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A Temporal Analysis of Cell Cycle and Morphogenetic Events in *Tetrahymena pyriformis* [2, 3]

Received 15 April 1978, *accepted* 30 August 1979

Synopsis. The relative duration of oral morphogenesis and DNA synthesis has been investigated by an approach which combines the methodology of the chemostat with a demographic analysis. Details of the method are presented. Cell generation times were varied under constant conditions of temperature and nutrient limitation while cells were maintained in balanced growth. Data collected at generation times of 3 to 14 h indicate that the morphogenetic sequence is unaltered and most closely approaches a time-dependent assembly process. DNA synthesis appears to be a cycle-dependent phenomenon and requires an interval which is proportional to the total cycle time. Thus, controls for oral morphogenesis and DNA synthesis do not appear to be tightly linked. These results are dependent upon the assumptions of the method and hypotheses tested.

In recent years oral morphogenesis or stomatogenesis of the ciliated protozoa has been examined with increased interest in an effort to establish phylogenetic relationships among related organisms (see e.g., Corliss 1968, Peck 1974, Small 1967). From such studies emerges the notion of a constitutive set of ordered morphological events. That is, one observes a progression or sequence of specific events (stages) for a given organism, and stage B, not only logically, but always follows stage A, etc. There is a clear impression that ciliate stomato-

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[3] An earlier report of material contained in this paper was presented at the 5th International Congress of Protozoology in New York on June 28, 1977.

genesis is a carefully prescribed developmental sequence and as such may be a "programmed" process. The possibility that this morphogenetic program is an intimate part of the cell cycle is worthy of our consideration.

Although many studies have detailed the morphological stages of stomatogenesis, there have been but a few which have directed attention to the time-course of events during this dynamic process (Frankel 1960, 1964, Holz 1960, Williams 1964). Further, while it has been well established that ciliates will have different generation times in different growth media (Wille 1972) or at different temperatures (Thormar 1959, Giese and McCaw 1963), no detailed analysis of the temporal events of stomatogenesis has yet been carried out under conditions of varying generation time [4]. One hint of what might be expected comes from Frankel's 1960 analysis of *Glaucoma chattoni*.

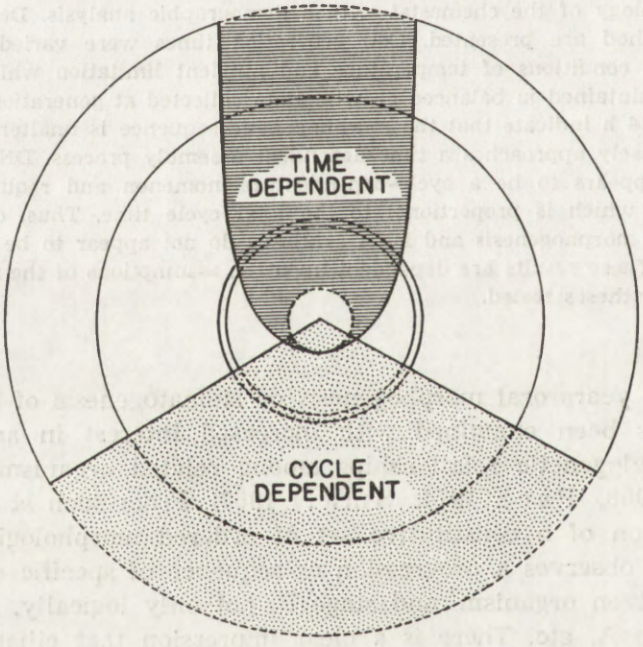

Fig. 1. Circular chronogram diagramatically represents time-dependent and cycle-dependent hypotheses. Perimeter distance represents real time-in-cycle. Note that time-in-cycle is identical for both conditions only at the double circle

[4] After the preliminary parts of this report were completed, we were informed of the work of Suhr-Jessen et al. which has subsequently been published (1977). The Discussion of the present paper has been revised to consider this important contribution.

Here he provides some data on the time of stomatogenesis as found in three different strains of *G. chattoni* which differed in their rates of growth. He concludes that "...the absolute duration of stomatogenesis is relatively constant and species-specific...."

While Frankel provides us with the suggestion that stomatogenesis may be a time and species dependent phenomenon, another alternative is that stomatogenesis is intimately linked with the cell cycle, and as cells move to longer generation times, these events "stretch" out in a proportional manner; the cycle-dependent hypothesis. In their simplest forms, the time-dependent and cycle-dependent hypotheses are diagrammed in Fig. 1. Although Mitchison (1974) discusses a variety of ways that cell life cycle events may be linked, the time- or cycle-dependent hypotheses will suffice for our present considerations.

We have developed a novel approach to this problem (but see footnote [4]) which employs the technology of the chemostat coupled with a demographic analysis of the data. In this way it was possible to collect data on one strain of *Tetrahymena pyriformis* grown at one temperature but with a variety of specific limiters to growth and generation times. We have ascertained absolute values for the duration of stomatogenic stages under these conditions. From this analysis it was possible to get an idea about the plasticity and limits of the controlling mechanism(s) of stomatogenesis. In addition, this method is also applicable to the analysis of other cellular events, and we present preliminary data here for a temporal analysis of DNA synthesis during the cell cycle.

Materials and Methods

Steady-state cultures of axenic *Tetrahymena pyriformis* W were grown at generation times (GT) of three to twenty-three hours, a 7 2/3-fold range of GT's. The three hour GT was accomplished in batch culture, and repetitive samples were taken to assure the steady-state conditions of exponential growth. Other GT's were achieved at 28°C in a conventional Novick and Szilard (1950) chemostat where nutrient limitation was by carbon, leucine or oxygen. Medium A of Holz et al. (1959) was used without glucose supplementation to achieve carbon limitation, with 1/10th leucine concentration for leucine limitation, and it was bubbled with 2% oxygen-balance nitrogen for oxygen limitation. In each case population kinetics were carefully monitored by cell counts made with a Coulter Counter (Model B) fitted with a 140 μm aperture.

Stages of stomatogenesis were quantitatively scored from Chatton-Lwoff silver impregnations (Corliss 1953) of cells taken from each experimental condition. All slides with poorly impregnated specimens were discarded regardless of the source of their imperfection. In every case, the orientation of specimens scored was rigorously controlled so both the proter buccal area as well

the region of potential or developing opisthe anlagen were clearly visible. All organisms not meeting this criterion were summarily discounted and are not reflected in our quantitative data. The seven stages of stomatogenesis (0 and I–VI) were after Frankel (1964).

In order to estimate the time of DNA synthesis (see below) average DNA contents were determined on replicate samples of approximately 5×10^6 cells by a modification of the Schmidt and Thannhauser (1945) method. Samples were taken from a steady-state, carbon-limited chemostat (as above), and data are presented in relative but arbitrary units. A detailed description of the analytical method as well as absolute estimates for *T. pyriformis* will appear elsewhere (Antipa et al. in preparation).

Analysis of Real Time-in-Cycle

As is the case in all asynchronous and exponentially expanding populations which do not exhibit cell death, the analysis of events for real time-in-cycle requires: (1) knowledge of the timing of the event, and (2) adjustment of raw data for the phenomenon of recruitment from behind. Since one dividing cell will give rise to two new cells at t_o, a growing population will contain a larger percentage of new cells than old cells. The characteristic and constant population skew which results has been described previously (e.g., Powell 1956, Bostock 1970, Barford and Hall 1976). Since the chemostat provides the opportunity to investigate a culture which remains in steady-state and asynchronous exponential growth (Kubitschek 1970), the population profile within a chemostat is also described by this characteristic population. It seems,

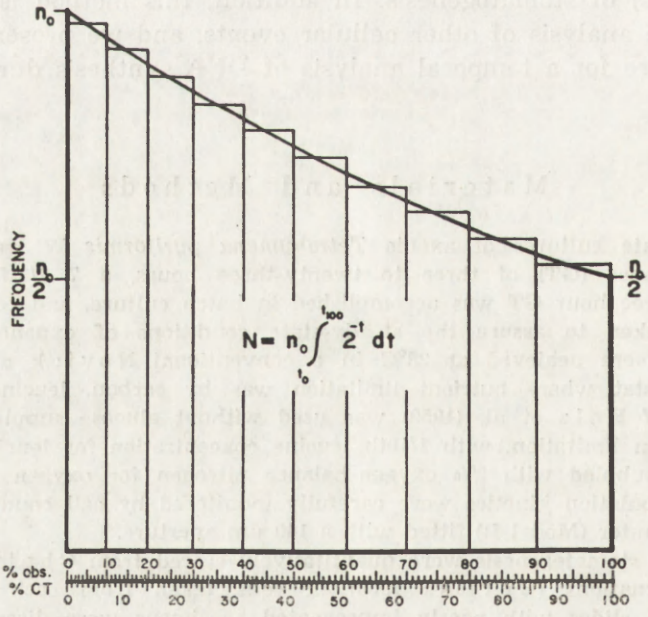

Fig. 2. Age distribution of idealized population after James (1974). Nomograph below allows for conversion of percent observed to percent of cycle time

appropriate, then, to review the population profile of an asynchronous, exponentially growing population (Fig. 2). Here we provide the breakdown of an idealized population, the formula which describes the population based on the logistic growth curve, and an iteration of this formula which provides a nomograph for direct conversion of appropriate raw data to time-of-cycle values.

Both unlimited steady-state growth in a batch culture and limited steady-state growth in a chemostat guarantee asynchronous growth, a necessary assumption for correction of data by the method described above. Further, Williams (1964), Prescott (1957) and others have demonstrated that individual generation times of *T. pyriformis* show but small variation from the mean population GT. Variability in GT's may be as small as 7%, and we have no reason to believe that variability under our chemostat conditions exceeds this estimate. One consequence of the "tightness" of GT's is that the theoretical population profile (Fig. 2) closely approximates what one finds in the experimental situation. We have assumed perfect agreement to the idealized populations in our computations which appear below; the small but inevitable variation from this in our experiments does not materially influence our results.

Methods of temporal analysis by the digestion of data from asynchronous cultures provides certain advantages over analysis of either single cell data or data derived from either synchronous or synchronized cultures. The main advantages are: (1) the ability to simultaneously acquire large numbers of organisms in all stages of morphogenesis, (2) the opportunity to examine organisms taken from a homogeneous environment, and (3) especially pertinent to this study, the compatibility of the analytical method to the experimental methodology of the chemostat. Not unexpectedly, such a method also has distinct disadvantages. The principal disadvantage lies in the fact that one must know the position of the temporal events being investigated within the total cycle time. In the case of stomatogenesis, since the terminal event (end of stage VI) is also coincident with the termination of the division cycle (by definition, Frankel 1964) and since the stomatogenic sequence is a continuous morphogenetic series, this particular problem is alleviated. One simply works backward in the analysis of the complete series.

When knowledge of the precise positioning of an event is not available, it is still possible to test data against theoretical considerations, and this is how we have dealt with our analysis of DNA synthesis. Again, knowledge of the characteristic population skew is essential, but in this case it provides a predictable effect on the average parameters of the culture, and this provides the opportunity for testing data against prediction. James (1974) has presented us with an important conceptual extention of the population skew in presenting his concept of the "unit cell." The unit cell refers to the integrated volume of an irregular cubic with dimensions of time-of-cycle, population number (both as in Fig. 2) and amount of substance per cell (e.g., DNA/cell). The integrated volume of this cubic represents the average per cell measurement such as those we have made on DNA. As a consequence, it is possible to make a series of assumptions, generate values based on those assumptions, and then compare experimentally derived data against these predicted values. We present one such argument in the discussion.

Results

Stomatogenesis

Data on the duration of stomatogenesis were collected from eight different experimental situations. Raw data were analyzed according to the methods described above, and a summary of these observations appears in Table 1. Perusal of these data immediately indicates that there is no major alteration in the course of stomatogenesis under these experimental conditions. In all cases, all six stages of oral development have been observed. Further, the sequence of stages does not appear to be greatly influenced by either the limiter to growth or the GT, as stages II, III and VI tended to be of short duration in each case while stages I, IV and V tended to be of longer duration. Stage III was generally of the shortest duration, and stages I, IV and V were longer and, on the average, of approximately similar duration. In general, the total time course of stomatogenesis required a smaller portion of the total cycle time as cells moved to longer generation times. Further, the morphological stages of stomatogenesis required approximately the same time regardless of the overall cell generation time.

DNA Content

The average per cell DNA content for *Tetrahymena* grown under carbon-limitation at generation times of 5.5 to 23 h is given in Table 2. Our measurements were unable to detect a significant change in DNA content over this four-fold range of GT's. The significance of this result is discussed below.

Discussion

We have been able to demonstrate that *T. pyriformis* can be maintained for extended periods in continuous exponential and steady-state growth at generation times of 5 to 23 h. Successful chemostat culture has been achieved with leucine, oxygen and carbon-limitation, and under each of these conditions, *T. pyriformis* performed admirably by maintaining a steady and constant titer while meeting conditions of the imposed generation time. None of the difficulties frequently associated with the chemostat culture of other organisms (Pirt 1975) such as cell death, wall growth or incomplete division proved to be of consequence in these experiments.

Table 1

Sample	Limiter	GT	n		Stomatogenic Stages 0	I	II	III	IV	V	VI	Duration of oral development
1	UL	3.0	1000	A	500	111	64	50	104	98	73	—
				B	41.8	11.0	6.4	5.8	12.5	12.5	10.0	58.2
				C	75.3	19.8	11.5	10.4	22.5	22.5	18.0	104.7
2	LEU	5.0	1000	A	687	78	38	25	74	37	61	—
				B	60.7	9.2	4.3	3.1	9.6	4.4	8.7	39.3
				C	182.1	27.6	12.9	9.3	28.8	13.2	26.1	117.9
3	LEU	5.2	1000	A	754	75	42	27	47	31	24	—
				B	68.3	9.0	5.6	3.1	6.5	4.3	3.2	31.7
				C	213.1	28.1	17.5	9.6	20.3	13.4	10.0	98.9
4	O_2	6.4	300	A	209	26	17	4	19	18	7	—
				B	61.8	10.2	6.8	1.4	8.5	8.0	3.3	38.2
				C	237.3	39.2	26.1	5.4	32.6	30.7	12.7	146.7
5	O_2	6.4	300	A	217	16	10	12	16	17	12	—
				B	65.0	6.2	3.8	5.0	7.0	7.6	5.4	35.0
				C	249.5	23.7	14.5	19.1	26.8	29.8	20.6	134.5
6	LEU	9.0	1000	A	837	39	21	21	30	34	18	—
				B	78.2	4.7	3.0	2.9	4.1	4.6	2.5	21.8
				C	422.3	25.4	16.2	15.7	22.1	24.8	13.5	117.7
7	LEU	9.5	1000	A	869	23	15	17	25	26	25	—
				B	82.3	2.9	2.3	2.1	3.3	3.6	3.5	17.7
				C	469.1	16.5	13.1	12.0	18.8	20.5	20.0	100.9
8	C	10.0	1000	A	816	43	24	21	30	52	14	—
				B	75.8	5.2	3.2	3.0	3.9	7.0	1.9	24.2
				C	454.8	31.2	19.2	18.0	23.4	42.0	11.4	145.2

Rows A, B and C are respectively: raw data, duration as percent of cycle time, and duration in minutes. Other abbreviations are as follows: GT — generation time in hours, n — number of specimens scored, UL — "unlimited" growth, LEU — leucine, O_2 — oxygen, C — carbon.

The ability to manipulate cell generation times in a specific medium and at a specified temperature has allowed us to perturb the "normal" time course of the cell division cycle and examine the relationship between temporal events of stomatogenesis and DNA synthesis. Under conditions of batch culture growth, for every round of DNA synthesis there is one sequence of oral morphogenesis; these events remain tightly coupled. It has been known, however, that conditions for heat shock synchrony of *Tetrahymena* result in the uncoupling of these processes,

and DNA synthesis persists while stomatogenesis is blocked (Zeuthen 1974, Lowy and Leick 1969). What we have found in this study is a temporal dissociation of events of DNA synthesis and oral morphogenesis under conditions of balanced growth.

Table 2

Sample	Limiter	GT	Relative DNA Content
1	C	5.5	0.421
2	C	8.5	0.416
3	C	10.0	0.411
4	C	14.0	0.432
5	C	23.0	0.403

Abbreviations as in Table 1

Stomatogenesis

The results of our experiments on stomatogenesis indicate that this morphological sequence of events takes place in approximately the same length of time regardless of the cell generation time. This suggests that stomatogenesis in *T. pyriformis*, or at least the associated morphological events, is a fixed sequence that resembles a time-dependent assembly process. Our data are summarized in a linear chronogram (Fig. 3 A) which indicates the variability in our data but clearly suggests the time-dependent nature of this process. An examination of Table 1 and Fig. 3 A indicates that the duration of stomatogenesis under conditions of either carbon or oxygen limitation tends to be longer than under leucine limitation. Carbon or oxygen limitation may both represent forms of energy limitation while leucine represents amino acid or nitrogen limitation. These differences may result in the discrepancies observed, and further experimentation should validate this secondary phenomenon associated with the timing of stomatogenesis.

Suhr-Jessen et al. (1977) have recently performed a remarkably similar experiment. They examined *T. pyriformis* GL in a chemostat culture with an unspecified limiter. They clearly establish that the cells are undergoing balanced growth with a single limiter at generation times of 4.9 to 17.2 h. In their study they also conclude that the course of stomatogenesis is a time-dependent phenomenon, in this case of approximately 91 m duration at 28°C. We have summarized their data in Fig. 3 B. It is evident that our data do not precisely agree with theirs, but this may be explained by either the different strains and media used or by some differences in the method of scoring. We would

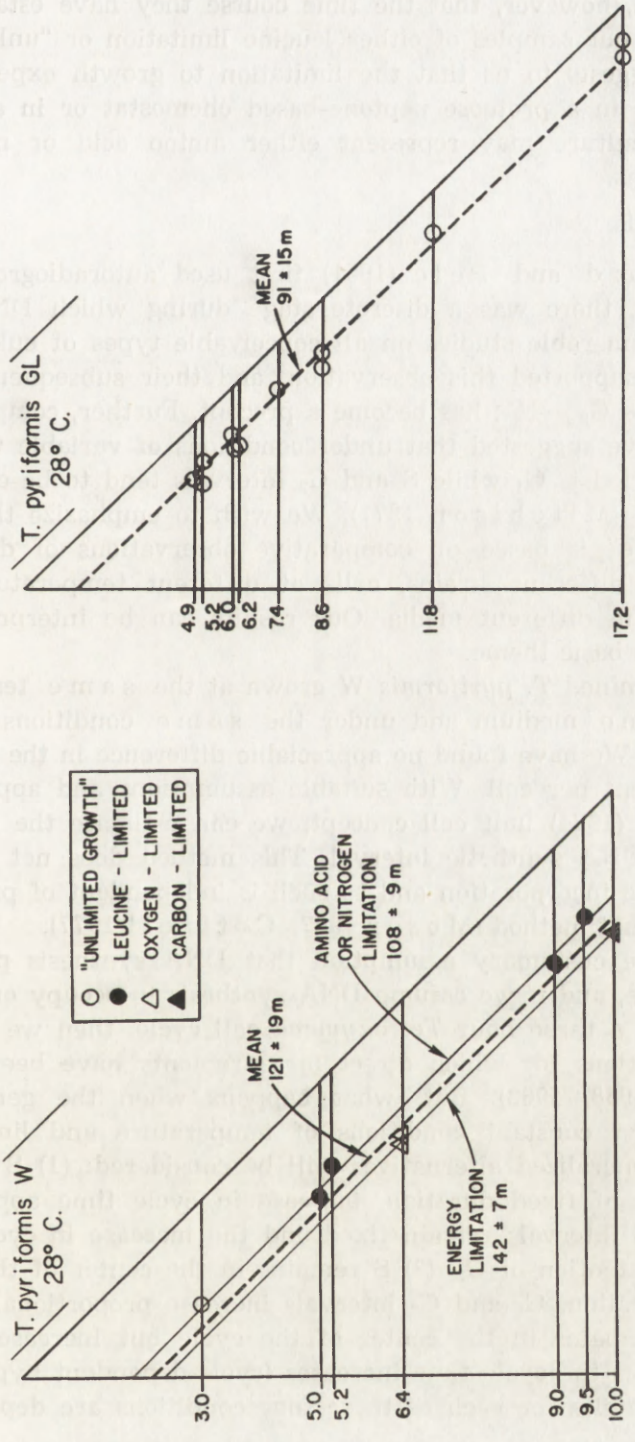

Fig. 3. Linear chronograms which indicate the onset of stomatogenesis (symbol) at the position observed within the total cycle time. Numerals at left of each figure indicate the GT in hours. A — data presented here. B — after Suhr-Jessen et al. (1977)

like to point out, however, that the time course they have established most closely fits our samples of either leucine limitation or "unlimited" growth. This suggests to us that the limitation to growth experienced by *T. pyriformis* in a proteose peptone-based chemostat or in an "unlimited" batch culture may represent either amino acid or nitrogen limiting conditions.

DNA Synthesis

Since Howard and Pelc (1951) first used autoradiography to demonstrate that there was a discrete stage during which DNA was synthesized, innumerable studies on all conceivable types of eukaryotic organisms have supported this observation, and their subsequent algorithm, G_1 — S — G_2 — M, has become a precept. Further, comparative investigations have suggested that under conditions of variable GT, the most flexible period is G_1 while S and G_2 intervals tend to be constant in their duration (Mitchison 1971). We wish to emphasize that this "general principle" is based on comparative observations of different cells or tissues, different strains, cells at different temperatures, or strains growing in different media. Our results can be interpreted to deviate from this basic theme.

We have examined *T. pyriformis* W grown at the same temperature, in the same medium and under the same conditions of nutrient limitation. We have found no appreciable difference in the average DNA content per cell. With suitable assumptions and application of the James (1974) unit cell concept, we can estimate the relative position of the DNA synthetic interval. This method does not require the use of isotope incorporation and as such is independent of problems associated with that method (Moser 1967, Cottrell 1977).

If we use the customary assumption that DNA synthesis proceeds at a uniform rate, and if we assume DNA synthesis to occupy one hour in the middle of a three hour *Tetrahymena* cell cycle, then we will be filling the conditions for which direct measurements have been made (McDonald 1958, 1962). But, what happens when the generation time varies under constant conditions of temperature and limiter to growth? Four generalized alternatives will be considered: (1) S and G_2 intervals remain of fixed duration, increase in cycle time appears in G_1, (2) G_1 and S intervals remain fixed and the increase in cycle time appears as an extention of G_2, (3) S remains in the center of the cycle and of fixed duration, G_1 and G_2 intervals increase proportionally, and (4) S interval remains in the center of the cycle but increases along with G_1 and G_2 as the cycle time increases (cycle-dependent hypothesis, see Fig. 1). Examples for each of these four conditions are depicted in

Fig. 4. Figure 5 demonstrates the effect the age distribution and assumptions have on the mean DNA content for asynchronous cultures under each of these four conditions. It will be seen that while each condition predicts identical values for the mean DNA content at the three hour GT, conditions 1 and 2 deviate in a predictable and systematic curvilinear fashion for other GT's while conditions 3 and 4 predict a constant DNA content across generation times. By this method it is not possible to distinguish between conditions 3 and 4.

Our data conform to the results predicted by conditions 3 and 4 (Fig. 6). Within the context of our experiment, it is possible to distinguish between these two cases with a simple labeling experiment. From Fig. 4 it will be seen that as cells grow at longer GT's under condition 3, fewer cells will be involved in S at any given time, while under condition 4, the same proportion is engaged in DNA synthesis at all GT's. As a consequence, it is possible to resolve between two situations by

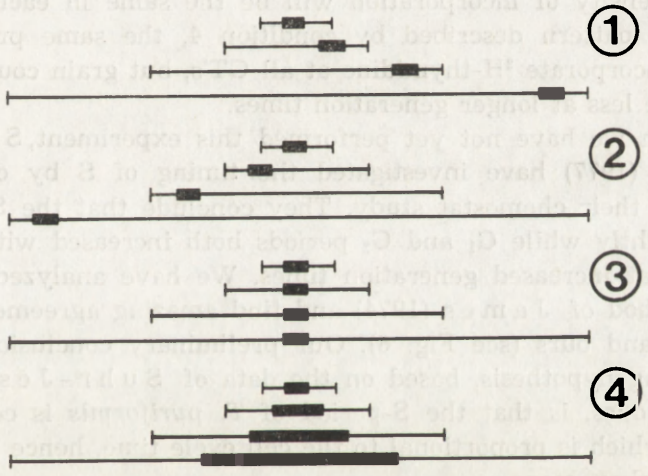

Fig. 4. Diagramatic representation of four possible conditions (1–4) for the S interval (blocks) at 3, 6, 12 and 24 h GT's. See text for further detail

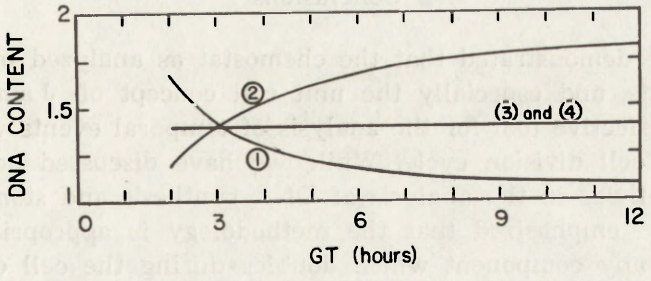

Fig. 5. Average DNA content as a function of generation time for each of the four conditions described in Fig. 4 and text

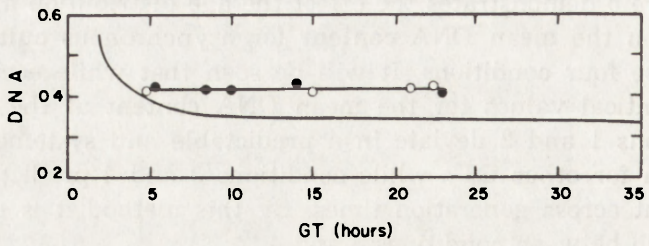

Fig. 6. Relative but arbitrary DNA content observed in a carbon-limited chemostat (solid circles) in this study and as derived from Suhr-Jessen et al. (1977) (open circles). Line without data points represents condition 1 (above) and is included for reference

looking at the relative numbers of cells incorporating ^{3}H-thymidine during a short pulse. If cells are growing according to condition 3, fewer cells will incorporate ^{3}H-thymidine at longer GT's than at shorter GT's, but the intensity of incorporation will be the same in each case. If S follows the pattern described by condition 4, the same proportion of cells will incorporate ^{3}H-thymidine at all GT's, but grain counts per cell will become less at longer generation times.

Although we have not yet performed this experiment, Suhr-Jessen et al. (1977) have investigated the timing of S by conventional methods in their chemostat study. They conclude that the S-period increased slightly while G_1 and G_2 periods both increased with the same proportion at increased generation times. We have analyzed their data by the method of James (1974) and find amazing agreement between their data and ours (see Fig. 6). Our preliminary conclusion and present working hypothesis, based on the data of Suhr-Jessen et al. (1977) and ours, is that the S-period of *T. pyriformis* is controlled in a manner which is proportional to the cell cycle time, hence it is a cycle dependent phenomenon.

Conclusions

We have demonstrated that the chemostat as analyzed by demographic methods, and especially the unit cell concept of James (1974), can be an effective tool for the analysis of temporal events which occur during the cell division cycle. While we have discussed here the use of these methods in the analysis of DNA synthesis and stomatogenesis, it should be emphasized that the methodology is appropriate for the analysis of any component which doubles during the cell cycle. Since the chemostat is ideally suited for the examination of balanced growth during which cellular components double, it is hoped that this approach will be put to use in other studies of cellular growth.

We observe that stomatogenesis most closely approaches a time-dependent phenomenon (Fig. 1), and in this conclusion we are in agreement with Frankel (1960) and Suhr-Jessen et al. (1977). DNA synthesis is found to approximate a cycle-dependent phenomenon (Fig. 1), and here we are supported by Suhr-Jessen et al. (1977). It becomes evident, then, that controls for stomatogenesis and DNA synthesis in *Tetrahymena* are not tightly coupled. It remains the task of future endeavors to elaborate on these circumstances.

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It is my pleasure to acknowledge the technical assistance provided by B. W. Swanson, M. Zarski and P. Szczepański during various phases of this project. The opportunity to examine the Suhr-Jessen et al. (1977) manuscript prior to its publication is especially appreciated; for this I am indebted to Dr. Leif Rasmussen.

RÉSUMÉ

Le temps relative de la morphogénèse de l'appareil oral et de la synthèse de l'ADN a été étudié par un procédé qui combine la méthodologie de chemostate avec l'analyse démographique. Les détails de cette méthode sont décrits. Le temps de duré des générations des cellules est variables dans les conditions thermiques et nutritives stables, quand les cellules sont maintenues dans la croissance équilibrée. Les données concernant les temps de génération entre 3 et 14 h montrent que la séquence morphogénétique reste intacte qu'elle approche à un processus dépendant du temps. La synthèse de l'ADN paraît être phénomène dépendant de la phase du cycle et elle nécessite un délai proportionnel au temps total du cycle. Donc, les mécanismes de contrôle de la morphogénèse de l'appareil oral et de la synthèse de l'ADN probablement ne sont pas étraitement liés entre eux.

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Tetratoxum unifasciculatum (Fiorent.) (*Ciliata, Entodiniomorphida*) I. Somatic and Adoral Infraciliature

Received 19 April 1979

Synopsis. Silver impregnation of *Tetratoxum unifasciculatum* (Fiorent) has revealed the occurrence of three somatic ciliary zones in this species. The structure of the adoral zone appeared to be the same as in *Cycloposthium* Bundle and *Tripalmaria* Gass. of the family *Cycloposthiidae.*

The genus *Tetratoxum Gass.*, comprising species from horse intestine, belongs to the family *Ditoxidae* according to Strelkov (1939), being separated from *Cycloposthiidae* Poche. Beside *Tetratoxum* the family *Ditoxidae* comprises the genera *Ditoxum* Gass., *Cochliatoxum* Gass. and *Triadinium* Fiorent.; the last one having body shape not characteristic of the family. The base for separation of the new family by Strelkov (1939) was the internal structure as well as the character of the adoral zone, not retractable inside to the body.

Performing the systematic revision of the family *Cycloposthiidae* Latteur and Dufey (1967) created the family *Spirodiniidae* which embraced three subfamilies. In the subfamily *Spirodininae* they placed the genus *Spirodinium* Fiorent (allocated by Strelkov to *Ophryoscolecidae*) and the genera ranged by Strelkov in his family *Ditoxidae,* also the genus *Tetratoxum* Gass. These authors accepted the number and shape of ciliary zones, so called additional ones, and the presence or lack of the skeleton as the base for distinguishing of the new family and subfamilies.

This investigation was supported by Committee of Cytobiology of the Polish Academy of Sciences.

The above mentioned different opinions on the system of *Entodiniomorphida* from horse intestine raised up the need of further studies, especially on the adoral ciliature of these ciliates.

Among *Entodiniomorphida* from horse intestine only the infraciliature of *Cycloposthiidae* has been studied till now. To this family belong two genera occurring in horse: *Cycloposthium* Bundle and *Tripalmaria* Gass. Detail description of infraciliature of *Cycloposthium edentatum* was given by Fernandez-Galiano (1959). Due to this author it is known that the adoral zone in *Cycloposthium* is composed of three clearly differring parts. The same character shows the adoral zone in *Tripalmaria dogieli* examined by Wolska (1978). In the family *Ditoxidae* the species *Triadinium caudatum* Fiorent. has been studied by Wolska (1969) but this species, being not typical of the family, is not suitable for comparison. Senaud and Grain (1972) investigated *Cochliatoxum periachtum* Gass. under the electron microscope. The protozoan was also silver impregnated but its adoral infraciliature was not described in detail.

The aim of this work was to investigate the somatic and adoral infraciliature of the typical representative of the family *Ditoxidae*, namely *Tetratoxum unifasciculatum* (Fiorent.) and to compare it with other, already investigated representatives of *Entodiniomorphida* from horse.

The structure of *T. unifasciculatum* was given by Gassovski (1918), Hsiung (1930) and Strelkov (1939). These authors described four somatic ciliary zones in this ciliate. In the present paper only schematic drawing of the ciliature is given, obtained after silver impregnation of the ciliates (Fig. 1).

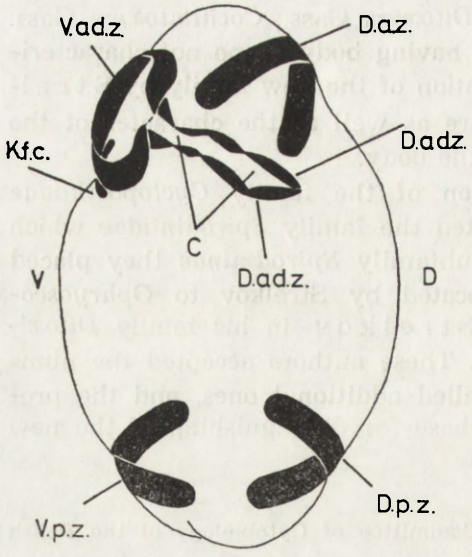

Fig. 1. Scheme of disposition of the ciliary zones, left side view. The protozoan is represented as transparent. Ventral side (V). Dorsal side (D). Dorsal anterior zone (D.a.z.). Dorsal posterior zone (D.p.z.). Ventral posterior zone (V.p.z.). Ventral part of the adoral zone (V.ad.z.). Dorsal part of the adoral zone (D.ad.z.). Kinetosomes of free cilia (K.f.c.). Cytostome (c)

Material and Methods

Content of the horse colon, obtained from the sloughterhouse in Rawicz just after killing a horse, was a source of the material. A sample of content, filtered through a gauze in order to remove larger food particles, containing protozoans was fixed in 10% formaldehyde during 24 h. After that time the protozoans were washed in water and a drop of condensed suspension was placed on steam heated object glass, mixed with a drop of warm gelatin (after Chatton and Lwoff) and spread in a thin film over the glass. Then the preparation was placed in a moist chamber in refrigerator. Next day the preparation was submerged into silver solution after Bielschowsky. Freshly prepared silver solution was diluted in proportion 1 : 1 with distilled water. The impregnation time was established experimentally, usually several seconds were sufficient. After that the preparation was quickly washed in distilled water and put into 10% formaldehyde till darkening. The preparation was then dehydrated and mounted in Canada balsam.

Results

Total ciliature of *Tetratoxum unifasciculatum* is composed of adoral ciliature and three somatic ciliary zones or additional zones. The somatic zones — dorsal anterior, dorsal posterior and ventral posterior ones are in form of a ribbons. Each of these ribbons forms an arch embracing the dorsal or the ventral margin of flattened body of the ciliate and reaches to about the middle of the left or the right body side (Pl. I 1, Fig. 1). Each somatic zone, being rounded at both ends, is composed of parallel rows of kinetosomes. The rows of kinetosomes are arranged densely and evenly spaced, being diagonal to the long axis of the zone. The kinetosomes of particular rows lay closely together, so under a low microscope magnification they look like uniform diagonal strips. In posterior zones almost all strips show thickenings in their mid-length giving an appearance of a thin discontinuous band running along the zone (Pl. I 1). In the anterior (dorsal) zone two such bands are visible (Pl. 1 3).

The adoral infraciliature is differentiated into three parts. At the ventral side there is a wide ribbon surrounding the border of an opening leading to a concavity directed toward the dorsal side and forming the vestibulum. Narrowed endings of this ribbon, are bent inward the concavity (Fig. 1). This ventral part of the adoral infraciliature is composed of dense, parallel and evenly arranged diagonal rows of kinetosomes, being similar to the somatic zones. Along this ribbon, as in the dorsal somatic zone, two dark bands are visible (Pl. I 3). The second part of the adoral infraciliature is in form of a narrow ribbon forming a loop

directed postero-dorsally. The endings of the dorsal ribbon butt against the endings of the ventral one. A narrow ribbon, forming the dorsal part of the adoral infraciliature is composed of short rows of kinetosomes. The kinetosomal rows are more loosely arranged than in the ventral part and particular kinetosomes are more intensely blackned (Pl. II 4). The dorsal ribbon of short rows runs in waves on the bottom of vestibulum surrounding a slit-like cytostome. The third part of the adoral ciliature constitutes a group of kinetosomes lying on the ventral side of the ciliate body and being slightly shifted to the right, near the posterior margin of the ventral part of adoral ciliature (Fig. 1, Pl. I 2, II 5).

Discussion

The ciliary zones in *T. unifasciculatum*, recognized after silver impregnation, show the same character as the ciliary zones of up to now examined *Entodiniomorphida* from horse intestine and cattle rumen. All zones are composed of diagonal rows of kinetosomes, parallel and situated in the same distances. Such arrangement of kinetosomal rows in ciliary zones was recognized by Noirot-Timothée (1960) in *Ophryoscolecidae* from cattle rumen and, somewhat earlier, by Fernandez-Galiano (1959) in *Cycloposthium edentatum* (fam. *Cycloposthiidae*) from horse. Recently, the same pattern of ciliary rows has been found in another representative of *Cycloposthiidae*, namely in *Tripalmaria dogieli* by Wolska (1978).

In *T. unifasciculatum* the membranellae are lacking in somatic zones as well as in the adoral one although the cilia in these zones are grouped into larger units, the syncilia, similarly as in other *Entodiniomorphida*. The adoral zone of *T. unifasciculatum* shows no particularities; its structure fits well the structure of the adoral zone characteristic of *Cycloposthiidae*. It may be characterized by different arrangement of kinetosomal rows and more intense impregnation of kinetosomes in the dorsal than in the ventral part. Such difference in structure between the ventral and the dorsal parts does not exist in *Ophryoscolecidae*. At the posterior border of the ventral part of adoral zone in *T. unifasciculatum* occurs a group of kinetosomes pertaining to a prominent cytoplasmic lip surrounding the adoral zone. Presence of this group of kinetosomes (free cilia kinetosomes) is a common character of *Cycloposthiidae* and *Ophryoscolecidae* (Fernandez-Galiano 1959, Wolska 1965, 1978). In comparison with *Cycloposthiidae* the dorsal part of the adoral zone in *T. unifasciculatum* is more strongly

developed and, what is more, the disjunction between the dorsal and the ventral parts, well visible in *Cycloposthiidae*, is not visible in this species. The dorsal and the ventral parts of the adoral zone develop from two separate primordia in *Cycloposthiidae* and *Ophryscolecidae* (Noirot-Timothée 1960, Wolska 1965). As *T. unifasciculatum* is concerned, the protozoans in course of division have not been found in the examined material, so the observations on the number of adoral zone primordia could not be made. In order to elucidate this problem further investigations are necessary as well as more comprehensive examination of this species under discussion with the aid of electron microscope.

RÉSUMÉ

L'imprégnation de *Tetratoxum unifasciculatum* (Fiorent.) à l'argent démontre la présence de trois zones ciliaires somatiques. La zone adorale se caractérise par la même composition que chez *Cycloposthium* Bundle et *Tripalmaria* Gass. de la famille des *Cycloposthiidae*.

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EXPLANATION OF PLATES I–II

Tetratoxum unifasciculatum, photographs of silver impregnated specimens

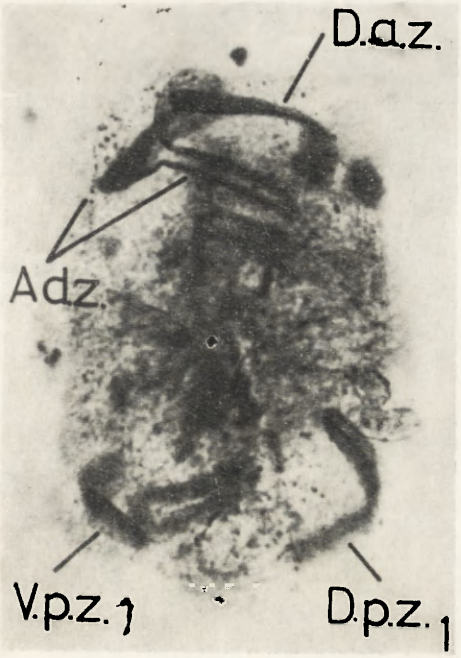
1: A general view. Dorsal anterior zone (D.a.z.). Dorsal posterior zone (D.p.z.). Ventral posterior zone (V.p.z.). Adoral zone (Ad.z). × 700

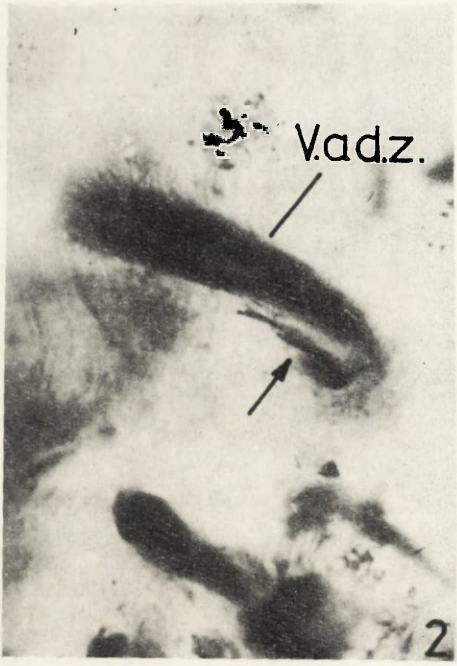
2: Ventral part of adoral zone (V.ad.z.). Kinetosomes of free cilia (arrow.) × 1700

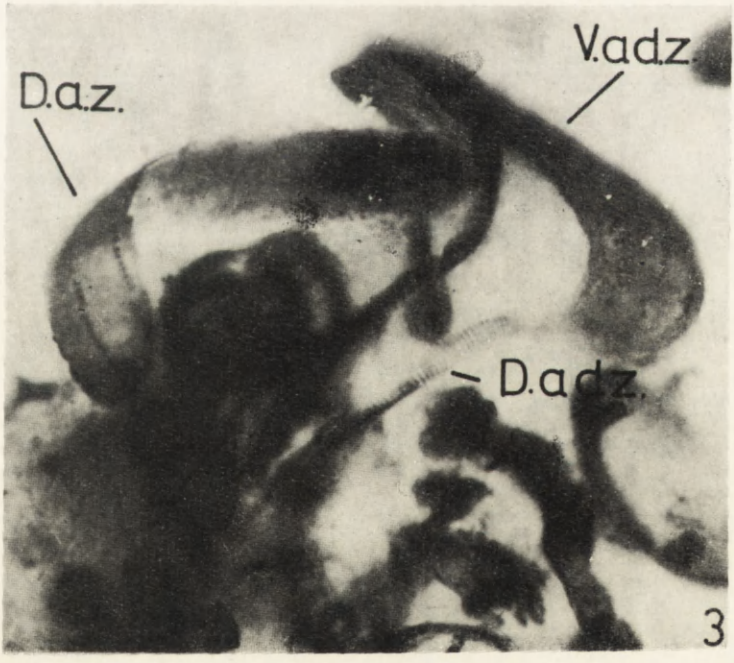
3: Dorsal anterior zone (D.a.z.). Ventral part of the adoral zone (V.ad.z.). Dorsal part of the adoral zone (D. ad.z.). × 1700

4: Dorsal anterior zone (D.a.z.). Dorsal part of the adoral zone (D.ad.z.). × 1700

5: Dorsal anterior zone (D.a.z.). Ventral part of the adoral zone (V.ad.z.). Dorsal part of the adoral zone (D.ad.z.). Kinetosomes of free cilia (arrow). × 1700







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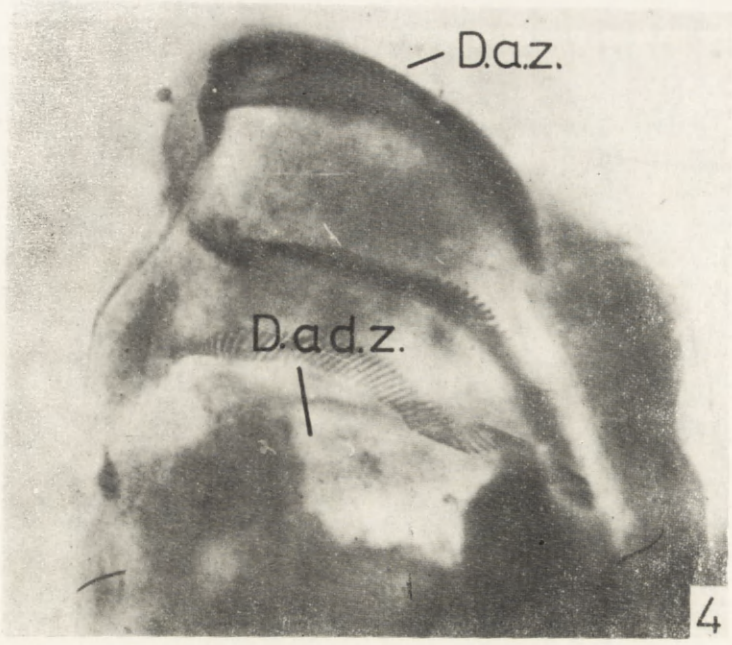

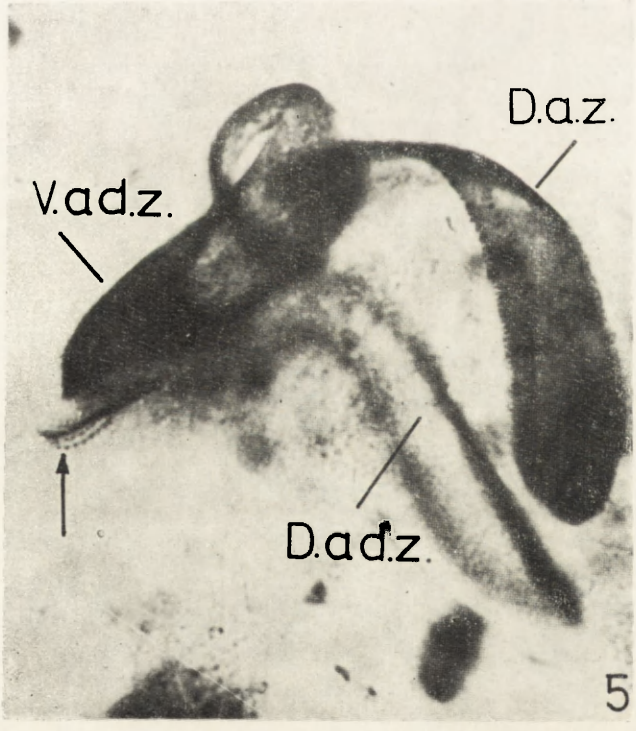

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Tetratoxum unifasciculatum (Fiorent.) (*Ciliata, Entodiniomorphida*) II. Electron Microscope Investigations

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Synopsis. The study has been performed on ultrastructure of the cortex and of the buccal apparatus of *Tetratoxum unifasciculatum*. It has been found that under cell membrane and epiplasm of bare parts of the body there is a layer of longitudinal microtubules arranged in bundles under which longitudinal bars of dense substance are situated. Short kinetosomes are dispersed in microfibrillar layer occurring under these bars. The kinetosomes of the ciliary zones are not grouped although the cilia join together and form syncilia. Under kinetosomes of ciliary zones spreads a net of dense substance in which strands parallel to kinetosomal rows are dominant. The structure of *T. unifasciculatum* cortex is similar to that of *Triadinium caudatum* and *Cochliatoxum periachtum* of the same family. Also the complex cytostome–cytopharynx shows the same character as in both mentioned species. A group of cilia called "free cilia" occurs also in *T. unifasciculatum*; their ultrastructure being the same as in other examined representatives of *Entodiniomorphida* and *Blepharocorythina*.

New concepts of the system of *Ciliata* have changed the position of *Entodiniomorphida* (de Puytorac et al. 1974, Corliss 1975, 1977, Jankowski 1973, Seravin and Gerassimova 1978). The opinions are concordant that *Entodiniomorphida* ought to be removed from former class *Spirotricha* and placed in more primitive one, but the rank of this group is still discutable as well as its supposed relations with *Blepharocorythina*. Also the system within *Entodiniomorphida* from horse intestine is differently considered by various authors (Wolska 1971). Thus, the need arises of more extensive studies on these ciliates. Recently, *Tetratoxum unifasciculatum*, the representative of the family *Ditoxidae* created by Strelkov (1939), has been examined using silver impregnation method (Wolska 1980). The aim of the present paper is to recognize the ultrastructure of the cortex and of the buccal apparatus of this species.

This investigation was supported by Committee of Cytobiology of the Polish Academy of Sciences.

Material and Methods

The material was taken from the content of horse colon in Rawicz sloughterhouse. Samples of content were taken just after killing the horse and put in vacuum flasks. Just after bringing to the laboratory the protozoans from intestinal content were prepared for embedding in Epon 812 according to the method of Grain (1966). Sections cut on ultramicrotome III LKB were placed on Formvar-coated grids and contrasted with uranyl acetate and lead citrate. Observations were made in Tesla BS 500 electron microscope. Semi-thin sections were made according to the procedure described earlier (Wolska 1978a).

Results

The body of *Tetratoxum unifasciculatum* is covered by single cell membrane spreading also over the cilia. Under this membrane in nonciliated regions there is a thin layer of structureless substance of medium electron density — the epiplasma (Pl. I 1, II 5). Somewhat deeper longitudinal bundles of microtubules are situated. In most cases the bundle comprises six microtubules lying at two levels by three (PI. I 1, II 5). Among bundles of microtubules there are vesicles comprising granular content or empty ones (Pl. I 3, II 4), some of them being opened outside (Pl. I 1). Under the bundles of microtubules there are longitudinal bars of dense substance taking exactly the same course as the microtubules (PI. I 1, 3, II 4, 6). The longitudinal bars are connected by thin transverse strands of dense substance (PI. I 1, 3, III 7). The longitudinal bars are underlayered by circular microfibrils (Pl. I 1, 2, II 4) with short nonciliated kinetosomes being dispersed among them (Pl. I 2, 3, II 4, 6). Moreover, the microfibrils proliferate the whole thickness of the ectoplast which, what is known in *Ditoxidae* (Strelkov 1939), does not surround the endoplasmic sac from all sides being limited to the anterior part of the body and partly to the right side. A compact strand of microfibrils occurs in the region of surface ribs on the ventral and on the dorsal body sides. This strand runs circularly at the base of ribs (Pl. III 8). The microfibrils delimit also the ectoplast from endoplast (endoplasmic sac) forming an inconspicuous layer here and there (Pl. Ill 7, 8), or well visible double layer (Pl. Ill 9). Bars of dense substance do not occur in cytoplasmic lips surrounding the zones of cilia and in the vault of vestibulum (Pl. Ill 10).

The ciliary zones, in form of ribbons, are composed of parallel evenly spaced rows of cilia (Pl. IV 12, 14). It has been already shown on silver impregnated total preparations (Wolska 1980). The kinetosomes have opened bases (Pl. IV 13). Structureless dense substance at the

base of kinetosomes slightly narrows lumen of the kinetosome cylinder and gives branchings to inside of the cell and to neighbouring kinetosomes. As a result, a complicated spatial net is formed spreading beneath the ciliary zones. In this intricate dense net the most pronounced are the strands running deeply along the rows of kinetosomes and being connected by transverse commissures (Pl. IV 14, VI 17 a). Derivative fibres get off from kinetosomes. Some of them are microtubular transverse fibres, the other ones are supposed to be feebly developed kinetodesmal fibrils (Pl. IV 14). The nematodesms, abundant in *T. unifasciculatum*, arise from the dense net. Some nematodesm are large and spread far behind the ciliary zones (Pl. IV 11, V 15). In places of large nematodesms origin dense substance of the net forms greater aggregations (Pl. IV 17) corresponding to the thickening of kinetosomal rows revealed in silver impregnated preparations (Wolska 1980). The nematodesm are especially numerous in the adoral zone (Pl. V 16). They form a timbering of cytopharynx in their further course. The cytoplasmic lips surrounding the ciliary zones are bent in their distal parts and form a characteristic pattern in sections (Pl. IV 12, X 28).

The cilia in *T. unifasciculatum* are joined into groups, similarly as in other *Entodiniomorphida*. For these groups the name syncilia has been admitted. Senaud and Grain (1972) have observed that in *Cochliatoxum periachtum* the axis of syncilium does not coincide with antero-posterior axes of kineties. The same phenomenon may be observed in *T. unifasciculatum* (Pl. IV 12).

At the ventral margin of the protozoan body, within the area of adoral zone lip, the cytoplasma is deeply ploughed and short deformed cilia are embedded in furrows (Pl. VI 18, VII 19, IX 25). In folds between furrows a layer of microtubular fibres occurs under cell membrane (Pl. VII 20). The kinetosomes of mentioned cilia (free cilia) have been already revealed in *T. unifasciculatum* by silver impregnation method (Wolska 1980). These cilia and folds occupy a small area in shape of a belt beginning at the right side of the body, overpassing ventral margin and spreading over the left side (cf. Wolska 1980). Left extremity of this structure is slightly bent backward and enters deeply the base of the adoral zone lip. So, in some sections this structure may be cut twice (Pl. VI 18, IX 25). The cilia are swollen or flattened and so short that they do not reach even the half height of the folds (Pl. VII 21). The axosomes are lacking in kinetosomes of this group of cilia but septum and axial granules are present (Pl. VII 19). The pattern of microtubular fibres is disturbed in most cilia. Most frequently the axial fibres can not be distinguished and the number of pairs of peripheric fibres differs from nine (Pl. VII 22 a). In some cilia

fibres are crowded disorderly (Pl. VII 22). Some cilia of this group, situated peripherically, retain normal pattern of fibres.

The cytostome is situated in the wall of a concavity (vestibulum) the inlet of which is surrounded by the ventral part of the adoral zone (Wolska 1980). In general the vestibulum is directed slightly to the back and to the dorsal right side of the body, but its particular segments deviate from this direction. The cytoplasm around the cytostome is slightly raised forming a rampart covered by short rows of cilia of the dorsal part of the adoral zone (Pl. VIII 23). Wide cytopharynx, folded in transverse and longitudinal sections, is directed to the dorsal back and to the right side (PI. VIII 24, IX 26, 27). Along the vault of the vestibulum run the folds similar to cytoplasmic lips surrounding somatic ciliary zones (PI. X 28, 30). The cytostome is provided with microtubules (rideaux de tubules) derived from the cilia neighbouring the cytostome (Pl. X 29). The wall of cytopharynx is composed of the layer of nematodesms, small groups of microtubules and a compact fibro-granular layer (Pl. XI 31, 32, XII 33). Outside to it there are numerous large nematodesms (Pl. XI 31, XII 33) originating from the dense net underlying the adoral ciliary zone. The microfibrils are interlaced among these nematodesms (Pl. XI 31, XII 33).

Discussion

Comparison of structure of *Cycloposthiidae sensu lato* examined under the electron microscope has shown that the cortex of *T. unifasciculatum* is the most similar to that of *Triadinium caudatum* (Wolska 1978 b). In nonciliated parts of the body under cell membrane in both species there is a thin layer of epiplasm, then the layer of microtubules arranged in bundles. Under them there are the bars od dense substance and finally the microfibrillar layer with short nonciliated kinetosomes dispersed without any order. Moreover, at the level of microtubules and partly at the level of bars, numerous vesicles are present in both species. Probably they secrete mucus visible sometimes on the cell membrane as a fuzz. The cortex of *T. unifasciculatum* differs only slightly from *Cochliatoxum periachtum* (Senaud and Grain 1972) by the presence of epiplasma layer and another shape of elements of dense substance situated between microtubular and microfibrillar layers. In *C. periachtum* they are in form of strongly developed plates instead of bars.

In *Tripalmaria dogieli* (Wolska 1978 a) all mentioned elements of the cortex are arranged in the same order but in addition to that there is a polysaccharide skeleton embraced by microfibrillar layer at the

right side of the body. Such skeleton is lacking in *T. unifasciculatum* as well as in *T. caudatum* and *C. periachtum*. *T. unifasciculatum* is the most different from *Cycloposthium bipalmatum* (Grain 1966). The latter does not show distinct splitting of microtubular layer in bundles or the dense substance in form of bars or plates. In *C. bipalmatum* the mucus vesicles do not occur but there is a polysaccharide skeleton in almost the whole body between the microtubular and microfibrillar layers. The kinetosomes in nonciliated body parts of *C. bipalmatum* have not been recognized.

Organization of the ciliary zones in *T. unifasciculatum* is the same as in other *Entodiniomorphida*. The cilia are grouped in syncilia, while in the level of kinetosomes grouping are lacking (Noirot-Timothée 1960, Grain 1966, Senaud and Grain 1972). The kinetosome cylinder in *T. unifasciculatum* is open similarly as in *T. caudatum* and *C. periachtum*, being thus different from *T. dogieli* and *C. bipalmatum*. It seems that the kinetosomes of *T. unifasciculatum* have the same associated fibres as *C. periachtum* and *T. caudatum*, namely transverse fibres and kinetodesmal ones. All kinetosomes of ciliary zones in *T. unifasciculatum* are included into a common net of dense substance formed by ramification of "manchets" surrounding the bases of kinetosomes. It is very similar to the arrangement of dense substance joining the kinetosomes in *T. caudatum* and *C. periachtum*. The connections between kinetosomes exist also in other *Entodiniomorphida* but dense substance has another form. The cytostome-cytopharynx complex in *T. unifasciculatum* has the same character as in *C. periachtum* and *T. caudatum*. The cytopharyngeal wall is composed of the layer of microtubules arranged in small groups and of the outer compact granular layer. This wall is folded and surrounded by nematodesms. Another structure has the wall of cytopharynx in *C. tripalmaria*.

In sum, the ultrastructure of the cortex and of the buccal apparatus in *T. unifasciculatum*, *C. periachtum* and *T. caudatum* is very similar. The genera *Tetratoxum*, *Cochliatoxum* and *Triadinium*, as well as *Ditoxum*, characterized by lack of skeleton, non retractible adoral zone and reduced ectoplast, have been taken out by Strelkov (1939) from the family *Cycloposthiidae* Poche and placed in a new family. The results of electron microscope investigation of the representatives of three of these genera uphold Strelkov's opinion. Simultaneously Strelkov (1939) transferred the genus *Spirodinium* Fiorent from the old family *Cycloposthiidae* to *Ophryoscolecidae* forming a new subfamily *Spirodiniinae* for it. Strelkov quotes some morphological characters of this genus approaching it to *Ophryoscolecidae*. Another opinion has been pronounced by Latteur and Dufey (1967). These authors relate *Spirodinium* to *Tetratoxum*, *Cochliatoxum*, *Triadinium* and *Ditoxum*

comprising all these genera in the subfamily *Spirodiniinae* within the new family *Spirodiniidae*. Further investigations on *Spirodinium*, in light as well as in electrons microscope, should give answer whether this genus ought to be placed within the family *Ophryoscolecidae* or within the distinct family *Spirodiniidae* (subfamily *Spirodiniinae*) together with *Tetratoxum*, *Cochliatoxum*, *Triadinium* and *Ditoxum*.

The "free cilia" in *T. unifasciculatum* have the same ultrastructure as in *Triadinium caudatum* (Wolska 1978 b) and *Tripalmaria dogieli* (Wolska 1978 a), as well as in the representatives of *Blepharocorythina* — *Ochoterenaia appendiculata* (Wolska 1978 c) and *Circodinium minimum* (Wolska 1979). Thus, "free cilia" are common structures of *Entodiniomorphida* and *Blepharocorythina*. Such generalization is justified because the kinetosomes of "free cilia" have been recognized in light microscope also in other representatives of *Entodiniomorphida* (Fernandez-Galiano 1959, Wolska 1965) and *Blepharocorythina* from horse intestine (Wolska 1971) beside the species mentioned in the present paper.

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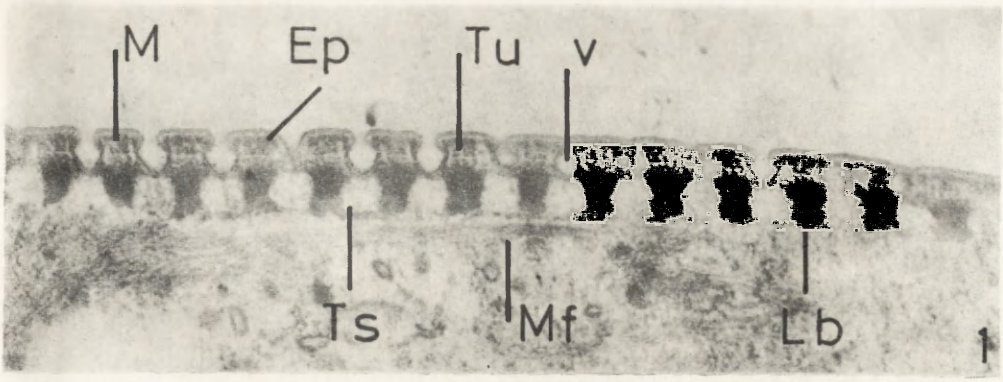
RÉSUMÉ

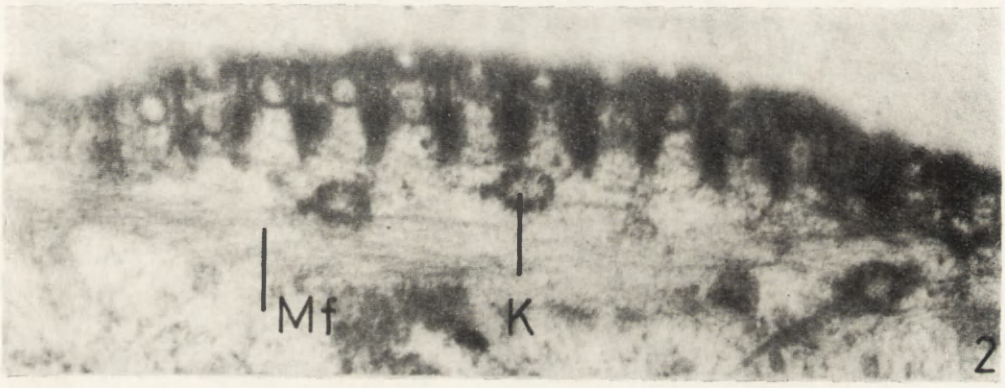
L'ultrastructure du cortex et de l'appareil oral a été étudiée chez le *Tetratoxum unifasciculatum* (Fiorent). Dans les parties du corps dépourvues de la ciliature on trouve, sous la membrane cellulaire et l'épiplasme, une couche des microtubules longitudinales disposées par faisceaux et, plus profondement, des baguettes longitudinales d'une substance dense. Au dessous de ce système caractéristique des baquettes les courts cinétosomes sont dispersés dans une couche microfibrillaire. Dans les zones ciliés les cinétosomes ne sont pas groupés en dépit du fait que les cils fusent en formant des syncils. Sous les cinétosomes des zones ciliés s'étend un réseau composé d'une substance dense avec la prédominance des bandes dirigées en parallèle par rapport aux rangs des cinétosomes. La structure du cortex chez *T. unifasciculatum* est pareille à celle du *Triadinium caudatum* et du *Cochliatoxum periachtum*, appartenants à la même famille. Le complexe cytostome–cytopharynx porte également le même caractère chez ces trois espèces. La groupe des cils appellés "free cilia" est présent chez *T. unifasciculatum*. Ils ont la même ultrastructure que chez les autres *Entodiniomorpha* et *Blepharocorythina* étudiés jusqu'à présent.

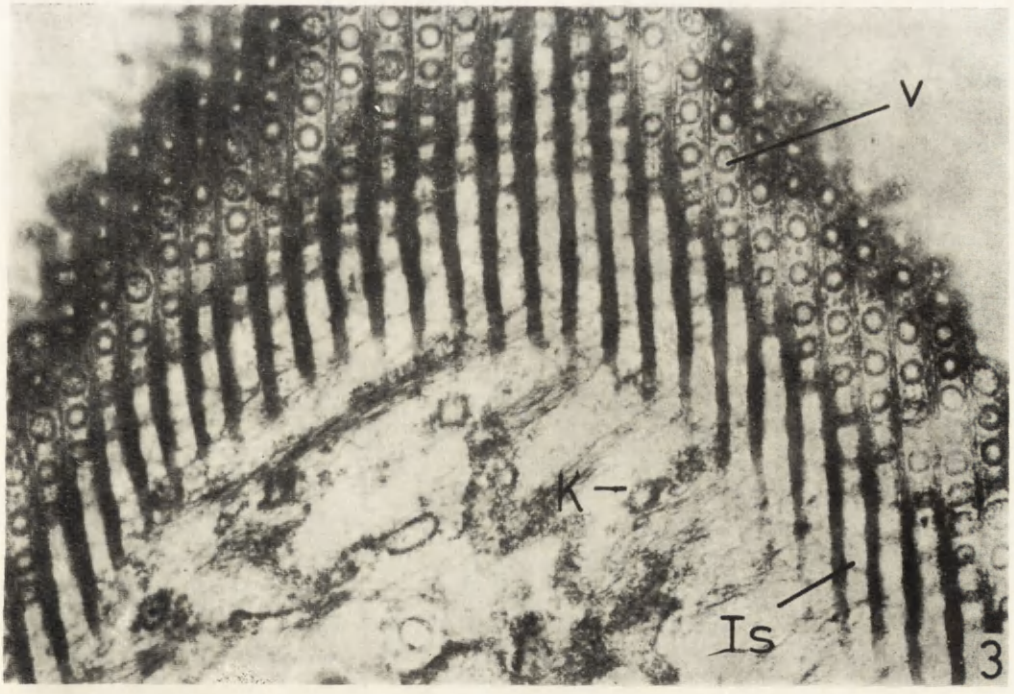
EXPLANATION OF PLATES I—XII

Tetratoxum unifasciculatum (Fiorent.), 1–22a and 28–33 — electronograms, 23–27 — microphotographs of semi-thin sections.
1: Transverse section perpendicular to body surface. Cell membrane (M), epiplasm (Ep), microtubules (Tu), vesicles (V), longitudinal bars (Lb), transverse strands (Ts), microfibrils (Mf). × 35 000
2: Section oblique to body surface. Microfibrils Mf), kinetosome (K). × 36 400
3: Section tangent to body surface, slightly oblique. Vesicles (V) with granular content or empty ones seen among microtubules, kinetosomes (K) dispersed in microfibrillar layer; transverse strand (Ts) of dense substance. × 23 000
4. Oblique section. Vesicles (V) among longitudinal microtubules; microfibrillar layer (Mf), kinetome (K). × 32 000
5: Transverse section. Microtubules (Tu), epiplasm (Ep), × 39 000
6: Section tangential to body surface. Vesicles (V), longitudinal bars (Lb), microfibrils (Mf), kinetosome (K). × 18 400
7: Transverse section. Transverse strands of dense substance (Ts); delimination of ectoplast from endoplasmic sac is seen. ×26 000

8: Transverse section through the region of ribs in the anterior body part. Strand of microfibrils (Mf) at the base of ribs. × 23 000
9: Microfibrils at the boundary of ectoplast and endoplasmic sac. Nematodesms in ectoplast (Nd). × 41 400
10: Section through the vault of vestibulum. × 39 000
11: Section through ectoplast. Numerous nematodesms. × 13 800
12: Section through somatic ciliary zone. Groups of cilia and direction of kineties is seen. × 6700
13: Longitudinal section through kinetosomes. Open bases of kinetosomes are seen. Strand of dense substance (arrow). × 19 200
14: Transverse section, slightly oblique, through kinetosomes of ciliary zone. Kinetodesmal fibres (Kd), transverse fibres (T). Double strands of dense substance running under kineties and transverse strands are visible. × 32 000
15: Large nematodesma originating from dense substance net. × 19 200
16: Section through adoral zone. Abundance of nematosms × 19 200
17: Mass of dense substance in the place of origin of nematodesma (arrow). ×33 600
17 a: Longitudinal section through kinetosomes. Mass of dense substance going to inside and to neighbouring kinetosomes (arrow). × 32 200
18: Section through "free cilia" region. Twice cut folds and cross-sections of some cilia are seen. × 19 200
19: Section through folds and "free cilia" near adoral zone. Adoral zone cilia (Ad.c.), kinetosomes of "free cilia" (K.f.c.) cytoplasmic lip of the adoral zone (C.l.). × 26 000
20: Section through folds separating "free cilia". Microtubules (arrow). × 33 600
21: Section through folds and deformed "free cilia". A very short cilium (arrow). × 24 000
22: Section through deformed cilia. Atypical pattern of fibres in cilia. × 49 000
22 a: The same. Cross-section through a cilium with 6 pairs of tubular fibres (arrow). × 49 000
23: Section in plain of cilia surrounding cytostome. Ventral side (V), dorsal side (D), × 1700
24: Section similar to the preceding nearer right side of the body. Ventral side (V), dorsal side (D), section of cytopharynx is visible. × 1700
25: Approximately sagittal section near left body side. "Free cilia" (arrow), ventral side (V), × 1700
26: Longitudinal oblique section near right body side. Cytopharynx directed postero-dorsally. Macronucleus is seen. Dorso-right side (D.R.). × 1700
27: Section similar to above one. Cilia of the anterior dorsal somatic zone (arrow). × 1700
28: Section through cytoplasmic lip of the somatic ciliary zone. × 19 200
29: Section through kinetosomes and originating from them microtubular fibres forming an equipment of cytostome. × 20 000
30: Section through vestibulum and cytopharynx. × 15 000
31: Section through vestibulum and cytopharynx. Outer nematodesms (arrow), microfibrill (Mf). × 10 000
32: Section through cytopharynx. Groups of microtubules (Tu), fibro-granular layer (arrow). × 19 200
33: Section through vestibulum and cytopharynx. Nematodesms and small groups of microtubules (arrow), outer nematodesms (Nd), microfibrils (Mf), "Rideaux de tubules" (R). × 28 000







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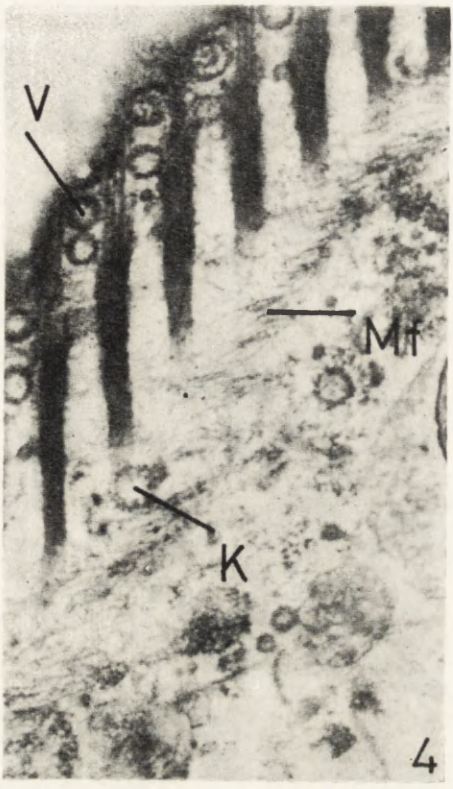

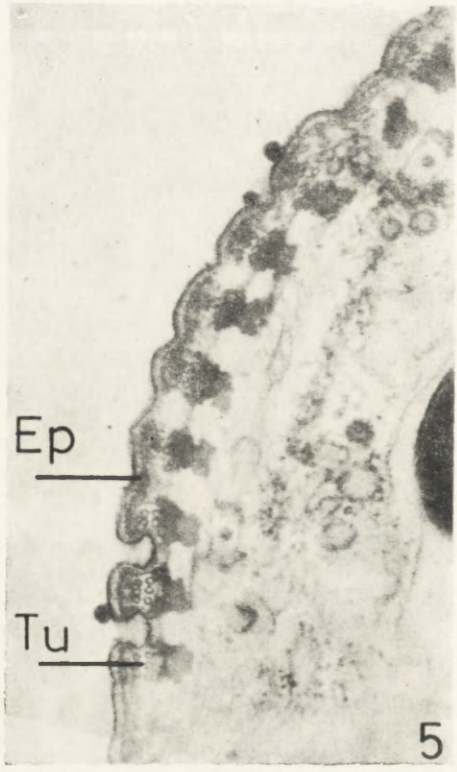

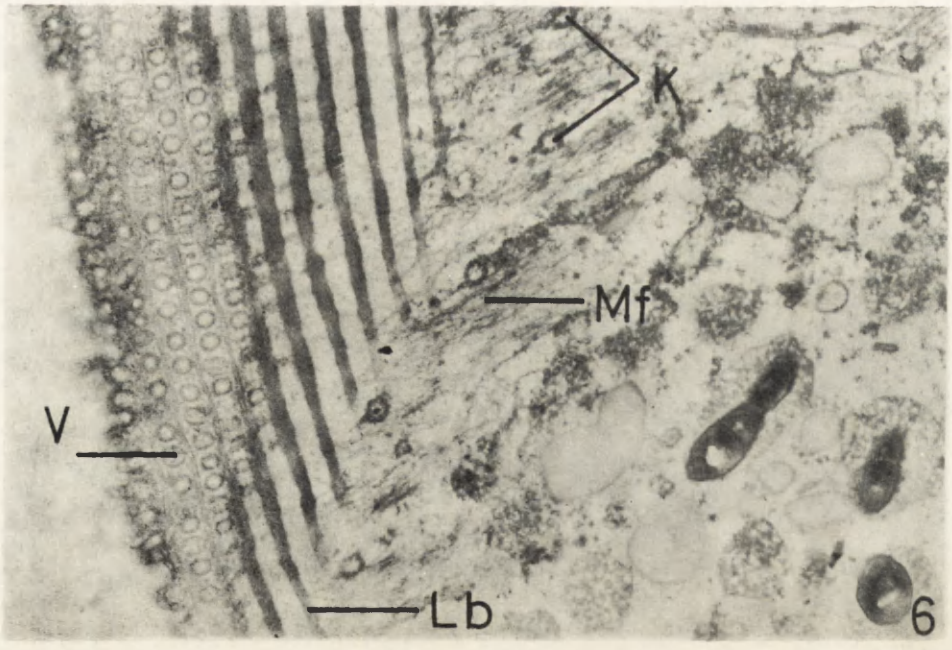

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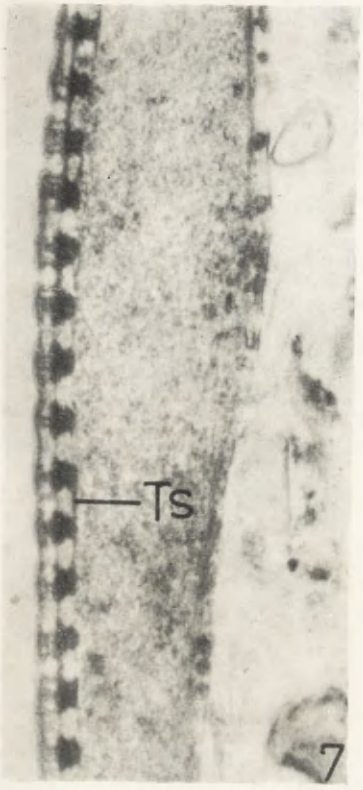

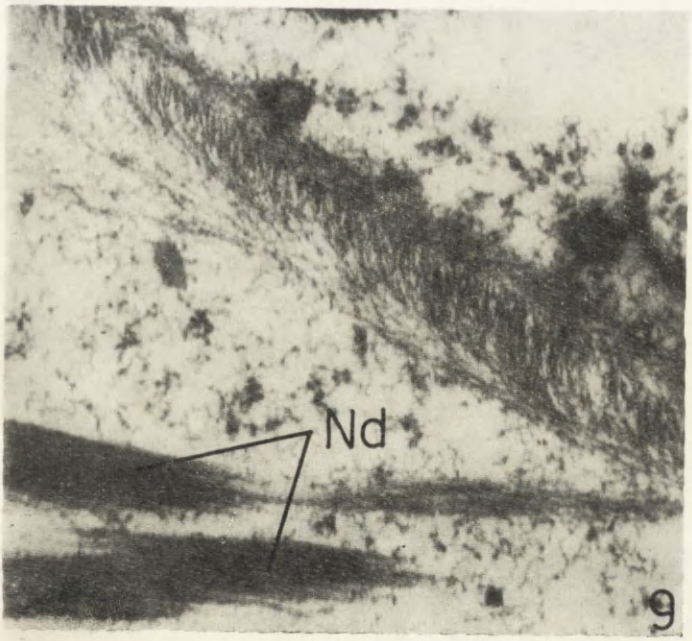

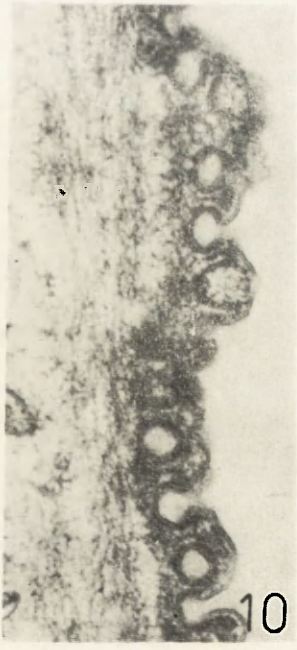

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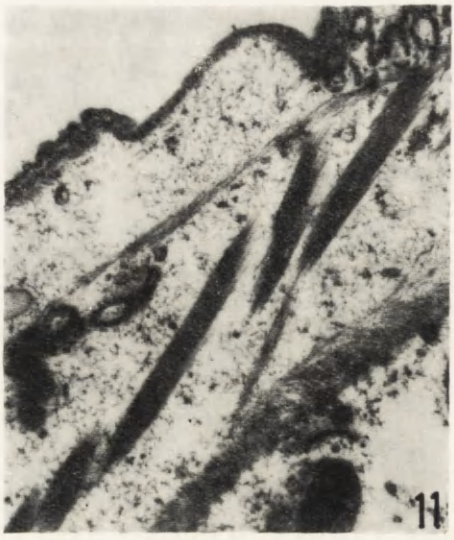

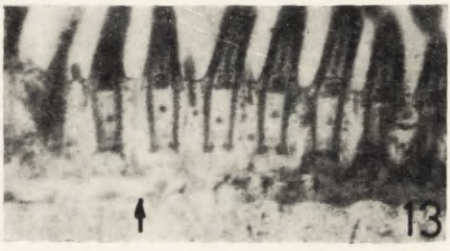

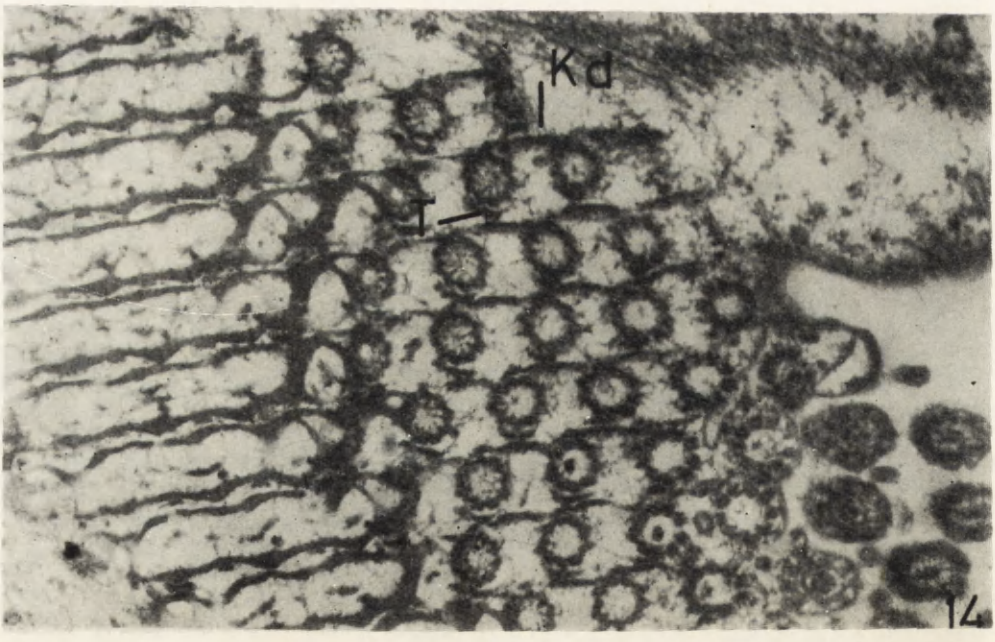

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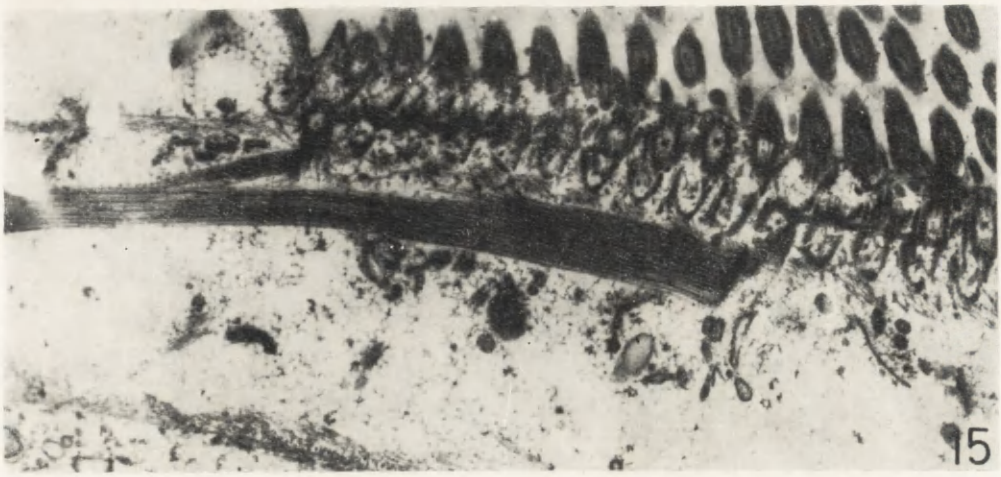

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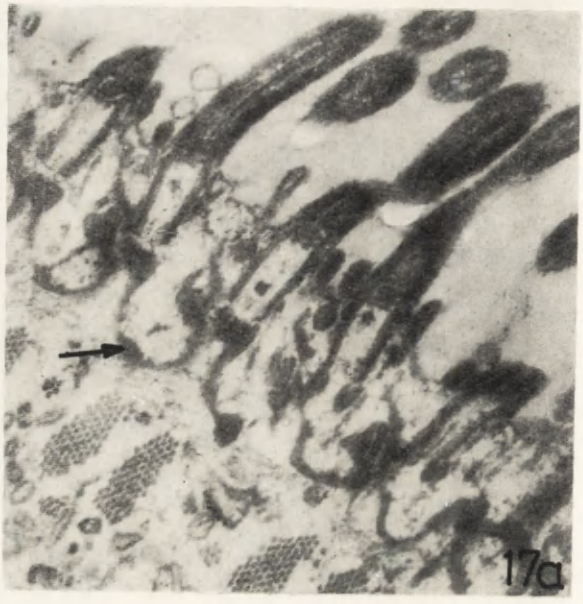

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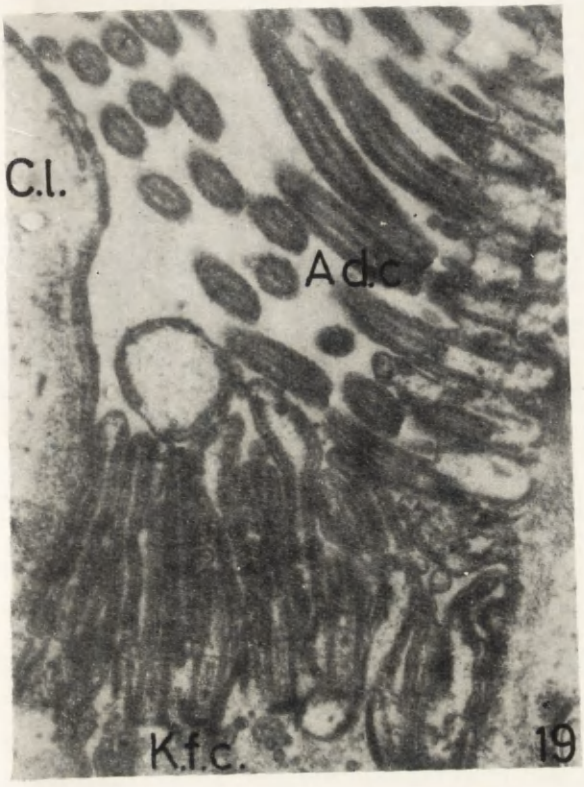

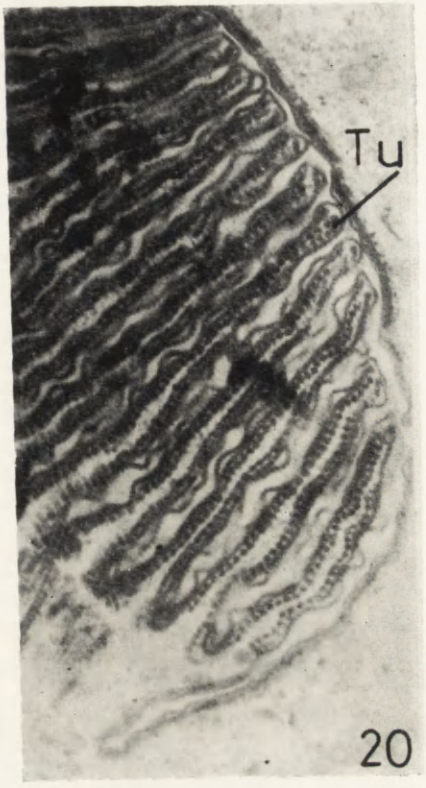

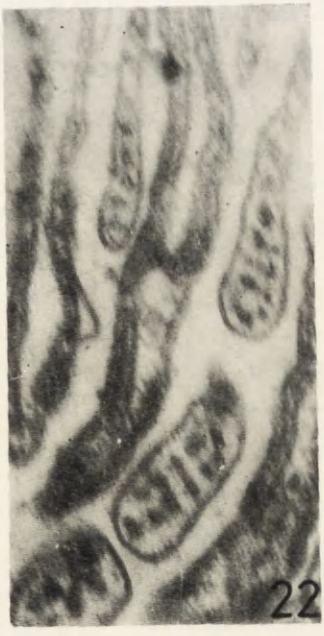

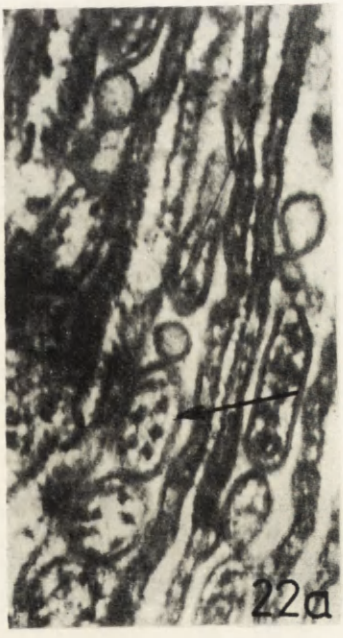

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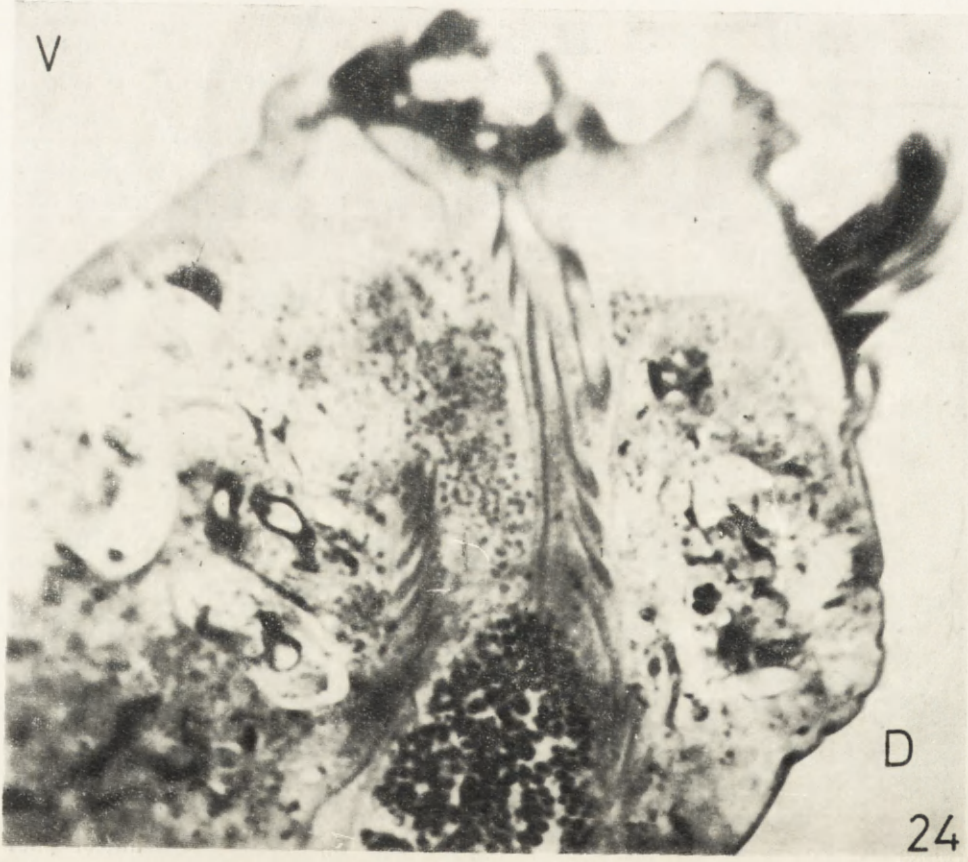

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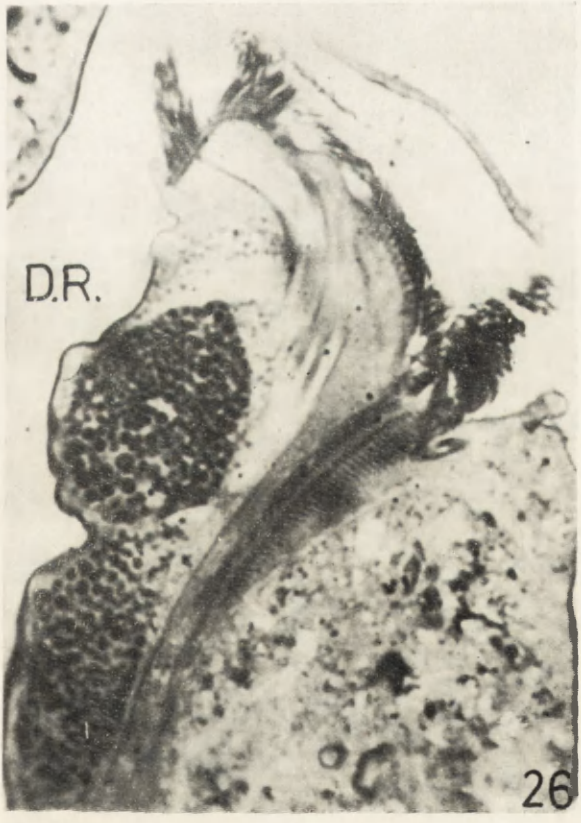

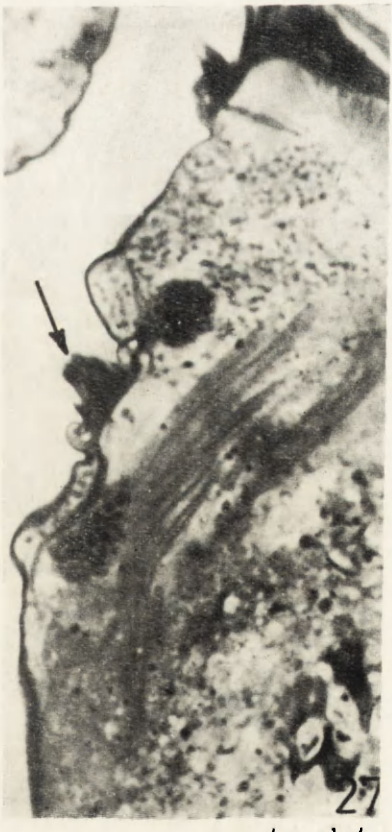

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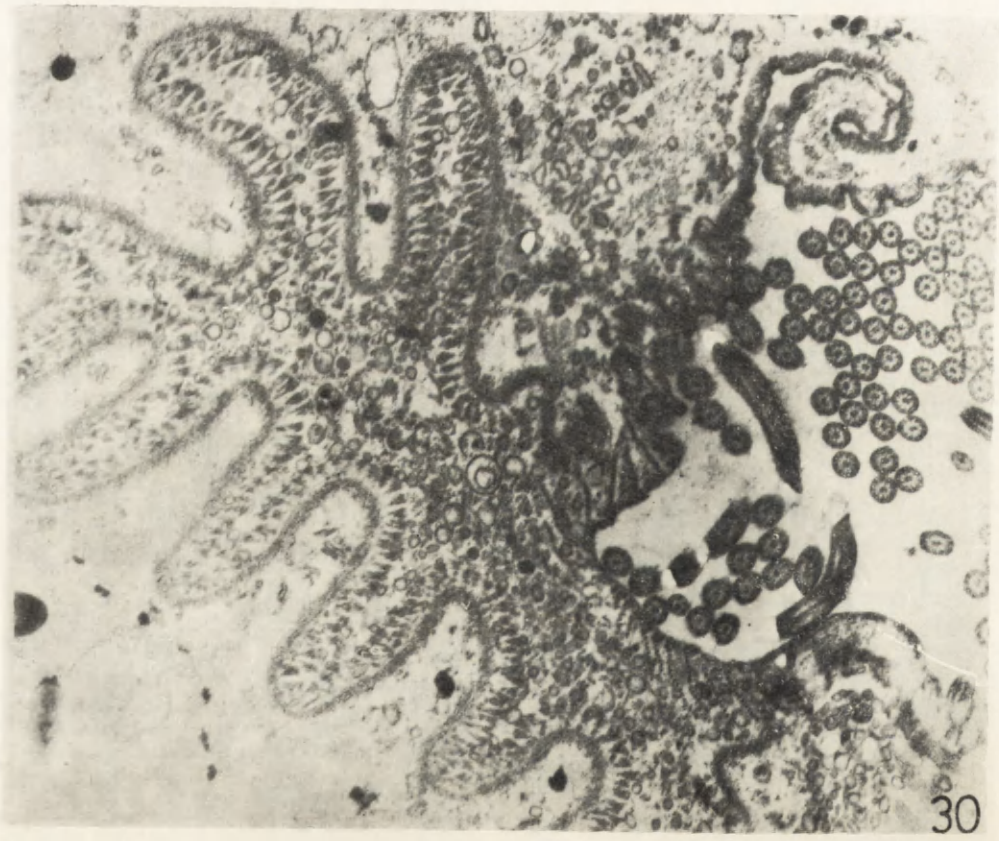

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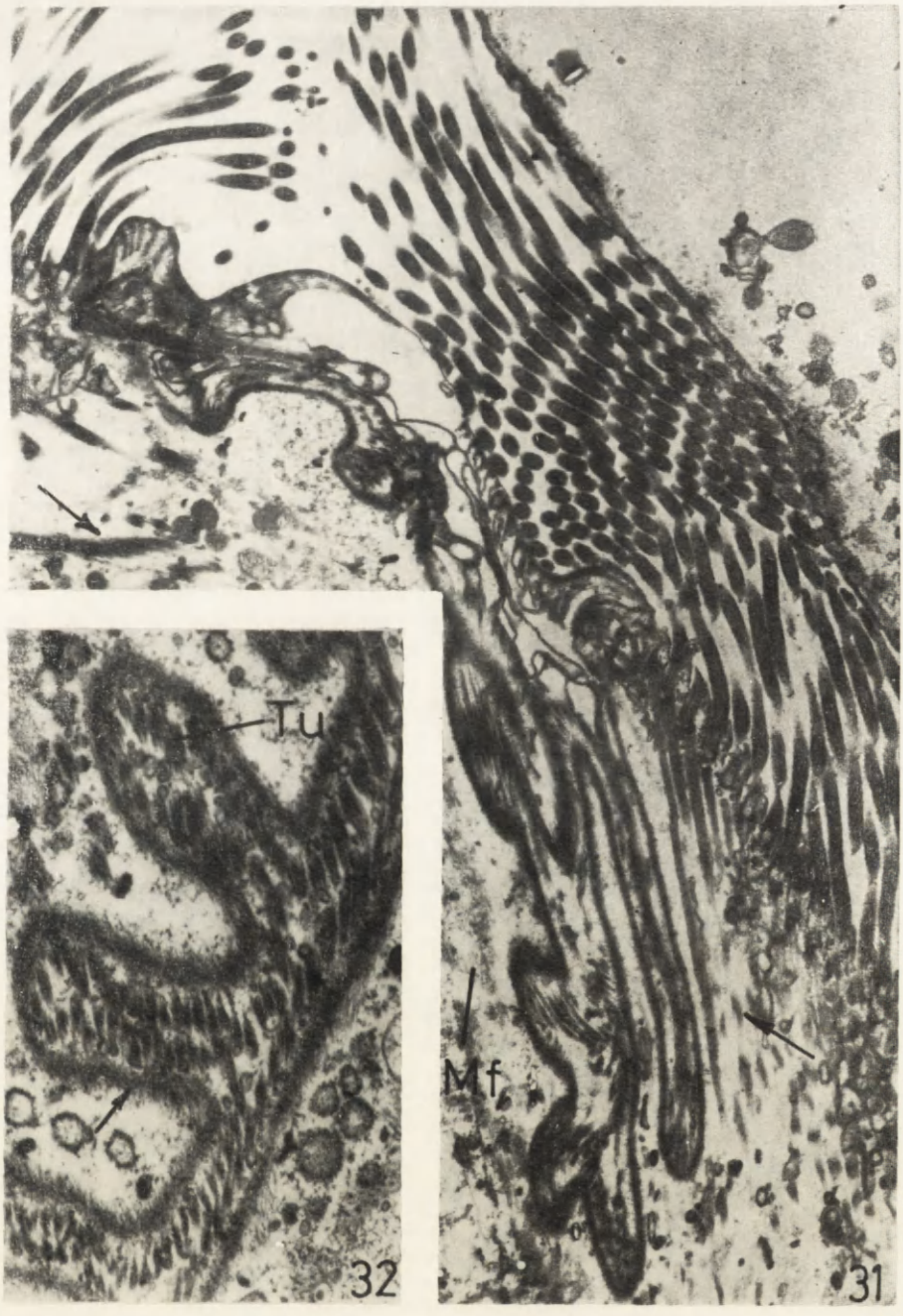

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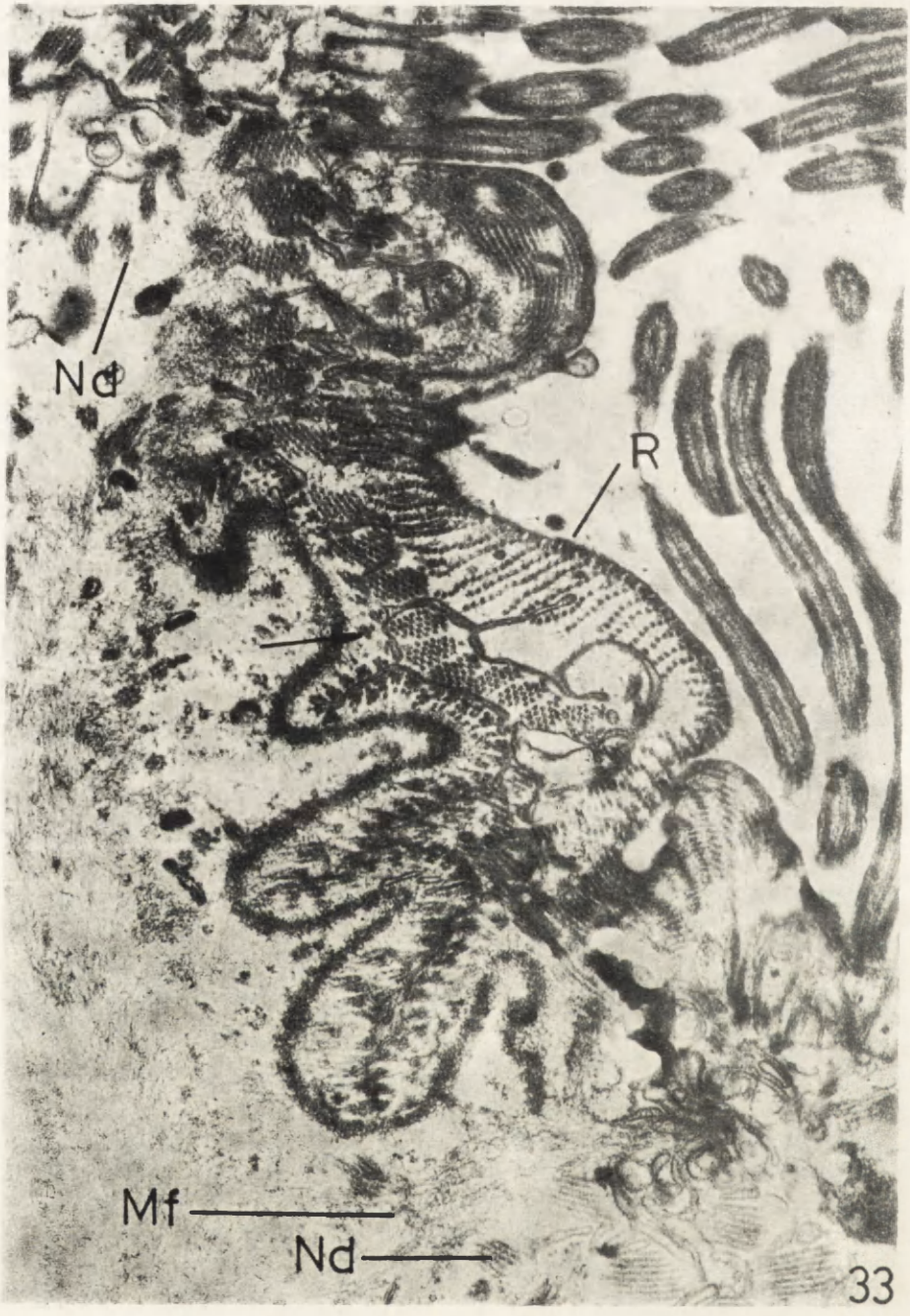

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Taxonomische Studien über die Ciliaten des Großglocknergebietes (Hohe Tauern, Österreich). VI. Familien *Woodruffiidae*, *Colpodidae* und *Marynidae*

Received 12 July 1979

Synopsis. Es wird die Morphologie, die Infraciliatur und das Silberliniensystem einiger neuer oder wenig bekannter *Colpodida* aus den Kleingewässern des Großglocknergebietes (Hohe Tauern, Österreich) beschrieben. Folgende neue Taxa werden errichtet: *Platyophrya citrina* nov. spec., *Platyophrya dubia* nov. spec., *Platyophrya hyalina* nov. spec., *Platyophrya procera* nov. spec., *Colpoda ovinucleata* nov. spec., *Colpoda rotunda* nov. spec. und *Colpoda variabilis* nov. spec. Die Infraciliatur und das Silberliniensystem von *Maryna ovata* weisen darauf hin, daß die *Marynidae* mit den *Colpodidae* eng verwandt sind.

In einer früheren Studie (Foissner 1978) habe ich mich eingehend mit der Systematik der *Colpodida* auseinandergesetzt und die Ordnung in zwei Unterordnungen aufgeteilt. Während der Untersuchungen über die Ciliatenfauna der Kleingewässer des Großglocknergebietes fand ich mehrere neue *Colpodida*, deren Morphologie und Infraciliatur Gegenstand dieser Arbeit ist. Für die schon damals hervorgehobene enge Verwandtschaft der Gattungen *Platyophrya* und *Cyrtolophosis* können nun weitere Belege beigebracht werden. Bei *Platyophrya hyalina* ist der Mund so wie bei *Cyrtolophosis mucicola* deutlich auf die Ventralseite verlagert und etwas eingesenkt. Vor kurzem fand ich in alpinen Böden außerdem eine *Cyrtolophosis* verwandte Art, bei der die linke Körperseite fast ganz unbewimpert ist, ähnlich wie bei der Gattung *Platyophrya* (s. Foissner 1978). Im gleichen Biotop entdeckte ich weitere neue Species der Genera *Platyophrya* und *Cyrtolophosis*. Ihre Beschreibung wird an anderer Stelle veröffentlicht. Diese Beobachtungen weisen darauf hin, daß ein Großteil der Arten der Familien *Woodruffiidae* und *Cyrtolophosididae* noch unbekannt ist.

Material, Methoden, Abkürzungen

Das Untersuchungsmaterial wurde in den Kleingewässern (Weidetümpeln, ausgedrückten Moosen von kleinen Bächen etc.) entlang der Großglockner-Hochalpenstraße gesammelt. Genauere Fundortangaben und ökologische Daten finden sich bei Foissner (1980). Darauf bezieht sich auch die Numerierung des Kleingewässers beim *Locus typicus* der neuen Arten.

Zur Darstellung der Infraciliatur und des Silberliniensystems verwendete ich die nasse Silberimprägnationsmethode von Corliss (1953) und das trockene Silberimprägnationsverfahren von Foissner (1976). Der Kernapparat wurde mit Orcein-Essigsäure, die Protrichocysten wurden mit Methylgrün-Pyronin (MP) angefärbt (Foissner 1979). Alle Arten wurden einer sorgfältigen Lebendbeobachtung unterzogen.

A — Gefressene Grünalgen
aM — Adorale Membranellen
Ba — Bakterien (?)
CV — Kontraktile Vakuole
D — Großer Dorsalsack
d — Kleiner Dorsalsack
Dr — Diagonalrinne
DV — Defäkationsvakuole
E — Kompakte Entoplasmaeinschlüsse
K — Gallertgehäuse
G — Kristalle
lP — Linke Polykinete
*m*K — Mycterale Kineten
NV — Nahrungsvakuole
P — Exkretionsporus der CV
pM — Parorale Membran
Pt — Protrichocysten
rP — Rechte Polykinete
T — Trichiten
V — Vestibulum
Vk — Vestibularkineten
Vn — Ventralnaht am Kiel
Z — Zoochlorellen

Beschreibung der Arten

Außer den unten beschriebenen Arten fand ich noch *Cyrtolophosis mucicola* Stokes, *Colpoda cucullus* Müller, *Colpoda inflata* (Stokes) und *Colpoda steinii* Maupas. Den Beschreibungen dieser Species durch Kahl (1930–35), Foissner (1978) u.a. habe ich nichts hinzuzufügen.

1. Familie *Woodruffiidae* Gelei

Platyophrya citrina nov. spec. (Abb. 1 a–d)

Diagnose: 40–60 µm große, etwa 2 : 1 abgeflachte, stark metabolische *Platyophrya*, deren Entoplasma leicht gelborange gefärbt ist. Ventrallinie sigmoid, Dorsallinie im proximalen Abschnitt meist stark gewölbt, so daß der Mund weit nach links verschoben wird. Rechts 7–9 Kineten mit je etwa 30 Basalkörperpaaren. Links 6–7 Kineten mit je ca. 12 Basalkörperpaaren, einschließlich der dicht unterhalb der 4–5 kleinen adoralen Membranellen liegenden Dikineten.

Locus typicus: Vereinzelt in einer tümpelartigen Verbreiterung eines reinen Bächleins (Tümpel 27) südlich des Fuschertörls (Großglockner-Hochalpenstraße, etwa 2300 m ü.d.M.).

Morphologie: Körperform variabel, aber sehr charakteristisch (Abb. 1 a, d). Unterschiedlich ist vor allem die Stärke der Wölbung der Dorsallinie. Körperquerschnitt proximal lang oval, distal kurz oval (Abb. 1 b, c). Mund von sehr zarten Trichiten umgeben. Makronucleus kugelförmig, mit länglichen Nucleolen und dicht anliegendem Mikronucleus. Dicht unter der Pellicula viele leicht zylindroide Protrichocysten (Abb. 1 a). Sie werden in Form von kleinen Tröpfchen abgesondert, die rasch zu einem umfangreichen, feinmaschigen Netzwerk verquellen (Foissner 1979). Kontraktile Vakuole subterminal, mit kurzem, breitem Röhrenporus. An der Stelle seiner Ausmündung ist das Tier stets leicht eingedellt. Entoplasma mit vielen, oft sehr großen, orangegrünen Einschlüssen (Abb. 1 a). Bewegung: langsam, torkelnd, wühlt in Algen- und Bakterienhäufchen. Nahrung: Algen. Manchmal häufig in Weidetümpeln.

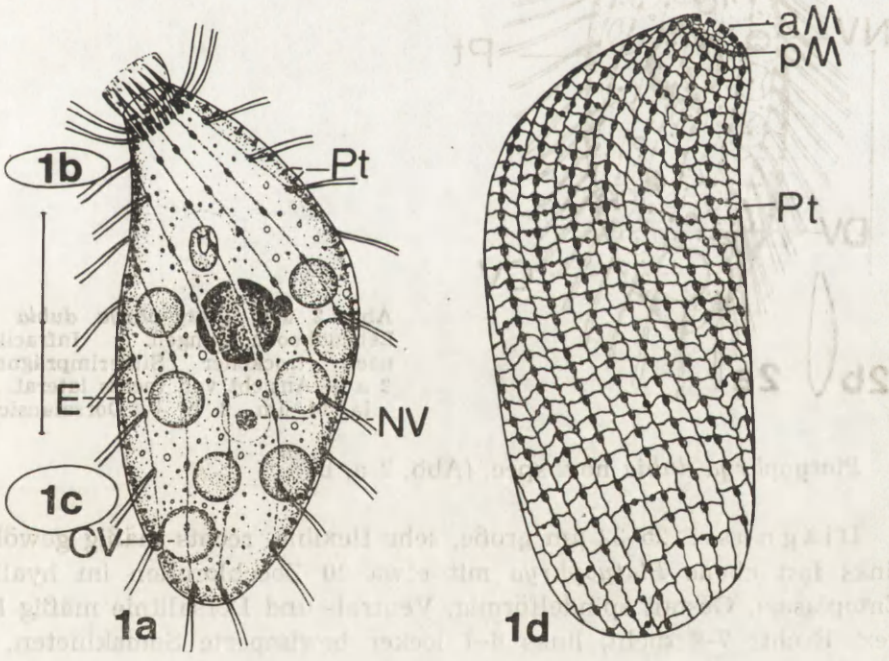


Abb. 1a-d. *Platyophrya citrina* nach Lebendbeobachtungen. Infraciliatur und Silberliniensystem nach trockener Silberimprägnation. 1 a — Ansicht von links lateral. Skala 20 µm. 1 b, c — Körperquerschnitt proximal und subterminal. 1 d — Infraciliatur und Silberliniensystem der rechten Seite

Somatische und orale Infraciliatur und Silberliniensystem (Abb. 1 d) gleichen weitgehend *Platyophrya vorax* (s. die bei Foissner 1978 in Abb. 3 noch als *Platyophrya sp.* bezeichnete *P. citrina*).

P. citrina bildet bereits wenige Stunden nach dem Fang leicht orange gefärbte, kugelförmige Cysten, die von einer sich mit MP intensiv blau färbenden Ectocyste und einer sich nicht anfärbenden Entocyste umgeben sind. Bei der Konjugation vereinigen sich die Partner mit dem Mund.

Diskussion: *P. citrina* steht *P. lata* Kahl, 1930–35 nahe, deren Dorsallinie eine ähnliche Form aufweist. Sie unterscheidet sich von dieser durch die Färbung, die Anzahl der Kineten, den Körperquerschnitt und die Größe.

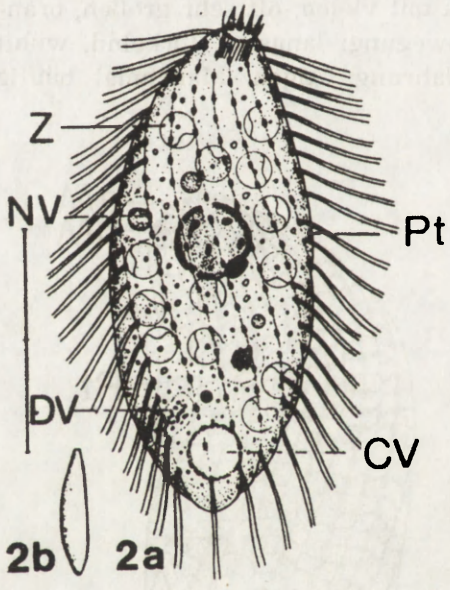


Abb. 2 a–b. *Platyophrya dubia* nach Lebendbeobachtungen. Infraciliatur nach trockener Silberimprägnation. 2 a — Ansicht von rechts lateral. Skala 14 µm. 2 b — Dorsalansicht

Platyophrya dubia nov. spec. (Abb. 2 a, b)

Diagnose: 25–32 µm große, sehr flexible, rechts mäßig gewölbte, links fast ebene *Platyophrya* mit etwa 20 Zoochlorellen im hyalinen Entoplasma. Gestalt spindelförmig. Ventral- und Dorsallinie mäßig konvex. Rechts 7–9 dicht, links 6–7 locker bewimperte Somakineten. 4–5 adorale Membranellen.

Locus typicus: Häufig in einem stark eutrophen Kleingewässer (Tümpel 52 a) östlich des Wallackhauses (Großglockner-Hochalpenstraße, etwa 2300 m ü.d.M.).

Morphologie: Mundeingang spaltförmig, fast apikal gelegen, von kurzen, *in vivo* schwierig erkennbaren Trichiten umgeben. Während der Nahrungsaufnahme kann er sich auf etwa das Doppelte seines normalen Durchmessers erweitern. Makronucleus kugelförmig, mit undeutlichen, länglichen Nucleolen, zentral gelegen. Mikronucleus ellipsoid, stark lichtbrechend, dem Makronucleus dicht anliegend. Pellicula durch die leicht rechtsspiralig verlaufenden Somakineten stark gekerbt. Cilien etwa 6 µm lang, am distalen Pol ein etwa 5 µm durchmessendes wimperloses Feld. Zwischen den Kineten zylindroide Protrichocysten. Kontraktile Vakuole leicht subterminal. Entoplasma farblos, mit fettig glänzenden, kugelförmigen Einschlüssen. Nur wenige Nahrungsvakuolen mit Algenresten. Meist einige unregelmäßig geformte Defäkationsvakuolen (?) mit orange gefärbten Einschlüssen (Abb. 2 a).

Die somatische und orale Infraciliatur sowie das Silberliniensystem gleichen weitgehend dem von *P. citrina* (s. oben).

Diskussion: Diese Species unterscheidet sich von *P. vorax* (s. unten) durch die geringere Größe, den stärker abgeflachten, spindelförmigen Körper, den mehr apikal gelegenen Mund sowie durch den Besitz von Zoochlorellen. Von *P. nana* Kahl, 1926 unterscheidet sie sich durch die Körperform, die kräftigere Kerbung der Pellicula und durch die Zoochlorellen. Da aber diese Merkmale bei *Platyophrya* sehr variabel sind, ist nicht auszuschließen, daß *P. dubia* nur ein Ökotyp einer der oben erwähnten Arten ist, wenn man die Zoochlorellen nicht als Specieskriterium gelten läßt. Von *Woodruffia viridis* Gelei, 1954, die ebenfalls Zoochlorellen besitzt, ist *P. dubia* klar zu trennen, da *W. viridis* bedeutend größer ist, über 30 Somakineten und etwa 10 adorale Membranellen besitzt (vgl. Grolière 1975).

Platyophrya hyalina nov. spec. (Abb. 3 a–c)

Diagnose: 36–45 µm große, sich nach distal verschmälernde, rechts konvexe und links konkave *Platyophrya* mit bisquittförmigem Körperquerschnitt und stark gekerbter Pellicula. Oralapparat auf die Ventralseite verlagert, mit 4–5 adoralen Membranellen. Makro- und Mikronucleus von einer deutlich erkennbaren Membran umgeben. Zwischen Chromatin und Membran körnchenartige, anfärbbare Strukturen (Bakterien?). Rechts 7–9 dicht, links 6–7 locker bewimperte Somakineten.

Locus typicus: Vereinzelt zwischen Moosen eines reinen Bächleins südlich des Heldendenkmales (Probenahmeort 26) (Großglockner-Hochalpenstraße, etwa 2300 m ü.d.M.).

Morphologie: *P. hyalina* ist auf der Höhe des weit nach ventral versetzten, merkbar eingesenkten, von zarten Trichiten gestützten Oralapparates am breitesten und verschmälert sich nach distal allmählich.

Das sich mit Orcein intensiv färbende Chromatin des Makronucleus wird in weitem Abstand von einer Membran umgeben, die auch den ellipsoiden Mikronucleus einschließt. Das ist typisch für *Platyophrya* (Foissner 1978), aber nicht bei allen Arten so klar zu sehen. Pellicula durch die leicht rechtsspiralig verlaufenden Somakineten stark ge-

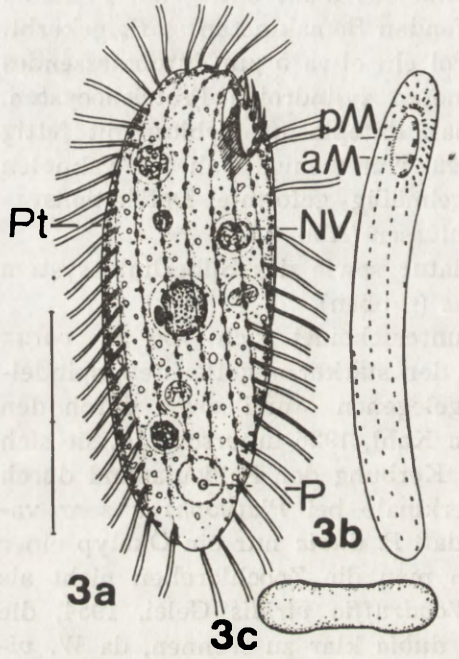


Abb. 3 a-c. *Platyophrya hyalina* nach Lebendbeobachtungen. Infraciliatur nach trockener Silberimprägnation. 3 a — Ansicht von rechts lateral. Skala 20 µm. 3 b — Ventralansicht. Skala 20 µm. 3 c — Querschnitt in Körpermitte

kerbt. Zwischen den Wimperreihen Protrichocysten. Kontraktile Vakuole deutlich subterminal, Exkretionsporus auf der rechten Seite, etwa 4 µm vom distalen Ende entfernt. Entoplasma meist mit vielen Nahrungsvakuolen mit Algen und farblosen Granula. Kriecht unruhig auf Detritushäufchen und am Oberflächenhäutchen des Sammelglases umher.

Somatische und orale Infraciliatur (Abb. 3 b) und Silberliniensystem ähnlich wie bei *P. vorax* (s. Foissner 1978). In Folge der starken Verlagerung des Mundes stoßen aber nur 3 Kineten an den rechten Mundrand, während es bei den anderen hier beschriebenen Arten stets 4–5 sind.

Diskussion: Unterscheidet sich hinsichtlich der Körperform von allen bisher bekannten Vertretern der Gattung. Die Stellung des Mundes, die starke Kerbung der Pellicula und die Größe ähneln *P. angusta* Kahl, 1926, bei der nach Buitkamp (1977) die adoralen Membranellen fehlen sollen.

Platyophrya procera nov. spec. (Abb. 4 a–c)

Diagnose: 45–81 µm große, lang ovale, etwa 2 : 1 abgeflachte, leicht kontraktile und metabolische *Platyophrya,* deren Pellicula durch die leicht rechtsspiralig verlaufenden Somakineten deutlich gekerbt wird. Dorsallinie konvex, Ventrallinie leicht konkav. Rechts 7–9 dicht, links 6–7 locker bewimperte Somakineten. Oralapparat auf die Ventralseite verschoben, mit 4–6 adoralen Membranellen.

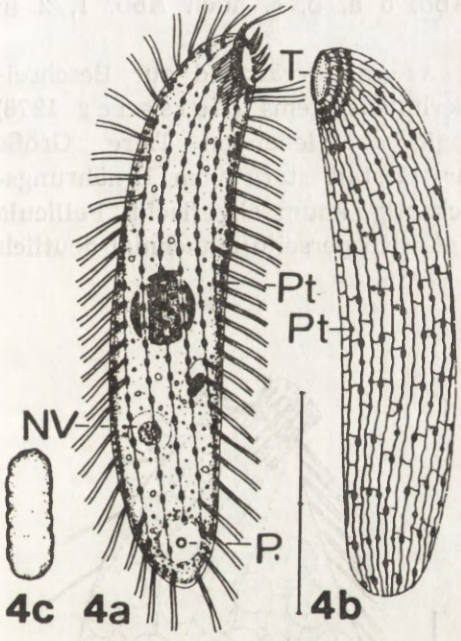


Abb. 4 a–c. *Platyophrya procera* nach Lebendbeobachtungen. Infraciliatur und Silberliniensystem nach trockener Silberimprägnation. 4 a — Ansicht von rechts lateral. Skala 30 µm. 4 b — Infraciliatur und Silberliniensystem der linken Seite. 4c — Körperquerschnitt

Locus typicus: Vereinzelt zwischen Moosen eines reinen Bächleins am Weg in das Rotmoos (Probenahmeort 4) (Großglockner-Hochalpenstraße, etwa 1200 m ü.d.M.).

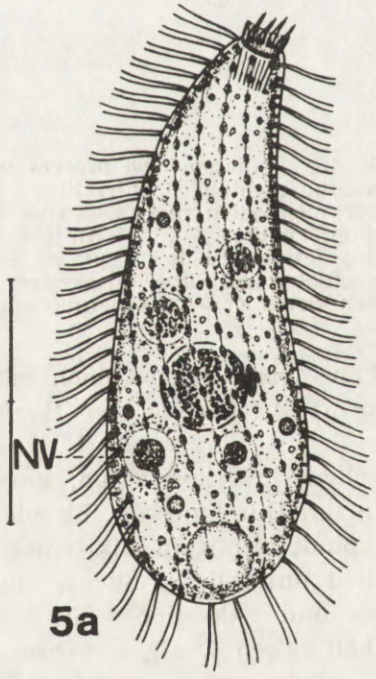
Morphologie: Oralapparat von schwierig erkennbaren, kurzen Trichiten umgeben. Makronucleus 8–10 µm im Durchmesser, mit vielen kleinen Nucleolen und dicht anliegendem, spindelförmigem Mikronucleus. Cilien der Dikineten lang und weich. Dicht unter der Pellicula rundliche Protrichocysten. Kontraktile Vakuole und Exkretionsporus leicht subterminal. Entoplasma sehr hyalin, enthält neben wenigen Nahrungsvakuolen mit Algenresten meist einige größere orange gefärbte Einschlüsse und mäßig viele farblose, glänzende Granula. Gleitet langsam auf Pflanzenresten und am Oberflächenhäutchen des Sammelglases. Somatische und orale Infraciliatur und Silberliniensystem sehr ähnlich wie

bei *P. vorax* (s. Foissner 1978). Basalkörper paarig angeordnet, auf der locker bewimperten linken Seite trägt aber nur ein Basalkörper eines Paares ein Cilium. Das trifft vielleicht auch auf die anderen hier beschriebenen Arten zu!

Diskussion: *P. procera* unterscheidet sich von den anderen Arten der Gattung, deren Mund auf die Ventralseite verlagert ist, durch die ungewöhnlich schlanke Körperform und den spindelförmigen Mikronucleus.

Platyophrya vorax Kahl, 1926 (Abb. 5 a, b, s. auch Abb. 1, 2 in Foissner 1978)

Morphologie und Diskussion: Ergänzend zur Beschreibung der Infraciliatur und des Silberliniensystems (Foissner 1978) hier noch Angaben zur Morphologie der lebenden Tiere. Größe 50–65 µm. Körper weich, verformbar. Gestalt stark vom Ernährungszustand abhängig. Trophont breit sackartig, kaum abgeflacht, Pellicula glatt (Abb. 5 b). Theront lang oval, linke Körperseite manchmal deutlich



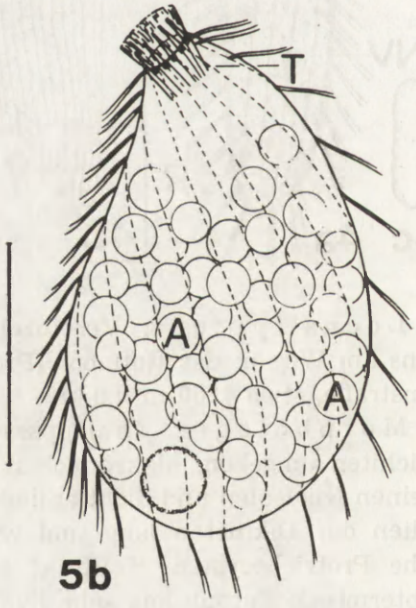


Abb. 5 a–b. *Platyophrya vorax* nach Lebendbeobachtungen. Infraciliatur nach trokkener Silberimprägnation. 5 a — Theront rechts lateral. Skala 25 µm. 5 b — Trophont links lateral. Skala 30 µm

abgeflacht, Pellicula durch die Wimperreihen kräftig gekerbt (Abb. 5 a). Mund leicht subpolar, von mäßig deutlichen Trichiten umstellt, kann bei der Nahrungsaufnahme stark erweitert werden. Makronucleus kugelförmig, fein granuliert, mit deutlicher, vom Chromatin leicht abgesetzter Membran, zentral bis subzentral gelagert. Mikronucleus spindelförmig, auffallend stark lichtbrechend, dem Makronucleus dicht anliegend. Zwischen den Wimperreihen rundliche Protrichocysten. Kontraktile Vakuole terminal, von vielen glänzenden Granula umgeben. Entoplasma farblos, stets mit vielen schollenartigen, farblosen Granula und einigen farblosen und orangeroten, fettig glänzenden, kugelförmigen Einschlüssen. Stark ernährte Individuen oft leuchtend grün durch gefressene Algen. Nach W e n z e l (1953) kommen auch Stämme mit Zoochlorellen vor. Bewegung rasch, mit dem proximalen Körperteil werden bohrende Bewegungen in Detritushäufchen durchgeführt.

2. Familie *Colpodidae* Ehrenberg

Tabelle 1

Biometrische Charakteristik von *Colpoda ovinucleata* und *Colpoda variabilis*. Kinetenzahl nach trocken versilberten Individuen. Alle anderen Werte nach naß imprägnierten Exemplaren

Parameter	Extremwerte (µm)	Mittelwerte (µm)	Anzahl der untersuchten Individuen
Länge	80–133	100.2	11
	72–91	76.9	16
Breite praeoral	20–53	42.9	9
	38–61	44.4	11
Länge vom proximalen Pol bis zum	28–40	33.0	6
Beginn des Oralapparates	17–31	23.0	11
Länge vom proximalen Pol bis zum	38–53	47.0	6
Ende des Oralapparates	31–42	37.1	9
Makronucleus	24–38×13–20	31.2×16.1	6
	15–20×15–20	18.1×16.7	8
Mikronucleus	4.0–4.2×2.7–3.0	4.05×2.8	4
	2.0–3.0×1.5–2.7	2.6×2.2	7
Länge der linken	20.0–22.6	21.2	4
Polykinete	17.0–22.6	19.2	10
Durchmesser des Exkretionsporus	3.0	—	1
	2.8–4.0	3.4	5
Gesamtanzahl der Kineten	80–85	81.9	8
	50–58	54.0	11
Anzahl der postoralen Kineten	19–26	22.5	9
	12–15	14.4	10

Colpoda ovinucleata nov. spec. (Abb. 6 a–g, 10–16, 18, Tab. 1).

Diagnose: *In vivo* 85–155 µm große, etwa 35 µm dicke, nierenförmige, rechts fast ebene, links deutlich gewölbte *Colpoda*, deren Dorsalsack distal noch eine kleinere sackartige Vorwölbung besitzt. Oralapparat mit großem, in der proximalen Körperhälfte gelegenem, halbmondförmigem Vestibulum und gerade zur Körperlängsachse orientierter linker Polykinete. Etwa 8 bewimperte Kineten an der Innenwand des Vestibulums. Makro- und Mikronucleus ellipsoid (etwa 2 : 1). Durchschnittlich 60 Somakineten und 22 postorale Kineten, die in der tiefen Diagonalrinne bis zum Exkretionsporus ziehen. Entoplasma mit plattenförmigen, glänzenden Kristallen und schwärzlichen Granula. Alle Cilien paarig.

Locus typicus: Häufig in stark eutrophen Kleingewässern (Tümpel 67) am Weg zur Pfandlscharte (Großglockner-Hochalpenstraße, etwa 2200 m. ü.d.M.).

Morphologie: Körperform variabel, meist deutlich nierenförmig, praeoral schmäler als postoral (Abb. 6 a, 10). Am Eingang zum Oralapparat, von dem eine hohlkehlenartige Rinne diagonal über die linke Seite bis zum distalen Pol zieht, tief eingebuchtet. Die distale Körperhälfte erscheint dadurch sackartig aufgewölbt. Am unteren Ende dieses Sackes wölbt sich noch ein kleinerer, sehr leicht deformierbarer Sack vor, in dem die kontraktile Vakuole liegt (Abb. 6 a, d, 10, 13, 14). Dieser Sack war in den Präparaten stets faltig deformiert und verschwindet auch *in vivo* nach Auflegen des Deckglases sehr schnell. Rechte Polykinete des Oralapparates aus unbekannten Gründen kaum imprägnierbar. Distale Hälfte des Vestibulumeinganges aufgewölbt, wodurch exakt dieselben Mundverhältnisse wie bei *Tillina canalifera* entstehen (s. Turner 1937). Makronucleus rechts des Vestibulums gelegen, mit netzartig angeordnetem Chromatin. Mikronucleus dem Makronucleus genähert und in nassen Silberpräparaten von einer auffällig dicken Membran umgeben (Abb. 6 a). Pellicula durch die Wimperreihen deutlich gekerbt. Sie ist zwischen je 4 Dikineten bläschenartig aufgewölbt. Darunter liegen die ca. 3 µm langen Protrichocysten, die nach dem Ausstoß eine dünne Hülle um das Tier bilden. Die kontraktile Vakuole entsteht durch Zusammenfließen vieler kleinerer Vakuolen. Exkretionsporus leicht subterminal; Cytopyge etwas oberhalb der kontraktilen Vakuole. Im Entoplasma Tausende, 3–8 µm große, gelbgrün glänzende, plattenförmige Kristalle verschiedener Form und Größe. Außerdem 1–3 µm große, unregelmäßig sphaerische, schwärzliche Granula, die besonders im Dorsalsack gehäuft auftreten. Die etwa 10 µm großen Nahrungsvakuolen enthielten Bakterien und Phytoflagellaten.

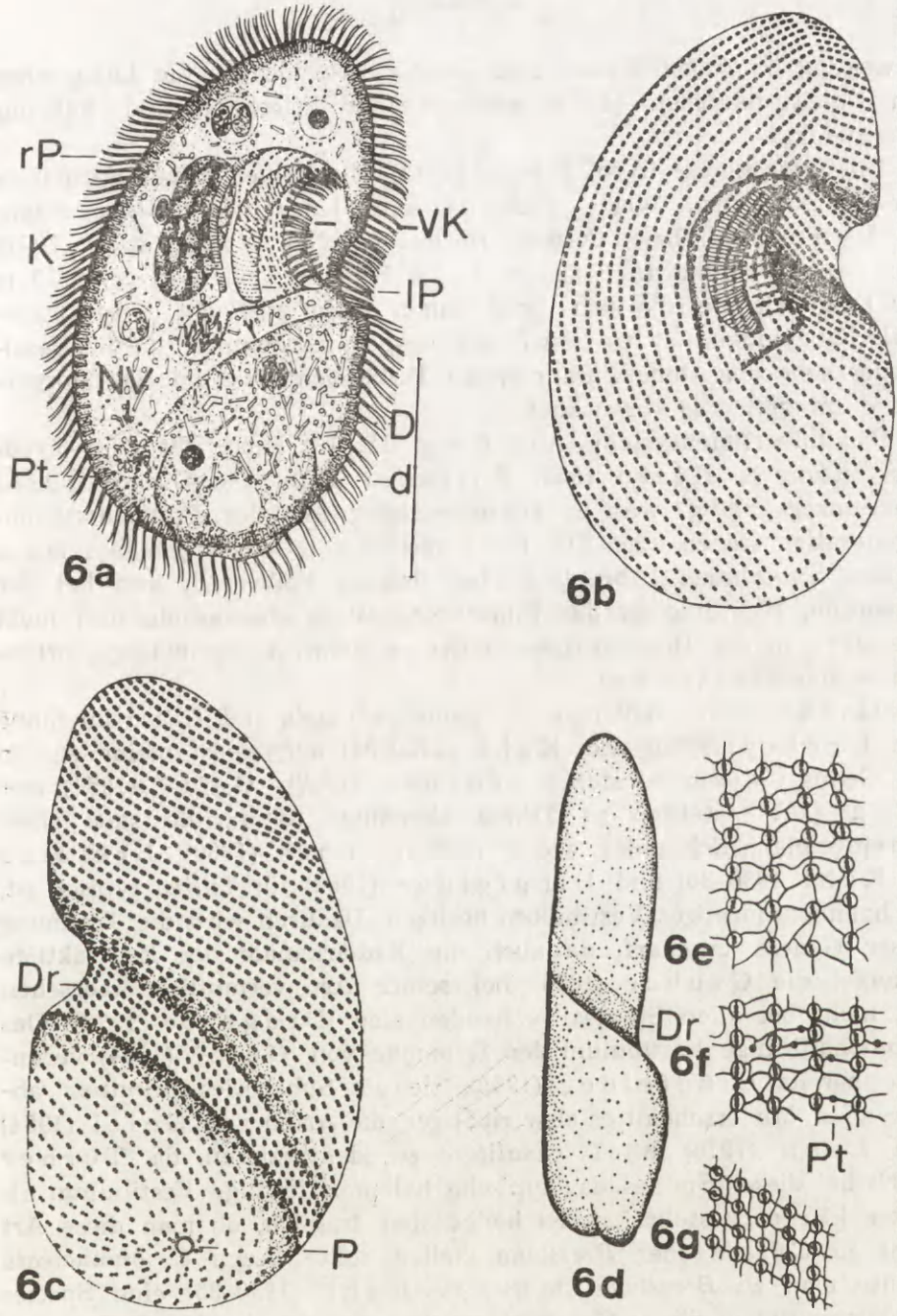


Abb. 6 a–g. *Colpoda ovinucleata*. 6 a — Ansicht von rechts lateral nach Lebendbeobachtungen. Skala 50 µm. 6 b — Infraciliatur ventral-rechts lateral. Nasse Silberimprägnation. 6 c — Infraciliatur links lateral. Nasse Silberimprägnation. 6 d — Schematisierter Längsschnitt nach Lebendbeobachtungen. 6 e, f — Teile der Infraciliatur und des Silberliniensystems der rechten Seite ohne und mit Protrichocysten. Trockene Silberimprägnation. 6 g — Teil der Infraciliatur und des Silberliniensystems im Bereich der Diagonalrinne. Trockene Silberimprägnation

Bewegung im freien Wasser rasch, unter Rotation um die Längsachse. An Detritushäufchen oft stillstehend oder kriechend und Nahrung einstrudelnd.

Die Infraciliatur (Abb. 6 b, c, 10–12, 14–16) gleicht mit Ausnahme der biometrischen Werte (Tab. 1) sehr jener von *Tillina magna* (s. Lynn 1976) bzw. *Colpoda spiralis* (s. Novotny et al. 1977). Von den durchschnittlich 22 postoralen Kineten verlaufen etwa 15 in der Diagonalrinne, die sich distal immer mehr ausflacht. Dieses postorale Kinetenband ist bis zum Exkretionsporus erkennbar, da die Basalkörper sehr eng stehen. Im distalen Polbereich sind die Basalkörperpaare unregelmäßig angeordnet.

Das Silberliniensystem (Abb. 6 e–g, 15, 18) entspricht dem Typus der Gattung (s. Klein 1929, Foissner 1978). Das Gitter ist meist sehr unregelmäßig, was in Zusammenhang mit der Protrichocystenregeneration stehen mag. Die Relationskörper dieser Organellen liegen in den Silberlinien (Abb. 6 f). Im distalen Polbereich und bei der praeoralen Nahtlinie ist das Silberliniensystem engmaschig und nicht orientiert. In der Diagonalrinne bildet es ziemlich regelmäßige, orthogonale Maschen (Abb. 6 g).

Diskussion: Faßt man die Genera *Colpoda* und *Tillina* im Sinne von Gruber (1880) und Kahl (1930–35) auf, so ist meine Art in das Genus *Colpoda* zu stellen. Turner (1937), Gelei (1954) und Lynn (1976) stellten zu *Tillina* allerdings Arten, die statt eines röhrenförmigen Schlundes, wie er nach Gruber (1880), Bresslau (in Kahl 1930–35) und Dingfelder (1962) für *Tillina* typisch ist, ein halbmondförmiges Vestibulum besitzen. Dadurch wird die Trennung dieser Genera unscharf, da auch die Radiärkanäle der kontraktilen Vakuole, die Gruber (1880) bei seiner Art vermutlich übersehen hat, nicht bei allen Species vorhanden sind (Dragesco 1972). Das halbmondförmige Vestibulum der *T. magna* von Gelei (1954) veranlaßte bereits Dingfelder (1962), sie als Subspecies *primitiva* abzutrennen. Mir erscheint es aber richtiger, die Arten von Gelei (1954) und Lynn (1976) mit *T. canalifera* zu identifizieren, da Turner (1937) bei dieser Species das typische halbmondförmige Vestibulum als Erster klar dargestellt hat. Es bleibt aber fraglich, ob man diese Art nicht zu *Colpoda* oder *Bresslaua* stellen sollte. Auch *C. ovinucleata* könnte man zu *Bresslaua* stellen, da Kahl (1930–35) eine Species (*Bresslaua sp.*) in dieses Genus einordnete, bei der das Vestibulum nur etwa die Hälfte des praeoralen Abschnittes einnimmt.

Innerhalb der Gattung *Colpoda* weist die neue Art nur zu *C. reniformis* Kahl, 1930–35 nähere Verwandtschaft auf. Von dieser 90–100 µm großen Moosform unterscheidet sie sich durch den Dorsal-

sack, das etwas größere Vestibulum, den mehr ellipsoiden Makronucleus, die weniger deutlichen Protrichocysten, das ungefärbte Entoplasma, die geringere Abflachung und die Entoplasmaeinschlüsse. Die Übereinstimmungen sind — wenn man eine gewisse Subjektivität der Beobachter miteinrechnet — allerdings beträchtlich. Sollte sich bei der Moosform von Kahl ein ähnlicher Dorsalsack finden, könnte man *C. ovinucleata* vielleicht mit dieser synonymisieren.

Colpoda rotunda nov. spec. (Abb. 7 a–d)

Diagnose: 130–170 µm große, sehr breit ovale, wenig abgeflachte und beim Oralapparat kaum eingebuchtete *Colpoda* mit kugelförmigem Makronucleus, dessen Nucleolen netzförmig angeordnet sind. Ein kugelförmiger Mikronucleus. Etwa 45 Kineten.

Locus typicus: Vereinzelt in einer Lithotelme (Tümpel 38) zwischen Knappenstube und Hochtor (Großglockner-Hochalpenstraße, etwa 2400 m ü.d.M.).

Morphologie: Stark ernährte Individuen fast kugelförmig. Am Eingang zum Vestibulum nur wenig, manchmal überhaupt nicht eingebuchtet. Oralapparat verhältnismäßig klein, gattungstypisch aufgebaut, stets vor der Körpermitte liegend. Pellicula glänzend, da zwischen je 2 Kineten 2–3 Reihen zylindroider Protrichocysten liegen (Abb. 7 d). Kontraktile Vakuole terminal, rechts der Medianen, von vielen leicht rötlichen Granula umgeben. Entoplasma dicht mit Nahrungsvakuolen mit Bakterien und unregelmäßig kugelförmigen, kompakten Einschlüssen gefüllt. Bewegung auffallend langsam, manchmal auf der Stelle kreisend. Vermehrung in Teilungscysten.

Wegen des seltenen Vorkommens konnte die Infraciliatur nicht genau studiert werden. Es sind ca. 45 Kineten vorhanden. Etwa 18 stoßen am Kiel zusammen, ca. 8 ziehen vom Oralapparat weg. Sie sind von den Somakineten der linken Seite nur undeutlich abgesetzt, da keine Diagonalrinne vorhanden ist. Silberliniensystem (Abb. 7 c) gattungstypisch ausgebildet (vgl. Foissner 1978).

Diskussion: Diese Species steht *Colpoda cucullus* nahe. Sie unterscheidet sich von dieser durch die Größe, die Struktur des Makronucleus, die geringere Einziehung beim Oralapparat, die größere Anzahl der Wimperreihen und die geringere Abflachung. Keines dieser Merkmale reicht für sich allein aus, um die Aufstellung einer neuen Art zu rechtfertigen, da *C. cucullus* sehr variabel ist (vgl. die Angaben von Roux 1901, Klein 1929, Kahl 1930–35, Burt 1940, Tuffrau 1952, Wenzel 1953, Gellért 1955, Vörösvary 1950, Reuter 1961, Hashimoto 1966).

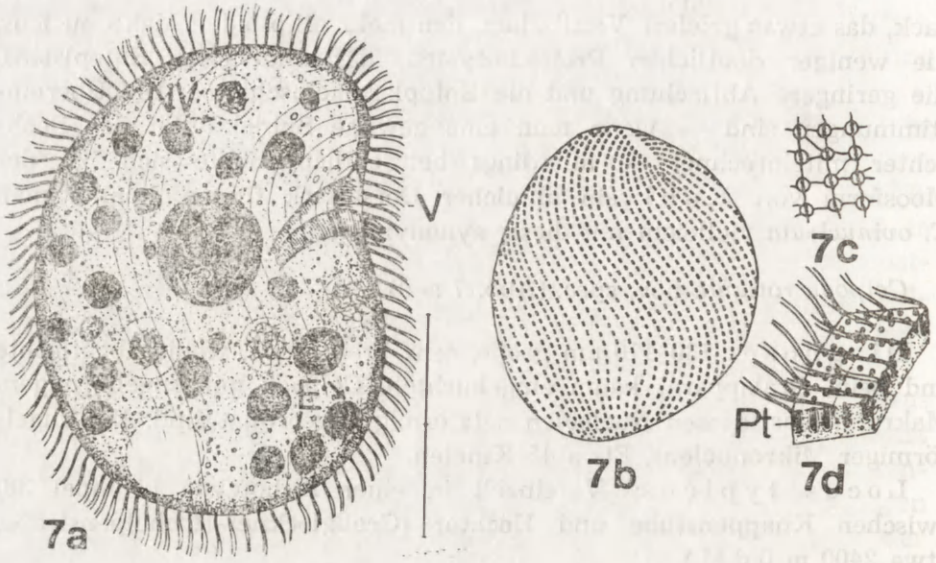


Abb. 7 a-d. *Colpoda rotunda* nach Lebendbeobachtungen (a, d) und trockener Silberimprägnation (b, c). 7 a — Ansicht von rechts lateral. Skala 75 µm. 7 b — Infraciliatur der Dorsalseite. 7 c — Teil der Infraciliatur und des Silberliniensystems. 7 d — Teil der Pellicula mit Protrichocysten

Colpoda variabilis nov. spec. (Abb. 8 a–g, 17, 19–24, Tab. 1)

Diagnose: *In vivo* 80–120 µm große, etwa 30 µm dicke, plump nierenförmige, rechts fast ebene, links deutlich gewölbte *Colpoda*, deren Dorsalsack durch die tief eingesenkte, bis zum distalen Körperpol reichende Diagonalrinne scharf akzentuiert ist. Oralapparat mit geräumigem, in der proximalen Körperhälfte gelegenem, trichterförmigem Vestibulum und leicht schräg zur Körperlängsachse orientierter linker Polykinete. Etwa 8 bewimperte Kineten an der rechten Innenwand des Vestibulums. Makronucleus kugelförmig, Mikronucleus ellipsoid. Durchschnittlich 40 Somakineten und 14 postorale, sehr eng nebeneinander verlaufende Wimperreihen. Alle Basalkörper sind paarig angeordnet. Protrichocysten sehr auffällig, verquellen nicht zu einer strukturlosen Hülle. Entoplasma mit vielen farblosen Granula. Ernährt sich von Bakterien und Phytoflagellaten.

Locus typicus: Häufig in einem stark eutrophen Kleingewässer (Tümpel 1) östlich des Wallackhauses (Großglockner-Hochalpenstraße, etwa 2200 m ü.d.M.).

Morphologie: Körperform sehr variabel (Abb. 8 a–c), meist plump nierenförmig, praeoral nur wenig schmäler als postoral, machmal praeoral weit vorspringend (Abb. 8 a). Am Eingang zum Oralapparat tief eingebuchtet. Von ihm zieht eine tiefe Rinne, die bei stark ernähr-

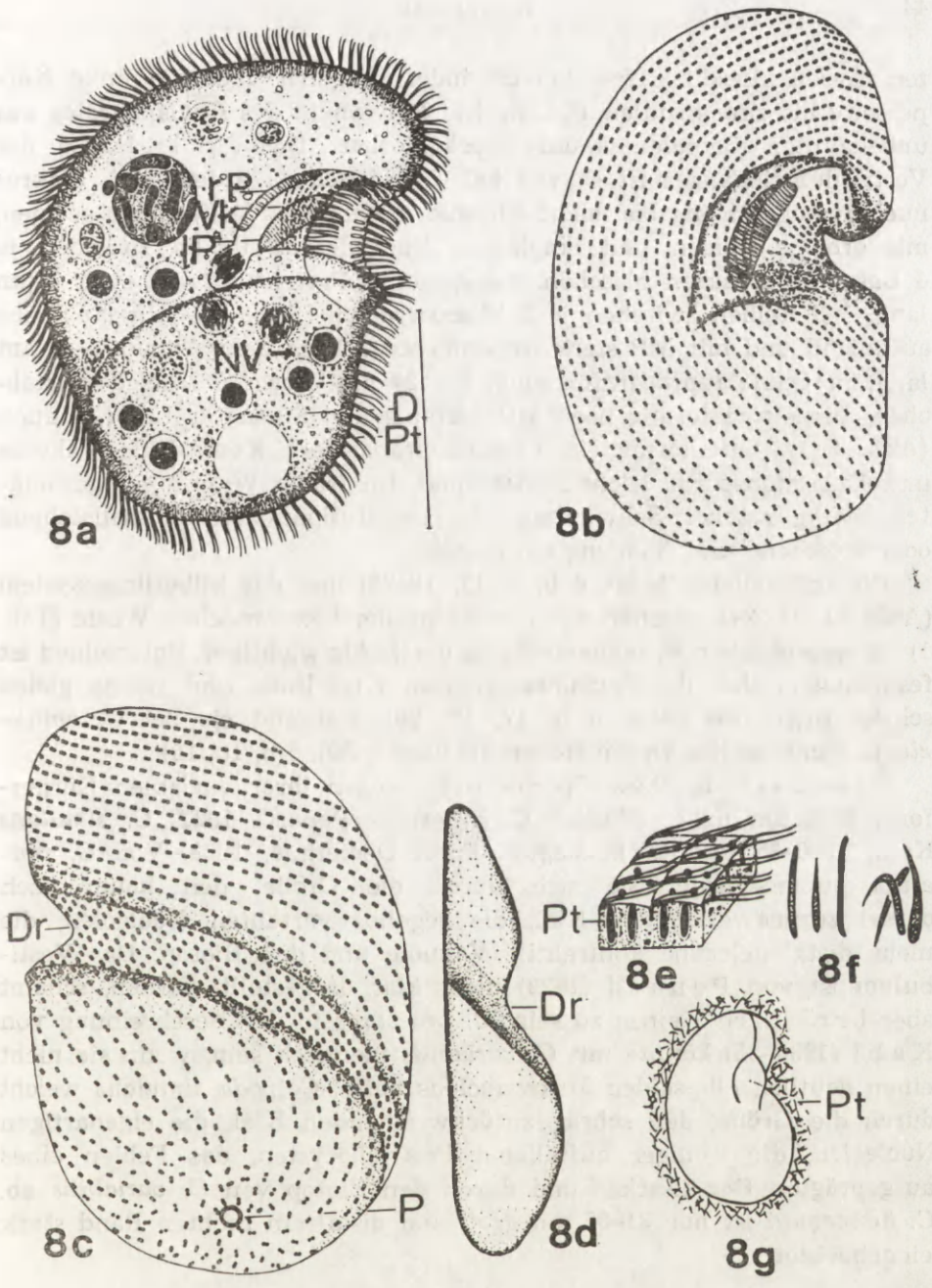


Abb. 8 a–g. *Colpoda variabilis*. 8 a — Ansicht von rechts lateral. Nach Lebendbeobachtungen. Skala 50 µm. 8 b — Infraciliatur ventral-rechts lateral. Nasse Silberimprägnation. 8 c — Infraciliatur links lateral. Nasse Silberimprägnation, Körperform nach Lebendbeobachtungen. 8 d — Schematisierter Längsschnitt nach Lebendbeobachtungen. 8 e — Teil der Pellicula mit Protrichocysten. 8 f — ausgestoßene Protrichocysten nach MP-Färbung. 8 g — Individuum mit Protrichocystenhülle

ten Tieren allerdings fast verschwindet, diagonal über die linke Körperseite bis zum distalen Pol. Rechte Polykinete des Oralapparates aus unbekannten Gründen kaum imprägnierbar. Die distale Hälfte des Vestibulumeinganges ist so wie bei *C. ovinucleata* aufgewölbt. Makronucleus kugelförmig bis leicht ellipsoid, rechts des Vestibulums gelegen, mit großen runden und länglichen Nucleolen. Pellicula zwischen je 4 Basalkörperpaaren bläschenartig gewölbt. Protrichocysten etwa 3 µm lang, 1–3 Reihen zwischen je 2 Wimperreihen (Abb. 8 e). *In vivo* sehr auffallend und mit MP stark rot anfärbbar. Nach dem Ausstoß 4–6 µm lang, in Opalblaupräparaten auch bis 20 µm. Form verschieden, stäbchen- bis kommaförmig nach MP-Färbung und Silbernitratimprägnation (Abb. 8 f), fadenförmig in Opalblaupräparaten. Kontraktile Vakuole und Exkretionsporus leicht subterminal. Im freien Wasser ein gewandter, mäßig rascher Schwimmer. An Detritushäufchen oft stillstehend oder kriechend und Nahrung einstrudelnd.

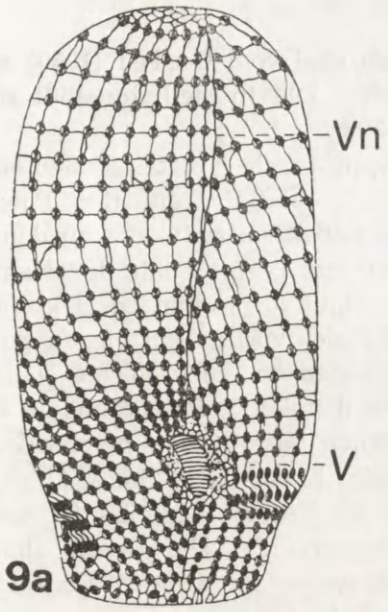
Die Infraciliatur (Abb. 8 b, c, 17, 19–22) und das Silberliniensystem (Abb. 17, 23, 24) gleichen mit Ausnahme der biometrischen Werte (Tab. 1) im wesentlichen *C. ovinucleata* (s. dort). Als wichtiger Unterschied ist festzuhalten, daß die Somakineten vom Kiel links und rechts gleich schräg wegziehen (Abb. 8 b, 17, 19, 20), während sie bei *C. ovinucleata* rechts steiler an ihn stoßen als links (Abb. 6 b, 10, 15).

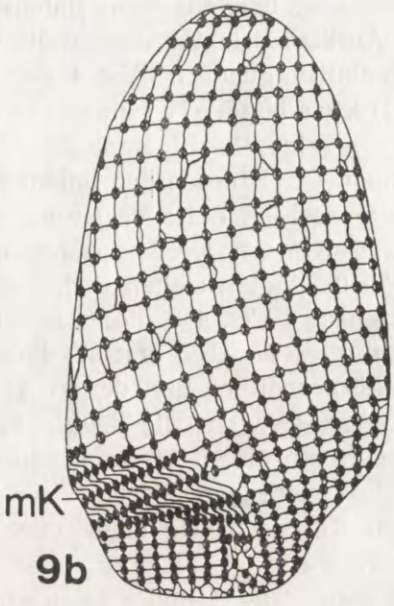
Diskussion: Diese Species steht wegen ihrer variablen Körperform 3 Arten nahe, nämlich *C. praestans* Penard, 1922, *C. simulans* Kahl, 1930–35 und *C. henneguyi* Fabre-Domergue, 1888. Von *C. praestans* unterscheidet sie sich durch die Größe, den kaum nach dorsal zurückweichenden Kiel, die regellos verteilten Nucleolen, die mehr distal gelegene kontraktile Vakuole und den Biotop. Das Vestibulum ist von Penard (1922) nicht klar dargestellt worden, scheint aber beträchtlich kleiner zu sein. *C. praestans* in der Beschreibung von Kahl (1930–35) könnte mit *C. variabilis* identisch sein, wenn sie nicht einen deutlich ellipsoiden Makronucleus hätte. *Colpoda simulans* weicht durch die Größe, den schräg zurückweichenden Kiel, die eigenartigen Nucleolen, die weniger auffälligen Protrichocysten, das Fehlen eines ausgeprägten Dorsalsackes und durch den Biotop von *C. variabilis* ab. *C. henneguyi* ist nur 31–65 µm groß und distal am rechten Rand stark eingebuchtet.

3. Familie *Marynidae* Poche

Maryna ovata Gelei, 1950 (Abb. 9 a-c, 25–30)

Morphologie: Den sorgfältigen Lebendbeobachtungen von Gelei (1950, 1954) und Dingfelder (1962) habe ich nichts hinzuzufügen. Die starke Formvariabilität (Fig. 9 a–c) kann ich bestätigen. Die





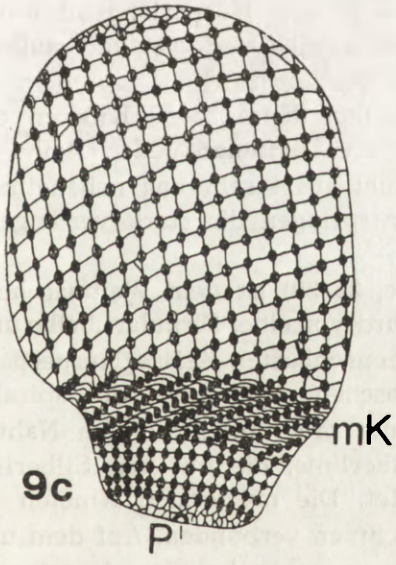


Abb. 9 a–c. *Maryna ovata* nach Lebendbeobachtungen (Körperform) und trockener Silberimprägnation (Infraciliatur, Silberliniensystem). 9 a — Ventralansicht. 9 b — Ansicht von rechts lateral. 9 c — Dorsalansicht

Infraciliatur und das Silberliniensystem sind vom Gelei (1950) nur in den Ansätzen richtig dargestellt worden. Die Infraciliatur läßt sich in 3 Abschnitte gliedern (Abb. 9 a–c, 25–29):

(1) Etwa 50 (Gelei gibt nur bis 36) praeorale Kineten stoßen entlang der ventralen, leicht sigmoiden Nahtlinie und des apikalen Poles zusammen. Die Kineten der linken Seite stoßen spitzwinkelig an den Kiel, die der rechten Seite verlaufen zuerst fast parallel mit der Nahtlinie. Etwa 4 Kineten werden durch den Mund abgeschnitten. Die folgenden 7–10 Kineten ziehen rechts des Mundes vorbei und biegen postoral leicht nach links. 4–5 dieser Kineten stoßen an die postorale Nahtlinie, während die anderen erst am Rand der distalen Polfläche enden. Einige Basalkörper dieser und der an sie seitlich anstoßenden mycteralen Kineten wandern auf die distale Polfläche hinaus. Die linke Abbiegung, die auch von einem Zusammenrücken der Kineten begleitet ist, bewirkt den linksspiraligen Verlauf der Wimperreihen. Die Cilien sind am ganzen Körper paarig angeordnet und stehen im distalen Drittel etwa doppelt so eng wie im proximalen Abschnitt.

(2) Links des Mundes beginnen die sehr dicht bewimperten mycteralen Kineten. Es sind ca. 15 schräg ($\sim$ 45°) zur Körperlängsachse orientierte Wimperreihen. Die letzten 2 Reihen sind verkürzt und stoßen an die durchlaufenden Kineten rechts des Oralapparates.

(3) An die mycteralen Kineten stoßen distal 25–30 Kineten zu je etwa 6 Basalkörperpaaren. Die distalen Dikineten jeder Reihe sind leicht vergrößert und bestehen vielleicht aus 4 sehr eng nebeneinander liegenden Basalkörpern. Von ihnen entspringen die stark verlängerten, sehr empfindlichen Caudalcilien.

Das Silberliniensystem (Abb. 9 a–c, 25–29) ist dem Weitmaschentyp zuzuordnen. Jedes Basalkörperapaar wird von einer Zirkularfibrille umgeben, von der 4 Silberlinien zu den benachbarten 4 Basalkörperpaaren ziehen. Die dadurch entstehenden Maschen sind wegen des spiraligen Kinetenverlaufes shombisch. Entlang der prae-und postoralen Nahtlinie verläuft eine nicht unterbrochene Silberlinie, die mit den Silberlinien der anstoßenden Somakineten kontaktet. Die mycteralen Kineten werden durch umgekehrt Z-förmige Silberlinien verbunden. Auf dem unbewimperten proximalen Pol breitet sich ein unregelmäßiges Engmaschengitter aus, das von den Silberlinien der Somakineten gebildet wird. Die distale Polfläche ist bis auf die oben erwähnten Basalkörperpaare unbewimpert und von einem feinmaschigen Silberliniennetz bedeckt. Im Zentrum dieser Polfläche liegt der Porus der kontraktilen Vakuole. Der Rand des Vestibulums wird von einem Engmaschengitter bedeckt. Die Oralstrukturen konnten nicht genau erkannt werden. Eine typische

Polykinete ist oft gut imprägniert, *in vivo* wurde auch eine zweite Polykinete festgestellt (vgl. Gelei 1950).

Diskussion: Meine Untersuchungen bestätigen die von Gelei (1954) und Dingfelder (1962) vorgenommene Einordnung der *Marynidae* in die *Colpodida* (vgl. Foissner 1978) und geben keine Hinweise dafür, daß diese Familie in die *Trichostomatida* einzuordnen wäre (Corliss 1977). Die von Dingfelder (1962) vorgeschlagene Ableitung der *Marynidae* von den *Colpodidae* kann ich voll unterstützen. Zu den von ihm angeführten homologen Merkmalen sind auf Grund der vorliegenden Untersuchungen folgende hinzuzufügen:

(1) Das Silberliniensystem entspricht dem Typus der *Colpodidae* (vgl. Foissner 1978).

(2) Die praeorale Nahtlinie ist homolog dem Kiel der *Colpodidae.* Die auffällige Länge dieser Nahtlinie zeigt, daß der Mund im Verlaufe der Evolution nach distal gewandert sein muß, zusammen mit den Kineten der Diagonalrinne.

DANKSAGUNG

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SUMMARY

The morphology, infraciliature, and silverline system of some new or little known *Colpodida* occurring in small water bodies of the Großglockner area (Hohe Tauern, Austria) is described. The following species are new to science: *Platyophrya citrina* nov. spec., *Platyophrya dubia* nov. spec., *Platyophrya hyalina* nov. spec., *Platyophrya procera* nov. spec., *Colpoda ovinucleata* nov. spec., *Colpoda rotunda* nov. spec., and *Colpoda variabilis* nov. spec. The infraciliature and the silverline system of *Maryna ovata* demonstrate that the *Marynidae* are closely related to the *Colpodidae.*

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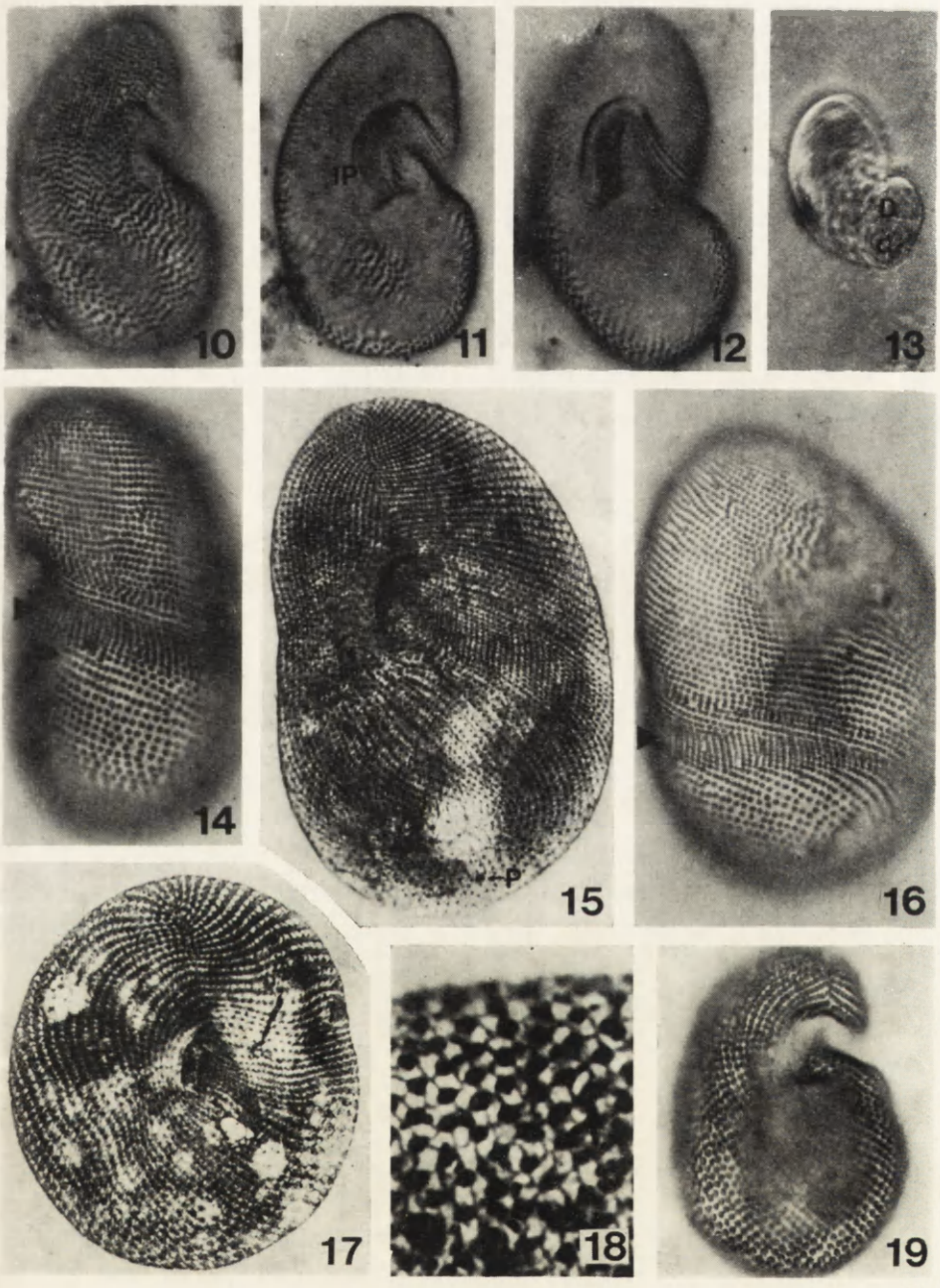
LEGENDEN ZU DEN TAFELN I–II

10–16, 18: *Colpoda ovinucleata*. Infraciliatur und Silberliniensystem nach nasser Silberimprägnation (10–12, 14, 16) und trockener Silberimprägnation (15, 18). 10, 11 — Ansicht eines Individuums von ventral-rechts lateral in verschiedener Fokushöhe. 12 — Ventralansicht. 13 — freischwimmendes Exemplar, Phasenkontrast, Ansicht von links lateral. 14 — Seitenansicht. Der Pfeil weist auf die Diagonalrinne. 15 — Ventralansicht. 16 — Ansicht von links lateral. 18 — Teil der Infraciliatur und des Silberliniensystems

17, 19–24: *Colpoda variabilis*. Infraciliatur und Silberliniensystem nach nasser Silberimprägnation (19, 20–23) und trockener Silberimprägnation (17, 24). 17 — Ansicht von ventral und proximal. 19 — Ansicht von ventral-rechts lateral. 20, 21 — Ansicht eines Tieres von ventral-rechts lateral in verschiedener Fokushöhe. 22 — Ansicht von links lateral. 23, 24 — Teile der Infraciliatur und des Silberliniensystems der rechten Seite. Die Pfeile weisen auf die Basalkörperpaare

25–30: *Maryna ovata*. Infraciliatur und Silberliniensystem nach trockener Silberimprägnation. 25 — Ventralansicht. Die Pfeile weisen auf die Ventralnaht. 26 — proximaler Pol. Der Pfeil weist auf die Ventralnaht. 27 — Dorsalansicht. 28 — distaler Körperteil. Der Pfeil weist auf eine Reihe vergrößerter Basalkörperapparate. 29 — Ansicht von rechts lateral. 30 — Lebendaufnahme im Phasenkontrast. Das lange Gallertgehäuse ist gut erkennbar



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A New Opalinid *Hegneriella mukundai* sp. n. from an Old World Hylid *Kaloula pulchra taprobanica* Parker from India

Received 21 May 1979

Synopsis. *Hegneriella mukundai* sp. n. having body dimensions 93.65 μm ± 2.71 μm × 58.05 μm ± 2.24 μm and nuclear dimensions 25.01 μm ± 1.74 μm × 17.72 μm ± 0.49 μm with eight to ten nucleoli, is described from *Kaloula pulchra taprobanica* Parker and also compared with those of *H. dobelli* Earl, 1971 and *H. cheni* Earl, 1972.

Since the erection of the genus *Hegneriella* by Earl (1971), which embraces only two species *H. dobelli* Earl, 1971 and *H. cheni* Earl, 1972, no other contribution to this genus has so far been made. The present study records a third species of this genus, namely *H. mukundai*, sp. n., which is its first occurrence in the Old World.

In the course of this investigation, 517 host animals, *Kaloula pulchra taprobanica* Parker were examined. Some harboured only flagellates, others mixed populations of *Zelleriella* spp., *Protoopalina* spp. and nyctotherans. Only four individuals had *Hegneriella mukundai* sp. n. Neither *Opalina* spp. nor *Cepedea* spp. were encountered in this host.

Materials and Methods

The host specimens *Kaloula pulchra taprobanica* Parker were collected from the district of Midnapore, West Bengal. The rectal contents of the hosts were emptied into drops of 0.65% saline on slides. Living opalinids were examined with the phase-contrast microscope. Part of the material was fixed in Schaudinn's and stained with iron hematoxylin, after Chen (1944). Silver impregnation after Corliss (1953) was used for the study of the infraciliature, even though iron alum hematoxylin stain served well for this purpose. Measurements were made on both living and stained opalinids, the composite drawing being made with the aid of a camera lucida and microphotographs.

Results

Typical trophozoites of *Hegneriella mukundai* sp. n. are oval; the posterior section is broader than the anterior; ventral margin is slightly concave towards the anterior side. The cell is uninucleate. The body measures 81.7–107.5×47.3–73.1 μm (see Fig. 1).

The body is uniformly ciliated with short (2.5–5.1 μm) cilia. The ciliary lines (14–19) run obliquely parallel to the anterioposterior axis, having interkinetal distances which average 1.7 μm anteriorly and 3.4 near the posterior pole.

The large, ovoid nucleus, ca. 25×18 μm, is central and placed obliquely to the longitudinal axis. Its variously-sized, round nucleoli are scattered in the nucleoplasm, numbering eight to ten (Fig. 2).

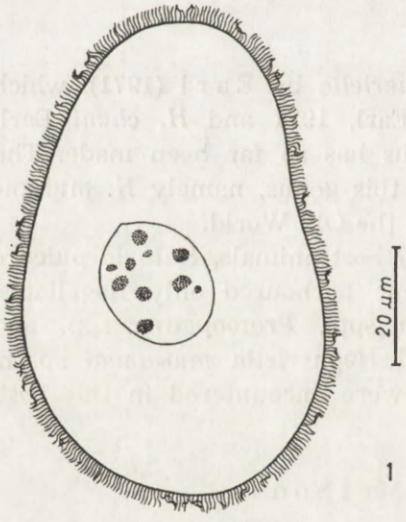

Fig. 1. *Hegneriella mukundai* sp. n.

Fig. 2. Prolate nucleus of *Hegneriella mukundai* sp. n. showing nucleoli (arrows)

Discussion

Both *Hegneriella dobelli* and *H. cheni* were described from the same anuran host: *Bufo valliceps*, but from Louisiana and Texas respectively. *H. dobelli* is broad and obliquely truncated at the anterior end, the posterior end being comparatively narrower, whereas *H. cheni* has

a broad not narrow posterior section. The nucleus of *H. cheni* is spindle-shaped, containing three nucleoli, while that of *H. dobelli* is large and prolate, containing 10 nucleoli, which may reflect difference in the number of chromosomes.

H. mukundai, inscribed to the memory of the reputed Indian protozoologist Prof. M. M. Chakravarty, has a single large nucleus, containing eight to ten nucleoli, and shows some resemblance to *H. dobelli,* though different in dimensions (see Table 1). Measurements of the three known species are compared in Table 1.

E a r l (1979) has commented, under the possible title of "unfinished business", that *H. cheni* was bypassed by the collector, C h e n, enigmatically, since, in his opinion C h e n (1948) was extraordinarily competent and an experienced cytologist familiar with *Zelleriella.*

Table 1

A comparative mensural data of *Hegneriella dobelli* Earl, 1971, *H. cheni* Earl 1972 and *H. mukundai* sp. n. in microns

		Range	Arithmetic mean	Standard deviation	Standard error	Coefficient of variation (%)
Length of the body	*H. dobelli*	62.0–133.0	98.2	23.10	2.3	24
	H. cheni	86.0–168.0	142.0	—	2.2	—
	H. mukundai	81.7–107.5	93.6	10.52	2.7	11
Width of the body	*H. dobelli*	50.0–75.0	61.0	14.00	1.4	23
	H. cheni	44.0–89.0	70.7	—	0.7	—
	H. mukundai	47.3–73.1	58.0	8.68	2.2	14
Length of the nucleus	*H. dobelli*	30.0–42.0	34.1	5.70	0.6	6
	H. cheni	27.0–50.0	36.6	—	0.7	—
	H. mukundai	17.0–37.4	25.0	6.77	1.7	27
Breadth of the nucleus	*H. dobelli*	13.0–21.0	16.2	4.20	0.4	4
	H. cheni	12.3–24.0	16.9	—	0.3	—
	H. mukundai	13.6–18.7	17.7	1.92	0.4	10
Length of the cilia	*H. mukundai*	2.5–5.1	3.6	0.80	0.2	21
Distance between the two ciliary lines in the anterior region	*H. mukundai*	—	1.7	—	—	—
Distance between the two ciliary lines in the posterior region	*H. mukundai*	—	3.4	—	—	—

ACKNOWLEDGEMENTS

Authors are greatly thankful to Dr. P. R. Earl for showing his great interest in this subject. Sincere thanks are also due to Professor Dr. Asok Ghosh, Head of the Department of Zoology, University of Calcutta, for providing necessary laboratory facilities.

RÉSUMÉ

Hegneriella mukundai sp. n., avec les dimensions du corps: 93.65 μm ± 2.71 μm × × 58.05 μm ± 2.24 μm, les dimensions du noyau: 25.01 μm ± 1.74 μm × 17.72 μm ± ± 0.49 μm, et avec 8–10 nucléoles, trouvée chez *Kaloula pulchra taprobanica* Parker, est décrite et comparée avec *H. dobelli* Earl, 1971 et *H. cheni* Earl, 1972.

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P. A. LALPOTU

Maupasella perionychis sp. n. a New Astomatous Ciliate from the Earthworm *Perionyx sansibaricus* in India

Received 15 May 1979

Synopsis: A new astomatous ciliate *Maupasella perionychis* sp. n. (sub. fam. *Hoplitophryoidea* Puytorac 1972) is described from the gut of an earthworm *Perionyx sansibaricus* in Maharashtra, India. It is characterized by a long ribbon like body measuring 95.8 μm × 18.9 μm on the average and with 26 rows of kineties on one side and 18–20 on other. This is the first record of an astomatous ciliate from earth worm of this region.

The genus *Maupasella* was erected by Cépède 1910 for a ciliate which had been earlier recorded by Maupas from the alimentary canal of an Algerian earthworm. Subsequently several forms have been described by different workers like Keilin (1920), Bhatia and Gulati (1927), Heidenreich (1935 a, b). Wichterman (1939), Katashima (1952), Puytorac (1952) and Lom (1959 a, b, c, 1961). Puytorac (1972) in his review of the astomatous ciliates included this genus in the subfamily *Maupasellinae* Cépède, 1910, family *Maupasellidae* Cheissin, 1930 under a new superfamily *Hoplitophryoidea*. He listed 16 species of this occasional change of direction or a progressive push forward, rotating along their long axis.

The body of the ciliate is typically elongated, flat and ribbon shaped, having more or less uniform width troughout (Fig. 1 *1*). The two ends are rounded, the anterior one being more broadly rounded and the posterior slightly narrower. At the anterior tip of the body is a typical spine-like outgrowth (about 3.5–4 μm long) which is characteristic and probably helps for attachment (Fig. 1 *2*). The body length is about five times the width. The body is covered by a fairly thick pellicle which is quite flexible permitting body movements in all directions. The cytoplasm appears granular and there is no apparent distinction into ecto and endo plasm. The cytoplasm around the macronucleus is vacuolar. The body is covered with a thick coat of cilia arranged in

closely set longitudinal lines. The cilia are uniform all over the body and are about 3–4 μm in length on the average.

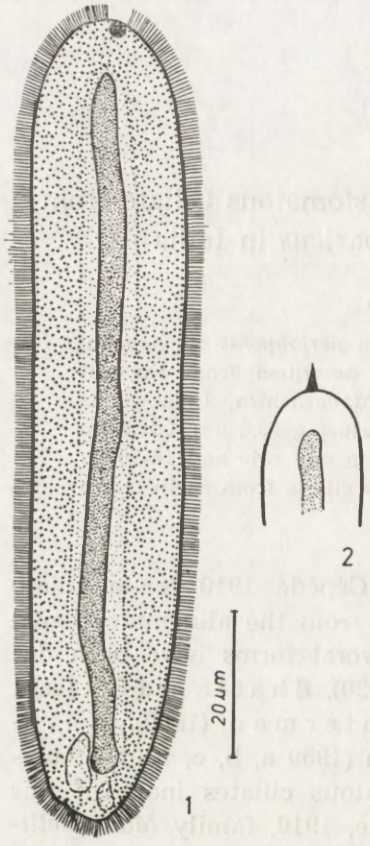

Fig. 1. *Maupasella perionychis* sp. n. *1* — Showing general morphology (based on Schaudin/Haematoxylin preparations), *2* — Showing the spine-like structure at anterior end (based on an organism stained with Lugol's solution

There are on an average 46 ciliary rows, 26 of them on the ventral side (Pl. I 3) and 20 on the dorsal side. The kineties converge at the anterior end on the ventral side on a small triangular unciliated area, from where arises the spine-like adhesive organ (Pl. I 3, 4). The kineties run backwards more or less parallel to one another and converge on a posterior suture coinciding with the posterior end of the body.

The macronucleus is extremely long, extending more than three-fourths of the length of the body. It is ribbon shaped and granular, with a smooth outline in most cases (Fig. 1 *1, 2*). The micronucleus could not be seen in any of the specimens genus from various annelids in different parts of the world. Form India Bhatia and Gulati (1972) were the only authors todate who have recorded this genus. They found *M. nova* in *Pheretima posthuma* and *Pheretima hawayana.*

Material and Methods

The ciliates described were found in the gut of an earthworm *Perionyx sansibaricus* during the course of a survey of the ciliate fauna of invertebrates of Maharashtra, State, India. The organisms were examined in detail in living condition in temporary wax-sealed preparations. Observations in the living condition were also made with the help of vital stains. Particularly neutral red and methylene blue were useful, besides Lugol's solution. Permanent preparations were made with Heidenhain's iron haematoxylin and phospho-tungstic acid haematoxylin, following fixation in Schaudinn's fluid. Silver impregnation was done by the dry method, after Klein (1958).

Description of *Maupasella perionychis* sp. n.
(Plate I 3, 4, 5)

The ciliate was found in the gut of the earthworm, *Perionyx sansibaricus* collected from the viscinity of Aurangabad, Maharashtra. The parasites were found mostly just above the middle of the body being particularly concentrated in the post-clitellar region. The infection was never heavy, even though the incidence was common.

In the living condition the ciliate moves about very slowly. The movement is mostly spiral around the same point with an occasional change of direction or a progressive push forward, rotating along their long axis.

The body of the ciliate is typically elongated, flat and ribbon shaped, having more or less uniform width throughout (Fig. 1 *1*). The two ends are rounded, the anterior one being more broadly rounded and the posterior slightly narrower. At the anterior tip of the body is a typical spine-like outgrowth (about 3.5–4 μm long) which is characteristic and probably helps for attachment (Fig. 1 *2*). The body length is about five times the width. The body is covered by a fairly thick pellicle which is quite flexible permitting body movements in all directions. The cytoplasm appears granular and there is no apparent distinction into ecto and endo plasm. The cytoplasm around the macronucleus is vacuolar. The body is covered with a thick coat of cilia arranged in closely set longitudinal lines. The cilia are uniform all over the body and are about 3–4 μm in length on the average.

There are on an average 46 ciliary rows, 26 of them on the ventral side (Pl. I 3) and 20 on the dorsal side. The kineties converge at the anterior end on the ventral side on a small triangular unciliated area, from where arises the spine-like adhesive organ (Pl. I 3, 4). The kineties run backwards more or less parallel to one another and converge on a posterior suture coinciding with the posterior end of the body.

The macronucleus is extremely long, extending more than three-fourths of the length of the body. It is ribbon shaped and granular, with a smooth outline in most cases (Pl. I 2). The micronucleus could not be seen in any of the specimens examined as it is probably very small and covered over by the micronucleus.

No contractile vacuoles were seen in any of the living or fixed organisms.

The dimensions of the organism are shown in Table 1.

Discussion

Of the various species listed by Puytorac (1972) this species comes close to *M. mucronata* (Cépède, 1910 Puytorac, 1954 in the number of the kineties i.e., about 46. However, the present species is much longer in size than *M. mucronata*, *measuring* 66.2–132.3 μm × 15.8–22.1 μm (95.8 μm × 18.9 μm) as against 30–90 μm × 48 μm. Particularly, the extremely elongated and narrow ribbon like body of the present species is characteristic with the length-breadth ratio being almost 5 : 1.

The ciliate described above from *Perionyx sansibaricus* appears to be similar to the elongated forms described by Bhatia and Gulati (1927) from two species of *Pheretima*, *P. posthuma* and *P. hawayana* in its body dimensions and in the length of the macronucleus. These authors have described that there are fine and closely set cilia disposed in 27 longitudinal rows which could be "counted at the broader end of the body". In the present organism there are about 26 rows on one side and 18–20 on the other. In view of these and the fact that the number and arrangement of the kineties is a major basis for taxonomic identity of the various species at present, the present organism is considered identical with the species described by Bhatia and Gulati as *M. nova* in spite of the fact that Bhatia and Gulati described the presence of many contractile vacuoles, while none are present in the present organism.

Table 1

Dimensions of *Maupasella perionychis* sp. n. (all measurements in microns)

Particulars	Minimum	Maximum	Average
Length of the body	66.2	132.3	95.8
Width of the body	15.8	22.1	18.9
Length of the macronucleus	53.6	110.3	80.3
Width of the macronucleus	3.0	4.8	3.5
Ratio of length of the body and width of the body	5 : 1		
Ratio of length of the body and length of the macronucleus	1.2 : 1		

Cépède (1910) gave the name *M. nova* for a parasite which had earlier been discovered by Maupas from the alimentary canal of an Algerian earthworm. Similar organisms were recorded later by several workers, including Bhatia and Gulati (1927). As Mackinnon and Hawes (1961) stated "for many years thereafter apparently

the same organism was remarked, always in oligochaetes, until a considerable number of distinct but related species had accumulated under Cépède's name. Unfortunately, Cépède did not identify his hosts and his description is defective in just those particulars by which the species now recognized are distinguished, so that it is impossible to tell which of these corresponds with that seen by the original author or, in many cases, those seen by his successors. Thus the name that is so common in the literature, *M. nova*, can no longer be applied to any species".

In view of the foregoing discussion it appears necessary not to apply the name *M. nova* to any species and hence this ciliate is designated as *Maupasella perionychis* sp. n. with *M. nova sensu* Bhatia and Gulati (1927) as its synonym.

Species: *Maupasella perionychis* sp. n.
Host: *Perionyx sansibaricus*
Habitat: Alimentary canal
Locality: Aurangabad, Maharashtra, India.

The type slides are deposited in the Protozoology Laboratory, Department of Zoology, Marathwada University, Aurangabad 431 004, India.

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RÉSUMÉ

Un nouveau Cilié astome *Maupasella perionychis* sp. n. (sous-famille *Hoplitophryoidea* Puytorac 1972) a été trouvé dans l'intestin du Lombric *Perionyx sansibaricus* à Maharashtra, aux Indes. Son corps allongé en forme de ruban mesure 95.8 μm × 18.9 μm en moyenne et possède 26 rangs des cinétosomes d'un côté et 18–20 de l'autre. C'est la première découverte d'un Cilié astome chez un lombric dans cette région.

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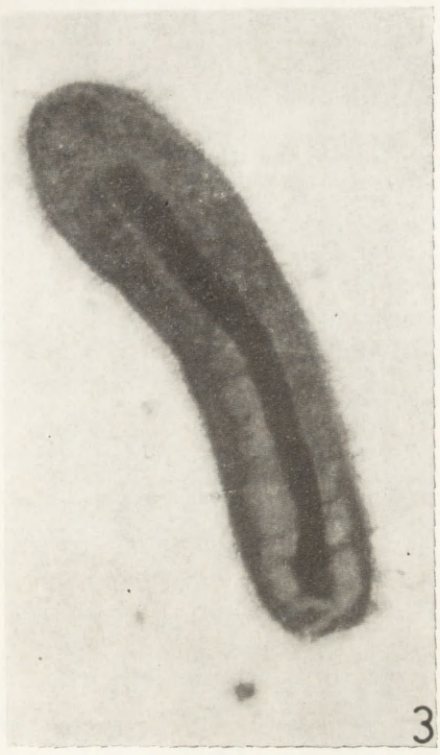
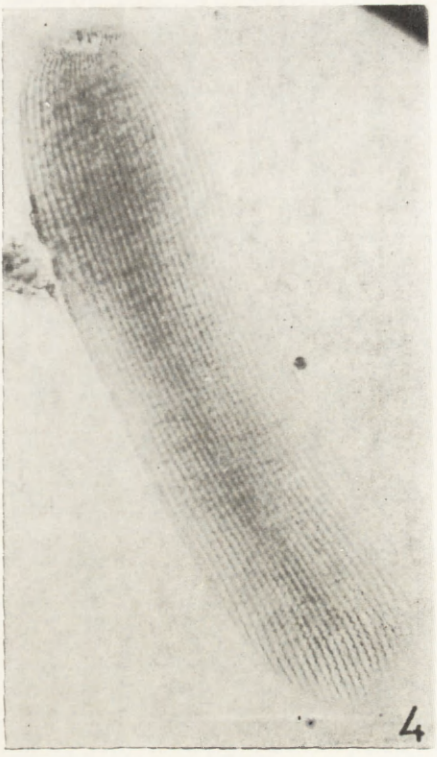
EXPLANATION OF PLATE I

Maupasella perionychis sp. n.
3: Showing arrangement of kineties in ventral view. (Based on silver impregnated preparations
4: Entire organism
5: The entire end magnified to show the triangular naked area





P. A. Lalpotu auctor phot.

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A New Septate Gregarine, *Actinocephalus bradinopygi* sp. n. from the Dragon Fly, *Bradinopyga geminata* Rambur [1]

Received 18 July 1979

Synopsis. The morphology and life-history of a new species of septate gregarine, *Actinocephalus bradinopygi*, sp. n. from the gut of the odonate, *Bradinopyga geminata* Ramb. is described.

The cephalonts reach 825 × 168 μm and the epimerite is complex. Cysts spherical, 800 μm in diameter and have an ectocyst 150 μm thick. Gametes isogamous, 10 μm in diameter. Spores oval, 17×5 μm, octozoic. Sporozoites spindle-shaped, 10×3μm.

While examining the local odonate fauna for gregarine parasites we frequently came across a septate gregarine belonging to the genus *Actinocephalus* parasitic in the gut of the dragon fly, *Bradinopyga geminata* Rambur. A perusal of the literature showed that there has so far been only one species, *A. sieboldi* (Kölliker, 1848) reported from the gut of the larvae and imago of *Aeschna* sp. and *Sympetrum vulgatum*, *S. pedemontum* collected at Würtzburg, Erlangen, Beyrouth. Gdańsk and Silesia (Poland) (Geus 1969). The present form differs from it in several respects and hence is considered a new species for which the name *Actinocephalus bradinopygi* sp. n. is proposed.

Material and Methods

The dragon flies were collected from the dairy farm area in Visakhapatnam (Andhra Pradesh, India) where they are found actively flying around a stream of water.. They were also collected from the Botany gardens in the Andhra

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University Campus at Waltair. The insects were trapped using a net and maintained singly in glass finger-bowls at the bottom of each of which a moist blotting paper was placed to keep the faecal matter passed out in a moist condition. There was no external indication of infection and hence all the insects collected were decapitated and the gut examined microscopically for the gregarines. Smears were prepared from the gut of the infected hosts, fixed in Schaudinn's fluid and stained with Ehrlich's acid haematoxylin. Cysts collected from the faecal matter were kept in 2.5% aqueous Potassium dichromate and kept in a moist chamber to observe gametogenesis and sporogony. Smears of the cysts showing gametes were fixed in Schaudinn's fluid and stained with Ehrlich's acid haematoxylin while those showing spores were fixed in Carnoy's fluid and treated according to Feulgen's technique. Material for sectioning was fixed in alcoholic Bouin's fluid, sectioned at 8 μm thickness and stained with Heidenhain's iron haematoxylin.

Observations

38 out of 60 adult *Bradinopyga geminata* Rambur were infected with a septate gregarine belonging to the genus *Actinocephalus.*

Trophozoites: No intracellular stage of development was observed. The smallest cephalont measured 316 × 44 μm and the largest measured 825 × 168 μm. The body of the cephalont is divisible into three parts, an anterior complex epimerite which is broad at the base and is drawn into a long tubular neck. The tip of the neck is expanded into a shallow cup, the margin of which is produced into 9–11 petaloid lobes (Fig. 1, *3*). When the epimerite was contracted, which was often the case in smear preparations, it appeared in the form of a bulb-like structure at the tip of the epimerite (Fig. 1, *2*). In fully grown cephalonts the epimerite measured 272 μm in length and is broadest at the base where it measured 60 μm. The neck which was tubular was 16 μm in width. The protomerite was hemispherical and measured 90×116 μm. The deutomerite was the longest part of the body and measured 540×125 μm and has conspicuous striations, which are more prominant along the sides (Fig. 1, *5*). The protoplasm in the deutomerite was more coarsely alveolated than that in the protomerite which was finely alveolated. The nucleus has a variable position in the deutomerite and may be situated either in the anterior, middle or posterior end.

Cephalonts attached to the gut epithelium showed that the lobes of the cup-shaped part of the epimerite are anchored in the epithelial cells of the midgut (Fig. 1 *1*). Some of the cephalonts were also seen moving about freely in the lumen of the gut, but it has not been possible to ascertain if the cephalonts can detach and reattach themselves to the gut epithelium. The largest sporadin observed measured 1300×

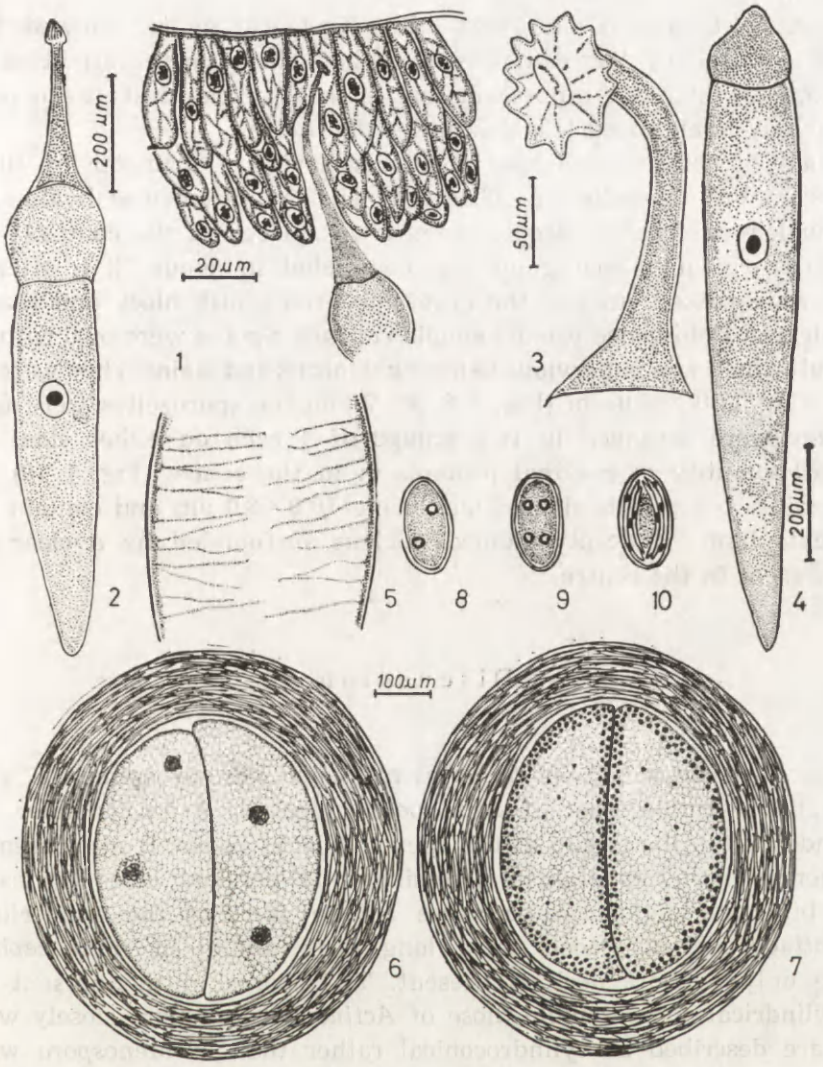

Fig. 1. *Actinocephalus bradinopygi* sp. n. *1* — Attachment, *2* — A cephalont — note the contracted bulb-like part of epimerite, *3* — An enlarged view of the epimerite, *4* — A sporont, *5* — A part of the deutomerite showing transverse striations, *6* — Cyst showing 4 nuclei — 2 in each of the two gametocytes, *7* — A multinucleate cyst showing peripheral arrangment of nuclei in the gametocytes, *8* — 2 nucleate sporoblast, *9* — 4 nucleate sporoblast, *10* — A sporocyst showing sporozoites

255 µm and was solitary. The deutomerite shows transverse striations which were more conspicuous than in the cephalonts.

C y s t s: Cysts were spherical having a diameter of 800 µm and were opaque white (Fig. 1 *6*, *7*). A gelatinous ectocyst 150 µm thick was

present. Cysts passed out along with the faecal matter showed 20–25 nuclei in each of the gametocytes. When the gamete formation was completed, which was approximately 48 h after the cysts were passed out, the cysts appeared transluscent.

Gametes: Gametes are isogamous having a diameter of 10 μm. No locomotor organelle was observed. The cytoplasm was hyaline and the nucleus which was deeply stained was placed in the centre.

Sporogony: Sporogony was completed in about 72 h after the cysts were passed out and the cysts appeared bluish black and float on the surface. Dehiscence was by simple rupture. Spores were oval with a rigid wall which was impervious to most chemicals and stains. They were oval measuring 17.0 × 5.0 μm (Fig. 1 *8*, *9*). When the sporozoites were formed they were arranged in two groups of 4 each on either side, with a small quantity of residual protoplasm in the centre (Fig. 1 *10*). The sporozoites are spindle-shaped measuring 10.0×3.0 μm and contain hyaline cytoplasm. A deeply stained nucleus surrounded by a clear halo was present in the centre.

Discussion

The form described in the present paper has an epimerite which generally resembles that of *Menospora* in having a long slender neck expanded into a large cup at the apex but lacks hooks. It also resembles the genus *Actinocephalus* in posessing 9–11 petaloid lobes (instead of 8–10 bifurcate digitiform processes at the apex of the epimerite as in *Actinocephalus*) placed on a long neck unlike in *Actinocephalus* where only a short neck is present. The spores in the present case are cylindrical and resemble those of *Actinocephalus* more closely where they are described as cylindroconical rather than of *Menospora* where they are crescentic. In the present work greater importance is attached to the presence or absence of spines in the epimerite, rather than the length of the neck of the epimerite and as such, the present form is placed in the genus *Actinocephalus*. *A. sieboldi* is the only species reported so far from odonate hosts (intestine of the larvae of *Aeschna* sp.). *A. sieboldi* reaches a size of 162–188×45 μm and the epimerite is composed of 4–6 sharp recurved hooks while in the present form the fully grown cephalonts reach 825×165 μm and the epimerite is different. The spores in *A. sieboldi* are described as biconical and the size is not given while in the present form they are cylindrical and measure 17.0×5.0 μm. In view of the above mentioned reasons the form descri-

bed in the present paper is considered new and the name *Actinocephalus bradinopygi* sp. n. after the host is proposed.

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RÉSUMÉ

La morphologie et le cycle d'évolution sont décrites d'une espèce nouvelle de segment grégarine, *Actinocephalus bradinopygi* sp. n. de l'odonate, *Bradinopyga geminata*, Ramb.

Les cephalontes sont 825 × 168 μm et l'épimerite est complexe. Les kystes sont sphériques, 800 μm dans diamètre, l'épaisseur du externe kyste mesure 150 μm, Gamètes similaire, 10 μm dans diamètre spores ovale, 17 × 5 μm. Octozoic. Sporozoite fusiforme, 10×3 μm.

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Kinetics of the Regeneration of Rhythmic Contraction Activity in *Physarum polycephalum* Drops

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Synopsis. The endoplasmic drops isolated from plasmodial veins of *Physarum polycephalum*, under appropriate culture conditions, regenerate rhythmic contraction activity characteristic of the intact plasmodium The time indispensable for resumption of contraction activity follows a normal distribution, while the kinetics of a trigger mechanism for this activity is an example of elementary process of Poisson's kinetics. The temperature dependence of the kinetics of a trigger mechanism shows a phase transition in the range of 16.6–18.4°C. Approximately, the same temperature transitions were observed for response of plasmodium to chemical stimuli and rate of pseudopodia extension (Ueda and Kobatake 1978). Since these results were interpreted as the existence of two distinct stages in plasmodium membrane activities, it is reasonable to suspect that plasmalemma of *Physarum polycephalum* is involved in a trigger mechanism.

Plasmodium of the slime mould *Physarum polycephalum* is a favourable model for the study of different aspects of motile activity (Kamiya 1959, Komnick et al. 1973). Among them, investigations of different oscillatory phenomena in its contraction behaviour, the nature of oscillations and their control are of special interest (Wohlfarth-Bottermann, in press).

Endoplasmic drops of *Physarum polycephalum* represent a unique

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model for investigation of *de novo* formation of contractile apparatus and regeneration of its function (Isenberg and Wohlfarth-Bottermann 1976). Moreover, the model can be easily obtained by puncturing the plasmodial veins. Endoplasm which fills out the veins is under hyperpressure and therefore it protrudes and forms a drop after puncture. The drops isolated from the veins, under appropriate culture conditions undergo a sequence of spontaneous, time-dependent differentiation processes which finally lead to a new migrating plasmodium. The sequence consists of regeneration of plasmalemma (Wohlfarth-Bottermann and Stockem 1970), endoplasm-ectoplasm transition which is accompanied with actin polymerization (Isenberg and Wohlfarth-Bottermann 1976), differentiation of actomyosin fibrils and regeneration of plasmalemma invaginations (Götz von Olenhusen et al 1979), *de novo* generation of contraction rhythmicity (Götz von Olenhusen and Wohlfarth-Bottermann 1979), and finally locomotion of plasmodium. Up to now, the problem of *de novo* generation of vitality of the endoplasmic drops was mainly restricted to the study of morphogenetic processes. The aim of the present study was to determine the kinetics of *de novo* generation of oscillating contraction activity of untreated drops and to postulate the localization of a trigger mechanism for the contractile activities in plasmodium.

Material and Methods

The slime mould *Physarum polycephalum* was cultured as described by Camp (1936). The endoplasmic drops (1–1.5 mm in diameter) were obtained by puncturing the veins of frontal region of freely migrating plasmodium with a steel needle.

Immediately after their generation from flowing endoplasm the drops were separated from plasmodium and placed in a moist chamber on plexiglass surface. Time dependent events in the contractile behaviour of endoplasmic drops were controlled tensiometrically using the "contact method" (Wohlfarth-Bottermann 1975) under isometric conditions of measurements. The drops collected during 8–10 h from the same plasmodium were counted as a single sample. The number of drops in the samples varied from 23 to 32.

The duration of plasmodium migration without feeding before the production of drops was controlled only in the experiments in which the influence of starvation on *de novo* generation of rhythmic contraction activity of drops was investigated. The measurements were taken in room temperature (about 21°C).

Effect of temperature on contraction behaviour of the drops was investigated in a chamber of which temperature was controlled with the aid a Peltier element.

Results

A typical tensiometer curve of the contraction behaviour of an endoplasmic drop is shown in Fig. 1. First minimum of visible oscillations was chosen as the start point of rhythmic contraction activity (stage S_3) of a drop. Duration of preliminary stage is marked as S_{12}.

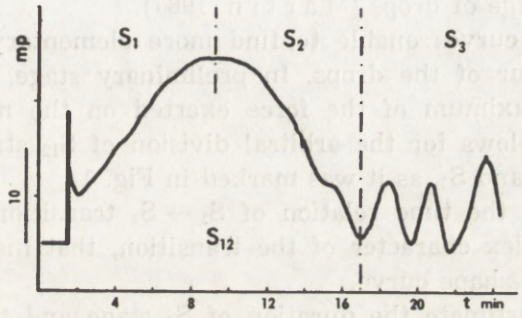

Fig. 1. A typical tensiometric curve of *de novo* generation of rhythmic contraction activity in an endoplasmic drop of *Physarum polycephalum*. Time of the drop generation is the starting poin of the measurement. Phases S_{12} and S_3 denote preliminary and rhythmic contraction activity stages respectively. S_1 and S_2 — subphases of the preliminary stage

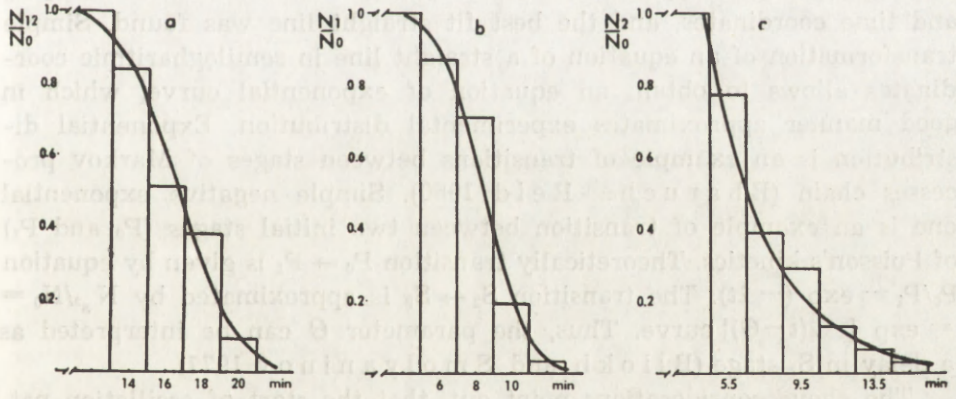

Fig. 2. The histograms of the kinetics of (a) $S_{12} \rightarrow S_3$ (b) — $S_1 \rightarrow S_2$ and (c) — $S_2 \rightarrow S_3$ transitions. N_0 — total number of the drops; N_{12}, N_1 and N_2 — number of the drops in S_{12}, S_1 and S_2 stages respectively. In the case of $S_2 \rightarrow S_3$ transition the drops in the sample were synchronized in respect to the begining of S_2 stage

Figure 2a presents an example of histogram of S_{12} stage duration, it means, of kinetics of the transition from preliminary stage (S_{12}) to oscillation pattern (S_3) of contraction activity. The sample consisted

of 31 drops and was measured in room temperature. The histogram is satisfactory approximated by S-shape curve of distribution function of normal distribution. The approximation curve was obtained on the basis of the best fit straight line on normal distribution control chart.

Normal distribution of the initiation of rhythmic contraction activity suggests that the start of oscillations against the time of the drops generation is a result of numerous independent events which additively lead to active stage of drops (M a r t i n 1967).

Tensiometric curves enable to find more elementary stages in contraction behaviour of the drops. In preliminary stage, the drops show characteristic maximum of the force exerted on the measuring device receptor. This allows for the arbitral division of S_{12} stage into two separate stages S_1 and S_2, as it was marked in Fig. 1.

Histogram of the time relation of $S_1 \rightarrow S_2$ transition (Fig. 2 b) still suggests a complex character of the transition, that means, is well approximated by S-shape curve.

In order to estimate the duration of S_2 stage and the time relation of $S_2 \rightarrow S_3$ transition the time point of the maximum of the force exerted on the measuring device receptor was chosen as a time zero for this distribution (Fig. 2 c). The attempt of approximation of this distribution by S-shape curve did not give a satisfactory result. With the purpose of attaining a good approximation the distribution was tested in $\ln N_2/N_0$ and time coordinates, and the best fit straight line was found. Simple transformation of an equation of a straight line in semilogharitmic coordinates allows to obtain an equation of exponential curve, which in good manner approximates experimental distribution. Exponential distribution is an example of transitions between stages of Markov processes chain (B h a r u c h a - R e i d 1960). Simple negative exponential one is an example of transition between two initial stages (P_0 and P_1) of Poisson's kinetics. Theoretically transition $P_0 \rightarrow P_1$ is given by equation $P_0/P_1 = \exp(-\lambda t)$. The transition $S_2 \rightarrow S_3$ is approximated by $N_{s^2}/N_0 = \exp[-\lambda(t-\Theta)]$ curve. Thus, the parameter Θ can be interpreted as a delay in S_2 stage (B l i o k h and S m o l y a n i n o v 1977).

The above considerations point out, that the start of oscillation pattern of contraction activity depends on numerous events in initial period (S_1) of the drops behaviour, while terminal one (S_2) is determined by trigger system, which kinetics can be described as a single Poisson's process.

Investigations of stochastic processes raise the question in what extent a sample can be regarded as representative for population. In other words, what conditions have to be fulfilled during collecting drops which make a sample. In order to give some impression concerning this pro-

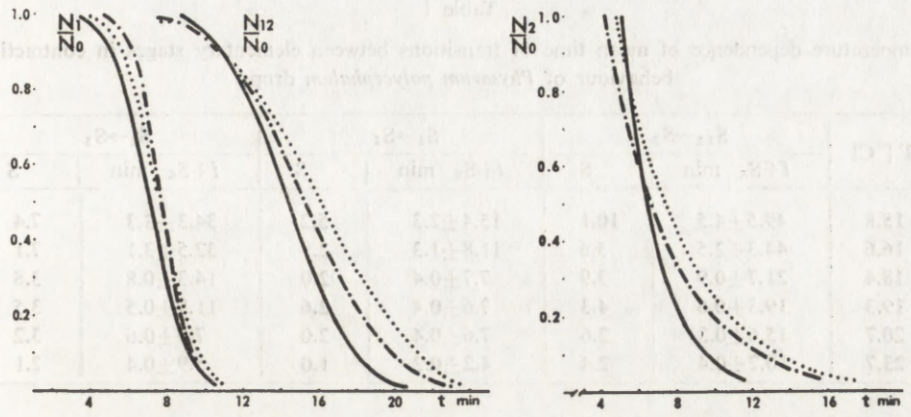

Fig. 3. The influence of the period of plasmodium starvation on the kinetics of the transitions between elementary stages in contraction behaviour of *Physarum polycephalum* drops. The curves approximate the histograms of $S_{12} \rightarrow S_3$, $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions for samples taken after 12 h ($N_o = 24$ drops — continuous line), 36 h ($N_o = 23$ drops, dot-dash line) and 60 h ($N_o = 32$ drops — dotted line) of starvation. N_{12}, N_1 and N_2 — number of the drops in S_{12}, S_1 and S_2 stages respectively

blem the kinetics of starting point of rhythmic contraction activity was investigated at different periods of plasmodium starvation. The drops were collected after 12, 36 and 60 h of starvation of the same plasmodium. The results are shown in Fig. 3. There are drawn the best fit curves for $S_{12} \rightarrow S_3$, $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions. The curves were obtained in the same manner as in the case of distributions shown in Fig. 2.

Since $S_{12} \rightarrow S_3$ and $S_1 \rightarrow S_2$ transitions follow a normal distribution, their means equality can be tested by Student's difference test (B r a n d t 1970). The hypothesis of equal means has to be rejected only in the case of $S_{12} \rightarrow S_3$ transition for samples taken after 12 and 60 h of starvation ($|t| = 2.88 > P_{0.05} = 2.01$). This result points that an intrinsic process or processes taking place in plasmodium during starvation can change parameters of distributions. It mainly concerns the terminal phase of $S_{12} \rightarrow S_3$ transition, thus, a postulated trigger system, which acts during $S_2 \rightarrow S_3$ transition. On the other hand it imposes conditions of sample making for investigation of the kinetics of a trigger system. Drops have to be collected from a single plasmodium no longer than during 12 h, because otherwise slight differences in parameters of negative exponential distribution would lead to S-shape of distribution function of $S_2 \rightarrow S_3$ transition.

The kind of kinetics of above mentioned transitions does not change

Table 1

Temperature dependence of mean time of transitions between elementary stages in contraction behaviour of *Physarum polycephalum* drops

T [°C]	$S_{12} \rightarrow S_3$		$S_1 \rightarrow S_2$		$S_2 \rightarrow S_3$	
	$\bar{t}+S_x$ min	S	$\bar{t}+S_x$ min	S	$\bar{t}+S_x$ min	S
15.8	49.5±4.5	10.1	15.4±2.3	5.2	34.3±3.3	7.4
16.6	44.3±2.5	5.6	11.8±1.3	2.9	32.5±3.1	7.1
18.4	21.7±0.9	3.9	7.7±0.4	2.0	14.3±0.8	3.8
19.3	19.3±0.6	4.3	7.6±0.4	2.6	11.9±0.5	3.5
20.7	15.6±0.3	2.6	7.6±0.4	2.0	7.9±0.6	3.2
23.7	10.2±0.4	2.4	4.2±0.2	1.0	5.9±0.4	2.1

S — standard deviation of the samples, S_x — standard error of the samples

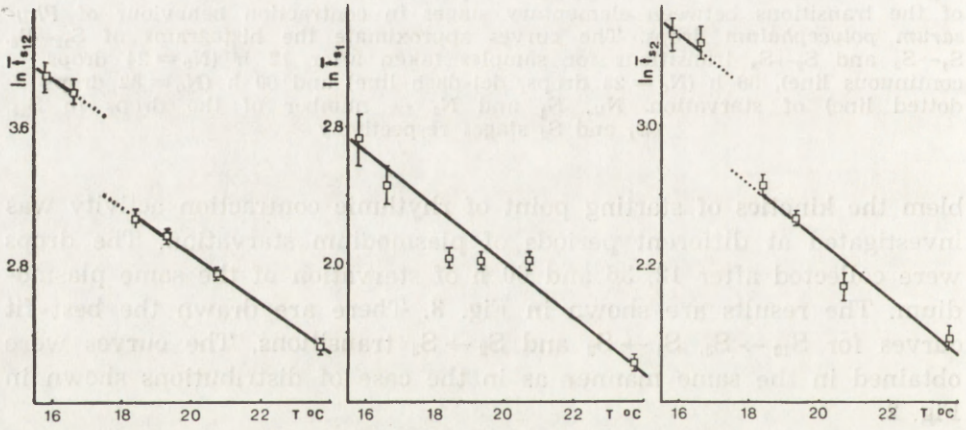

Fig. 4. Temperature dependence of mean time of $S_{12} \rightarrow S_3$, $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ transitions (see Table 1) plotted in semilogharithmic coordinates

with temperature, that means, they preserve their S-shape or, respectively, negative exponential character. It was tested at 18.4, 19.3, 20.7, and 23.7°C. However, there exist conspicuous differences between mean time and standard deviations of all transitions (Table 1).

Figure 4 shows the same data plotted as ln $\bar{t}s_{12}$, s_1, s_2 against temperature. There is a clear discontinuous change of the mean time values of $S_{12} \rightarrow S_3$ and $S_2 \rightarrow S_3$ transitions, while there is no such in $S_1 \rightarrow S_2$ transition. The mean time of initiation of rhythmic contraction activity against the time of drops generation ($\bar{t}s_{12}$), and the time of intervention of a trigger mechanism ($\bar{t}s_2$), are decreased by factor 1/2 in the range of 16.6–18.4°C.

Discussion

The presented results concerning kinetics of *de novo* generation of rhythmic contraction activity of the endoplasmic drops ($S_{12} \rightarrow S_3$ transition) confirm a complexity of processes which finally lead to contraction behaviour characteristic of the intact plasmodium. The complexity is reflected by normal distribution of this transition. In preliminary stage the drops undergo very quick process of plasmalemma regeneration by means of membrane vesiculation (Wohlfarth-Bottermann and Stockem 1970). The organization of the contractile gel reticulum of a drop and regeneration of plasmalemma invaginations is over after about 5–10 min (Isenberg and Wohlfarth-Bottermann 1976, Götz von Olenhusen et al. 1979). Then, the drops regenerate rhythmic pattern of contraction activity (Götz von Olenhusen and Wohlfarth-Bottermann 1979).

On the basis of listed above sequence of time-dependent processes the S_1 stage of the drops (Fig. 1) with mean time of 7.6 ± 0.4 min at room temperature can be interpreted as complex of processes of regeneration of plasmalemma, actin transformations and fibrilogenesis. It is additionally confirmed by S-shape curve (Fig. 2 and Fig. 3) of $S_1 \rightarrow S_2$ transition. Simultaneously, S_2 seems to be a stage, when contractile apparatus of the drops, from morphogenetic point of view, is ready for initiation of contraction activity and is waiting for a stimulus. Thus, $S_2 \rightarrow S_3$ transition shows the kinetics of a trigger mechanism which stimulates the contractile system of *Physarum polycephalum*. The kinetics of a trigger, as it is shown in Fig. 2 and Fig. 3, is represented by a single Poisson's process. Elementary stages of Poisson's kinetics were also found in a process of fibroblasts spreading (Bliokh and Smolyaninov 1977). However, the relationship between actomyosin fibrilogenesis and generation of a force (Fig. 1) at the preliminary stage of a drop remains obscure at the present time.

The study of kinetics of $S_{12} \rightarrow S_3$ and $S_1 \rightarrow S_2$ transitions and of a trigger mechanism against the time of plasmodium starvation was done in order to define the homogeneity of the samples. Period of starvation was chosen arbitraly as a factor influencing many inner processes taking place in plasmodium, which can disturb the kinetics of investigated transitions. The obtained results point out that the kinetics of a trigger system is sensitive to the period of plasmodium starvation. Simultaneously, more or less "pure" kinetics of a trigger system can be investigated when drops are collected from the single plasmodium no longer than during 12 h.

The distinct differences between mean time of *de novo* generation of rhythmic contraction activity are observed with temperature variations (Table 1 and Fig. 4). In the range of 16–24°C, mean time of $S_{12} \rightarrow S_3$, $S_1 \rightarrow S_2$ and triggering of oscillations decrease exponentialy (Fig. 4) with the increase of temperature. However, the curves for $S_{12} \rightarrow S_3$ and a trigger mechanism show discontinuous decreasing by factor 1/2 in range of 16.6–18.4°C. It means that the trigger mechanism of *de novo* generation of oscillation contraction activity has two distinct stages. A phase transition between these stages is caused by temperature variations.

Discontinuous changes in magnitude of the response of plasmodia to chemical stimuli and the rate of pseudopodia extension caused by temperature variations were found by Ueda and Kobatake (1978). The transition temperature for these phenomena is about 15°C. The results were interpreted as a conformational change of the surface membrane of *Physarum polycephalum* at transition temperature.

Plasmalemma was earlier suspected to be a site of a trigger mechanism which acts via changes in ionic permeabilities and/or electrogenic ion pumps. However, still there is a lack of clear experimental evidence of this hypothesis (Wohlfarth-Bottermann in press). Conformity of transition temperatures for chemotactic phenomena, rate of pseudopodia extension and time of intervention of a trigger mechanism might suggests that plasmalemma of *Physarum polycephalum* is involved in a trigger system, but the present results give no background to formulate any hypothesis explaining the mechanism of this system.

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My thanks are due to Prof. K. E. Wohlfarth-Bottermann, Head of the Institute of Cytology and Micromorphology, University of Bonn for giving necessary facilities to carry out the tensiometric measurements and for the helpfull discussions.

RÉSUMÉ

Les gouttes d'endoplasme isolées des veines du plasmodium de *Physarum polycephalum*, maintenues dans les conditions ambiantes favorables, recupèrent l'activité contractile rythmique qui est caractéristique pour un plasmodium intact. La variation du temps nécessaire pour la restitution de l'activité contractile suit une distribution normale, tandis que la cinétique du mécanisme qui déclenche cette activité représente le cas d'un processus élémentaire

obeissant à la cinétique de Poisson. La dépendence de la cinétique du mécanisme déclencheur de la température manifeste une transition de phase entre 16.6 et 18.4°C. Une transition thermique approximetivement pareille était observée pour la réponse du plasmodium à la stimulation chimique et la vitesse d'extension des pseudopodes (Ueda and Kobatake 1978). Ces résultats sont interprétés comme dus à la présence de deux stades distinctes de l'activité de la membrane plasmodiale, on peut donc raisonablement supposer que la plasmalemma de *Physarum polycephalum* est engagée dans le fonctionnement du mécanisme déclencheur.

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Cytochalasin B Induced Inhibition of Food Ingestion in *Dileptus anser* (*Ciliata, Gymnostomata*)

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Synopsis. The influence of different concentrations of cytochalasin B (CB) on *Dileptus anser* was studied. The physiological observations and ultrastructural studies were carried out. The inhibitory effect of this drug on food vacuole formation process was stated. The possible mechanism of CB action was discussed.

The cytochalasins are a group of fungal metabolites which have been found to have inhibitory effects on a variety of cell processes. The effects of CB are primarily of two types: (1). It has been proposed that it causes the disintegration of microfilaments and myofilaments (Schroeder 1970, Bluemink 1971, Spooner et al. 1971, Wessels et al. 1971, Spooner 1973, Axline and Reaven 1974, Copeland 1974, Sachs et al. 1974, Williamson 1975). Some biochemical studies have postulated a direct interaction of cytochalasins with actin and/or myosin (Spudich 1972, Spudich and Lin 1972, Hartwig and Stossel 1976). (2). Other authors, however, have not accepted these conclusions and have proposed alternative explanation such as membranotropic effects for the mechanism of cytochalasins action (Hauschka 1973, Rathke et al. 1975, Weber et al. 1976).

Cytochalasin B (which has beeen used in most studies) has been found to inhibit phagocytosis in various cells (Williams and Wolf 1971, Zigmond and Hirsch 1972, Axline and Reaven 1974, Nilsson et al. 1973, Hoffman et al. 1974, Rothstein and Blum 1974, Nilsson 1977). Since the food ingestion in carnivorous *Ciliate* — *Dileptus anser* involves both: membrane and filament activity and each of these structures is mainly involved in different stage of the process, the observations of CB influence on different stages of endocytosis can help in the elucidating of the way of CB action. On

the other hand the observations of physiology and ultrastructure of the cell after CB treatment may explain the mechanisms involved in the process of food ingestion in *Dileptus*.

Material and Methods

Dileptus anser O. F. M. was used in this study. The cells were cultured in Pringsheim solution and fed every other day with *Colpidium colpoda*. For experiments the cells before feeding were selected. They were washed in 2 mM phosphate buffer pH 7.1 overnight. Stock solution of 10 mg of CB (Serva) in 1 ml of DMSO was prepared. The CB solutions used in experiments were prepared by using 1mM phosphate buffer pH 7.1. The experiments were carried out at room temperature. 5–20 μg/ml CB was added to the samples containing about 20 cells each. The appropriate solutions of DMSO (0.5–2%) and phosphate buffer were used as control media. After 5, 30, 60 min and 24 h of CB, DMSO, or buffer treatment *Colpidia* were added in order to test the food ingestion ability of *Dileptus* cells. The number of cells containing food vacuoles and cells without vacuoles after 5 min or 30 min exposure to the prey was counted. The experiments have been repeated ten times and the mean number (in per cent) of the cells with, and without vacuoles was calculated. The observations of the single, living cells treated with CB and exposed to the prey were also carried out.

In order to test the influence of CB on the ultrastructure of *Dileptus* the preparations of CB treated and control cells for electron microscopy were made. The fixation method of Golińska (1978) was used. The sections obtained by using LKB ultramicrotome were contrasted with uranyl acetate and lead citrate and examined in JEM 100 B electron microscope.

Results

The treatment of *Dileptus anser* cells with CB causes the inhibition of endocytosis. The effect is stronger in higher concentrations of the drug (Table 1, 2) and it is more distinct when the data obtained during 5 min exposure to the food are taken into account (Table 1). This observation indicates that the complete inhibition of food ingestion does not occur, but the process is much slower then in control cells. The rate of endocytosis in DMSO treated cells is little slower than in buffer medium, however, it is significantly higher than in CB treated cells. No significant differences are seen in the food ingestion ability depending on different time of CB treatment except of 24 h exposure to the drug, when the complete inhibition of vacuoles formation occurs.

The observations of the behaviour of living *Dileptuses* treated with CB have revealed that only the second step of endocytosis is inhibited

Table 1

The percentage of cells of *Dileptus anser* containing food vacuoles (+) and without food vacuoles (−) after different time of CB, DMSO or phosphate buffer treatment. Colpidia added for 5 min

Time of treatment	Medium													
	CB Concentration (μg/ml)						DMSO Concentration (%)						Buffer	
	20		10		5		2		1		0.5			
	+	−	+	−	+	−	+	−	+	−	+	−	+	−
5′	11.1	88.9	19.8	80.2	25.5	74.5	86.5	13.5	70.0	30.0	88.5	11.5	95.0	5.0
30′	10.0	90.0	17.0	83.0	30.0	70.0	80.0	20.0	84.6	15.4	85.6	14.4	97.2	2.8
60′	6.3	93.1	19.6	80.4	25.5	74.5	86.1	13.9	87.5	12.5	86.0	14.0	93.3	6.7
24 h	0.0	100.0	0.0	100.0	18.2	81.8	85.0	15.0	75.0	25.0	100.0	0.0	100.0	0.0

Table 2

The percentage of cells of *Dileptus anser* containing food vacuoles (+) and without food vacuoles (−) after different time of CB, DMSO or phosphate buffer treatment. Colpidia added for 30 min

Time of treatment	Medium													
	CB Concentration (μg/ml)						DMSO Concentration (%)						Buffer	
	20		10		5		2		1		0.5			
	+	−	+	−	+	−	+	−	+	−	+	−	+	−
5′	40.9	59.1	52.1	47.9	56.0	44.0	100.0	0.0	95.6	4.4	97.5	2.5	100.0	0.0
30′	40.0	60.0	42.5	57.5	65.4	34.6	95.2	4.8	88.8	11.2	94.6	5.4	100.0	0.0
60′	33.7	66.3	52.6	47.4	64.2	35.8	96.9	3.1	93.7	6.3	100.0	0.0	100.0	0.0
24 h	0.0	100.0	—	100.0	16.0	84.0	91.5	8.5	92.9	7.1	100.0	0.0	94.9	5.1

by this agent. It means that the predators do not loose their ability to capture the prey cells, but they can not ingest them.

The studying of the ultrastructure does not reveal very distinct differences between the microfibrillar ring which holds together small nemadesmata of the internal basket of oral apparatus in CB treated and control cells (PI. I 1, 2) and in microfibrillar layer situated between ecto-and endoplasm in the whole body of *Dileptus*.

Discussion

The effects of CB on food ingestion in *Dileptus anser* can be due to its action either on the membrane or on the microfilaments involved in food vacuole formation. The observations of the living cells after CB treatment have revealed that the process of opening of the mouth and vacuole formation is effected by CB. It has been suggested that in opening of mouth the microfilament ring in which the internal nemadesmal basket is anchored, can play a role (Tołłoczko 1979). It has been also postulated by the same author that the new filament material appears during food ingestion in the area of the oral apparatus. On the other hand a lot of membrane material in form of smooth, elongated vesicles has been observed in endoplasm inside the nemadesmal basket of *Dileptus anser* (Golińska 1978). These vesicles are probably the pool of the membrane which is used during vacuole formation similar as in *Paramecium* (Allen 1974). Since no vacuole formation was observed in CB treated Dileptuses the fusion of the vesicles with the cytostomal membrane does not occur after CB treatment. On the contrary, in noncarnivorous Ciliates *Paramecium* and *Tetrahymena* enormously big vacuoles were formed in CB treated cells, but they were not able to separate (Nilsson 1973, Nilsson et al. 1973, Tołłoczko 1977). These observations suggest that some CB sensitive mechanism acts in *Dileptus* prior to the vacuole formation, whereas in *Paramecium* and *Tetrahymena* it appears during food vacuole separation. It can be assumed that it could be microfilament action. This assumption is supported by many observations that CB acts on many filamentous structures (see Introduction). On the other hand, the observation that CB acts on food ingestion already after 5 min treatment is in agreement with studies of Hauschka (1973) who has postulated partitioning of CB in hydrophobic regions of the cell membrane. Since ultrastructural differences between CB treated and untreated Dileptuses are not clearly seen — the most possible explanation is that the effects of CB are due to its influence first on the membrane and then on microfilaments action, but without causing distinct differences in their

ultrastructure. Similar data have been obtained by Bohatier and Kink (1977) who observed unnormal function of regenerated, normally built oral apparatus, when cyclohexymide was applicated during regeneration of *Dileptus anser*. Rathke et al. (1975) have observed no microfilament disintegration during primary phase of CB action. Kurkinen et al. (1978) have also observed the influence of this drug on both: membrane phenomena and actin containing microfilaments in cultured fibroblasts.

The hypothesis of Weber et al. (1976) that cytochalasins induce the changes in plasma membrane and these changes cause as a second step the changes in microfilaments seems to be the most probable explanation of CB action on food ingestion process in *Dileptus*.

ZUSAMMENFASSUNG

Der Einfluss von diversen Konzentrationen von Cytochalasin B (CB) auf *Dileptus anser* wurde unterschut. Physiologische und ultrastrukturalen Forschungen wurden durchgefürt. Die Hemmung von Ernahrungsvakuolenbildung nach der CB Behandlung wurde festgestellt. Möglicher Mechanismus von CB-aktion wurde beschprochen.

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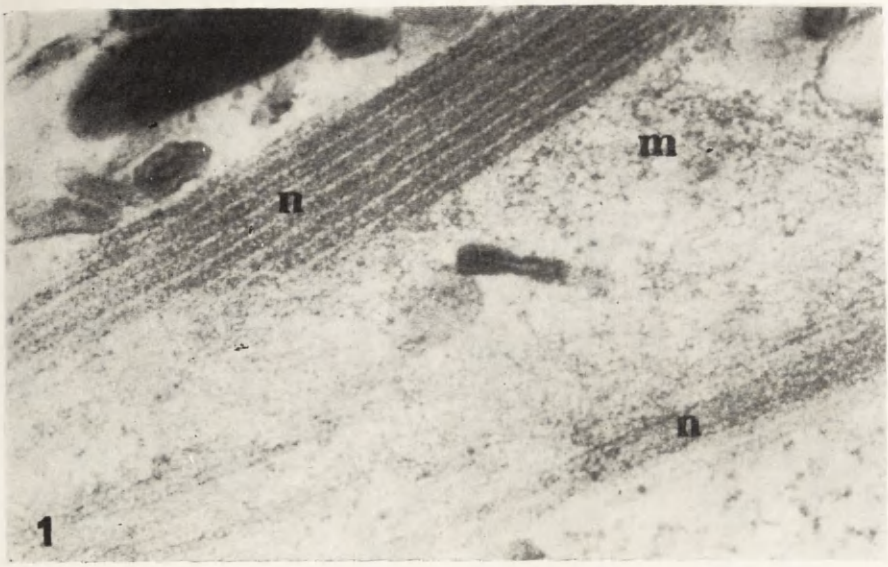
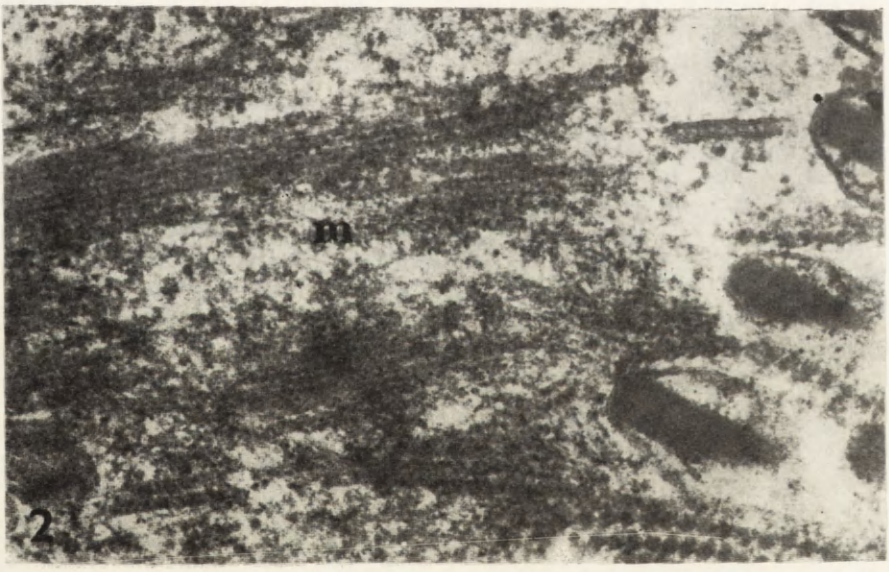
EXPLANATION OF PLATE I

Fragments of microfibrillar ring in which nemadesmal basket is anchored, n — nemadesma, m — microfilaments

1: Control cell of *Dileptus anser* (×54 000)
2: *Dileptus anser* after 1 h treatment with 10 μg/ml CB (×54 000)





B. Tołłoczko auctor phot.

In preparation:

S. Fabczak: Electrical Properties of Cell Membrane in Protozoan, *Stentor coeruleus*. I. Modification of Resting Membrane Potential by Extracellular Ions — S. Fabczak: Electrical Properties of Cell Membrane in Protozoan *Stentor coeruleus*. II. Effect of Temperature on Internal Membrane Potential — A. Wasik and J. Sikora: Effects of Cytochalasin B and Colchicine on Cytoplasmic Streaming in *Paramecium bursaria* — B. Tołłoczko: Effect of Colchicine on Food Ingestion in *Dileptus anser* — Z. Sayers: Motile Behaviour of *Amoeba proteus* under Various Ionic Conditions — W. Kłopocka and A. Grębecki: Motory Independence of Pseudopodia in Freely Moving *Amoeba proteus* — M. Cieślawska: Dynamics of the Ending Veins in Plasmodia of *Physarum polycephalum* — J. Kołodziejczyk and A. Grębecki: Dynamics of the Frontal Margin of Plasmodia of *Physarum polycephalum* — B. Skoczylas and D. Kucharczyk: Reproducibility of *Paramecium tetraurelia* strain 51 S and *Paramecium octaurelia* strain 299 S in Mass Cultures — V. Golemansky and D. Kuldjieva: *Eimeria garzettae* sp. n. (*Coccidia*: *Eimeriidae*) in the Little Egret (*Egretta garzetta* L.) from Bulgaria — M. Mukherjee and D. P. Haldar: Observations on *Eimeria glossogobii* sp. n. (*Sporozoa*: *Eimeriidae*) from a Fresh Water Teleost Fish — А. Ковальчук: Быстрый метод изготовления постоянных препаратов инфузорий в экспедиционных условиях.

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Cena prenumeraty krajowej: rocznie zł 200,—, półrocznie zł 100,—

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— do 10 czerwca na II półrocze roku bieżącego.
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