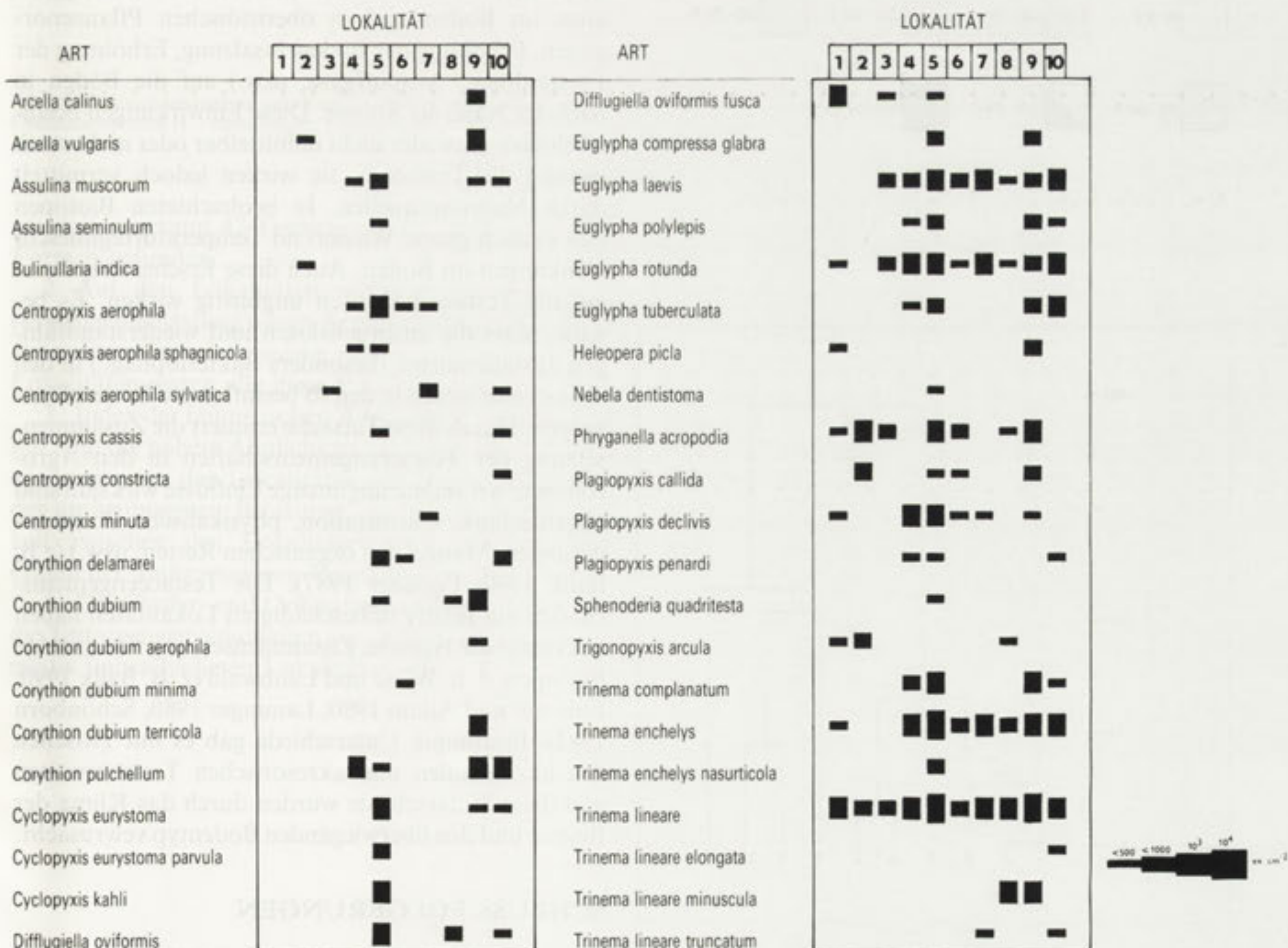

Abb. 2. Abundanzvergleich bei einzelnen gefundenen Testaceenarten auf studierten Lokalitäten (1-10 – Lokalitätsnummern).

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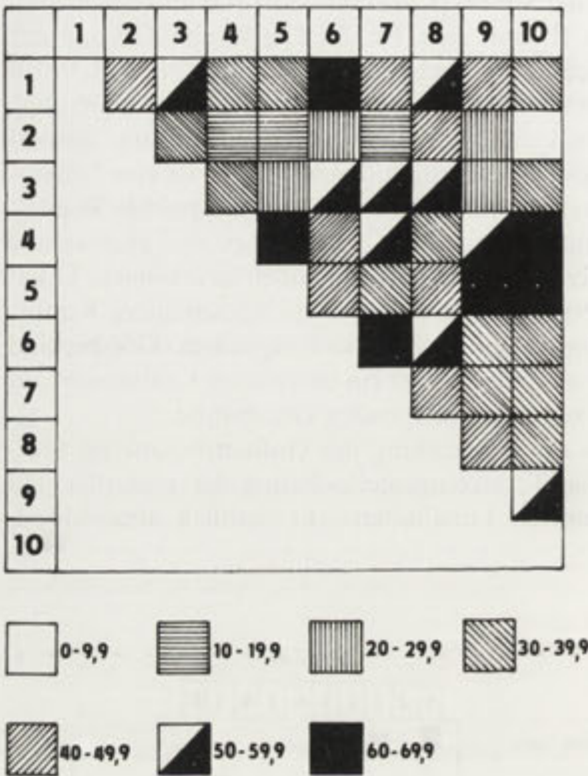

Abb. 3. Index faunistischer Ähnlichkeit nach Sorensen.

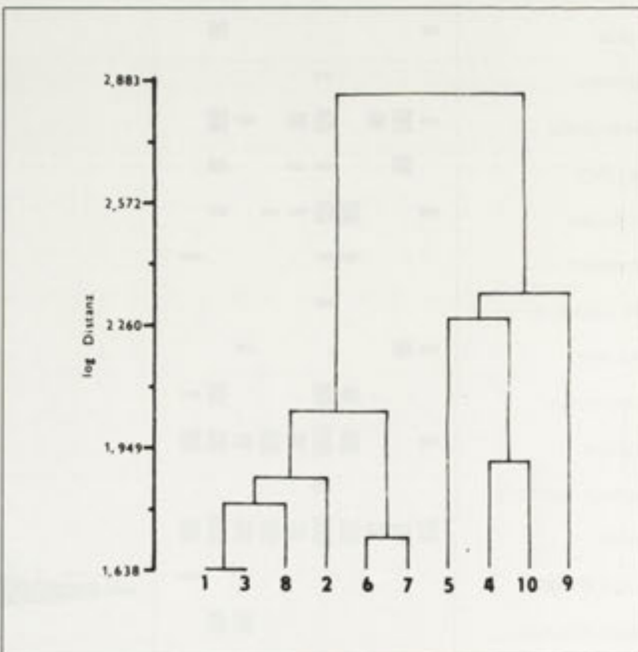

Abb. 4. Clusteranalyse der Testaceenzönosen aus studierten Lokalitäten.

Im dreidimensionalen Ordinationsraum wurden die Testaceengemeinschaften aus gestörten und ungestörten Stellen sehr gut und deutlich abgegrenzt. Die Darstellungen der Testaceengemeinschaften aus ungestörten Lokalitäten belegen einer kleineren Teil des Ordinationsraumes als die Darstellungen der Gemeinschaften aus gestörten Lokalitäten. Diese Erscheinung weisst darauf hin, dass die Testaceengemeinschaften aus den ungestörten Lokalitäten höhere Verwandschaft als die aus den gestörten Stellen haben (Abb. 5).

DISKUSSION

In der Literatur existieren keine Angaben über den Einflüss des Strassenverkehrs auf die Testaceenzönosen aus den Böden. Niedrige Abundanzwerte, niedrige Artenzahl und spezifische Artenstruktur sind wahrscheinlich ein Resultat kombinierter Einwirkungen des Strassenverkehrs (Konzentration von schweren Metallen im Boden und in oberiridischen Pflanzenorganen, Erhöhung der Bodeneinsalzung, Erhöhung der Einstaubung, Auspuffgase, usw.) auf die Böden in nächster Nähe der Strasse. Diese Einwirkungen beeinträchtigen entweder nicht unmittelbar oder nicht stark genung die Testaceen, sie wirken jedoch vermittelt durch Nahrungsquellen. In beobachteten Biotopen gibt es auch grosse Wasser- nd Temperaturregimeschwankungen im Boden. Auch diese Erscheinung kann auf die Testaceenzönosen ungünstig wirken. Es bewirkt, dass die anspruchslosen und wiederstandfähigen Testaceenarten (besonders Bakteriophage) in den Testaceenzönosen in den so beeinflussten Böden überwiegen. Durch diese Tatsache erinnert die Zusammensetzung der Testaceengemeinschaften in den Agrozönosen, wo andere ungünstige Einflüsse wirksam sind (Agrotechnik, Chemisation, physikalische Bodenänderungen, Mangel den organischen Resten, usw.) (z B. Balik 1990; Foissner 1987). Die Testaceengemeinschaften auf relativ unbeschädigten Lokalitäten haben in Prinzip die typische Zusammensetzung für einzelne Biotopen, d. h. Wiese und Laubwald (z. B. Balik 1990; Foissner und Adam 1980; Laminger 1980; Schönborn 1982). Bestimmte Unterschiede gab es nur zwischen den akzidentalen und akzesorischen Testaceenarten und diese Unterschiede wurden durch das Klima der Region und den überwiegenden Bodentyp vewrusacht.

SCHLUSS FOLGERUNGEN

1. Auf 10 Lokalitäten mit unterschiedlichem Grad der Strassenverkehrseinwirkung in Warschau (Polen)

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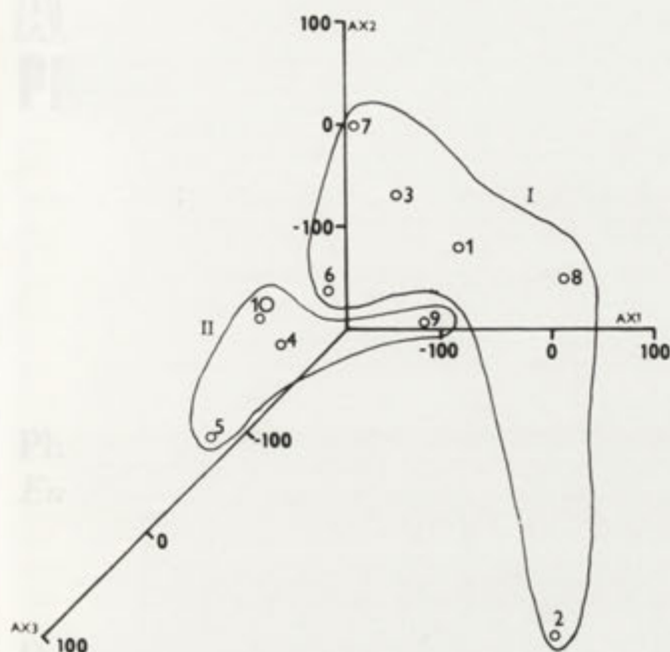

Abb.5. Ordinationsanalyse von Ergebnissen im dreidimensionalen Ordinationsraum (I – beeinflusste Lokalitäten, II – unbeeinflusste Lokalitäten).

wurden insgesamt 42 Testaceenarten, Varietäten und Formen gefunden.

2. Auf den Lokalitäten mit der höheren anthropogenen Belastung waren Artenzahl, Abundanz und Artendiveritätsindex niedrig. Auf den unbeeinflussten Lokalitäten waren alle diese Parameter hören.

3. Index der faunistischen Ähnlichkeit nach Sorensen zeigt die höhere Ähnlichkeit zwischen den Testaceenzönosen aus den Lokalitäten mit ähnlichem Grade der anthropogenen Belastung und niedrige Ähnlichkeit zwischen den Lokalitäten mit verschiedenem Grade der anthropogenen Belastung.

4. Die Cluster- und Ordinationsanalysen sonderten die Testaceengemeinschaften aus den beschädigten und relativ unbeschädigten Lokalitäten ab.

5. Die Strassenverkehrseinwirkung auf die Zönosen der Bodentestaceen ist wahrscheinlich indirekt. Dienegativen Strassenverkehrseinwirkungen (Bleikumulation, Einsalzung, Einstaubung, usw.) wirken auf die Testaceen durch die Nahrungskette.

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Phototactic and Polarotactic Responses of the Photosynthetic Flagellate, *Euglena gracilis*

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Summary. Action spectra of the phototactic orientation have been calculated from fluence rate response curves for green and dark bleached *Euglena gracilis*. Instead of assaying cell populations, the spectra are based on cell tracking of individual flagellates using a fully automatic, real time image analysis system. Both spectra show the activity of a typical blue light receptor. In a horizontal cuvette under polarized irradiation from above the cells orient perpendicular to the E-vector at low fluence rates (6000 lx) and parallel at high fluence rates (30000 lx). The low fluence rate response was also found in monochromatic radiation both at 380 and 460 nm.

Key words. *Euglena gracilis*, phototaxis, polarotaxis, orientation

INTRODUCTION

The unicellular photosynthetic flagellate, *Euglena gracilis*, uses a number of external chemical and physical parameters (Häder 1988, Nultsch and Häder 1988) to orient in its environment. While some motile microorganisms have been found to orient in the water column with the aid of thermal (Mizuno et al. 1984, Poff 1985) and chemical (Berg 1985, Macnab 1985) gradients, the magnetic field of the earth (Frankel 1984, Esquivel and de Barros 1986) and even electrical currents (Mast 1911), *Euglena* mainly orients with respect to light (Diehn et al. 1977, Häder et al. 1981, 1986, Colombetti et al. 1982, Lenci et al. 1983) and gravity (Brinkmann 1968, Häder 1987).

In addition to a weak photokinetic effect (Wolken and Shin 1958) and both step-up and step-down photophobic responses (Shimmen 1981, Doughty and Diehn 1983, 1984), the most important light response is phototaxis in this organism (Mast 1911, Häder et al. 1981), defined as directed movement with respect to the direction of the incident light beam. Negative phototaxis (away from the light source) was found at fluence rates above 12.65 W m^{-2} and was extremly precise above 126.5 W m^{-2}. At low fluence rates (< 1.4 W m^{-2}) a less pronounced positive phototaxis (toward the light source) was observed (Diehn 1973a,b, Colombetti et al. 1982, Häder et al. 1981).

A number of action spectra have been published for the phototactic orientation of *Euglena*; however, some of them have been measured using a so-called phototaxigraph (Bound and Tollin 1967, Diehn 1969) which – despite of its name – measures photoaccumulations of cells in a light beam which can be brought about by photokinesis, phobic responses, preferential settling on the glass wall in light and also phototaxis. All action spectra determined so far are based on mass movement techniques, assaying the behavior of a population

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rather than individual cells (Gössel 1957, Checcucci et al. 1975,1976).

The photoreceptor is thought to be a flavoprotein arranged in a paracrystalline array in the paraflagellar body (PFB), a swelling of the emerging flagellum inside the reservoir (Ghetti et al. 1985, Doughty and Diehn 1980, Benedetti and Checcucci 1975). This is supported by fluorometric studies which have indicated the presence of flavin type chromophores in the PFB (Benedetti and Lenci 1977). Spectroscopic analysis of isolated flagella with the PFB still attached indicated that in addition to flavins one or more pterins are present in the PFB (Galland et al. 1990). This finding was further supported by biochemical analysis of the PFB proteins and their chromophoric groups (Brodhun and Häder 1990). During the last years pterins have been found several times in *Euglena gracilis* (Böhme et al. 1980, Juknac et al. 1988).

According to the shading hypothesis the detection of light direction was explained by a periodic shading of the photoreceptor when a lateral light beam is intercepted by the stigma during rotation. However, several pieces of evidence contradict a periodic shading mechanism in *Euglena* (Häder et al. 1986, Häder 1987). An alternative explanation is that the cells detect the light direction using a dichroic arrangement of their photoreceptor pigments. Diehn (1969) concluded from measurements of the phobic response in polarized light, that the pigments in the shading device are dichroically oriented. Creutz and Diehn (1976) found that *Euglena* moved perpendicular to the polarization plane. The three–dimensional orientation of the absorbing vectors of the photoreceptor pigments within the PFB was illustrated with respect to the cell's axes (Häder 1987). The vector of maximal absorption of the chromophore deviates clockwise 30° off the long axis of the cell. Seen from the front end, the plane of maximal absorption deviates 60° counterclockwise from the flagellar plane. The aims of this paper are to characterize the wavelength dependence of phototaxis on a single cell basis and to analyze the polarotactic reaction of green and dark bleached *Euglena* under monochromatic and white light of different fluence rates.

MATERIALS AND METHODS

Cultures of the unicellular green flagellate *Euglena gracilis* strain Z were grown in 100 ml Erlenmeyer flasks in 40 ml of a medium described previously (Checcucci et al. 1976). The cells were kept in a temperature–controlled room (20° C) under constant white light from Osram L36W/32 fluorescent lamps (600 lx). Dark bleached cells were grown under the same conditions but in flasks wrapped light tight in aluminum foil. Normally the behavior was analyzed with cells in their original medium and at their normal concentration. If it was necessary to concentrate the organisms a technique modified from Kessler (1985) was used: the cells were kept in a test tube with a loose cotton wool plug submerged at the top end. Due to their negative gravitactic orientation the cells moved upwards and through the plug so that motile, responsive organisms concentrated at the top. Too dense cultures were diluted with fresh sterile medium.

The swimming behavior was studied in a glass cuvette (40 x 40 x 0.17 mm^3 inner dimensions) located on the stage of an inverted microscope (Leitz Labovert) with monitoring infrared light produced with a cut–off filter. A real time image analysis system described by Häder and Lebert (1985) was used to track the organisms and analyze their direction of movement. The image of the organisms was recorded by an infrared sensitive video camera (Sony AVC3250), digitized in real time and stored in a RAM (read and write memory) with a resolution of 512 x 256 picture points (pixels) with 64 possible grey levels. A Z80–based microcomputer had access to the video memory and was used for further processing. A program written in ASSEMBLY language for real time execution was capable to find an organism at random, determine its outline (Grant and Reid 1981, Berns and Berns 1982) and calculate its center of gravity (centroid). This process was repeated for a predetermined number of times in consecutive images to follow one organism at a time. The deviation angles from the light direction were calculated and the data stored in a disk file for further statistical analysis (Batschelet 1965, 1981, Mardia 1972). From these raw data histograms were calculated with a resolution of 64 bins using PASCAL programs.

The actinic white light was produced with a 250 W quartz halogen slide projector (Prado, Leitz, Wetzlar, FRG). The beam was collimated with a 200 mm focal length lens. The fluence rate was varied by inserting absorptive neutral density filters (Schott and Gen.) and measured with a thermophile (Kipp and Zonen, CA 1) connected to a microvoltmeter (Keithley, type 155). Monochromatic light was obtained with interference filters with 8 – 14 nm halfband widths (Schott and Gen., Mainz, FRG). Polarized light was produced by using polarizing film filters.

RESULTS

The action spectrum for positive phototactic orientation was measured by determining the tracks of individual cells. It is based on fluence rate response curves at individual wavelengths. In a random orientation 50% of the cells move in the two quadrants towards the light source while in perfect orientation 100% of all cells move within these two quadrants; therefore a medium value of 75% was chosen to calculate the action spectrum (Fig. 1). It has a major maximum at 380 nm and a second at 450 nm. Two additional maxima are found at 420 and 495 nm. In order to determine the role of the chloroplast pigments in photoperception or shading, the action spectrum was also measured for dark bleached cells (Fig. 2). It is similar to the one for green *Euglena* cells, but there is a striking difference: dark grown cells need lower fluence rates for the same response in the visible part of

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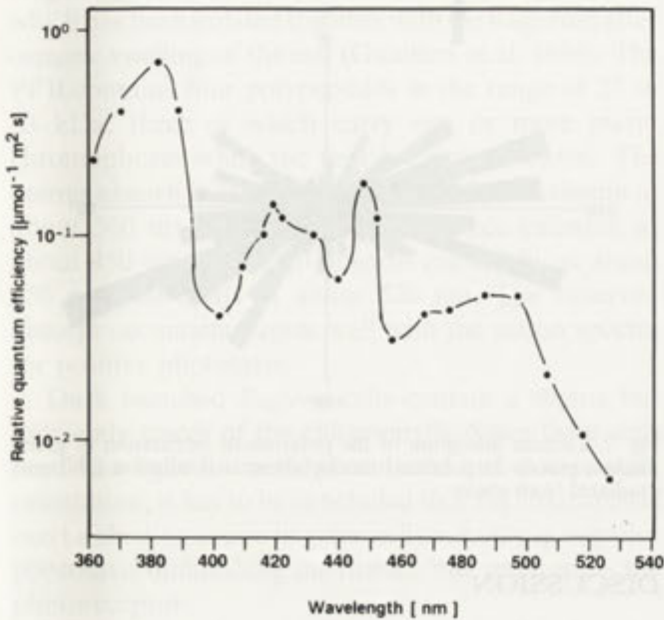

Fig. 1. Action spectrum of phototactic orientation in green *Euglena gracilis* based on fluence rate response curves measured in individual cells

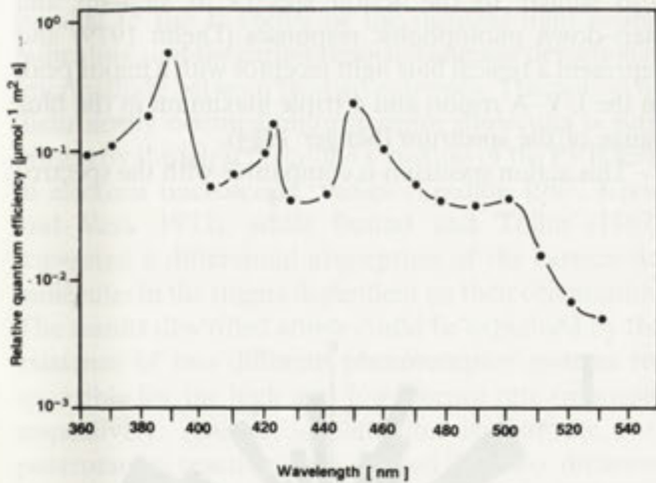

Fig. 2. Action spectrum of phototactic orientation in dark grown (bleached) *Euglena gracilis* based on fluence rate response curves measured in individual cells

the spectrum. This does not hold for the ultraviolet region, where the cells need the same fluence rates as green *Euglena*.

The polarotactic orientation was measured by irradiating the cells coaxially with the measurement beam using a half silvered mirror. In white actinic light at about 6000 lx *Euglena* showed a strong orientation perpendicular to the polarization plane (Fig. 3). At higher fluence rates (30000 lx) the organisms moved parallel to the polarization plane (Fig. 4). The cross-over point between low and high intensity reactions was at about 10000 lx, but differed between different cultures. In the intermediate range between parallel and perpendicular orientation the cells moved at some angle between the two extremes, often 30°.

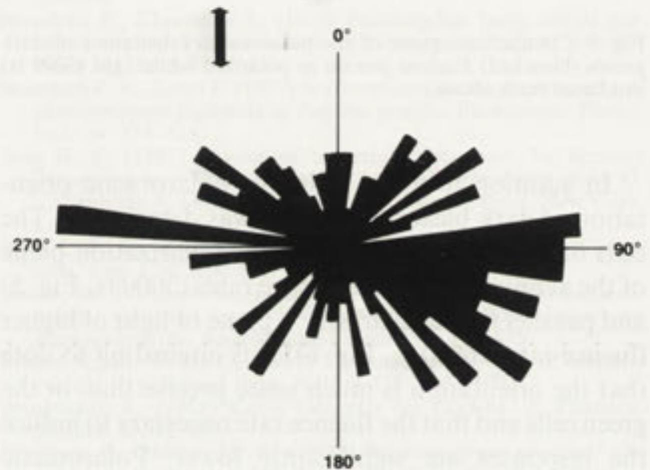

Fig. 3. Circular histogram of the polarotactic orientation of green *Euglena gracilis* in polarized white light (6000 lx) irradiated from above

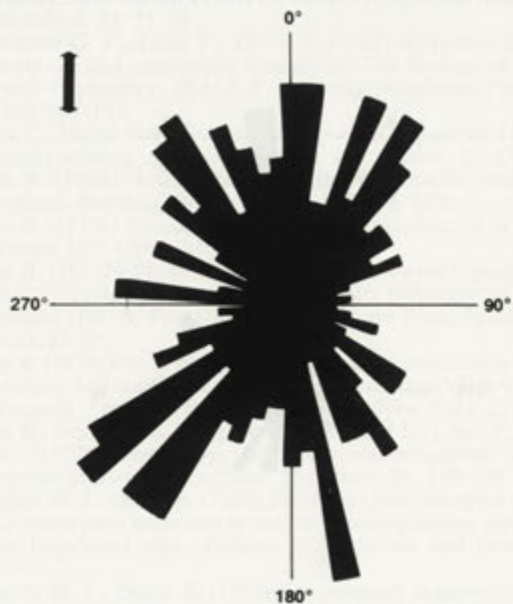

Fig. 4. Circular histogram of the polarotactic orientation of green *Euglena gracilis* in polarized white light (30000 lx) irradiated from above

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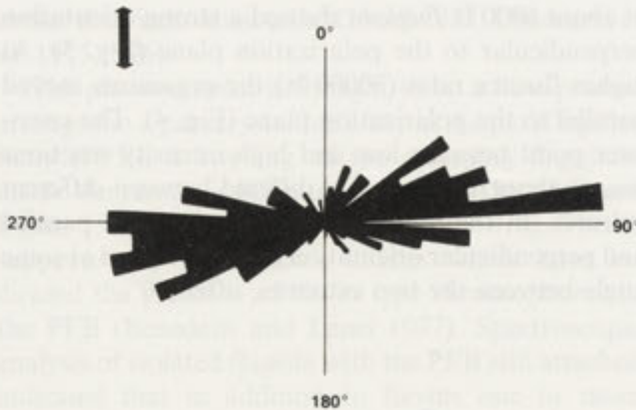

Fig. 5. Circular histogram of the polarotactic orientation of dark grown (bleached) *Euglena gracilis* in polarized white light (3000 lx) irradiated from above

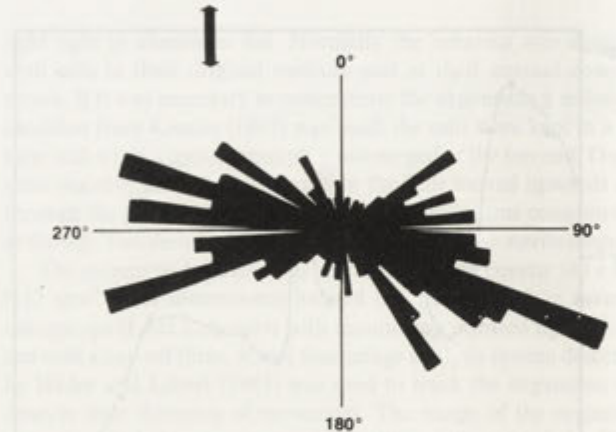

Fig. 7. Circular histogram of the polarotactic orientation of green *Euglena gracilis* in polarized monochromatic irradiation (380 nm) irradiated from above

In addition to green cells, the polarotactic orientation of dark bleached *Euglena* was determined. The cells oriented perpendicular to the polarization plane of the actinic light at low fluence rates (3000 lx, Fig. 5) and parallel to the polarization plane of light of higher fluence rates (6000 lx, Fig. 6). It is interesting to note that the orientation is much more precise than in the green cells and that the fluence rate necessary to induce the responses are significantly lower. Polarotactic orientation was also determined in green cells at 380 and 460 nm, since at these wavelengths maxima were found in the action spectra of the phototactic reaction. At both wavelengths a polarotactic orientation perpendicular to the E–vector was found (Fig. 7, 8).

DISCUSSION

The action spectra for positive phototaxis based on fluence rate response curves in green and dark grown (bleached) *Euglena gracilis* were measured using individual cell tracks. They are similar to those determined previously using population techniques (Gössel 1957, Bound and Tollin 1967, Diehn 1969). They are also similar to the action spectra of step–up and step–down photophobic responses (Diehn 1979) and represent a typical blue light receptor with a major peak in the UV–A region and a triple maximum in the blue range of the spectrum (Senger 1984).

This action spectrum is compatible with the spectro-

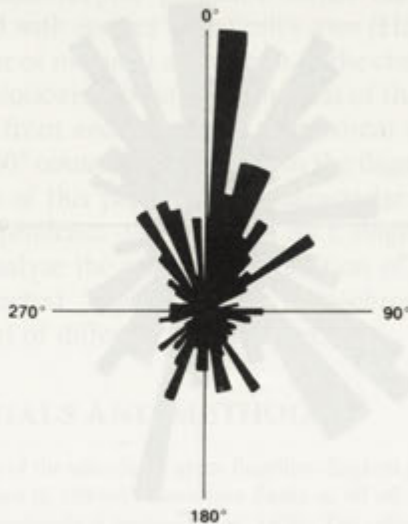

Fig. 6. Circular histogram of the polarotactic orientation of dark grown (bleached) *Euglena gracilis* in polarized white light (6000 lx) irradiated from above

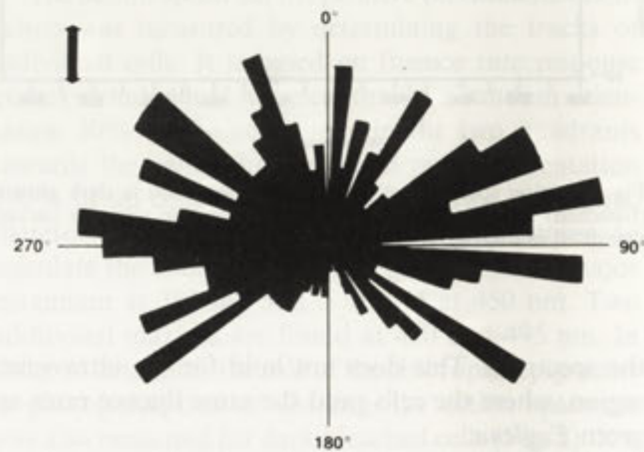

Fig. 8. Circular histogram of the polarotactic orientation of green *Euglena gracilis* in polarized monochromatic irradiation (460 nm) irradiated from above

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scopic and biochemical characterization of the PFB which has been isolated together with the flagellum after osmotic swelling of the cell (Gualtieri et al. 1986). The PFB contains four polypeptides in the range of 27 to 33 kDa, three of which carry one or more pterin chromophores while the fourth carries a flavin. The pterins absorb in the UV–A region with a maximum at about 360 nm and show a fluorescence emission at about 450 nm; the flavin absorbs maximally at about 450 nm and emits at about 520 nm. The observed absorption maxima agree well with the action spectra for positive phototaxis.

Dark bleached *Euglena* cells contain a stigma but have only traces of the chlorophylls. Since these cells need less light for both phototactic and polarotactic orientation, it has to be concluded that the chloroplasts exert a shading action in green cells not only in negative phototaxis diminishing the fluence rate received by the photoreceptor.

Since the cells respond to polarized light, the chromophores in the photoreceptor molecules need to be oriented dichroically. At an intermediate fluence rate the cells were found to orient 30° clockwise from the E–vector of the polarized light (Häder 1987). In this report we show that at low fluence rates the orientation is perpendicular and at high fluence rates parallel to the E–vector of the incident light beam, indicating that the earlier reported angular deviation is intermediate between the two extremes. The notion of dichroically oriented photoreceptor molecules is supported by the paracrystalline structure of the PFB seen in electron microscope studies (Leedale 1967, Kivic and Vesk 1972), while Bound and Tollin (1967) suggested a differential absorption of the carotenoid molecules in the stigma dependent on their orientation. The results described above could be explained by the existence of two different photoreceptor systems responsible for the high and low fluence rate response, respectively. Another explanation is that the two polarotactic reactions are caused by two different absorbing vectors within the receptor molecules as suggested by Johansson and coworkers (1979). However, the two absorption vectors in the isoalloxazine ring differ by 30° which is not compatible with the parallel and perpendicular orientation, respectively, of the cells found during the two light regimes. In addition, the vectors absorbing at 380 and 450 nm should also differ by about 30°, which our results with monochromatic radiation indicate is not the case. The results also indicate that the two photoreceptor molecules involved – flavins and pterins – are not oriented perpendicular to each other and that both mediate the two different polarotactic responses.

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Phototactic Orientation in *Dictyostelium discoideum* Amoebae

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Summary. Amoebae of *Dictyostelium discoideum* strain AX-2 have been found to show a precise phototactic orientation only during the stationary phase of the growth while they did not respond to light in the early logarithmic phase. The sensitivity was not as high as in slugs and the threshold in white light was found at about 10^{-4} W m^{-2}. The cells show a positive phototaxis at low fluence rates and a negative one at higher fluence rates; the cross over point was found at about 10^{-1} W m^{-2}. Toward the end of the stationary phase the negative phototaxis to a high irradiance (62 W m^{-2}) reversed into a positive phototaxis, while the positive phototaxis (1.6 x 10^{-2} W m^{-2}) remained stable during the whole stationary phase. Artificial ultraviolet radiation (from a transilluminator) drastically affected photoorientation and motility in *Dictyostelium discoideum* amoebae within about 15 min.

Key words. *Dictyostelium discoideum*, amoebae, phototaxis, orientation.

INTRODUCTION

The cellular slime mold *Dictyostelium discoideum* undergoes a pronounced life cycle alternating between a unicellular amoebal and a multicellular pseudoplasmodium (slug) stage (Bonner 1982). As many other motile microorganisms (Häder 1987, Nultsch and Häder 1988) both stages of the organism responds to a number of external stimuli in order to find and stay in a microhabitat of suitable conditions for growth and spore discharge.

In its slug stage *Dictyostelium discoideum* has been found to respond exclusively positive phototactically (Bonner et al. 1950). The action spectrum extends throughout most of the visible spectrum (Francis 1964, Poff et al. 1974, Poff and Häder 1984). The mechanism of slug phototaxis is based on a lens effect: the cylindrical multicellular organism has a refractory index sufficient to result in an efficient focusing of a parallel light beam to the rear side (Francis 1964, Häder and Burkart 1983). This lens effect is destroyed in the ultraviolet region below 300 nm due to a high internal absorption so that the slugs show a negative phototaxis in this spectral region (Häder 1985). Fisher and Williams (1981) assume that the focused light at the far side of the slug results in the production of a slug turning factor, an unknown molecule of low molecular weight, to which the organism responds negative chemotactically. A model by Poff and coworkers (1986) tries to explain the mechanism of phototactic orientation by the optical properties of the slug tip which is the only photosensitive portion of the organism. This model also accounts for the bimodal orientation right and left of the light beam found in some strains (Fisher and Williams 1982, Poff et al. 1986). In addition, *Dictyostelium discoideum* slugs are extremely temperature sensitive and respond either positive or negative thermotactically in a temperature gradient (Poff and Skokut 1977, Bonner 1982).

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Amoebae of *Dictyostelium discoideum* are also phototactic (Hong et al. 1981) but the direction of movement depends on the fluence rate of the incident light beam. In analogy to the bimodal orientation in slugs, some *Dictyostelium discoideum* amoebae strains show a pronounced multimodal orientation (Häder et al. 1983b, Fisher et al. 1985). In addition to phototaxis, amoebal *Dictyostelium discoideum* show also thermotaxis (Hong 1983) and two types of chemotaxis using cAMP (Gerisch 1982, Raven et al. 1985) and folate (Pan et al. 1972, Konijn 1975) as attractants.

Ultraviolet radiation has been found to affect a number of developmental steps in *Dictyostelium discoideum* (Ford and Deering 1979, Ohnishi et al. 1982, Häder 1983a). In addition, motility in slugs is drastically affected by artificial ultraviolet radiation as well as phototactic orientation (Häder 1983b). These phenomena have also been found under solar ultraviolet radiation (Häder and Häder 1989). The aim of this paper is to characterize the degree of phototactic orientation during the amoebal development and to demonstrate that also amoebal phototaxis and motility are prone to ultraviolet inhibition.

MATERIALS AND METHODS

All experiments were carried out with the unicellular amoebae of *Dictyostelium discoideum* stain AX-2. The cells grew in rotary shake cultures at 100 rpm at about 23°C in a peptone-yeast extract-glucose medium as previously described (Häder and Poff 1979a) with added streptomycin (250 μg/ml). For the irradiation experiments the cells were harvested and resuspended in fresh medium at a density of 2.5×10^5 cells per milliliter. Aliquots of 10 ml were transferred into open Petri dishes (90 mm in diameter) which were placed on the stage of a light microscope (BH-2, Olympus, Japan). The movement of the organisms was observed using dark field infrared irradiation in order not to disturb the photoorientation of the cells (Hader et al. 1987).

The image of the moving cells was recorded by a CCD camera and the video signal was digitized at a spatial resolution of 512x512 pixels with 256 possible grey levels each (Matrox digitizer card PIP-1024, Quebec, Canada) which was plugged into an IBM AT compatible microcomputer (Tatung 7000, Taipei, Taiwan). The software developed to track motile microorganisms allows real time digitization with an A/D conversion of all pixels every 40 ms. Since the organisms move far slower than flagellates, for which the tracking routine was originally developed, delay periods of 60 s were inserted between digitization of individual snapshots. Four images were taken in sequence, stored in a dedicated video memory and displayed on an analog monitor in pseudocolor graphics. The computer had access to the image in memory and could both read and write pixels at random.

The software package had been written in the computer language C (Häder and Vogel in press). All time consuming procedures such as manipulation of image pixels, outline detection and calculation of centroid were written in ASSEMBLY Language (80286). The algorithm starts by scanning the first image from the top left corner. The outline routine is based on the chain code algorithm (Freeman 1961) which allows each cell position to be described uniquely by its edge vectors; it is very fast and allows to reduce the amount of information drastically. Several algorithms for calculating the circumference, area and centroid have been developed (Freeman 1974, 1980). The search is continued until all organisms in the whole frame have been analyzed. The centroids and areas of all organisms are stored in an array. In the next frame (60 s later) the positions of all organisms are determined as described above; however the stored centroids from the previous frame are used as starting points. If the previous centroid is found not to be within the area of the organism's new position or the close surrounding the cell is regarded as lost. Equally all organisms leaving the field of view are discarded. The positions and areas of all found organisms are stored in a second array and the process is repeated for the third and fourth digitized image, overwriting the second array. When two organisms meet, the direction of movement is no longer defined; therefore, when a sudden increase in the area of an organism is encountered this organism is discarded. Upper and lower limits for the area allow to distinguish cells from debris in the image.

The movement vector can be calculated for each individual organism from the raw data stored in the two arrays, and the value can be stored in terms of the deviation angle from a predetermined stimulation direction (defined as 0°). Since the time elapsed between the first and final frame is known from the built-in hardware clock of the computer, the individual speed of movement of all cells can be calculated from the individual distances moved.

Circular histograms for the direction distribution as well as the velocity distribution with a resolution of 64 sectors of 5.6° each are calculated using subsequent programs written in a higher level language (Turbo Pascal, Borland) (Häder 1988). Rayleigh tests are performed to analyze the directedness of the moving organisms (Batschelet 1965, 1981, Mardia 1972). The direction of movement is determined using a Fast Fourier Analysis which also allows smoothing of the raw histograms and Inverse Fourier Analysis reconstructs the smoothed histograms (Häder and Lipson 1986).

The actinic white light irradiation was produced using a 250 W slide projector equipped with a 24V quartz halogen bulb (Kindermann, Universal). The fluence rates were adjusted using neutral density filters (Schott and Gen., Mainz, FRG). The fluence rates were measured with a luxmeter (Gossen, Mavolux digital, Erlangen). Ultraviolet radiation was produced from an inverted transilluminator (312 nm filter, Bachofer, Reutlingen, FRG) and measured using an Optronics (model 742) double monochromator spectroradiometer.

RESULTS

Histograms of the direction of movement of *Dictyostelium discoideum* amoebae in the stationary growth phase show a pronounced positive phototactic effect. The precision of orientation has been quantified using the Rayleigh test (Batschelet 1965, 1981) which yields an r-value: a value close to zero describes a random orientation and a value close to one signals an almost perfect orientation. At an irradiance of 10^{-3}W m^{-2} an r-value of 0.43 was found for *D. discoideum* amoebae (Fig. 1a). At an irradiance of

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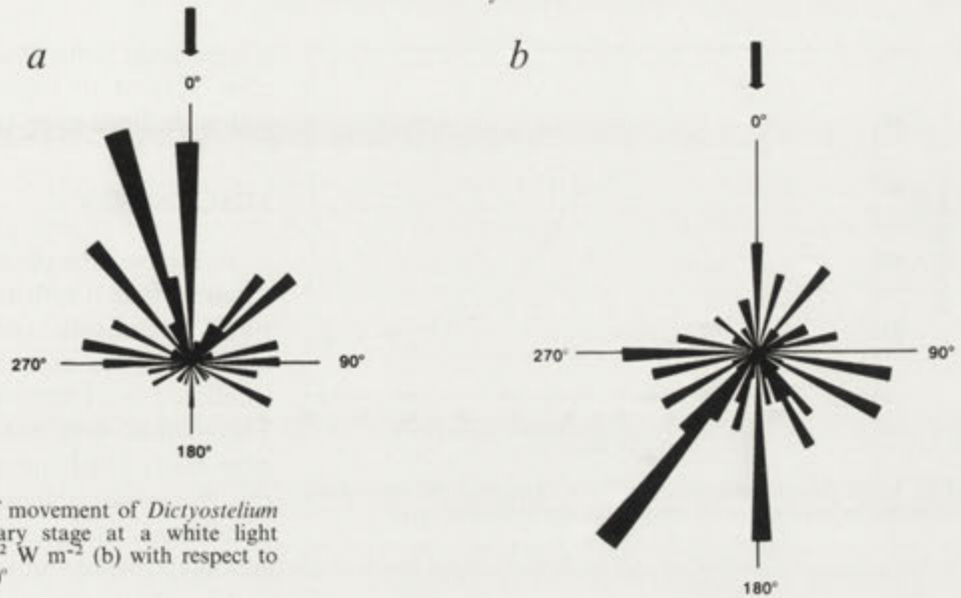

Fig. 1. Histogram of the direction of movement of *Dictyostelium discoideum* amoebae in the stationary stage at a white light irradiance of 10^{-3} W m^{-2} (a) and 10^{2} W m^{-2} (b) with respect to a lateral light beam impinging from 0°

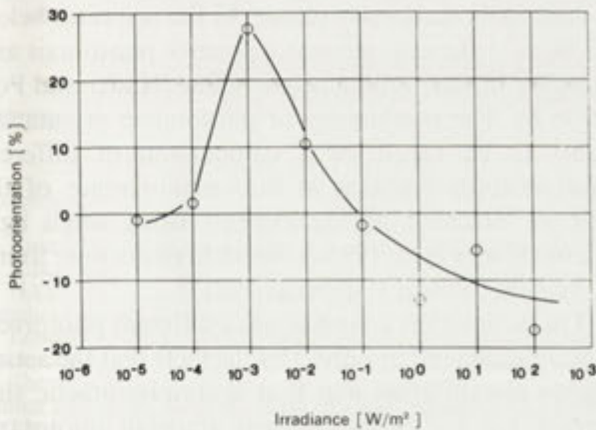

Fig. 2. Dependence of the phototatic orientation (r-value, ordinate) on the white light fluence rate (W m^{-2}, abscissa) of *Dictyostelium discoideum* amoebae in the stationary stage

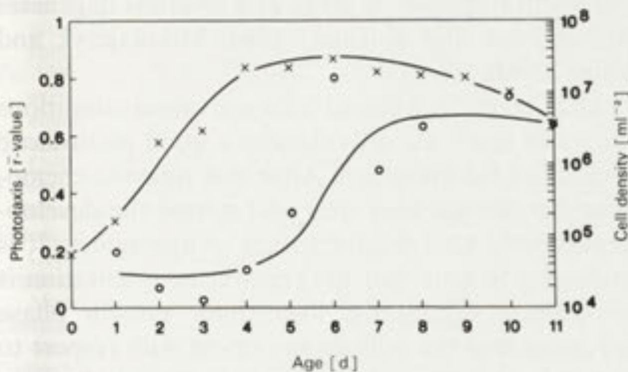

Fig. 3. Dependence of the degree of positive (closed circles) and negative (crosses) phototactic orientation (r-value, left ordinate) on the culture age (abscissa, days) in comparison to the concentration of cells (right ordinate)

10^{2} W m^{-2} a negative phototaxis was determined (Fig. 1b) and the orientation was less pronounced, quantified with an r-value of about 0.18. The fluence rate-response curve measured using white light shows a threshold at about 10^{-4} W m^{-2} (Fig. 2). At 10^{-3} W m^{-2} the positive phototactic orientation was maximal and reversed into a negative one at about 10^{-1} W m^{-2}. The negative phototactic orientation reached a plateau at about 10^{2} W m^{-2}. When measured in the logarithmic phase, the orientation was almost random (data not shown). The dependence of the degree of orientation on the culture age is shown in figure 3: The comparison with the cell concentration indicates that the onset of positive (at 1.6×10^{-2} W m^{-2}) phototactic orientation slightly lags after the beginning of the stationary growth phase. This is also true for the negative phototaxis. It even stays high, when the cell density starts to decline. The motility of the cells is equally high during the logarithmic and most of the stationary phases and decreases only when the cell density declines (Fig. 4).

When exposed to ultraviolet radiation, motility was found to decrease even after short exposure times (data not shown). In order to quantify this behavior, the percentage of motile cells was determined using the real time automatic image analysis which is also capable of determining whether an organism is motile or not during the predetermined observation period. Under the influence of ultraviolet radiation motility of *Dictyostelium discoideum* amoebae decreased drastically within 15 min (Fig. 5). Simultaneously, the degree of orientation was also affected (histograms not shown). When quantified using the Rayleigh test

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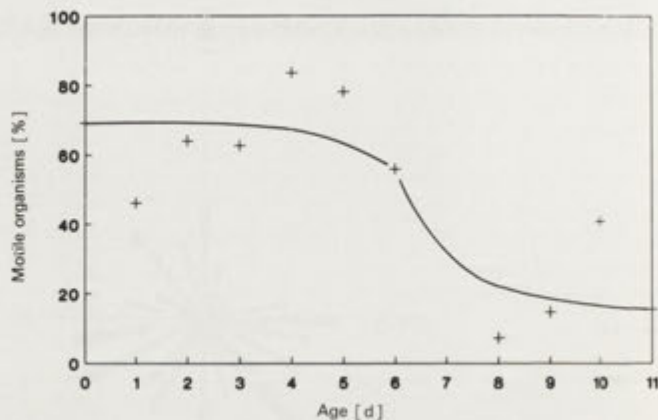

Fig. 4. Percentage (ordinate) of motile *Dictyostelium discoideum* amoebae in dependence of the culture age (abscissa, days)

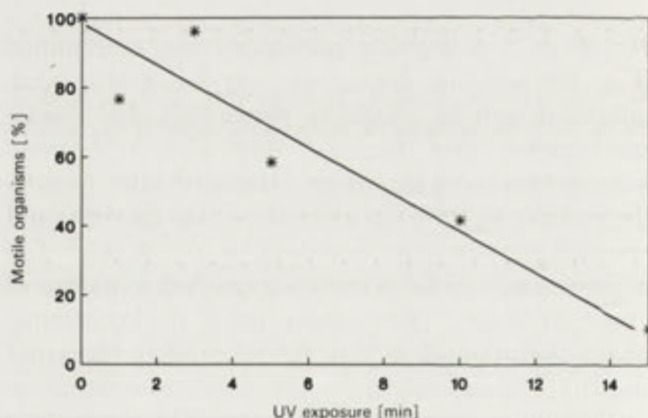

Fig. 5. Percentage of motile *Dictyostelium* amoebae (ordinate) in dependence of the exposure time to ultraviolet radiation (abscissa, min)

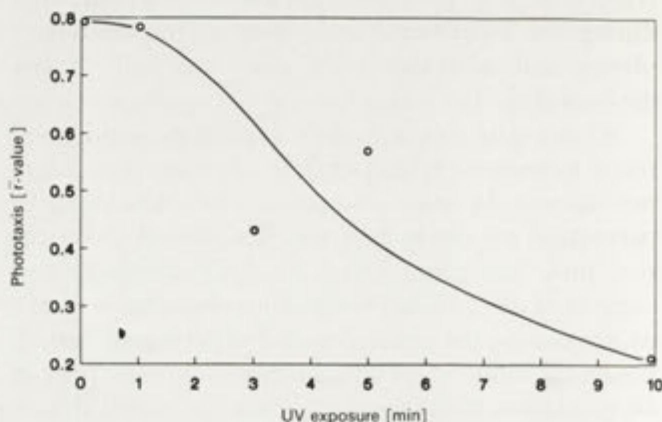

Fig. 6. Degree of phototactic orientation of *Dictyostelium discoideum* amoebae (r-value, ordinate) in dependence of the exposure time to ultraviolet radiation (abscissa, min)

a significant reduction in the r-values was found even after very short exposure times. Phototactic orientation declined even faster than motility (Fig. 6).

DISCUSSION

In the past the phototactic orientation of amoebae was determined with an indirect method based on light trap experiments: cells are attracted by the light scattered from cells and agar particles within the irradiated field from outside (Häder and Poff 1979a,b). This method does not allow to determine the fluence rates easily which induce positive or negative phototaxis. The results obtained with the direct measurement of cell tracks indicates that the amoebae are as sensitive as slugs in white light (Poff and Häder 1984).

The phototaxis experiments performed with the light trap method described above used AX-2 amoebae in their early stationary phase. At fluence rates below 0.1 W m^{-2} the cells showed a positive phototaxis and at higher fluence rates a negative one (Häder and Poff 1979a,b). The mechanism of phototactic orientation seems to be based on a comparison of different photoreceptor readings at the circumference of the cells as indicated by experiments using small light beams (Häder et al. 1983a). Similar results were found in *Amoeba proteus* (Grębecki 1981).

The notion that amoebae use a different photoreceptor than slugs is supported by the facts that the action spectra are different and that a nonphototactic slug mutant has an almost normal amoebal phototaxis (Häder et al. 1980). Action spectra for amoebal phototaxis of several, genetically different strains indicated that multiple photoreceptors are involved in the light response (Häder et al. 1988). The action spectrum clearly differs from those found for photomovement responses in green and colorless flagellates (Mikołajczyk and Kuźnicki 1984, Mikołajczyk and Walne 1990).

After being transferred into starvation conditions (on water agar) the cells showed a good phototactic orientation for about 2 h. After that time the chemotactic mechanism took over and started the developmental cycle with the formation of aggregates. It is interesting to note that the phototactic orientation is non-existent during the logarithmic growth phase indicating that the cells do not orient with respect to light as long as they find sufficient bacteria to feed on. Only when subjected to starvation light guides the cells toward the surface; but they are prevented from moving into the too bright sunlight by the negative

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phototaxis. Shortly afterwards chemotaxis toward cAMP takes over and the single amoebae start to aggregate.

The sensitivity toward ultraviolet radiation is even more pronounced in amoebae than in slugs which may be due to the fact that in the multicellular pseudoplasmodia there is a massive mutual shading while the single amoebae are fully exposed. Phototactic orientation is even faster affected by ultraviolet radiation than motility indicating that it is a direct effect perhaps affecting the photoreceptor molecule or an element in the sensory transduction chain rather than an indirect effect mediated by a decreased motility.

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ACTA PROTOZOOLOGICA

Changes in the Structure and Nutrition Preference of the Protozoa Community in Standing Water Developed from Running Water

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Summary. The development of *Ciliata* communities was investigated under running water and standing water conditions. The composition of a community changes as a result of discharge changes. Changes are the most significant in the increase of the biomass quantity.

Key words. Protozoa community, running water – standing water, biomass.

INTRODUCTION

Though most protozoologists agree with the empirical observation that protozoa are mostly eurytopic according to their ecological demands, opinions differ on the distribution of water discharges. This must be the reason why specialists pay relatively little attention to investigations of *Protozoa*, which live in running water. During the last three years I have been tracing, with the help of the ASCA program, to what extent this topic has been investigated. In almost one and a half million papers examined by ASCA only 378 dealt with free living *Ciliata* from freshwater. That this topic has become the focus of interest is shown by the fact, that last year the number of publications on running waters has doubled. Unfortunately, the majority of investigations on the level of supraindividual organizations do not make use of numerical data, but hypothesis.

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Most of the papers I know (in manuscript), dealing with the environmentally damaging effects of the Bös (1819 riv. km) hydro– electric power plant – which is planned to be built on the Danube – are based only on hypothesis. This fact has prompted me to deal with the question numerically in my investigation on what is bound to happen to the organisms when the water flow slows down and stops. These investigations, as shown by the title, aim to examine the changes in the structure and nutrition preference of the *Protozoa* community in standing water developed from running water.

There is a branch of the Danube near to our institute, which was found suitable for my short–term investigations (Fig.1). The sidebranch at Göd, as a hydrological unit, has already been amply investigated. Several papers have been concerned with its water chemistry, hydrology and its comparison with the main stream (Bothar 1972, Dvihally 1962, Dvihally and Kozma 1964, Nosek and Bereczky 1981, Oertel 1982). The aim of our investigations was not, therefore to reveal the hydrological conditions of the branch, but to observe the formation of the planktonic *Ciliata* community, which has been disconnected from the main stream.

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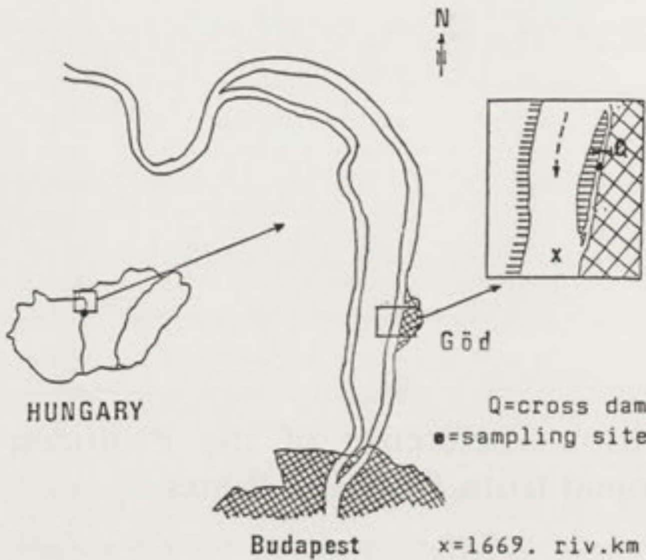

Fig. 1. Location of the sampling site in a sidebranch of the river Danube at Göd

MATERIALS AND METHODS

The sidebranch of Göd, which is nearly 900 m long (1668.9–1669.8 river km), is isolated from the main stream by a crosswise dam. This dam has become rather damaged in recent years, so the connection with the main stream which occurred earlier at 370 cm water level has today been brought about at 200 cm. Just behind the dam a 2.7 m deep dip has been formed (Oertel 1982). This field was selected as a sampling site, because the effects of the disconnection from the main stream were the earliest to appear here (Fig.1).

The investigations have disclosed that the time of sampling could not be unequivocally planned in advance. The samples were collected on the basis of water level forecasts on the period between September 14 and October 4,1988. Water was filtered from September 14 to October 4 from the side branch seven times, each time 100 l water through a net of 20μ mesh size. Both living and fixed materials were studied by our own method (Bereczky 1985) and by protargol impregnation as modified by Wilbert (1974). Focussing on our main task, only the process of disconnection was observed without paying any attention to environmental factors. Changes of the *Ciliata* community were measured statistically (Southwood 1978) as follows:

Constancy K_i $(A_i/M).100$

A_i – number of samples in which the species i occurs
M – total number of samples

Dominance D_i $(n_i/N).100$

n_i – individual number of species
N – total individual number in the sample

Diversity (Shannon–Weaver)

$$H'' = -\frac{n_i}{N}\log\frac{n_i}{N}$$

Evenness

$$E = \frac{H''}{\log_2 s}$$

s – number of species

Similarity index (Sørensen) for species

$$C_S = 2S_j/(S_a{}^{-}S_b)$$

Sj – sum of the smaller individual numbers of the common species
N_a – total individual number in sample a
N_b – total individual number in sample b

Similarity index (Sörensen) for abundance

$$C_N = 2N_j/(N_a + N_b)$$

N_j – sum of the smaller individual numbers of the common species
N_a – total individual number in sample a
N_b – total individual number in sample b

The biomass value of species not known to us from the tables of Czorik (1968) were calculated by the following formula:

$$V = \frac{h}{6}(b_1 + 4b_2 + b_3)$$

V – volume
h – body length
b_1 – lower area
b_2 – area of the middle cross-section
b_3 – upper area

In our investigations the constancy values are related to period. Since *Ciliata* of short generation time are able to spread considerably, it was thought reasonable to calculate constancy for 21 days.

The dominance relationship was studied because of the changes in nutrition types. The dynamics of changes were traced by examining diversity, evenness, the two types of similarity index and biomass values. The investigations were begun on September 14, but were, however, stopped two days later for 10 days because a smaller flood created a relatively intensive connection between the main stream and the sidebranch. Examinations could finally be accomplished during the period between September 26 and October 4 when water level sank (Fig.2).

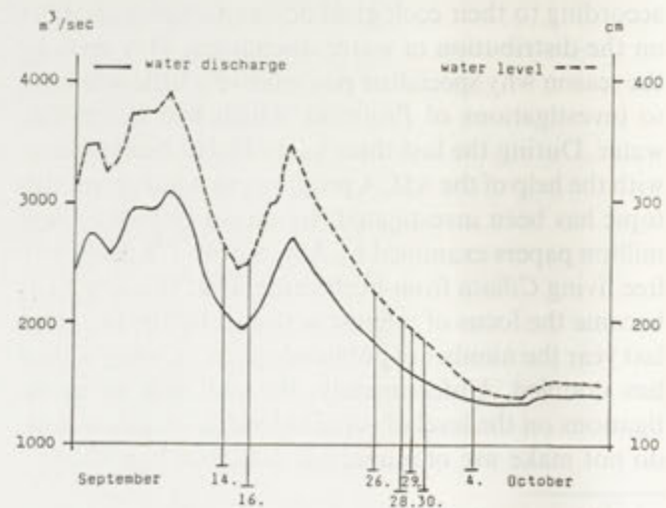

Fig. 2. Water level and water discharge and the time of sampling (1988)

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RESULTS

Samples were taken for control from the main stream three times. They contained 43 *Protozoa* species, the majority being *Ciliata*. A total of 62 species were collected from the sidebranch. The number of common species was 26. The taxa were mostly euplanktonic, eurytopic. Neither *Amoeba* nor *Testacea* played a significant role in forming the plankton, as indicated by their low constancy and similarly low dominance values (Table 1).

Table 1

Constancy and dominance in the sidebranch at Göd
(14.09-04.10,1988)

Species	Constancy	Dominance						
		14.09	16.09	26.09	28.09	29.09	30.09	4.10
Amoeba proteus (Pallas, 1766)	1	—	—	0.4	—	—	—	—
Amoeba sp.	3	—	—	0.4	—	—	0.8	0.9
Arcella discoides (Ehrenberg, 1843)	1	—	—	0.4	—	—	—	—
A. hemisphaerica (Perty, 1852)	2	0.3	—	1.8	—	—	—	—
A. vulgaris (Ehrenberg, 1832)	3	—	—	0.9	0.3	0.3	—	—
A. vulgaris f. *undulata* (Deflandre, 1928)	1	—	—	0.4	—	—	—	—
Cyphoderia ampulla (Ehrenberg, 1940)	1	—	—	1.3	—	—	—	—
Difflugia limnetica (Levander, 1900)	1	—	—	0.4	—	—	—	—
D. lobostoma (Leidy, 1879)	2	—	—	3.1	—			
Actinobolina radians (Stein, 1867)	1	—	—	—	—	—	—	1.8
Aspadisca lynceus (O. F. Müller, 1773	1	—	—	1.3	—	—	—	—
Astylozoon fallax (Engelmann, 1862)	3	0.3	—	—	—	—	0.4	0.4
A. vagans (Stiller, 1941)	2	—	—	—	—	—	0.8	1.8
Codonella cratera (Leidy, 1877)	3	8.0	11.4	0.9	—	—	—	—
Coleps hirtus (O. F. Müller, 1876)	4	2.0	4.6	0.9	1.6	0.8	—	—
C. hirtus v. *lacustris* (Fauré-Fremiet, 1924)	5	5.7	3.4	3.5	0.6	2.8	1.6	4.1
Condylostoma vorticella (Ehrenberg, 1883)	3	—	—	0.4	—	—	0.8	0.4
Cyclidium sp.	3	—	—	—	0.3	0.3	5.7	0.9
Didinium nasutum (O. F. Müller, 1773)	4	0.6	0.6	—	2.2	—	0.4	6.8
Enchelys simplex (Kahl, 1929)	2	—	—	—	—	—	2.1	1.8
Epistylis pyriformis (Perty, 1852)	3	—	—	—	0.3	—	0.4	0.4
E. rotans (Šves, 1897)	4	0.8	—	0.4	0.3	—	0.8	1.8
Euplotes patella f. planctonicus (Kahl, 1932)	1	—	—	—	—	—	—	0.4
Frontonia atra (Ehrenberg, 1833)	3	—	0.6	0.4	—	—	1.2	—
Gonostomum affine (Stein) Sterki, 1878)	1	—	—	0.9	—	—	—	—
Halteria cirrifera (Kahl, 1932)	1	0.3	—	—	—	—	—	—
Hastatella radians (Erlanger, 1890)	1	—	—	—	—	—	—	0.4
Holophrya nigricans (Lauterborn, 1894)	1	—	—	—	—	—	—	6.4
H. simplex (Schewiakoff, 1893	3	—	—	0.4	0.6	—	0.8	0.9
Holophrya sp.	4	0.3	1.1	—	—	3.-4	7.3	8.2
Monodinium balbianii (Fabre-Domergue, 1888)	5	0.6	2.8	1.8	13.3	18.3	10.9	13.2
Nassula ornata (Ehrenberg, 1833)	2	—	0.6	—	—	—	0.4	—
Nassula sp.	1	—	—	—	—	—	1.2	—
Onychodromus grandis (Stein, 1859)	3	—	—	1.8	0.6	0.8	—	—
Oxytricha fallax (Stein, 1859)	1	—	—	0.9	—	—	—	—
Phascolodon vorticella (Stein, 1859)	3	—	—	—	7.6	17.7	20.6	7.8
Prorodon teres (Ehrenberg, 1833)	1	—	—	—	—	—	—	1.8
Pseudochilodonopsis algivora (Kahl, 1931)	2	—	—	0.4	—	0.3	—	—
Stentor niger (O. F. Müller, 1773)	1	—	—	—	0.3	—	—	—
S. polymorphus (O. F. Müller, 1773)	5	0.8	0.6	0.9	—	0.3	1.2	4.1
Stokesia vernalis (Wenrich, 1929)	5	2.3	4.6	1.8	10.7	5.3	16.2	1.8
Strobilidium conicum (Kahl, 1932)	3	—	1.1	2.2	0.9	0.8	—	—

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Table 1 (cont.)

S. gyrans (Stokes, 1887)	1	1.1	—	—	—	—	—	—
S. humile (Penard, 1922)	5	4.5	14.8	16.7	5.4	9.0	1.2	0.4
S. velox (Fauré-Fremiet, 1924)	5	1.1	5.1	—	23.3	12.1	6.9	2.3
Strobilidium sp.	1	0.3	—	—	—	—	—	—
Strombidium viride (Stein, 1859)	4	—	1.7	—	4.1	6.5	4.4	0.4
Strombidium sp.	2	—	—	—	—	0.8	1.2	—
Stylonychia mytilus complex	2	—	—	0.4	—	0.3	—	—
Tintinnidium fluviatile (Stein, 1863)	5	32.8	36.0	35.2	16.1	0.3	—	1.4
Tintinnopsis cylindrata (Kofoid et Campbell, 1929)	1	—	—	0.4	—	—	—	—
Tintinnopsis sp.	3	33.9	4.0	0.9	—	—	—	—
Uroleptus piscis (O. F. Müller, 1773)	1	—	—	—	0.3	—	—	—
Urocentrum turbo (O. F. Müller, 1786)	1	—	—	—	—	0.3	—	—
Urotricha farcta (Clap. et Lachm., 1859)	4	0.3	—	—	0.3	3.4	2.8	4.6
Vorticella convallaria (Linnaeus, 1758)	1	—	—	—	—	0.3	—	—
V. incisa (Stiller, 1932)	3	0.8	—	1.8	1.6	5.3	—	—
V. margaritata (Fromentel, 1874)	4	—	—	0.4	0.3	0.6	2.0	13.2
V. mayeri (Fauré-Fremiet, 1920)	1	—	—	0.4	—	—	—	—
V. microscopica (Fromentel, 1874)	3	—	0.6	0.4	—	1.4	—	—
V. nebulitera (O. F. Müller, 1773)	5	0.6	1.7	9.7	3.5	3.1	3.2	7.8
V. similis (Stokes, 1887)	5	2.6	4.6	5.3	3.1	5.1	4.4	2.3
Zoothamnium ramosissimum (Sommer, 1950	1	—	—	—	—	—	—	0.9

Constancy = occurence of species (%) 0- 20% constancy 1; 21- 40% ,, 2; 41- 60% ,, 3; 61- 80% ,, 4; 81-100% ,, 5

Dominance = individual number (%)

Among *Ciliata* the high constancy value often coincided with high dominance. In the process of running water becoming standing water, the dominance relationships of species change. This must be particularly emphasized in the euplanktonic *Strobilidium* species and *Tintinnidium fluviatile*. The latter is the dominant species of the main stream, mostly feeding on Diatoma. During this time 65–70% of the phytoplankton was made up by Diatoma in the main stream (15—19 million/l; personal communication by Kiss). At the beginning of investigations the abundant *Tintinnidium* decreased after having been totally disconnected from the main stream and was replaced, surely because of the changes in the competitive conditions, by *Epistylis rotans* and *Stentor polymorphus*.

A similar change occurred in the individual number of other planktonic organisms, e.g., of *Strobilidium humile*, which earlier was constant. *Urotricha farcta*, however, which is known as a typical inhabitant of dammed waters, was characterized by an increase in individual numbers, when disconnected from the sidebranch. The community, formed after a flood, was composed of many accessory species, showing the unstable state. Earlier similar values were found for the entire year, when carrying out investigations in the sidebranch (Nosek and Bereczky 1981). Changes in the individual number of dominant species clearly suggest the direct effects of minor floods and also modifications after a rise in water level (Table 2).

Table 2

Individual number changes of dominant species in the sidebranch (10^3 ind/m^3)

	14.09	16.09	26.09	28.09	29.09	30.09	04.10
Tintinnidium fluviatile	460	252	320	204	6	—	18
Monodinium balbianii	8	20	16	196	390	162	174
Phascolodon vorticella	—	—	—	96	378	306	102
Strobilidium velox	16	36	—	296	258	102	30
Stokesia vernalis	32	32	16	136	114	240	24

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Table 3

Changes of similarity values regarding Protozoa species during the study period (Sørensen)

	14.09.	16.09.	26.09.	28.09.	29.09.	30.09.	04.10.
14.09.	—	0.68	0.48	0.56	0.53	0.53	0.54
16.09.	—	—	0.44	0.56	0.56	0.56	0.49
26.09.	—	—	—	0.50	0.57	0.41	0.39
28.09.	—	—	—	—	0.70	0.63	0.63
29.09.	—	—	—	—	—	0.55	0.53
30.09.	—	—	—	—	—	—	0.81
04.10.	—	—	—	—	—	—	—

Table 4

Changes of similarity values regarding the abundancy of Protozoa during the study period (Sørensen)

	14.09.	16.09.	26.09.	28.09.	29.09.	30.09.	04.10.
14.09.	—	0.55	0.46	0.32	0.15	0.13	0.15
16.09.	—	—	0.61	0.46	0.22	0.19	0.18
26.09.	—	—	—	0.42	0.28	0.17	0.21
28.09.	—	—	—	—	0.56	0.50	0.39
29.09.	—	—	—	—	—	0.59	0.38
30.09.	—	—	—	—	—	—	0.53
04.10.	—	—	—	—	—	—	—

It is possible to compare two habitats by the coefficients of similarity. Similarity was calculated between the dates of calculating constancy. The number of species (Table 3) does not express the rate of changes or of abundance (Table 4). The similarity index which refers to September 14 and September 26 (the starting dates) shows the essential changes of the community as the result of disconnection from the main stream. However, the *Protozoa* community can change not only due to the effects of the chemical and physical components of the environment, but as suggested by Cairns (1962), also due to the direct, mutual effect of species. This mutual effect, as it is known, involves competition, predation and production of such materials, by which given species can control the growth of others. It is highly probable that these materials under standing water conditions exert more effect than those in running water.

Though the composition of species changes, the number of taxa which can be collected remains on a relatively constant level. This is demonstrated in Table 5, listing not only the diversity and evenness values, but also the changes in the number of species and that of individuals. Although the effect of a minor flood can be detected in the composition of a community, as regards the number either of species or individuals, regeneration very soon occurs.

Shortly after disconnection a high number of species was accompanied by a still higher number of individuals. On September 26 thehigh species number detected was shown by those species which had drifted from the sediment and from the periphyton. The impact of the flood is shown by the relatively low (0.69) evenness index.

The increase of biomass can best characterize the effects of changes having occurred due to disconnection on September 29. This increase took place between September 30 and October 4, i.e. in only four days

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Table 5

Changes of diversity (H), evenness (E), number of species (S) and individualis (N), as well the biomass in the sidebranch at Göd in the period of 1988 Sept. 14-Oct. 4. (*Amoeba, Testacea, Heliozoa, Ciliata, Suctoria*)

	14.09.	16.09.	26.09.	28.09.	29.09.	30.09.	04.10.
H	2.75	3.19	3.58	3.42	3.71	3.58	4.20
E	0.62	0.75	0.69	0.75	0.78	0.81	0.86
S	22	19	36	24	27	27	30
N (10 ind/m^3)	1404	700	908	1268	2130	1482	1314
biomass (mg/m^3)	0.8182	0.3758	0.6827	0.7307	0.9858	0.6215	4.0316

Table 6

Percentage distribution of nutrition types in the study period

	14.09.	16.09.	26.09.	28.09.	29.09.	30.09.	04.10.
A %	45.60	72.00	72.20	75.40	64.50	69.20	39.70
A+B %	43.80	16.00	7.90	2.50	9.00	4.00	4.10
B %	0.60	0.00	3.50	0.60	2.50	9.70	16.90
P %	9.70	11.40	7.00	19.90	22.20	16.20	34.20
O %	0.30	0.60	9.20	1.60	· 1.70	0.80	5.00

A = alga, A+B = alga+bacterium, B = bacterium, P = predatory, O= omnivorous.

and became more than sixfold. It can be particularly attributed to the increase in the individual number of *Stentor polymorphus* and to the development of *Epistylis* colonies. The latter does not show such changes in the number of individuals, because such colonies are regarded only as a single individual in protozoological practice. Naturally, this is not the way of evaluating biomass, because a well-developed *Epistylis* colony sometimes contains 10–50 individuals. So such colonies can practically form even half of the estimated biomass. Suddenly increased biomass – living organisms – on stored retained waters can pose manifold problems in the oxygen balance of the water. On the one hand, there is an increase in the oxygen demand used for respiration, while on the other, there is need of additional oxygen to eliminate the dead organic material damaged by the mechanical effect of turbines in water power plants.

Table 6 shows the percentage rate of nutrition types. During the investigation algae, algae and bacteria, bacteriavorous, predatoryand omnivorous *Protozoa* were found to be present in the sideb-ranch. Until disconnection practically only algal and algivorous and bacteriavorous dominance was noted. A rearrangement in nutrition preference occurred simultaneously with the change in the standing water character and the increase of the biomass. While in the adjoining part of the main stream and the sidebranch, according to available nutriments, the preferred nutrition unanimously included algae, then as sampling ended the rate of bacterium eaters and predators increased at the expense of the latter.

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Some Enzymes of the Starch Degradation Pathway in Rumen Protozoa with Special Reference to *Ophryoscolex caudatus*

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Summary. Amylase and maltase activity of enzyme preparations from *Entodinium caudatum*, *Ophryoscolex caudatus*, *Ostracodinium obtusum bilobum* and large rumen protozoa fraction were compared. The enzyme preparation from *O. caudatus* had the highest amylase activity but the maltase activity of this preparation was very low. Maltose phosphorylase and hexokinase activity of the preparation from *O. caudatus* were examined. The production of glucose phosphates and glucose from maltose by the action of maltose phosphorylase markedly increased in the presence of inorganic phosphate. The production of glucose phosphates from glucose by action of hexokinase markedly increased in the presence of ATP. *O. caudatus* gets its energy probably directly by maltose phosphorylation.

Key words. Rumen Protozoa, *Ophryoscolex caudatus*, enzymes.

INTRODUCTION

The degradation of starch is an important feature of the rumen metabolism and several species of bacteria (Marounek and Bartoš 1986) and protozoa (Coleman 1986) are active in this fermentation. One function of the Entodiniomorphid protozoa is to remove starch grains from the fluid phase in the rumen and make them unavailable for bacterial attack (Coleman 1980). Michałovski et al. (1986) found a loss of ingested starch of 52 to 65% from the cells of *Entodinium caudatum* 24 h after feeding the cultures. All the protozoal species tested including *Ophryoscolex caudatus* contain α–amylase (Coleman 1986) and α–D–glucosidase (Williams et al. 1984).

The purpose of the present investigation is to compare the activity of amylase and maltase (an α–D–glucosidase) in enzyme preparations from some rumen protozoa including *O. caudatus* and to examine the activity of maltose phosphorylase and hexokinase in *O. caudatus*.

MATERIAL AND METHODS

Preparation of Protozoa Suspensions and Enzyme Preparations

E. caudatum and *O. caudatus* were grown in vitro as described by Coleman (1978a) and washed by centrifugation (Coleman 1978b) in salt solution D (containing, g/l: K_2HPO_4, 6.3; KH_2PO_4, 5.0; NaCl, 0.65; dried $CaCl_2$, 0.045; $MgSO_4 \cdot 7H_2O$, 0.09; pH 6.8) or in 0.5% NaCl (*O. caudatus* for phosphorylase assay). The large protozoa fraction was prepared from ovine rumen content by the procedure of Coleman and Sandford (1979). *Ostracodinium obtusum bilobum* was grown as monoculture in the ovine rumen as described by Coleman and Sandford (1979). Protozoal suspensions from the rumen were washed in salt solution D as described by Coleman and Sandford

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(1979, 1980). The protozoa were finally resuspended in salt solution D or distilled water (for phosphorylase assay) so that a high protozoal concentration was obtained. Concentrated protozoal suspensions were disrupted by sonication (Coleman 1978b) and the particulate matter and any unbroken cell material was removed by centrifugation (7000 g, 20 min, 4°C). The supernatant was used as the enzyme preparation. If necessary it was stored under N_2 at -20°C.

Amylase and Maltase Assay

Amylase was assayed by following the appearance of reducing sugars during incubation at 39°C using soluble starch as substrate. In an introductory study of suitable conditions the reaction mixture contained 0.8 ml 0.1M ββ'- dimethylglutarate buffer (DMG), 0.07 to 0.5 ml 0.2 or 2% starch solution, 0.05 to 0.1 ml of the enzyme preparation from large rumen protozoa and distilled water to give a final volume of 1.5 or 1.6 ml. Controls containing distilled water instead of starch solution or enzyme preparation were used in the incubations. Samples (0.1 ml) were removed initially and at intervals (10 to 30 min) for reducing sugars estimation (Nelson 1944) and for glucose estimation by the glucose oxidase method (Huggett and Nixon 1957). Results were corrected for values obtained initially and in absence of enzyme. The rate of the reaction was measured at eight substrate concentrations and the kinetic constants (V_{max}, K_m) were determined from a double reciprocal plot of rate against substrate concentration. In the comparative experiment the reaction mixture contained 0.8 ml DMG (pH 5.5), 0.5 ml 2% starch solution, 0.1 ml of the enzyme preparation and 0.2 ml distilled water. The reaction continued for 60 min. The results were calculated as though all the reducing sugar was maltose.

The reaction mixture for maltose assay contained 0.8 ml DMG (pH 5.5), 0.1 ml 0.1M maltose, 0.1 ml enzyme preparation or distilled water (so that final volume was 1.0 ml). Samples (0.1 ml) were taken initially and after 30, 60, 90 and 186 min incubation at 39°C for glucose estimation.

Experiment on Maltose Phosphorylase and Hexokinase Assay

The reaction mixture for maltose phosphorylase assay contained 0.1 ml [U-^{14}C] maltose (10 μCi/ml), 0.1 ml DMG (pH 6.5), 0.1 ml enzyme preparation from *O. caudatus*, 0.05 ml 0.1M KH_2PO_4 or 0.05 ml 0.1M ATP or distilled water to the final volume of 0.4 ml. The reaction mixture for hexokinase assay contained 0.05 ml [U-^{14}C] glucose (25 μCi/ml), 0.1 ml DMG (pH 6.5), 0.1 ml enzyme preparation from *O. caudatus*, 0.05 ml 0.1M ATP or distilled water to the final volume of 0.4 ml. Samples of approximately the same volume were taken with Pasteur pipettes initially and after 1, 3 and 6 h of incubation at 39°C. The removed samples were heated immediately to 100°C for 2 min and then were spotted at 2.5 cm intervals along the origin of a Whatman No. 1 filter paper. The material was chromatographed in solvent system B for 24 h (Coleman 1969) which separated glucose, maltose and glucose phosphates. The samples were chromatographed together with radioactive markers: [U-^{14}C] glucose, [U-^{14}C] maltose, [U-^{14}C] glucose-1-phosphate and [U-^{14}C] glucose-6-phosphate. The radioactivity content of the identified spots was determined by a multiple-flow chromatogram scanner (Baird and Tatlock, Chadwell Heath, Essex, U.K.). The results were expressed as the relative percentage of the ^{14}C content of the spots of glucose phosphates or glucose from the ^{14}C content of all identified spots from a single sample.

Experiment on Maltose Phosphorylase Assay

The reaction mixture contained 0.1 ml [U-^{14}C] maltose (10μCi/ml) 0.1 ml DMG (ph 6.5), 0.05 ml 10% K-phosphate buffer (pH 6.5) or distilled water and 0.1 ml enzyme preparation from *O. caudatus*. The reaction was allowed to continue for 5.5h at 39°C and then it was stopped by heating to 100°C for 2 min. The incubation medium was added on the top of a column containing Dowex 2 [acetate]. Sugars were eluted with distilled water and then the glucose phosphates were eluted with 1N HCl. The radioactivity present in eluates of 1 ml volume was quantified in an automatic flow counter (Nuclear Chicago Corp.) as described by Coleman (1969). The eluate containing the highest quantity of glucose phosphates was evaporated by vacuum exsiccation. The dry content of the tube was dissolved in 2 drops of distilled water and then spotted on a Whatman No. 1 filter paper impregnated with 0.01M $Na_2B_4O_7$ (Coleman 1969). The sample was chromatographed together with radioactive markers: [U-^{14}C] glucose-1-phosphate and [U-^{14}C] glucose-6-phosphate in solvent system C for 96 h (Coleman 1969). The results were expressed as the relative percentage of ^{14}C determined in the eluates containing glucose phosphates from the ^{14}C content of all eluates.

Other Procedures

The number of protozoa was estimated by the method of Coleman (1958). The protein content of the enzyme preparations was estimated by the method of Lowry et al. (1951).

Chemicals

^{14}C compounds were supplied by Amersham International plc, Amersham, Bucks, U.K. The following substrates were used: soluble starch (Hopkin and Williams, Chadwell Heath, U.K.); maltose (BDH Chemicals, Poole, U.K.).

RESULTS

Suitable conditions for the amylase assay were examined using the enzyme preparation from the large rumen protozoa fraction. This fraction contained *Isotricha intestinalis, Polyplastron multivesiculatum, O. caudatus, Diploplastron affine* and a few protozoa of the genus *Entodinium*. A linear increase

Table 1

Protozoal number (x 10^6/ml) and protein content (m/ml) of the enzyme preparations

Species	Protozoal number	Protein content
Entodinium caudatum	1.72	4.81
Ophryoscolex caudatum	0.191	3.14
Ostracodinium obtusum bilobum	1.28	17.96
Large rumen protozoa	0.545	7.98

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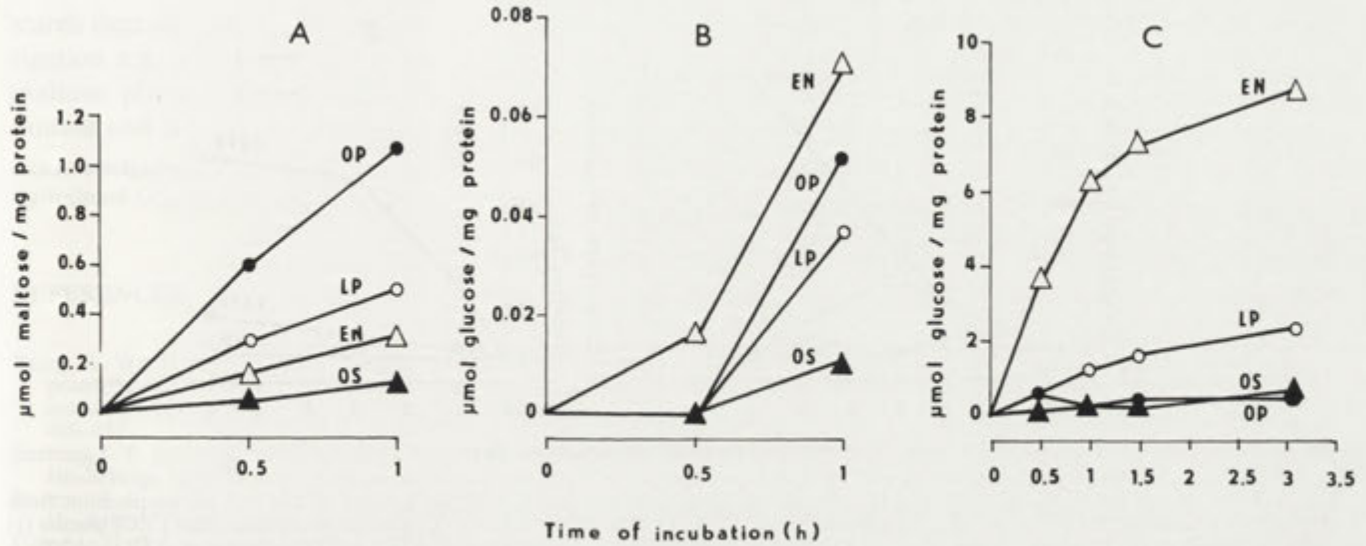

Fig. 1. Comparison of amylase and maltase activity of enzyme preparations from rumen protozoa: A. production of reducing sugars from soluble starch, B. production of glucose from soluble starch, C. production of glucose from maltose. *Entodinium caudatum* (EN), *Ophryoscolex caudatus* (OP), *Ostracodinium obtusum bilobum* (OS), large rumen protozoa fraction (LP)

of reducing sugars concentration was observed during 1 h incubation with soluble starch. The optimal pH for the release of reducing sugars was 5.5 and oxygen had not an influence on amylolytic activity (results not shown). The kinetic constants of amylase in this preparation were: $K_m = 1.06$ g starch /1, $V_{max} = 0.885$ μmol maltose / h /mg protein.

In the comparative experiment the production of reducing sugars and glucose from soluble starch by four different enzyme preparations was followed. The protozoal number and protein content of the compared preparations were estimated (Table 1). The highest concentration of reducing sugars per mg protozoal protein was liberated after 1 h incubation by the enzyme preparation from *O. caudatus*. However, the preparation from *O. caudatus* liberated less glucose from starch than the preparation from *E. caudatum* (Fig. 1 A,B). These results suggested the absence of maltase activity in *O. caudatus*. This suggestion was examined in the following comparative experiment by using maltose as substrate. Fig. 1 C shows that *O. caudatus and Ostracodinium obtusum bilobum* had a very low activity of maltase but *E. caudatum* had a comparatively high maltase activity. The low activity of maltase and the high amylolytic activity suggested a direct utilisation of maltose as a source of energy by *O. caudatus*.

The phosphorylation of malttose by maltose phosphorylase is accompanied with the liberation of glucose according the equation: maltose + orthophosphate = β–D–glucose–l–phosphate + D–glucose (Barman 1969a). The released glucose could be phosphorylated by hexokinase (glucokinase) according the equation: ATP + D–glucose = ADP + D–glucose–6–phosphate (Barman 1969a).

In an attempt to obtain evidence on possible presence of maltose phosphorylase and hexokinase activity in *O. caudatus* [^{14}C] maltose was incubated with the enzyme preparation and the amount of [^{14}C] glucose and [^{14}C] glucose phosphates produced was determined. The amount of glucose liberated increased markedly from almost nothing in the presence of added phosphate suggesting the presence of maltose phosphorylase in the protozoal preparation (Fig. 2 A). Fig. 2 A shows that [^{14}C] glucose could not be detected after 3 h incubation in the presence of ATP suggesting that the glucose was rapidly phosphorylated by hexokinase. To confirm the presence of hexokinase activity [^{14}C] glucose was incubated with the preparation from *O. caudatus* and the production of [^{14}C] glucose phosphates in the presence and absence of added ATP was compared. Fig. 2B shows that there was a marked increase in production of glucose phosphates in the presence of added ATP. In the presence of ATP less [^{14}C] glucose phosphates were produced from [^{14}C] maltose than from [^{14}C] glucose (Fig. 2B). Probably ATP was first degraded to phosphate which was used then in the phosphorylation of maltose.

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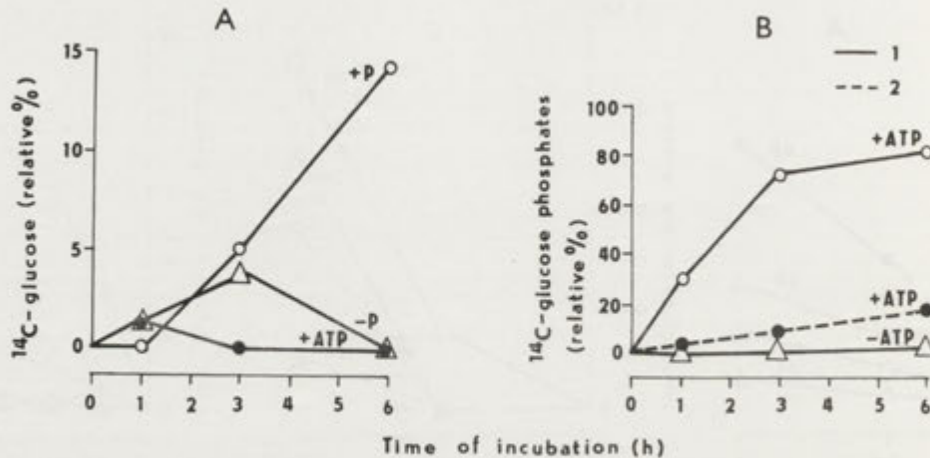

Fig. 2. Effect of inorganic phosphate and ATP on maltose phosphorylase and hexokinase activity of the enzyme preparation from *Ophryoscolex caudatus*: A. production of [^{14}C] glucose from [^{14}C] maltose, B. production of [^{14}C] glucose phosphates from [^{14}C] glucose (1) and [^{14}C] maltose (2). Presence of phosphate (+P), absence of phosphate (– P), presence of ATP (+ATP), absence of ATP (–ATP)

The following experiment made by incubating the *O. caudatus* preparation with [^{14}C] maltose in the absence and presence of inorganic phosphate resulted in the production of 1.0 and 10.2 relative % of [^{14}C] glucose phosphates, respectively. The marked increase of the production of glucose phosphates in this experiment and of glucose in the other experiment (Fig. 2A) in the presence of phosphate confirm the presence of maltose phosphorylase in the protozoal preparation. The glucose phosphates produced in the presence of phosphate were shown to be a mixture of glucose-1-phosphate and glucose-6-phosphate showing that phosphoglucomutase was present.

DISCUSSION

In the present experiment *O. caudatus* had a very low activity of maltase although Williams et al. (1984) have found a higher α-D-glucosidase activity in *O. caudatus* than in *E. caudatum*. α-Glucosidase catalyzes the hydrolysis of a variety of α-D-glucopyranosides (Barman 1969b). The enzymes of *Dasytricha ruminantium*, *Epidinium ecaudatum* and *E. caudatum* which hydrolyze maltose appear to be true maltases and not general α-glucosidases (Bailey and Howard 1963). The difference between the results of Wiliams et al. (1984) and those obtained in the present experiment can be explained by the use of different protozoal strains or by the specificity of maltases in rumen protozoa. Coleman (1986) showed that the concentration of amylase varied more than 20-fold between 12 species of rumen protozoa. Differences between values of enzyme activity in the present paper and those given by Williams et al. (1984) and Coleman (1986) could be due to assay or strain differences.

The finding of the comparatively high activity of amylase and the low activity of maltase in *O. caudatus* suggested that this ciliate may get its energy by maltose phosphorylation. The pathway of starch degradation in a few species of rumen protozoa has been studied in more detailed. Although there is some α-glucan phosphorylase and maltose phosphorylase activity in *E. caudatum* the major route of starch utilisation is by hydrolysis to maltose and then glucose followed by phosphorylation of the latter to glucose-6-phosphate (Coleman 1969). In *Epidinium ecaudatum caudatum* the two phosphorylases are more active than in *E. caudatum*. Glucose phosphates are probably produced from starch by the action of the phosphorylase and from glucose in the presence of hexokinase (Coleman and Laurie 1976). In *P. multivesiculatum* the phosphorylase pathway is the more important. Starch is degraded probably to glucose-1- phosphate, which is isomerized to glucose-6-phosphate (Coleman and Laurie 1977). The glucose-6-phosphate is in rumen protozoa synthesized into polysaccharide or broken down by glycolysis (Coleman 1986).

The present investigation has shown a high activity of amylase, maltose phosphorylase and hexokinase in *O. caudatus*. These results suggest that most of the starch is probably hydrolysed to maltose followed by its phosphorylation by maltose phosphorylase to glucose-1-phosphate which is probably isomerized to glucose-6-phosphate. Glucose liberated from maltose is phosphorylated in the presence of hexokinase to glucose-6-phosphate. The proposed pathway of

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starch degradation in *O. caudatus* needs further investigation e.g. optimum pH and kinetic constants of maltose phosphorylase, hexokinase, phosphoglucomutase and α–glucan phosphorylase.

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The Small Intestine in Experimental Giardiasis of Rats. Morphological Changes and Mast Cell Response

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Summary. Three–week old rats were orally infected with 10.000 *Giardia muris* cysts. The elimination of the parasite and the histological changes in the jejunum were studied for an observation period of 56 days. During the whole period the parasites could be observed in an intraluminar position in the jejunum (trophozoites) and in the feces (cysts). The morphological changes in the jejunum were only minor, consisting in a transient change in villus–crypt ratio, goblet cell increase, focal infiltration with lymphocyte like cells and plasma cells. Of particular interest was the proliferation of mucosal mast cells observed from 28 to 56 days post infection. It was concluded that the rat might serve as a model for longstanding *Giardia* infections accompanied by minor pathological changes in the gut and proliferation of mucosal mast cells. In addition to the well documented mucosal mast cell proliferation during a helminth infection, this study indicates that also a protozoan parasite is capable of inducing a mucosal mast cell response.

Key words. *Giardia muris*, giardiasis, small intestine, mast cells response.

INTRODUCTION

Giardiasis is endemic in every country in the world and recently the clinical and epidemiological evidences for a marked pathogenicity of *Giardia intestinalis* in man have increased. Despite recent investigations on *G. intestinalis* and giardiasis many aspects of the pathophysiology remain to be answered. The clinical spectrum of giardiasis varies widely, including varying degrees of malabsorption. There is evidence that this disorder is not due to the villous atrophy (Tewari and Tandon 1974, Levinson and Nastro 1978, Hartong et al. 1979) and that the morphological changes of the jejunal mucosa may be slight and nonspecific. On the other hand it is unknown why some patients remain asymptomatic. A correlation of symptoms with the parasite's infecting dose, parasite strain variability, host susceptibility, and host–related factors has been suggested.

To study these problems experimental models have been developed in mice and Mongolian gerbils (Roberts–Thompson et al. 1976, Craft 1982, Belosevic et al. 1983). We found that the rat model of giardiasis induced experimentally is characterized by a long–term chronic infection without negative stools and spontaneous elimination of the parasite (Majewska and Kasprzak 1983, Majewska 1985).

The aim of the present study was to observe the morphological changes of the small intestine of rats infected experimentally with *Giardia* and to further define the *G. muris*–rat model.

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MATERIALS AND METHODS

P a r a s i t e. *Giardia muris* cysts were obtained from the same source as in the previous investigations, i. e. from fresh feces of cyst-passing rats infected experimentally with cysts (Majewska and Kasprzak 1983). In brief, *Giardia* cysts were purified by a slight modified procedure of Meyer (1979) and described elsewhere (Majewska and Kasprzak 1983). The final pellet was resuspended in tap water and cysts were counted in a hematocytometer chamber. The volume of the inoculum was 0.1 ml and the number of cysts was 10.000. The number of viable cysts calculated using the Bingham and Meyer excystation procedure (1979) was about 1000 (10%) and determined by the eosin-exclusion method was about 9500 (95%).

A n i m a l s. Three-week-old weanling outbred Wistar rats of either sex, weighing 25-30 g, from the sixth *Giardia*-free generation were used. The non-infected and infected animals were housed in separate cages and fed on standard pellet diet and water ad lib.

P a r a s i t o l o g i c a l e x a m i n a t i o n. Fresh feces were collected daily and suspended in tap water. A representative number of smears examined microscopically (at least 21 per time after infection) was prepared. The number of cysts was estimated semiquantitatively as + (few cysts in whole preparation), or + + and + + + (1 to 5 cysts and over 5 cyst per microscopic field at total magnification 300x respectively).

A u t o p s y. Ten-centimeter segments of jejunum were removed, dissected longitudinally and rolled on a stick ("Swiss-roll" method by Mollenbeek and Ruitenberg 1981).

H i s t o l o g i c a l e x a m i n a t i o n. One "Swiss-roll" was fixed in Carnoy's solution and embedded in paraffine. The sections were stained with haematoxylin and eosin for conventional histological assessment. The cellular infiltrations were differentiated by using the Unna-Pappenheim method. The Periodic Acid Schiff (PAS) stain was used to demonstrate goblet cells and mucus. Another "Swiss-roll" of the jejunum was fixed in formalin acetic acid (Enerbäck 1966a,) and embedded in paraffine. The sections were stained with toluidine blue to demonstrate both the mucosal mast cells and connective tissue mast cells as well as the intraepithelially located globular leucocytes. In haematoxyline-osin stained, well-oriented sections the intestinal architecture was assessed by measurements of the villus crypt ratio, the villus configuration and the cellular infiltration in the lamina propria.

E x p e r i m e n t a l d e s i g n. The animals were inoculated intragastrically by the inoculation of 10.000 cysts each. The rats were divided in five groups each consisting of three infected and two non-infected animals, and killed on day 7, 14, 28, 42 and 56 post infection (p. i.).

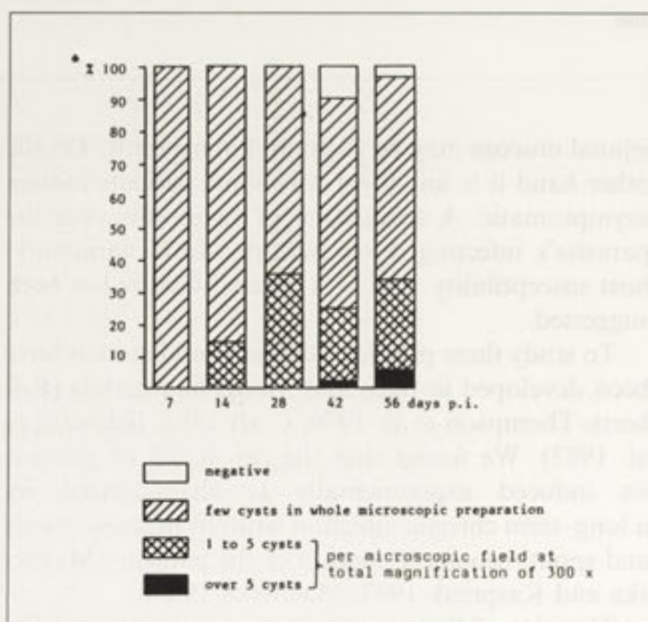

Fig. 1. Cyst excretion rate at the authopsy days of rats infected with *G. muris;* *the percentage of preparations in particular classification (= cyst excretion rate) was calculated from a representative number of preparations.

RESULTS

Parasitological examinations

Daily microscopic control of feces revealed that the infection was of long duration; all the rats were infected throughout the whole observation period. The pattern of cysts excretion showed little variability; after a 4 to 8 days period all the rats were persistently excreting cysts. The number of cysts in the feces was not high (in most samples designated as "+"), but it rose by the second week and remained so through the whole observation period. In Fig. 1 the data obtained at the autopsy days are given to allow a direct comparison with the histological results.

Histological examinations

The histopathological changes of jejunum are surveyed in Table 1. In the groups of rats killed on day 7 p. i. the intestinal architecture of the jejunum was normal (Fig. 2). The villi were mostly finger-shaped and covered with a single layer of epithelium. Also the lamina propria was normal (Fig. 2); there was little infiltration with only few lymphocytes and with mast cells of the connective tissue mast cells type. The PAS stained sections demonstrated a normal number of goblet cells in a characteristic position. In the Peyer's patches the germinal centers were of low activity. In contrast to the lack of abnormalities in the intestinal architecture, a significant PAS-positive reaction of the mucus at the apical side of the epithelial cells (Fig. 5) and some morphological signs of a hyperplasia of the crypts – which were equal in length with the villus height – were observed. In the mucus over the villi many *Giardia* trophozoites could be observed.

At day 14 p. i. slight changes in a few villi, which were broadened, and some hyperplasia of the intestinal glands – deeper with flattening of the epithelial cells – were found. Furthermore, marked activation of the germinal centers in the Peyer's patches was observed.

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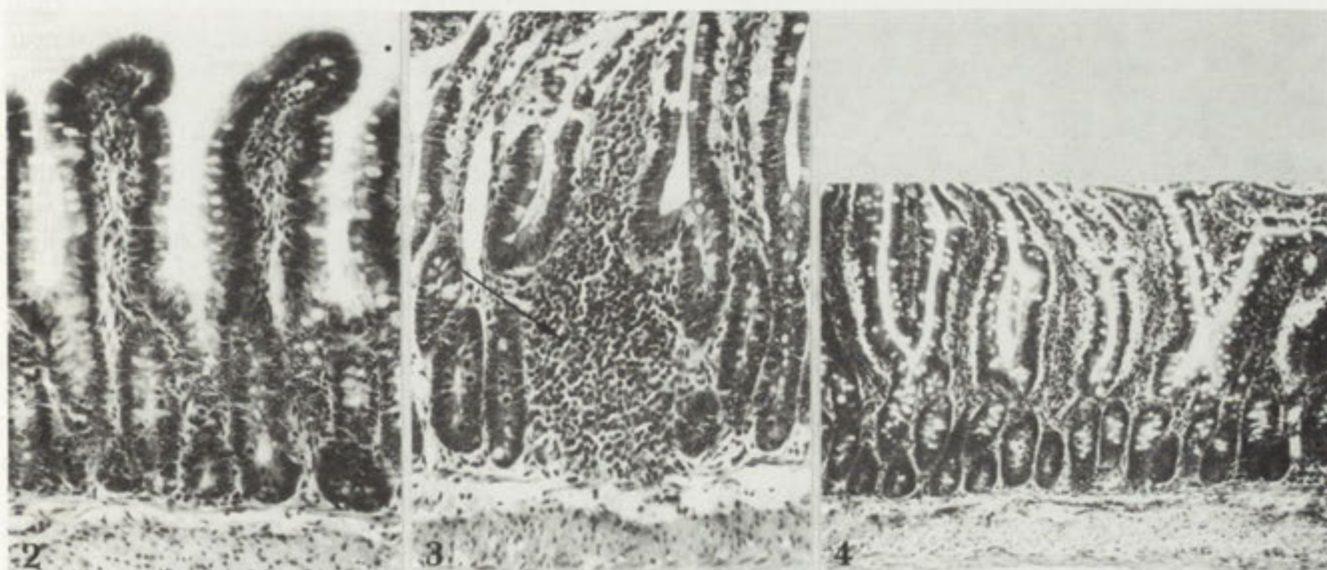
Table 1

Survey of changes in the jejunum of rats infected experimentally with *Giardia muris*

Days post-infection	Villi	Intestinal glands	Lamina propria	Payper's patches	*Giardia* trophozoites (intraluminar)
7	Finger-like. Goblet cells normal in location and number	Hyperplasia	No changes	Low activity of germinal centres (GC)	Numerous
14	Few villi broadened. Increased number of goblet cells near crypts	Hyperplasia	Slight increase of mucosal mast cells (MMC) numbers	Activation of GC	Numerous
28	Some villi shortened. Proliferation of entero-cytes. Goblet cells located near the crypts	Hyperplasia	Slight infiltration of lymphocyte-like cells and plasma-cells. Numbers of MMC the same as on day 14 p. i.	Prominent activation of GC	Single
42	Some villi broadened, shortened. Gobled cells accumulated near the crypts	Normal, only few with sings of hyperplasia	Slight infiltration of lymphocyte – like cells and plasma-cells. Significant increase in numbers of MMC	Prominent activation of GC	Single
56	Most villi of normal architecture	Normal	Slight fibrosis. Single cellular infiltration with lymphocyte-like cells and plasma-cells. Significant increase in MMC numbers	Low activity of GC	Very few



Figs. 2-4. Preparations of jejunum, stained with haematoxylin-eosin. 2 – Day 7 p. i.; normal architecture, numerous *Giardia* trophozoites in micus. 100x. 3 – Day 28 p. i.; focal infiltration with lymphocyte-like cells (arrow). 80x. 4 – Day 56 p. i.; minor cellular infiltration with lymphocyte-like cells and plasma-cells and slight fibrosis. 50x.

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Adjacent to the Peyer's patches mast cells with the morphological characteristics of mucosal mast cells were observed (Fig. 8). In the lamina propria small infiltrations composed of lymphocyte–like cells were present. Many trophozoites of *Giardia* were found between the villi.

Figs. 5-7. Preparations of jejunum, Periodic Acid Schiff stain. 5 – Day 7 p. i.; marked PAS-positive reaction of mucus (arrow.) 60x. 6 – Day 28 p. i.; marked PAS-positive reaction of goblet cells (arrow). 80x. 7 – Day 56 p. i.; marked PAS-positive reaction of the goblet cells located also in crypts (arrow). 60x.

In the group of rats sacrificed on day 28 p. i. slight focal changes were observed; the changes concerned mainly a shortening of individual villi and the hyperplasia of the intestinal glands (Fig. 3). The goblet cells were agglomerated chiefly at the base of the villi, close to the intestinal crypts. The PAS–positive reaction of the goblet cells were very marked (Fig. 6). Focal infiltration of lymphocyte–like cells (Fig. 3) and some eosinophils were observed. In the number of mucosal mast cells, aggregated mainly close to the Peyer's patches the germinal centers were prominently activated, and close to the patches the numbers of mucosal mast cells were increased (Fig. 9). Some trophozoites were observed between the villi.

On day 42 p. i. most of the villi were finger–shaped but a few showed some deformation (they were shorter and broader). In the lamina propria focal infiltrations of lymphocyte–like cells and plasma cells were present. The intestinal glands were smaller and of more regular shape. The goblet cells showed a very marked PAS–reaction. In the jejunum a significant increase in the number of mucosal mast cells, aggregated mainly close to the Peyer's patches, was observed. In the patches the germinal centers were clearly activated. Single trophozoites were present in the lumen between the villi.

In rats killed on day 56 p. i. most villi were of normal architecture, and only a few showed deformation and were shortened. The intestinal glands were of normal shape and size. The goblet cells were also located near the crypts and showed a marked PAS–positive reaction (Fig. 7). In the lamina propria a small cellular infiltration (lymphocyte–like cells and plasma cells) and a slight fibrosis were seen (Fig. 4). Also, at that observation day the number of mucosal mast cells was increased (Fig. 10). Intraluminar *Giardia* trophozoites were only occasionally seen.

DISCUSSION

The experimental infection with *G. muris* in rats under *Giardia* free conditions is, contrary to the infection of conventionally reared animals, characterized by a long duration; the infection persisted throughout the whole observation period. The number of parasite cysts in the feces was not high but rose by the second week and remained so thereafter. We found a similar pattern of infection in the previous experiments (Majewska and Kasprzak 1983, Majewska

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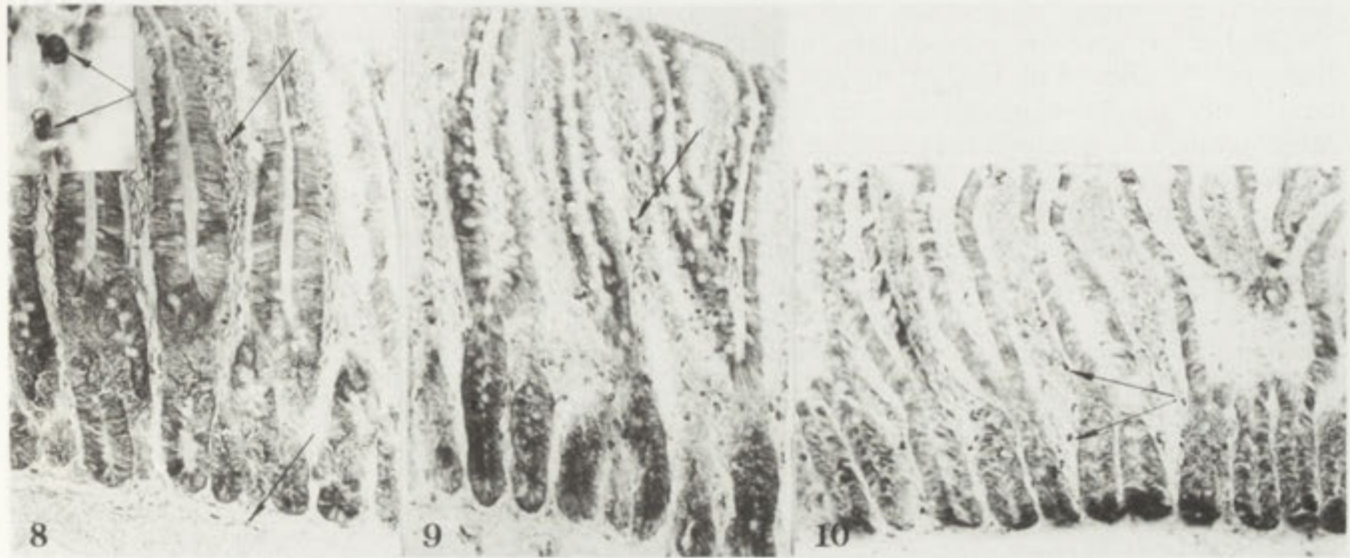

Figs. 8-10. Preparations of jejunum, stained with toluidine blue. 8 – Day 7 p. i.; single musocal mast cells (arrows). 60x. Insert 300x. 9 – Day 28 p. i.; increased number of mucosal mast cells (arrow). 80x. 10 – Day 56 p. i.; numerous mucosal mast cells. 80x.

1985). Snider et al. (1982) observed a prolonged infection with *G. muris* in mice of the C3H/He lineage and found that the ability to clear the infection was dependent on the sex, the age at infection time, and the environmental conditions during rearing of the young mice (SPF vs conventional). Roberts–Thomson et al. (1980) and Belosevic et al. (1982) also found that the course of a *G. muris* infection in various strains of mice differed in intensity and duration of cysts excretion.

In experiments conducted hitherto by other authors, the peak of cyst excretion was in the initial period of infection, the cysts being undetectable after several weeks (Bemrick 1962, Roberts–Thomson 1976, 1980, Nair et al. 1981, Gillon et al. 1982, Craft 1982). The experimental rat model described in the present study is characterized by a prolonged parasite infection, without spontaneous elimination of the parasite or even gradual decrease in cyst excretion. In spite of the fact that the cyst excretion remained high until the end of the observation period, the number of intraluminar trophozoites based on assessment in histological sections of the small intestine decreased rapidly at 28 days post–infection.

The changes in the small intestine observed in this experiment differ from those in man (Yardley et al. 1964, Nath et al. 1974, Ridley and Ridley 1976, Hartong et al. 1979) or mice (Roberts–Thomson et al. 1976, Ferguson et al. 1980, Gillon et al. 1982). In our studies the changes were slight and focal, the lining epithelium was normal, and the cellular infiltrations in the lamina propria were slight and focal.

The intrinsic observation in this study was the significant increase in numbers of mast cells with morphological characteristics of mucosal mast cells after prolonged parasite infection. In the study of jejunum biopsies from patients with giardiasis Kocięcka et al. (1984) observed that the numbers of mucosal mast cells and their locations were normal although the jejunum morphology was abnormal.

Mucosal mast cells form a subpopulation of thymus–dependent mast cells (Ruitenberg and Elgersma 1976); in the rat they lack heparine (Tas and Berndsen 1977). So far mucosal mast cells have been observed in both experimental and clinical infections with helminths. In a patient with trichinellosis a marked increase of mucosal mast cells in the jejunum was described (Gustowska et al. 1983). The observation authenticates the data from experimental *Trichinella* infections in rats (Ruitenberg et al. 1979, Vos et al. 1983), mice (Ruitenberg and Elgersma 1976) and monkeys (Teppema et al. 1981) as well as *Nippostrongylus* infections in rats (Befus and Bienenstock 1979, Nawa and Miller 1979) and *Hymenolepis* infections in rats (Hindsbo et al. 1982) and mice (Andreassen et al. 1978).

In the tissue invading nematodes *Trichinella* (Ruitenberg et al. 1979) and *Nippostrongylus* (Nawa and Miller 1979) mucosal mast cells were observed both in the stroma and the epithelial lining, the latter ones being referred to as globule leucocytes (Ruitenberg et al. 1979). In the lumen–dwelling cestode *Hymenolepis*,

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however, an increase of mucosal mast cells was observed in the rat without an increase in globule leucocytes (Hindsbo et al. 1982). Until now it is not clear whether the globule leucocytes represents a separate population of metachromatic cells or whether they are derived from mucosal mast cells (Vos et al. 1983). The present study shows that mucosal mast cells can also proliferate during a non–helminth infection. Furthermore, this mucosal mast cell proliferation is not accompanied by an increase in globule leucocytes. The possibility that this mucosal mast cell response was due to a accidental infection with helminths present in the animal house can be ruled out.

Our studies indicate that also the rat may be used as a model in studying intestinal pathomorphology of *Giardia* infections. In contrast to the murine model the rat may be used for observation of host–parasite relationship in long–standing infections. Of particular interest is the observation that this protozoan infection is accompanied by the proliferation of mucosal mast cells.

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Note sur Difflugia humilis sp. n. (*Protozoa: Rhizopoda: Testacea*)

Note on *Difflugia humilis* sp. n. (*Protozoa: Rhizopoda: Testacea*)

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Résumé. Dans cette note, nous décrivons unee spéce nouvelle de Thecamoebien *Difflugia humilis* sp. n. observé parmi les plantes aquatiques et les Sphainges en Irlande et en Belgique.

Mots clés. *Difflugia humilis* sp. n., les plantes aquatiques, Sphaignes, Irlande, Belgique.

Summary. In this note, we describe a new species of Testate Amoeba *Difflugia humilis* sp. n. from Aquastic plant and Sphagnum from Ireland and Belgium.

Key words. *Difflugia humilis* sp. n., aquastics plant, Sphagnum, Ireland, Belgium.

Au cours d'une étude sur les Thecamoebiens d'Irlande (Beyens et Chardez 1984), dans un prélèvement de sapropèle fixé au formol, nous avions observé une espèce particulière. Vue en trop peu d'exemplaires sous forme de thèques vides, nous ne l'avions pas retenue dans nos listes, nous l'avions toutefois consignée dans nos cahier as de laboratoire.

Depuis, nous avons retrouvé cette espèce parmi des Sphaignes immergées en Belgique, ce qui nous a permi d'en faire une étude plus complète, objet de cette note.

Difflugia humilis sp.n. (Fig. 1 b, 2-9).

Cette espèce très petite, semble habiter de préférence des niches écologiques riches en Diatomées, dans des eaux claires, à faible pH: variant de 4,3 à 5.

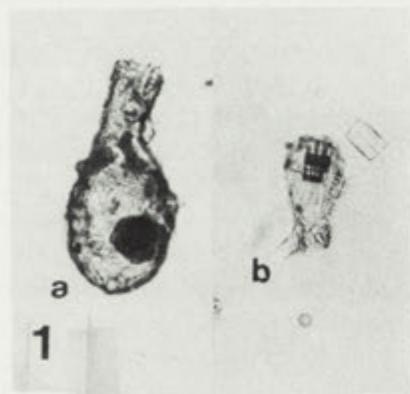

Fig. 1
a) *Difflugia bacillifera* Penard
b) *Difflugia humilis* sp. n. (X 200)

Fig. 1 Diagnose: Thèque allongée ou ovale, section transversale plus ou moins circulaire, transparente, les flancs convergent vers le pseudostome et forme un col plus ou moins net, ou brusqument tronqués, sans col apparent. Le pseudostome est circulaire et étroit, souvent irréguilier dans ses contours.

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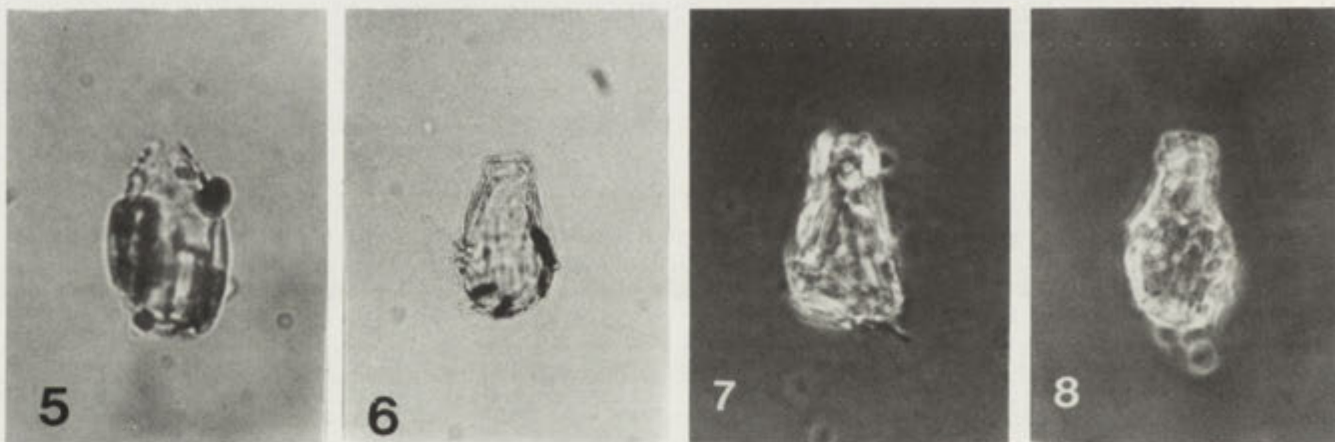
Fig. 2 à 8. *D. humilis* sp. n. (X 400)



La thèque est formée d'un vernis organique d'origine endogène, consolidée par un revêtement externe constitué de Diatomées, qui la recouvrent entièrement, lui confèrent un contour irrégulier, conséquence d'une certaine variation intraspecrfique. (Fig. 2-8).

Fig. 2-8. Dimensions: Hauteur = 40-60 μm, Diamètre = 22- 36,5, pseudostome = 8-15 μm

Moyennes (N = 10): H = 46,98 μm, D = 27,45, pseudostome = 9,37 μm.

Fig. 9. Le cytoplasme est trés claire, finement ponctué, les pseudopodes de type „Lobé" consistent le plus souvent en une émission formant un seul pseudopode (Fig. 9).

Le corps du Rhizopode, est souvent massé au niveau du col ou du pseudostome et n'accupe que les 2/3 du volume interne de la thèque.

Une pulsole située généralement vers le milieu du corps. La progression par reptation est lente.

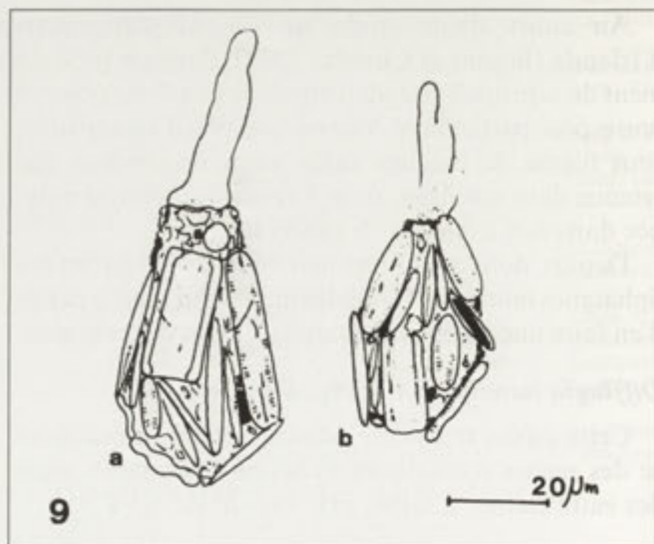


Fig. 9. *D. humalis* sp. n.: émissions pseudopodiques. (Les figures 9 b et 5, montrent des exemples de spécimens dont le col est absent, ou peu apparent).

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Un seul noyau, sphérique d'un diamètre de 9,5 á 10 μm.

Ecologie: Dépôs de fond parmi les plantes aquatiques et parmi les mares à Sphaignes les toubières et les sédiments riches en Diatomées.

Répartition: Irlande, Région d'Allihies; Sapropèle d'une zone marécageuse d'un étang, parmi une flore principalement constituée de *Juncus acutiflorus, Ranunculus flammula* et *Eleocharys sp.* pH 4,5. (Leg. Beyens).

Belgique: Plusieurs stations en Hautes Fagnes; Fanges Wallones: mares à Sphaignes immergées pH 5.

Préparation Type: n° V20, Cotypes : n° Y15 et M18; déposées dans les collections de la Faculté des Sciences Agronomiques de l'Etat, Gembloux.

DISCUSSION

L'extrême petite taille de cette nouvelle espéce, ne nous permet pas de l'assimiler à *Difflugia bacillifera* Penard avec qui elle présente certaines affinités telles que: le revêment de la thèque et l'écologie.

En effet la différence de taille est absolument trop importante *D. bacillifera* a une hauteur variant de 90 à 160 μm (Fig. 1a, b). L'étude du noyau, montre égalament une différence de diamétre considérable 30 μm chez *D. bacillifera* et 9,5 à 10 μm chez *D. humilis*.

Outre les dimensions qui varient quelque-peu chez *D. humilis*, les principales variations intraspécifiques se situent au niveau du col, qui n'est pas toujours présent ou visible. Ce phénomène est dû à la présence de Diatomées, souvent de grande taille masquant quelquefois l'ouverture du pseudostome.

Les Diatomées utilisées par *D. humilis*, sont d'espèces et de genres variés, toujours nombreux dans les tourbières et parmi les plantes aquatiques.

D. humilis utilise uniquement des valves ou des frustules non remaniées.

Cette nouvelle espèce, n'est certainement pas rare, mais sa taille réduite et souvent dissimulée par son revêtement la rend peu visible, elle apparait comme une espèce typique des biotopes riches en Diatomées, oû on la rencontre a côté d'espéces caractéristiques de ces milieux: *Difflugia rubescens, Difflugia bacillifera, Difflugia dujardini, Centropyxis aculeata* et ses variétés, *Netzelia oviformis*, ainsi que de nombreuses espèces de *Nebela, Quardulella* et *Euglypha*.

Avec *Difflugia pulex* Penard, c'est certainement une des plus petites espèces du genre.

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ACTA
PROTOZOOLOGICA

Le Genre *Cyphoderina* Schlumberger, 1845 (*Protozoa: Rhizopoda: Testacea*)

The Genus *Cyphoderia* Schlumberger, 1845 (*Protozoa: Rhizopoda: Testacea*)

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Résumé. Dans cette note monographiques, nous proposons une révision des espéces du Genre *Cyphoderia,* basée sur la forme générale et la disposition des écailles de la thèque Nous décrivons également deux espèces nouvelles pour la Science: *Cyphoderia venustus* sp. n. et *C. ventricosa* sp. n.

Most clès. Genre *Cyphoderia, C. venustus* sp. n. *C. ventricosa* sp. n., *Testacea,* Révision taxonomique.

Summary. In this monographical note a revision of species of the genus *Cyphoderina* Kind is proposed. The differenciation characters between them are pointed, such as morphology of tests disposition of scales, measurements and ecology. Two new species *Cyphoderia venustus* sp. n. and *C. ventricosa* sp. n. are also described.

Key words. Genus *Cyphoderia, C. venustus* sp. n. *C. venricosa* sp. n. *Testacea,* taxonomic revision.

Depuis 1845, date de la création du Genre *Cyphoderia* par Schlumberger et l'importante étude sur les Rhizopodes par Leidy en 1879, de nombreux auteurs ont singalé et étudié beaucoup d'espéces de ce genre important;

Plusieurs classifications ont été établies ou proposées, Husnot (1942), changeant le nom de l'espèce la plus commune „*ampulla*" en „*communis*", divise le Genre en deux „sous-genres" *Parvialveolata* et *Latialveolata;* comportant cinq espèces et dix variétés. Cette façon de voir trop artificielle, ne fut pas retenue.

D'une façon plus simple et plus judicieuse, Wailes et Penard (1911), avainet divisé l'ensemble des espèces en trois groupes basés sur la morphologie générale et les types de structure de la thèque, distinguant les thèques de section circulaire, composées d'écailles imbriquèes ou non et les théques de section circulaire, composées d'écailles imbriquées ou non et les thèques de section plus ou moins triangulaires ou irrégulières.

Nous basant en grande partie sur ce principe, nous proposons une révision des espèces existantes, avec la description de deux espèces nouvelles.

Diagnose de Genre

Le Genre *Cyphoderia* est caractérisé par une thèque allongèe dont le col est recourbé suivant l'axe lon-

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gitudinal à la partie antèrieure. Le fond est arrondi, acuminè ou mammeloné suivant l'espećce.

Le revêtement est constitué de petites écailles siliceuses circulaires ou elliptiques biconvexes, régulièrement imbriquées suivant l'espèce. Ces écailles sont incrustées dans un vernis organique transparent, jaunâtre ou brunâtre suivant l'âge.

La disposition des écailles présente plusieurs types d'imbrication:

a) jointives; les écailles se recouvrent partiellement par les bords,

b) non jointives; les écailles sont plus ou moins espacées.

Le pseudostome ne posséde pas de lévre.

Les caractéres descriptifs d'une espèce doivent être aussi simple que possible, c'est pourquoi il nous semble important, sans refaire les diagnoses des espèces décrites, d'en résumer simplement les critères essentiels qui caractérisent chaque espèce: Ces caractères sont les suivants: Section transversale de la thèque, forme du fond et du col, forme des écailles et leur disposition, enfin, la longueur totale moyenne (les dimensions extrêmes sont reprises dans le Tableau 1.)

Systématiquement, le Genre *Cyphoderia* se place dans la Classe des *Filosea* Leidy, 1879, Sous-classe des *Testaceafilosia* De Saedeleer, 1934, Ordre des *Gromiida* Claparede et Lachmann, 1859, Sous-orde des *Euglyphina* Bovée, 1985.

Le Genre comporte 11 espèces, 9 variétés et1 forme actuellement décrites, rassemblées dans la Famille des *Cyphoderiidae* De Saedeleer.

L'espèce type est *Cyphoderia ampulla* (Ehrenberg) Leidy, 1879.

Dans son ensemble, le Genre est essentiellement aquatique au sens large, la majorité des espèces se rencontrent dans le sapropèle, la vase et les sédiments du fond des étangs, lacs, mares et ruisseaux, également dans les Sphaignes et les Mousses humides. Certaines espèces semblent plus fréquentes dans les Mousses et le périphyton des plantes aquatiques. Quelques espèces sont psammophiles ou psammobiontes strictes et vivent dans le mésopsammon supralittoral des Mers et Océans.

Les espèces

Groupe I: Sections transversale de la thèque circulaire. Ecailles jointives.

Tableau 1

Genre *Cyphoderia* Schlumberger, 1845. Dimensions exstrèmes des espèces

	L	D	Ps	E
Cyphoderia ampulla	80-130	40-80	16-28	1.2-2
Cyphoderia ampulla v. *major*	180-260	65-90	16-30	3-4
Cyphoderia ampulla v. vitrae	87-120	32-50	12-18	2
Cyphoderia ampulla v. *vitrae* f. *papillosa*	67-120	32-50	12-18	2
Cyphoderia ampulla v. *papillată*	110-120	43-49	14-16	2
Cyphoderia ampulla v. *crassa*	100-120	38-75	16-28	1.5-2
Cyphoderia ampulla v. *thomassi*	80-100	38-50	14-16	1.5-2
Cyphoderia ampulla v. *bicornis*	140	90	20	?
Cyphoderia loevis	35-50	21-25	8-10	?
Cyphoderia myosorus	125-130	?	?	2.3-3
Cyphoderia perlucidus	50-58	25-27	10-11	0.6-0.9
Cyphoderia schonborni	123-144	81-91	21-23	?
Cyphoderia venustus	45-58	20-24	8-10	0.8-1
Cyphoderia ventricosa	70-80	40-43	12-14	0.12-0.6
Cyphoderia trochus	100-115	45-55	18-20	2.5-3
Cyphoderia trochus v. *imbricata*	100-125	30-40	15-18	2.5-3
Cyphoderia trochus v. *amphoralis*	87-153	38-52	13-17	3-5
Cyphoderia trochus v. *palustris*	100-125	30-40	15-18	3-3
Cyphoderia calceolus	150-185	65-80	19-32	2-3
Cyphoderia compressa	82-112	30-46	10-18	1.5
Cyphoderia littoralis	38-51	18-26	7-12	?
Cyphoderia littoralis v. *shimodensis*	38-51	?	?	?

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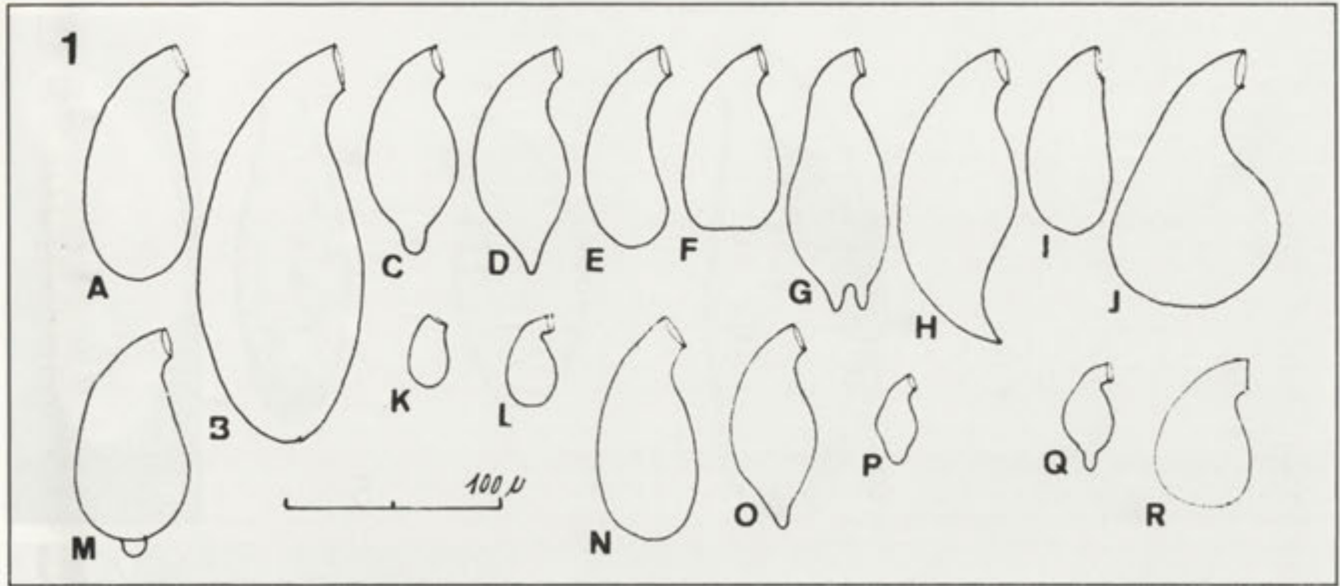

Fig. 1. Ensenble de silhouettes vues de profil. A – *C. ampulla*, B – *C. ampulla* v. *major*, *C. ampulla* v. *vitrae*, D – *C. ampulla* v. *papillata*, E – *C. ampulla* v. *crassa*, F – *C. ampulla* v. *thomasi*, G – *C. ampulla* v. *bicornis*, H – *C. calceus*, I – *C. compressa*, J – *C. schonborni*, K – *C. loevis*, L – *C. perlucidus* M – *C. trochus*, N – *C. trochus* v. *amphoralis*, O – *C. trochus* v. *palustris*, P – *C. littoralis*, Q – *C. venustus*, R – *C. ventricosa*.

Cyphoderia ampulla (Ehrenberg) Leidy, 1879

Syn.: *Difflugia ampula* Ehrenberg, 1840, *Cyphoderia margaitacea* Schlumberger 1845 *Cyphoderia communis* Husnot, 1943, *Euglypha curvata* Perty, 1852

Caractéristiques: Fond de la théque arrondi, col plus ou moins recourbé, écailles circulaires, longuer 120 μm. Aquatique au sens large. Cosmopolite.

Cyphoderia ampulla v. *major* Penard, 1902

Caractéristiques: Fond de la thèque arrondi, col plus ou moins recoubé, écailles circulaires, Longueur 220 μm. Aquatique principalement dans les grands fonds.

Cyphoderia ampulla v. *vitrae* Wailes et Penard, 1911

Caractéristiques: Fond de la théque arrondi ou mammeloné, col plus ou moins recoubé, écailles non visibles, longueur 90 Um, principalement dans les Mouses et les Sphaignes.

Cyphoderia ampulla v. *vitrae* f. *papillosa* Wailes, 1915

Caractéristiques: Diffère de la variété par la présence d'un prolongement caudal, pointu ou arrondi. Longueur 95 μm.

Cyphoderia ampulla v. *papillata* Wailes et Penard, 1911

Caractéristiques: Fond de la théque arrondi mammeloné, col plus ou moins recourbé, écailles circulaires, longueur 120 μm. Principalement dans les Mousses et les Sphaignes.

Cyphoderia ampulla v. *crassa* (Husnot) Thomas, 1955

Caractéristiques: Cette variété se distigue de l'espèc e par les flances de la théque, qui suivent une courbure presque parallèle lui donnat un aspect plus trapu, le col est généralement assez peu recourbé. Longueur 110 μm.

Cyphoderia ampulla v. *thomasi* Chardez, 1956

Caractéristiques: Le fond de la théque est plat, col peu recoubé, aquatique au sens large. Longueur 90 μm.

Cyphoderia ampulla v. *bicornis* Stepanek, 1963

Caractéristiques: Fond de la théque possédant deux prolongements en forme de cornes creuses, col fortement recourbé, longeur 140 μm. Aquatique.

Cyphoderia loevis Penard, 1902

Caractéristiques: Fond de la théque arrondi, parfois muni de quelques particules minérales, col très peu recourbé, Lonqueur 40 μm. Aquatique.

Cyphoderia myosorus Penard, 1908

Caractéristique: Fond de la théque acuminé en pointe creuse, col peu recourbé, écailles ovales ou elliptiques, longuer 125 μm. Aquatique.

Cyphoderia perlucidus Beyens et Chardez, 1986

Caractéristiques: Fond de la théque arrondi, col fortement recourbé, longuer 54 μm. Thèque toujourst rés transparente. Principalement dans les Mousses et le périphyton des plantes aquatiques.

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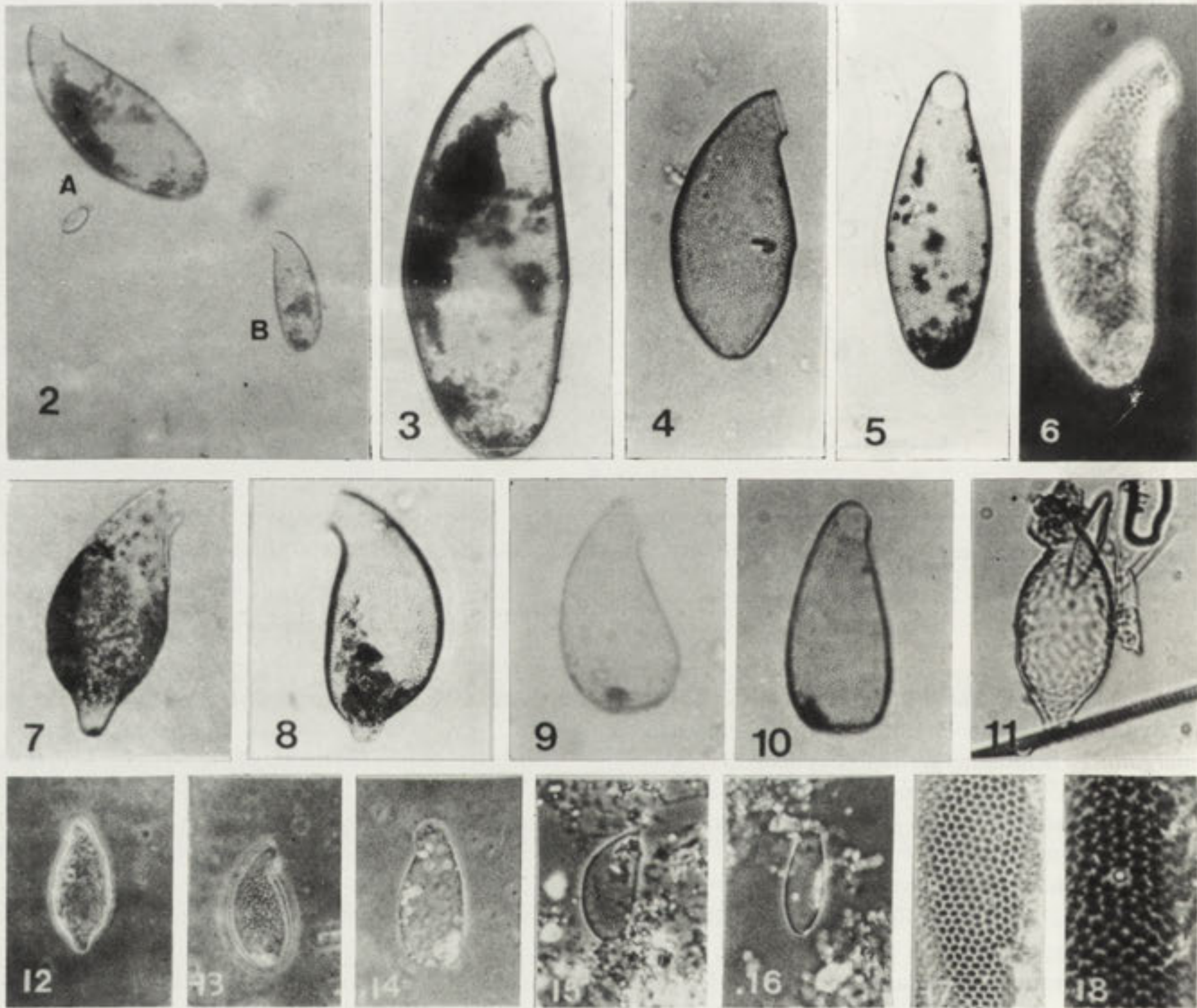

Figs. 2–18. Les espéces du geure *Cyphoderia*. 2A – *C. ampulla* v. *major*, 2B – *C. ampulla* (X100), 3 – *C. ampulla* v. *major* (X400), 4 – *C. ampulla* (X400), 5 – *C. ampulla* (vue de face (X400), 6 – *C. ampulla* v. *crassa* (X400), 7 et 8 – *C. ampulla* v. *vitrae* (X400), 9 – *C. ventricosa* (X400), 10 – *C. ampulla* v. *thomasi* (X400), 11 – *C. venustus* (X600), 12 – *C. venustus* (X400), 13 – *C. perlucidus* (X400), 14 – *C. loevis* (X400), 15 – *C. compressa* (X400), 16 – *C. compressa* (vue de face X400), 17 – Ecailles juinitives (X1000), 18 – Ecailles superposées. (X1000)

Cyphoderia venustus: sp. n.

Diagnose: Théque allongée, de section transversale circulaire, le fond est plus ou poins ogival, terminé par un prolongement mammelliforme quelquefois assez long et étroit. Le col est court, fortement recourbé et bien net. Le revêtement est classique, constiué d'écailles circulaires jointives plus grandes sur le corps qu'au niveau du col et fond de la théque.

Dimensions moyennes: longuer 53 μm, diamétre 22 μm, hauteur du col 9,5 μm diamétre du pseudostome 8,5 μm, écailles 0,9 μm. Cette nouvelle espèce est actuellement connue de Mousses épigées humides de la région d'Olafsik (alt 324 m) Islande, et du sapropèle de l'étang de Virelle, Belgique. Dans une première étude (1969), nous avions figuré cette espèce sans la nommer (fig. 38 pl. 7).

Cyphoderia ventricosa sp. n.

Diagnose: Thèque trapue, ventrue, de section transversale circulaire à fond arrondi. Le col est cours, fortement recourbé, présentant un pseudostome circulaire situé sur le même plan que la face ventrale. La structure de la thèque est constitutée de petites écailles elliptiques non jointives, disposéed sans orde.

Dimensions moyennes: longuer 75 μm, diamètre 42 μm, pséudostome 13 μm, ecailles 0,12 X 0,6 μm. Cette nouvelle espèce à été observée dans le sapropèle vaseux

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du fond des étangs Epioux et Stavelot (Belgique).

Groupe II: Section transversale de la thèque circulaire. Ecailles superposées.

Cyphoderia trochus Penard, 1890

Caractéristiques: Fond de la théque acuminé ou mammeloné, col ovalisé latéralement Pseudostome ovale, longueur 100 µm. Aquatique. Cette espèce est susceptible de comporter 3 variétés.

Cyphoderia trochus v. *imbricata* (Penard) Deflandre-Riguaud, 1958

Syn.: *Cyphoderia ampulla* v. imbricata Penard, 1905

Caractéristiques: Fond de la thèque acuminè, col peu recoubé, longuer 110 µm. Aquatique.

Cyphoderia trochus v. *amphoralis* Wailes et Penard, 1911

Caractéristiques: Fond de la théque arrondi, col peu recourbé, longuer 120 µm. Aquatique.

Cyphoderia trochus v. *palustris* Penard, 1902

Caractéristiques: Fond de la thèque mammelonè, col plus ou moins recourbé, longueur 110 µm. Aquatique et dans les Sphaignes.

Groupe III: Section transversale de la thèque non circulaire plus ou moins irréguliére. Ecailles jontives.

Cyphoderia calceolus Penard, 1902

Caractéristiques: Fond de la thèque acuminée en pointe, section plus ou moins triangulaire, col peu recourbé, longueur 170 µm. Aquatique.

Cyphoderia compressa Golemansky, 1979

Caractéristiques: Fond de la thèque arrondi, section ovale, comprimée latéralement col peu recourbé pseudostome ovale, longueur 45 µm. Espèce psammobiotane stricte.

Cyphoderia littoralis v. shimodensis Sudzuki, 1979

Les dimensions et écailles n'ont pas été décrites, cette variété proche de l'espèce devrait être revue.

Species incertea

Cyphoderia ampulla v. *penardi* Kourov, 1925
Cyphoderia truncata Schulze, 1875
Cyphoderia euglyphoides Husnot, 1943

Species excludendae

Cyphoderia communis Husnot, 1943
Cyphoderia communis v. *tubulosa* Husnot, 1943
Cyphoderia communis v. *strangulata* Husnot, 1943
Cyphoderia communis v. *mamillaria* Husnot, 1943
Cyphoderia communis v. *magna* Husnot, 1943
Cyphoderia communis v. *acuminata* Husnot, 1943
Cyphoderia communis v. *acuminata* f. *flexa* Husnot, 1943
Cyphoderia trochus v. *longicollis* Husnot, 1943
Cyphoderia trochus v. *longicollis* Husnot, 1943
Cyphoderia trochus v. *umbonata* Husnot, 1943
Cyphoderia ampula v. *minor* Chardez, 1972

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ACTA
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Urostyla thompsoni Jankowski, 1979 a Little Known Marine Ciliate (*Ciliophora, Hypotrichida*)

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Summary. The morphology and some basic morphogenetic characteristics of a little-known marine hypotrich of the genus *Urostyla* from the Baltic coast are presented. The species corresponds to the brief description of an antarctic hypotrich given by Thompson (1972) and named as *Urostyla thompsoni* by Jankowski (1979)

Key words. *Urostyla thompsoni*, marine ciliate.

INTRODUCTION

Urostyla fhomsoni has been includet into a numerical taxonomic study of Urostylina presented earlier in the same journal (Wiackowski 1988). Now, a detailed description of the species based on protargol stained specimens is given.

MATERIALS AND METHODS

The ciliate was found in a culture of *Prorodon rabbei* Czapik, 1965 which had been maintained for many years in the Department of Hydrobiology, Jagiellonian University. The culture was originally established from a sample of psammon of the brackish Lake Ptasi Raj (Baltic coast near Gdańsk). See Czapik and Jordan (1976) for a description of the site.

Prorodon rabbei, a histophagous ciliate, was fed with pieces of living *Enchytreidae* once a week. The small *Urostyla* living in the same Petri dishes fed on an unidentified colourless flagellate and bacteria growing on the remains of the worm tissue. Since tentative clonal cultures were not successful, study was based on the preparations made with the specimens tak directily from the „wild" culture.

The ciliates were stained with protargol according to the method of Wilbert (1975). A mixture of Bouin's fluid with saturated mercuric chloride (1:1) was used as fixative. The terminology of Borror and Wicklow (1983) was adopted for the description of ciliary structures.

RESULTS

General body form

The morphology of an interphase specimen is shown in Figs. 1–5. The width to length ratio is about 1:2. The cell has an elliptical shape. The ciliate is distinctly dorso-ventrally flattened with the ventral side somewhat concave. The macronucleus (Ma) consists of about 40 oval fragments 9–18 μm long. From 5 to 8 micronuclei (Mi) per cell could be seen. A diameter of a single micronucleus is about 3 μm. The contractile vacuole is typically located on the left side of the cell. The cytoplasm of the living ciliates is transparent with a slight yellowish shade.

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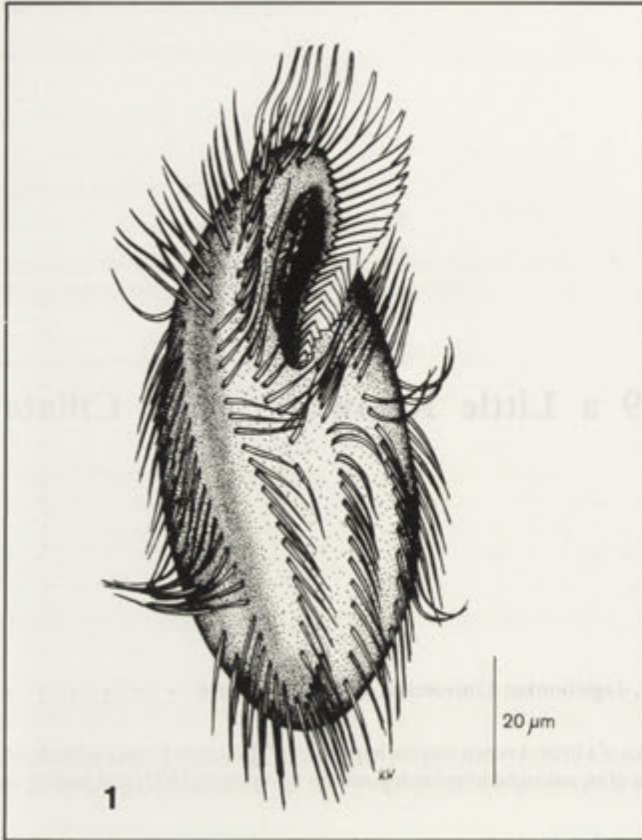

Fig. 1. General picture of *Urostyla thompsoni* based on live and protargol stained preparations.

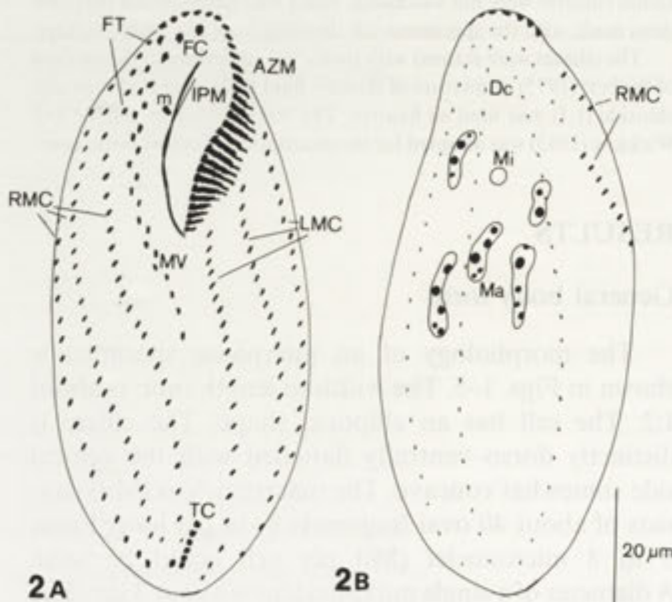

Fig. 2. Cortical anatomy of *Urostyla thompsoni*. Camera lucida drawings of potargol impregnated specimen as seen from the ventral (a) and dorsal (b) sides.

Ciliary structures

The buccal ciliature has the form typical of the majority of hypotrichs (Fig. 4). The adoral zone of membranelles (AZM) extends along the left side of the peristome and curves around the anterior border of the organism. It does not extend onto the right side of the cell. The longest adoral membranelles are located in the middle of the peristome length. The undulating membrane system (UM) consists of two relatively long paroral membranelles. The inner paroral membranelle (IPM) consists of a single row and the outer (OPM) of a double row of kinetosomes. There is a single malar cirrus (m) close to the OPM.

The midventral cirri (MV) have a typical form: a double row of cirri organized in a zigzag line (Figs. 2 and 3) in the anterior half of the organism. The MV are distinctly shortened. They end with a single row of 4–5 cirri just below the midpoint of the cell. A row of about 4–5 fronto-terminal (FT) or "migratory" cirri can be seen on the right side of the MV close to the AZM. There are 3 slightly enlarged (hypertrophied) frontal cirri (FC) in the anterior region (Fig. 4). The row of transverse cirri (TC) consists of about 6 cirri.

Most of the specimens have three marginal rows of cirri on the left and right sides (LMC and RMC). The dorsal surface ciliature (Dc) usually consists of three kineties. However, three specimens with 5 and two with 4 dorsal kineties were noted among 30 specimens studied (Fig. 6). Caudal cirri are absent.

Cortical morphogenesis

The most important features of the morphogenetic process are as follows:

1. The origin of the oral primordium is typical of most urostyline hypotrichs. The new kinetosomes appear in patches on the left side of some MV cirri in the central part of the ventral surface (Fig. 7).

2. The old AZM is completely disorganized and the new one reproduced "de novo" at least during physiological reorganization morphogenesis (Fig. 11).

3. The new marginal rows develop from primordia formed in each of the old marginal row on both sides of the cell. Thus, each of the existing marginal rows participates directly in the development of a new one (Figs. 8, 11).

4. Dorsal primordia develop within each of the existing dorsal kineties on two levels for proter and opisthe respectively.

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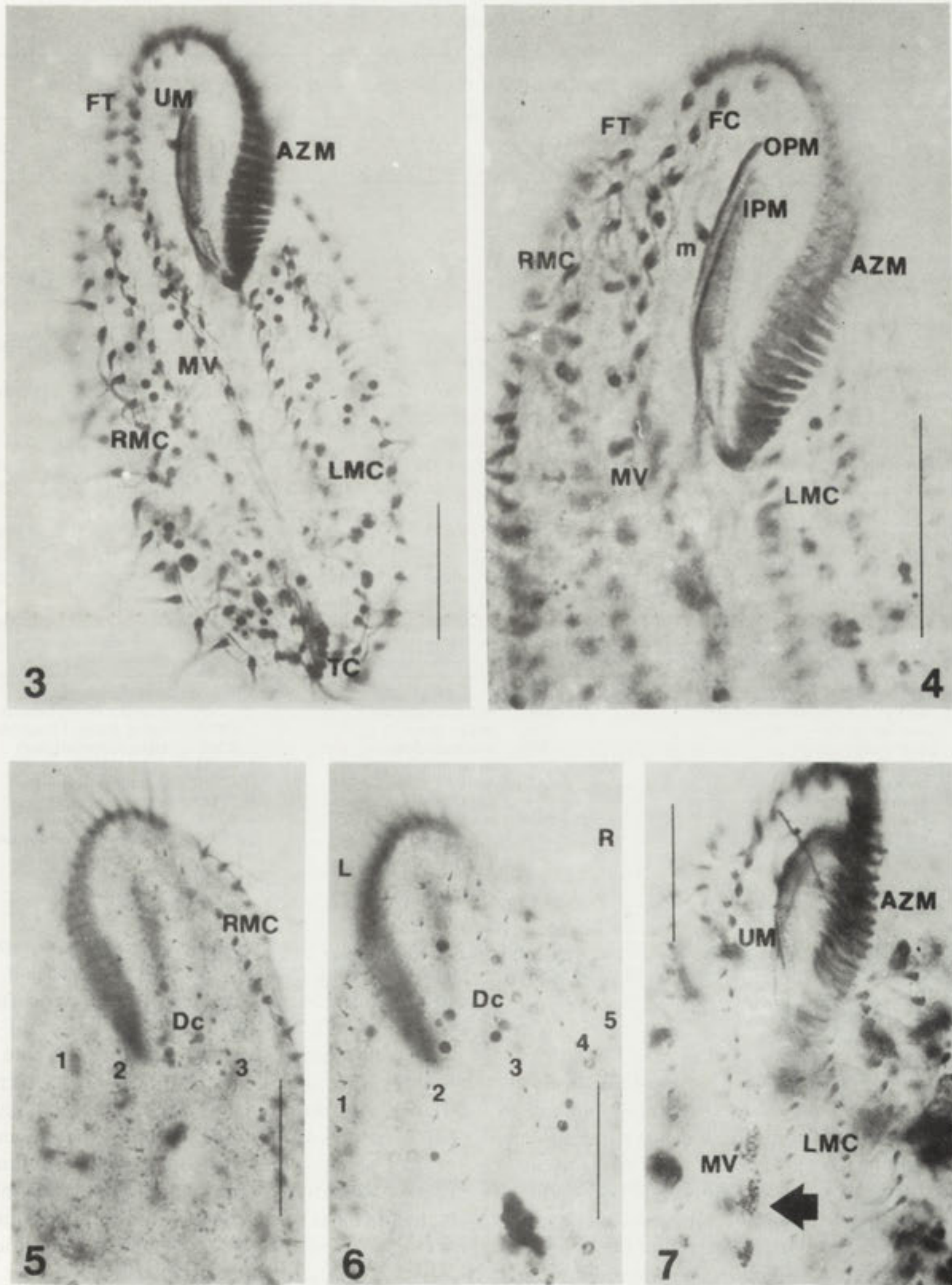

Figs. 3–7. *Urostyla thompsoni*. 3 – General aspect of the ventral side of protorgol-impregnated specimen. Scale, 20 µm. 4 – Peristome and frontal region of the ventral sufrace of protargol-impregnated specimen. Scale, 20 µm. 5 – Dorsal side of a typical specimen. Note three kineties of dorsal cilia (Dc.) Scale, 20 µm. 6 – A specimen with 5 dorsal kineties. Scale, 20 µm. 7 – The first sign of cortical morphogenesis: new kinetosomes appear in patches on the left side of some MV cirri in the middle of the cell (arrow). Scale, 20 µm.

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Table 1.

Basic biometric characteristics of *Urostyla thompsoni* Jankowski, 1979

	x̄	MD	SD	CV	Min	Max	N
Distances (μm):							
Total length	89.9	89.9	9.99	0.11	69.0	106.1	30
Maximal width	46.3	46.6	5.57	0.12	35.0	60.0	30
Peristome length	37.2	38.0	2.98	0.08	30.0	41.5	30
Distance between anterior end of the OPM and cytosystome	28.5	28.8	2.25	0.08	24.0	33.0	30
Length of the longest adoral membranelle	6.7	6.8	0.60	0.09	6.1	8.2	10
Number of:							
Adoral membranelles	26.0	26.0	1.72	0.07	22	29	30
Fronto-terminal cirri	4.3	4.0	0.61	0.14	3	5	29
Transverse cirri	6.1	6.0	0.70	0.11	5	8	27
Left marginal rows	3.5	3.0	0.73	0.21	3	5	30
Right marginal rows	3.1	3.0	0.31	0.10	3	4	30
Dorsal kineties	3.3	3.0	0.64	0.20	3	5	30
FVT streaks	11.8	12.0	1.19	0.10	10	14	30
Cirri in the posterior MV row	4.2	4.0	0.96	0.23	3	7	30
Macronoclear fragments	40.8	41.5	8.18	0.20	30	54	6

AZM	adoral zone of membranelles	LMC	left marginal cirri	N	number of specimens
CV	coefficient of variation	m	malar cirrus	OPM	outer paroral membranelle
Dc	dorsal cilia	Ma	macronucleus	RMC	right marginal cirri
FC	frontal cirri	Max	maximum value	R	right side
FT	fronto-terminal cirri	MD	median	SD	standard deviation
FVT	fronto-ventro-transverse complex	Mi	micronucleus	TC	transverse cirri
IPM	inner paroral membranelle	Min	minimum value	x̄	arithmetic mean
L	left side	MV	midventral cirri	UM	undulating membrane

5. The reorganization of the ventral surface ciliature is regularly accompanied by that of dorsal kineties.

DISCUSSION

The ciliate presented above is almost identical with the picture of an antarctic *Urostyla* published by Thompson (1972). Thompson did not name his species, presenting it as an unidentified (probably new) *Urostyla* from the Antarctic. Jankowski (1979), on the basis of Thompson's communication exclusively, presented a formal taxonomic description of the species, giving it the name *Metaurostyla thompsoni*. Genus *Metaurostyla* being no longer valid (Jankowski 1980), the species should be referred to as *Urostyla thompsoni* Jankowski, 1979. It was mentioned neither by Hemberger (1982) nor by Borror and Wicklow (1983).

The ciliate described in the present paper is strikingly similar to the antarctic *Urostyla*. The differences between the two forms concern the number of left and right rows of marginal cirri. Thompson's species has 45 rows on each side. The number of dorsal kineties is different, too. Here the difference is more pronounced, since Thompson (1972) reported about 7 dorsal kineties in the antarctic species. However, taking into account the observed variation in the two characters (Table 1), and on the basis of the only available morphological criteria, the species presented here is identified as *Urostyla thompsoni* Jankowski, 1979.

The ciliate resembles *Urostyla marina* Kahl, 1932 redescribed by Borror (1979). The absence of fronto-terminal cirri in *U. marina* is the main difference between these two forms.

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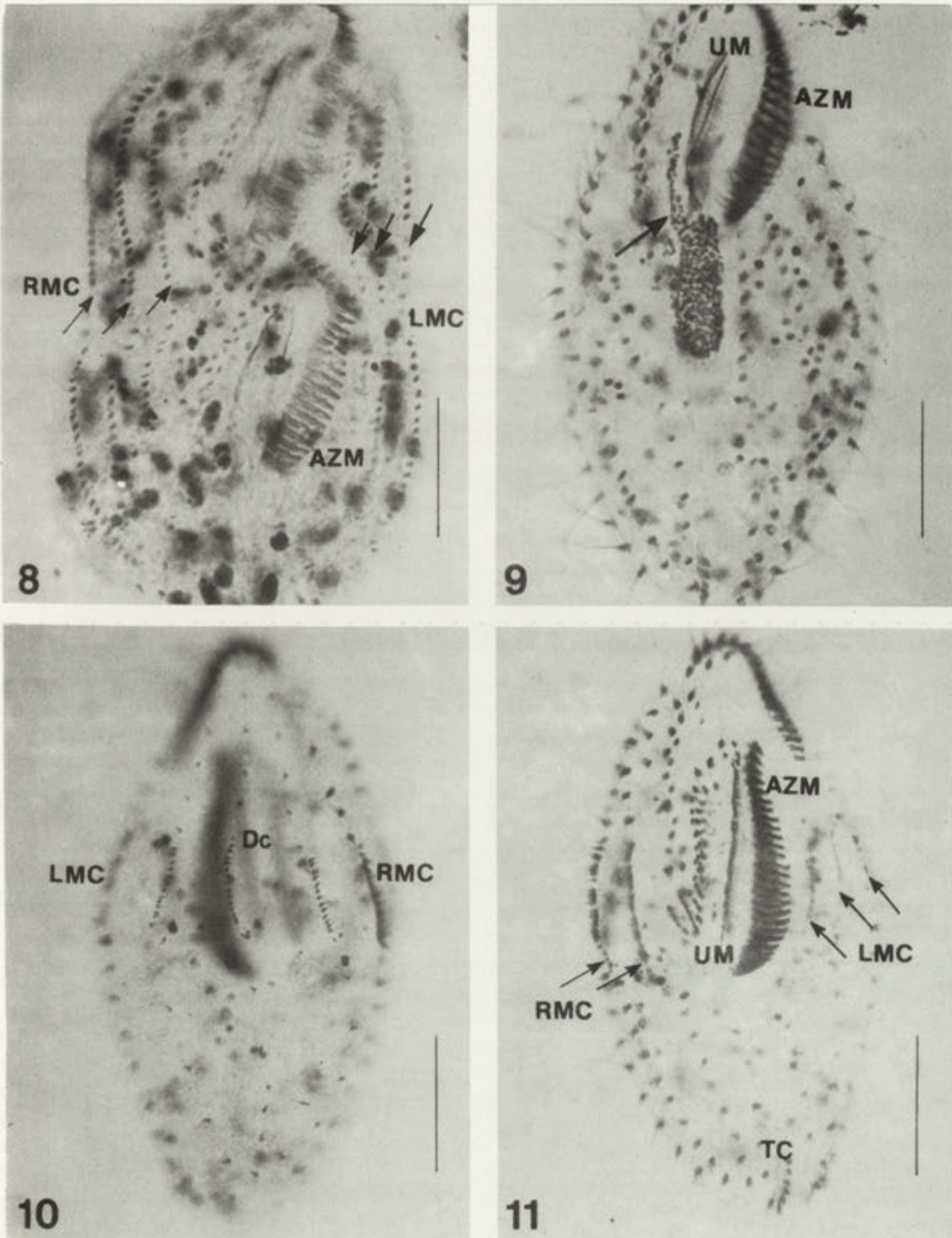

Figs. 8–11. *Urostyla thompsoni*. 8 – A late stage of divisional morpogenesis: the two sets of cortical structures for proter and opisthe are ready. Note that each new marginal row is formed inside the previously existing one (arrows). Scale, 20 μm. 9 – An early stage of reorganizational morphogenesis: a single anarchic field of kinetosomes (oral primordium) is formed in the central region of the ventral surface. A row of kinetosomes in the right anterior corner of the oral primordium is the future fronto-ventro-transverse primordium (FVT). Scale, 20 μm. 10 – An intermediate stage during reorganization: development of dorsal primordia. The primordium of a new dorsal kinety is formed inside each previously existing dorsal kinety. Scale, 20 μm. 11 – Ventral side of the same specimen as in fig. 10. A new AZM is formed parallel to the old one, which is gradually resorbed. Marginal primordia develop in each previously existing row (arrow). Scale, 20 μm.

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Morhpological Differentiation of Caprine *Sarcocystis* Species: Evidence of Occurrence of *Sarcocystis hircicanis* in India

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Summary. In a total of 288 goats examined 146 (50.69%) were positive for sarcocysts, involving the musculature of oesophageal, cardiac, skeletal, ocular and diaphragm. of these samples, two types of cysts, morphologically distinct in their shape and size were recorded. The oesophageal cysts which were isolated frequently were thinner, elongated with one end pointed and the other rounded and measured 550 – 615 μm in length and 55 – 85 μm in with suggesting their similarity with *Sarcocystis hircicanis*. Another type of cysts which were mostly observed in the cardiac muscle were thick–walled, maggot–like to spindle shaped with both ends rounded and measured 225 to 285 μm in length and 75 to 135 μm in width with the cyst wall showing distinct striations. These were indistinguishable with the descriptions of *S. capracanis*. This appears to be the first report of the occurrence of *S. hircicanis* in India.

Key words: *Sarcocystis capracanis, S. hircicanis*, goat, parasite.

INTRODUCTION

Srivastava et al. (1985) described two types of morphologically distinct cysts which were isolated from muscle samples of heart and oesophagus of naturally infected indigenous goats. Although the morphological differentiation was quite distinct but due to the fact that dogs acted as the final host for both the types of sarcocysts the authors concluded those to be the two forms of *Sarcocystis capracanis*. However, the detail descriptions of *S. hircicanis* reported by Aryeetey et al. (1980) and Heydorn and Holzner (1983) has now become available and therefore, it was thought to be of interest to re–examine the results of Srivastava et al. (1985) in the light of the new informations on *S. hircicanis*.

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During the recent years caprine sarcocystosis has generated a great deal of interest amongst the parasitologists which led to the speciation of various species of *Sarcocystis* infecting goats. The first attempt in this direction was made by Fischer (1979) who described the caprine *Sarcocystis* species as *S. capracanis* causing a very severe morbidity and mortality in experimentally infected goats.

Subsequently, Aryeetey et al. (1980) described a three layered pellicle in the schizonts and also the presence of two types of cysts viz. the sarcosysts with primary cyst wall having protrusion which were mostly straight and forming thick striated cyst wall and a thin–walled cyst without striated cyst wall and protrusions. The later form was thought to be second *Sarcocystis* species which was later designated as the *Sarcocystis hircicanis* by Heydorn and Holzner (1983) with dogs as the final hosts for the both the species. They further opined that *S. capracanis* and *S. hircicanis*

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were thus morphologically distinct on the basis of their dimension, shape and size at various stages of the development.

Recently, Srivastava at al. (1985) also observed two distinct types of sarcocysts from cardiac and oesophageal musculature of goats with distinct variations in their shape and size but owing to the non–availability of the morphological details of *S. hircicanis* considered these to be *S. capracanis.*

MATERIALS AND METHODS

The materials and methods of Srivastava et al. (1985) was following throughout the present study. Interamuscular cysts isolated from various organs of autopsied goats were washed thrice with physiological saline solution and examined unstained in the watch glasses for the study of their shapes and sizes. The morphological variations of sarcocysts isolated from different organs recorded and measurements of freshly isolated unstained cysts were done with the ocular micrometer.

RESULTS AND DISCUSSION

Detailed morphological studies of *Sarcocystis* isolated from musculature of different organs revealed two distinct types of cysts having detectable variations in their morphology. The first category of cysts were thick–walled in the majority some what spherical or maggot–like in appearance but few cysts were spindlshaped with rounded tips at one end or on both the ends. The cyst wall was thick, white opaque in colour when examined fresh was found lying on the long axis of muscle fibres measuring 225 – 285 μm in length and 75 – 195 μm in width. The cyst wall was striated, septate and inside the sarcocystis cavity there was a distinct septation. The cystic compartments were filled with metrocytes and mature bradyzoites. Morphological features were identical to the descriptions of *S. capracanis* (Fescher, 1979). Such cysts were isolated from the cardiac musculature and were shown in the photomicrographs No. 1–3 of Srivastave et al., (1985).

The second type of sarcocysts were thinner and elongated, their cyst wall was opaque white in colour with only one end pointed, and filamentous, while the other end was rounded. The cyst wall in such samples were thin, faintly striated, and the septation was not distinct in the sarcocystis cavity which was found completely filled up with mature merozoites and metrocytes. Such sarcocysts measured 550 – 615 μm in length and 55–85 μm in width and were comparatively more fragile in nature than the thick walled cysts. The morphological features were identical to those of *S. hicricanis* described by Heydorn and Holzner (1983).

The presence of these two distinct types of cysts with detectable morphological variations observed in the present study suggested the occurrence for prevalence of both the species of caprine *Sarcocystis* in India. Through this report the authors attempted to correct the earlier findings of Srivastava et al., (1985) in which the presence of two morphologically distinct types of sarcocysts from the cardiac and oesophageal musculature was described and illustrated as two forms of *S. capracanis.*

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