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# Morphology and Evolution of Intestinal Parasitic Flagellates of the Far-Eastern Roach *Cryptocercus relictus*

*Synopsis.* Intestinal flagellates from the hindgut of the Far-Eastern wood-feeding roach *Cryptocercus relictus* were studied. The flagellate fauna of *Cr. relictus* includes: *Barbulanympha cryptocerci, Urinympha cirrata, Trichonympha major, T. ussuriensis, T. lutea, Leptospironympha variabilis, L. popularis, L. lepida, Bispironympha unica, Eucomonympha nana* (order *Hypermastigida*), *Saccinobaculus gloriosus, S. spatiatus, S. scabiosus, Notila proteus ussuriensis, Oxymonas nana ussuriensis* (order *Oxymonadida*), *Hexamita cryptocerci* (order *Diplomonadida*). Morphological descriptions of all these species are given. Some questions of the origin, distribution and evolution of flagellates of the orders *Oxymonadida* and *Hypermastigida* in both roaches and termites are discussed.

## Contents

## I. Introduction

All basic principles of the theory of evolution have been successfully developed mainly for *Metazoa*. For advancing a synthetic theory, it is, however, essential to study peculiarities of the evolution at the cellular level of organization, i.e., in *Protozoa*. It would be of great theoretical interest to reveal in *Protozoa* both the general principles by which evolution is accomplished in the entire animal kingdom, and

original features inherent to unicellular organism (Dogiel 1954, 1965, Poljansky 1965, 1969, 1970, 1971, 1972 and oth.).

Among *Protozoa*, specialized parasites are most convenient for evolutionary studies, because their evolution proceeds mainly in parallel with that of their hosts (see reviews by Dogiel 1964; Michajłow 1960, 1968). Therefore, in certain cases, one may estimate the evolutionary history of specialized parasites basing upon data about evolution of their hosts. A vivid example of such an investigation is V. A. Dogiel's study (1946) on the evolution of ciliates of the family *Ophryoscolecidae* living in the stomach of ruminants.

This paper deals with evolution of specialized parasitic flagellates inhabiting the hindgut of termites and wood-feeding roach *Cryptocercus*. This fauna of flagellates, belonging to the orders *Oxymonadida* and *Hypermastigida* (Honigberg et al. 1964), is large and diverse. It includes about 55 genera and 300 species. These flagellates inhabit only two taxa of insect hosts: roaches of the genus *Cryptocercus* (*Blattodea, Cryptocercidae*) and lower termites (*Isoptera, Mastotermitidae, Calotermitidae, Hodotermitidae, Rhinotermitidae*). The majority of the flagellate genera are specialized either for termites or for roaches. There exist, however, three genera whose representatives occur in both host groups. These are *Oxymonas* (order *Oxymonadida*) as well as *Leptospironympha* and *Trichonympha* (order *Hypermastigida*).

Lower termites and the wingless roach *Cryptocercus* have a similar diet consisting exclusively of cellulose which they obtain by feeding on wood. The alimentary canal of these insects lacks cellulose-splitting enzymes. The flagellates which abound in the posterior part of the intestine of termites and *Cryptocercus* contain, however, cellulase and participate in the digestion of food by their hosts (Cleveland 1923, 1926, Cleveland et al. 1934). Close symbiotic interrelations between these animals is a form of mutualism and they have been studied biochemically (for references, see reviews by Honigberg 1967, Hungate 1955). In terms of the general concept of parasitism (Dogiel 1964), the association between xylophagous insects and flagellates can, however, be regarded as a host–parasite association and the flagellates can be termed "parasitic" in the broader sense of the word. According to this concept, parasites are organisms which use other organisms as their environment and source of food, at the same time relinquishing to their hosts partly or completely (as in this instance) the task of regulating their relations with the external environment (Dogiel 1964). The author will use the term "parasitic" for the flagellates of *Cryptocerus* to emphasize the latter peculiarity, which is of great significance for studying their evolution.

Only lower termites and *Cryptocercus* have specific symbiotic flagellates of the orders *Oxymonadida* and *Hypermastigida*. The parasitic protozoan fauna of other representatives of the order *Blattodea*, including *Blatta, Blattella, Periplaneta* and *Panesthia*, known in good detail, has a different composition and shows another kind of physiological relationships with the host than that of the genus *Cryptocercus*

(Kudo 1926, Grassé 1926, Chen 1933, Bishop 1933, Kidder 1937, Yamasaki 1939). This also applies to the parasite fauna of higher termites (fam. *Termitidae*) as compared to lower representatives of the order (Kirby 1931, 1932 a, 1941, Grassé et Noirot 1959).

The problems of evolution of the orders *Oxymonadida* and *Hypermastigida* are here considered in relation with our study of the species composition and speciation of the intestinal flagellates of the Far-Eastern roach *Cryptocercus relictus*. Our interest to this parasite fauna is due to the following reasons.

(1) Up to now, symbiotic flagellates similar to those from lower termites have been found only in *Cryptocercus punctulatus* (Cleveland et al. 1934). Investigation of this fauna in other *Cryptocrecus* species could shed light on the origin, distribution and evolution of flagellate species within the orders *Oxymonadida* and *Hypermastigida*.

(2) As known from entomological evidence, recent relict roaches — *Cryptocercus punctulatus* and *Cr. relictus* — have been developing independently since the Tertiary time in conditions of complete geographical isolation (the former species in North America, and the latter, in the Far-East of Asia) (Bei-Bienko 1950). This makes possible to follow the formation of their respective parasite faunas.

(3) The hypothesis, first proposed by American authors (Cleveland et al. 1934), that the systematic similarity of the parasitic fauna of the roach and that of termites is a mere reflection of the phylogenetic relation of their hosts, was supported unreservedly by other investigators of this parasite group. Their opinions varied, however, as for the question of which host should be considered primary. Some of the authors (Cleveland et al. 1934, Kirby 1937, 1949, Grassé 1952) considered cockroaches (the orders *Protoblattodea* and *Blattodea*) to be the primary hosts, while others (Dogiel 1965) assumed that such primary hosts were termites. Entomological data on the phylogenetic relation between termites and cockroaches were rather contradictory, and only recently new convincing evidence as to their relations became available.

(4) Information about phylogeny of lower termites and species composition of their intestinal flagellates permits to compare the evolutionary processes of symbionts in them and in *Cryptocercus*.

(5) The study of oxymonads and hypermastigids, which belong to most highly organized protozoa, is of interest from the cytological viewpoint.

(6) A study of the evolution and of its factors in a specific group of parasitic flagellates would contribute to elucidate some general questions of the theory of evolution, as that of speciation and rate of evolution in various animal groups. A study of the evolution of oxymonads and hypermastigids, of which those inhabiting roaches reproduce both sexually and asexually while those living in termites, only asexually, would elucidate the most debatable question about the significance of sexuality for the progressive evolution.

Practically, our tasks were as follows:

(1) To compare morphologically and systematically the flagellate faunas in two roach species — *Cryptocercus relictus* and *Cr. punctulatus*.

(2) To compare stages of the sexual process in flagellates from the Far-Eastern roach with similar stages from the North-American host.

(3) To elucidate the question of the primary host of flagellates of the orders *Oxymonadida* and *Hypermastigida* in the light of new evidence about the close phylogenetic relation of termites and roaches.

(4) To define a possible way of evolution of the parasite fauna of *Cryptocercus relictus* and its determining factors.

(5) To compare the evolution of flagellates in two host groups using as an example the genus *Trichonympha* whose species occur in both termites and roaches.

Some preliminary results of this study have been published elsewhere (Bobyleva 1967, 1969, 1973 a, b).

## II. Material and Methods

Far-Eastern wood-feeding roaches were collected in the Suputinsky forest reserve (Primorye Territory, USSR). In the Ussuri taiga, they form colonies dwelling in trunks of fallen and partially rotten trees. The roaches are active at night while in daytime they hide in the passages which they gnaw out in the wood. Using a strong knife, we splitted the rotten trunk and caught the insects startled by daylight. The roaches belonging to different colonies were kept in separate terrariums-All the roaches we had caught were indentified as *Cryptocercus relictus* Bei-Bienko and our identi. fication was checked and approved by the author who first described the species, the late Professor G. I. Bei-Bienko.

The morphology of flagellates was studied both in fixed material (total preparations and sections. and *in vivo*. Smears of the roach hindgut content were fixed according to Nissenbaum, Schaudinn, and Bouin, and stained with Heidenhain's hematoxylin, Feulgen's reagent, methyl-green - pyronin-mixture, or impregnated, with protargol according to Dragesco (1962) and Uhlig (1968). Intestinal faunas of 314 adult individuals and nymphs of various age were examined on total preparations. For sections, the roach hindgut content was fixed with Zenker's fluid containing formalin, Bouin's, Champy's, Flemming's, Benda's, Carnoi's fluids, or 4% formalin, and embedded in paraffin on celloidine plates according to Peterfi. The sections, 3 or 5 μm thick, were stained by the same methods as for smears.

Living flagellates were studied using a phase-contrast device. A drop containing flagellates was placed on a slide and mixed with one of the three solutions:

(1) salt solution for insect cells (Roskin and Levinson 1957),

(2) Ringer's solution for poikilotherms (Roskin and Levinson 1957),

(3) 0.6% sodium chloride solution.

Thereupon the drop was covered with a coverslip sealed with vaseline. In such a chamber the flagellates survived two to four hours and could be observed using even 90× immersion objective. Judging by the survival time, the solution for insect cells proved to be the most suitable medium for the flagellates.

Characterization of flagellate species from *Cryptocercus relictus* includes various measurements of the body and of some organelles as well as meristic characters. Only individuals which had well preserved their body shape after fixation and staining were chosen for measurement. Usually

the same standard measurements as Cleveland used to describe the intestinal flagellates of *Cryptocercus punctulatus* were made (Cleveland et al. 1934). The results of measurements were treated biometrically. Tables accompanying the description of each species include only variation indices of the given characters: the limits (min and max) and the arithmetical mean with its standard error. The characteristic contains also meristic characters, some of which are constant (number of chromosomes, number of atractophores) while others are more or less variable in different species (number of flagellate rows, number of parabasals and number of axostyles).

Holotypes of species of intestinal flagellates of *Cryptocercus relictus* are kept in the collection of the Laboratory of cytology of unicellular organisms at the Institute of Cytology in Leningrad.

## III. Fauna of Intestinal Flagellates of the Wood-Feeding Roach
### *Cryptocercus relictus*

### 1. Systematic composition of the fauna

Order *Hypermastigida* Grassi et Foà

Fam. *Hoplonymphidae* Light

(1) *Barbulanympha cryptocerci* Bobyleva
(2) *Urinympha cirrata* Bobyleva

Fam. *Trichonymphidae* Grassi

(3) *Trichonympha major* Bobyleva
(4) *T. ussuriensis* Bobyleva
(5) *T. lutea* Bobyleva

Fam. *Eucomonymphidae* Cleveland et al.

(6) *Eucomonympha nana* Bobyleva

Fam. *Spirotrichonymphidae* Grassi

Subfam. *Macrospironymphinae* Cleveland and Day

(7) *Leptospironympha variabilis* Bobyleva
(8) *L. popularis* Bobyleva
(9) *L. lepida* Bobyleva
(10) *Bispironympha unica* Bobyleva

Order *Oxymonadida* Grassé

Fam. *Oxymonadidae* Kirby
Subfam. *Saccinobaculinae* Kirby

(11) *Saccinobaculus gloriosus* Bobyleva
(12) *S. spatiatus* Bobyleva
(13) *S. scabiosus* Bobyleva
(14) *Notila proteus* Cleveland, ssp. *ussuriensis* Bobyleva

Subfam. *Oxymonadinae* Kirby

(15) *Oxymonas nana* Cleveland, ssp. *ussuriensis* Bobyleva

Order *Diplomonadia* Wenyon

Fam. *Hexamitidae* Kent
(16) *Hexamita cryptocerci* Cleveland et al.

## 2. Description of species

Flagellates found in *Cryptocercus relictus* and closely related species inhabiting *Cr. punctulatus* are compared using largely light microscopical structural evidence. However, taking into account modern concepts of various flagellate structures, we use some terms introduced by electron microscopists.

When several species of flagellates belonging to one genus are described, a brief morphological characteristic is also given, but not in the case when only one species of a genus is described.

### *Barbulanympha cryptocerci* Bobyleva, 1973
#### (Fig. 1, Pl. I, II)

Body acorn-shaped. Anterior body end (the rostrum) carries, immediately behind the cap, two identical flagellated areas separated by two strips of cytoplasm free from flagella (Fig. 1, A, C, Pl. I 1). The flagellar areas are semiconical (Fig. 1 B, C, Pl. I 3). Within the areas, flagella are arranged in longitudinal rows (Fig. 1 C). There are about 60 rows in each area but only median ones reach the apical region.

A parabasal-axostylar complex is closely associated with each flagellated area. Underneath the rows of flagella there are semi-conical parabasal plates from which parabasal threads with suspended parabasal bodies differentiate (Fig. 1 A, Pl. I 2, 3, II 5, 6). The threads of the parabasals extend from the plates at the level of the posterior end of the flagellated areas (Fig. 1 A). Parabasal bodies are shaped as smooth strands reaching down to the middle of the nucleus (Pl. I 2). The nucleus is surrounded by about 60 parabasals.

The axostyle apparatus consists of fine fibrils, starting at the base of the cap, which extend between the flagellated areas along the strips of cytoplasm (Fig. 1 C, Pl. I 4). Behind the flagellated areas, they join with each other forming a fibrillar ring (Fig. 1 B, C, Pl. I 3, 4) Some axostyle fibrillae leave the ring and go down into the cytoplasm of the post-rostral zone (Fig. 1 B). The structure of the axostyle apparatus revealed in *B. cryptocerci* by protargol impregnation is consistent with that in some *Barbulanympha* species from *Cryptocercus punctulatus* as revealed by electron microscopy (H o l l a n d e et V a l e n t i n 1967).
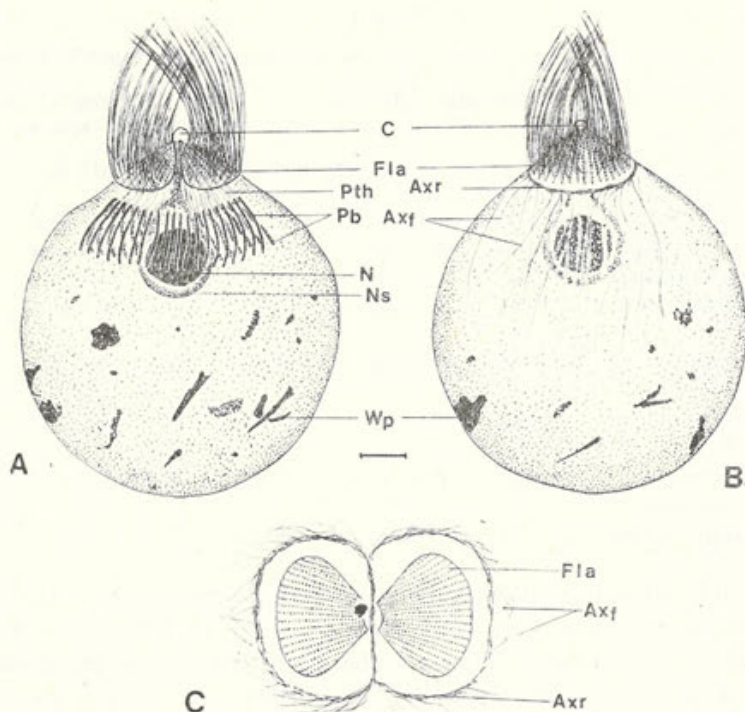
Fig. 1. *Barbulanympha cryptocerci* Bobyleva. A — Two identical flagellated areas separated by a strip of cytoplasm free from flagella are clearly visible, B — One flagellated area is seen, C — Vertical view of two flagellated areas and axostyle apparatus. Drawings (Fig. 1–19, 23–30) are made with the aid of a camera lucida and each drawing is an attempt to reproduce certain features of a particular stained or living organism rather than to put together or to reconstruct the features of an ideal organism

The nucleus lies beyond the flagellated areas inside a nuclear sleeve (Fig. 1 A; Pl. I 2). One can count 24 chromosomes during mitosis (Pl. II 7, 8). The cytoplasm of the postrostral zone contains numerous wood particles (Fig. 1).

The size of the body and of some organelles are given in Table 1.

Differential diagnosis:

Cleveland et al. (1934) described the following four species of *Barbulanympha* from *Cryptocercus punctulatus*:

|  | Average size of the body (in μm) | Number of chromosomes |
|---|---|---|
| *B. ufalula* | 285 × 204 | 50 ± 2 |
| *B. laurabuda* | 201 × 143 | 40 |
| *B. coahoma* | 114 × 76 | 32 |
| *B. estaboga* | 154 × 114 | 16 |

Table 1

Sizes (in μm) of the Body and of some Organelles of *Barbulanympha cryptocerci* and *Urinympha cirrata*

| Characters | Barbulanympha cryptocerci N = 124 | | | Urinympha cirrata N = 88 | | |
|---|---|---|---|---|---|---|
| | min | M±m | max | min | M±m | max |
| Length of body | 47.5 | 95.6±2.1 | 152.0 | 68.4 | 138.7±3.8 | 180.5 |
| Maximum body width | 38.0 | 67.5±1.5 | 116.5 | 9.5 | 16.0±0.4 | 20.9 |
| Length of flagellated area | 5.7 | 10.3±0.1 | 15.2 | 3.8 | 4.0±0.2 | 5.7 |
| Distance from anterior end to nucleus | 9.5 | 17.3±0.2 | 19.0 | 5.7 | 10.3±0.1 | 17.1 |
| Transversal diameter of nucleus | 9.5 | 15.7±0.5 | 21.9 | 5.7 | 9.1±0.6 | 15.2 |
| Length of parabasal body | 13.3 | 15.6±0.3 | 22.8 | 7.6 | 10.5±0.3 | 17.1 |
| Length of flagellum | 28.5 | 45.6±0.3 | 57.0 | 17.1 | 25.2±0.6 | 30.4 |
| Number of parabasals | | 60 | | 12 | 12 | 12 |
| Number of chromosomes | 24 | 24 | 24 | 12 | 12 | 12 |

As shown by subsequent investigation of sexual processes in these flagellates, the figures given for the two former species referred to diploid zygotes, while *B. coahoma* and *B. estaboga* proved to be different stages of the life cycle of the same species: "*B. estaboga*" was an asexual cell while "*B. coahoma*" was a zygote which not yet underwent meiosis. Revising earlier materials, Cleveland (1953) identified his *Barbulanympha* species by their respective haploid numbers of chromosomes:

B. *ufalula*      — 26 chromosomes
B. *laurabuda* — 20        ,,
B. *coahoma*   — 16        ,,
B. *wenyoni*   — 12        ,,

In this work Cleveland gives, however, no sizes for the body and its organelles, unlike as for the previously described zygotes of *B. ufalula* and of *B. laurabuda*. No measurements are also quoted for the new species, *B. wenyoni*, discovered only in roaches from Pacific Coast regions (California).

The above described species, *B. cryptocerci* from *Cryptocercus relictus*, is smaller than the zygotes of *B. ufalula*, *B. laurabuda* and *B. coahoma*. Its average body size is 96 μm×68 μm. The respective figures for *B. cryptocerci* and *B. wenyoni* are unfortunately lacking. We consider the chromosome number of 24, in *B. cryptocerci*, as haploid, but it is not impossible that we make the same mistake as Cleveland, and that the respective cells would turn out to be zygotes.

*Urinympha cirrata* Bobyleva, 1973
(Fig. 2, Pl. III)

Body long and narrow, with maximum width at the nucleus level (Fig. 2, Pl. III 9). Besides flagellates with elongate and narrow bodies, the roach intestine con-

tains also pyriform specimens (Pl. III 10). The flagellates assume such shape before division or at the entry into the sexual process.

At the anterior end of the body, behind the cap, identical triangular flagellated areas lie opposite to each other (Fig. 2, Pl. III 9). They are separated by strips of cytoplasm free from flagella (Fig. 2).
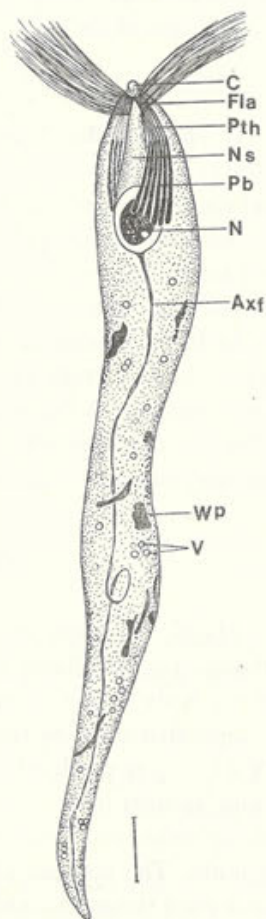
Fig. 2. *Urinympha cirrata* Bobyleva. Lateral view of the entire organism. Two symmetrical flagellated areas separated by a strip of cytoplasm free from flagella are clearly visible

Under each flagellated area parabasal threads with parabasal bodies differentiate from the parabasal plates. The parabasals are arranged in two antimeric groups around the nucleus. Each group contains six parabasal bodies (Fig. 2, Pl. III 11).

The axostyle apparatus is represented by a bundle of fine tightly packed fibrils which runs down to the posterior end of the body (Fig. 2, Pl. III 11). A similar bundle of fibrils ("endoplasmic thread") was found in *Hoplonympha natator* (Light 1926). Both light and electron microscopic evidence (Cleveland et al. 1934, Hollande and Carruette-Valentin 1971) show however, that in *Urinympha talea* from *Cryp-*

Fig. 3. *Trichonympha major* Bobyleva. Lateral view. Optical longitudinal section of anterior portion of the cell

and the rest of the body is maintained solely due to the rostral tube, a structure in the centre of the rostrum. Our observations made on three species of *Trichonympha* from *Cryptocercus relictus* show, however, that:

(1) The cell pellicle is not interrupted in the circular fissure (Fig. 3) (vital observations using a phase contrast device); this is in good agreement with Cleveland's data (1960) and the results of electron microscopy (Grimstone and Gibbons 1966).

(2) The flagellar grooves bifurcate at the level of the circular fissure and are not interrupted between the rostrum and the postrostrum (Pl. V 19).

(3) In the internal layer of ectoplasm there is no real fissure, but only an interruption between rostral and postrostral kinetosomes, due to which an optical effect of a circular fissure is attained (Fig. 3). This also agrees with electron microscopic evidence (Hollande et Carruette-Valentin 1971). It is not unlikely that the interruption between the kinetosomes of rostral and postrostral flagella provides flexibility of the anterior end of the body when a protozoan moves through the dense contents of the roach intestine.

In the postrostrum of trichonymphs from termites, right behind the circula-fissure, Kirby (1932 b) found a denser (as compared to next regions) portion of cytor

plasm. He suggested that this was a thick ectoplasmic strand connecting the rostral tube and the postrostral part of the body. This interpretation is closely associated with his idea that the ectoplasm between the rostral and postrostral portions of the body is interrupted by a circular fissure. Such a dense region at the beginning of the postrostrum exists also in trichonymphs from the Far-Eastern roach (Fig. 3, Pl. V 15). However, we found no ectoplasmic strand in this region. We are inclined to think that this part of the postrostral ectoplasm seems denser because it contains twice as many rows of flagella than that of the rostrum and fibrils of the nuclear sleeve (Fig. 3, Pl. IV 13, V 15). The body diameter at the levels of the rostrum and of dense region of the postrostrum being approximately the same, this is likely to be an optical effect due to closer arrangement of flagella rows and fibrils of nuclear sleeve.

The parabasal apparatus consists of parabasal threads and parabasal bodies suspended on them. The shape, number and position of the parabasals are significant taxonomic characters of species of the genus.

The nucleus of *Trichonympha* belongs to the "chromosomal" type, characterized by chromosome spiralization persisting throughout the interphase. The position of the nucleus in respect to the flagellated area and parabasals varies in different species. In trichonymphs from *Cryptocercus* the nucleus is enclosed in a special nuclear sleeve. Cleveland (1949) describes this as a firm structure composed of two symmetric semiconic halves. The anterior end of the nuclear sleeve commences under the outer cap while the posterior end is closely adjacent to the nuclear membrane. The surface of the sleeve is strengthened by numerous longitudinal ridges. The nuclear sleeve of three species of *Trichonympha* from the Far-Eastern roach differs in its structure from that of trichonymphs from the North-American roach. This structure is flexible and consists of thin fibrils that come off the base of the rostral tube and come down the dense region of the postrostrum. They are twisted in endoplasm, then are again untwisted and become longitudinal and enclose the nucleus (Fig. 3, Pl. IV 13, 14). Our three species of *Trichonympha* from *Cryptocercus relictus*, as well as trichonymphs from *Cr. punctulatus*, possess 24 chromosomes.

The cytoplasm of the non-flagellated area contains mainly numerous wood particles, but in some species, however, there occur also various spherical inclusions.

### *Trichonympha major* Bobyleva, 1973
(Fig. 3, 4, Pl. IV, V)

Comparatively large trichonymphs with an elongate, spindle-shaped body. The flagellated area occupies a half of the body. There are 42 rows of flagella on the rostrum (Pl. V 16). The rostral tube is conical (Fig. 3, 4, Pl. IV 13, V 15).

Parabasal bodies are shaped as left-hand coiled strands making 3–5 turns (Pl. IV 13). They are attached to parabasal threads at the level of the nuclear sleeve and
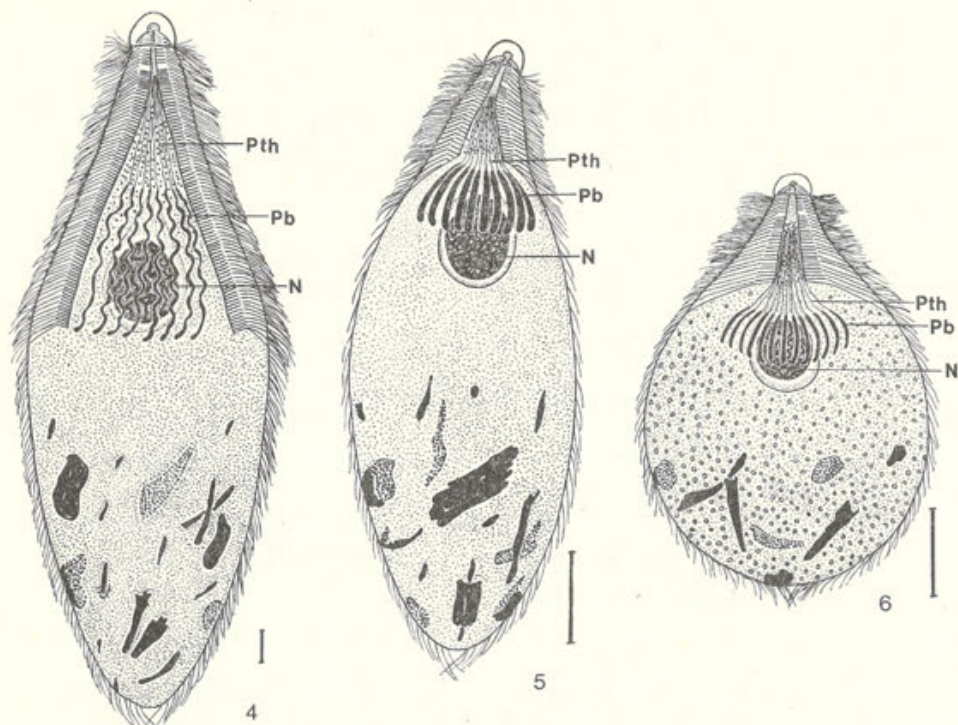
Fig. 4–6. 4 — *Trichonympha major* Bobyleva, 5 — *Trichonympha ussuriensis Bobyleva*, 6 — *Trichonympha lutea* Bobyleva. Lateral views of entire organisms

their rear end reaches the level where the flagellated area terminates (Fig. 4, Pl. IV 12). About 60 parabasals surround the nucleus.

The nucleus, encased in the sleeve, lies within the flagellated body part (Fig. 4, Pl. IV 12).

The endoplasm of the postrostral portion contains numerous large granules that become smaller as they approach the rostral tube.

The sizes of the body and of some organelles are given in Table 2.

Differential diagnosis:

*T. major* is closely related to *T. acuta* and *T. okolona*, trichonymphs from the intestine of the North-American roach. *T. major* is larger ($167 \times 48$ μm) than *T. acuta* ($138 \times 56$ μm) and *T. okolona* ($107 \times 36$ μm) and has more rows of flagella in the rostrum (44) than the species from *Cryptocercus punctulatus* (24 in *T. acuta*, 22 in *T. okolona*).

In all the three species the nucleus lies within the flagellated body part. However, in *T. acuta* and *T. okolona* it is localized in the midzone whereas in *T. major* it is situated closer to the end of the flagellated area, so that the rear end of the flagellated area, of the nucleus and of the parabasals is approximately at the same level.

Table 2

Sizes (in μm) of the Body and of some Organelles of Three Species of *Trichonympha* from *Cryptocercus relictus*

| Characters | T. major N = 88 | | | T. ussuriensis N = 80 | | | T. lutea N = 68 | | |
|---|---|---|---|---|---|---|---|---|---|
| | min | M±m | max | min | M±m | max | min | M±m | max |
| Length of body | 138.2 | 166.6±1.9 | 205.2 | 41.8 | 67.5±1.0 | 83.6 | 34.2 | 44.5±3.1 | 58.9 |
| Maximum width of body | 29.5 | 47.9±1.1 | 80.8 | 11.4 | 24.8±0.6 | 38.0 | 24.7 | 33.4±2.3 | 38.0 |
| Length of rostrum | 9.5 | 11.5±0.1 | 13.3 | 3.8 | 4.9±0.1 | 7.6 | 3.8 | 4.4±0.2 | 5.7 |
| Width of rostrum at base | 9.5 | 12.9±0.1 | 17.1 | 3.8 | 7.9±0.2 | 11.4 | 3.8 | 7.2±0.4 | 9.5 |
| Length of flagellated area | 45.6 | 67.5±1.0 | 95.0 | 7.6 | 13.3±0.2 | 19.0 | 7.6 | 11.4±1.9 | 17.1 |
| Distance from anterior end to nucleus | 39.9 | 50.5±C.8 | 66.5 | 11.4 | 15.3±0.3 | 22.8 | 9.5 | 11.4±0.6 | 13.3 |
| Transversal diameter of nucleus | 14.7 | 17.6±1.2 | 22.8 | 7.6 | 9.5±1.4 | 13.3 | 5.7 | 8.2±0.3 | 9.5 |
| Length of parabasal body | 19.0 | 28.5±2.6 | 38.0 | 7.6 | 8.0±0.6 | 9.5 | 5.7 | 7.2±0.8 | 9.5 |
| Number of parabasals | | 60 | | | 20 | | 28 | 30 | 31 |
| Number of flagellar rows on rostrum | 42 | 42 | 42 | 28 | 28 | 28 | 23 | 23 | 23 |
| Number of chromosomes | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 |

The parabasal apparatus of *T. major* is closer to that of *T. okolona* than to that of *T. acuta*. The arrangement and the number of parabasals are the same in both former species but the shape of parabasal bodies differs. In *T. okolona* the strands are slightly wavy and are 19 μm long while in *T. major*, they are coiled and 29 μm long.

### *Trichonympha ussuriensis* Bobyleva, 1973
### (Fig. 5)

Small flagellates resembling *T. major* by the body shape. The flagellated area occupies only one-third of the body. The rostrum carries 28 rows of flagella. The rostral tube is cylindrical.

Parabasal bodies are represented by smooth strands that come off parabasal threads at the level where the flagellated area ends, and extend nearly to the middle of the nucleus. Parabasals (20 in number) hang in an umbrella-like manner over the nucleus. The nucleus lies beyond the flagellated area.

The sizes of body and of some organelles are given in Table 2.

Differential diagnosis:

By its appearance and body size *T. ussuriensis* resembles *T. parva* from the North-American roach. The average body size of *T. ussuriensis* is $68 \times 18$ μm and that of *T. parva*, $59 \times 18$ μm. The length of the flagellated area is 13 μm in *T. ussuriensis* and 9 μm in *T. parva*. The rostrum of *T. ussuriensis* carries 28 rows and that of *T. parva* 22 rows of flagella.

The parabasal apparatus of *T. ussuriensis* differs from that of *T. parva*. In *T. ussuriensis* the parabasal bodies are thin and elongated (about 8 μm in length). They lie over the nucleus, reaching only the middle of it. In *T. parva* the parabasals are small (about 4 μm in length), sausage-like, and are grouped around the nucleus.

By localization of parabasals in relation to the nucleus, *T. ussuriensis* is close to *T. algoa* from *Cryptocercus punctulatus* as well as to *T. lighti* and *T. subquasilla* from termites. In these trichonymphs parabasal bodies hang over the nucleus but they differ in shape from the parabasals of *T. ussuriensis*.

### *Trichonympha lutea* Bobyleva, 1973
### (Fig. 6, Pl. V 17, 18)

The smallest of all known trichonymphs. The body is pear-shaped (Fig. 6). The flagellated area is restricted to the anterior third of the body. The rostrum carries 23 rows of flagella. The rostral tube is conical. The depth of the external ecto-plasmic layer increases from the front to the rear end of the rows of flagella (Fig. 6).

Parabasal bodies vary in number from 28 to 31 and are grouped around the nucleus. They have the shape of smooth thin strands with their distal ends slightly

bent towards the nucleus (Fig. 6, Pl. V 17, 18). The nucleus lies beyond the flagellated area.

The cytoplasm of the non-flagellated region is filled with numerous yellow spherules.

The sizes of the body and of some organelles are given in Table 2.

Differential diagnosis:

By its body shape *T. lutea* resembles *T. sphaerica* from termites of the genus *Zootermopsis*, but the latter are giants as compared to *T. lutea*. Among the trichonymphs from the North-American roach, *T. chula* seems to be most closely related to *T. lutea*. The average size of *T. chula* is $80 \times 34$ μm and that of *T. lutea*, $45 \times 35$ μm. In both species the flagellated area is short — 13 μm in *T. chula* and 11 μm in *T. lutea*.

The form of parabasal bodies and their localization around the nucleus are similar in the two species. The number and size of parabasals, however, differ in *T. chula* and *T. lutea*. The latter species has at average 29 parabasals, each about 8 μm in length. In *T. chula*, there are about 40 parabasal bodies, each about 14 μm in length.

The two species differ also in the number of rows of flagella. There are 23 rows of flagella on the rostrum of *T. lutea* and 15 rows on that of *T. chula*. They differ in body shape: *T. lutea* are pyriform while *T. chula* are elongated.

## *Eucomonympha nana* Bobyleva, 1973
### (Fig. 7, 8, Pl. VI 20, 21, VII 24–26)

The whole body is covered with flagella. Rows of flagella start from the top of the rostrum, right under the cap, and disperse radially; they are meridional in the rostrum and helical in the postrostrum (Fig. 7, Pl. VI 21). Their number varies from 39 to 48. In the centre of the rostrum there is a jug-like rostral tube (Fig. 7, Pl. VI 21). A small non-flagellated ring of the body surface exists between the rostrum and the postrostrum; this is likely to be an analog of the circular fissure of *Trichonympha* (Fig. 7, Pl. VI 20, 21). Rostral flagella are longer than postrostral ones. The ectoplasm of *Eucomonympha*, like the ectoplasm of the flagellated area in *Trichonympha*, is subdivided in two layers: an outer lobulated one and an inner kinetosome-bearing one.

The parabasal apparatus consists of fine parabasal filaments lining each row of flagella in the postrostrum, and of small rounded dictyosomes arranged chainwise along flagellar spirals (Fig. 8, Pl. VII 24–26). The parabasal apparatus found in *E. nana* after protargol impregnation corresponds to that in *E. imla* (from *Cryptocercus punctulatus*) revealed by electron microscopy (Hollande et Carruette-Valentin 1971).

The axostyle apparatus consists of thin fibrils extending from the base of the rostrum into the depth of the endoplasm. The axostyles frequently interlace behind the nucleus making up a common bundle (Pl. VI 20).
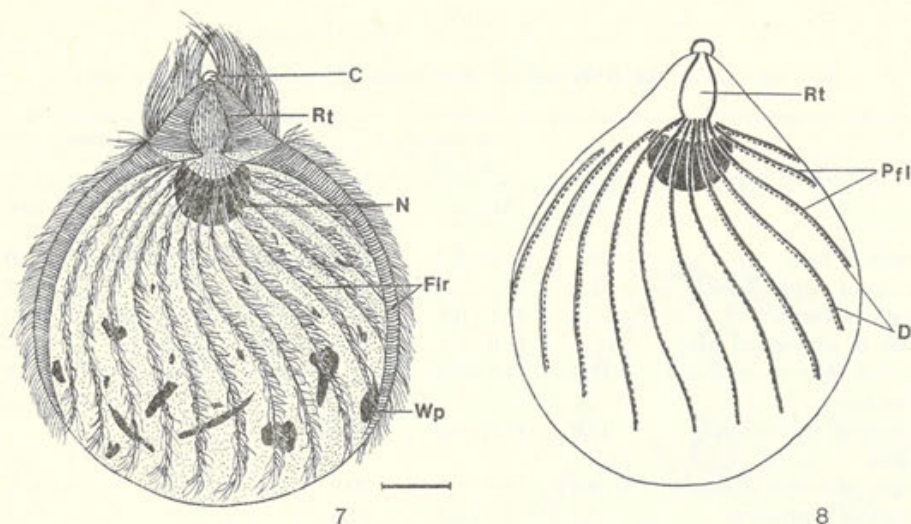
Fig. 7, 8. *Eucomonympha nana* Bobyleva. 7 — Lateral view of the entire organism, 8 — Diagrammatical picture of the arrangement of parabasal filaments and dictyosomes in *Eucomonympha nana*

A cup-shaped nucleus enclosed in a nuclear sleeve lies at the base of the rostrum (Fig. 7, Pl. VI 20, 21). Mitotic nuclei show $22 \pm 2$ chromosomes.

The endoplasm is packed with numerous particles of wood.

Peculiarly, the flagellates of the genus *Eucomonympha* exist in the roach intestine as two concomitant morphological forms of which one is free moving in the gut lumen and the other attached to the intestinal wall. The free forms have a rounded body and are stuffed with striking amounts of food. The attached forms are elongate and always free of wood particles. All intermediate stages between these two extreme morphological forms can be found. The physiological significance of such dimorphism and the mechanism of attachment of flagellates to the intestinal wall are not known. The coexistence of two morphological forms was first reported for *E. imla* from *Cryptocercus punctulatus* (Cleveland et al. 1934). We found it also in *E. nana* (Pl. VI 20, 21).

The sizes of the body and of some organelles are presented in Table 3.

Differential diagnosis:

*E. nana* (from the Far Eastern roach) differs from *E. imla* (from the North-American host) mainly by its smaller size of the body and of all organelles as well as by the number of chromosomes. The largest specimens of *E. nana* scarcely reach the minimum size of *E. imla*. The average size of *E. nana* is $72 \times 53$ μm, and that of *E. imla* — $138 \times 123$ μm. The average length of the rostrum in *E. nana* is 14 μm while in *E. imla*, 17 μm. At average there are 44 rows of flagella in *E. nana* and 84 in *E. imla*.

Table 3

Sizes (in μm) of the Body and of some Organelles of *Eucomonympha nana*

| Characters | Free forms N = 92 | | | Attached forms N = 55 | | |
|---|---|---|---|---|---|---|
| | min | M±m | max | min | M±m | max |
| Length of body | 49.4 | 72.2±1.3 | 100.7 | 57.0 | 86.7±2.0 | 133.0 |
| Maximum width of body | 32.3 | 53.2±2.3 | 89.3 | 32.3 | 40.3±1.6 | 47.5 |
| Length of rostrum | 13.3 | 14.1±0.1 | 15.2 | 15.2 | 16.7±0.6 | 19.0 |
| Width of rostrum at base | 7.6 | 11.0±0.1 | 15.2 | 19.0 | 21.9±1.0 | 24.7 |
| Distance from anterior end to nucleus | 11.4 | 14.4±0.3 | 17.1 | 16.0 | 19.9±0.5 | 21.1 |
| Transversal diameter of nucleus | 13.9 | 19.0±0.6 | 20.9 | | | |
| Length of rostral flagellum | 9.5 | 13.9±1.1 | 20.9 | | | |
| Length of postrostral flagellum | 5.7 | 9.5±0.8 | 11.4 | | | |
| Number of flagellar rows | 39 | 44 | 48 | | | |
| Number of chromosomes | | 22±2 | | | | |

A similar difference in the size of the body and of the organelles is observed when attached forms of both species are compared. During mitosis in *E. nana* approximately 22±2 chromosomes are seen, whereas in *E. imla* there are about 50 chromosomes.

### The genus *Leptospironympha* Cleveland et al., 1934

Flagellates with flagella arranged in two bands arising from the same point at the anterior end of the body (Pl. VII–IX). In the rostrum the flagellar bands are almost meridional while in the postrostrum they are helical and make 1.5–10 clockwise turns. In the rostrum, flagella are arranged in rows parallel to the wall of the rostral tube. In the helical portions of the bands, the flagella are inserted in short rows arranged across the bands and parallel to the long axis of the body. The number of flagella in a row is one of the taxonomic species characters within the genus. Rostral flagella are a little longer than postrostral ones.

The parabasal apparatus consists of dictyosomes following the course of the flagellar spirals.

The nucleus is enclosed into a nuclear sleeve. The shape of the nucleus and its position in the postrostrum are variable.

The ectoplasm as a rule contains bacteria. The endoplasm is filled up with wood particles.

*Leptospironympha variabilis* Bobyleva, 1973
(Fig. 9, 10, Pl. VI 22, 23, VIII 31)

Comparatively large flagellates. The body widens backwards attaining the maximum width in its posterior third. The cap lies in a groove of the cytoplasm (Fig. 9, Pl. VI 22, 23). There are 26 rows of flagella in the rostrum (Pl. VIII 31). In the post-
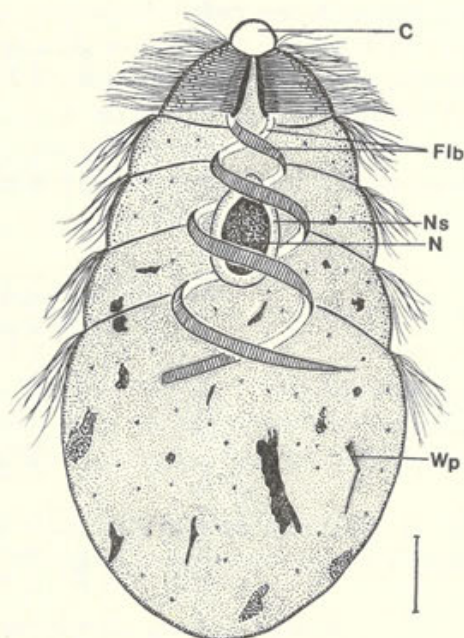


Fig. 9. *Leptospironympha variabilis* Bobyleva.
Lateral view of the entire organism

rostrum, flagellar bands make 2.5–3 turns; they reach the posterior end of the body. Each row of flagella crossing the spiraling portion of a band contains 12 flagella.

The parabasal apparatus is represented by dictyosomes lying on both sides of each flagellar band (Fig. 10).



Fig. 10, 11. Diagrammatical pictures showing the arrangement of dictyosomes along flagellar bands in *Leptospironympha variabilis* (10) and — in *Leptospironympha popularis* (11)

10

11

Table 4

Sizes (in µm) of the Body and of some Organelles of Three Species of *Leptospironympha* from *Cryptocercus relictus*

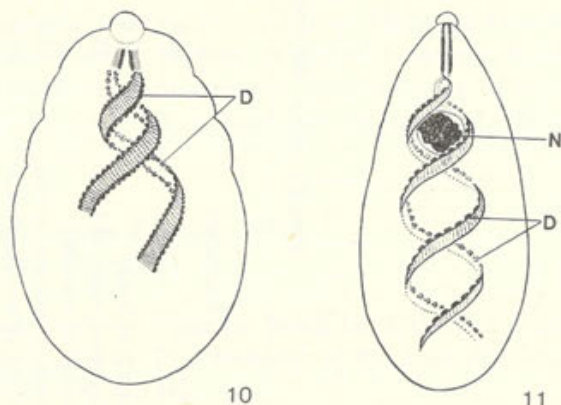| Characters | *L. variabilis* N = 133 | | | *L. popularis* N = 115 | | | *L. lepida* N = 78 | | |
|---|---|---|---|---|---|---|---|---|---|
| | min | M±m | max | min | M±m | max | min | M±m | max |
| Length of body | 38.0 | 82.3±1.3 | 125.4 | 34.2 | 53.8±0.8 | 76.0 | 20.3 | 37.2±1.1 | 56.5 |
| Maximum width of body | 28.5 | 45.8±0.6 | 72.2 | 13.3 | 25.1±0.4 | 32.3 | 7.6 | 11.8±0.9 | 17.1 |
| Length of rostrum | 3.8 | 7.6±0.2 | 11.4 | 5.7 | 7.8±0.1 | 3.5 | 3.8 | 5.3±0.2 | 7.6 |
| Width of rostrum at base | 15.2 | 24.7±1.7 | 32.3 | 7.6 | 11.4±0.2 | 17.1 | 5.7 | 7.2±0.6 | 13.3 |
| Length of non-flagellated region of body | 19.0 | 38.5±0.6 | 58.3 | | | | | | |
| Distance from anterior end to nucleus | 9.5 | 16.6±0.2 | 26.6 | 9.5 | 11.3±0.1 | 13.3 | 9.5 | 11.7±0.1 | 13.3 |
| Transversal diameter of nucleus | 5.7 | 9.8±0.2 | 13.3 | 9.5 | 12.1±0.2 | 17.1 | 3.8 | 3.8±0.6 | 5.7 |
| Number of turns of each flagellar band in postrostrum | 2.5 | 3 | 3 | 4 | 5 | 5 | 6 | 7 | 8 |

A drop-like nucleus is situated at the level of the second turn of the flagellar bands (Fig. 9, Pl. VI 22, 23).

The sizes of the body and of some organelles are given in Table 4.

Differential diagnosis:

By its appearance, *L. variabilis* resembles *L. wachula* from the North-American roach. *L. variabilis*, however, differs from *L. wachula* by a larger size of its body and of its organelles. Thus the average specimens of *L. variabilis* (82 μm) are larger than the largest specimens of *L. wachula* (60 μm). These species differ also in the position of their nuclei: in *L. variabilis*, the nucleus lies at the level of the second turn of the flagellar bands, while in *L. wachula*, at the base of the rostrum.

The dictyosomes of *L. variabilis* are arranged on both sides of the flagellar bands whereas in *L. wachula* they form a single row along the flagellar bands.

### *Leptospironympha popularis* Bobyleva, 1973
#### (Fig. 11, Pl. VIII 27–29)

The body gradually widens backwards. There are 18 rows of flagella in the ros trum. In the postrostrum, the flagellar bands reach the end of the body spiraling in 4–5 turns (Pl. VIII 27, 28). Each row of flagella crossing a flagellar band consists of 6 flagella.

Small oval dictyosomes form one row along each flagellar band (Fig. 11, Pl. VIII 29). An irregular shaped nucleus lies in the region of the second coil of the flagellar bands (Fig. 11).

The sizes of the body and of some organelles are given in Table 4.

Differential diagnosis:

By its appearance *L. popularis* is close to *L. eupora*, the type species of the genus The differences between these species are as follows:

*L. popularis* is larger than *L. eupora*. The average specimens of *L. popularis* are about 54 μm in length and those of *L. eupora*, about 34 μm. There are 18 rows of flagella on the rostrum of *L. popularis* and 14 rows on that of *L. eupora*. In the postrostrum, the flagellar bands make approximately the same number of turns in both species (4–5 in *L. popularis* and 3.5–5 in *L. eupora*), but the coils of the spiral are tighter in *L. eupora* than in *L. popularis*.

### *Leptospironympha lepida* Bobyleva, 1973
#### (Pl. IX 32–36)

Small flagellates with a long narrow body. Flagellar bands reach the end of the body, making 6–8 turns in the postrostrum. The diameter of the coils decreases towards the posterior body end (Pl. IX 33, 36).

Parabasal apparatus is same as in *L. popularis* (Pl. IX 35).

An elongate nucleus with two constrictions lies at the level of the second-fourth turns of the flagellar bands (Pl. IX 34).

The sizes of the body and of some organelles are presented in Table 4.

Differential diagnosis:

By its body shape *L. lepida* resembles *L. rudis* from the North-American roach. *L. lepida* is, however, a smaller species as compared to *L. rudis*. The average length of the body in *L. lepida* is 37 μm and in *L. rudis* — 50 μm. The flagellar bands of *L. lepida* make a smaller number of turns in the postrostrum (6–8 as compared to 8–10 in *L. rudis*). The species under comparison also differ in the shape of the nucleus. In *L. lepida* the nucleus is elongated with two constrictions and in *L. rudis* it is spherical.

<div align="center">

*Bispironympha unica* Bobyleva, 1969
(Fig  12–14, Pl. X 37–39)

</div>

The body shape is elongated with a rounded posterior end. The body evenly widens backwards attaining the maximum width in its last third (Fig. 12, Pl. X 37). The anterior end of the body is covered with the cap. Two identical flagellar bands arise from under the cap. In the rostrum they are arranged meridionally, opposite to each other, and slightly curved in the longitudinal plane. In the postrostrum the flagellar bands take a spiral course and make only one turn; the direction of their coiling is clockwise (Fig. 12, Pl. X 37–39). Figure 13 shows several aspects of the flagellar bands appearing when the flagellate is examined laterally at various angles. In the rostrum, flagella are arranged in parallel rows running along the band and packed so densely that they seem to fuse into a continuous stripe. We failed to count the number of rows of flagella in the rostrum.

In the spiralling portion of the bands, the flagella are inserted in pairs oriented across the band. Owing to the fact that the bands are only slightly helical, each pair of flagella makes almost a straight angle with the longitudinal axis of the body (Fig. 14, Pl. X 37–39). In the ectoplasm, both flagella of a pair stick together making a bundle (=fasciculus). However, at the surface of the body they become separate again (Fig. 14).

In the spiralling portion of the bands, flagella exist only behind the level of the middle or even the end of the nucleus (Fig. 12, Pl. X 37, 38). A short non-flagellated area thus exists between the rostrum and the flagellated part of the bands.

Numerous parabasal bodies are arranged in pairs along the spiral part of each flagellar band (Fig. 12, 14, Pl. X 37–39), 15 to 21 pairs of dictyosomes accompany each flagellar band. No axostyles have been found.

A rounded or slightly oval nucleus lies at the base of the rostrum. It is enclosed in the nuclear sleeve (Fig. 12).

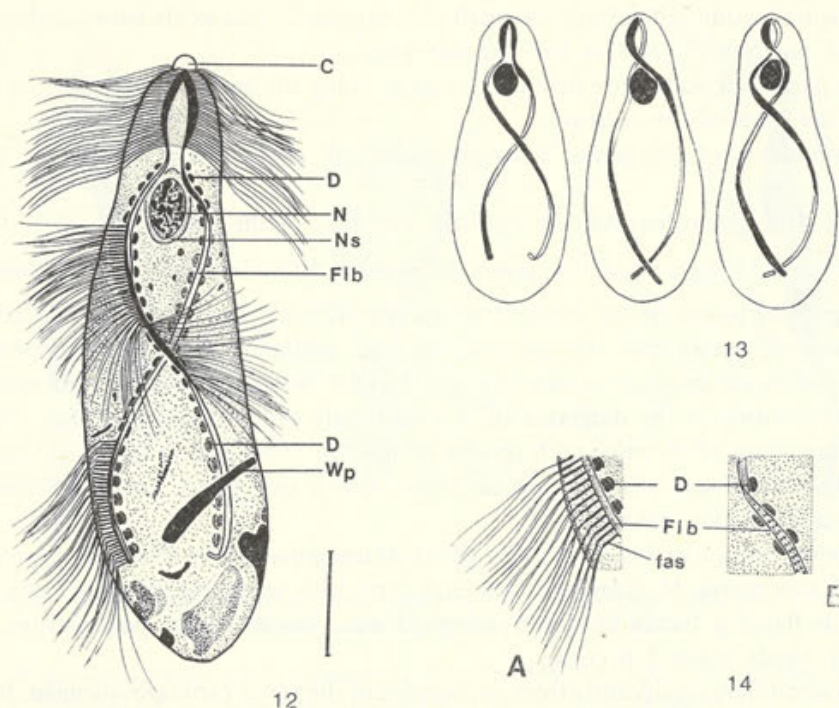The sizes of the body and of some organelles are given in Table 5.

Fig. 12–14. *Bispironympha unica* Bobyleva. 12 — Lateral view of the entire organism, 13 — Diagram of several aspects of flagellar bands when the flagellate is examined laterally at various angles, 14 — Portion of flagellar band in the postrostrum. A — Lateral view, B — Vertical view

Table 5

Sizes (in μm) of the Body and of some Organelles of *Bispironympha unica*

| Characters | min | M±m | max |
|---|---|---|---|
| Length of body | 39.9 | 51.1±0.4 | 66.5 |
| Maximum width of body | 13.3 | 16.7±0.2 | 24.7 |
| Length of rostrum | 5.7 | 7.2±0.01 | 7.6 |
| Width of rostrum at the base | 9.5 | 12.3±0.1 | 17.1 |
| Distance from anterior end to nucleus | 7.6 | 8.9±0.1 | 9.5 |
| Transversal diameter of nucleus | 5.7 | 5.9±0.1 | 7.6 |
| Length of rostral flagellum | 13.3 | 14.8±0.1 | 17.1 |
| Length of postrostral flagellum | 9.5 | 10.5±0.1 | 13.3 |

Diagnosis of the genus *Bispironympha* Bobyleva, 1969

Flagellates of the family *Spirotrichonymphidae*, subfamily *Macrospironymphinae*, with two identical flagellar bands. In the rostrum, the flagellar bands lie meridionally, opposite to each other, and are slightly curved in the longitudinal plane. In the postrostrum, the flagellar bands are coiled but make only one turn: the direc-

tion of spiralization is clockwise. A small non-flagellated area exists between the rostral and postrostral parts of the flagellar band.

The parabasal bodies are arranged in pairs along the spiral bands. The nucleus lies at the base of the rostrum.

Composition of the genus: a single species, *B. unica*, which is also the type species.

Host — *Cryptocercus relictus* from the Far East of the USSR.

Differential diagnosis of the genus *Bispironympha* and of the species *B. unica*:

Flagellates found in the hindgut of the Far-Eastern roach and designated as *Bispironympha unica* may be assigned to the subfamily *Macrospironymphinae*. This is due to the presence of two identical flagellar bands which are coiled and arranged according to the diagnosis of this subfamily (Cleveland and Day 1958). The comparison of *B. unica* with species of the five other genera of the subfamily *Macrospironymphinae* allowed the conclusion that a new genus should be created to accomodate this species.

*B. unica* cannot be assigned to the genus *Macrospironympha*. The only representative of this genus, *M. xylopletha*, is a large form with an almost spheroid body and two wide flagellar bands lined with identical inner bands. In the postrostrum, the flagellar bands make 5–6 coils.

*B. unica* differs significantly from any species of the genus *Leptospironympha*. In all species of this genus, flagellar bands in the postrostrum make more than two coils.

The flagellates of the remaining three genera — *Spirotrichosoma*, *Apospironympha* and *Colospironympha* — have flagellar bands of two types — primary and secondary, the latter resulting from the former. This character argues against their proximity to the described species.

### The genus *Saccinobaculus* Cleveland et al., 1934

Flagellates bearing four flagella on the anterior end of the body. A ribbon-like axostyle passes along the whole body but never protrudes from the cell. The posterior end of the axostyle is surrounded by the axostyle sheath, the shape of which varies in different species of this genus.

The shape of the body is extremely variable, ranging from spherical to elongated. This variation is due to the undulating movements of the axostyle which is mainly responsible for the movement of the protozoan.

The nucleus lies near the anterior end of the cell. The nucleus, the axostyle and the flagella are firmly interconnected.

### *Saccinobaculus gloriosus* Bobyleva, 1973
### (Fig. 15, Pl. XI 44)

Small flagellates. The axostyle sheath widens in the mid-portion taking a bulb-like shape. There is a ring on the bulb-shaped area of the sheath. The proximal and distal
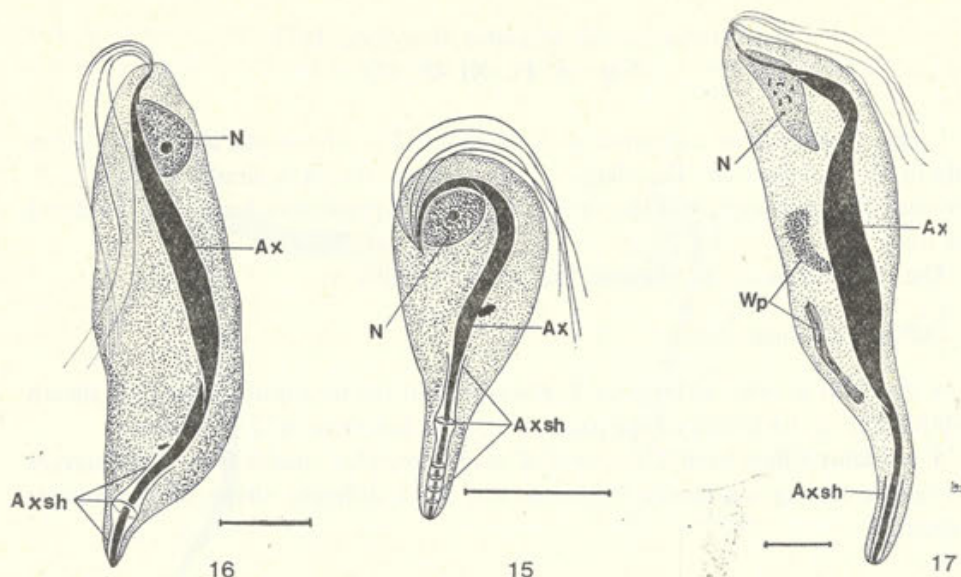
Fig. 15–17. 15 — *Saccinobaculus gloriosus* Bobyleva, 16 — *S. spatiatus* Bobyleva, 17 — *S. sca-biosus* Bobyleva. Lateral views of entire organisms

ends of the sheath are closely adjacent to the axostyle. The distal end of the axostyle sheath often has four rings resembling those of the bulb-shaped area.

The results of measurements of *S. gloriosus* are given in Table 6.

Table 6

Sizes (in μm) of the Body and of some Organelles of Three Species of *Saccinobaculus* from *Cryptocercus relictus*[1]

| Characters | S. gloriosus N = 10 | | | S. spatiatus N = 10 | | | S. scabiosus N = 10 | | |
|---|---|---|---|---|---|---|---|---|---|
| | min | M | max | min | M | max | min | M | max |
| Length of body | 22.8 | 29.8 | 38.4 | 41.8 | 53.9 | 78.6 | 77.9 | 89.5 | 98.8 |
| Width of body | 7.6 | 8.7 | 10.4 | 13.3 | 14.8 | 23.6 | 13.3 | 18.6 | 22.8 |
| Length of axostyle sheath | 13.3 | 14.0 | 15.2 | 13.3 | 16.0 | 17.1 | 11.4 | 13.9 | 15.2 |
| Width of axostyle | | 1-2 | | | 4 | | | 8 | |
| Transversal diameter of nucleus | 3.8 | 4.1 | 5.7 | 5.7 | 8.0 | 11.4 | 5.7 | 6.0 | 7.6 |

[1] For measurement, specimens were selected that had an elongate shape of the body, with the axostyle only slightly bent.

Differential diagnosis:

*S. gloriosus* can be easily differentiated from all other species of the genus due to its small size. The average size of *S. gloriosus* is 30 μm. The other species are two or three times larger.

### *Saccinobaculus spatiatus* Bobyleva, 1973
(Fig. 16, Pl. XI 41, 42)

Larger flagellates as compared to *S. gloriosus*. The ribbon-like axostyle is 4 μm wide in the mid-portion. The shape of the axostyle sheath is similar ot that in *S. gloriosus*. The mid-portion of the sheath bears a well-pronounced ring, but the distal end does not.

The dimensions of *S. spatiatus* are given in Table 6.

Differential diagnosis:

*S. spatiatus* is twice as large as *S. gloriosus*, but the length of its axostyle sheath with respect to the length of the axostyle itself is less than in *S. gloriosus*.

*S. spatiatus* differs from *S. lata* and *S. ambloaxostylus*, species from *Cryptocercus punctulatus* having essentially the same size, by a different shape of its axostyle apparatus.

### *Saccinobaculus scabiosus* Bobyleva, 1973
(Fig. 17 Pl. XI 43)

Large flagellates. A wide ribbon-like axostyle is about 8 μm large in the mid-portion. The posterior end of the axostyle is encased in a sheath resembling in shape the sheath of a sword. The wall of the sheath is closely adjacent to the axostyle and shows an external cross-striation. As a rule. the nucleus is elongate and contains few nucleoli.

The sizes of the body and of some organelles are given in Table 6.

Differential diagnosis:

*S. scabiosus* can be easily differentiated from other species of the genus due to its large size and the shape of axostyle sheath.

### *Notila proteus* Cleveland, ssp. *ussuriensis* Bobyleva, 1973
(Fig. 18)

Large flagellates with a wide ribbon-like axostyle. The posterior end of the axostyle has no sheath. The cytoplasm contains numerous granules. Each of the four flagella is fastened in the depth of the cytoplasm to peculiar cytoplasmic bodies.

Differential diagnosis:

This flagellate resembles by its appearance *N. proteus* as described by Cleveland (1950 b) but differs from it by the absence of granules in the axostyle. According

to Cleveland, axostyle granules are characteristic to *N. proteus*. This enables us to regard flagellates found in *Cryptocercus relictus* as a new subspecies of *N. proteus*.
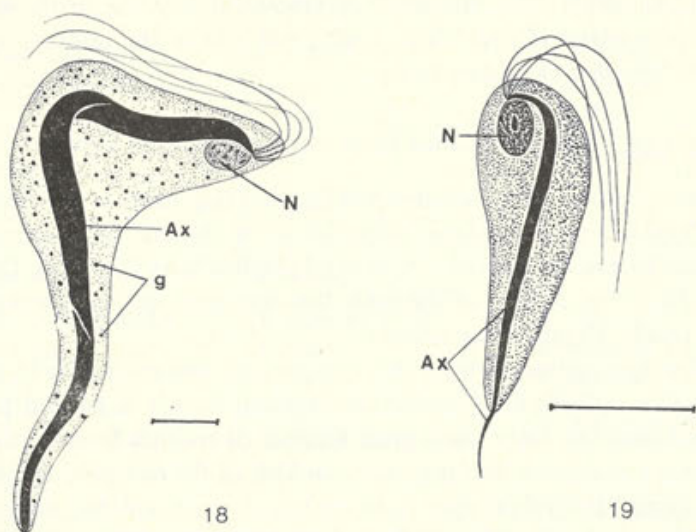


Fig. 18. *Notila proteus* ssp. ussuriensis Bobyleva. Lateral view of the entire organism
Fig. 19. *Oxymonas nana* ssp. *ussuriensis* Bobyleva. Lateral view of the entire organism

*Oxymonas nana* Cleveland, ssp. *ussuriensis* Bobyleva, 1973
(Fig. 19, Pl. XI 45)

Small flagellates. By their body size they are close to *Oxymonas nana*. Averags specimens are $21 \times 9$ μm long. Large numbers of these flagellates were found in the gut lumen. The lumen forms of the flagellate had no stalk which in attached forms is used to pierce the intestinal wall of the host. The posterior end of the ribbon-like axostyle protrudes from the flagellate's body and has no rings.

The nucleus is spherical and contains a single nucleolus. It is attached to the anterior end of the axostyle but not enclosed in a nuclear sleeve. The cytoplasm contains no inclusions.

Differential diagnosis:

Judging by Cleveland's (1950 a) illustrations the flagellates described here are rather close to *O. nana*. The flagellates from *Cryptocercus relictus* differ, however, from *O. nana* by lack of rings which surround the posterior end of the axostyle of *O. nana*.

In *Oxymonas* from *Cr. relictus*, the cytoplasm contained no light-brown granules characteristic of *Oxymonas* from *Cr. punctulatus*. Therefore we presently regard these flagellates as a subspecies of *Oxymonas nana*.

## *Hexamita cryptocerci* Cleveland et al. 1934

The intestinal fauna of *Cryptocercus relictus* was found to contain a small flagellate by all characters alike *Hexamita cryptocerci* discovered in the intestine of *Cryptocercus punctulatus* (Cleveland et al. 1934) and in the intestine of a roach of the genus *Panesthia* (Kidder 1937).

### 3. Comparison of Flagellate Faunas from two *Cryptocercus* Species

The species composition of the intestinal fauna of flagellates of the North-American roach, *Cryptocercus punctulatus*, is presented in the first and second columns of Table 7 according to the data of Cleveland (1950 a, b, 1953, 1966 a, Cleveland et al. 1934). The third column of the table lists flagellate species discovered in the Far-Eastern roach, *Cryptocercus relictus*.

Before drawing conclusions from this comparison, one must bear in mind some peculiarities of the specific composition of intestinal faunas in natural populations of roaches and termites. Only when great number of roaches from various regions of the habitat are examined, one may have an idea of the complete composition of the parasite fauna. Individual specimens within a roach colony, entire colonies, or even all colonies of one region of the habitat tested may lack some of the flagelates generally typical for the species of roach (Cleveland et al. 1934). This is due

Table 7

The Parasite Fauna of Roaches of the Genus *Cryptocercus*

| Cr. punctulatus | | Cr. relictus |
|---|---|---|
| Appalachian area | Pacific coast area | Suputinsky Reservation |
| 1 | 2 | 3 |
| *Barbulanympha laurabuda* | *B. laurabuda* | *B. cryptocerci* |
| *Barbulanympha ufalula* | *B. ufalula* | |
| *Barbulanympha estaboga* | *B. estaboga* | |
| | *B. wenyoni* | |
| | *Rhynchonympha tarda* | |
| *Urinympha talea* | *U. talea* | *U. cirrata* |
| *Idionympha perissa* | | |
| *Trichonympha acuta* | *T. acuta* | *T. major* |
| *Trichonympha okolona* | *T. okolona* | *T. ussuriensis* |
| *Trichonympha algoa* | *T. algoa* | *T. lutea* |
| *Trichonympha lata* | *T. lata* | |
| *Trichonympha chula* | *T. chula* | |
| *Trichonympha parva* | *T. parva* | |
| | *T. grandis* | |

Table 7 (continued)

| 1 | 2 | 3 |
|---|---|---|
| *Eucomonympha imla* | *E. imla* | *E. nana* |
| *Leptospironympha eupora*<br>*Leptospironympha wachula* | *L. rudis* | *L. variabilis*<br>*L. popularis*<br>*L. lepida* |
|  |  | *Bispironympha unica* |
|  | *Macrospironympha*<br>    *xylopletha* |  |
| *Prolophomonas tocopola* | *P. tocopola* |  |
| *Saccinobaculus ambloaxostylus*<br>*Saccinobaculus lata* | *S. ambloaxostylus*<br>*S. lata* | *S. gloriosus*<br>*S. scabiosus*<br>*S. spatiatus* |
| *Notila proteus* | *N. proteus* | *N. proteus ussuriensis* |
| *Paranotila lata* | *P. lata* |  |
| *Oxymonas nana* | *O. nana* | *O. nana ussuriensis* |
| *Monocercomonoides globus* | *M. globus* |  |
| *Hexamita cryptocerci* | *H. cryptocerci* | *H. cryptocerci* |

to the special mode of infection of hosts by flagellates with peculiarities of the co-lonial mode of life of the host. Young roaches become infected with flagellates from nymphs of various ages during the molting period by eating feces containing protozoa. The hatching and the molt of nymphs occur simultaneously and only in summer. The absence of one or a few flagellate species in a roach parental pair in the course of formation of a new colony will thus be transmitted to all subsequent generations in this colony. A host does not need the whole set of flagellates typical for a given species for normal life, since a few species of xylophagous flagellates (or even one species, under experimental conditions) are enough for successful splitting of cellulose.

Such deviations of the fauna composition from the typical one were repeatedly reported by Cleveland for the North-American roach. Thus the intestinal flagel-late faunas of *Cryptocercus punctulatus* from two geographically separated North-American regions — the Western (Pacific coast area) and the Eastern (Appalachian area) — differ somewhat in their generic and specific compositions (see Table 7). Cleveland observed deviations in the flagellate compositions not only in roaches from two isolated regions but also in roach colonies from different habitats of one area as well as in different colonies from one habitat. Thus *Macrospironympha xylopletha* was found only in roaches in three states of the Pacific coast area and was absent in five other states examined.

Similar differences in the composition of flagellate faunas in termites from different colonies and different habitats were reported by Kirby (1947).

We also observed cases of irregular distribution of flagellates among *Cryptocercus relictus* on the territory of the Suputinsky Reservation. *Bispironympha unica*, for instance, was discovered only in roaches from two colonies on the Ginseng Hill. *Saccinobaculus scabiosus* and *Notila proteus ussuriensis* were found only in roach colonies on the Grabovaya Hill. Individual differences in the composition of flagellate faunas among roaches of a single colony usually consisted in absence of *Bispironympha unica, Leptospironympha lepida, Saccinobaculus scabiosus, S. spatiatus, Notila proteus ussuriensis*. The individuals of one colony also showed different intensities of infection with some flagellate species. In certain individuals, *Trichonympha major* were predominant, whereas in other roaches of the same colony, *Trichonympha popularis* or *Barbulanympha cryptocerci* or *Eucomonympha nana* prevailed.

Due to these deviations in the composition of the intestinal fauna of roaches one must examine a large number of hosts from different areas of their habitat to determine the fauna composition typical for a given roach species in general.

Table 7 shows that *Cryptocercus punctulatus* and *Cr. relictus* bear almost the same genera but different species of flagellates. Small differences in the generic composition of flagellates from the two host species are not essential. Some genera which lack from *Cr. punctulatus* (e.g., *Bispironympha*) or from *Cr. relictus* (e.g., *Macrospironympha*) are analogs of each other and usually are systematically related. The numbers of flagellate genera in the North-American roaches from two areas of their habitat and in the Far-Eastern roaches are related as 13 : 14 : 10.

The different species composition of the flagellates in the intestine of *Cryptocercus punctulatus* and *Cr. relictus* shows that flagellates possess a host specificity. Specificity, as a varied degree of adaptation of the parasite to the host, has been elaborated in the process of historical development of the host-parasite system; specificity can be defined as the affinity of the parasite to one host species or a group of species (Dogiel 1964). Basing solely on their occurrence in roaches, the flagellates of the two *Cryptocercus* species seem to show strict specificity to a definite host species. The only exceptions are *Hexamita cryptocerci* and *Monocercomonoides globus* which have wider specificity. Besides *Cryptocercus*, they occur in other *Blattodea* (Kidder 1937). It should, however, be born in mind that mutual transfaunation experiments between *Cryptocercus punctulatus* and *Cr. relictus* are needed to prove the supposed strict specificity of their flagellates.

The comparison of faunas from the North-American roaches originating from two areas of its habitat and the Far-Eastern roach by the number of species (22 : 24 : 16) shows that the composition of flagellates in *Cr. punctulatus* is more diverse than in *Cr. relictus*. This could be accounted for by two circumstances. First, flagellates from the North-American roach have been studied in more detail and their hosts have been collected in many points of their habitat. All Far-Eastern roaches investigated by us had been collected in the relatively small area of the Suputinsky

Reservation. Taking into account possible local deviations from the generalized pattern of infection of the roach species, we may assume that the list of flagellates from *Cryptocercus relictus* would be supplemented if the host is examined in other points of its habitat (Kurentzow 1959). Second, the contemporary composition of the parasite fauna of the Far-Eastern roach is likely to be somewhat impoverished since *Cryptocercus relictus* is a Tertiary relict (Bei-Bienko 1950). Many parasitologists who studied the fauna of relict animals indicated disappearance of some of their parasite species (Dogiel 1964). This is probably due to the relict living conditions of the host itself. In this connection, Cleveland's data about gradual disappearance of *Macrospironympha xylopletha* from the fauna of *Cr. punctulatus* are of interest. In the beginning of his investigations, Cleveland recorded this flagellate in roaches from three states — Washington, Oregon and California (Cleveland et al. 1934). Twenty years later, *Macrospironympha xylopletha* could be still found only in roaches from a small site — Crescent (California) (Cleveland 1956 a).

In conclusion, it should be noted that flagellates of the order *Hypermastigida* predominate over representatives of the order *Oxymonadida* in the parasite faunas of both *Cryptocercus* species.

## IV. Evolution of Flagellates Living in Termites and Roaches

### 1. Phylogenetic Parallelism in the Evolution of *Cryptocercus* and of their Parasitic Flagellates

In result of numerous studies of correlation between the evolution of parasites and of their hosts (see reviews by Dogiel 1964, Michajłow 1968), a conclusion has been drawn that the parasites, particularly highly specific and specialized ones, evolve in parallel with their hosts in time and space. This general principle of evolution of parasites and of their hosts is, however, often disturbed by various ecological factors. The evolution of intestinal flagellates from wood-feeding roaches and of the genus *Cryptocercus* itself is one more illustration of parallel evolution of parasites and of their hosts.

According to Bei-Bienko (1950), the genus *Cryptocercus*, the only one in the family *Cryptocercidae* (*Blattodea*) includes three species — *Cr. punctulatus* Scudder, 1862, *Cr. relictus* B.-Bienko, 1935 and *Cr. primarius* B.-Bienko, 1938. *Cr. punctulatus* lives in North America in two separated areas — Eastern (Appalachian area) and Western (Pacific coast area). *Cr. relictus* occurs on the territory of the Primorye region of the Soviet Far East. *Cr. primarius* is found only in the highlands of the Central China. Thus, all the three species are geographically far separated though living in similar conditions and having a similar mode of life.

Contemporary roaches of the genus *Cryptocercus* are Tertiary relicts (Bei-Bienko 1950). This conclusion is drawn from the following arguments:

(1) Primitive organization of these roaches. Being an intermediate form between *Cr. punctulatus* and *Cr. relictus*, *Cr. primarius* has more primitive features in comparison with the other two species.

(2) Distribution of these roaches in close connection with the fragments of the arctotertiary type flora. The latter was prevailing in the Northern hemisphere in the Tertiary. At present, however, it can be found only in North America and in the Far East, i.e., in places where *Cryptocercus* species occur.

(3) Separation of the areas of distribution.

(4) Absence of close relations with other groups of *Blattodea*.

In the light of this evidence and the paleographic data on the geological relationship between the continents of Asia and of North America (for example, see Krishtofovitch 1932), it can be suggested that in the Tertiary period a vast territory of the North hemisphere was occupied with forests of moderate climate. Only one *Cryptocercus* species occurred here; by its morphology and mode of life it was similar to the contemporary *Cr. primarius*. Changes in the climate and, consequently, in the flora, which began in the Palearctic by the end of the Tertiary period rendered the environmental conditions unfavourable for *Cryptocercus*. Fragmentation of the flora and separation of the continents af Asia and of North America led to isolation of some *Cryptocercus* populations and favored their divergence (Fig. 20). "The
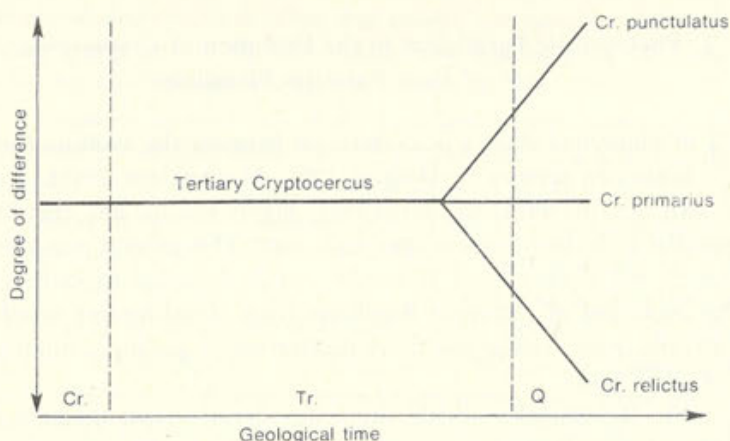


Fig. 20. Phylogram of the genus *Cryptocercus* (by data of Bei-Bienko 1950)

evolution of *Cr. relictus* and *Cr. punctulatus* proceeded independently from a morphological type which was evidently widespread in the Tertiary and which is now represented by *Cr. primarius*" (Bei-Bienko 1950, p. 336).

*Cryptocercus relictus* and *Cr. punctulatus* which have been separated geographically since the Tertiary, contain intestinal flagellates specific for each host species. From our viewpoint, the only explanation of this fact is that since the Tertiary,

both *Cryptocercus* and its intestinal flagellates developed in parallel. Speciation in the flagellates was influenced by the geographic isolation of the hosts which permitted a divergence of characters of the parasite. Accumulation of these changes led to more profound morphological alterations which permitted to classify the flagellates as independent species. This type of speciation is called geographical, being one of the main ways of species formation in the animal kingdom. Inasmuch as the time of host differentiation from the ancestral species — the Tertiary *Cryptocercus* — is known, one may determine the speciation rate for the parasites. The time of formation of strictly specific flagellates in *Cryptocercus punctulatus* and *Cr. relictus* amounts to about 25 million years, since the onset of divergence of the Tertiary *Cryptocercus* dates back to the Tertiary Neogen. The rate of evolution estimated by the rate of formation of new taxons, proved to the same in the hosts and the parasites: geographically isolated populations of the Tertiary *Cryptocercus* as well as those of their parasites both attained the level of independent species only.

## 2. Phylogenetic Relationships Between Faunas of Roaches and Termites

The hypothesis that intestinal parasites of lower termites and of roaches of the genus *Cryptocercus* trace their origin to some common ancestral flagellates is now beyond doubt. This hypothesis, speculative at the time, was first advanced by Cleveland et al. 1934. It has been supported, on one hand, by evidence of morphological proximity of the flagellates from the two host groups (Cleveland et al. 1934), and on the other hand, by absence of cross infection with flagellates between roaches and termites (Cleveland et al. 1934, Cleveland and Nutting 1955, Nutting 1956), which argues against the possibility of secondary infection of one of the hosts from the other. Recent data on the phylogenetic proximity between termites and roaches not only support this hypothesis but also permit to elucidate the obscure point of what the primary host of symbiotic flagellates could have been. Most entomologists now maintain that the *Isoptera* descended directly from the stem of *Blattodea* in the late Paleozoic and early Mesozoic (Fig. 21) (see, for instance, Krishna 1970, Sharov 1968). We shall discuss here only evidence which enables us to define the representatives of *Blattodea* from which the termites have differentiated.

A detailed comparison of the morphology of most primitive representatives of the orders *Isoptera* and *Blattodea* — the termite *Mastotermes darwiniensis* and the roach *Cryptocercus punctulatus* —shows that these insects are similar in their organization (McKittrick 1965). Symbiotic bacteria (=bacteriocytes) characterizing all roaches were found as well in primitive termite *Mastotermes darwiniensis* (Grassé et Noirot 1959). The immunological analysis of the intestinal contents in *Cryptocercus punctulatus* and *Zootermopsis nevadensis* revealed a number of similar antigenes in these insects (Ablin and Ritter 1967). This allows to assume that contem-
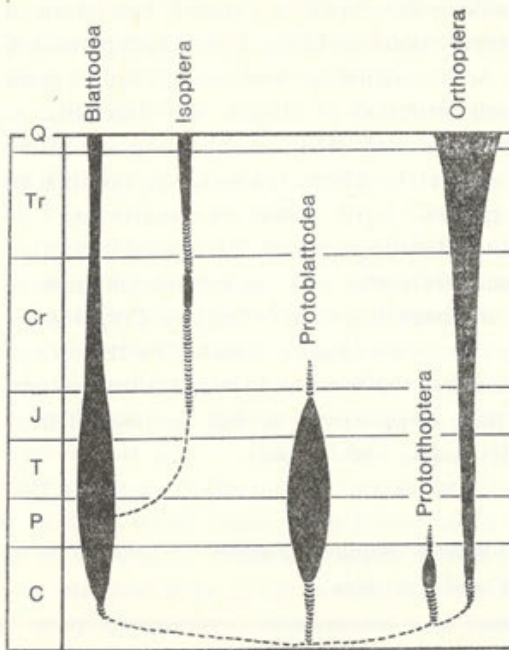
Fig. 21. Phylogrammatical scheme of the orders *Blattodea* and *Isoptera* (according to S h a r o v 1968)

porary relict roaches (*Cryptocercus*) are surviving representatives of the stem of *Blattodea* from which the termites diverged.

Data on the phylogenetic proximity of termites and roaches allow the conclusion that primary hosts of symbiotic flagellates of the orders *Oxymonadida* and *Hypermastigida* were roaches (probably similar in their organization and biology to the now-living *Cryptocercus*), while termites are their secondary hosts.

### 3. Analysis of Evolutionary Pathways of Flagellates in Termites and Roaches as Illustrated by the Genus *Trichonympha*

Supposing that *Blattodea* are primary hosts of the flagellates, the systematic resemblance of the faunas of lower termites and of *Cryptocercus* may be regarded as a consequence of parallel evolution of the hosts and of their parasites. The divergence of termites from the stem of Paleozoic *Blattodea* was followed by that of their parasites. It would be of interest to trace whether the principle of evolutionary parallelism between the hosts and their parasites is retained in the course of subsequent separate evolution of flagellates in *Cryptocercus* and in lower termites.

This can be done using flagellates of the genus *Trichonympha*. The choice of this genus is due to the following considerations:

(1) The genus *Trichonympha* is common to parasitic faunas of both termites and roaches and is widespread among its hosts. *Trichonympha* occur in the Far-

Eastern and North-American roaches and in three of the four families of lower termites (the exception is the family *Mastotermitidae* containing only one species).

(2) The genus *Trichonympha* contains many species. It includes 31 species among which 10 inhabit *Cryptocercus* and 21, the termites.

(3) Differences between *Tirchonympha* species are very distinct.

(4) The supposed phylogenetic proximity of trichonymphs from roaches and termites is supported by immunological investigations (Ablin and Ritter 1967). The immunological comparison of the intestinal flagellates of *Cryptocercus punctulatus* and of *Zootermopsis nevadensis* revealed a common antigenic factor which, according to Ablin and Ritter, depends on the presence of trichonymphs in the intestine of both hosts. The agglutinin against *Trichonympha* from *Cryptocercus* was found to be reactive against trichonymphs from *Zootermopsis* and vice versa.

A comparison of the phylogenetic scheme of the hosts with the distribution of parasites among its various branches enabled us to gain some insight into the divergence of the host and of their parasites. The presence of strictly specific trichonymphs in *Cryptocercus punctulatus* as well as in *Cr. relictus* indicates that trichonymphs which inhabited the Tertiary *Cryptocercus* changed in parallel to its divergence into two independent species. Thus, the evolution of the Tertiary *Cryptocercus* and of its trichonymphs is strictly and completely parallel.
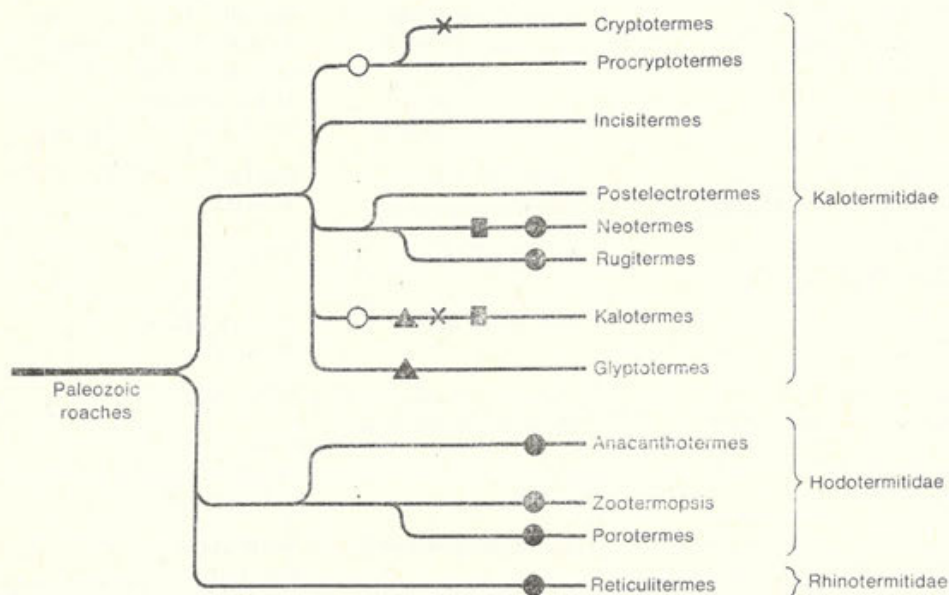


Fig. 22. Phylogram of lower termites (according to Krishna 1970) (contains only genera of termites inhabited by *Trichonympha* species). *Trichonympha* dwelling in various genera of the host, o — *T. corbula*, × — *T. subquasilla*, ▲ — *T. chattoni*, ■ — *T. zeylanica*. *Trichonympha* dwelling in various species of one genus of the host, ● — *T. sphaerica, T. saepicula, T. agilis, T collaris, T. campanula, T. magna, T. peplophora, T. turkestanica*

Considering the distribution of trichonymphs in termites (their phylogramm, drawn according to Krishna 1970, is given in Fig. 22), we can distinguish two stages of their combined evolution. At the first stage, the termites differentiated from the Paleozoic roaches and diverged into families; in parallel to this, separate species of the genus *Trichonympha* formed. Hence, different species of *Trichonympha* exist in all evolutionary lines of lower termites: *Calotermitidae, Hodotermitidae* and *Rhinotermitidae*. At this stage, there was a parallelism in the evolution of the hosts and the parasites: the divergence of hosts was followed by that of parasites. At the second stage, further differentiation of lower termites into subfamilies and genera occurred but it was not accompanied by parallel phylogenetic differentiation of *Trichonympha* species. We believe that an analysis of specificity of trichonymphs toward their hosts within lower termites would substantiate this viewpoint.

As seen from Table 8, based mainly on Kirby's data (1932 b, 1937, 1944), trichonymphs from the intestine of termites show no strict host specificity. Most spe-

Table 8

Distribution of Flagellates of the Genus *Trichonympha* in Termites

| Species of the genus *Trichonympha* | Host | Host's distribution |
|---|---|---|
| *T. chattoni* Duboscq et Grassé, 1927 | *Glyptotermes irridipennis* (and other 12 species of *Glyptotermes*) *Kalotermes milleri* *Kalotermes schwartzi* | Australia, Java, Shri-Lanca (Ceylon), Fiji, Philippines, Costa-Rica, East Africa (Uganda), North America (Florida) |
| *T. zeylanica* Duboscq et Grassé, 1927 | *Kalotermes militaris* *Kalotermes obscurus* | Shri-Lanca Ceylon), Australia |
| *T. divexa* Kirby, 1944 | *Kalotermes* sp. | South Africa |
| *T. tabogae* Kirby, 1932 | *Kalotermes tabogae* | Panam's isthmus (Panama) |
| *T. sphaerica* Duboscq et Grassé, 1927 | *Zootermopsis angusticollis* *Zootermopsis nevadensis* | North America (California) |
| *T. subquasilla* Kirby, 1932 | *Cryptotermes clevelandi* *Kalotermes immigrans* | Panam's isthmus (Panama), Galapagos islands |
| *T. lighti* Kirby, 1932 | *Kalotermes emersoni* | North America (Mexico) |
| *T. saepicula* Kirby, 1932 | *Rugitermes kirbyi* *Rugitermes panamae* | Panam's isthmus (Panama, Costa-Rica) |
| *T. quasilla* Kirby, 1932 | *Kalotermes perezi* | Panam's isthmus (Costa-Rica) |

Table 8 (continued)

| Species of the genus *Trichonympha* | Host | Host's distribution |
|---|---|---|
| T. corbula Kirby, 1944 | *Procryptotermes* sp. *Kalotermes longus* *Kalotermes costaneiceps* | Madagascar |
| T. teres Kirby, 1944 | *Neotermes meruensis* | East Africa (Tanganyica) |
| T. agilis Leidy, 1877 | *Reticulitermes flavipes* (and other 4 species of *Reticulitermes*) | Europe, Japan, North America |
| T. minor Grassi et Foà, 1911 | *Reticulitermes lucifugus* | Europe |
| T. ampla Kirby, 1944 | *Kalotermes occidentis* | North America (Mexico) |
| T. collaris Kirby, 1932 | *Zootermopsis angusticollis* *Zootermopsis nevadensis* | North America (California) |
| T. campanula Kofoid et Swezy, 1919 | *Zootermopsis angusticollis* *Zootermopsis nevadensis* *Zootermopsis laticeps* | North America (California, Arizona) |
| T. magna Grassi, 1917 | *Porotermes adamsoni* *Porotermes grandis* | Australia |
| T. peplophora Kirby, 1944 | *Neotermes howa* | Madagascar |
| T. turkestanica Bernstein, 1928 | *Anacanthotermes murgabicus* (and other 4 species *Anacanthotermes*) | Turkestan (USSR); Egypt, India |
| T. globulosa Perez-Reyes et Lopez-Ochoterena, 1965 | *Incisitermes marginipennis* | North America (Mexico) |
| T. paraspiralis Perez-Reyes et Lopez-Ochoterena, 1965 | *Incisitermes marginipennis* | North America (Mexico) |

cies of *Trichonympha* were found to be specific only to the host genera. Thus, *Trichonympha agilis* inhabits five termite species of the genus *Reticulitermes*, widespread in Europe, Japan and North America. Some trichonymph species are peculiar to a number of genera of termites belonging to the same family. An excellent example is *Trichonympha chattoni*, found in 13 termite species of the genus *Glyptotermes* and in two species of the genus *Kalotermes*. It must be specially mentioned that the

hosts of this species of *Trichonympha* are cosmopolitans. Among trichonymphs inhabiting termites there are also species dwelling in only one host species. *Trichonympha lighti*, for example, is known to inhabit only *Kalotermes emersoni*. Kirby however, maintains that the cases of apparent strict specificity of trichonymphs to one
host species must be ascribed solely to the incompleteness of our knowledge of intestinal faunas of great number of termites.

It can also be concluded from Fig. 22 that when a single species of *Trichonympha*
inhabits a number of related genera of termites, this species must have been present
in the common ancestor of these host genera. Thus, *Trichonympha corbula*, *T. subquasilla* must have been living even in the late Cretaceous, i.e., at the time of divergence of the evolutionary lines leading to the actual genera *Cryptotermes* and *Glyptotermes*. It is likely that *Trichonympha chattoni* and *T. zeylanica* already existed in
Mesozoic termites before the differentiation of the genera *Neotermes*, *Kalotermes*
and *Glyptotermes*. Specialists in the phylogeny of lower termites suggest that all
now living subfamilies and genera of termites differentiated in the late Cretaceous
(Emerson 1955, Krishna 1970). Consequently, the geological age of some trichonymph species, which occur in a number of host genera, amounts approximately to
100 million years. Other trichonymph species, e.g., *Trichonympha agilis*, apparently
specific to one host genus, must have been present in the ancestral form of this genus
before it differentiated into various termite species, i.e., at least in early Tertiary.
The age of these trichonymph species probably amounts to about 75 million years.

This analysis indicates that, contrary to the situation peculiar to roaches, in the
case of termites divergence of the host did not encourage divergence of its parasites.
A question arises why was the parallelism in the evolution of the host and of the
parasites discontinued at the stage of phylogenetic differentiation of termites into
subfamilies and genera. What could be the explanation of the evolutionary inertnesof termite-dwelling trichonymphs which did not follow the phylogenetic differen.
tiation of their hosts? A possible cause of this phenomenon will be discussed later ons

## V. Sexuality among Flagellates of the Orders
### *Oxymonadida* and *Hypermastigida*

### 1. Sexual Process in Flagellates from *Cryptocercus relictus*

Flagellates from the hindgut of *Cryptocercus punctulatus* regularly undergo a sexual process during the molting period of the host. Peculiar features of this process
have been studied in detail in the numerous investigations by Cleveland (review:
Cleveland (1956 b). Molting nymphs of *Cryptocercus relictus* were also
found to contain flagellates at different stages of the sexual process. All species
inhabiting the intestine of the Far-Eastern roach showed sexual stages. However,
due to the lack of material, the whole process has never been traced in a single spe-

cies of flagellates. To interpret and to identify the stages observed, we used Cleveland's (1956 b) schemes of sexual cycles in related species of flagellates living in *Cr. punctulatus*. Since the faunas of the North-American and Far-Eastern roaches have an identical generic composition, it may be assumed that the general pattern of sexual processes in congeneric species of flagellates will be similar, despite their provenance from different host species. According to Cleveland, the sexual proceses are alike in different flagellate species belonging to one genus and inhabiting the same host species (*Cr. punctulatus*).

We now turn to the description of the sexual stages found in flagellates from *Cr. relictus*.

### *Barbulanympha cryptocerci* and *Urinympha cirrata*
### (Fig. 23, Pl. XII 46)

These flagellates were found in the intestine of freshly-molted nymphs at the zygote stage. The zygote is spherical and immobile, with strongly thickened ectoplasm. The flagella are packed in two intracytoplasmic groups and surrounded by a light zone. Such zygotes are called pseudocysts since they have no true cyst wall. The pseudocyst
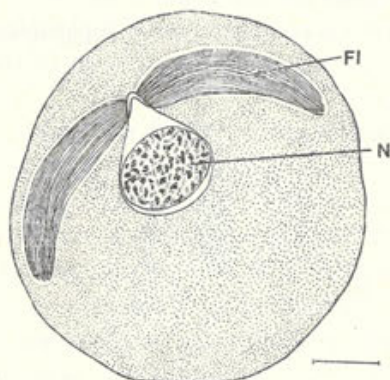


Fig. 23. *Barbulanympha cryptocerci* Bobyleva. Pseudocyst

stage coincides in time with shedding of the nymph's exoskeleton. When the chitinous intima of the gut is shed, a part of the pseudocysts is discharged with it; thereupon they are eaten by hatching nymphs or by nymphs not molting at that time. A dense layer of ectoplasm seems to protect the pseudocyst from environmental factors.

### Species of the Genus *Trichonympha*
### (Fig. 24, 25)

In *Trichonympha major*, *T. ussuriensis* and *T. lutea*, various stages of gametogenesis in cysts were observed. The cells are spherical, surrounded by a thick cyst membrane. *T. major* has cysts about 34 μm in diameter; *T. ussuriensis* and *T. lutea*,

about 16 μm. A most peculiar mitotic division of the gametocyte nucleus leads to formation of the nuclei of gametes. This division is accompanied by cytoplasmic reorganization during which all organelles of the gametocyte dedifferentiate and new ones arise in gametes. Figure 24 presents cysts of *T. ussuriensis* during formation of
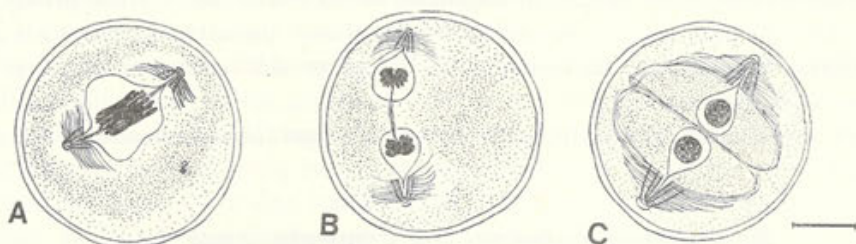
Fig. 24. Different stages of gametogenesis in cysts of *Trichonympha ussuriensis* Bobyleva. A — Prophase, B — Telophase, C — Almost completely formed gametes

gametes. The nuclei are in prophase (Fig. 24 A) and in telophase (Fig. 24 B). Rostra are already formed in the future gametes. Cysts of such stages were found in the intestine of nymphs shedding its exoskeleton. The molted nymphs (with soft and light chitine) contain cysts in which formation of the extranuclear organelles of the gametes is completed but cytoplasmic division is proceeding. In Fig. 24 C one can see a cyst containing two gametes of the trichonymphid type with oppositely directed
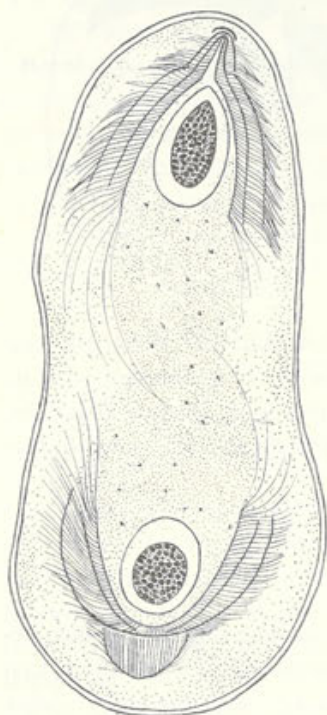
Fig. 25. *Trichonympha major* Bobyleva. The cyst ready to excyst

rostra. The cyst shown in Fig. 25 is elongated; the gametes of *T. major* are ready to excyst and cytokinesis is being completed.

## Eucomonympha nana
### (Pl. XII 47–49)

Different fertilization stages of these flagellates were seen. At the stage presented in Pl. XII 47, 48 the gametes have already fused, but remnants of the male gamete — a rostral tube with parabasal filaments — are still distinctly visible. The male pronucleus migrated toward the stationary female pronucleus. Then, fusion of the pronuclei which starts with a contact of their nuclear membranes was observed (Pl. XII 47, 48). The microphotograph 49 shows the resulting zygote. This cell has only one large nucleus, but the rostral tube of the male gametes is still visible. Unlike *E. imla* from *Cryptocercus punctulatus* (Cleveland 1956 b) in *E. nana* from *Cr. relictus* the fusion of cytoplasm of the gametes is not accompanied by discarding of the male gamete organelles. They are likely to resorb so slowly that remain to be seen even in the zygote.

## Species of Leptospironympha
### (Fig. 26–28, Pl. XIII 50, 51)

In *Leptospironympha variabilis* and *L. popularis*, stages of gametocyte formation, gametogenesis and fertilization were observed. A cell entering the sexual process transforms into a gametocyte, in which the spiralling flagellar bands undergo destruction. Desintegration of the flagellar bands starts with migration of some flagellar rows towards the posterior end of the cell (Fig. 26, Pl. XIII 50, 51) where they are completely resorbed. Only the rostral portion of each flagellar band is retained. Thereupon, the gametocyte encysts and gametes form inside the cyst (Fig. 27). Gametogenesis is accompanied by reorganization of the extranuclear organelles. After the molting of the roach is completed the gametes leave the cyst and fertilization occurs. Figure 28 shows the anterior half of a zygote of *L. popularis* at the moment of fusion of the pronuclei, which starts with a contact of nuclear membranes.

## Species of Saccinobaculus
### (Fig. 29, 30, Pl. XIII 52, 53)

*Saccinobacolus gloriosus, S. spatiatus* and *S. scabiosus* were observed at different stages of fertilization. Not only fusion of the nuclei but also that of axostyles is typical for the copulation of these flagellates. Figure 29 shows *S. gloriosus* which is larger than the asexual cell and has eight flagellae, two nuclei and one axostyle. The nuclear membranes are in close contact. The next Figure (30) shows a zygote which
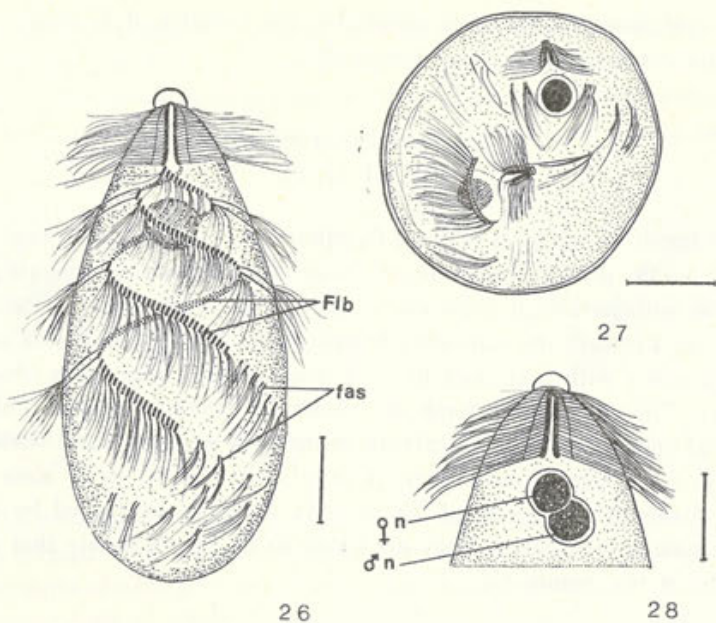
Fig. 26–28. *Leptospironympha popularis* Bobyleva. 26 — Gametocyte, 27 — Gametogenesis in cyst.
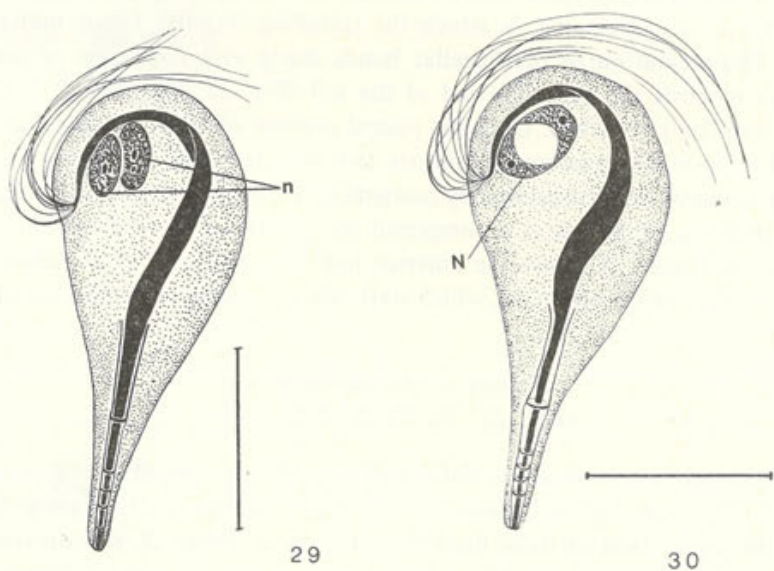28 — Anterior part of *L. popularis* cell. Fusion of pronuclei



Fig. 29, 30. *Saccinobaculus gloriosus* Bobyleva. 29 — Fusion of pronuclei. 30 — Zygote

has an oval nucleus with crescentic poles. A zygote of *S. spatiatus* with not yet fused axostyles of the gametes is presented in the micrograph 53 (Pl. XIII). *S. scabiosus* (Pl. XIII 52) occurred at the stage of fusion of pronuclei. The cell has eight flagellae (out of focus), two nuclei and one axostyle. Here we probably have a case of autogamy.

### Notila proteus ussuriensis
### (Pl. X 40)

These flagellates were found at the prezygotic stage (Cleveland 1950 a). The cell showing in Pl. X 40 has a double set of flagellae (out of focus), two nuclei and one axostyle.

\*

\*     \*

The comparison of some separate stages of the sexual process found in flagellates from the Far-Eastern roach with respective stages in flagellates from the North-American roach shows that they are similar in both morphology and time relationship to the host's molting. Such similarity permits the assumption that in both host species the sexual processes follow the same general pattern. Some minor differences, however, exist at certain stages of the process. Thus, in *Eucomonympha nana*, unlike *E. imla*, the male gamete organelles are not discarded during fusion of the dimorphous gametes but gradually resorbed in the zygote cytoplasm. In *Saccinobaculus spatiatus* from *Cryptocercus relictus*, the pronuclei fuse earlier than the axostyles, while in all species of this genus from *Cr. punctulatus* the sequence is inverse: fusion of axostyles precedes that of gametic nuclei. The above differences are, however, too insignificant to bring the sexual process of congeneric flagellates from the two host species out of the common general pattern.

## 2. Is Sexuality in Oxymonadida and Hypermastigida
## Primary or Secondary?

The studies of Cleveland on the sexual cycles in flagellates from *Cryptocercus punctulatus* stimulated a search for the sexual process in flagellates from termites. Thorough investigations by a number of scientists however, failed to find sexuality in the intestinal flagellates of most termites which showed only asexual reproduction throughout the life cycle.

The presence of the sexual process in flagellates from *Cr. punctulatus* and its absence in closely related flagellates from termites raise a question of whether sexuality or on the contrary, asexuality is primary in this group of parasites. Two opinions on this question exist, both closely allied to two different concepts of the primary

host of the flagellates. Considering Cleveland's results from the standpoint of genetic consequences of the sexual process, the American investigators Hawes (1963) and Sonneborn (1957) supposed that the flagellates of *Cryptocercus punctulatus* exhibit stages of progressive degradation of the sexual process. Their hypothesis is that ancestral flagellates, which inhabited the intestine of the common roach-like ancestor of both termites and *Cryptocercus*, possessed sexuality. Subsequently, the sexual process was retained, though somewhat degraded, in the line of roaches, and completely lost in the line of termites.

On the other hand, some Soviet authors (Dogiel 1965, Poljansky 1971) who considered the parasitism of flagellates in termites primary and that in *Cryptocercus* secondary, supposed the great diversity of gamogony and meiosis in flagellates from *Cr. punctulatus* to reflect a kind of evolutionary instability of these phenomena. They supposed that these events are comparatively recent and that the sexual process arose in flagellates of *Cryptocercus, de novo*, when flagellates, which were primarily asexual, passed from termites to roaches.

The modern concept of the origin of termites directly from the stem of *Blattodea*, now shared by the majority of entomologists, as well as our own findings about the existence of sexual processes in flagellates from *Cryptocercus relictus*, provide further indirect evidence in favour of the former opinion, that of primarity of the sexuality in the orders *Oxymonadida* and *Hypermastigida*.We may assume that flagellates of the Tertiary *Cryptocercus* possessed sexuality since it exists in flagellates from both *Cr. punctulatus* and *Cr. relictus*. Ancestral flagellates populating the intestine of paleozoic roaches probably also showed sexuality.

This also makes likely the assumption of Hawes and Sonneborn that flagellates from termites lost their sexual process in the course of evolution. The reason for such loss may be another mode of transmission of the flagellates in termites as compared to roaches.

In flagellates from *Cryptocercus* the sexual process is intimately connected with transmission of symbionts from one host specimen to another (Cleveland et al. 1934). During the sexual process, cysts and pseudocysts are formed. They are discharged into the external medium at the moment of molt of the host. Young roaches hatched from eggs become infected with flagellates by eating cysts together with wood particles. Similarly, the number of symbiotic flagellates is increased in the intestine of nymphs. Consequently, a strict correlation between the time of hatching of young roaches and molting of nymphs, on one hand, and a close connection between the molting period in hosts and the onset of the sexual process in flagellates, on the other hand, ensure transmission of flagellates among members of a roach colony.

The mode of transmission of symbionts in termites is different. The flagellates are transmitted from host to host via proctodeal feeding typical of termites (Andrew 1930). Young termites and nymphs of various age lick off anal excreta containing flagellates. During the molt of a termite, all its flagellates die and are eliminated to-

gether with the old intima of the gut. As soon as the molt is completed, nymphs re-gain their flagellate fauna through proctodeal feeding from non-molting nymphs. The ability of flagellates to get "mumifical" at the exit from the hindgut helps them to pass uninjured through the digestive tract of the new host specimen (Dogiel 1956). Quick reproduction of symbionts in molted nymphs is provided for by mi-totic flares among newly acquired flagellates (Andrew and Light 1929). Consequ-ently, transmission of flagellates among termites is accomplished exclusively via proctodeal feeding of the hosts.

Flagellates of termites reproduce asexually during the whole life cycle, i.e., from the time of acquisition of infection in a termite up to its next molt. For the over-whelming majority of flagellates, the sexual process is not known. However, Cle-veland succeeded to discover hormone-induced sexuality in some species. These are *Trichonympha magna* from *Porotermes adamsoni* (1965 a), some species of *Pseudo-trichonympha* from the family *Rhinotermitidae* (1965 b) and all species of flagellates from the primitive *Mastotermes darwiniensis* (1966 b, c). According to Cleveland, the sexual process was seen very unfrequently a few hours prior to ecdysis. No cysts were formed in the course of the process, which in some features resembled that described for *Barbulanympha* (Cleveland 1953). At ecdysis, all flagellates including those which underwent a sexual process were eliminated from the host. Since a molt-ing termite does not feed and the only way of flagellate transmission among hosts is proctodeal feeding, the possibility of survival and transmission of zygotes to new host specimens is negligible. In essence, the above peculiarities of the host's biology make gamogony in flagellates useless. The rare cases of seuxuality among flagellates from termites are likely to reflect the ancestral ability of flagellates to respond to the molting hormone of the host. This supposition is based on experimental evidence of the ecdysone effect on flagellates from the North-American roach (Cleveland et al. 1960), demonstrating a direct relation between the onset of the sexual process and the titre of the molting hormone.

As pointed out by Cleveland (1965 a) and Honigberg (1970) the transmis-sion mechanism of flagellates in termites would render sexuality unimportant in terms of the ultimate advantages that it could confer upon the symbionts. It can be suggested that elaboration of a perfect mechanism of host-to-host transmission of flagellates was the main trend in the evolution of this parasite-host system. From this point of view transmission of flagellates by proctodeal feeding in termites is more reliable than that by cysts observed in *Cryptocercus*. The mode of transmission by proctodeal feeding guarantees preservation of symbionts among hosts. The estab-lishment of this mode, however, resulted in the loss of the sexual process which ceased to be a stage indispensable for completion of the life cycle of the parasite.

The feeding habits of *Cryptocercus* have favored a mechanism of transmission of symbionts by cysts. In the evolution of flagellates from roaches, the sexual process was accordingly retained as a stage indispensable for completion of the life cycle of a parasite.

## 3. Possible Relation between Mode of Reproduction
### and Character of Evolution in Flagellates

In the previous chapter we demonstrated a correlation between the evolution of flagellates of the genus *Trichonympha* and of their hosts within two groups of insects — *Cryptocercus* and lower termites. The main ,onclusion was that in roaches, the trichonymphs evolved strictly and completely in parallel to their hosts, whereas in termites this evolutionary parallelism was discontinued at the stage of phylogenetic differentiation of the host into subfamilies and genera.

Approximately since the Tertiary period, the trichonymphs from roaches and those from termites underwent different evolutionary changes. Trichonymphs which inhabited the Tertiary *Cryptocercus* evolved by divergent speciation, which proceeded in parallel to the speciation of the hosts. Trichonymphs populating the lower termites demonstrated since the Tertiary a high degree of stability of species in time. This resulted in a sharp widening of the range of hosts inhabited by each flagellate species, due to phylogenetic differentiation of termites into subfamilies and genera and conservation of the existing trichonymph species.

A connection seems to exist between the character of evolution of trichonymphs and the absence or presence of the sexual process in their life cycle. No sexual process occurs in the overwhelming majority of trichonymphs from termites. The only exception is *Trichonympha magna*. However the data of Cleveland (1965 a) show that sexuality in *T. magna* is of little value from the standpoint of biparental inheritance and evolution. This only corroborates the hypothesis about the secondary loss of the sexual process in flagellates inhabiting termites.

Since the sexual process is the source of genotypic variation arising through genetic recombination its loss must have led the respective species of *Trichonympha* onto the road of very restricted evolutionary changes. The exclusively agamic reproduction must have resulted in standardization of the progeny whose monotonous genotypes were of little value for evolution.

Trichonymphs of *Cryptocercus* which had retained the sexual process in the course of their evolution, had a greater evolutionary potential as compared to trichonymphs of termites, owing to stronger recombination possibilities of the gene pool during the sexual process.

Consequently, the differences in the character of evolution between phylogenetically related trichonymphs from *Cryptocercus* and those from lower termites seem to be caused by transition of the flagellates populating termites to exclusively agamic reproduction.

We have considered the evolution of only one genus *Trichonympha*, and thus we cannot insist that loss of the sexual process has influenced in the same way the character of evolution of other representatives of the termite intestinal fauna. For example, rapid instantaneous speciation related to polyploidy is possible in some flagellates (for instance, in the polyploid series of species of *Spirotrichosoma* from

the termite *Stolotermes ruficeps* (Cleveland and Day 1958). It is not unlikely that other types of specific differentiation that are still unknown also exist in protozoa reproducting only agamically.

Summary

Intestinal flagellates from the hindgut of the Far-Eastern wood-feeding roach *Cryptocercus relictus* were studied. Some questions of the origin, distribution and evolution of flagellates of the orders *Oxymonadida* and *Hypermastigida* in both roaches and termites are discussed.

The flagellate fauna of *Cr. relictus* includes: *Barbulanympha cryptocerci, Urinympha cirrata, Trichonympha major, T. ussuriensis, T. lutea, Leptospironympha variabilis, L. popularis, L. lepida, Bispironympha unica, Eucomonympha nana* (order *Hypermastigida*), *Sacinobaculus gloriosus, S. spatiatus, S. scabiosus, Notila proteus ussuriensis, Oxymonas nana ussuriensis* (order *Oxymonadida*), *Hexamita cryptocerci* (order *Diplomonadida*). Morphological descriptions of all these species are given.

The comparison of the faunas of the North-American roach (*Cryptocercus punctulatus*) studies by Cleveland and of the Far-Eastern roach has revealed identicas generic but different specific compositions of their flagellates. As *Cr. punctulatul* and *Cr. relictus* developed as independent species under conditions of prolonged geographical isolation (Bei-Bienko 1950), the strict specificity of their flagellates toward the host species must have arisen by parallel divergent evolution of both the Tertiary *Cryptocercus* and of their intestinal parasites. In this case the evolution of the host and its parasites was strictly and completely parallel. Speciation in flagellates, based on the geographic isolation of the hosts, is one of the common ways of species formation in the animal kingdom. The rate of evolution, estimated by the rate of formation of similar taxons, was found to be identical in the host and its parasites.

Our data support the hypothesis that primary hosts of flagellates of the orders *Oxymonadida* and *Hypermastigida* were paleozoic *Blattodea,* from which termites diverged in the late Paleozoic or early Mesozoic. It is likely that contemporary relict roaches of the genus *Cryptocercus* are survivors of this ancient group of *Blattodea*.

Hormone-induced sexual processes exist in flagellates from *Cryptocercus relictus*. They proceed similarly to those in flagellates from *Cr. punctulatus*. The hypothesis about the primarity of sexuality over asexuality among of the orders *Oxymonadida* and *Hypermastigida* is discussed and found likely. Asexuality of flagellates from lower termites appears a secondary phenomenon.

Using the genus *Trichonympha*, common to intestinal faunas of both termites and roaches, evolutionary pathways of flagellates from two host groups — termites and roaches — were compared. The evolution of trichonymphs from two *Cryptocercus* species — *Cr. relictus* and *Cr. punctulatus* — went in parallel to that of their

hosts. On the other hand, no parallelism has been observed in evolution of termites and their trichonymphs since the Tertiary i. e., the time of phylogenetic differentiation of termites into subfamilies and genera.

The flagellates of the genus *Trichonympha* from two host groups underwent different evolutionary changes for about the same period of time. The evolution of trichonymphs from *Cryptocercus* was a divergent speciation. At the same time in termites *Trichonympha* species showed resistance and stability. The evolutionary inertness of trichonymphs of lower termites seems to be determined by transition of the flagellates to an exclusively agamic mode of reproduction.

РЕЗЮМЕ

Проведено изучение видового состава фауны жгутиконосцев, обитающей в заднем отделе кишечника дальневосточного лесного таракана *Cryptocercus relictus*. Это исследование представляло интерес как для решения частных вопросов о происхождении, распространении и эволюции жгутиконосцев отрядов *Oxymonadida* и *Hypermastigida*, так и для обсуждения ряда общих вопросов эволюционной теории.

(1) Описана фауна жгутиконосцев, состоящая из 10 видов отряда *Hypermastigida* и 3 видов и 2 подвидов отряда *Oxymonadida*. Уточнен ряд морфологических деталей в строении этих простейших.

(2) Сравнение паразитофаун североамериканского таракана (*Cr. punctulatus*), по данным Кливленда, и дальневосточного (*Cr. relictus*), показало, что они имеют идентичный родовой состав жгутиконосцев, но разный видовой состав. На основе данных о формировании *Cr. punctulatus* и *Cr. relictus* как самостоятельных видов (Бей-Биенко 1950) показано, что строгая специфичность жгутиконосцев к виду хозяина возникла в ходе параллельной дивергентной эволюции третичного *Cryptocercus* и населяющих их кишечник жгутиконосцев, при этом в ходе эволюции хозяев и паразитов наблюдается строгий и полный филогенетический параллелизм. Видообразование у жгутиконосцев протекало на основе географической изоляции хозяина и представляет собой один из универсальных способов видообразования в животном мире. Темп эволюции, определяемый по скорости образования новых таксонов, у хозяина и парзитов оказался одинаковым.

(3) Уточнена гипотеза о первичных хозяевах жгутиконосцев отрядов *Oxymonadida* и *Hypermastigida*. Предполагается, что ими были палеозойские тараканы, от которых в позднем палеозое или раннем мезозое дивергировали термиты. Возможно, современные реликтовые тараканы рода *Cryptocercus* являются теми выжившими до наших дней представителями ствола таракановых, от которых эволюировали термиты.

(4) У жгутиконосцев из *Cr. relictus* обнаружен половой процесс, который происходит во время линьки хозяина и протекает сходно с таковым у жгутиконосцев *Cr. punctulatus*. Обсуждается гипотеза о первичности явления сексуальности среди жгутиконосцев отрядов *Oxymonadida* и *Hypermastigida* и о состоянии асексуальности у жгутиконосцев, обитающих в низших термитах, как вторичном явлении. Утрата полового процесса у жгутиконосцев, обитающих в термитах, вероятно, произошла в ходе эволюции в связи с формированием особого способа заражения термитов жгутиконосцами.

(5) На примере рода *Trichonympha*, общего для паразитофаун термитов и тараканов, проведено сравнение путей эволюции жгутиконосцев из двух групп хозяев — термитов и тараканов. Показано, что жгутиконосцы этого рода претерпели разные эволюционные изменения примерно за одинаковый период времени. Эволюция трихонимф из двух видов

тараканов *Cryptocercus* — *Cr. punctulatus* и *Cr. relictus* протекала параллельно с эволюцией их хозяев, при тесной связи филогенезов хозяина и паразитов. В эволюции трихонимф из низших термитов, начиная с третичного периода — времени филогенетической дифферен-цировки термитов на подсемейства и роды, не наблюдалось параллелизма в эволюции па-разитов и хозяев. Высказывается предположение, что нарушение принципа параллелизма в эволюции паразита и хозяина у жгутиконосцев из термитов связано с выпадением полового процесса из их жизненного цикла.

(6) Эволюционный путь трихонимф из *Cryptocercus* — это путь дивергентного видооб-разования. Эволюционный путь трихонимф из термитов связан с устойчивостью и стабиль-ностью видов во времени. Возможно, эволюционная инертность трихонимф, обитающих в кишечнике низших термитов, обусловлена переходом жгутиконосцев к исключительно агамному способу размножения.

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## EXPLANATION OF PLATES I–XIII

Pl. I. *Barbulanympha cryptocerci*. Total preparations. Impreganation according to Uhlig.

1,2 — Lateral view, 1 — focus onto two identical flagellated areas separated by a strip of cytoplasm free from flagella, 2 — focus onto atractophore, nucleus and parabasal bodies. 3, 4 — vertical view of the anterior end of the body, 3 — focus on to axostyle ring, 4 — focus onto axostyle fibrils at the base of the cap

Pl. II. *Barbulanympha cryptocerci*. 5, 6, 7, 8 — total preparations, impregnation according to Uhlig. 7, 8 — sections, Heidenhain's hematoxylin

5 — Anterior part of the body, focus onto parabasal plates and atractophore, 6 — vertical view of the flagellated areas, parabasals and nucleus, 7, 8 — mitosis, 7 — prophase, 8 — anaphase

Pl. III. *Urinympha cirrata*. Total preparations. Impregnation according to Uhlig

9, 10 — lateral view of the entire organism, 9 — specimen with elongated and narrow body, 10 — pyriform specimen, 11 — lateral view of anterior part of the body, focus onto one flagellated area, one group of parabasal bodies and axostyle bundle

Pl. IV. *Trichonympha major*. Total preparations; impregnation according to Uhlig

12 — lateral view of the entire organism, 13, 14 — anterior end of the body, 13 — focus onto nuclear sleeve with nucleus, 14 — focus onto surface of the nuclear sleeve

Pl. V. 15, 16, 19 — *Trichonympha major*. 15 — total preparation, impregnation according to Dragesco; anterior end of the body, focus onto dense region of ectoplasm. 16, 19 — sections; Heidenhain's hematoxylin, 16 — transversal section at the level of rostrum, 19 — longitudinal section at the level of circular fissure. 17, 18 — total preparations, impregnation according to Dragesco, 17 — lateral view of parabasal bodies of *Trichonympha lutea*. 18 — vertical view of parabasal bodies of *Trichonympha lutea*

Pl. VI. Total preparations; impregnation according to Uhlig. 20, 21 — *Eucomonympha nana*, 20 — attached form, 21 — free form, 22, 23 — *Leptospironympha variabilis*, lateral view of the entire organism

Pl. VII. *Eucomonympha nana*. Total preparations; impregnation according to Uhlig. Vertical view

24 — focus onto flagellar rows, 25 — focus onto parabasal filaments, 26 — focus onto dictyosomes.

Pl. VIII. *Leptospironympha popularis* 27–30, *L. variabilis* — 31. Total preparations, impregnation according to Uhlig. 27, 28 — lateral view, focus on to flagellar bands. 29 — lateral view, focus onto dictyosomes. 30 — vertical view, focus onto flagellar bands at the base of cap. 31 — vertical view of the rostrum

Pl. IX. Total preparations; impregnation according to Uhlig (34 — Heidenhain's hematoxylin) 32 — lateral view of *Leptospironympha popularis* and *L. lepida*. 33–36 — *Leptospironympha lepida*. 33, 36 — focus onto flagellar bands, 34 — focus onto nucleus, 35 — focus onto dictyosomes

Pl. X. 37–39 — *Bispironympha unica*, total preparations; impregnation according to Uhlig. Focus onto flagellar bands and nucleus. 40 — *Notila proteus ussuriensis*, total preparation Heidenhain's hematoxylin, fusion of pronuclei.

Pl. XI. Total preparations, impregnation according to Uhlig. Lateral view. 41, 42 — *Saccinobaculus spatiatus*, 43 — *S. scabiosus*, 44 — *S. gloriosus*, 45 — *Oxymonas nana ussuriensis*

Pl. XII. Total preparations, impregnation according to Uhlig. 46 — pseudocyst of *Urinympha cirrata*, 47, 48 — the stages of fusion of pronuclei in *Eucomonympha nana*, 49 — zygote of *Eucomonympha nana*.

Pl. XIII. Total preparations, impregnation according to Uhlig. 50 — gametocyte of *Leptospironympha variabilis*, 51 — gametocyte of *L. popularis*, 52 — fusion of nuclei in *Saccinobaculus scabiosus*, 53 — zygote of *S. spatiatus*

## Abbreviations on the Text-figures and Plates

A — Atractophore, Ax — Axostyle, Axf — Axostyle fibrils, Axr — Axostyle ring, Axsh — Axostyle sheath, C — Cap, Cf — Circular fissure, D — Dictyosomes, Dr — Dense region of ectoplasm, Exl — External lay of ectoplasm, Fla — Flagellar area, Flb — Flagellar band, Flr — Flagellar row, fas — fasciculus of flagella, G — Grooves in external layer of ectoplasm, g — granules, Inl — Internal layer of ectoplasm, N — Nucleus, n — pronucleus, Ns — Nuclear sleeve, Pb — Parabasal body, Pfl — Parabasal filament, Pp — Parabasal plate, Pth — Parabasal thread, Rt — Rostral tube, V — Vacuoles, Wp — Wood particle

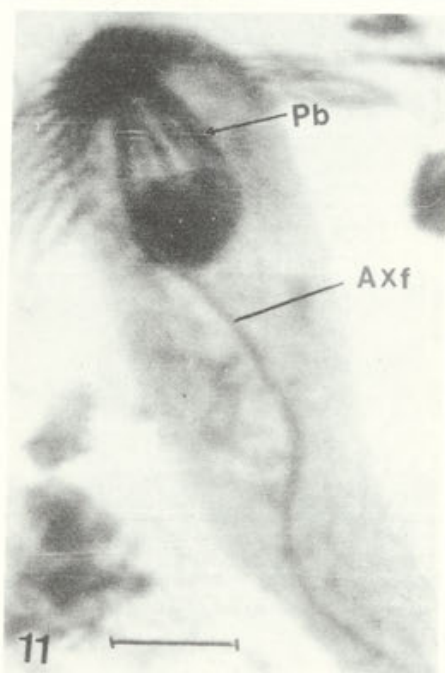The scale in all text-figures and plates corresponds to 10 $\mu$m
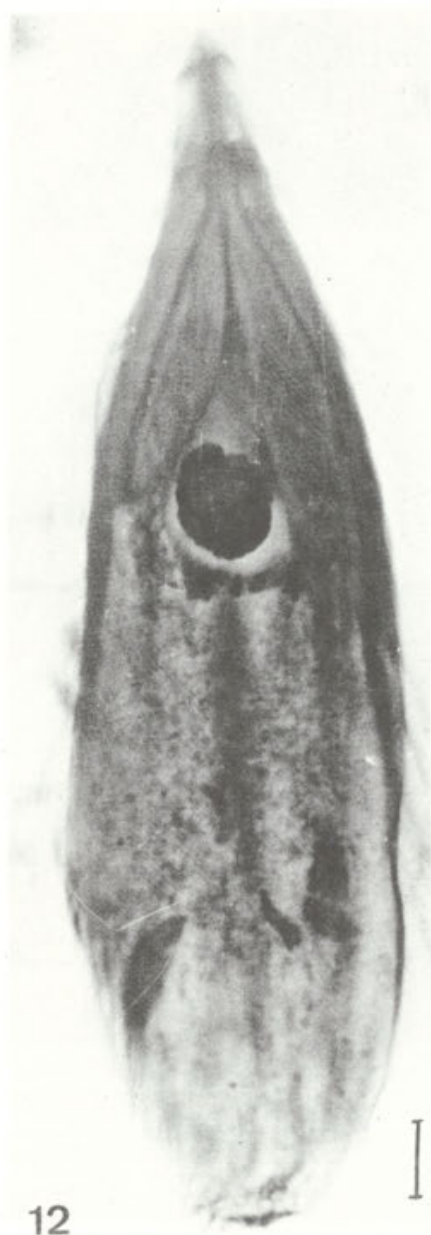
PLATE I



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PLATE II
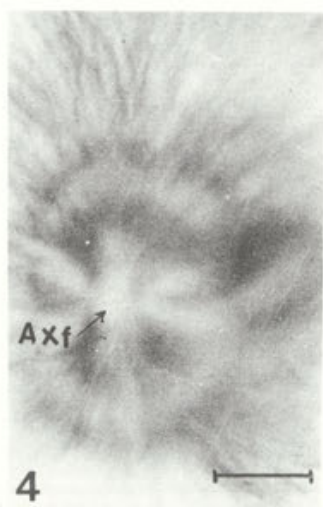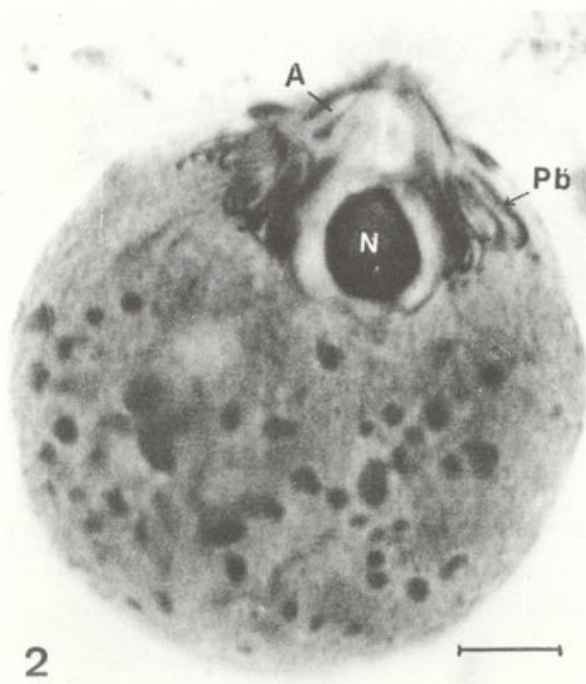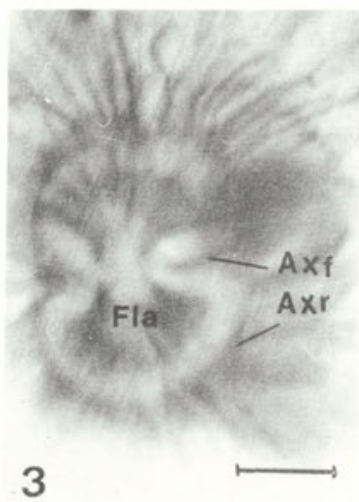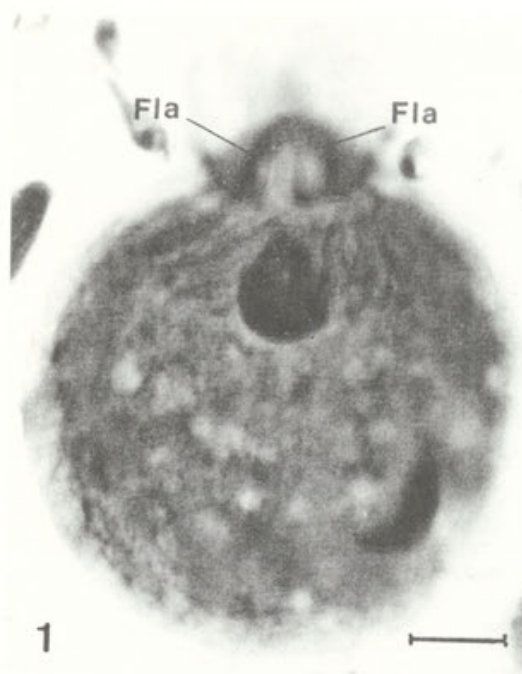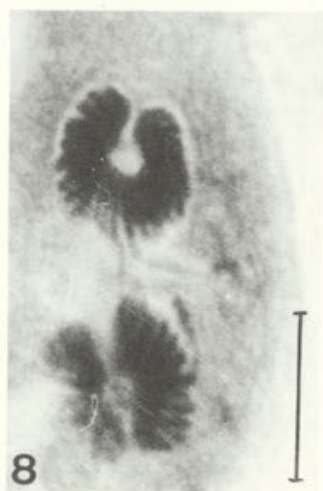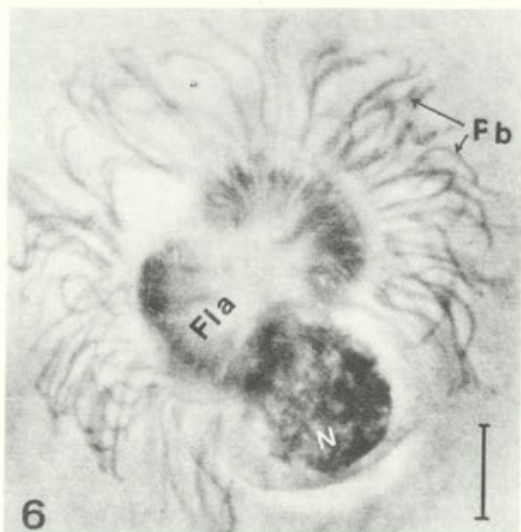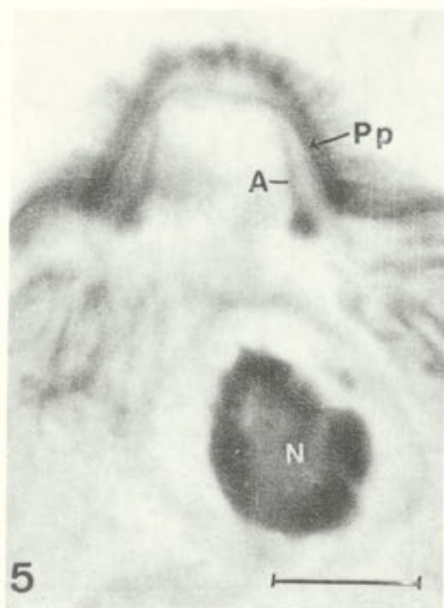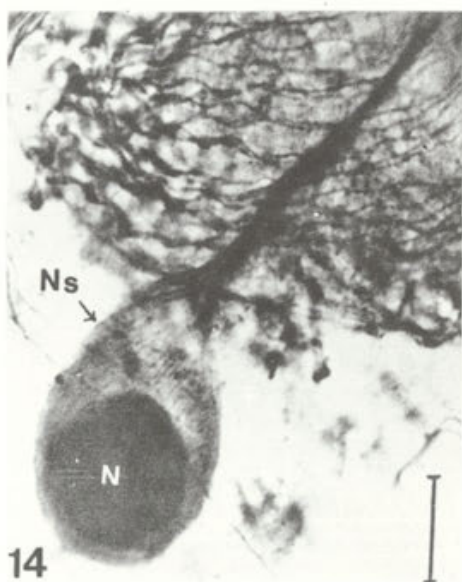








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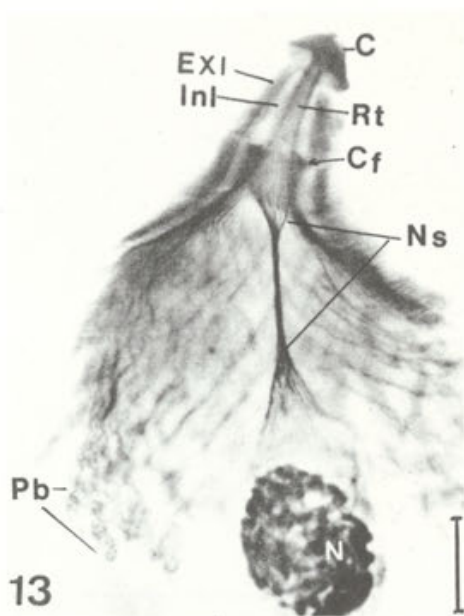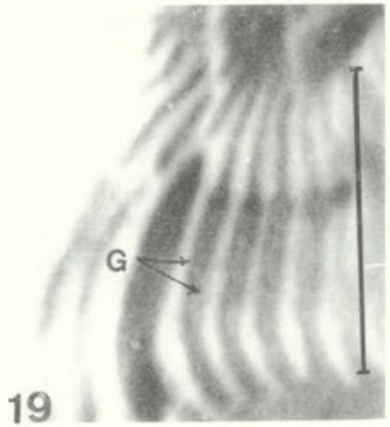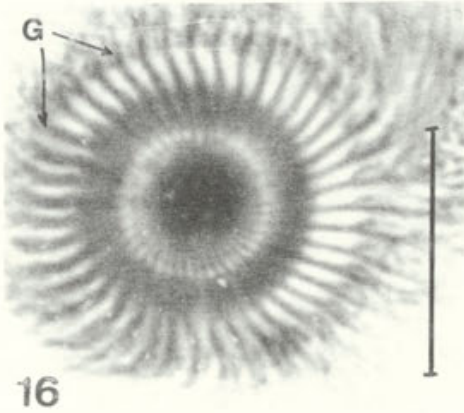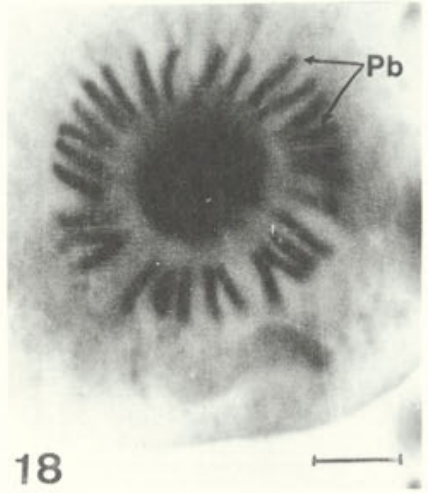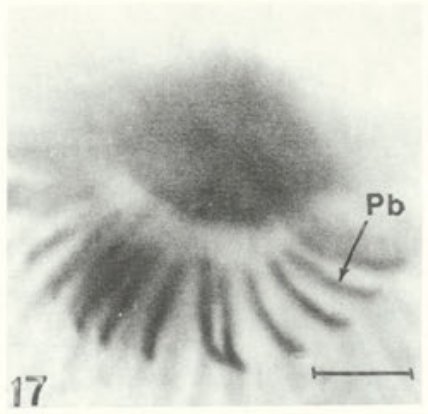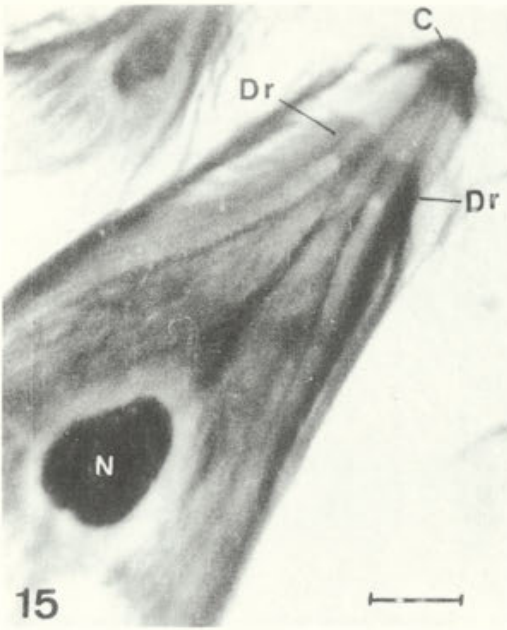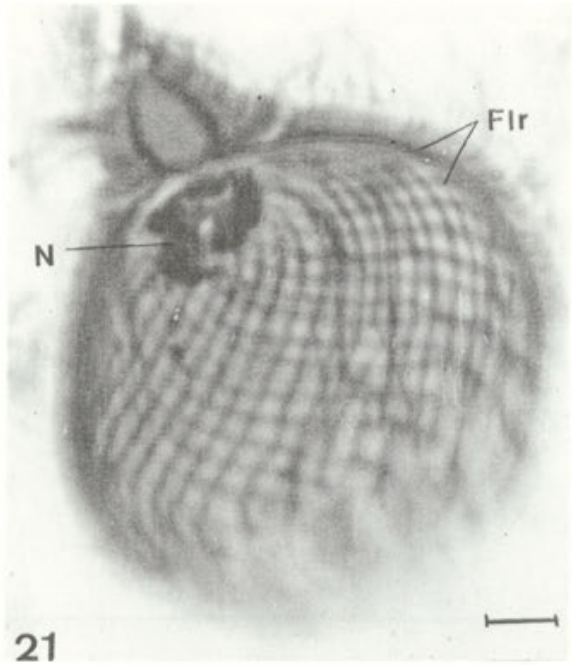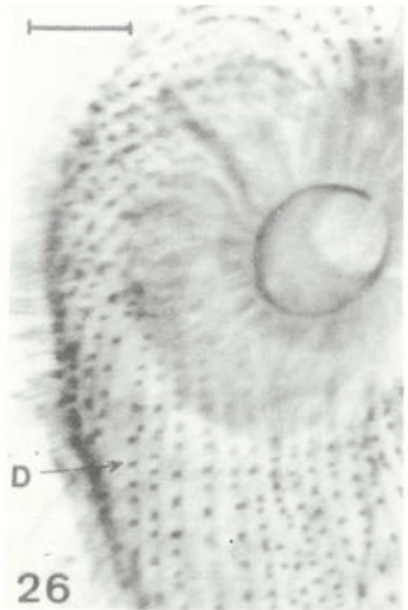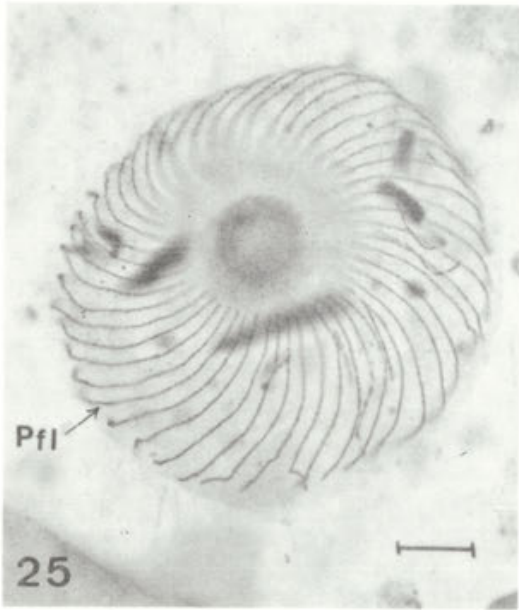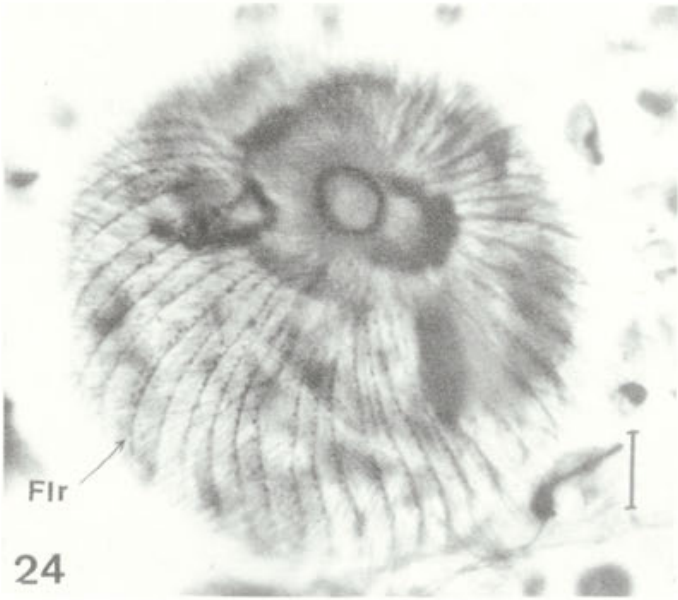auctor phot.

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PLATE V
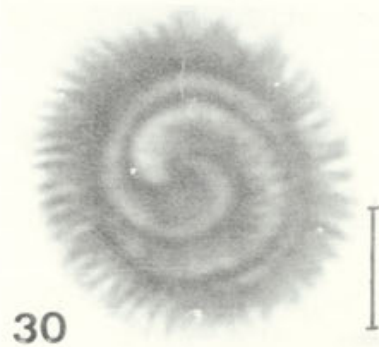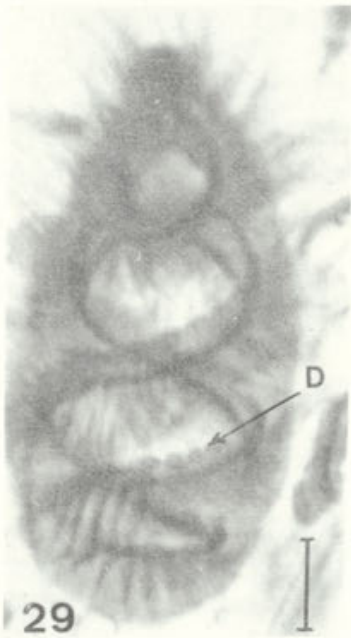


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PLATE VI

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PLATE VIII



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PLATE XI



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PLATE XII



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PLATE XIII
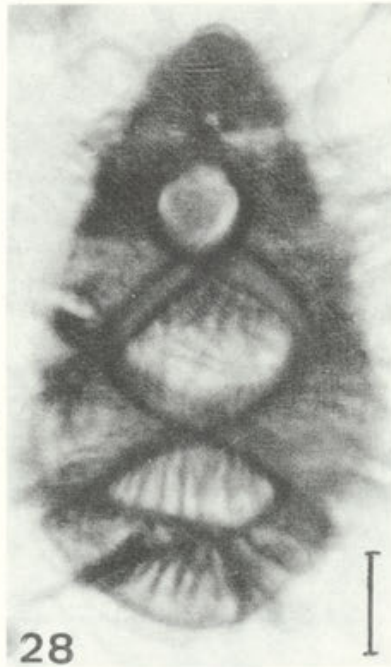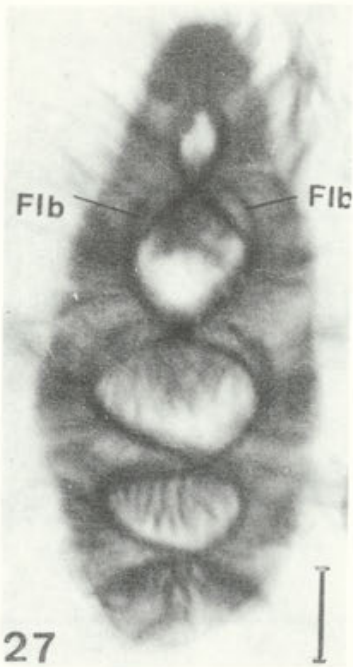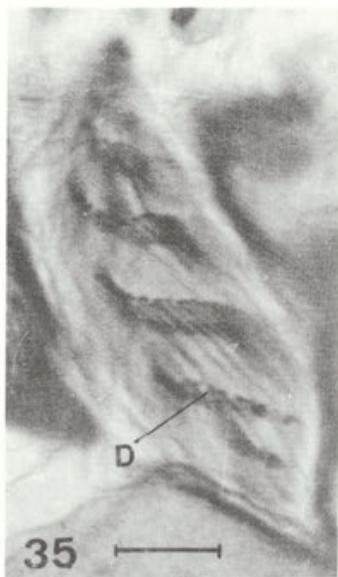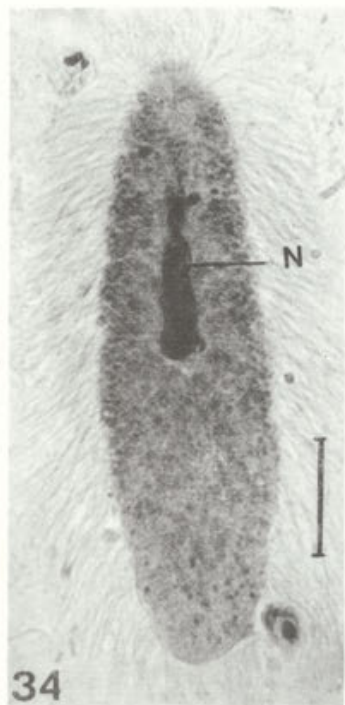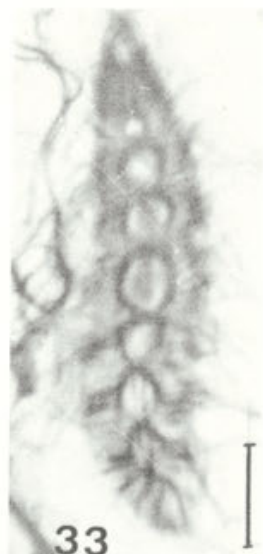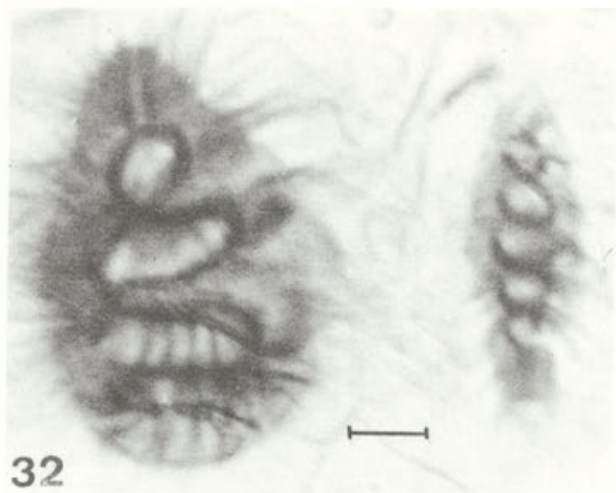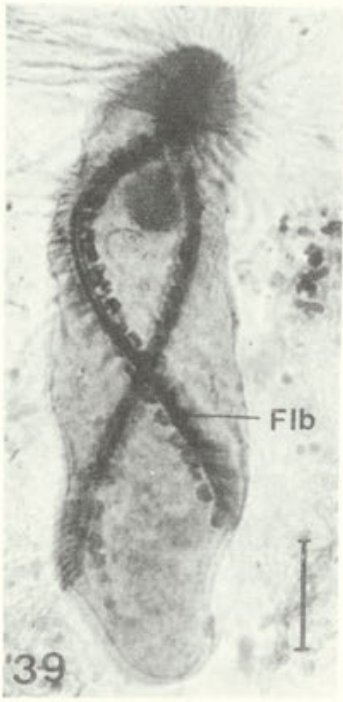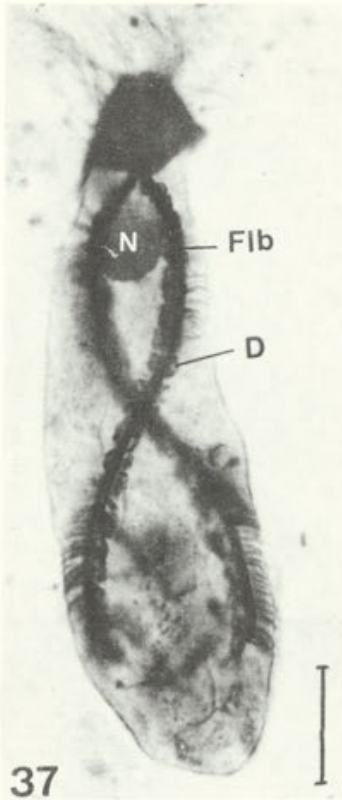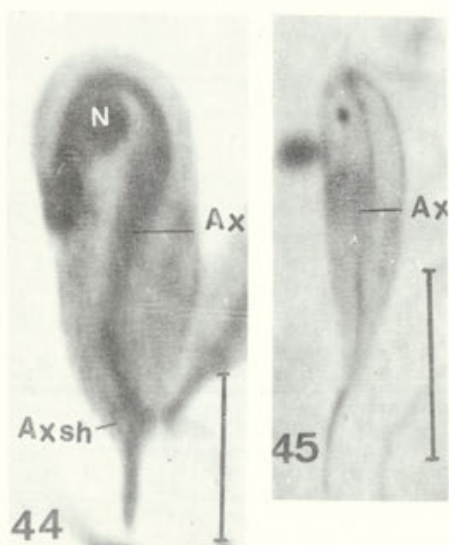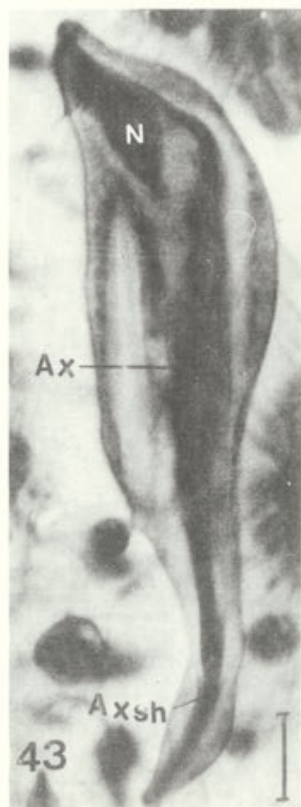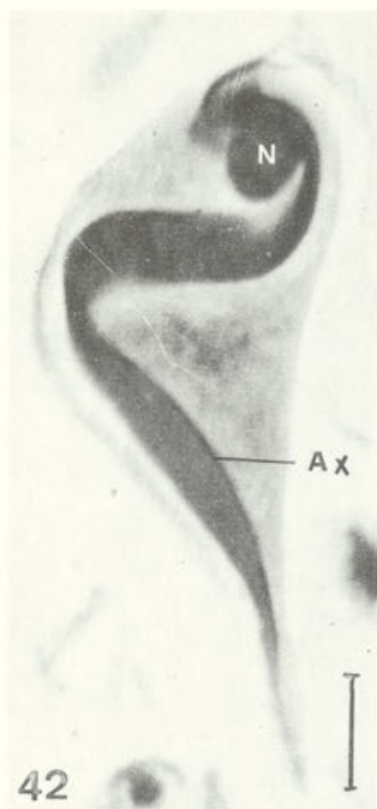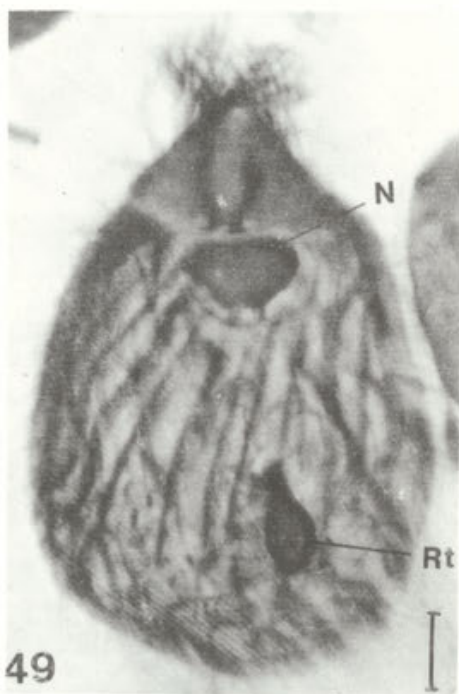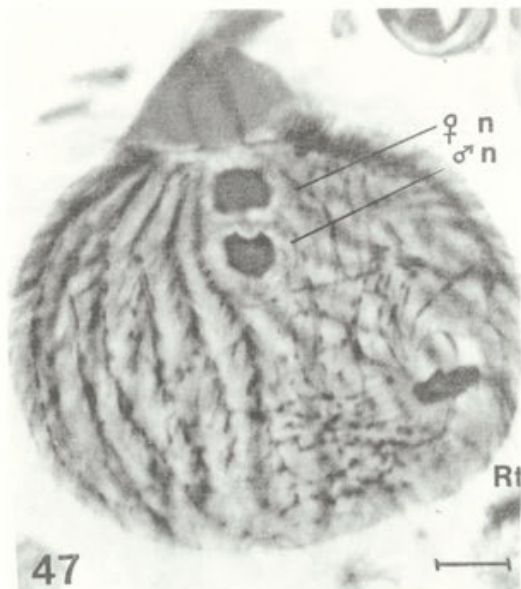


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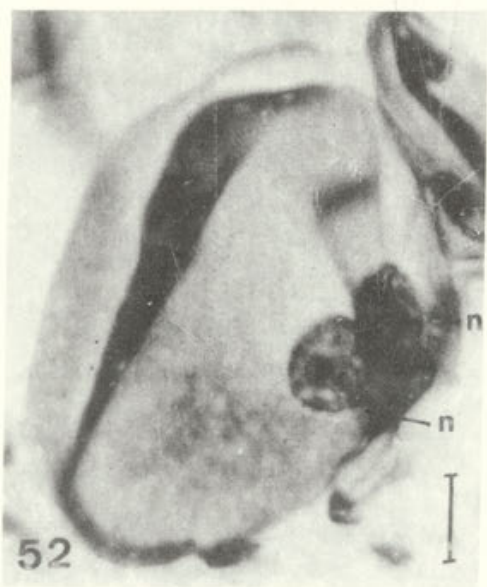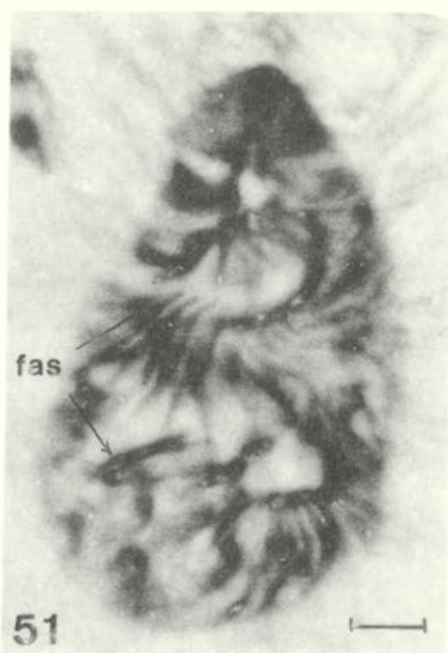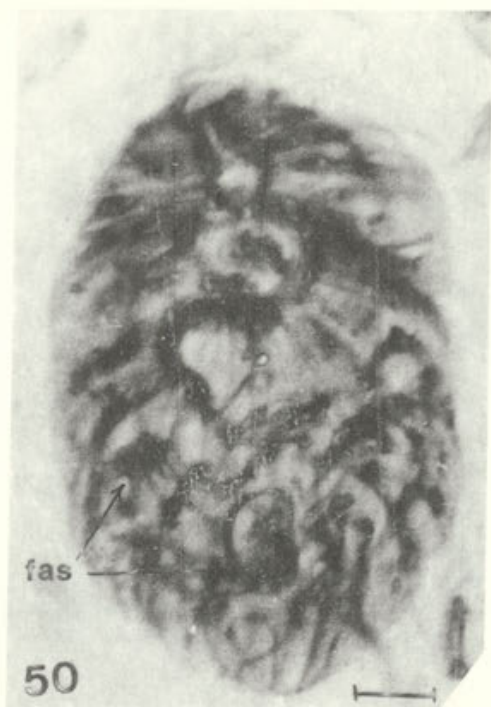
Phyllis Clarke BRADBURY

## *Conidophrys pitelkae*, a New Species of Pilisuctorian from Cuticular Hairs of *Crangon crangon* (Linneus)

*Synopsis*. A new species of *Conidophrys* is described from shrimp at Roscoff, thereby expanding the family (*Conidophryidae*) to include ecto-symbionts of decapod *Crustacea*. *Conidophrys pitelkae's* size and shape, as well as the shape of its macronucleus differ somewhat from previously described species. Protargol impregnations of tomitogenesis show a direct continuity between the infraciliature of the adult and the more complex infraciliary pattern of the tomite. Protargol also reveals a blind-ending tubule opening on the tomite's ventral surface. This tubule has never been reported from *Conidophrys*, and its function is unknown.

## Introduction

Pilisuctorian ciliates spend most of their lives perched on cuticular hairs of *Crustacea*, growing at the expense of their hosts (Chatton et Lwoff 1934, 1935). Their structure is so specialized that they cannot be placed with confidence in any existing order of ciliates, although Chatton et Lwoff (1936), who first described the family, tentatively placed them in the *Trichostomatida*. Although rarely noted, probably they are widespread since *Conidophrys pilisuctor*, the more common species, has been reported from France (Chatton et Lwoff 1934, 1936), England (Jones and Khan 1970, Mohr and LeVeque 1948), Scandinavia (Fenchel 1965) and California (Mohr and LeVeque 1948). The family has been characterized as parasites of edriophthalmic *Crustacea*, but now the discovery of *Conidophrys* on shrimp means that the definition of the family must be enlarged.

*Conidophrys* completes its life cycle on a single host. A ciliated migratory stage, the tomite, fastens by its cytostome to the tip of a cuticular hair, encysts, and loses its cilia. The encysted ciliate, now the feeding individual (trophont), grows in length and after achieving a certain size, begins to form a tomite at the free end of its body. As one tomite completes its differentiation and separates from the tomont, another begins to form directly beneath the first so that eventually tomonts may have three or four tomites stacked one above the other. The tomites are released one at a time,

and each settles on a cuticular hair and repeats the cycle. No sexual stages have ever been observed.

In the summer and fall of 1973 a species of *Conidophrys* was observed on the cuticular hairs of *Crangon crangon* at Roscoff and Penpoull. Because it differs from previously described species in certain structural characteristics and also by its choice of host, it is herein described as a new species and named *Conidophrys pitelkae* for Dr. Dorothy R. Pitelka.

## Materials and Methods

At the Station Biologique de Roscoff, *Crangon* was collected on the incoming tide by dip net. Shrimp were pinched in half by forceps and each half examined under low magnification for *Conidophrys*. Fragments of exoskeleton with infected hairs were fixed in 3% glutaraldehyde in sea water and later impregnated with protargol (K i r b y 1950). Living organisms were observed by phase contrast microscopy.

## Observations

### The living organism

Perhaps the dearth of descriptions of species of *Conidophrys* is due to their immobility and inconspicuous appearance. On a relatively large crustacean like a shrimp, *Conidophrys* can easily escape notice, especially as infections on adult shrimp are usually sparse, 20 ciliates being a relatively heavy infection. The mature trophonts on the tips of their hairs are attenuated inverted pyramids, always completely motionless, long and slender, with a colorless granular cytoplasm and without cilia or any other obvious indication of their taxonomic class. Each trophont has a water expulsion vacuole usually in diastole, in the proximal half of its body. (In this report, the adjectives, proximal and distal, refer to the orientation of the ciliate relative to the host.) Only under high magnification is the cyst wall easily visible. Trophonts vary in length from tiny tear-drop-shaped, recently settled and transformed tomites (30 μm) (Fig. 3) to long, slender, predivision forms (100 μm). However, on adult hosts, by far the most common form of *C. pitelkae* is the tomont with a single tomite forming (Fig. 1, 2). Probably the tomont continues feeding during reproduction, because even after a tomite leaves the cyst, the cytoplasm of the tomont seems to elongate to replace the released tomite. Growth stops at the host's molt, and tomonts with four or five tomites in their distal extremities may be observed during the last stages of molting. The cyst itself has no pore or operculum. During excystment the tomite's water expulsion vacuole expands, and the cilia on its ventral surface (proximal to the tomont) beat strongly. The tomite appears to push its dorsal surface

against the distal cyst wall until finally the wall breaks and the tomite's dorsal surface bulges through the rent. However, the tomite does not just pop out. One end slips out first. Chatton et Lwoff (1936) have determined that in *C. pilisuctor* the same end of each tomite escapes first, and since the tomite swims in this direction, this end is anterior.
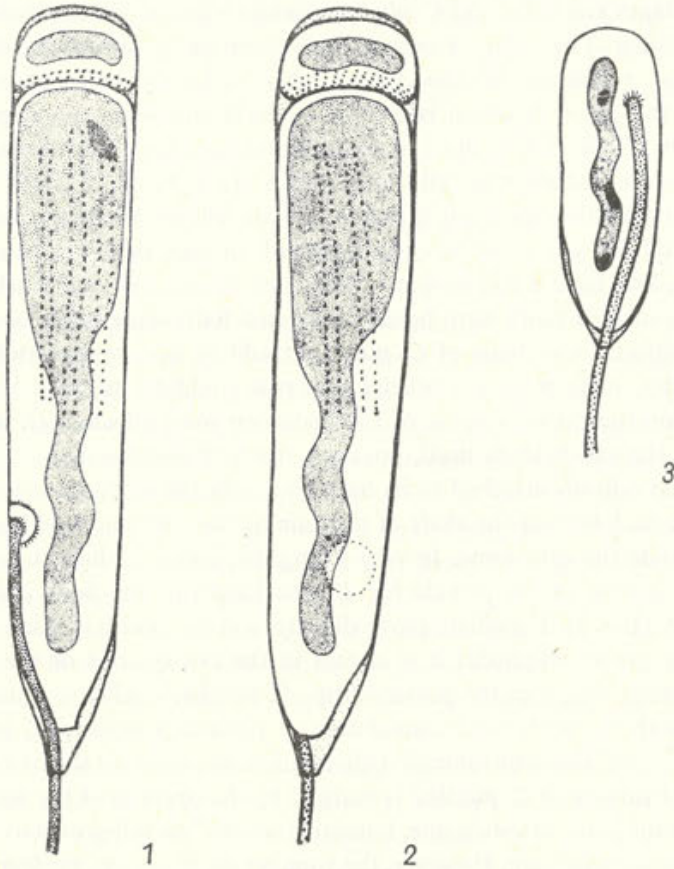


Fig. 1–3. 1 — A left lateral view of a tomont of *Conidophrys pitelkae*. The cytosome attached to the host's cuticular hair marks the ventral surface. A tomite has been formed on the tomont's distal surface but not yet released. 2 — A right lateral view of the same tomont. The water expulsion vacuole is near the proximal end of the body. 3 — A very young trophont with a serpentine macronucleus containing endosomes and a cytostome in the distal end of the body

The empty cysts remain on the cuticular hairs, and occasionally a single tomite may be seen in an otherwise empty cyst, implying that the entire tomont divides into tomites without leaving a residuum. The cyst itself is thin and transparent and remains so after protargol impregnation. The living ciliate fills the entire cyst except for a small cone at the proximal end which surrounds the cuticular hair. After fixa-

tion the organism shrinks causing the unaffected cyst to form loose folds around its body. Living tomonts bearing at least one tomite ranged from 80 to 145 μm in length and 18 to 27 μm in width, but fixed animals had shrunk 40–45%, averaging 70 μm by 12.5 μm.

Supposedly, pilisuctorians are fixed on secretory hairs and feed on the secretion that otherwise would be released by the hair. *Crangon crangon* is covered with hairs of various shapes and sizes, and *C. pitelkae* seems to attach to hairs that differ visibly from one another. The ciliate is easiest to discover on the downward-directed hairs outlining the periphery of the pleura. Not all of these hairs are alike, but by far the most common is a long stout bristle with two opposing lateral rows of barbs. *C. pitelkae* is most likely to fix on the apex of this bristle. However, it seems most improbable that all these bristles are secretory. In only one instance did an infected bristle appear any different than its fellows. When the old exoskeleton was slipped off the new exoskeleton in a premolt shrimp, the inner base of an infected hair seemed to have a cell or some substance associated with it while the bases of all the uninfected hairs were bare. Uninfected hairs were never observed giving off any secretion. Individuals of *C. pitelkae* could be observed scattered along the row of bristles, or four or five bristles in a row could be infected. When the very short hairs on the dorsal surface of the abdomen were infected, *C. pitelkae's* cyst extended to the exoskeleton itself, covering the hair completely.

The ciliate is firmly attached to its hair. Probably the strength of the attachment is due to the enclosure of the shaft of the hair by the cyst since the hair itself stops short just inside the cytostome. In very young trophonts the hair runs in a straight longitudinal groove in the pellicle for 2/3 the length of the body before it enters the cytostome (Fig. 3). The ciliate grows distally, and the mouth is gradually displaced posteriad. In grown trophonts it is always in the last quarter of the body and in a few specimens almost at the posterior tip. *C. pitelkae* is always completely colorless, never with the posterior accumulation of pigment visible in *C. pilisuctor*.

By convention, the cytostome of a ciliate identifies its ventral surface. The opposite or dorsal surface of *C. pitelkae* is marked by the opening of the water expulsion vacuole near the plane of cytostome. Lateral "furrows" on full-grown living trophonts represent the infraciliature. However, the younger the trophont, the less likelihood of observing an infraciliature either in living or silver impregnated material. When, after silver impregnation, the infraciliature first becomes visible, in young growing trophonts, it appears as very delicate thin lines, unlike the appearance of kineties in mature trophonts or in other ciliates. Chatton et Lwoff (1936) have described the almost instantaneous metamorphosis of the tomite to the young trophont after attachment, and they also were mystified by the immediate disappearance of the actively beating ciliature and the conspicuous infraciliature. As in other ciliates probably some form of the infraciliature persists during the growth of the trophont, but the trophont itself is never ciliated. The segments of its kineties that migrate into the tomite become ciliated during tomitogenesis.

Drawings of *C. pilisuctor* and *C. guttipotor* (Chatton et Lwoff 1936) indicate that these species are shorter and stouter than *C. pitelkae*. The hairs on isopods and amphipods to which *C. pilisuctor* and *C. guttipotor* attach, bend, perhaps owing to the ciliate's weight or perhaps for some physiological reason. The bodies of the ciliates themselves are slightly flexed, the ventral surface being convex and the dorsal surface concave. In contrast, *C. pitelkae* is longer and proportionately more slender than the species on edriophthalmic *Crustacea*. Its body is long and attenuated without a concave or convex surface. The hair to which it attaches does not bend and consequently the ciliate is borne at a right angle to its host following the natural orientation of the hair.

Protargol impregnations

Protargol impregnations of *C. pitelkae* confirm that the infraciliature of this species, like that of *C. pilisuctor* and *C. guttipotor* (Chatton et Lwoff 1936) is confined to the right and left surfaces of the ciliate (Fig. 1, 2). The left surface has a field of 6 kineties, arranged in 3 pairs more or less parallel to the ciliate's longitudinal axis. On the right surface there is a field of 4 kineties (2 pairs) also oriented longitudinally. The distal portion of these 2 fields form the tomite's ciliature during tomitogenesis, perhaps the only function of the infraciliature in the trophont.

Although the cytostome is very small, some differentiation can be discerned after protargol impregnation. The close-fitting junction of the hair and the cytostome is outlined by a dark deposit. The cytostome forms a chamber sunk in the cytoplasm, circular in outline, sometimes with dark lines radiating from the junction. The circle has a dark border and sometimes thin rays radiate from it into the cytoplasm (Fig. 1, 3). The nature of the food and how it is ingested is still a mystery. Recognizable food vacuoles have never been observed. When after long compression under a coverslip, an organism degenerates, its cytoplasm rapidly becomes vacuolate, but how these vacuoles arise is also unknown.

Protargol impregnations of the trophont's macronucleus reveal that it is a slender serpentine column extending almost the entire length of the body and increasing in length as the organism grows. Several large endosomes are scattered along its length (Fig. 3). With the beginning of tomitogenesis, the shape of the macronucleus changes markedly, thickening along its length and fanning out dorso-ventrally in the distal half of the body. Therefore in protargol preparations, the shape and extent of the macronucleus appears to vary according to the surface of the ciliate that is uppermost. The macronucleus observed edge-on resembles the serpentine column of the young trophont, and only by focussing optical sections through it, can its extent be estimated. The macronucleus observed broadside occupies almost the whole width of the distal half of the ciliate (Fig. 1, 2).

During tomitogenesis the endosomes disappear, and fine lines oriented more or less longitudinally appear within the macronucleus. Macronuclear division is a simple scission of its distal extremity. The tomite's macronucleus is therefore a rod with

tapering ends. The micronucleus is difficult to recognize in the young trophont, but during tomitogenesis a large spindle-shaped micronucleus is easily visible close to the left surface of the distal tip of the macronucleus (Fig. 2).

Tomitogenesis

Chatton et Lwoff (1936) have described a complicated morphogenesis of the tomite in *C. pilisuctor*, based on the observation of many living specimens. The following description of tomitogenesis and the infraciliature of the tomite in *C. pitelkae* is based on protargol-impregnated tomites developing or retained within the cyst.

The first visible sign of beginning tomitogenesis is the rupture of the kineties in the same plane in each of the two fields. The distal fragments of the two fields, the anlagen of the tomite's infraciliature, incline toward the ventral surface (Fig. 4 A, B).
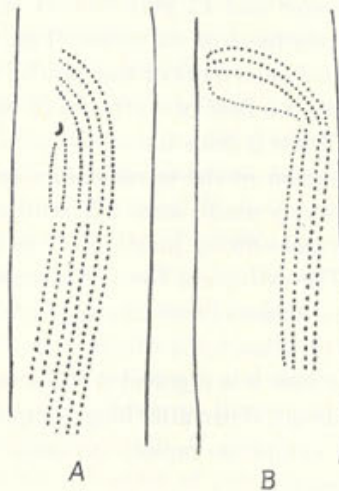


Fig. 4. A — The left lateral field of kineties early in tomitogenesis. The anlage of the tomite's left lateral ciliature has separated from the tomont's infraciliature and begun to migrate to a position normal to the direction of the tomont's kineties. A granule can be seen distal to kineties 9 and 10. B — The kineties of the right lateral field of the same organism, showing that the right anlage of the tomite's ciliature moves into position first. Kinety 1 has diverged from its fellows

The distal tips of the kineties in each field converge to a point and the kineties are no longer so obviously paired. In the right field of kineties, the most ventral kinety of the four is now the most proximal. It separates from its fellows along all its length except for its distal end which remains close to the distal ends of the other three kineties. Instead of 2 pairs the field is now divided 1 and 3, and the cytoplasm in the gap begins to invaginate. In the left field the ventral pair of kineties is always a little shorter than the others. A granule just distal to it impregnates with protargol. The cytoplasm distal and dorsal to the short pair begins to invaginate, carrying this pair and the middle pair of kineties on the invaginating surface. The third pair remains

at the edge of the growing depression. These infoldings of the cytoplasm are the first signs of the physical separation of the tomite from the tomont. The anlage of the right field is the first to become perpendicular to the tomont's long axis, while the anlage of the left field remains for some time at only a slight angle to the kineties from which it originated (Fig. 4 A, B). After both new fields are at 90° to the tomont's nfraciliature and cytoplasmic fission is well-advanced, the schema for the disposition of the kineties in the tomite can be discerned (Fig. 5).
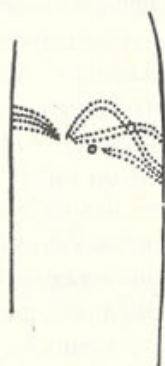


Fig. 5. Later in the morphogenesis of the tomite of *Conidophrys pitelkae* when both anlagen of the ciliature have lost all connection with the tomont's infraciliature and are now in position for the tomite's subsequent allometric growth

Allometric growth of the cytoplasm and the kineties change the final pattern that is achieved, but each kinety in the tomite can be traced back to a kinety from the left or right field (Fig. 6). In the tomont the kinety on the right nearest the cyto-
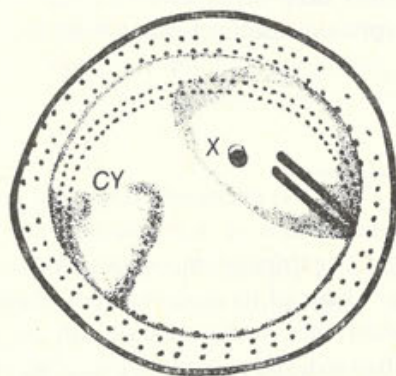


Fig. 6. Ventral view of the tomite of *Conidophrys pitelkae*. The tube is marked X, and the cytostome is Cy

stome gives rise to the kinety at the right of the tomite's cytostome, and by definition is kinety 1. Diverging very early in tomitogenesis from its three companions, in the mature tomite it originates in the depression around the cytostome. In a few tomites faint dark-staining rays extend from kinety 1 toward the cytostome. Kinety 1 itself proceeds across the floor of the depression to the lateral rim of the organism where it veers posteriad and runs along the right edge of the tomite to end posterior-

ly. The other 3 kineties form a ribbon around the right side of the tomite, and where the 3 are joined by kinety 1 the ribbon is 4 kineties wide. On the left side only kineties 5 and 6, the pair most distant from the cytostome, form a ribbon. Kineties 7 and 8, still distinctly paired, run along the anterior wall of the oral depression and into another depression at the posterior, over its floor and along its posterior wall, apparently ending at the tomite's posterior rim. Kineties 9 and 10 are medial, originating in the posterior third of the body on the floor of the posterior depression. Originating from the shortest pair in the field, they are now only a few micra long, but more intensely impregnated than the other kineties (Fig. 6). They end next to kineties 7 and 8 at the tomite's posterior rim. The dark granule first observed early in tomitogenesis, just distal to kineties 9 and 10, differentiates into a tiny tube that plunges into the cytoplasm at a right angle to the ventral surface. Although its walls do not impregnate with protargol, its blind end impregnates very intensely. It retains its spatial relationship with kineties 9 and 10 and is completely separate from the cytostome described by Chatton et Lwoff (1936), a deeper, more conspicuous invagination some distance anterior to it. Because it is only a micron or two in diameter, probably only electron microscopy will give a clue to its function.

In summary, the mature tomite is a small, somewhat flattened, hemisphere (25 μm by 12 μm in life), encircled by a fringe of cilia formed from kineties 2, 3, and 4 on the right and 5 and 6 on the left. Its water expulsion vacuole is posterior, a little to the right of kineties 9 and 10. The micronucleus is at the right of the origin of kineties 9 nad 10. The elongate oval or rod-shaped macronucleus is oriented anteroposteriorly at the right of the micronucleus. The tomite's ventral surface has two rather deep depressions separated by a prominent ridge of cytoplasm, the anterior depression leading to the cytostome, and a posterior heavily ciliated depression (Fig. 6).

## Discussion

The two previously described species of *Conidophrys* differ from one another in minor details of their structure and in their hosts, *C. pilisuctor* being on amphipods and *C. guttipotor* on isopods. Although *C. pitelkae sp. n.* resembles these two species, the shape of its macronucleus, the slightly different proportions of its body, and its occurrence on decapods justify its description as a new species. When it was first observed, the possibility that it was *C. pilisuctor* on a new host was considered. *Conidophrys* has never been reported from Roscoff. Chatton et Lwoff (1936) state that species of amphipods and isopods that in other regions show heavy infections of *Conidophrys*, at Roscoff are never infected. A cursory examination of amphipods and isopods from the same habitat as infected *Crangon* revealed no *Conidophrys*. Therefore it seems unlikely that the shrimp at Roscoff are infected by the *Conidophrys* of edriophthalmic *Crustacea*, and so the definition of the genus must be expanded to include species found on decapods.

The diet and manner of feeding in *Conidophrys* has yet to be explained. Chatton's et Lwoff's assertion (1936) that *Conidophrys* feeds on the secretions of special cuticular hairs has not been tested. With the exception of a *Conidophrys* from *Jaera ishiosetosa* (Jones and Khan 1970) (described from formalin-fixed collections of the isopods), *Conidophrys* is always attached to a hair of its host. Since individuals are always enclosed in a cyst, the inference is that the ciliate draws up substances from the host through the hair and grows at the expense of its host. Whether these substances are secretions normally released into the water or another of the hosts' internal fluids is yet to be determined. Jones and Khan (1970) offer no suggestions as to how the species on *J. ischiosetosa* feeds. They describe it as attached to the host's exoskeleton by a thick stalk which never encloses a hair. Although in some respects, their drawings resemble *C. guttipotor* from the isopod, *Spheroma serratum*, the existence in their species of a cytopharynx distal to the infraciliature is a significant structural difference from all other species of *Conidophrys*.

Besides *Conidophrys*, two other ciliate parasites of *Crustacea*, the apostomes, *Synophrya* (Chatton et Lwoff 1935) and *Terebrospira* (Bradbury and Goyal 1974) also feed and grow while encysted. *Synophrya*, an invader of the gills of portunid crabs, is surrounded by a stout cyst wall as well as host cuticle, yet it enlarges at the expense of its host to become several hundred micra in diameter. *Terebrospira* dissolves galleries through the endocuticle of shrimps while it is surrounded by a thin cyst wall. The infraciliature of these genera like that of *Conidophrys* is dedifferentiated, but in the case of the apostomes, the kineties are meridional (Bradbury et al. 1974, Chatton et Lwoff 1935) rather than restricted to two fields. A significant difference in structure between encysted feeding apostomes and pilisuctorians is the complete lack of a cytostome in the former. However, the apostomes are surrounded by their food while *Conidophrys*' only contact with its putative food supply is at the hair. Protargol stains of this junction indicate a complexity which only the electron microscope can resolve.

## Species Diagnosis of *Conidophrys pitelkae*

Life cycle: trophonts and tomonts on the cuticular hairs of *Crangon crangon* (Linneus). Migratory tomites differentiated singly and freed singly from apex of the tomont to reinfect host or spread infection to new individuals.

Trophont: body — an elongate slender cone, enclosed in thin transparent cyst, attached proximally by cytostome to cuticular hair of host. Four short, straight, unciliated kineties, longitudinally oriented on trophont's middle right surface. Similar field of six kineties oriented longitudinally in middle left lateral surface. Macronucleus: long, serpentine, with conspicuous endosomes.

Tomont: trophont transforms *in situ*. Kinetal fields divide transversely. Distal fragments of both fields orient at right angles to parent kineties, migrate to tomite differentiating on tomont's distal surface.

Macronucleus: long, thickened. fan-shaped at distal end. Spindle-shaped micronucleus at left distal end of macronucleus.

Tomite: flattened hemisphere with curved surface dorsal. Two large ventral depressions, anterior leads to cytostome. Kineties 2, 3, 4, 5, 6 form ciliary fringe partly encircling tomite's lateral surface. Kineties 1, 7, 8, 9, 10 form ventral ciliature. Tubule (function unknown) opening at surface of anterior wall of posterior depression. Rod-shaped macronucleus oriented antero-posteriorly, micronucleus in posterior half of body at left of macrcnucleus.

## RESUMÉ

La description d'une nouvelle espèce de *Conidophrys* sur une crevette de Roscoff étend le parasitisme externe de cette famille (*Conidophryidae*) aux Crustacés décapodes. Les dimensions et la forme de *Conidophrys pitelkae*, de même que la forme de son macronoyau, diffère sensiblement de celles des espèces dècrites précédemment. Des imprégnations, au Protargol, de la tomitogenèse font apparaître une continuité directe entre l'infraciliature de l'adulte et l'organisation infraciliaire, plus complexe, du tomite. Le Protargol révéle également l'ouverture d'un tubule aveugle sur la surface ventrale du tomite. Ce tubule n'avait jamais été signalé chez les *Conidophrys* et sa fonction est inconnue.

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## *Drilocineta perionyxi* sp. n. (*Hysterocinetidae*) a New Thigmotrich Ciliate from the Earthworm *Perionyx excavatus* E. Perrier of India

*Synopsis.* A new thigmotrich ciliate belonging to the genus *Drilocineta* Raabe, 1972 obtained from the posterior part of the intestine of the oligochaete *Perionyx excavatus* E. Perrier occurring in Barrackpore, Dist. 24-Parganas, West Bengal, India is described in this paper. It is named *Drilocineta perionyxi* sp. n. This is the first record of the genus in India.

## Introduction

The family *Hysterocinetidae* Diesing, 1866 contains some interesting endoparasitic thigmotrich ciliates inhabiting the posterior part of the alimentary tract of certain molluscs and oligochaetes. While revising the family *Hysterocinetidae*, Raabe (1972) created the genus *Drilocineta* designating *Drilocineta libyodrili* (de Puytorac 1968) as its type species and included *Drilocineta pontodrila* (Wichterman 1942). The present paper deals with a new species of *Drilocineta* obtained from the posterior one third of the intestine of the earthworm *Perionyx excavatus* E. Perrier occurring in Barrackpore, West Bengal, India. This is the first record of the genus in India. The type specimens are deposited in the National collection of Zoological Survey of India, Calcutta.

## Materials and Methods

One hundred and fourteen specimens of *Perionyx excavatus* collected from Barrackpore, Dist. 24-Parganas, West Bengal, India during November 1971 to September 1973 were dissected and examined. Approximately 80% of the worms were found to be infected with a thigmotrich ciliate. They were examined alive in physiological saline. For making permanent preparations the isolated specimens were fixed in Schaudinn's fluid and stained in Heidenhain's iron-haematoxylin. Klein's dry silver method was employed to observe the ciliary rows. All the measurements were taken with the aid of a calibrated ocular micrometer.

## Description

Body is elongated (Fig. 1) measuring 85–195 μm (average 125 μm) in length
and 43–85 μm (average 47 μm) in breadth. The anterior end is bluntly pointed and
slightly curved. The posterior end is truncated. The dorsal side is slightly convex
and the ventral side is incurved. At the antero-ventral region an inverted "U"-shaped
sucker with unequal ams is present. The long arm of the sucker measures 20–22 μm
and the short one 12–15 μm. The skeletal fibres within the sucker are feebly developed
and arranged in close rows parallel to arms lengthwise. In between the arms of sucker
there are five to six short ciliary rows. The long ciliary rows number from 20–22
(Fig. 2) on the ventral side and 28–30 on the dorsal side of the body. Ciliation is
dense and uniform in general. At the posterior end is located the transverse peristome
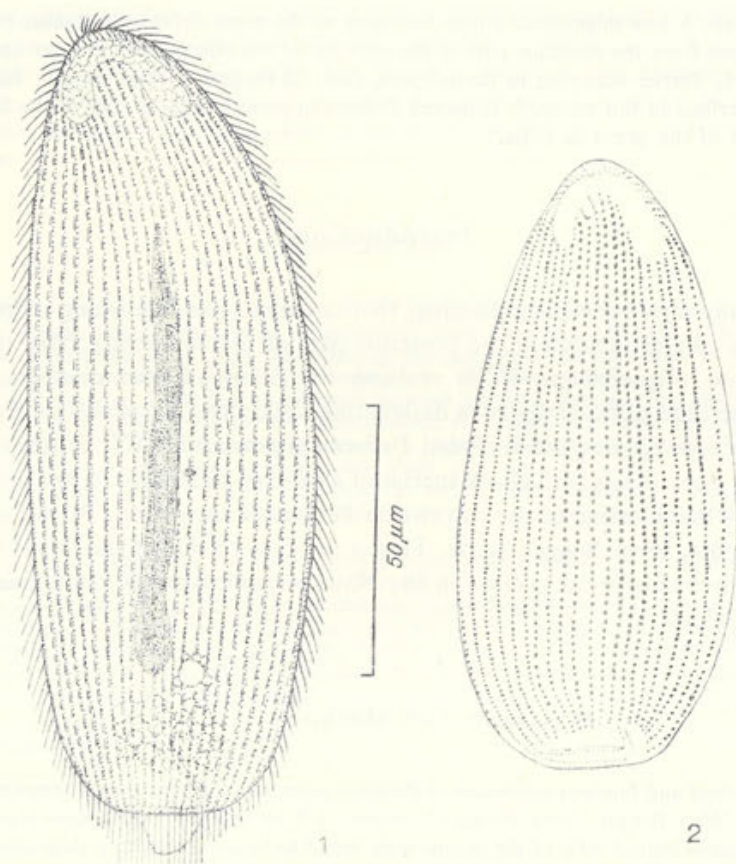


50 μm

1

2

Fig. 1–2. *Drilocineta perionyxi* sp. n. 1 — ventral view (nuclear apparatus, contractile vacuole
and food vacuoles are visible). 2 — the arrangement of ciliary rows and buccal ciliature on
the ventral surface of ciliate

bearing cilia of 7–9 μm long and a small undulating membrane. The cytopharynx measures 7–10.5 μm in length. A contractile vacuole with three to four accessory vacuoles is present just below the macronucleus. The macronucleus is long, measuring 33–99 μm (average 64 μm) in length and 3.5–6.5 μm (average 5 μm) in diameter with its posterior end rounded and anterior one acuminated. It is placed median and lies on the longitudinal axis of the body. The micronucleus is single, 2–2.5 μm in diameter and lies by the side of macronucleus (Fig. 1). The posterior part of the body contains 15–20 food vacuoles congregated below the macronucleus.

Species: *Drilocineta perionyxi* sp. n.
Host: *Perionyx excavatus* E. Perrier
Habitat: Intestine (posterior part)
Type locality: Barrackpore, West Bengal, India
Holotype: Z. S. I. Reg. No. Pt. 1750
Paratypes: Z. S. I. Reg. Nos. Pt. 1751, 1752, 1753, 1754

## Diagnosis of *Drilocineta perionyxi* sp. n.

Body elongated, size 125 μm (85–195 μm) by 47 μm (43–85 μm), anterior end bluntly pointed and curved, posterior end truncated, dorsal side slightly convex and ventral side incurved, an inverted "U"-shaped sucker of unequal arms, long arm 20–22 μm, short one 12–15 μm, skeletal fibres within sucker feebly developed, five to six short ciliary rows in between arms of sucker, 20–22 long ciliary rows on ventral and 28–30 on dorsal side, transverse peristome at posterior end with a small undulating membrane, cytopharynx 7–10.5 μm, food vacuoles congregated at the posterior part, single contractile vacuole with three to four accessory vacuoles below the macronucleus, macronucleus elongated with rounded posterior and acuminated anterior ends, 33–99 μm by 3.5–6.5 μm, placed median and parallel to linear axis of body, micronucleus 2–2.5 μm diameter located by the side of macronucleus. Paraite of the posterior part of intestine of *Perionyx excavatus* E. Perrier.

## Discussion

The long macronucleus, the short ciliary rows between the arms of sucker and the small congregation of food vacuoles in the posterior part of the form described above render it to be placed in the genus *Drilocineta* Raabe. The inverted "U"-shaped sucker and the lower number of ciliary rows on the ventral and dorsal sides differ the present species from *D. libyodrili* (de Puytorac) having the heart-shaped sucker and the greater number of ciliary rows (24–28 on the left side and ca. 80 on the right one) reported from *Libyodrilus violaceous* Beddard from Gabon, Africa.

The form and size of sucker and dimensions of both components of nuclear apparatus differ *D. perionyxi* sp. n. from *D. pontodrila* (Wichterman), characterized

by "V"-shaped sucker with unequal arms measuring 41 and 34 μm respectively, macronucleus 65 μm long and 10 μm wide and micronucleus 3×4 recorded from the marine earthworm *Pontodrilus bermudensis* Beddard from Tortugas, Florida, USA.

Hence the present form is described as a new species and named *Drilocineta perionyxi* sp. n.

### RÉSUMÉ

On décrit un nouveau cilié appartenant à *Thigmotricha*, genre *Drilocineta* Raabe, 1972, trouvé dans la partie postérieure de l'intestin de l'oligochète *Perionyx excavatus* E. Perrier provenant de Barrackpore, Dist. 24-Parganas, West Bengal, Indes. On lui a donné le non de *Drilocineta perio-nyxisp.* n. C'est la prémière fois qu'on note la présence de ce genre aux Indes.

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A. K. MANDAL and K. N. NAIR

# Myxobolus eeli sp. n. (Myxobolidae) a New Myxosporidium from Indian Spiny Eel Mastacembelus armatus (Lacepede)

*Synopsis.* A new species of *Myxobolus* (*Protozoa*: *Myxosporidia*) parasitizing the intestinal wall of the Indian Spiny eel, *Mastacembelus armatus* (Lacepède), is described in this paper. It is named *Myxobolus eeli* sp. n.

## Introduction

In March–April 1972 out of the thirty eight specimens of *Mastacembelus armatus* (Lacepede) collected from Champahati, 24-Parganas, West Bengal, India, four specimens are found to be infected by a new myxosporidian parasite of the genus *Myxobolus*. The infection is localized on the intestinal wall in the region just posterior to the stomach. From the body cavity of the same host occurring at Hyderabad, India, Qadri and Kumari (1965) described *Myxobilatus mastacembeli*. The type slides of the new species are deposited in Zoological Survey of India, Calcutta.

## Material and Methods

The cysts from the intestinal wall of the fish were removed and the liberated spores were examined in physiological saline. All measurements were taken from the fresh spores. Lugol's iodine was used for observing the iodinophilous vacuole as well as the polar filaments. For making permanent preparations the spores were fixed in Schaudinn's fluid and followed by staining in Heidenhain's iron-haemotoxylin. Spores were also stained with Leishman's stain.

## Description

Cysts are small, more or less oblong in shape, creamy-white in colour, measuring 329–400 μm in length and 200–300 μm in breadth and located in the internal wall of the intestine just below the stomach. Spores are subspherical in front view (Fig. 1)

and lenticular in lateral view (Fig. 2), measuring 10.5–14.0 μm in length (average 12.5 μm), 8.2–11.5 μm in breadth (average 9.75 μm) and 6.5–7.5 μm in thickness (average 7.0 μm). The spore wall is smooth having a uniform thickness 1–1.2 μm, with two to three triangular folds connecting the sporoplasm. The sutural line at the middle of the prominent ridge (Fig. 2) is distinct. Polar capsules, two in number,
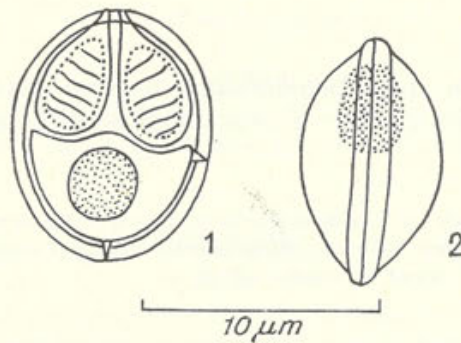


10 μm

Fig. 1. Front view of *Myxobolus eeli* sp. n.
Fig. 2. Lateral view of *Myxobolus eeli* sp. n.

are pyriform, equal in size, convergent and measuring 4.5–5.5 μm in length and 2.5–3.0 μm in breadth. Each capsule possesses a separate foramen at the apex of its terminal tube. The filament inside the polar capsule is small with six coils and in extruded condition measures 28.0–32.0 μm in length. The sporoplasm is comparatively big, more or less hemispherical in shape with three little projections, measuring 7.0 μm diameter and located below the polar capsules. The iodinophilous vacuole in the sporoplasm is prominent, circular and measures 2.5–3.0 μm in diameter.

Type host: *Mastacembelus armatus* (Lacepede)
Habitat: Inner wall of intestine
Type locality: Champahati, Dist. 24-Parganas, West Bengal, India.
Holotype: Z. S. I. Reg. No. Pt. 1587

Diagnosis of *Myxobolus eeli* sp.n.

Spore is sub-spherical in front view, lenticular in lateral view. Average size of spore is 12.5 μm (10.5–14 μm) by 9.75 μm (8.2–11.5 μm) by 7 μm (6.5–7.5 μm), spore wall smooth 1–1.2 μm with two to three triangular folds, polar capsules two, equal, pyriform, convergent, size 4.5–5.5 by 2.5–3.0 μm. Polar filament is with six coils and in extended condition measures 28.0–32.0 μm. Sporoplasm is more or less hemispherical having 7 μm diameter. Iodinophilous vacuole measures 2.5–3.0 μm in diameter, parasitic on the intestinal wall of *Mastacembelus armatus* (Lacepede).

# Discussion

Fourteen determined and one undertermined species of *Myxobolus* have so far been reported from various Indian fishes. Southwell (1915) reported one undetermined species of *Myxobolus* from *Rasbora daniconius* (Hamilton). Subsequently Southwell and Prashad (1918) described *M. nodularis* from the same host. Chakravarty (1939) described *M. calbasui* from *Labeo calbasu* (Hamilton), *L. rohita* (Hamilton) and *Cirrhina mrigala* (Hamilton) and *M. mrigalae* from *Cirrhina mrigala*. (Hamilton). The same author (1943) described *M. clarii* from *Clarius batrachus* (Linnaeus) and *M. catlae* from *Catla catla* (Hamilton), *Labeo rohita* (Hamilton) and *Cirrhina mrigala* (Hamilton). In 1948, Chakravarty and Basu described another species *M. bengalensis* from *Catla catla* (Hamilton). Tripathi (1952) while reviewing the protozoan parasites of Indian fishes described *M. indicum* and *M. sphericum* from *Cirrhina mrigala* (Ham.), *M. branchialis*, from *Barbus sarana* (Ham.), and *M. barbi* from *Barbus ticto* (Ham.), Bhatt and Siddiqui (1964) described *M. aligarhensis* and *M. ophicephali* from *Ophicephalus punctatus* (Bloch). From the same host Ray Chaudhuri and Chakravarty (1970) described *M. punctatus* from West Bengal. Karamchandani (1970) described *M. batae* from *Labeo bata* (Hamilton).

The species described here resembles *M. sphericum* Tripathi, 1952 in having triangular folds in the spore wall but differs in size (10.5–14×8.2–11.5 μm Vs. 9–9.5×7.2 μm) and shape of the spore. It is closely allied to *M. intestinalis* Kudo, 1929 described from *Pomoxis sparoides* Gilbert in shape and size of the spore. But the present one can easily be differentiated from *M. intestinalis* in having smaller polar capsules (6.5–7.5 μm Vs. 7.5–8.0 μm), lesser number of coils in the polar filaments (6 Vs. 10–12) and larger sporoplasm. Therefore, the species under report is considered as new and named *Myxobolus eeli* sp. n.

## RÉSUMÉ

Les auteurs décrivent une nouvelle espèce de *Myxobolus* (*Protozoa*: *Myxosporidia*) parasite du paroi de l'intestin d'une anguille indienne *Mastacembelus armatus* (Lacepède). Ils lui donnen le nom *Myxobolus eeli* sp. n.

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## Données complémentaires sur l'ultrastructure de *Neobursaridium gigas* Balech, 1941 (*Ciliatea, Hymenostomatida, Peniculina*)

*Synopsis.* L'auteur fait une courte description ultrastructurale de *Neobursaridium gigas* recontré au Brésil, en complétant les observations de ses prédécesseurs et insistant plus particulièrement sur l'analogie de ce Cilié avec quelques espèces de *Paramecium*. L'occurrence de glycogène au sein de l'endoplasme est signalée et son destin au cours de situations non favorables à l'animal est discuté. La présence de bactéries endonucléaires, son mode de pénétration et son action sont rapidement examinés. La disposition du matériel chromatique en gros et en petits corpuscules est également traitée.

## Introduction

Appuyé sur l'observation d'exemplaires de *Neobursaridium gigas* provenant d'Uganda, Nilsson (1969) fait une excellente étude de l'ultrastructure de cet Infusoire. On note toutefois que quelques compléments à son travail sont nécessaires, surtout en ce qui concerne les éléments permettant l'analogie de ce protozoaire par rapport à plusieurs espèces de *Paramecium*.

Dans une note antérieure, Kattar (1972 a) signale la présence de *Neobursaridium gigas* au Brésil, donc sa redécouverte en Amérique du Sud, et fait une courte description de l'animal, tout en soulignant les ressemblances et les différences des spécimens brésiliens par rapport aux exemplaires de l'Argentine et de l'Afrique.

## Matériel et Méthodes

Les *Neobursaridium* étudiés au microscope électronique provenaient d'une souche obtenue à Minas Gerais (Brésil) et qui sont depuis 1971 cultivés en laboratoire. Il s'agit d'une "race" amicronucléée, dont les spécimens présentent en moyenne 600 μm de longueur.

La fixation a été assurée par la glutaraldéhyde à 4% dans le tampon phosphate à pH 7.0, pendant 15 min, suivie d'une post-fixation au tétroxyde d'osmium à 1%, dans même tampon, pendant 60 min. Le matériel a été enrobé dans la gélose à 1.3% et inclus définitivement dans l'araldite. Les coupes ultrafines ont été contrastées par l'acétate d'uranyle et le citrate de plomb selon Reynolds, et examinées au microscope Philips sous une tension de 80 Kv.

## Résultats

La couche alvéolaire corticale est très épaisse et détermine des sillons profonds dans lesquels les cils sont partiellement logés. À l'intérieur de ces alvéoles se trouvent les crêtes ectoplasmiques très développées. Les crêtes ectoplasmiques, les alvéoles et les cils sont recouverts par la membrane unitaire (Pl. I 1).

Les cinétosomes, généralement disposés par couples, sont ciliés et se situent entre les alvéoles pelliculaires. Ils se disposent en lignes longitudinales ou cinéties. On observe juste autour de la région distale du cinétosome un anneau pelliculaire fusionné. Cet anneau peut envelopper soit seulement un cinétosome, soit une paire de cinétosomes. À proximité de la paire de cinétosomes se trouve l'ouverture du sac parasomal qui est une invagination digitiforme de la pellicule (Pl. VI 8, 9).

Tout au long des cinéties, au niveau des crêtes ectoplasmiques, courent sous la pellicule les fibrilles cinétodesmales (Pl. I 1). Sur des coupes longitudinales ces fibrilles se présentent allongées et avec des stries périodiques transversales; elles sont plus amincies à l'une des extrémités et s'attachent au cinétosome postérieur de chaque paire (Pl. II 2). Sur des coupes transversales on observe à l'intérieur de chaque crête ectoplasmique généralement cinq fibrilles cinétodesmales superposées, les quatre inférieures étant à peu près de la même épaisseur et la supérieure étant moins épaisse (Pl. I 1). En partant également des cinétosomes, en direction de l'endoplasme, on peut voir des fibrilles tubulaires rétrociliaires (Pl. I 1).

Les trichocystes, du type *Paramecium*, s'implantent perpendiculairement sous la pellicule entre les alvéoles. Ils ont à peu près la forme d'une carotte (Pl. III 3, IV 4). Sur des coupes longitudinales ils se présentent avec un corps et une pointe cu tête. La pointe (Pl. IV 5), très osmiophile, présente approximativement 2.2 µm et se montre dense aux électrons; elle a la forme d'un clou et se revêt d'une coiffe ou capuchon dont l'ultrastructure révèle sur des coupes transversales une série de structures internes semblables à des dents (Pl. V 6, 7). Cette coiffe ainsi que le corps du trichocyste sont recouverts, à leur tour, par une membrane qui ne s'applique pas sur toute la longueur du trichocyste et le sépare du cytoplasme adjacent. On reconnaît donc, sur des coupes transversales de la pointe, une capsule pourvue de structures internes sous forme de dents et un tube contenant à son intérieur une substance intratubulaire plus contrastée que la substance capsulaire. L'ensemble est recouvert par une membrane unitaire vacuola ire. Entre les couches capsulaire et tubulaire on observe un espace qui se présente en clair sur les électromicrographies.

Dans le trichocyste adulte le corps se présente peu contrasté sur les électromicrographies parce que la substance dont il est formé n'a aucune affinité par l'osmium, tandis que chez les trichocystes jeunes il se montre très osmiophile (Pl. IV 4).

Dans le cytoplasme de *Neobursaridium gigas* se rencontrent des mitochondries, du reticulum endoplasmique, des granules de glycogène, des ribosomes, des gouttelettes de lipides.

Les mitochondries se présentent approximativement sous une forme ovale et mesurent en moyenne 1.2 $\mu$m de longueur par 0.5 $\mu$m de largeur; leurs microvilli ont un diamètre de 200 Å (Pl. II 2, IV 5). Elles sont trés abondantes et, bien qu'elles soient distribuées sur toute la masse du cytoplasme, elles tendent à se concentrer préférablement dans sa région plus périphérique.

Le reticulum endoplasmique granuleux se présente sous la forme de saccules applatis isolés, entourés de ribosomes. Ces saccules, trouvés surtout aux environs de la superficie de l'endoplasme parmi les mitochondries, ne se disposent pas l'un sur l'autre, empilés ou organisés en amas réguliers comme chez beaucoup d'autres protozoa'res (Pl. VII 10, 11).

Le reticulum endoplasmique agranuleux est moins fréquent.

On rencontre abondamment dans le cytoplasme des granules dont le diamètre oscille autour de 100 Å et qui sont probablement des réserves alimenta'res de glycogène (Pl. IV 5, VII 10, 11). Ils sont disséminés dans presque tout le cytoplasme mais se disposent plus particulièrement à côté du reticulum endoplasmique rugueux et autour de quelques trichocystes. Il faut bien remarquer que dans le cytoplasme des bactéries ingérées et contenues dans les vacuoles digestives du Cilié, on peut également constater la présence de ces granules interprétés comme du glycogène.

Les gouttelettes de graisse, dont le diamètre oscille de 0.5 à 1 $\mu$m, ne se préservent pas facilement dans le matériel inclus dans l'araldite. Elles prennent des formes irrégulières avec une tendance à la sphéricité (Pl. VI 9). Elles se présentent avec une consistence homogène, sont très osmiophiles et se montrent avec une coloration foncée sur le matériel examiné au microscope photonique avant l'inclusion. Dans les coupes de matériel inclus dans les résines, les gouttelettes de graisse sont moins vivement contrastées. Elles sont plus abondantes dans l'endoplasme situé entre le macronoyau et la cavité buccale. Enveloppant certaines gouttelettes on peut voir une mince membrane plus contrastée. Certaines vésicules, apparemment vides, représenteraient probablement des espaces occupés préalablement par des lipides extraits au cours de l'inclusion.

Les vacuoles digestives sont recouvertes par une simple membrane unitaire; à leurs environs on observe de nombreuses mitochondries et des fragments du reticulum endoplasmique. La Planche IX 15 montre une vacuole digestive au début de son développement; les bactéries contenues à son intérieur, vues sur des coupes transversales ou longitudinales, sont pratiquement intactes.

Dans la cavité buccale ou infundibulum on voit deux péniculi et un quadrulus (Pl. VI 8, 9, VII 10, 11). Chaque péniculus est formé par quatre rangées de cinétoso-

mes ciliés. Les cinétosomes qui constituent le quadrulus se disposent anarchiquement. De chaque péniculus partent de longues fibrilles rétrociliaires connectant chaque péniculus avec la rangée externe de cinétosomes (Pl. VI 8). Il faut noter que seuls les cinétosomes de la quatrième et de la huitième rangées des péniculi portent des fibrilles rétrociliaires. C'est justement dans cette région où l'on peut observer le mieux les sacs parasomaux et l'anneau pelliculaire fusionné qui recouvre distalement le cinétosome.

Toujours dans l'endoplasme on signale la présence des faisceaux de fibrilles dont les éléments constitutifs ne sont pas très nets (Pl. III 3, V 7), ainsi que des tubules à parois membraneuses dont le diamètre oscille autour de 60 Å (Pl. I 1).

Le macronoyau est enveloppé par la double membrane pourvue de pores de 650 Å de diamètre (Pl. VIII 12, 13, 14). À son intérieur se trouvent des corpuscules denses aux électrons et un réseau fibreux moins dense. Les corpuscules denses sont les uns gros (0.7 μm de diamètre) et correspondent sûrement aux nucléoles; plus nombreux sont les petits corpuscules (0.06 à 0.1 μm de diamètre) assimilables à de la chromatine. En plus, on trouve parfois des bactéries endonucléaires longues de 1.2 μm et larges de 0.4 μm. Autour de ces bactéries et à leurs environs n'existe plus de matériel chromatique, c'est-à-dire de gros ou de petits corpuscules électrodenses (Pl. VIII 14).

Aucune coupe passant sur les vacuoles contractiles n'a été obtenue.

## Discussion

Au point de vue de l'ensemble de l'ultrastructure nos observations sur *Neobursaridium gigas* est en accord avec les travaux de Nilsson (1969) et Dragesco (1968). Il est donc certain que *Neobursaridium* se rapproche de *Paramecium* particulièrement par l'ultrastructure des trichocystes, du cortex et des péniculi. En plus, les systèmes fibrillaires présentent une analogie frappante avec ceux de *Paramecium aurelia*, espèce bien étudiée par Jurand et Selman (1969).

La présence de glycogène dans le cytoplasme de *Neobursaridium* semble due à l'ingestion de bactéries. En fait Estève (1969) a démontré cela chez *Paramecium caudatum*. Accumulées dans le cytoplasme de *Neobursaridium gigas*, ces réserves alimentaires seraient responsables de la coloration laiteuse à jaunâtre de l'animal. En effet quand les *Neobursaridium* sont soumis à un jeûne prolongé ils deviennent transparents à cause probablement de la perte ou utilisation des réserves glycogéniques.

L'occurence de bactéries endonucléaires attire toujours l'attention du chercheur. La littérature protistologique est très riche en observations à ce sujet, mais on ne connaît pas encore le type précis d'association réalisée entre les deux organismes. Puytorac et Kattar (1969) chez *Helicoprorodon multinucleatum* et Kattar (1972 b) chez *Pseudoprorodon arenicola* accusent également la présence de bactéries à l'in-

térieur du macronoyau de ces ciliés où l'on peut voir nettement une lyse de la chroma-
tine. Nilsson (1969) indique chez les *Neobursaridium* africains une intense invasion
de bactéries intranucléaires. Chez les spécimens brésiliens examinés au microscope
électronique nous n'avons observé que de rares bactéries. Il nous semble que ces
microorganismes auraient pénétré à l'intérieur du noyau à travers les pores nuclé-
aires, parce que nous n'avons constaté aucune lyse de la membrane nucléaire.

La distinction du matériel macronucléaire en granules ou corpuscules gros et
granules ou corpuscules petits est très évidente sur nos électromicrographies. Ils
ressemblent d'ailleurs assez bien aux corpuscules de *Paramecium caudatum* et de
*Blepharisma intermedium*. Seshachar (1964) chez *Blepharisma intermedium* inter-
prète les corpuscules petits comme des fragments ramifiés dans toutes les directions
à l'intérieur du nucléoplasme. Nous croyons toutefois que chez *Neobursaridium*
les corpuscules petits sont indépendants l'un de l'autre au sens qu'ils ne constituent
pas de fragments d'un cordon ou filament.

## SUMMARY

The author presents a brief ultra-structural description of *Neobursaridium gigas* collected in
Brazil, in addition to the observations made by his predecessors and emphasizes the analogy of this
Ciliate with some *Paramecium* species.
The occurrence of glycogen in the endoplasm is mentioned and the destiny of this substance
in the unfavourable situations is also discussed.
The presence of endonuclear bacterias, the way of penetration and their action are briefly exa-
mined.
The disposition of the chromatic material in small bodies and larger bodies is also treated.

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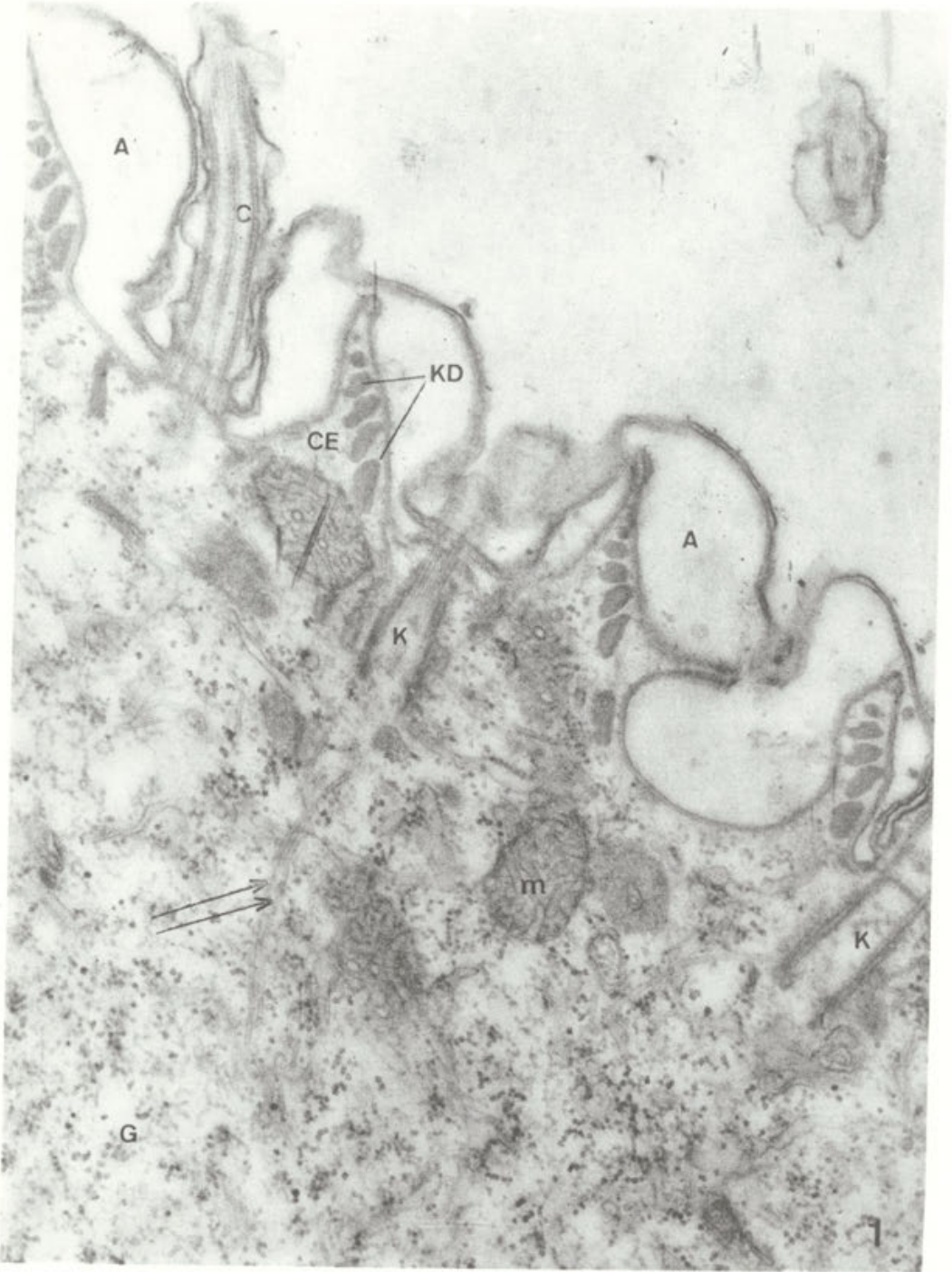
## EXPLICATION DES PLANCHES I–IX
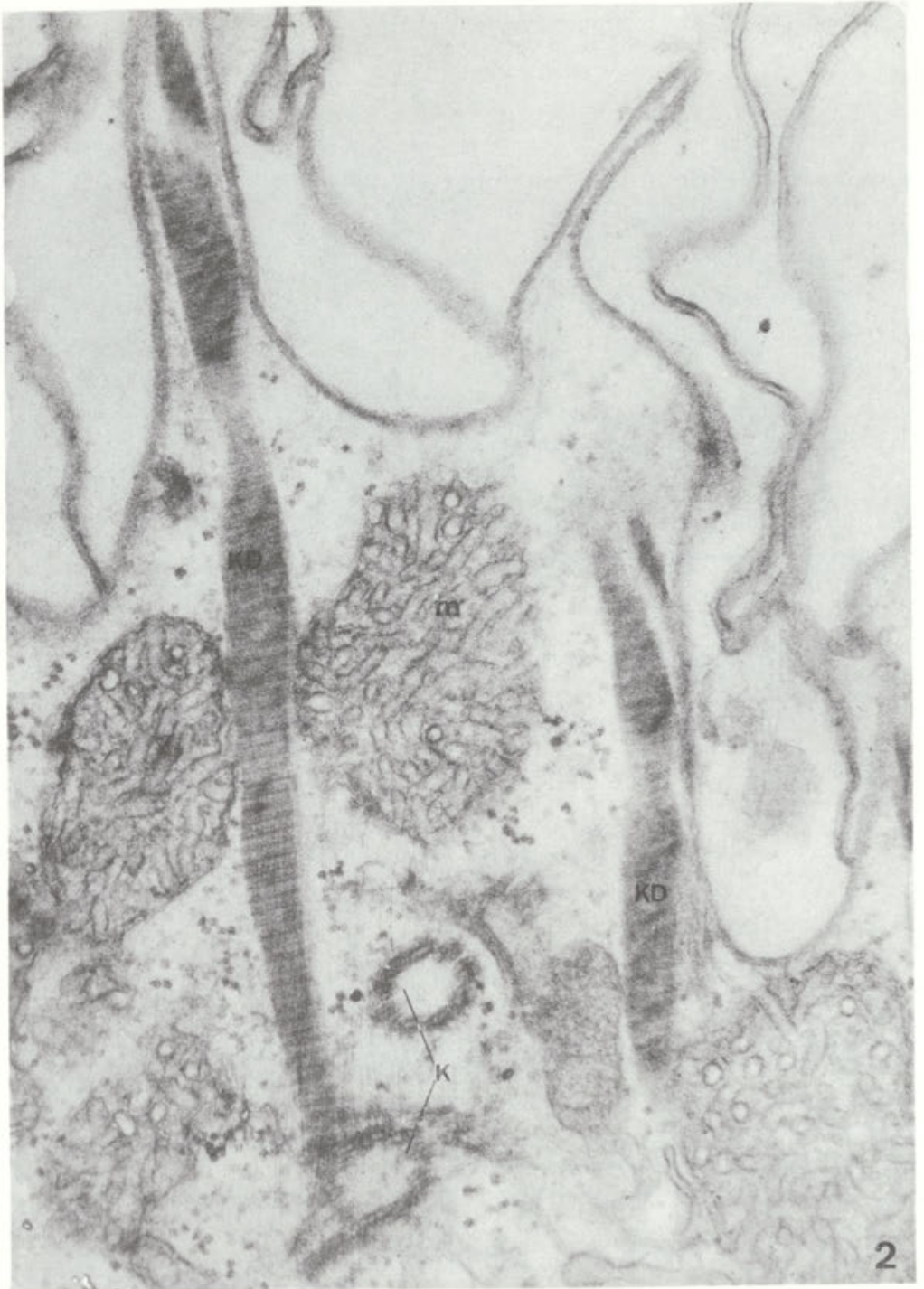
L'ultrastructure de *Neobursaridium gigas* Balech, 1941

1: Coupe légèrement oblique montrant les crêtes ectoplasmiques, les alvéoles pelliculaires et les fibrilles cinétodesmales. Les flèches indiquent quelques tubules à paroi membraneuse. × 47 900
2: Coupe à peu près longitudinale de fibrilles cinétodesmales, montrant la périodicité des bandes transversales. Le cinétosome postérieur est en rapport avec une fibrille cinétodesmale × 75200
3: Des trichocystes adultes coupés longitudinalement. Noter le point d'implantation de la pointe contre la pellicule entre les alvéoles et des faisceaux de fibrilles dans l'endoplasme (flèche). × 20500
4: Coupe longitudinale d'un trichocyste adulte (à gauche) et d'un trichocyste jeun (àdroite). Remarquer que le corps du trichocyste jeun est fortement osmiophile. × 14500
5: Détail, sur coupe longitudinale, de la pointe d'un trichocyste adulte. × 47500
6: Coupe transversale intéressant la pointe de deux trichocystes. × 65200
7: Coupe transversale passant par la pointe et le corps de plusieurs trichocystes adultes. La flèche indique un faisceau de fibrilles dans l'endoplasme. × 32600
8: Coupe au niveau de l'infundibulum mettant en évidence les péniculi, des fragments du quadrulus et deux rangées de fibrilles rétrociliaires connectant les cinétosomes de la quatrième et de la huitième cinéties des péniculi. Entre les cinétosomes on observe l'ouverture de quelques sacs parasomaux. × 7000
9: Coupe de l'infundibulum intéressant les péniculi. Observer les anneaux fusionné set l'ouverture d'un sac parasomal (flèche) à côté des cinétosomes. × 13000
10: Coupe à peu près longitudinale passant par le péniculus montrant les cils recouverts par la membrane unitaire et leurs microtubules. Dans l'endoplasme on observe les rideaux de microtubules connectés aux cinétosomes, des fragments de reticulum endoplasmique et du glyogène. × 39300
11: Coupe oblique au niveau d'un péniculus mettant en évidence des cinétosomes et les rideaux de microtubules. Chaque cinétosome est enveloppé distalement par un anneau pelliculaire fusionné. Noter l'abondance de granules de glycogène et les fragments du reticulum endoplasmique. × 39300
12: Coupe à travers le macronoyau, montrant la chromatine constituée par de gros et de petits corpuscules. × 10000
13: Électromicrographie du macronoyau avec sa double membrane nucléaire pourvue de pores (flèche). × 26700
14: Détail du macronoyau à l'intérieur duquel on observe trois bactéries. Remarquer l'absence de matériel chromatique aux environs des bactéries. × 47900
15: Coupe au niveau d'une vacuole digestive au début de son développement contenant à son intérieur des bactéries intactes. × 14500

Abréviations: A — alvéoles pelliculaires, Af — anneau pelliculaire fusionné, B — bactérie, CE — crête ectoplasmique, ER — reticulum endoplasmique granuleux, FR — fibrille rétrociliaire, G — glycogène, K — cinétosome, KD — fibrille cinétodesmale, LI — gouttelette de lipide, m — mitochondrie, M — macronoyau, P — péniculus, PS — sac parasomal, Q — quadrulus, R — rideau de microtubules, T — trichocyste, TC — corps de trichocyste, TP — pointe de trichocyste, TF — coiffe de trichocyste, VD — vacuole digestive
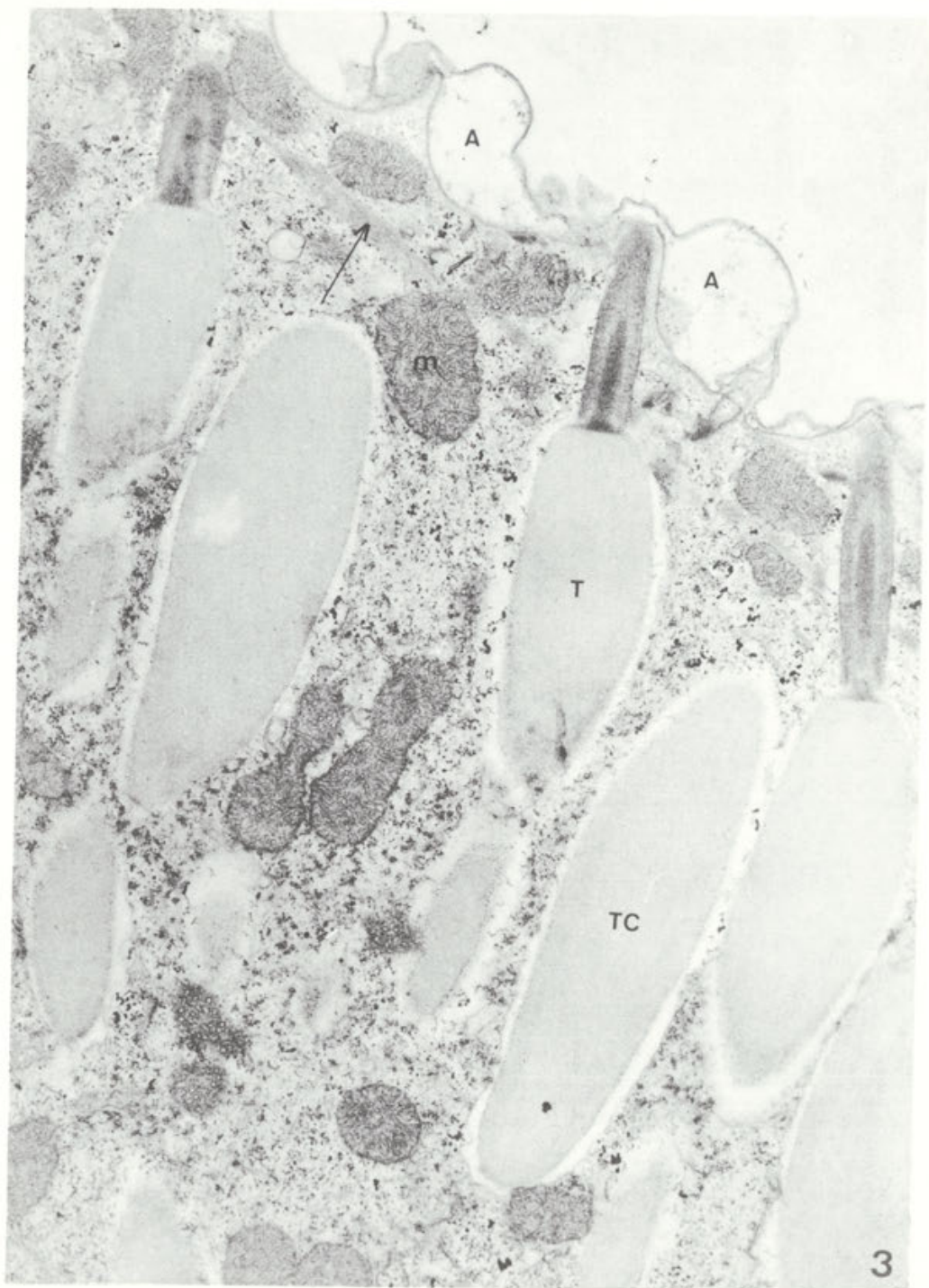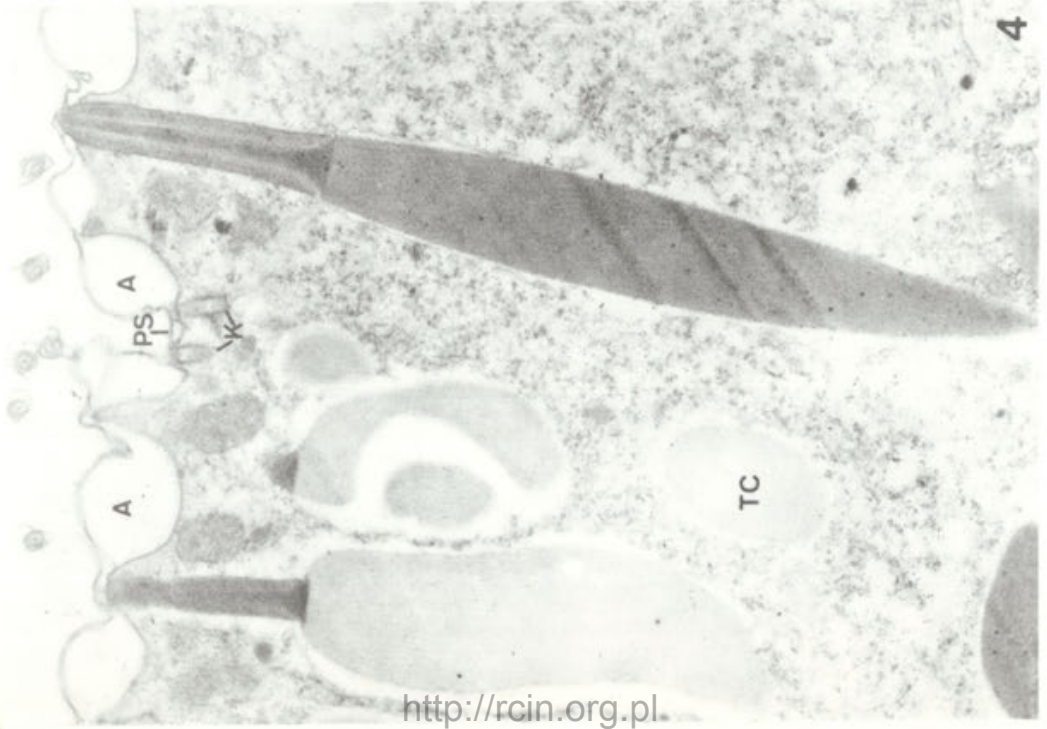
PLATE I



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PLATE II



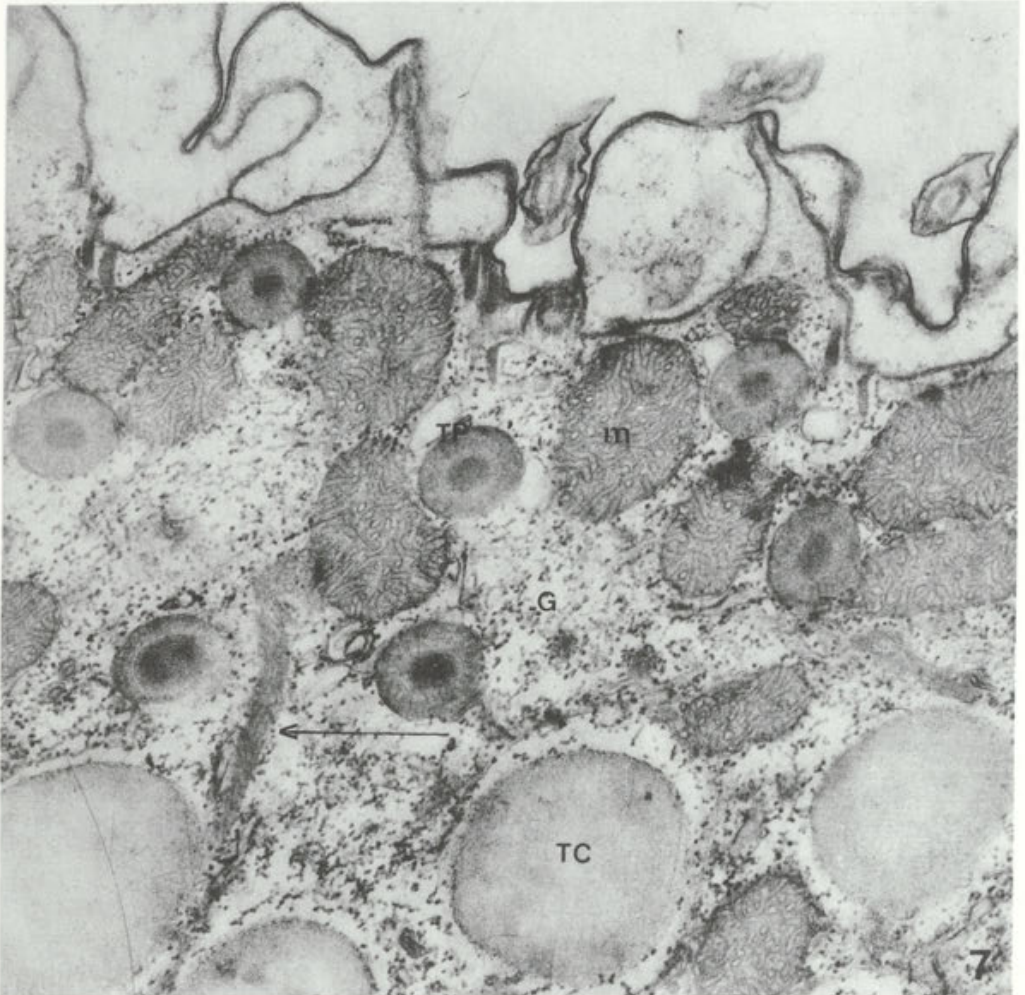M. R. Kattar

PLATE III



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PLATE IX

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PLATE V

PLATE VI

PLATE VIII

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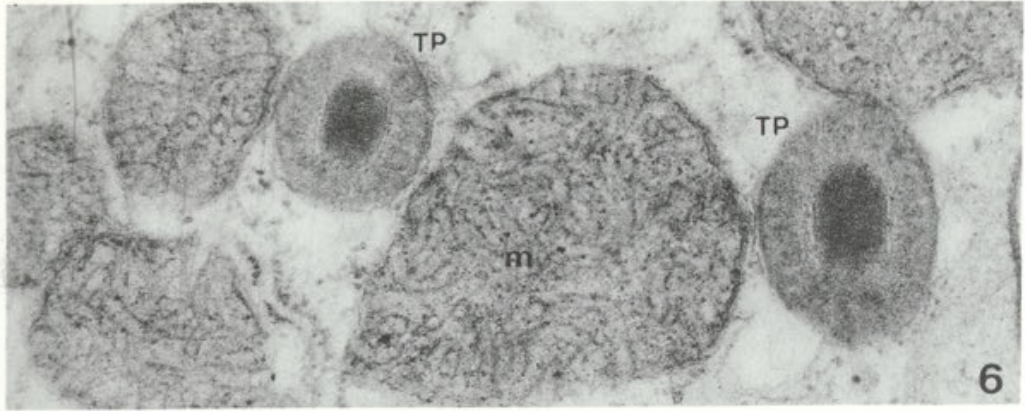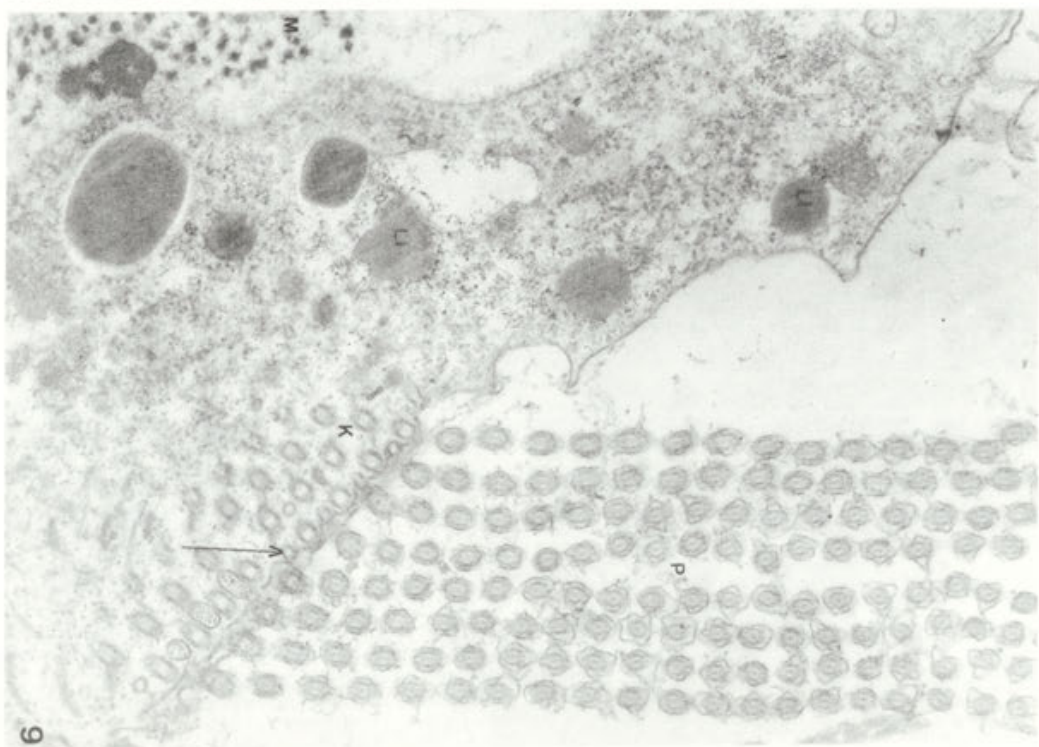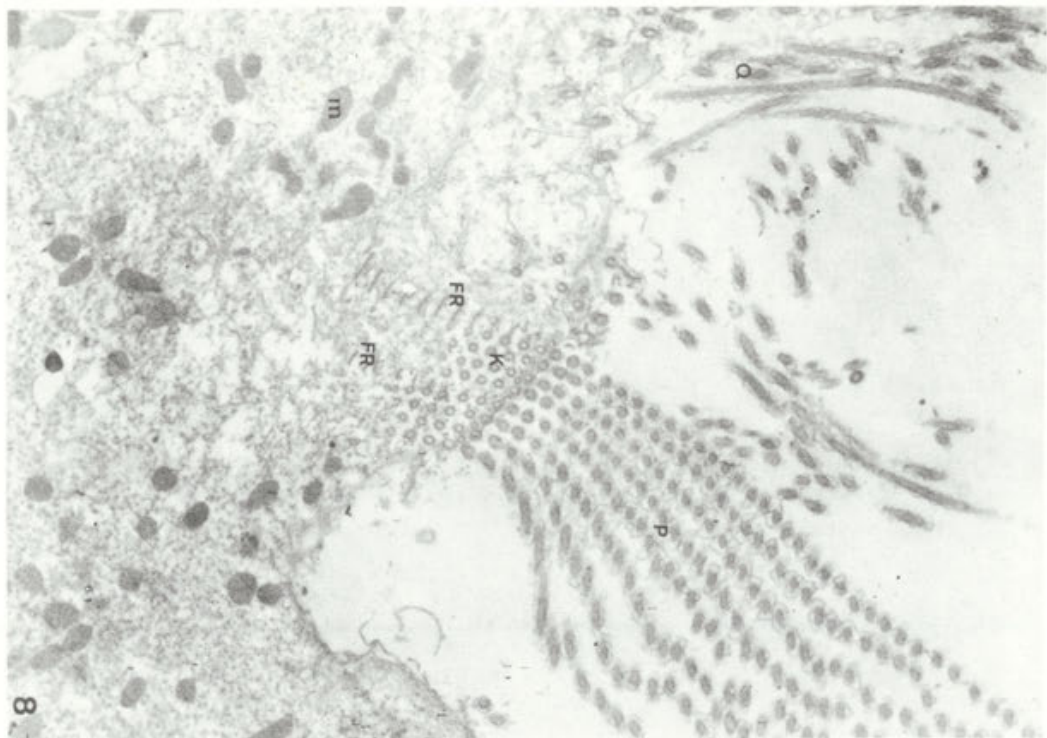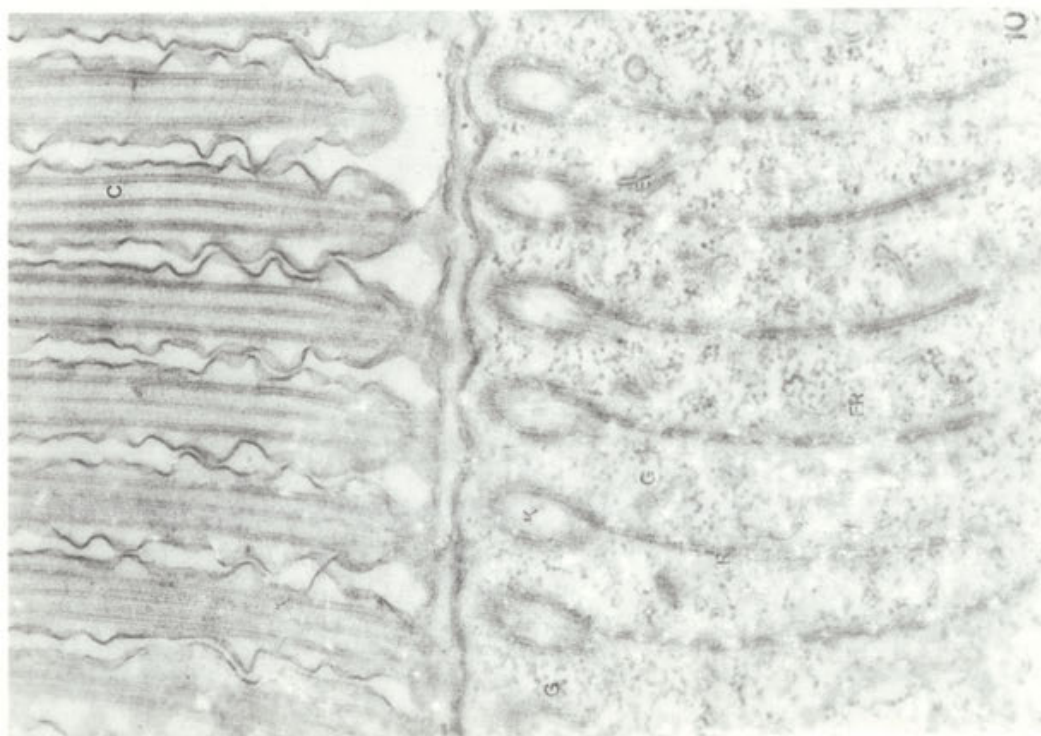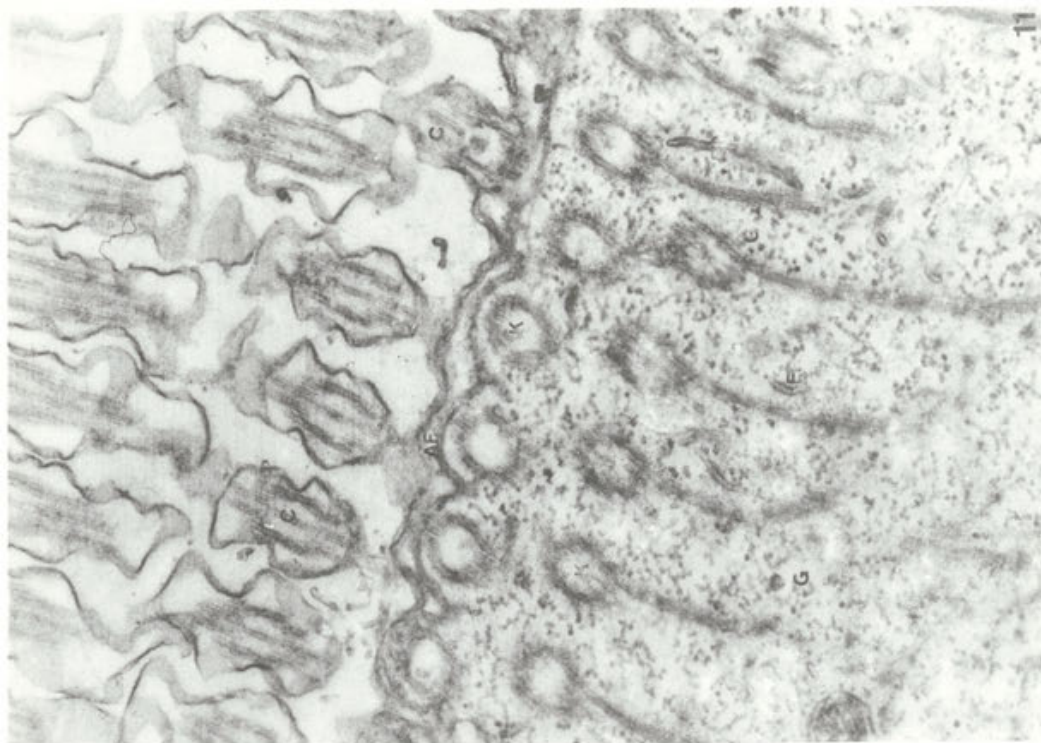
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# Stages in Sporogony of *Plistophora debaisieuxi* Jírovec (*Microsporidia*)

*Synopsis*. Six distinct steps are characterized in the sporogony of *Plistophora debaisieuxi* according to ultrastructures: (1) The sporont grows from a transformed diplokaryon to a multinucleate plasmodium with nuclei in finger-like protrusions. ER-lamellae grow from one single to several in parallel layers. The outer membrane is thin, adherent 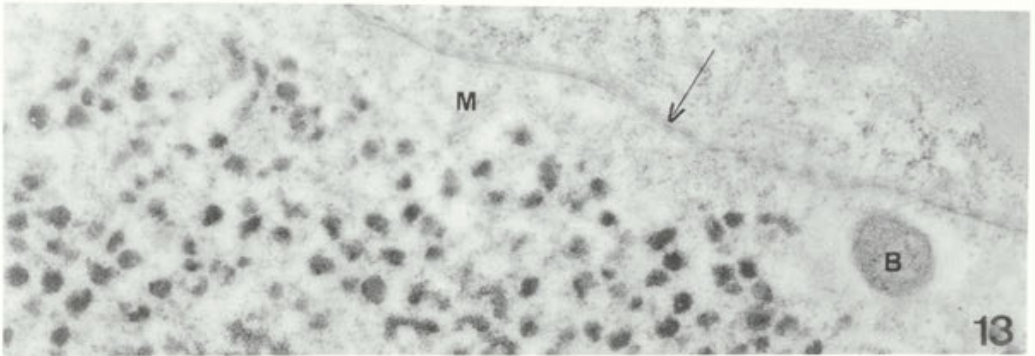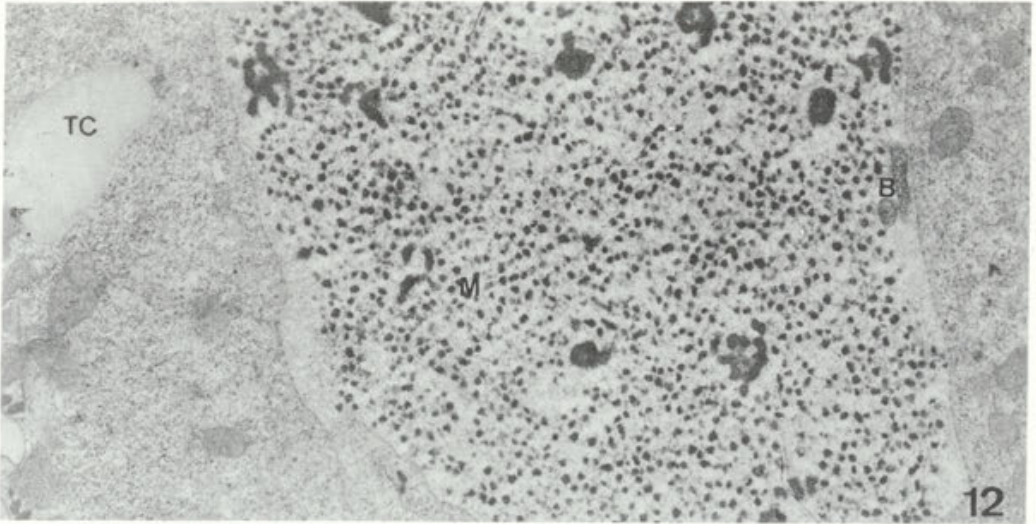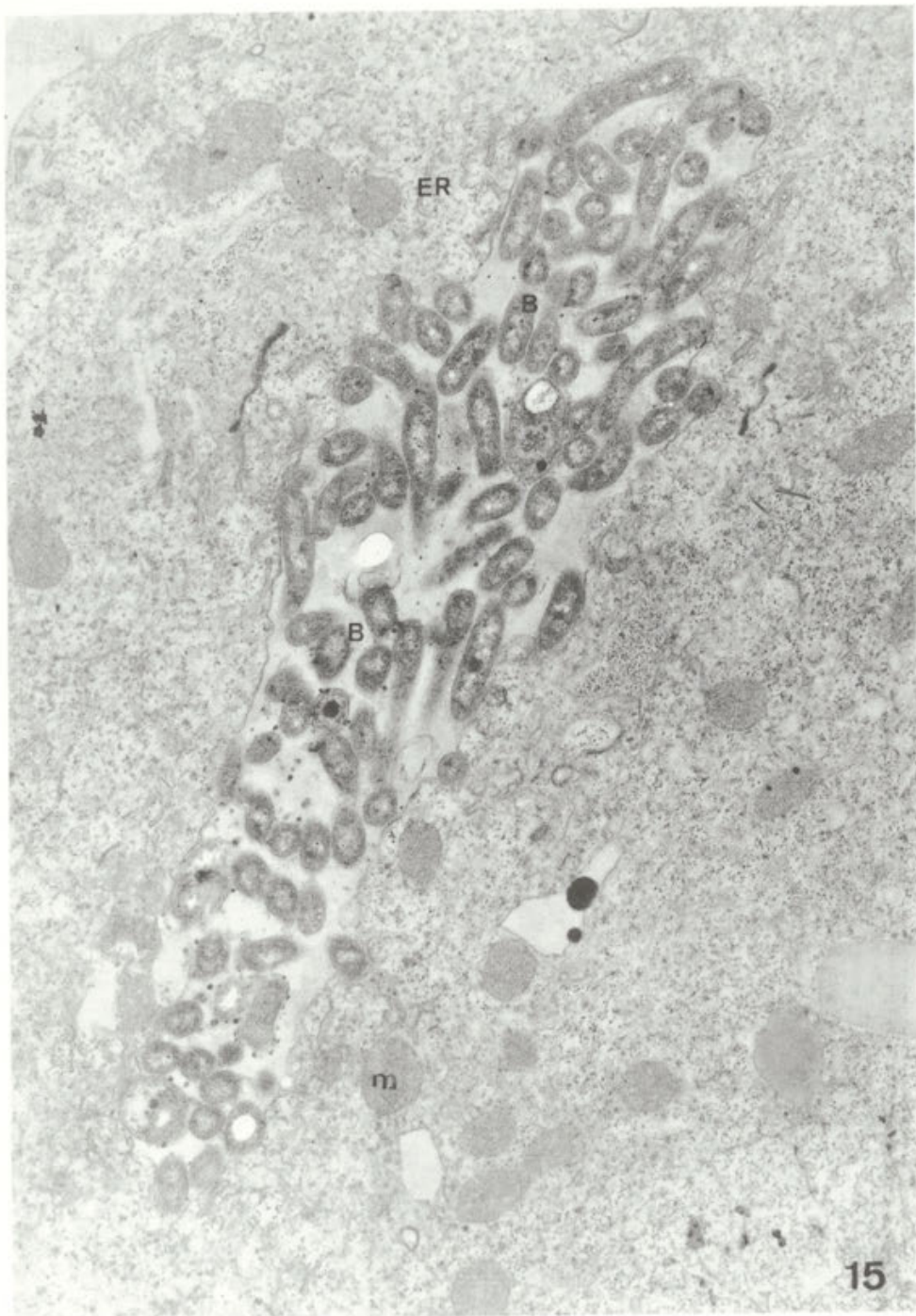to protrusions. A dense deposit is formed outside on their basal part. (2) The prosporoblast is still connected with the central mass. ER-lamellae form a multiple cap on the nucleus. On the surface of the outer membrane are hemispherical EM-dense coagula. (3) The young sporoblast is still connected with the central mass, its polar filament begins to form. On the surface the coagula change to a fleece of tubular protrusions. (4) The mature sporoblast is free, its polar filament is completed and coiled posteriorly. A posterior body takes part in its construction and it remains active till maturation. It is spongioid in structure and differs from Golgi's complex. (5) The young spore is shrunken after fixation. Its outer membrane has a fleece of tubular protrusions. The posterior body (posterosome) is in a vacuole. Outside, the host tissue is digested and a periparasitic vacuole appears. (6) The mature spore is rigid, oval with the electron transparent layer under the surface wall. On the surface the fleece is maintained originating in a honeycomb-like layer. The interior of the spore is inapparent.

## Introduction

The genus *Plistophora* is characterized by a sporogony where the sporont develops into many (more than 16) spores. Facing many new descriptions this definition given by Kudo (1924) needs a revision. But the old generic definition is good enough for the present study of some ultrastructures of the microsporidian *Plistophora debaisieuxi* Jírovec, 1943, a pathogen of blackfly larvae widely distributed in breeding places of the *Simuliidae*.

Stages of sporogony are the only frequent stages in tumor-like cysts in the invaded fatbody lobes. Their histology was well studied and depicted by Debaisieux and Gastaldi (1919). Vávra (1963) described tubular projections on the surface of the

spore walls and Maurand (1973) confirmed his observation. In his dissertation Maurand defined the sporogony as the last steps of development after the diplokaryon stage. First sporonts are uninuclear, later they form multinuclear plasmodia. Nuclei of the plasmodia move to the periphery and lose their dense, compact appearance. Then they break after observations of Maurand into 16 to 32 sporoblasts which maturate separate into oval spores.

The definition of what are stages in sporogony, which of them are first and which later depends very much on the estimation of the authors. In this paper we will differentiate some fundamental stages in sporogony of *P. debaisieuxi* which would be helpful to authors working with microsporidia in comparative studies of sporogony in other species.

## Material and Methods

Studies were performed on infected larvae of *Odagmia ornata* with prominent white elongate cysts of the microsporidian in the fat body of the posterior segments. Living and motile larvae were cut open under the dissecting microscope. Cysts with the parasite were extracted from the tissues. One third of the cyst was used for preparation of a wet smear for identification of the pathogen. The remaining tissue was brought into a drop of 4% glutaraldehyde in cacodylate buffer and after the performed diagnosis this material was transfered into a vial with surplus gluteraldehyde and fixed at 4°C over night. After washing in changed cacodylate buffer for 8 h it was refixed in 1% osmic acid in cacodylate buffer for another 8 h. After washing of the material it was embedded in Vestopal W and Durkupan after the procedures indicated by the producers. Material was cut on a Tesla ultramicrotome with glass knife and studied in a Tesla BS 613 electron microscope after staining with uranyl acetate and lead citrate.

The primary smear was used for studying in optical microscope after fixation with methyl alcohol and staining with Giemsa stain. One part of the material was hydrolysed with 0.1% hydrochloric acid at 90°C for 30 sec to 1 min and after washing with tap water was stained with Giemsa for selective staining of the nuclei inside the spores. Other material was smeared on cover slips, fixed in Bouin's liquid and stained with Heidenhain's iron hematoxylin.

## Results

### Stages of Sporogony in the Light Microscope

The starting point for the sporogony of *Plistophora debaisieuxi* is the end of the large autogamic diplokaryon stages with well visible chromosomes as described by Debaisieux and Gastaldi (1919). The next step. the sporont is a spherical cell, 10–15 μm in diameter with a large nucleus and chromosomes arranged in a coil which is crossed in the centre by an achromatic bundle (Pl. I 2). The nucleus does not come to a quiescent form, the chromosomes remain formed for most of the subsequent divisions. Binuclear, quadrinuclear and multinuclaer plasmodia can be seen

in the mass of developmental stages, with well formed dividing figures. After the third or fourth division of nuclei these proceed to the surface of the plasmodium and one part of them protrude from the surface as spherical protrusions (Pl. I 1). These preceed the rest of the plasmodium and are shed off first. The remaining nuclei are closed in finger-like protrusions which form morula-like groups where the protrusions are connected in the centre with the remaining cytoplasm of the plasmodium (Pl. III 6). At the end the protrusions separate from each other and change to oval sporoblasts which are free in host cells. There are no visible differences of structures during the development of sporonts to sporoblasts. The sporoblasts are not closed in any common membrane except the host cell. In mature cells the special staining reveals one single large nucleus in the central part. In most spores the polar filament is well visible as a coil in the posterior end of the spore. In the anterior end of the spore the vacuole which does not stain with Giemsa, represents the polaroplast. The posterior vacuole is in young spores occupied by a metachromatic granule of irregular shape, about half of the size of the nucelus.

### Stages of Sporogony in the Electron Microscope

Ultrastructures of the dividing sporonts of *P. debaisieuxi* during the first three divisions are very poor, but subsequent stages present specific morphological features and may be divided into six steps (Table 1).

(1) Sporonts represented by plasmodia before the formation of finger-like protrusions are cells with a thin membrane on the surface. with a large nucleus with one or several nucleoli of different size. Only one or two lamellae of the endoplasmic reticulum surround the nucleus which has a thin membrane. There is no visible difference in structure of the cytoplasm between the host cell and this stage of the parasite. Mitochondria are present only in the host cell. After the third cycle of nuclear divisions finger-like protrusions are formed (Pl. II 4).

(2) During this procedure a multiple endoplasmic reticulum with flat lamellae and parallel arrangement is formed around the nucleus. This remains during the rest of sporogony. At the same time an electron-dense coagulum is formed on the surface of the outer layer of the membrane (Pl. II 5). On the lateral and distal part it breaks into flat, hemisphaerical particles evenly distributed over the surface. For this stage we propose the term prosporoblast.

(3) The young sporoblast, the next step, is characterized by formation of the primordia of the polar filament. The ER-lamellae broaden in the distal part, some of them change into vesiculae in the distal part of the "fingers" and a special structure, the posterosome or posterior body is formed. It is a spongioid structure of tubules with many interconnections (Pl. III 6, IV 7, V 8) which are partially filled with some EM-dense substance. They do not communicate with ER-lamellae or vesicles, they have not the typical arrangement of the Golgi complex. When it first appears it is almost a spherical structure closed up by a surface-layer of tubules. On its outer margin

Table 1

Distribution of Structures in the Sporogony of *Plistophora debaisieuxi*

| | Sporonts | Prosporoblasts | Young sporoblasts | Mature sporoblasts | Young spores | Mature spores |
|---|---|---|---|---|---|---|
| Outer membrane | thin | thin | thick | thick | | |
| Fleece | | | . | +++ | +++ | ++++ |
| Chitinous layer | | | | | | ++++ |
| ER-lamellae | | | | | | |
|   Flat | . | ++++ | ++++ | +++ | +++ | |
|   Vesicular | | . | ++ | + | + | |
| Posterosome | | | ++++ | +++ | +++ | ... |
| x-group | | | ++ | . | | |
| Polar filament | | | + | ++++ | ++++ | ++++ |
| Polaroplast | | | | | ++ | ++++ |
| Plasmodium | ++++ | | | | | |
| Fingers | | ++++ | ++++ | | | |
| Free stages | | | + | ++++ | ++++ | ++++ |
| Proteolysis of host tissue | | | | | ++++ | ++++ |
| Shrinkage | | | | | ++++ | |

Intensity of the development of the structures: . — first appearance, + — under development, ++ — formed +++ — functional, ++++ — full function, ... — present but not demonstrated.

(Pl. IV 6, V 7) the dark substance concentrates in a system which helps to build the polar filament. There is no analogy in structure of the tubules and of the polar filament, only the dark substance sticks to the newly built filament. All the rich system of flattened and vesicular lamellae of the endoplasmic reticulum persists. The electron-dense substance spreads again over the surface and tubular projections of the wall form a dense fleece on the surface. Their basis comes from a honeycomb-like structure on the covering membrane of the sporoblast and the elongated tubules are fixed to the surface in several points. With further growth of this stage which is still connected with the central plasmatic area of the plasmodium, the tubules deteriorate and open into empty shells (Pl. VI 10). The orientation of the future spore is well fixed at this time: the posterior pole with the posterosome is distal, the anterior pole proximal to the centre of the rosette (Pl. III 6).

(4) The mature sporoblast breaks the connection with the central plasmatic area and becomes free. Remains without nuclei are autolysed and disappear. In the interior, the polar filament is finished, with its basal cap separated by a narrow

plasmatic bridge from the outer membrane of the spore. In fact, there is no connection of the polar filament with the polar cap and spore membrane, which may explain the process of extrusion and opening of the spore. The filament is coiled posteriorly, the number of coils is varying from individual to individual. The posterosome is located in the centre of the coil, still functional, with the dark substance in the interior of the tubules, spongioid in its organization (Pl. V 8, 9). The outer membrane of the mature sporoblast is thick and dense, not shrunken. Tubules of the fleece are empty shells with C-like cross sections. First signs of a digested halo appears around that stage (Pl. VI 10).

(5) The young spore is characterized by a shrunken outer wall, its nucleus is almost inapparent. The posterosome is well differentiated in a vacuole where the dense pulp remains as a spongioid framework (Pl. V 8, VI 10).

(6) The mature spore is rigid and pear shaped to oval, the outer membrane is covered with a dense growth of tubules and honeycomb like structures which are curled in the digested parasitophorous vacuole. An electron-transparent thick layer is deposed under the outer membrane. It corresponds with the chitinous layer. With the hardening of the spore wall, the internal structures are not well visible. In the spores the posterior vacuole which is the seat of the posterosome, is broken and does not remain in sections. Around each spore there is a sheet of the host cytoplasm which closes up the tubular fleece on the spore surface (Pl. VII 13).

## Discussion

With the complexity of its structures, *Plistophora debaisieuxi* is a special case among the microsporidia. This special complexity allows to define subsequent steps in sporogony, steps which occur in all microsporidia, but have not rich morphological characterization in other species. We will not discuss in this study the host–parasite relationship which is the object of another study.

The membranes of the sporont. During the growth and differentiation there is no evidence of any persistent membrane which may represent a persistent envelope of the plasmodium and later a vesicle forming the wall of the pansporoblast. When buds protrude from the plasmodium and grow to sporoblasts, the outer membrane follows the morphological changes and stick to the finger-like protrusions as their single and own membrane. The parasite is embedded into the cytoplasm of the host cell and all communications of nutrients are so ideal that on both sides there are no differences in ultrastructures (except the mitochondria in the host) and the membrane is very thin.

With further maturation a qualitative change is observed during the young spore when the former stretched wall is shrunken and folded after fixation with glutaraldehyde-osmic acid. The permeability of the wall has very much changed and does not allow the exchange of outer and internal osmotic pressure. The spore wall smo-

othens again during spore maturation when the thick chitinous wall is deposed in the outer membrane. Whereas former changes of the wall proceed rather slowly, this chitinous layer seems to be deposed very fast and there are no intermediary stages. The only preparation for this change are multiple vesicles which are deposed under the wall of the young spore. At the time when the definite spore wall is formed, they disappear.

The tubular structures, the fleece on the surface of the prosporoblasts, sporoblasts and spores, are characteristic for *P. debaisieuxi* but they may occur also in other mcrospo ridia. The material for them is provided by the parasite and is deposed first in a continuous layer on the surface of the stages. The mechanism of tubule formation is not clear but may be connected with a secretion through the wall, perhaps by pores mentioned by Liu and Davies (1973) in *Thelohania bracteata* spore walls. The formation of some stalagmites by coagulation of substances from liquids may be armodel of this procedure. At the time when digestive processes outside the young spoce form the parasitophorous vacuole, the tubules are not digested. The tubular fleece is analogous to mucus deposits on spores of other microsporidia.

Lamellae of the endoplasmic reticulum appear first as solitary structures encircling the nucleus. They form multiple layers with a parallel arrangement of the flattened type. Later a system of vesicular ER appears beside of the first. It is first concentrated in the posterior part of the sporoblast, later they are spread over the whole sporoblast and spore till formation of the chitinous layer.

The polar filament appears first in the young sporoblast. Its formation is connected with the function of what we propose to call the posterior body or posterosome. This spongioid structure of anastomosing tubules is adherent to the newly formed filament coil, on its surface it produces in the first period a foamy network which is very similar to what was shown in spores of a *Metchnikovella* by Richards and Sheffield (1970) (Pl. III 6). The electron dense mass which is coagulated around the coiled part of the filament is also visible on cross sections of the other end near the polar cap. The organization of the polar filament is that known from other studied cases. In some figures signs of a granulation — longitudinal as well as circular — of the filament can be recognized. The polar cap of the filament is separated from the pole of the spore wall by a narrow plasmatic layer and there is no visible permanent indication of the point where the spores open for the extruded filament. The polar cap is cup-like and the neck of the polar filament is turned up in a collar (Pl. VI 10, 12). There is an indication of a conical plug in the neck. Some authors tried to differentiate microsporidia of identical spore size and shape with the use of the angle of the arrangement of the coil of the polar filament and the number of cross sections of the filament. This feature may need much more investigation. In our sections the number of coils in the sporoblast and spores varied from 7 to 20 and it is not possible to show any general development of this feature during the maturation of the spore.

The authors who studied the ultrastructures of microsporidian spores indicate in accordance with Vávra (1965) and Sprague and Vernick (1969) the Golgi

apparatus as the source of the polar filament. As a matter of fact there is no evidence of a typical Golgi apparatus in microsporidia. It is absent in vegetative stages and the structure occurring in sporoblasts and spores is far from the well known lamellar arrangement of endoplasmic reticulum with lateral vesicles and its polarized orientation to the nucleus. Liu and Davies (1972 a), did not find a typical Golgi complex as the source of the polar filament of *Thelohania bracteata* in frozen-etched sections. They found only expanded sacs located around the ascendent part the polar filament and these sacs change later into lamellar structures of the polaroplast. In our material of *P. debaisieuxi* we find the posterior body or posterosome as a structure which was not defined nor studied by any author. Maurand (1973) has this structure in most ultrathin sections of spores, in *Thelohania bracteata, T. contejeani, T. moenadis, Nosema orthocladii, N. infirmum* and *Stempellia simulii*. He takes it for a Golgi complex. In one case, he takes another fragment of this system, appearing in the other side of the spore for a mitochondrium (his Fig. 97). The question of the mitochondria in microsporidia is still open. The posterosome is neither one nor the other. It appears at the time when the polar filament is formed but it remains in the sporoblast and spore during the whole maturation and seems to be functional. It has no distinct outer membrane and this makes it different from mitochondria. The anastomosing branched spongioid structures of tubules remains as subspherical mass and later it is more "transparent" as if there had formed a vacuole in which the dark pulp of the tubules is well visible (Pl. VI 12). Evidence of this structure in at least 7 microsporidia by Maurand makes it a normal structure for microsporidian spores. It is signalized from *T. bracteata* but Liu and Davies (1972 b) do not mention it. But a study of their freeze-etched materials reveal this structure on Figs. 1, 2 and 5 under the designation of ER, endoplasmic reticulum. The fate of the posterosome in mature spores is not clear. They usually break one part of the section in the posterior part and it does not stay (Pl. VII 13). It is just in the area where the posterosome is located.

The nucleus and the polaroplast of the sporoblasts and spores is less prominent, this may depend on fixation. Concerning the chromosomes and the presence of the nucleus, methods with optic microscopy are more efficient than EM methods. Compared with what can be seen in wet mounts and Giemsa smears, it is peculiar how much the procedures in the plasmodium and pansporoblast differ in the EM from wet mounts. It is well presented when we compare Pl. I 1 and Pl. III 6. Surface buds, irregular and spherical, differ very much from deep finger-like parts of the pansporoblast. After all, the surface buds are the first series of separation of sprooblasts which makes the original number of nuclei and sporoblast in sporogony so irregular. A second interesting experience is that spores in pansporoblasts are not formed by a deliberate process. From the first period of the plasmodium, the anterior poles of future spores are oriented to the centre and posterior poles to the periphery of the pansporoblast. There are no remains of nuclear material or other structures of the plasmodium in the central area. During maturation the cementation of the spore wall cau-

ses shrinking of the stage under outer pressure and without active transport of the content through the membrane. This impermeability is present also during spore maturation, but the incrustation of the wall with chitin resists to the pressure and does not show the shrinking.

It is interesting that all nutrition of the parasite before cementation occurs in a "friendly" way, without symptoms of cell damage. Only at late stages during spore maturation symptoms of proteolytic destruction of the host cell are visible. The sheaths of host tissue around each spore remind very much the structures characterizing the genus *Tuzetia* (Maurand 1973) and the definition of this genus must be carefully studied again.

## SOMMAIRE

La sporogonie de *Plistophora debaisieuxi* est dividé en six stades distinctes charactérisés par leur ultrastructure. (1) Le sporonte se develope d'un diplokaryon transformé dans un plasmode multi-nucleé, avec les noyaux déposés dans les protrusions spheriques. Lamelles du R. E. s'augmentent d'une seule couche a une groupe déposée en bloc. La membrane extérieure est fine, elle adhere a tous les protrusions. Une masse dense se depose sur la membrane extérieure dans la partie basaledes projections. (2) Le prosporoblaste est lié a la masse centrale du plasmode. Ses lamelles du R. E. produisent un bonnet de plusieurs couches sur le noyau. Sur la membrane exterieure se forme une couche de cogaulations hemisphériques. (3) Le sporoblaste jeune qui est aussi lié a la masse centrale, commence a former le filament polaire. Les coagulations sur l'exterieur se transforment dans une couche de tubules. (4) Le sporoblaste mure est séparé du plasmode. Son filamen polaire est complet et enroulé au postérieur. Le posterosome participe a la construction du filament et reste active jusqu'a la spore mure. C'est une structure spongieuse, différente du complex Golgi. (5) La spore jeune est deformée et comprimée apres la fixation. La membrane externe est couverte d'un poil des tubules. Le posterosome est renfermé dans une vacuole. Autour du stade est dissolu une vacuole periparasitaire. (6) Spore mure est ovoide et résistente, avec la couche transparente de matiere chitineuse. Le contenu de la spore est peu visible.

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## EXPLANATION OF PLATES I–VII

1: Mature spores of *Plistophora debaisieuxi* with visible oblique coil of the polar filament. In the centre a plasmodium buding into sporoblasts distributed irregularly over the surface. Phase contrast. 2000 ×

2: Autogamic diplokaryon stages before sporogony. Typical transversal bundles divide both chromosomal groups. Bouin, Heidenhain. 7000 ×

3: A sporogonial plasmodium with three dividing nuclei and two retarded ones. Bouin, Heidenhain. 7000 ×

4: The sporont stage with distinct nucleus (N) and nucleolus, and a single ER-lamella (E) in the host cell (h). 22 000 ×

5: The prosporoblast of *P. debaisieuxi* with the cap of ER-lamellae (E) over the nucleus which divides by mitosis (A). On the covering membrane there are electron-dark coagula on the distant part (O). n — nucleus of the host cell. 15 700 ×

6: Section of the pansporoblast with finger-like protrusions, in the stage of young sporoblasts. The central plasmatic remains (R) have vesicular ER-lamellae. In the sporoblasts we find ergastoplasmic lamellae (E), the posterosomes (P) and the polar filament in the process of formation (see left lower corner). The tubular protrusions on the surface form the fleece. Between the sporoblasts is the system of the host cell with mitochondria (m) and host ER-lamellae. 16 200 ×

7: The young sporoblast with the tubular fleece on the thickened covering membrane. N — nucleus, E — flattened lamellae of the endoplasmic reticulum, V — vesicular ER-lamellae. The posterosome (P) show the black secretion in the tubules and the polar filament close by (F). Another specialized lamellar system (X) is connected with the posterosome. 40 000 ×

8: Cross section of two pansporoblasts: a with sporoblasts and b with mature spores. In the centre (a) cytoplasm of the host cell with mitochondria. Sporoblasts have ER-lamellae, the polar filament and the posterosome. Two are of the "young" type, with smooth membrane. Others are of the cementing, shrunken type of young spores. The pansporoblast with mature spores (b) shows the spores with the long tubular fleece and the digested periparasitic vacuole. The host cell produces the thin membranous envelope of each spore. The nucleus of the host cell is excentrical (h). 6800 ×

9: A mature sporoblast of *P. debaisieuxi* with smooth surface. Tubular fleece (T) on its surface. The nucleus (N) with nucleolus. ER-lamellae and cross sections of the polar filament. On the posterior pole the posterosome (P). Outside the host cell with mitochondria (m). 18 200 ×

10: A young spore during cementation of the covering membrane and resulting shrinking of the body. Visible are coils of the polar filament, the posterosome (P) in a vacuole and empty shells of the fleece. The polar filament (F) ends in the polar cap which is separated from the spore wall by a plasmatic layer. In the opening of the filament there is a darker plug. 20 200 ×

11: Tangential section of a mature spore with structures of the tubular fleece. 21 300 ×

12: Polar cap of a young spore with the ending of the polar filament. This is turned up in a collar (F). 31 400 ×

13: A mature spore of *P. debaisieuxi* with shells of the fleece. The thick chitinous layer of the spore wall is under the covering nembrane. Internal structures not well fixed, showing the polar filament and a thick wall closing up the polaroplast. 29 500 ×

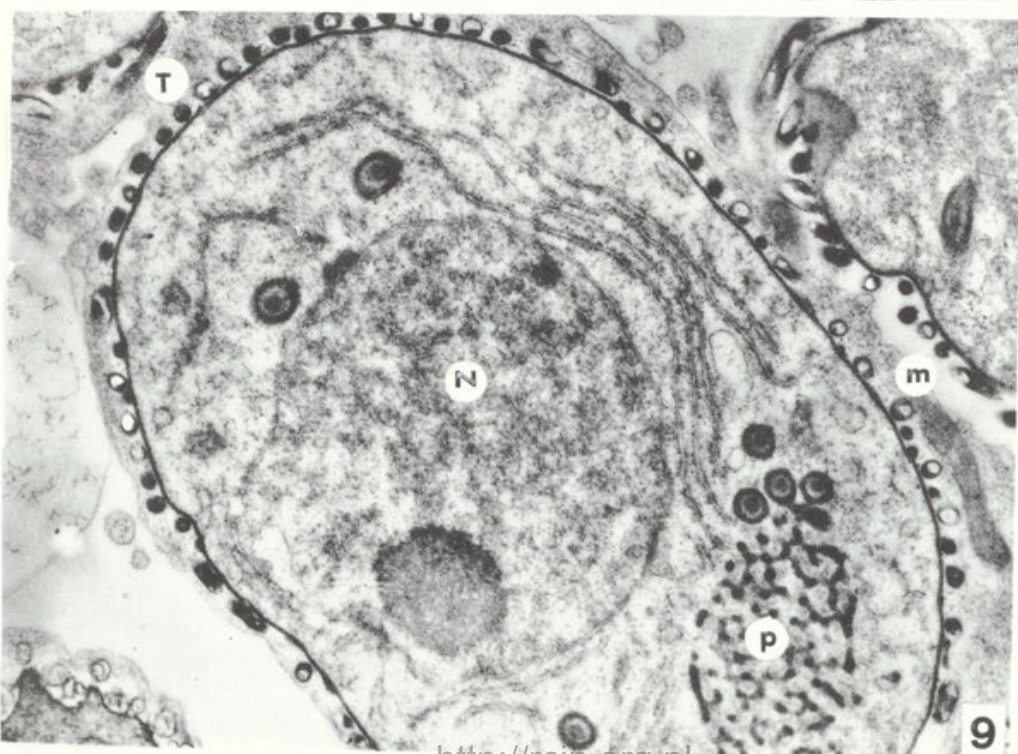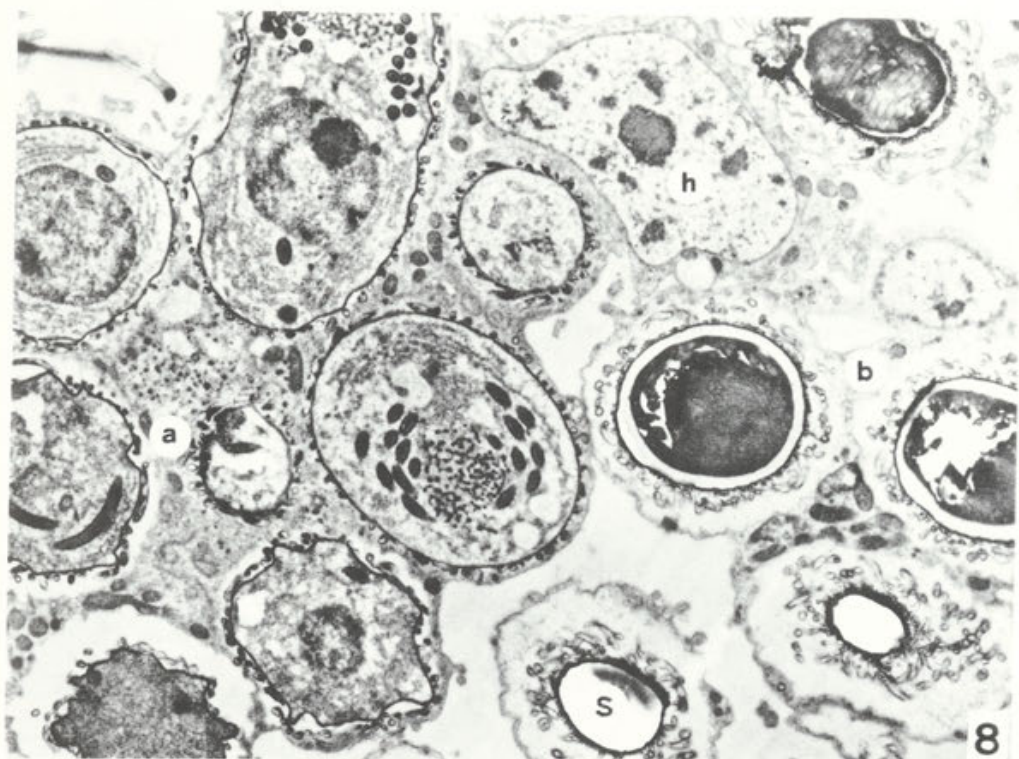On insert: Cross section of an adult spore with the posterior vacuole resistent to cuting 8 700 ×
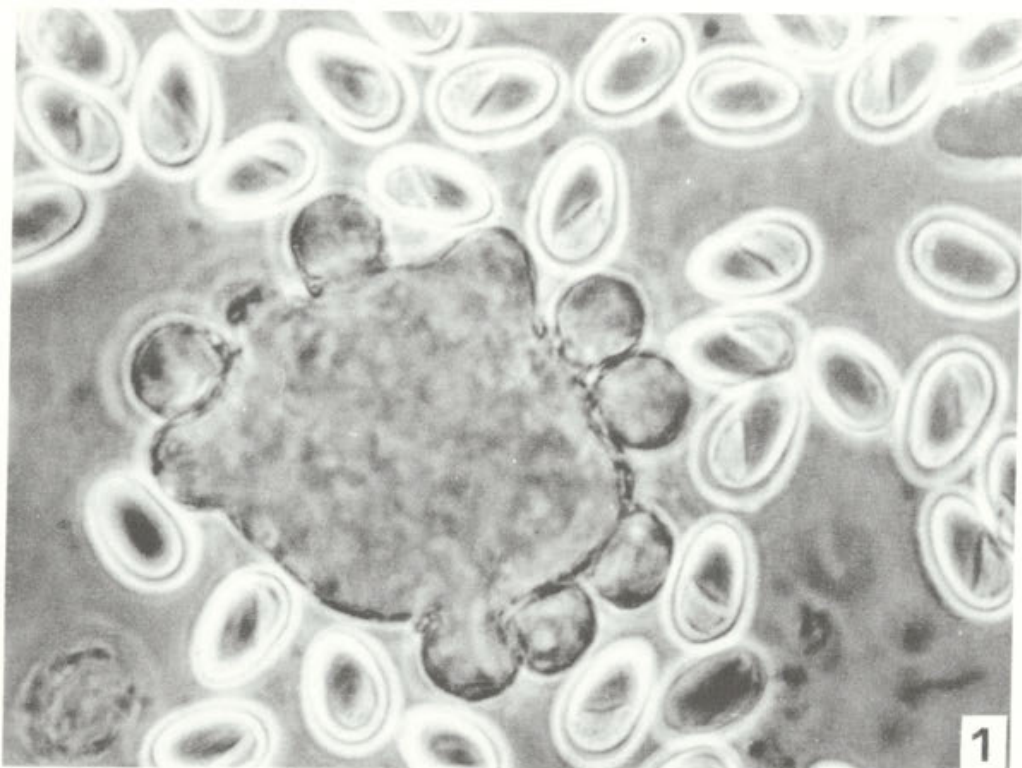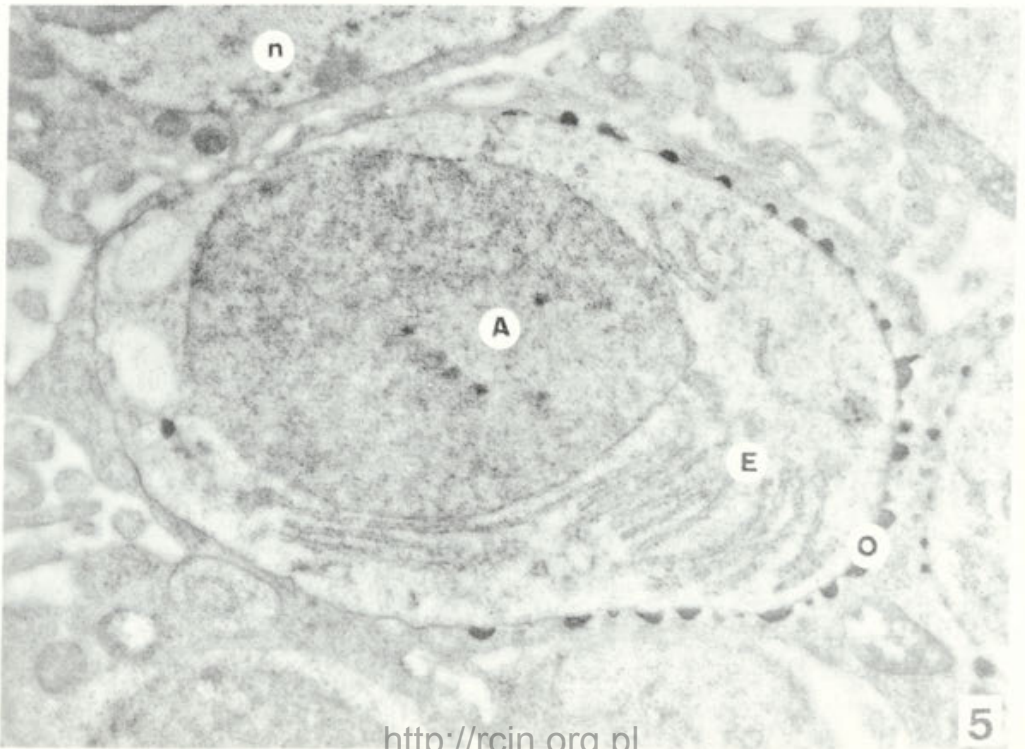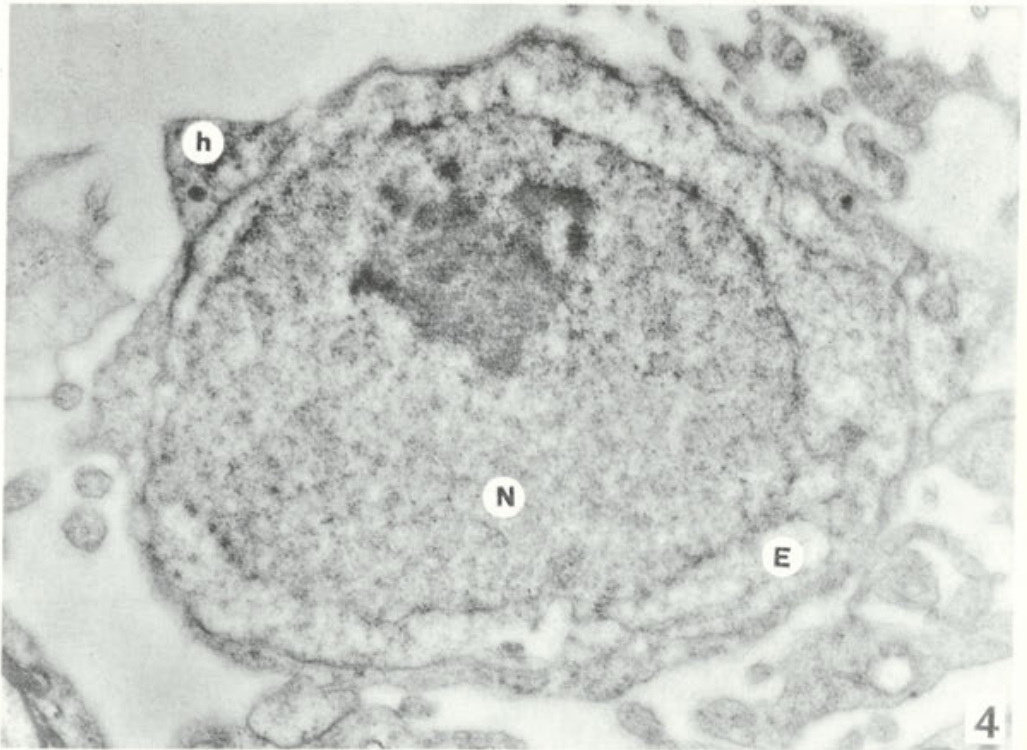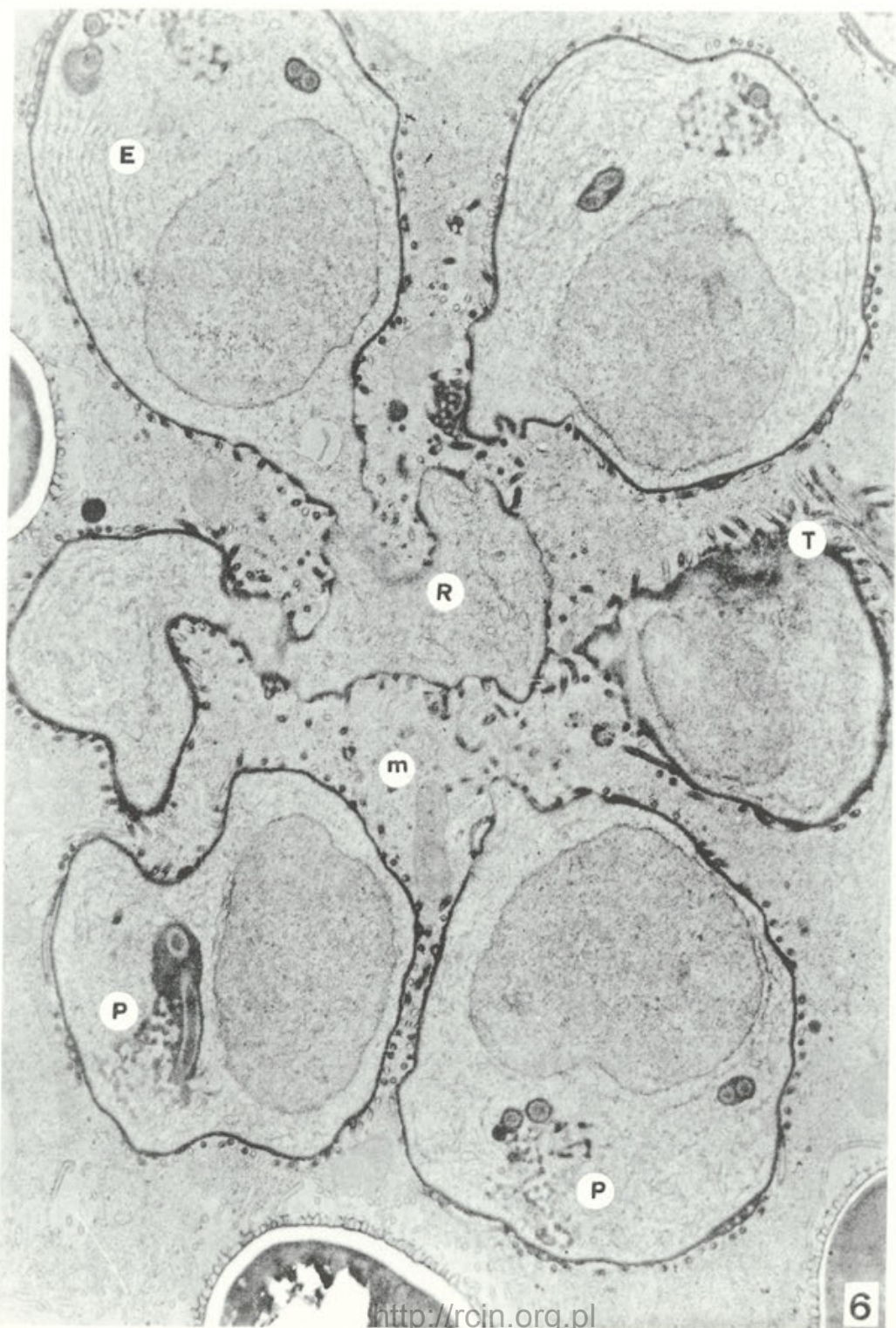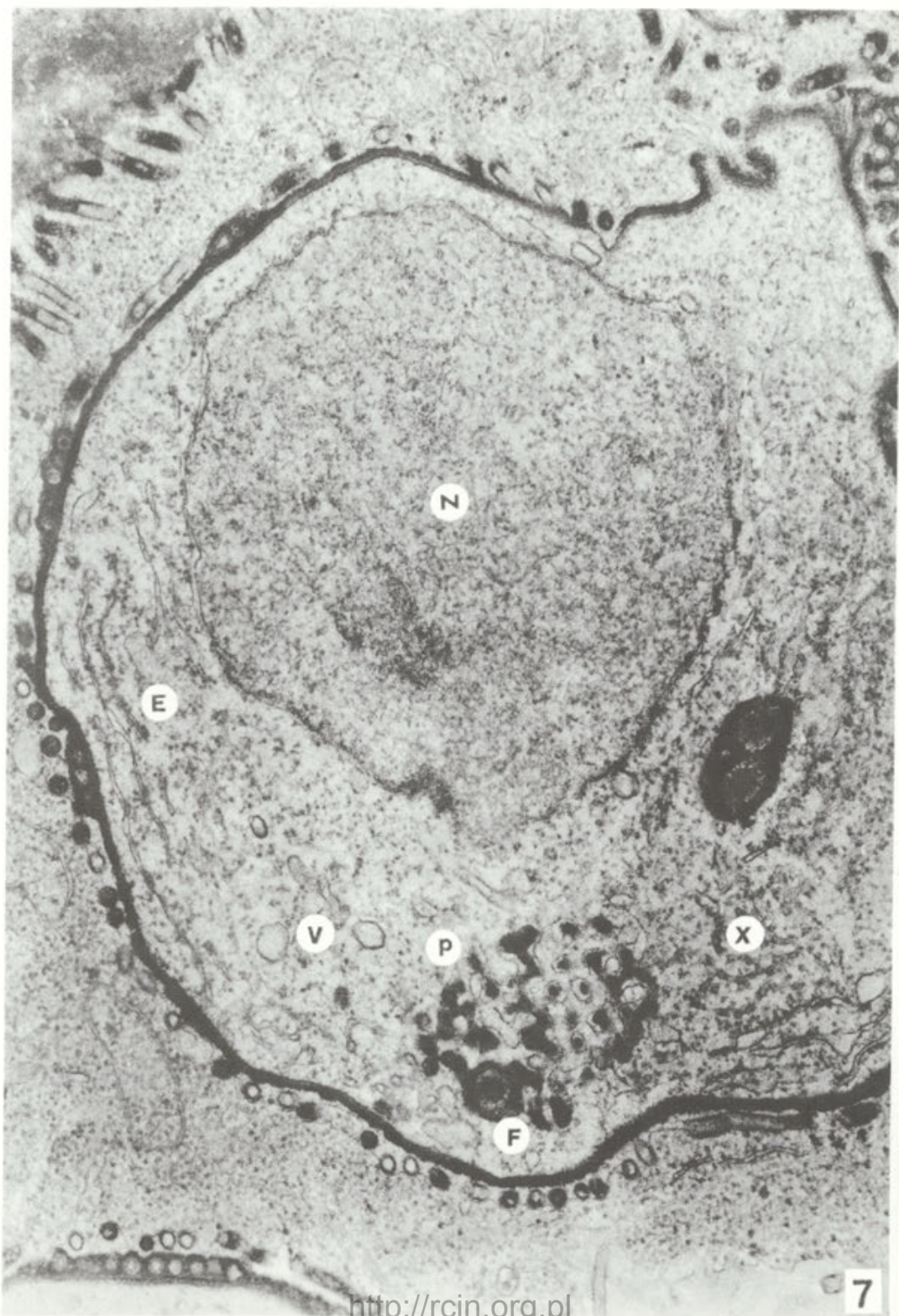
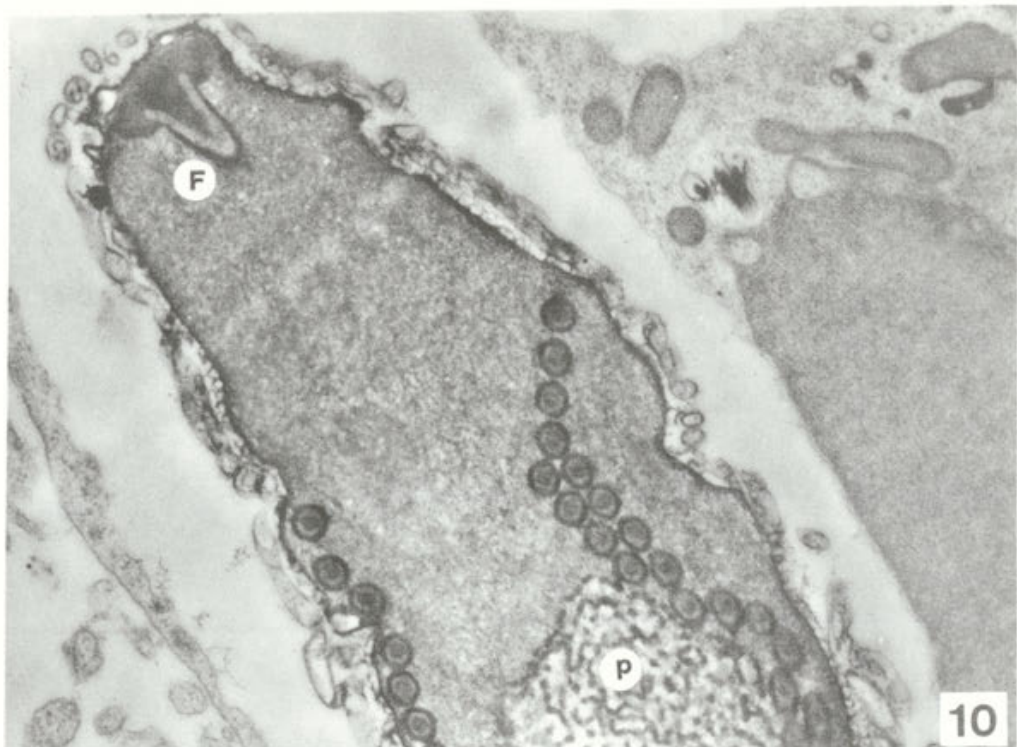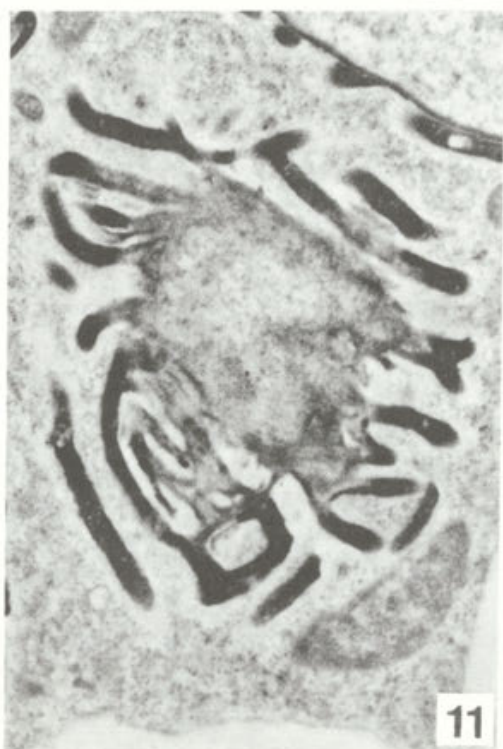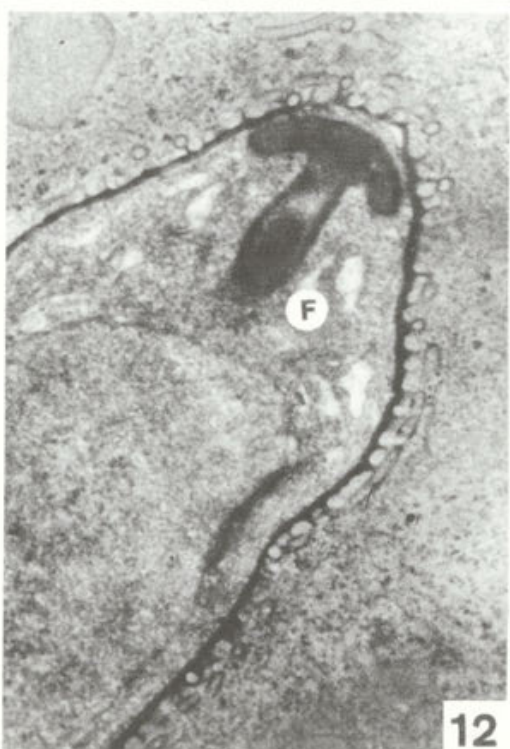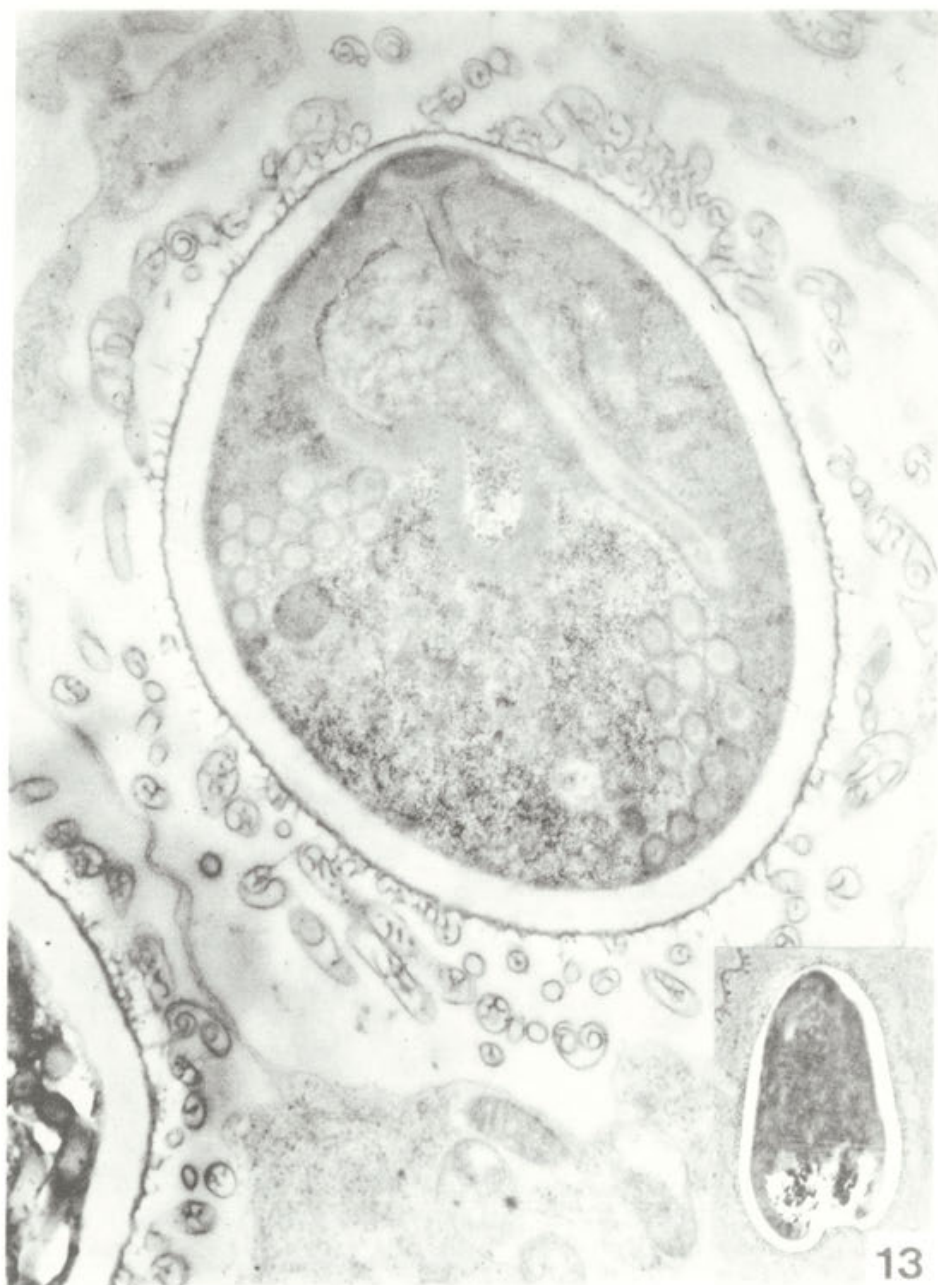PLATE II

J. Weiser et Z. Žižka

PLATE III

J. Weiser et Z. Žižka

auctores phot.

PLATE IV

J. Weiser et Z. Žižka

auctores phot.

J. Weiser et Z. Žižka

auctores phot.

PLATE VI

J. Weiser et Z. Žižka

auctores phot.

Ф. Г. АГАМАЛИЕВ и Р. М. БАГИРОВ
F. G. AGAMALIEV and R. M. BAGIROV

# Суточные вертикальные миграции инфузорий (микробентос, планктон, перифитон) Каспийского моря

## Diurnal Vertical Migrations of Microbenthic, Planctonic and Periphytonic Ciliates of the Caspian Sea

*Синопсис.* Суточные вертикальные миграции инфузорий микробентоса, планктона и перифитона Среднего и Южнего Каспия изучались в разные сезоны года. В микробентосе вертикальные миграции псаммофильных инфузорий изучены на трех типах песка (мелкий, гетерогенный, средний, крупный). У всех изучаемых групп инфузорий миграция в верхние горизонты начинается с второй половины дня, а миграция вглубь с рассветом. В результате в холодное и бедное светом время года инфузории пребывают в поверхностных слоях грунта и воды дольше чем в теплое время года. Солнечная погода вызывает более усиленную миграцию бентических инфузорий в нижние горизонты песка, а также горизонтальное перераспределение (миграция в тень камней, под водоросли и др).

## Введение

Вопросам вертикальной миграции морских и пресноводных организмов посвящено очень много работ. Однако, причины суточных вертикальных миграций, связаных с чередованием светлого и темного времени суток выяснены еще недостаточно. Одни авторы согласно сводке Константинова (1967) объясняют миграции как реакцию фототропизма, другие — как результат взаимодействия фото- и геотропизма. А многие другие авторы (Николаев 1952, Мантейфель 1960, 1972) указывают, что в вертикальных миграциях большое значение могут иметь биологические и биоценотические фак-

торы, в частности уход жертв от хищников утром в нижние неосвещенные слои. Все эти гипотезы основывались материалами наблюдений за много-клеточными организмами.

Что же касается одноклеточных организмов, в частности инфузорий, то их вертикальные миграции (особенно суточные вертикальные миграции) в различных географических районах изучены еще недостаточно. Как известно инфузории широко распространены в водоемах всех континентов земного шара и служат биологическими индикаторами загрязненности водоемов, а также являются пищей для молоди многих промысловых рыб. Помимо этого, многие виды их постоянно используются в качестве объектов для цитологических, физиологических, биохимических и др. исследований. По-этому изучение экологии инфузорий различных географических районов земного шара представляет большой теоретический и практический интерес.

Изучение вертикальной миграции инфузорий особенно усилилось после выхода работ Uhlig (1964, 1965), разработавшего надежную методику экстра-гирования псаммофильных инфузорий из грунта. В работах Fenchel and Jansson (1966), Fenchel (1967, 1968 a, b, 1969), Агамалиева (1967 a, b, 1970, 1972, 1973), Бурковского (1968, 1971), Petran (1968), Margalef (1963, 1968), Beers and Stewart (1967, 1969), Морозовская (1971), Заика (1972, 1973), Hartwig (1973) подробно рассматриваются вопросы вертикальной миграции фауны в целом и отдельных видов инфузорий в грунте и толще воды. Однако, суточные вертикальные миграции инфузорий до настоящего времени не изу-чались. В этой области можно назвать только работы Заика (1972), в которой даются суточные вертикальные миграции нескольких видов планктонных инфузорий.

В настоящей работе излагаются основные результаты изучения суточных вертикальных миграций всех экологических групп инфузорий (микробентос, планктон, перифитон) Каспийского моря.

### Методика работы и краткая экологическая характеристика района исследования

Работа проводилась в течение 1969–1973 гг. в различных участках Среднего и Южного Каспия (на разрезах: Махачкала, Дербент, Худат, Хачмас, Устье р. Куры, Куринская коса, Ленкорань, Форт-Шевченко, Песчаный, Бекташ, Куули-маяк) заливах западного и восточного побережья, а также островах Бакинского и Апшеронского архипелагов. Суточные наблюдения проводились, в основном, на четырех разрезах-Махачкала, устье р. Куры, Бекташ, Красно-водский залив и частично на побережье Апшеронского полуострова. На осталь-ных разрезах были собраны только качественные пробы. Последние в микро-

бентосе и перифитоне были собраны путем соскабливания поверхностных слоев грунта и неподвижных естественных, а также искуственных объектов. Количественные пробы микробентоса собирались поршневой трубкой (диаметр 2 cm) послойно: 0–1, 1–2, 2–3 cm; и далее с интервалами в 1 cm до глубины 20 cm. Для изучения суточной вертикальной миграции инфузорий отбор проб проводился в том же месте 6 раз в сутки (16, 20, 24, 4, 8 и 12 часов). В общей сложности изучено свыше 1000 проб, из них 250 количественных. Последние изучались не позднее, чем через 1 час после взятия. Для выделения инфузорий из песка был применен метод Улига (Uhlig 1964, 1965), описание которого дается в наших предыдущих работах (Агамалиев 1970).

Планктонные пробы были собраны обыкновенной сетью (мельничный газ No. 75) и батометром Нансена. Изучение вертикальной миграции планктонных инфузорий производилось на 5 суточных станциях по стандартным горизонтам 0–5, 5–10, 10–25, 25–50, 50–70, 70–100 m. Суточные наблюдения проводились через каждые 4 часа. Всего было собрано и обработано 320 проб, из них 200 количественных. Подсчет инфузорий производился в пробах воды объемом 5 ml без сгущения, в счетной камере, при трех-четырех повторностях.

Суточные и сезонные изменения фауны перифитонных инфузорий изучались на экспериментальных пластинах, подвешенных на стандартных глубинах 0–5, 5–10, 10–15, 15–20, 20–25 m. Максимальная-продолжительность экспозиции пластин в каждом сезоне составляла 10 дней. Всего было обработано 200 пластинок, обследование которых производилось каждые 4 часа.

Одновременно брали образцы песка для механического анализа и для определения содержания в нем органических веществ, а также проводили измерения некоторых гидрологических и гидрохимических показателей морской и интерстициальной воды (температуры, солености, содержания кислорода и др.).

На исследованной нами акватории Среднего и Южного Каспия (включая заливы и острова) температура прибрежных поверхностных слоев воды варьирует от 5 до 28°C. Прогрев воды обычно начинается с марта, а в мае, температура воды на поверхности в среднем достигает уже 13–15° в средней, 16–21° в южной части моря. В августе средняя температура воды повсеместно выше 25°.

Соленость воды в Среднем и Южном Каспии варьировала от 6.8‰ до 13.0‰. В восточных мелководных заливах моря она достигает 14.5‰.

Следует отметить, что отдельные географические районы Среднего и Южного Каспия отличаются друг от друга по содержанию органического вещества в грунте, по зернистости песка и по степени прибойности.

Зона западного побережья Среднего Каспия открыта и очень мало защищена от действия морского прибоя. Вода в этой зоне Каспия, чистая грунт состоит из мелкого (с примесью крупной фракции на Дербентском и Махачаклинском участках) песка с ракушей различной величины. На глубинах

25–50 m грунт представляет собой заиленный песок и песчаный ил. Органическое вещество в грунте данного района варьирует от 0.30 до 0.86%.

Район западного побережья Южного Каспия хорошо защищен от действия прибоя. Берег довольно сильно расчленен рядом наносов, а в море находится целый архипелаг грязевулканических и других островов. Южнее расположены устье р. Куры и Куринская коса, переходящие в прибойную Ленкоранскую зону. Грунты данного района представлены чаще всего мелкими, слегка заиленными песками и ракушечными илами. Сапробность грунта составляет в среднем 0.50–0.67% органического вещества. Модальный размер песчинок варьирует в пределах от 0.05 до 1.3 mm.

Восточный берег Среднего Каспия отличается от западного берега целым рядом климатических и гидрологических особенностей. Здесь в более глубоководных зонах широко распространены жесткие грунты (ракушечники, гравий, камни). В прибрежных зонах можно выделить 4 типа грунтов: песок, ракуша, илистая ракуша и твердый грунт. Песчаные грунты обычно распространены на мелководной полосе района исследования. Она простирается в основном до глубины 25 m. Эти грунты местами распространены до 50–100 m глубины (разрез Казахский залив). Органическое вещество в песках Среднего Каспия составляло от 0.24 до 0.67%. Модальный размер песчинок 0.78–3.00 mm.

Что касается восточного побережья Южного Каспия, то этот район характеризуется сильной изрезанностью береговой линии, широким распространением песчаных грунтов и наличием многочисленных заливов и бухточек. Наряду с песчаным грунтом на некоторых участках (Красноводский и Балханские заливы) данного района преобладают илистые и илисто-ракушечные грунты. Органическое вещество в грунтах юго-восточного Каспия варьирует от 0.38 до 2.70%. Модальный размер песчинок составляет 0.05––1.3 mm. Как видно из изложенного, отдельные географические районы несколько отличаются друг от друга. Однако, для суточных наблюдений были выбраны почти равномерные участки и в связи с этим в работе даны общие средние цифровые (суточные) данные по всей территории.

Результаты

В исследуемом районе было обнаружено свыше 300 видов инфузорий микробентоса, планктона и перифитона (обрастаний). Однако, при изучении суточных вертикальных миграций инфузорий в грунте и в толще воды учитывались лишь 130 часто встречающихся видов, образующих массовые популяции и относящихся к 18 семействам. Из них 57 видов относится к микробентосу, 30 видов — к планктону и 43 вида к перифитону (см. Табл. 1). Как

Таблица 1

Table 1

Состав фауны изученных экологических групп инфузорий Среднего и Южного Каспия

A Quantitative Composition of Species within Ecological Groups of Cilliates in the Middle and South Caspian Sea

| Семейства<br><br>Family | Число родов<br><br>No. of genera | Число видов  No. of species | | |
|---|---|---|---|---|
| | | Микро-бентос<br>Micro-benthos | План-ктон<br>Plancton | Перифитон<br><br>Periphyton |
| *Enchelyidae* Ehrbg. | 6 | 6 | 3 | 3 |
| *Colepidae* Ehrbg. | 1 | 2 | — | 2 |
| *Trachelocercidae* Kent | 3 | 7 | — | — |
| *Amphileptidae* Bütschli | 2 | 5 | 1 | 3 |
| *Loxodidae* Bütschli | 2 | 4 | — | — |
| *Didiniidae* Poche | 2 | 1 | 2 | — |
| *Spathidiidae* Kahl | 2 | 2 | — | — |
| *Dysteriidae* Clap. et Lachm. | 3 | 1 | 1 | 4 |
| *Frontoniidae* Kahl | 3 | 3 | 2 | — |
| *Pleuronematidae* Kent | 4 | 4 | 3 | 5 |
| *Spirostomatidae* Stein | 3 | 4 | — | — |
| *Condylostomatidae* Kahl | 1 | 2 | — | — |
| *Folliculinidae* Dons | — | — | — | 3 |
| *Halteriidae* Clap. et Lachm. | 3 | 1 | 4 | — |
| *Tintinnidae* Kofoid-Cambell | 1 | — | 1 | — |
| *Codonellidae* Kent | 2 | — | 5 | — |
| *Oxytrichidae* Ehrb. | 9 | 7 | 4 | 3 |
| *Euplotidae* Ehrb. | 2 | 6 | 4 | 4 |
| *Aspidiscidae* Stein | 1 | 2 | — | 1 |
| *Vorticellidae* Stein | 3 | — | — | 15 |
| Всего<br>Total | 53 | 57 | 30 | 43 |

видно из таблицы, в микробентосе (мезопсаммоне) основная часть фауны инфузорий падает на долю семейств *Enchelyidae, Trachelocercidae, Amphileptidae, Oxytrichidae* и *Euplotidae*, в планктоне — *Halteriidae, Codonellidae, Oxytrichidae* и *Euplotidae*, а в перифитоне — *Pleuronematidae, Vorticellidae, Dysteriidae* и *Euplotidae*.

Представители указанных семейств были обнаружены во все сезоны года, преимущественно в самых верхних слоях воды и грунта. В Табл. 2 даются результаты гранулометрического анализа грунтов Среднего и Южного Каспия.

Таблица 2
Table 2

Гранулометрическая характеристика грунтов Среднего и Южного Каспия

Granulometric Characteristic of Grounds of the Middle and South Caspian Sea

| Названия грунта Ground types | Размер зерен Granule diameter (mm) |
|---|---|
| Ил Sloam | 0.01–0.03 |
| Песчаный ил Sandy sloam | 0.03–0.05 |
| Песок Sand | |
| Очень мелкий Very fine | 0.05–0.08 |
| Мелкий Fine | 0.12–0.4 |
| Средний Medium | 0.5–0.7 |
| Крупный Coarse | 0.8–1.3 |
| Гравий    Gravel | |
| Мелкий Fine | 1.3–3.0 |
| Средний Medium | 3.0–6.0 |
| Крупный Coarse | 6.0–10.0 |

Псаммофильные инфузории (микробентос)

Изучение фауны инфузорий в различных типах грунтов показало, что илистые грунты и гравий наиболее бедны. Что касается очень мелкого песка, особенно илистого, то он может испытывать различную степень уплотнения и в верхних слоях лежать более рыхло, чем в нижних. По мере уплотнения грунтов инфузории все в меньшей степени проникают в его толщу. Поэтому при изучении суточной вертикальной миграции инфузорий указанные грунты (ил, гравий и очень мелкий песок) не рассматривались.

Вертикальные миграции инфузорий микробентоса были изучены, в основном, на трех типах песка (мелкий гетерогенный, средний, крупный). Следует отметить, что вертикальные миграции инфузорий всех экологических групп (микробентос, планктон, перифитон) носят суточный характер. Начинаются они с наступлением вечерних сумерек и заканчиваются с приближением рассвета. По-видимому, это связано с комплексным влиянием температуры, освещенности, солености, газового режима, волнения и др.

Температура относится к числу наиболее универсальных экологических факторов. При измерении температуры свободной морской и интерстициальной воды было выявлено, что температура последней зависит от температуры свободной морской воды, температуры воздуха, степени инсоляции и испарения. Она испытывает очень значительные суточные колебания. Как видно
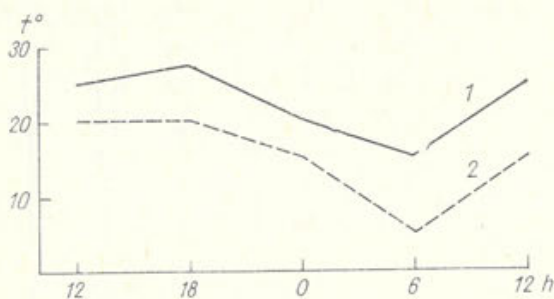
Рис. 1. Суточные колебания температуры морской и интерстициальной воды (1 — морская, 2 — интерстициальная на глубине 4–5 cm в мелком гетерогенном песке); ось ординат — температура, ось абсцисс — время суток
Fig. 1. Daily fluctuations of temperature in sea and interstitial water (1 — sea water, 2 — interstitial water at a depth of 4–5 cm in fine heterogenic sand). Ordinate — temperature, abscissa — time in hours

на Рис. 1, температура в морской и интерстициальной воде в мелком гетерогенном песке на глубине 4–5 cm изменяется параллельно. Глубокие слои песка (25–30 cm), напротив, почти не прогреваются при ярком солнечном освещении, за исключением зоны уреза воды и отдельных мелких лагун, где наблюдаются суточные изменения температуры даже на этой глубине. При продолжительной пасмурной погоде суточных изменений температуры в интерстициальной воде почти не наблюдается. В местах, где проводились опыты, было определено также содержание растворенного кислорода в свободной морской и интерстициальной воде мелкого гетерогенного песка. В первой оно составляло 5.82–6.75 $cm^3/l$, а во второй (при тихой погоде) — 3.14–4.17 $cm^3/l$ (суммарные данные для слоя песка глубиной от 0 до 10 cm). При штормовой погоде содержание $O_2$ в интерстициальной воде песка значительно увеличивается (в среднем до 5.09 $cm^3/l$). Указанные факторы, в первую очередь, определяют вертикальное распределение инфузорий.

Характер суточных вертикальных миграций инфузорий оказался несколько различным в разных типах песка, а также в разные сезоны года.

В пробах мелкого гетерогенного песка встречаются в основном микропоральные и эврипоральные виды инфузорий. К числу массовых форм здесь можно отнести *Holophrya simplex*, *Lacrymaria coronata*, *Trachelocerca coluber*, *Tracheloraphis prenanti*, *Litonotus lamella*, *Remanella rugosa*, *Mesodinium pulex*, *Paraspathidium fuscum*, *Frontonia marina*, *Pleuronema coronatum*, *Anigsteinia clarissima*, *Condylostoma arenarium*, *Holosticha manca*, *Euplotes raikovi*, *Aspidisca caspica*, и др. Все эти виды были обнаружены в различное время суток и во все сезоны года.

На Рис. 2 представлены количественные данные по суточной динамике вертикального распределения инфузорий в мелком гетерогенном песке в разные сезоны года. Из рисунка видно, что во все сезоны в темное время суток
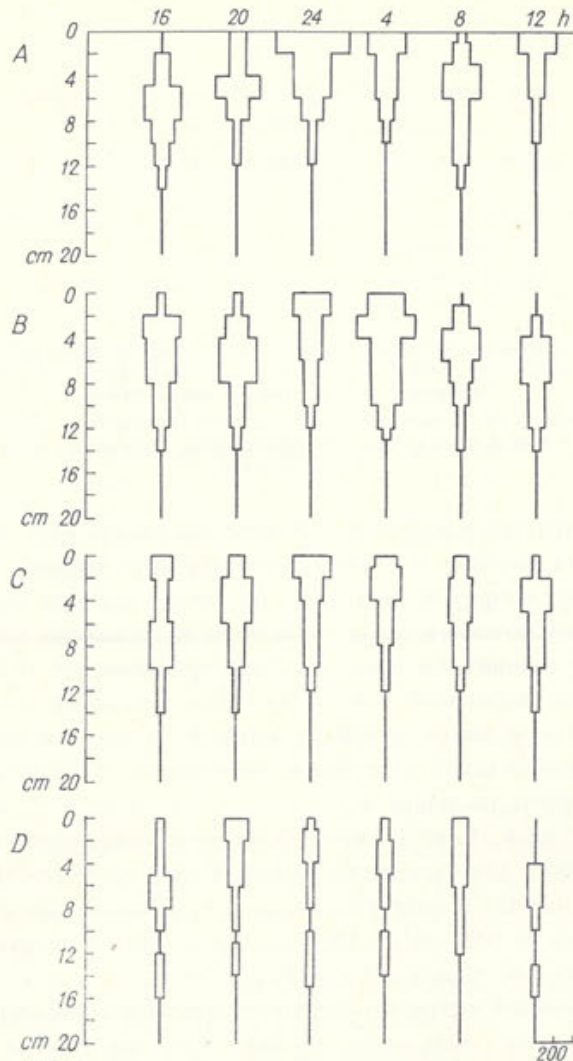
Рис. 2. Суточные вериткальные миграции инфузорий в мелком гетерогенном песке (Mo = 0.25–0.4 mm) в разные сезоны года A — весна, B — лето, C — осень, D — зима (для ясной погоды); ось ординат — глубина в сантиметрах, ось абсцисс — время суток. Отрезок в правом нижнем углу графика представляет 200 экземпляров инфузории

Fig. 2. Daily vertical migrations of ciliates in fine heterogenic sand (Mo = 0.25–0.4 mm) in various seasons. A — spring, B — summer, C — autumn, D — winter (sunny weather). Ordinate — depth in centimetres, abscissa — time in hours. Scale in right lower corner of diagram represents 200 specimens of ciliates

инфузории численно преобладают в верхних слоях грунта. Весною и летом ночью (в 24 и 4 часа) основная часть фауны сосредоточивается в слоях 0–2 и 0–4 cm. Осенью и особенно зимой выход инфузорий на поверхность в ночное время наступает раньше (к 20 часам), но выражен слабее; инфузории в темное вре-

мя численно преобладают осенью в слоях 0–2 и 1–4 cm, зимой в слоях 0–4 и 0–5 cm. Следует отметчить, что в темное время суток имеет место не только концентрация инфузорий в самом поверхностном слое грунта, но и частичный переход некоторых видов (*Holophrya simplex, Frontonia marina, Pleuronema coronatum* и др.) из грунта в толщу придонной воды.



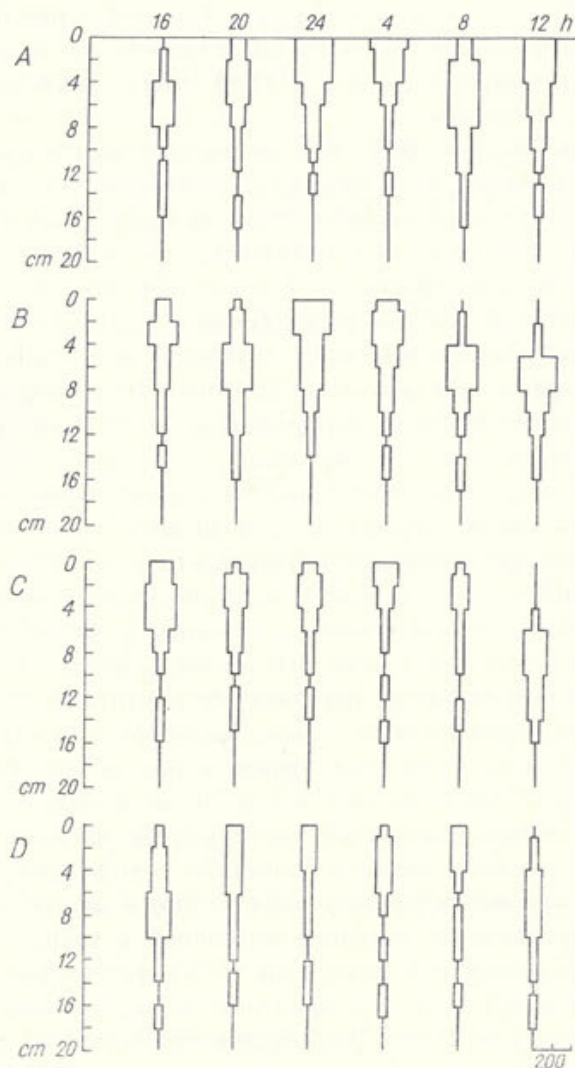Рис. 3. Суточные вертикальные миграции инфузорий в среднезернистом песке (Mo = 0.5–0.7 mm) в разные сезоны года A — весна, B — лето, C — осень, D — зима (для ясной погоды); ось ординат — глубина в сантиметрах, ось абсцисс — время суток. Отрезок в правом нижнем углу графика представляет 200 экземпляров инфузории

Fig. 3. Daily vertical migrations of ciliates in medium sand (Mo = 0.5–07 mm) in various seasons. A — spring, B — summer, C — autumn, D — winter (sunny weather). Ordinate — depth in centimetres, abscissa — time in hours. Scale in right lower corner of the diagram represents 200 specimens of ciliates

С рассветом основная масса инфузорий перемещается в более глубокие горизонты, причем зимой это происходит позже (после 8 час. утра, Рис. 2). Во все сезоны года в светлое время суток инфузории численно преобладают в средних слоях песка (2–4, 4–8 cm).

Общая численность инфузорий в мелком гетерогенном песке резко падает с глубиной. Весною, летом и осенью ниже 14 cm инфузории почти не встречаются. Зимой инфузории проникают в более глубокие слои (до 16 cm). Здесь чаще других встречаются виды родов *Trachelocerca*, *Tracheloraphis*, *Trachelonema*, *Remanella*, *Anigsteinia*.

В среднезернистом песке (Рис. 3) суточные вертикальные миграции носят в общем тот же характер, что в мелком гетерогенном песке. Весной и летом в темное время суток (24 и 4 часов) 60–70% особей инфузорий обнаруживаются в поверхностных слоях песка (0–1, 0–4 cm). Осенью и зимой ночной поверхностный максимум более растянут в глубину (0–6 cm). Фауна в этих слоях представлена в основном видами родов *Prorodon*, *Lacrymaria*, *Coleps*, *Litonotus*, *Uronema*, *Euplotes*, *Aspidisca*. В нижних слоях песка обнаруживаются главным образом микропоральные и некоторые эвритопные виды инфузорий (*Tracheloraphis prenanti*, *Loxophyllum helus*, *Paraspathidium fuscum*, *Remanella rugosa*, *Kentrophoros uninucleatum*, *Anigsteinia clarissima*).

В светлое время суток максимум численности инфузорий смещается в глубину сильнее, чем в мелком песке — до 6–8 cm летом и 6–10 cm осенью. Максимальная глубина проникновения инфузорий в грунт в среднем песке доходит до 16–17 cm весной, летом и осенью и до 18 cm зимой и сравнительно мало меняется в течение суток. В осеннее и особенно в зимнее время суточные миграции вообще выражены слабее, чем весной и летом.

Интересная и еще не вполне понятная черта вертикальных миграций инфузорий в мелком и среднем песке — выход на поверхность максимума численности инфузорий около 12 час. дня, причем только весной (Рис. 2 и 3). Этот выход кратковременный поскольку в 8 и 16 часов максимум численности приходится, как обычно для светлого времени суток, на более глубокие слои. Может быть, это явление связано с положительным фототаксисом обильных весной зеленых жгутиконосцев, которыми питаются многие инфузории.

Суточные вертикальные миграции инфузорий в крупнозернистом песке (Рис. 4) характеризуются теми же чертами, что и в среднезернистом, но средняя и максимальная численность инфузорий здесь ниже и глубина проникновения их в грунт больше (до 20 cm). Это обусловлено большими размерами пор, лучшей циркуляцией воды, большим содержанием кислорода (5.93 cm³/l) в интерстициальной воде и более равномерным распределением пищевых объектов (аэробные бактерии, одноклеточные водоросли и фотосинтезирующие жгутиконосцы). Фауна состоит в основном из неспецифичных видов инфузорий. Специфичные (интерстициальные) виды в данном песке составляли около 10% (*Tracheloraphis prenanti*, *Loxophyllum setigerum*, *Remanella rugosa*,

*Kentrophoros uninucleatum, Gruberia binucleata* и др.). Ночной максимум по-преж-
нему сохраняет поверхностное положение, кроме зимы, когда он несколько
смещен в глубину (1–4, 2–6 cm). Дневной максимум в крупном песке смещается
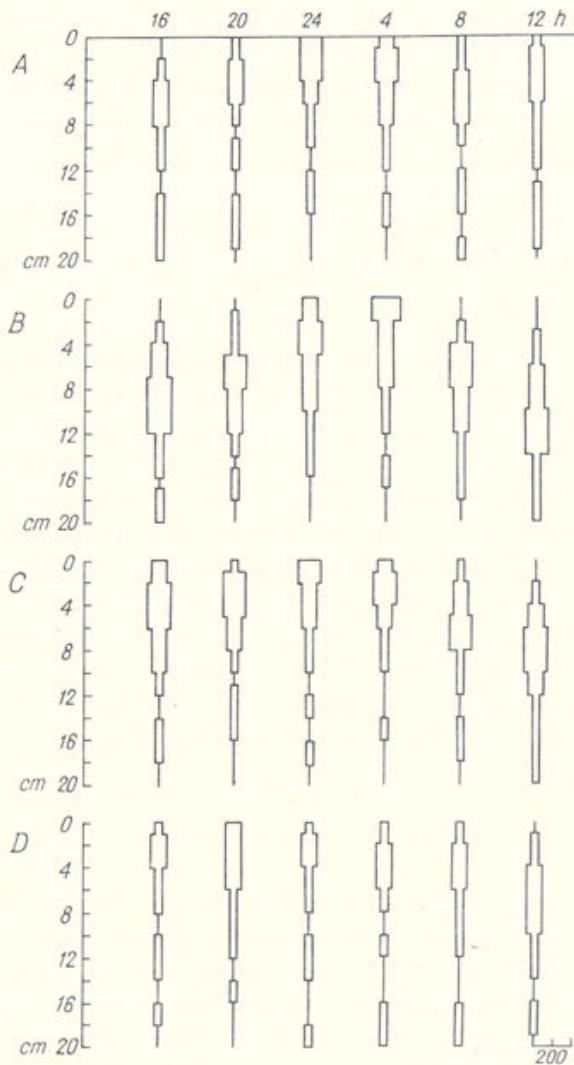в глубину еще сильнее, чем в среднем песке и приходится весной на слои



Рис. 4. Суточные вертикальные миграции инфузорий в крупнозернистом песке (Mo =
0.8–1.3 mm) в разные сезоны года A — весна, B — лето, C — осень, D — зима (для
ясной погоды); ось ординат — глубина в сантиметрах, ось абсцисс — время суток. Отрезок
в правом нижнем углу графика представляет 200 экземпляров инфузории
Fig. 4. Daily vertical migrations of ciliates in coarse sand (Mo = 0.8–1.3 mm) in various seasons.
A — spring, B — summer, C — autumn, D — winter (sunny weather). Ordinate — depth
in centimetres, abscissa — time in hours. Scale in right lower corner of the diagram represents
200 specimens of ciliates

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4–8 cm, летом на слои 6–14 cm и осенью — 2–10 cm. Зимой вертикальные суточные миграции выражены слабо, а летом — наиболее сильно, что, вероятно, связано с сильным прогревом грунта в этот сезон. Весенний 12-часовой, максумум у поверхности выражен в крупном песке слабо.

Для выяснения влияния инсоляции на суточные миграции инфузорий нами проводилось сравнение их вертикального распределения на одних и тех же участках литорали в одно и то же время суток (12 час. дня), но в солнечные и пасмурные дни. В результате было выявлено, что во всех типах песка летом при солнечной погоде дневная миграция инфузорий в нижние слои грунта выражена гораздо сильнее, чем в пасмурную (Рис. 7 a, b, c, I). В мелководных районах (0.3–0.5 m), где на песке много детрита или отдельные скопления водорослей, а также камни, в жаркую погоду наблюдается "пятнистое" распределение инфузорий. Вероятно, это объясняется тем, что при жаркой солнечной погоде часть фауны уходит вглубь песка, а часть укрывается под детритом, водорослями и в тени камней. Виды, уходящие вглубь песка, состояли исключительно из специфичных (интерстициальных) видов инфузорий (*Trachelocerca binucleata*, *Tr. coluber*, *Tr. prenanti*, *Tr. teissieri*, *Remanella rugosa*, *R. granulosa*, *Kentrophoros uninucleatum*, *Anigsteinia clarissima*, *Gruberia binucleata* и др.). Виды, перемещающиеся в основном в горизонтальной плоскости и собирающиеся под камнями и водорослями — в основном эвритопные, неспецифичные такие как *Prorodon binucleatus*, *Lacrymaria coronata*, *Coleps pulcher* виды семейства *Amphileptidae*, *Frontoniidae*, *Pleuronematidae*, *Oxytrichidae* и *Euplotidae*.

Как видно из Рис. 7, в пасмурную погоду (во всех типах песка) основное скопление инфузорий не только ночью, но и днем обнаруживалось в поверхностных слоях песка. Так, для мелкого гетерогенного песка максимум численности (в 12 час. дня) приходился на слои 1–6 cm, среднего песка — 0–7 cm, для крупного песка — 0–8 cm (см. Рис. 7 a, b, c, II).

## Планктонные инфузории

Суточные вертикальные миграции планктонных инфузорий были изучены на 30 видах. Доминирующими были виды семейств *Halteriidae*, *Codonellidae*, *Oxytrichidae* и *Euplotidae*. Остальные виды встречались в малых количествах.

Весною в планктоне в массовом количестве отмечались истинно пелагические виды инфузорий (*Tintinnididae*, *Codonellidae*). В мае температура в поверхностном слое воды колебалась от 18 до 20°C, глубже (20–25 m) она была порядка 6–10°C. В этом сезоне утром (8 час.) основное количество инфузорий находилось в средних слоях воды (10–25 m), где численность их составляла около 2.5 млн. экз/m³. Около 12 часов дня (при температуре 20°C) основная масса инфузорий кратковременно выходила в самые поверхностные слои воды (0–5 m), а к 16 часам снова мигрировала в 25-метровый горизонт (Рис. 5).

Около 20 часов, с приближением темноты, наблюдалось передвижение инфузорий в верхние горизонты, а к 24 часам на всех станциях максимальное количество инфузорий отмечалось в поверхностном десятиметровом слое воды. Однако уже в 4 часа утра начинается миграция инфузорий в глубину: их максимум приходится на слои 10–25 m.

Летом продолжается прогревание верхнего слоя воды, которое, благодаря вертикальной циркуляции, постепенно охватывает и более глубокие слои. Так, в конце июля и начале августа температура поверхностного слоя воды
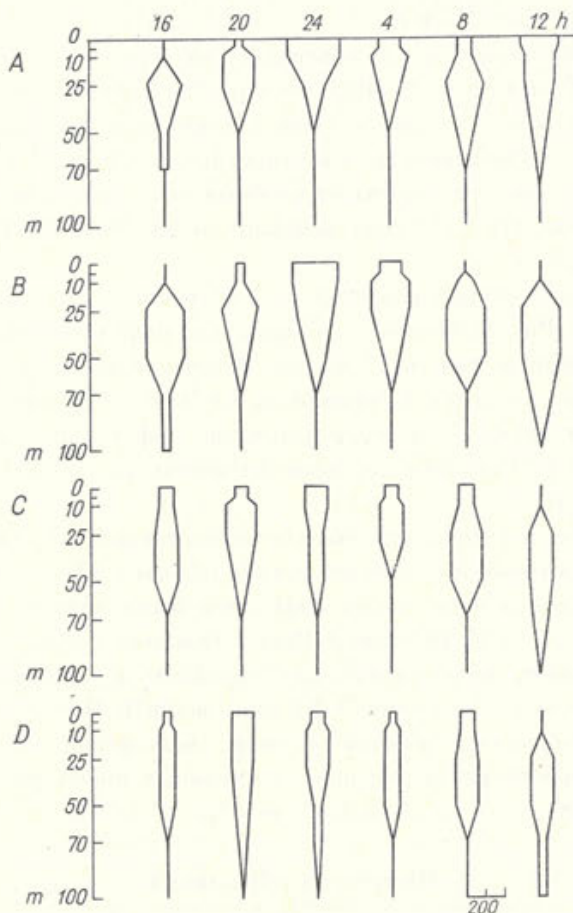


Рис. 5. Суточные вертикальные миграции планктонных инфузорий Каспийского моря в разные сезоны года A — весна, B — лето, C — осень, D — зима (для ясной погоды) ось ординат — глубина в метрах, ось абсцисс — время суток. Отрезок в правом нижнем углу графика представляет 200 экземпляров инфузории

Fig. 5. Daily vertical migrations of planctonic ciliates in the Caspian Sea in various seasons. A — spring, B — summer, C — autumn, D — winter (sunny weather). Ordinate — depth in metres, abscissa — time in hours. Scale in right lower corner of the diagram represents 200 specimens of ciliates

была 26–28°C, более глубоких слоев (25–50 m) — порядка 10–12°C. В этом сезоне удлиняется день и усиливается инсоляция.

В это время года в планктоне образуют массовые популяции *Enchelyodon sulcatus, Prorodon marinus, Mesodinium pulex, Didinium balbianii, Uronema marinum, Pleuronema coronatum, Cyclidium bergeri, Strombidium calkinsi, Tintinnopsis tubulosa, Holosticha manca, Euplotes balteatus* и другие малоспецифичные виды. Общий характер суточной вертикальной миграции инфузорий (Рис. 5) такой же, как весной; однако кратковреленный поверхностный максимум численности в 12 час. дня летом отсутствует. Дневной максимум приходится на большие глубины, чем весной (25–50 m).

В конце октября температура поверхностного слоя воды колебалас в пределах 16.2–18.7°C. Глубже (25–50m) температура равнялась 6–8°C.

Осенью и особенно зимой, в связи с сокращением продолжительности дня, уменьшением освещенности поверхностных слоев воды и началом штормовых ветров, видовое разнообразие и общая численность инфузорий уменьшается в 2–3 раза. Из планктона исчезают виды *Didinium, Uronema, Strobilidium, Tintinnopsis* и др.

В осенне-зимний период вергикальные миграции планктонных инфузорий выражены слабо (Рис. 5). Отмечается лишь уход инфузорий из поверхностных слоев около середины дня (в 12 часов). В течение круглых суток основная масса инфузорий находится в горизонтах 10–50 m. По сравнению с другими сезонами, в этот период зона сосредоточения инфузорий растянута по глубине, что, вероятно, связано с частыми штормовыми ветрами и интенсивной циркуляцией воды.

Сравнительное изучение вертикального распределения планктонных инфузорий при пасмурной и солнечной погоде (летом в 12 часов) показало, что при ясной погоде основные массы инфузорий были обнаружены на глубине 25–50 m (см. Рис. 7, d I). *Didinium balbianii, Frontonia marina, Cristigera minuta, Strombidium marinum,* виды семейства *Codonellidae* и *Tintinnididae* образовывали здесь массовые популяции (2–2.5 млн. экз/m³). При пасмурной погоде днем (12 час.) сохранялся "ночной" (точнее, свойственный 4 часам утра) характер вертикального распределения планктонных инфузорий: максимум их численности приходился на слой 10–25 m (Рис. 7, d, II).

## Инфузории обрастаний

Суточные изменения численности перифитонных инфузорий были в основном изучены на экспериментальных пластинках, подвешенных через каждые 5 m до общей глубины 25 m. Одновременно с этим проводились суточные наблюдения на гидротехнических сооружениях и отдельных сваях. Эксперименты проводились исключительно в защищенных от прибоя районах Среднего и Южного Каспия. В суточных наблюдениях учитывались лишь под-

вижные виды инфузорий, для которых мыслима вертикальная миграция. Обработка материала показала, что через сутки после погружения пластинки в воду на её поверхности обнаруживаются подвижные инфузории. Самое быстрое по времени (в течение 6 часов) оседание инфузорий на пластинки происходило летом. Зимой пластинки обрастали медленно, инфузории здесь появлялись только на вторые сутки.

Во все сезоны видовое разнообразие и численность подвижных форм инфузорий максимальны на третьи сутки, в течение которых и проводились суточные наблюдения.

Обработка материалов показала, что несмотря на кажущуюся трудность миграции перифитонных инфузорий на значительные расстояния сквозь толщу воды, эти миграции реально существуют и выражены вполне четко. Очевидно, инфузории в течение суток дважды уплывают с одних субстратов и оседают на других, находящихся на большей или меньшей глубине (Рис. 6).
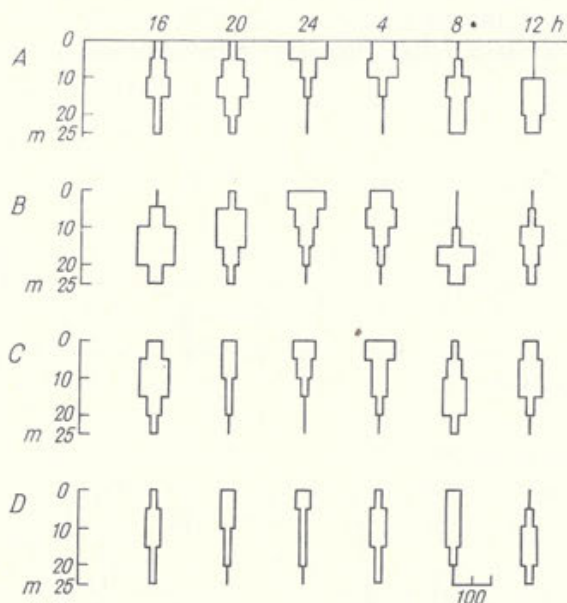


Рис. 6. Суточные вертикальные миграции перифитонных инфузорий Каспийского моря в разные сезоны A — весна, B — лето, C — осень, D — зима (для ясной погоды); ось ординат — глубина в метрах, ось абсцисс — время суток. Отрезок в правом нижнем углу представляет 100 экземпляров инфузории

Fig. 6. Daily vertical migrations of periphytonic ciliates in the Caspian Sea in various seasons. A — spring, B — summer, C — autumn, D — winter (sunny weather). Ordinate — depth in metres, abscissa — time in hours. Scale in right lower corner of the diagram represents 100 spe cimens of ciliates

Весной, при температуре воды 17–19°C, в темное время суток (24 и 4 часа) основная масса подвижных инфузорий была обнаружена на пластинках, на-

ходящихся в слоях воды 0–5 и 5–10 m от поверхности. Массовыми формами здесь оказались *Enchelys marina, Prorodon binucleatus, Coleps tesselatus, Cyclidium bergeri, Uronema marinum* и др. Утром и днем (8 и 12 час.) инфузории, напротив, численно преобладали на пластинках, находящихся в гороизнтах 10–15, 15–20 m (см. Рис. 6). Вечером (16 и 20 час.) наблюдалась промежуточная картина.

Летом (в июле) при температуре воды 27–28°C на пластинках массового развития достигали *Hypotricha*, (виды *Holosticha, Oxytricha, Euplotes, Aspidisca*). Общий характер их суточных миграций — тот же, что у весенних форм: утренняя миграция на более глубоко расположенные субстраты и вечерняя — в сторону поверхности моря (Рис. 6).

Осенью и зимой, с понижением средней температуры воды (16.5 и 4.5°C), численность инфузорий в перифитоне уменьшается. Чаще всего на пластинках обнаруживаются *Litonotus lamella, Loxophyllum helus, Trochilioides recta, Dysteria monostyla, Chlamydodon trioqetrus, Condylostoma arenarium f. proturostyla, Aspidisca leptaspis, A. fusca* и др.

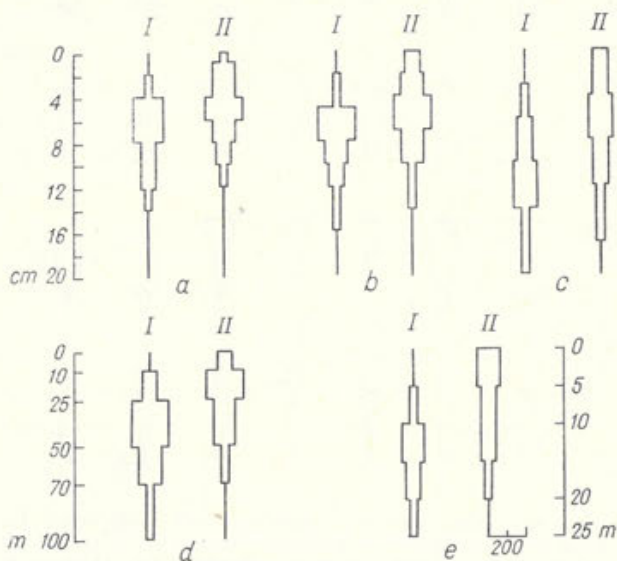В эти сезоны, в связи с сокращением светлого периода, "ночной" характер



Рис. 7. Изменение характера дневного (12 час.) вертикального распределения инфузорий летом при солнечной (I) и пасмурной погоде (II). (a — мелкий гетерогенный песок, b — средний песок, c — крупный песок, d — планктон, e — перифитон); ось ординат a–c — глубина в сантиметрах, d, e — глубина в метрах. Отрезок в правом нижнем углу графика представляет 200 экземпляров инфузории

Fig. 7. Changes in daily (12 h) vertical distribution of ciliates in summer, during sunny (I) and cloudy (II) weather (a — fine heterogenic, b — medium, c — coarse sand, d — plancton, e — periphyton). Ordinate — depth in centimetres (a–c) and in metres (d, e). Scale in right lower corner of the diagram represents 200 specimens of ciliates

распределения инфузорий преобладает в течение почти круглых суток. Дневной уход инфузорий в глубину выражен слабо и заметен осенью в 8 и 16 час., а зимой — в 12 час. (Рис. 6). При обработке серии проб, взятых с гидротехнических сооружений и деревянных свай в разное время суток (днем и ночью)-были получены те же результаты, что и на экспериментальных пластинках. В темное время суток основная часть перифитонных инфузорий находилась в верхних участках субстрата (сваи), а днем массовые популяции их обнаруживались в нижних участках (в горизонтах 5–10, 10–15 m). Что касается сидячих форм инфузорий, то изменения их численности в течение суток, естественно, не наблюдалось.

Результаты изучения вертикального распределения перифитонных инфузорий в пасмурную и солнечную погоду (летом в 12 час.) показало, что в первом случае основная масса инфузорий накапливается в 0.5-метровом горизонте, а во втором случае наибольшая численность инфузорий зарегистри рована в нижних горизонтах субстрата (10–15 m) (Рис. 7 e, I, II). Некоторы виды, как *Holosticha manca, Oxytricha tricornis, O. aeruginosa, Euplotes harpa, E. raikovi, Diophrys scutum, Aspidisca fusca, A. caspica* в указанных слоях образовывали массовые популяции.

## Обсуждение

Исследования суточных вертикальных миграций инфузорий микробентоса, планктона и перифитона (обрастаний) показали, что они различаются в отдельные сезоны года. У всех трех экологических групп инфузорий наблюдается в общем сходная реакция на действие факторов внешней среды (в частности, на свет). Так, в светлое время суток основная масса инфузорий всех трех экологических групп мигрирует вглубь грунта или воды, а в темное время суток возвращается в самые верхние слои. Однако, при тщательной обработке проб, взятых в различное время суток, выясняются некоторые вариации в вертикальных миграциях инфузорий в микробентосе, планктоне и перифитоне.

В микробентосе (псаммоне) амплитуда вертикальных миграций инфузорий различается в отдельных типах песка; она тем больше, чем крупнее песок. В мелком гетерогенном песке весной, летом и осенью в ночные часы иногда достигается очень высокая численность инфузорий в отдельных слоях (особенно верхних). Зимой, в связи с падением температуры, общая численность инфузорий во всех слоях значительно уменьшается, общая глубина проникновения инфузорий в грунт увеличивается, а дневные и ночные максимумы численности становятся менее четкими. Видимо, это связано с менее интенсивными суточными миграциями в холодное время года. В среднем и особенно крупном песке общая численность инфузорий меньше, а глубина их

проникновения в грунт больше, чем в мелком. Вертикальные миграции и здесь лучше выражены в теплое время года по сравнению с холодным.

Изучение суточных вертикальных миграций планктонных инфузорий в отдельные сезоны года показало, что весной и летом, мигрируя в вечерние часы из нижних горизонтов воды в верхние, инфузории к 24 и 4 часам образуют плотное скопление в верхнем 10-метровом слое. В светлое время суток основное скопление инфузорий обнаруживается глубже — в слоях 10–25, 25–50 m. Осенью и зимой как и в микробентосе, общее количество планктонных инфузорий несколько сокращается, а разница в количестве инфузорий между отдельными горизонтами уменьшается. Суточные сдвиги вертикального распределения становятся менее выраженными.

Как в микробентосе, так и в планктоне и периферитоне подъём инфузорий в верхние горизонты начинается уже со второй половины дня, когда интенсивность света в глубинных слоях уменьшается. В 20 часов значительное количество инфузорий, в зависимости от сезона года, находится в средних или даже в самом поверхностном слое. Самый ранний подъём инфузорий наблюдается осенью и зимой. В эти сезоны в 20 часов максимальное количество инфузорий находится уже в верхних горизонтах. Например, в мелком гетерогенном песке осенью в 20 часов основное скопление инфузорий обнаруживается в слоях 2–6 cm, а зимой — в самом поверхностном слое песка. Аналогичное явление наблюдается в средних и крупных песках. Сходная картина была обнаружена в планктоне, а также в перифитоне. Осенью в 20 час. основная масса планктонных инфузорий держится в слое 10–25 m, а зимой — 0–15 m. В перифитоне, как осенью, так и зимой, инфузории в 20 час. численно преобладают в горизонте 0–10 m.

Спуск инфузорий в нижние горизонты начинается со второй половины ночи. В отдельные сезоны года уже в 4 часа ночи большое количество инфузорий опускается в нижние горизонты. Самый ранний спуск инфузорий наблюдается летом. В это время в 8 часов утра в микробентосе основная масса инфузорий находится в слое 3–6 cm (в мелком гетерогенном песке) и 4–8 cm (в среднем и крупном песках), в планктоне — на глубине 25–50 m, а в перифитоне — в 15–20-метровом горизонте. Весной утреннее опускание инфузорий начинается несколько позже, а осенью и зимой — еще позже (Рис. 2–6) и приблизительно совпадает по времени с рассветом.

Следует отметить, что ночное распределение инфузорий в зависимости от зернистости грунта, а также от сезонов года несколько меняется. Весной в мелком гетерогенном песке инфузории в основном скапливаются в слое 0–2 cm, а в средних и крупных песках — в более толстом слое (0–4, 1–4 cm). В планктоне образуется скопление в верхнем 10-метровом горизонте, а в перифитоне их основное скопление обнаруживается в слоях 0–5, 5–10 m. Глубже численность инфузорий в 2–3 раза уменьшается, а в самых нижних горизонтах встречаются лишь единичные экземпляры.

Летом основное скопление инфузорий ночью наблюдается также в верхних горизонтах. Однако, в отличие от весеннего сезона, здесь инфузории несколько более рассредоточиваются и занимают более толстые слои грунта и воды с лишь постепенным увеличением количества инфузорий к поверхности (см. Рис. 2–6).

Осенью и особенно зимой в ночные часы максимальное количество инфузорий часто наблюдается не в самом поверхностном слое, а в следующем за ним, и заметно растянуто по глубине. Вероятно, это связано с тем, что на суточную ритмику миграций накладывается действие других факторов (температура, движение воды).

Таким образом, свет, по-видимому, является основным фактором, стимулирующим суточные вертикальные миграции как бентических, так и планктонных и перифитонных инфузорий. Наряду с этим, пища также может являться фактором, имеющим большое значение в суточных вертикальных миграциях. В частности, именно обилием пищи (фотосинтезирующих жгутиковых) мы склонны объяснить необычный кратковременный возврат многих инфузорий в самые поверхностные слои песка (Рис. 2–4) и воды (Рис. 5) около 12 час. дня, наблюдаемый только весной, т.е. во время "цветения" жгутиковых.

В пользу представления, по которому свет является основным фактором, регулирующим суточные вертикальные миграции инфузорий псаммона, планктона и перифитона, говорит и тот факт, что при пасмурной погоде дневная миграция инфузорий вглубь грунта или воды выражена гораздо слабее, чем при ясной погоде. Днем здесь сохраняется характер вертикального распределения инфузорий, свойственный ранним утренним часам (Рис. 7).

Вертикальное распределение инфузорий в грунте Fenchel and Jansson (1966) и Fenchel (1967, 1969) объясняют влиянием сероводорода и ухудшением кислородного режима с увеличением глубины. Несомненно, эти факторы лимитируют границы суточных миграций, т.е. определяют их диапазон. Однако, сами миграции вызываются, без сомнения, периодическими факторами. Другими факторами, определяющими диапазон суточных миграций, могут быть размеры пространств между частицами песка, характер температурного режима и распределение пищевых объектов в отдельных слоях грунта. Известно, что многие инфузории (бентические и планктонные) питаются одноклеточными водорослями (диатомовыми и др.), а также фотосинтезирующими жгутиконосцами. Как показал (Faure-Fremiet (1950 a, b, 1951), эти формы сами способны к вертикальным миграциям в грунте под воздействием приливно-отливного ритма и, вероятно, также и других факторов. Таким образом, одним из факторов, обусловливающих вертикальные миграции инфузорий, могут быть суточные или иные вертикальные миграции пищевых организмов.

Большой интерес представляет, наконец, сам факт способности перифитонных ползающих, т.е., казалось-бы, мало подвижных инфузорий к значи-

тельным суточным миграциям. Последние, несомненно, связаны с уплыванием их с одних субстратов, перемещением их в толще свободной воды и оседанием на гругие субстраты. Этот факт ранее не был известен.

Как отмечено выше, до настоящего времени литература посвященная суточной вертикальной миграции инфузорий почти отсутствует. Только в работе Заика (1972) (по Атлантическому океану и Средизменому морю), приводятся суточные вертикальные миграции ряда видов планктонных инфузорий. Данные этого автора показывают, что в Средиземном море максимум плотности микрозоопланктона приходится на глубину 20–30 m, независимо от времени суток, а в Атлантическом океане (Северо-западное побережье Африки) два максимума в слое 10–20 m и 50–75 m. Несколько иная картина отмечается в Каспийском море, где в зависимости от сезонов года численность инфузорий в слоях значительно меняются. Так, весной и летом основное скопление инфузорий обнаруживается в средних слоях воды (10–25 m), а осенью и зимой зона сосредоточения инфузорий становится растянутой и основная их масса находится в горизонтах 10–50 m.

В остальных работах приводятся только вертикальные распределения бентических и планктонных инфузорий. В замечательных работах Fenchel and Hartwig (по Балтийским и Северным морям) помимо общей фауны микробентоса детально разбирается ветикальное распределение отдельных видов инфузорий.

Сравнение полученных нами данных с данными этих авторов и с данными Бурковского (1968, 1971) (по Белому морю), Petran (1968) (по румынскому побережью Черного моря), Margalef (1963, 1968), (по Средиземному морю), Beers and Stewart (1969), (по Тихому океану) показывают, что несмотря на разное географическое положение этих морей, вертикальное распределение бентических инфузорий подчиняется общей закономерности: с увеличением глубины происходит значительное обеднение фауны. Однако при детальном сравнении вертикального распределения некоторых, массовых форм инфузорий указанных районов обнаруживаются некоторые различия в степени их проникновения в грунт и встречаемости в отдельных слоях. Так, в песках Белого моря максимальная глубина проникновения инфузорий доходит до 6 cm. Ниже 6 cm инфузории почти отсутствуют. Что касается численности инфузорий в отдельных слоях, то некоторые формы, как *Mesodinium, Remanella, Diophrys* образуют массовые популяции в самом верхнем слое (0–0.5 cm). Некоторые представители *Oxytrichidae* и *Trachelocercidae* в большом количестве встречаются на глубинах 0.5–2 cm. В Каспийском море указанные инфузории обнаруживают максимальное развитие в слоях 0–4 cm. В некоторых районах Каспия (западное и восточное побережье Южного Каспия) инфузории своего максимального развития достигают в слоях 0–6 cm.

Сравнение наших данных с данными Hartwig (1973) показывает, что максимальное проникновение инфузорий в глубь грунта в Северном море

больше (25–30 cm), чем в Каспийском море. Граница проникновения инфузорий вглубь грунта в песках Каспия доходит до 18–20 cm. Однако при сравнении вертикальной миграции отдельных групп инфузорий (в частности *Trachelocercidae*) обеих морей можно заметить, что в весенний и летний сезоны основная их масса (60–90%) концентрируется в слое 0–5 cm. Осенью и зимой большинство особей зарегистрированы в слоях 5–10 и 10–20 cm в Северном море и 5–13, 13–20 в Каспийском море (мелкий и крупный песок).

Данные Petran показывают, что инфузории в песках Черного моря проникают до глубины 8–10 cm. Некоторые формы — *Condylostoma remanei*, *Anigsteinia clarissima*, *Loxophyllum setigerum*, *Diophrys scutum* и *Frontonia marina* в большом количестве встречаются в слое 1–2 cm и особенно в слое 0–1 cm. *Pleuronema coronatum*, *Mesodinium pulex* доминируют в основном на глубине 5–6 cm. В отличие от Черного моря, в Каспии *P. coronatum* свое максимальное развитие получает в самом верхнем слое (0–1 cm), а *M. pulex* — в слое 2–3 cm. Вертикальное распределение некоторых массовых форм инфузорий (*Litonotus lamella*, *Condylostoma arenarium*, *Anigsteinia clarissima*, *Frontonia marina*, *Pleuronema coronatum*, *Diophrys scutum*, *Aspidisca fusca*) в Балтийском и Каспийском морях носит почти аналогичный характер. Указанные виды своего максимума достигают в слоях 0–1 и 1–2 cm.

При сравнении результатов полученных Margalef, Beers and Stewart, Морозовской и Заика по планктонным инфузориям прибрежных вод Испании (восточное побережье), Калифорнии (Тихий океан), Черного, Азовского морей и Каспия были отмечены аналогичные положения во всех отмеченных географических районах. Инфузории, в основном, типичные их формы (виды, родов *Tintinnopsis*, *Codonella*) наибольшей численности обнаруживают в самых поверхностных слоях прибрежных участков воды (0–10 m). В зависимости от сезонов года зоны максимального скопления инфузорий, меняются в пределах от 0.5 до 15 и 10–50 m.


Резюме

В разные сезоны года изучены суточные вертикальные миграции инфузорий микробентоса, планктона и перифитона Среднего и Южного Каспия на материале 130 часто встречающихся видов (57 видов микробентоса, 30 видов планктона и 43 вида перифитона). В микробентосе изучены вертикальные миграции псаммофильных инфузорий на трех типах песка (мелкий гетерогенный, средний, крупный).

Во все сезоны года и во всех типах песка в гемное вермя суток (24 и 4 часа) основное скопление инфузорий наблюдается в поверхностном 1–2 cm слое песка, а в светлое время суток — на несколько сантиметров глубже.

В холодное время года (осенью и зимой) общая численность инфузорий

уменьшается, зона их вертикального распределения становится более вытянутой, а их суточные миграции — менее выраженными. Сходная картина обнаружена при изучении планктонных и подвижных перифитонных инфузорий: вечером они мигрируют из нижних горизонтов воды в верхний и к 24 и 4 часам образуют максимум численности в верхнем 5–10 m горизонте. В светлое время суток основное скопление инфузорий обнаруживается в горизонтах 5–10, 10–25, 25–50 m. Миграция подвижных перифитонных инфузорий осуществляется сквозь толщу воды, путем их уплывания с одних субстратов и оседания на другие.

Как в микробентосе, так и в планктоне и перифитоне миграция инфузорий в верхние горизонты начинается со второй половины дня (16–20 час.), а миграция их вглубь — с рассветом и, видимо, связана с изменением освещенности. Весной и летом вечерний подъём инфузорий начинается позже, а утренний их спуск — раньше, чем осенью и зимой. В результате в холодное и бедное светом время года (осенью и зимой) инфузории пребывают в поверхностных слоях грунта или воды около 16 часов, весной — около 12 часов, а летом — около 6–7 часов.

В солнечную погоду смещение максимума численности инфузорий в нижние горизонты песка или воды днем выражено сильнее, чем в пасмурную. Кроме того, инсоляция вызывает и горизонтальное перераспределение бентических инфузорий (миграция их в тень камней, под водоросли и т.п.), в результате чего их распределение в поверхностных слоях грунта становится резко неравномерным.

## SUMMARY

Vertical daily migrations of microbenthic, plantonic and periphytonic ciliates of the Middle and South Caspian sea have been studied in various seasons using 130 abundant species (57 microbenthic, 30 planctonic and 43 periphytonic). In the microbenthos, vertical migrations of psammophilic ciliates have been studied in three types of sand (fine heterogeneous, medium, and coarse). In all seasons and in all types of sand, the main mass of the ciliates occurred in the superficial 1–2 cm. thick layers of the sand during the night (24 and 4 h), and in several centimeters deeper layers during the day. In cold seasons (autumn and winter), the general abundance of the ciliates decreases, the zone of their vertical distribution widens, and their daily migrations become less prominent. The daily migrations of the planctonic and of the motile periphytonic ciliates follow essentially the same pattern although the migration amplitude is here much greater (meters and dozens of meters). In the evening, they migrate upwards and produce by 24 until 4 h a superficial maximum in the upper 5–10 m thick water layer. During the day, the main mass of ciliates is in the 5–10, 10–25, and 25–50 m deep layers. The migration of motile periphytonic ciliates must occur through the water thickness, i.e., they leave one substrata and settle onto others.

In the microbenthos, the plancton and the periphyton, the upward migration of the ciliates begins during late afternoon (16–20 h), and the downward migration, at dawn, and both seem to be influenced by luminosity changes. In spring and summer, the upward evening migration begins later, and the downward morning migration earlier than in autumn and winter. In result, during

the cold and light-poor seasons, the ciliates remain in the surface layers of sand or water about 16 h whereas in spring — some 12 h, and in summer — only 6-7 h.

The downward migration of the ciliates during sunny days is stronger as compared to dull days. Insolation also causes horizontal re-distribution of the psammophilic ciliates as their migration into the shadow of stones, under masses of algae etc. This results in highly uneven distribution of these ciliates in the sand on sunny days.

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# Helical Nature of the Continuous Ciliary Beat of *Opalina*

*Synopsis*. High speed cinemicrographs taken with Nomarski interference contrast and phase contrast optics revealed that the cilia of *Opalina* beat with a continuous traveling helical wave pattern. This observation differs from the generally accepted discontinuous concept. In avoiding movements and in ciliary reversals the cilia just change the directional orientation of the axis of the helix, and simply continue to beat with this continuous traveling helical wave.

In comparison with the relatively complete description of the form of beat attained in studies of flagella (Brokaw 1971, 1972, Brown 1945, Gittleson and Jahn 1968, Holwill 1966, Votta et al. 1971), the description of the movement of cilia are quite inadequate. There are many reasons for this — most cilia are extremely small and the beat appears to be variable. In addition, unspecialized cilia when functioning normally during locomotion, are difficult to be directly observed and analyzed under the microscope because of their small size, their high angular velocity, their density of distribution and their optical properties which are almost identical with those of the cytoplasm as well as those of the surrounding fluid. All of these characteristics create many optical illusions and visual problems.

It is not mere coincidence that all previous observations on the form of ciliary activity were made on highly specialized organelles (e.g., cirri) with comparatively slow movement, of considerable size and usually of a compound nature instead of the unspecialized cilia used for locomotion. As a result, nearly all classical work on ciliary beat had been made on the abfrontal cirri of *Mytilus* by Gray (1928, 1930), Kinosita and Kamada (1939) and by Yoneda (1960, 1962). Gray designated the form of beat of the abfrontal cirri of *Mytilus* to consist of two separate discontinuous movements; a rapid effective stroke, and a slower recovery stroke along the same plane. This planar discontinuous beat pattern had been regarded as the only form of ciliary movement since the publication of Gray's monograph in 1928, and this idea had never been challenged until lately.

Utilizing a rapid fixation technique to avoid the rapid movement of locomotory cilia, Párducz (1967) came to the conclusion that the ciliary beat for *Paramecium*

and *Opalina* was discontinuous, but differed from the previous concept in that the
recovery stroke was three-dimensional, returning to the starting position by tracing
a curved arc similar to that of the letter "D". Jahn and Bovee (1968) suggested
the possibility of a three-dimensional recovery stroke and a bending of the cilia in
the effective stroke. Compared with the classical concept, the only discrepancy ap-
peared to center on the three-dimensional nature of the recovery stroke. Prior to
1968 (Kuźnicki et al. 1968 a, b) the discontinuous nature of the ciliary beat had
never been questioned. It was only after the introduction of the technique of direct
observation of ciliary activity in *Paramecium* that the possibility of a continuous
(and possibly helical) pattern of ciliary beat was suggested and started to cast some
doubts on the generally accepted discontinuous theory.

Sleigh (1960, 1962, 1968) stated that the ciliary beat of *Opalina* was a simple
planar effective and recovery stroke, in accordance with Gray's postulation. Other
than the work performed by Párducz (1967), the only other investigation on the
ciliary activity of *Opalina* was performed by Tamm and Horridge (1969, 1970),
with results identical to that reported by Párducz (1967), but in greater detail.
No direct analysis on the form of beat of freely swimming *Opalina* had ever been re-
ported until now.

It is clearly seen in the high speed movies and in subsequent analysis that both
the surface and profile views of the cilia on the same row of attachment show undu-
latory (propagatory) waves. Frame by frame tracings illustrate the fact that there
are two simultaneous undulatory waves propagating on the same cilium; traveling
at right angles to one another, and 90° out of phase. Geometrically, this constitutes
a continuous traveling helix. The fact that live organisms under normal free swim-
ming conditions were used in our analysis. as opposed to specialized compound
cirri (Gray 1928, 1930) and fixed specimens (Párducz 1967, Tamm and Horridge
1969, 1970), helps to enhance the quality and authenticity of this presentation. It
should be stressed, however, that not all ciliates locomote with this continuous helical
wave pattern. It had been shown conclusively in our laboratories (Boggs et al.
1970, Cheung et al. 1974) that the cilia of *Spirostomum ambiguum* beat with a typical
discontinuous pattern; a planar effective stroke, followed by an angularly deviated
recovery stroke.

The purpose of this research paper is to show that the cilia of a free swimming
*Opalina*, contrary to previous reports by Sleigh (1960, 1962, 1968), Párducz
(1967) and Tamm and Horridge (1969, 1970), beat with a continuous traveling
helical wave pattern.

## Materials and Methods

### Biological Material and Medium

*Opalina obtrigonoidea* were obtained from the rectum of common grass frogs pur-
chased from biological supply firms in Southern California. The organisms were

isolated out, washed and then resuspended in Naitoh's *Opalina* medium (Naitoh 1964). The organisms were normally used immediately, although they could be kept alive for at least six days in the medium.

Naitoh's *Opalina* physiological medium consists of:

| | |
|---|---|
| NaCl | 60 mM |
| KCl | 5 mM |
| CaCl$_2$ | 0.1 mM |
| MgCl$_2$ | 1 mM |
| Tris buffer | 10 mM |

with the pH adjusted to 7.8

Optical Equipment

Camera

A Redlake Locam high speed camera (Model 162–4DC) was used. This camera was capable of operating up to 400 frames per second. The speed of the movies were determined on each film by an internal timing light which was projected onto the edge of the film.

Microscope

A Zeiss Universal Research microscope was used. This microscope was equipped with Nomarski interference contrast, phase contrast and dark field optics. The general magnification of optical setup is $625\pm20\times$.

Light Source

A 100 Watts tungsten halogen light source was used.

Films

Eastman Kodak Ektachrome Commercial 7225 ECO film was used for color work, while Eastman Kodak $4\times$ Negative 7224 $4\times$N film was used for black and white. Framing rates of up to 400 frames per second could be used with black and white work

Movie Analyzer

Analysis of the high speed movies were made by repeated viewing at various speeds (2-24 FPS) with a flickerless L. W. Photo-optical analyzer, and also by frame-frame tracing and analysis. Tracings were made of selected sequences, either by direct reflection onto tracing paper, or by utilizing the rear window tracing technique. Black and white prints were made from selected frames for analytical purposes.

Method

*Opalina* suspended in 20 μl of Naitoh's medium was used each time to ensure uniformity. Vaseline mount preparations were made to prevent evaporation and to provide enough space for the *Opalina* to swim about freely.

http://rcin.org.pl

High speed movies were made of *Opalina* in Naitoh's medium with Nomarsk[i] interference contrast and occasionally with phase contrast optics. The movies obtained were later analyzed for the form of ciliary beat.

## Results

It can be observed in analysis of the films that the pattern of ciliary movement in *Opalina* is continuous. There is NO differentiation into effective and recovery patterns, and NO recovery stroke has ever been observed or recorded in the 6000 feet of available movies. Analysis of the movies also confirm the fact that the cilia of freely swimming *Opalina* beat with a traveling helical wave, propagating from base to tip along the entire length of the cilia. The propagation of such helical waves is illustrated in Pl. I, II and Fig. 1, 3. Plate I is a print which is composed of 24 successive frames of a movie sequence. It shows clearly the sequence of events in one cycle of a cilium in profile. Figure 1 is a superimposed frame-by-frame tracing of Pl. I. The tracings show clearly the propagation of the peak of the traveling wave along the entire length of the cilium, and that the ciliary beat is not differentiated into two discontinuous (effective and recovery) strokes. The nature of such a wave is discussed in detail by Jahn and Bovee (1968) to account for the movement patterns of certain blood protists, and was also described by Kuźnicki et al. (1970) in a report on the existence of a continuous helical ciliary beat in *Paramecium multimicronucleatum*. Geometrically, a traveling helical wave can be looked upon as consisting of two simultaneous undulatory waves (as shown in actual tracings in Fig. 3) propagating from base to tip along the entire length of the cilium, traveling at right angles to one another and 90° out of phase. The three-dimensional and helical nature of the wave can only be determined by considering tracings that are made from simultaneous surface and side cilia beating simultaneously on the same row of attachment. Such a consideration is shown clearly in Fig. 2 and 3. Figure 2 shows a drawing of the surface of a typical *Opalina*. Areas A and B indicate the location of tracings A and B of Fig. 3. Figure 3 A and B show clearly the simultaneous propagation of two different undulatory traveling waves, traveling at right angles to one another, and 90° out of phase. Such a combination constitutes a traveling helix.

The frequency of beat of such continuous traveling waves is about 25–30 cycles per second. It could be seen that even before one wave had ended at the tip of the cilium, a new wave had already started at the base of the cilium. The speed of propagation of the peak of the waves can reach as high as 250 μm per second, depending on the speed of forward propagation of the organism under consideration. The average length of the cilia of *Opalina obtrigonoidea* is $12\pm2$ μm and is long enough to show at least one complete wave, as can be seen in Pl. I, II and Fig. 1, 3. Usually there is about $1-1\frac{1}{4}$ wave in a cilium at any given time.

Metachronal waves can be seen easily in free swimming *Opalina*. The wave crests form a right-wound spiral on the uniform surface of the rather long cilia ($12\pm2$ μm).
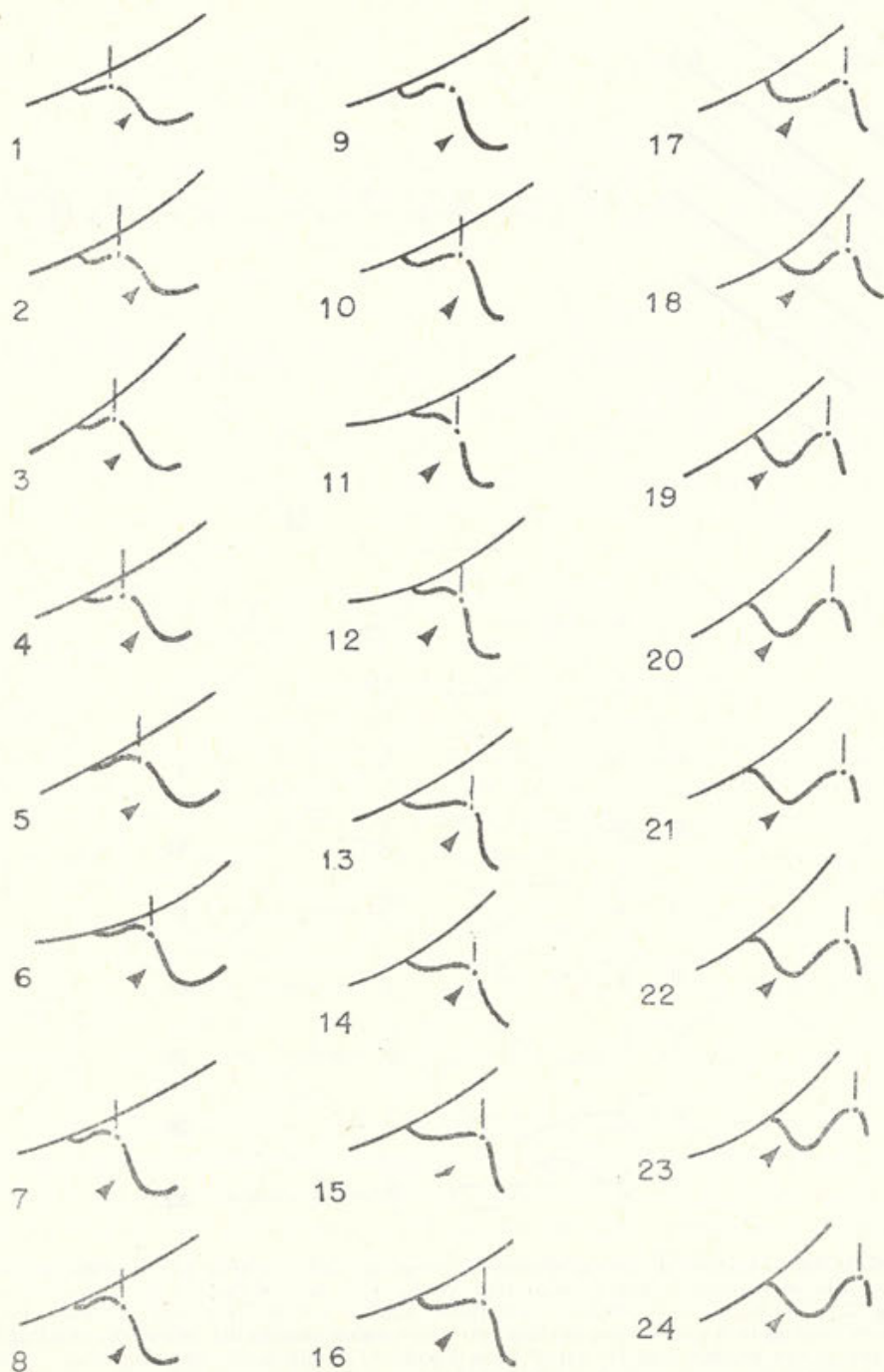
Fig. 1. Actual superimposed tracings of the cilium under consideration in Pl. I. Note the propagation of the peak of the wave along the entire length of the cilium throughout the entire 24 frame sequence. The markers are identical to the ones used in Pl. I
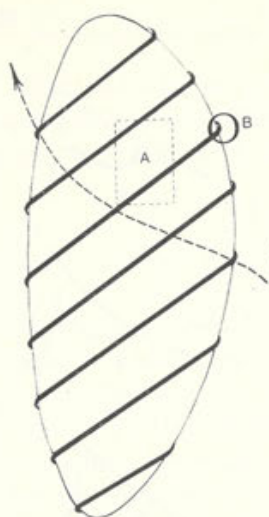
Fig. 2. A drawing of the surface of a typical *Opalina*. Areas A and
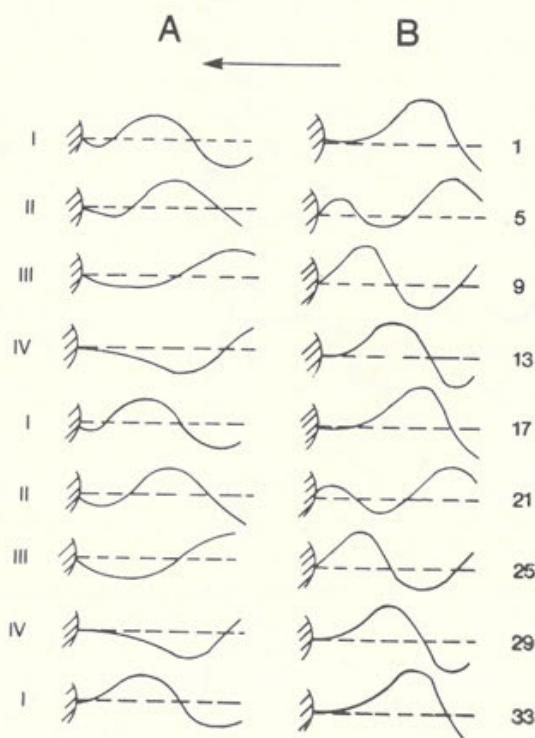B indicate the locations of the tracings A and B of Fig. 3



Fig. 3. Actual tracings of the movements of a surface and a profile cilium which are on the
same row of attachment. The exact location of the attachment of the two cilia are indicated in
Fig. 2. A and B are chosen from the same frames and the sequence of events is simultaneous. The
ciliary tracings in A and B serve to show two simultaneous undulatory waves, traveling at right
angles to one another, and 90° out of phase. Combined geometrically, they constitute a traveling
helix. The frames are indicated on the right (1 through 33 at intervals of 4) and the sequence of
events on the left (I through IV). The arrow indicates the direction of forward propagation of
the organism

High speed movies (taken at 400 FPS) viewed at 16–24 frames per second enable one to see such waves clearly. The metachrony conforms to the symplectic type of the Knight-Jones convention (1954) in that the cilia move in the direction of the wave travel. The velocity of the metachronal waves can go as high as 200 μm per second, originating from the anterior and progressing regularly towards the posterior end of the organism. Such metachronal waves can be seen clearly in Pl. II.

*Opalina* occurred in abundance in the rectum of frogs. Observations show that they bump into one another readily. As a result, turning movements and ciliary reversals are extremely common. In turning and avoiding movements, as well as in ciliary reversals, the cilia just change the direction of orientation of the axis of the helix, and simply continue to beat with the traveling helical wave. This avoiding turning movement consists of a rotation of the cilia about their points of attachment to the cell surface, and the rotation can be in either direction from the original position. In the case of ciliary reversal, the axis of the cilia rotates a full 180° from the original position, thus propelling the organism in the reverse direction.

## Discussion

In order to overcome the problems present when observing and analyzing the movment of cilia, previous investigators had either used specialized compound cirri or devised a special technique to stop the action and movement of the cilia; either partially by using a quieting reagent, or completely by utilizing the rapid fixation technique. Their conclusions were drawn on predications and speculations; predicting that the stopped ciliary movement will give them an idea of the original continuity and sequence of beat, and speculating that the chemicals that killed the organisms so drastically would not hinder the original ciliary action at the moment of death. Such a rapid fixation technique is extremely complicated and sophisticated and the results obtained can be considered very illustrative and suggestive, but can never be considered conclusive. The only acceptable conclusion is the one that is obtained by using unspecialized cilia moving in their normal non-viscous environment, without the application of any means to interfere with their normal live behavioral patterns.

It was a generally accepted concept that high speed cinemicrography could overcome the problems described previously. Such a technique had been utilized in Jahn's laboratory (1965, 1967, 1968) and had yielded important results in studies with flagella, but had been much less rewarding with cilia. Brokaw (1966) stated: "Studies on the nature of the control system re-emphasize the need for better methods of photographing the behavior of individual cilia, so that cilia, as well as flagella, can be used in experimental approaches".

Since the publication of Gray's work (1928, 1930), the planar discontinuous pattern of ciliary beat had been considered the basic pattern for ciliary movement,

and not much work had been done in this field until the perfection of the rapid fixation technique by Grębecki (1964), Párducz (1967) and by Tamm and Horridge (1969, 1970). Other than the postulation described by Sleigh (1960, 1962, 1968), the only other description for the ciliary beat of *Opalina* was proposed by Párducz (1967) and by Tamm and Horridge (1969, 1970). The pattern was considered to be discontinuous, with the forward effective stroke going in one direction in a planar manner, and the slower recovery stroke returning to the original position by tracing a pathway similar to that of the curved arc of the letter 'D', as shown in Fig. 4.
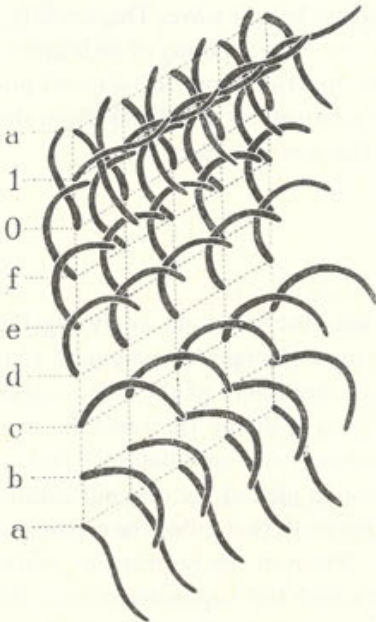


Fig. 4. Model proposed by Párducz (1967) to account for the three-dimensional recovery stroke of *Opalina*

Analyses of the high speed cinemicrographs taken with the aid of Nomarski interference contrast and phase contrast optics show that the ciliary beat pattern of *Opalina* differs remarkably from the three-dimensional discontinuous stroke pattern as proposed by Párducz, and also differs completely from the classical idea of Gray (as shown in Fig. 5) and Sleigh (as shown in Fig. 6).

At first glance, the cilia of a freely swimming *Opalina* appear to undulate in a planar manner similar to that of sperm tails (Gray 1955) or the posterior flagella of *Ceratium* (Brokaw 1962, Jahn et al. 1963). Careful analysis of the cilia on both surface and side views (as demonstrated in Fig. 2 and 3) indicate that ciliary beat is actually three-dimensional. This three-dimensional traveling helical wave is distally directed, and exerts a locomotory force from base to tip along the entire length of the cilia; thus, propelling the organism to move in a direction opposite to that of the propagatory waves (Fig. 7). Tracings of the surface and profile ciliary beats
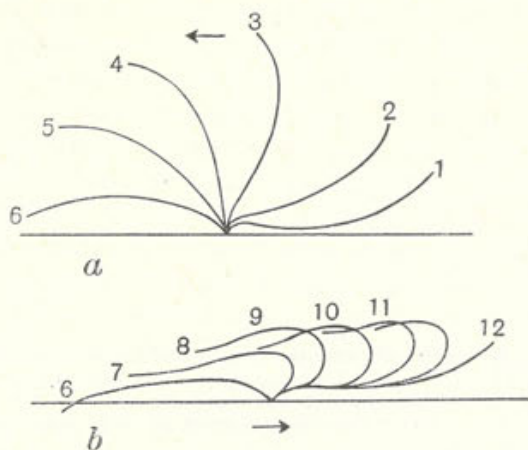
Fig. 5. A diagram of the movement of the abfrontal cirri of the beat pattern of *Mytilus* (Gray 1928). a — the effective stroke (1 through 6), b — the recovery stroke (6 through 12)
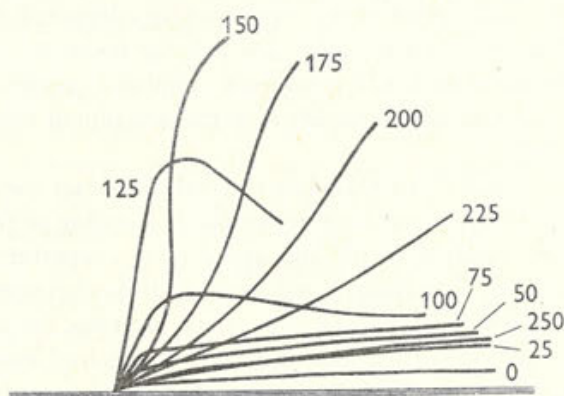


Fig. 6. A diagram of a tracing of the ciliary movement of *Opalina* as reported by Sleigh (1968)

on the same row of cilia (as shown by tracings A and B in Fig. 3) indicate that such a helical wave really consists basically of two simultaneous propagatory waves, traveling at right angles to one another, and 90° out of phase. The continuous nature of such a helical wave is very obvious. NO differentiation into separate discontinuous movements is noted, and NO recovery strokes have ever been observed.

An *Opalina* that is swimming straight forward is seen to be coated with transverse lines which move progressively backward towards the posterior end. These lines are the crests of the metachronal waves, and they can be seen easily when viewing the high speed movies and as shown in Pl. II. The wave crests are supposed to consist of many cilia moving together in their effective strokes (Tamm and Horridge 1970). However, no distinction into effective and recovery strokes had ever been noted in

Fig. 7. A drawing illustrating the nature of a continuous traveling helical wave in an individual cilium. The wave moves from base to tip along the entire length of the cilium. This drawing was previously used by J a h n and B o v e e (1968) to explain the movement of some blood protists

the cinemicrographs. Careful analyses show that the metachronal wave crests are actually the peaks of the traveling helical waves. The peaks of such traveling helical waves travel along the entire length of the cilia. Since the cilia are all distally directed, the peaks of the helical waves are moving towards the posterior end. Under such circumstances, the metachrony is still considered symplectic as the beat of the cilia (which is continuous and distally directed) is in the direction of travel of the meta-chronal waves.

It was generally accepted that when ciliary reversal takes place the cilia just change the directions of the effective and recovery strokes, depending on the new direction of forward movement. Analysis of the high speed movies revealed that this is not the case, as the distinction into effective and recovery strokes has never been observed in *Opalina*. In turning movement, as well as in ciliary reversals, the cilia just change the direction of orientations of the axis of the traveling helical waves, and the cilia simply continue to beat with this helical wave. The avoiding turning movement basically consists of a rotation of the cilia about their points of attachment to the cell surface, and the rotation can be in either direction from the original position. In the case of a ciliary reversal, the axis of the cilia rotates a full 180° from the original position, thus propelling the organism to move in the reverse direction.

One very basic question can arise concerning the existence of such a continuous traveling helical wave. Why is it that scientists in the last few decades could mistake this continuous helical wave of *Opalina* to be discontinuous, and only partly three-dimensional, if at all? The primary reason to account for this is the absence of the high speed cinemicrographic techniques to record the fast and complicated movements of individual cilium for analysis. The density of distribution of the cilia, their high angular velocity and the lack of contrast between the cytoplasm and the cilia actually made observations and analysis of the ciliary activity on the cell surface impossible. Microscopy had always been called upon to help to examine and analyze

minute objects and details, the fine structural elements of which were distinguished only by very slight differences in their refractive index. The introduction of the Nomarski interference contrast optics served to enhance the previously negligible differences in the refractive index and helped tremendously in the visualization of such fine structural details. Also, the Nomarski optics had a very limited depth of field. This optical property could serve to negate the neighboring interfering effects of the densely packed cilia. Phase contrast optics were sometimes used to complete the general picture if depth of field was desired. The use of such optics in combination with high speed cinemicrography can help to solve most of the problems that plagued previous investigators. The availability of a high speed movie camera that can perform readily at 400 frames per second (or even up to 6000 FPS) as compared with the maximum running speed of only up to 40–60 frames per second in the older models, makes high speed cinemicrography an indispensable tool in recording and analyzing ciliary activity.

It had been demonstrated in the same laboratory at the University of California at Los Angeles that the cilia of *Paramecium multimicronucleatum* (Kuźnicki et al. 1968, 1970), *Tetrahymena pyriformis* (Preston 1972), *Opalina ranarum* (Cheung et al. 1973 c), *Opalina obtrigonoidea* (Cheung et al. 1973 a, b) and *Colpidium* (Wilson 1974) all beat with the continuous traveling helical waves. It should be stressed, however, that by reporting the existence of such a wave pattern in *Opalina*, we are NOT implying that all ciliates locomote in this manner. It had been shown in our laboratories at the University of California at Los Angeles and also at the California Institute of Technology that the cilia of *Spirostomum ambiguum* beat in the generally accepted discontinuous manner (Boggs et al. 1970, Cheung and Winet 1974). The ciliary beat of *Spirostomum* is differentiated into two separate movements: a planar effective stroke and a slower three-dimensional recovery stroke, tracing a slightly angularly deviated path that is similar to the curved arc of the letter 'D', and is in accordance with the idea proposed by Párducz (1967) for other ciliates. In view of the available evidence, it is obvious to conclude that the ciliary beat pattern is not the same in all ciliates.

The distinction between cilia and flagella has never been clear when certain organisms of the traditional ciliate and flagellate subphyla are considered. It was popularly believed that the internal structures and the biochemistry of cilia and flagella might be different, because of the supposed difference in basic movement pattern.

The following ciliates, *Opalina ranarum* (Cheung et al. 1973 c), *Opalina obtrigonoidea* (Cheung et al. 1973 a, b), *Paramecium multimicronucleatum* (Kuźnicki et al. 1970), *Tetrahymena pyriformis* (Preston 1972) and *Colpidium* (Wilson 1974), have all been proven to beat with a continuous traveling helical wave. Such a type of wave is also the basic beat pattern of *Ceratium, Trichomonas, Chilomonas, Trachelmonas, Rhabdomonas* and *Menoidium* (Jahn nad Votta 1972, Votta et al. 1971), and such organisms are classified as flagellates (Jahn and Jahn 1949). It had also been shown earlier in our laboratory that the flagellates *Polytomella* (Git-

tleson and Jahn 1968), *Mastigamoeba, Peranema, Petalomonas* and *Trichomonas* all locomote with the planar discontinuous effective and recovery stroke pattern which is supposedly typical of ciliates. Such facts serve to illustrate that the usage of the form of beat as a means to identify and classify ciliates and flagellates is not reliable, and the beat patterns of ciliates and flagellates are not definitive.

It had been proven that both cilia and flagella contain the 9+2 proteinaceous fibers as a fundamental structure, and are of the same basic diameter as well as function (Manton 1952, 1956, Afzelius 1969). Recent research had confirmed that any differences that might be present were probably negligible, and that the cilia and flagella were basically identical biochemically, structurally and physiologically (Jahn et al. 1965, 1972, Grimstone 1966, Allen 1967, Allen and Eckert 1969, Afzelius 1969, Gibbons 1960, Frey-Wyssling 1965). The only other popularly held differences between cilia and flagella had been that of length, number, density of distribution and the form of beat. It has been clearly shown that the form of beat is not definitive and exceptions to the other supposed differences are not uncommon. It is well known that in the termite flagellate *Trichonympha*, the flagella are short and numerous and are of two distinctly short lengths (Jahn and Jahn 1949). Their uniform and dense distribution, their abundance in number and their short lengths resemble cilia more than flagella.

Frey-Wyssling (1965) recognized the great similarity of cilia and flagella, and he suggested the adoption of the term UNDULIPODIA to denote cilia and flagella collectively. With all the available evidence cited above, the collective term appears to be a logical term to use when referring to cilia and flagella, and deserves more credit and acceptance than it has received so far.

RÉSUMÉ

L'régistrement cinématographique à haute cadence pris avec le contraste d'interférence de Nomarski et le contraste de phase démontre que les cils de l'*Opalina* en battant suivent la forme d'une onde hélicoïdale qui se déplace de façon continue. Cette observation est différente par rapport à la conception généralement admise d'un battement discontinu. Pendant les mouvements de recul et pendant le rebroussement ciliaire, les cils ne changest que l'orientation de l'axe des leurs hélices et poursuivent simplement leur battement avec l'inde helicoïdale.

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## EXPLANATIONS OF PLATES I–II

Pl. I. A composite print of portions of 24 successive frames of a selected movie sequence. The focus is on one particular profile cilium in motion. The movie sequence was taken with Nomarski interference contrast optics at 400 FPS on a freely swimming *Opalina* in Naitoh's *Opalina* medium. This sequence was chosen to show one profile cilium going through one complete cycle of its beat in a continouus traveling pattern, as observed in one plane.

←is a marker indicating the same position in the frame, with reference to a point on the body of the organism,! is a marker placed close to the cilium under consideration, with the dot of the marker alongside the peak of the wave

Pl. II. Two successive prints showing the surface view of a freely swimming *Opalina* as opposed to the profile view of Pl. I. The metachrony is shown clearly. Note the continuity of the wave propagation and the absence of a recovery stroke

The general magnification of optical setup is 625±20 ×

PLATE I

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auctores phot.

PLATE II
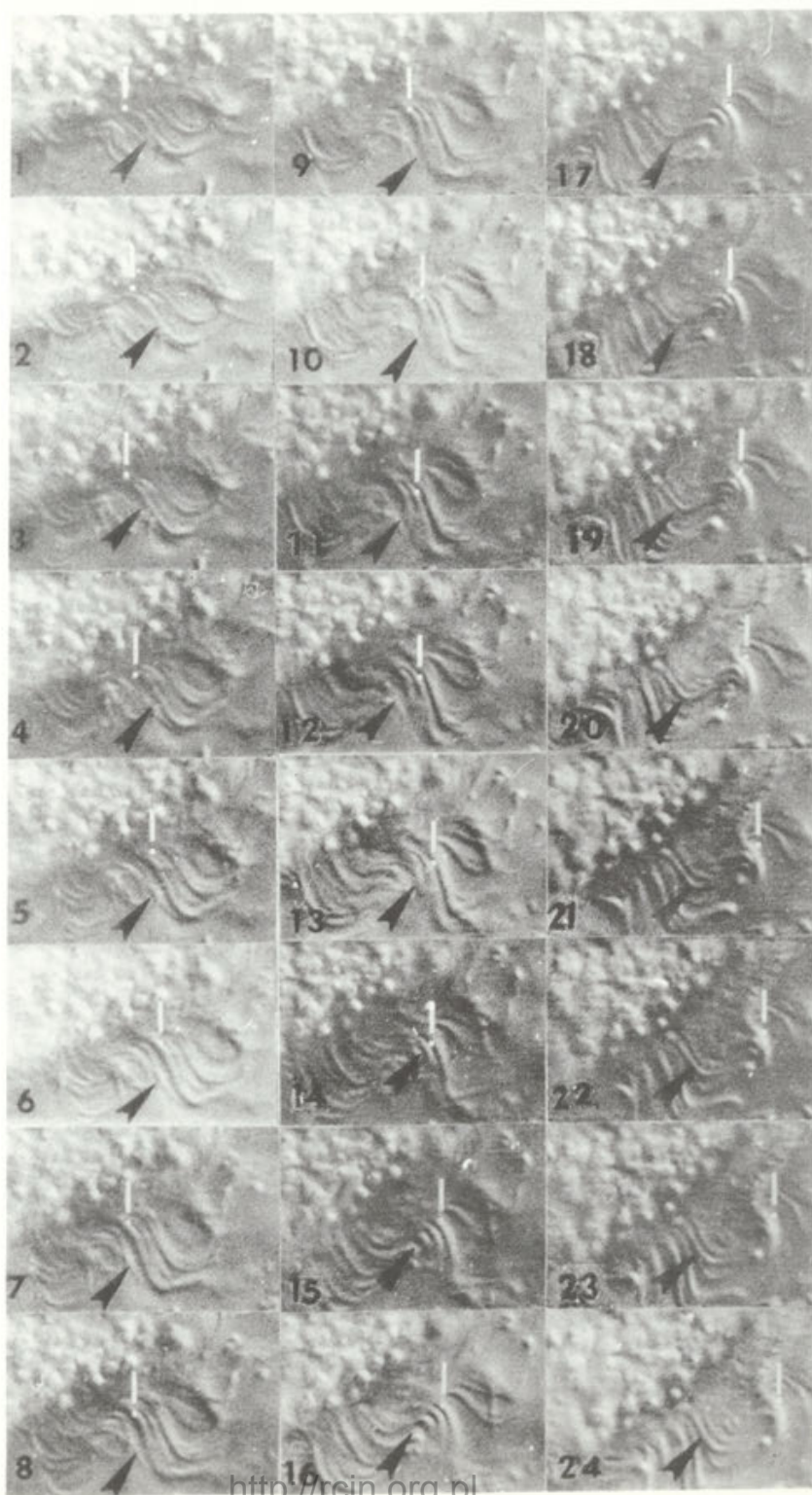


A. T. W. Cheung et T. L. Jahn            auctores phot.

E. MIKOŁAJCZYK

## The Biology of *Euglena ehrenbergii* Klebs. I. Fine Structure of Pellicular Complex and its Relation to Euglenoid Movements

*Synopis.* The examination of the pellicular complex of *Euglena ehrenbergii* was carried out by means of light, scanning and transmission electron microscope. The body is covered by a very thick (1–1.2 μm) layer of mucus. Beneath the mucus layer, the typical tripartite plasmalemma is present. Periplast does not form continuous layer. It is cut in each strip within the pellicular notch. In this area periplasts of the neighbouring strips are connected with a fan of 8–11 fibrils. Under the groove, periplast splits in the large and little tooth. The large tooth is cut on 0.05–0.08 μm pieces. 3–6 microtubules are found in each strip. In spite of so developed architecture of the periplast it does not prevent the cell from wide euglenoid movements. The presence of contractile elements in the cytoplasm between teeth within pellicular complex is supposed.

### Introduction

The biology of *Euglena ehrenbergii* Klebs, 1883, one of the largest euglenoids, is not so well known as *Euglena gracilis* or many other species. This ribbon-shaped organism is included into the group of euglenas characterized by flexible, metabolic body (Chu 1946, Conrad and van Meel 1952, Pringsheim 1956, Heimpel 1972), whose ultrastructure have never been studied in detail.

It is generally accepted that the intensity of euglenoid movements is parallel to the pliability of the periplast (Hamburger 1911, Günther 1927, Chadefaud 1937, Chu 1946, Pringsheim 1948, 1956, Leedale 1964, 1966, 1967, Mignot 1965, 1966). The elasticity depends on the thickness, compactness and architecture of this part of cytoplasm. Besides, the arrangement and quantity of striations also influences the intensity of body movements (Günther 1927, Chu 1946, Mignot 1965).

The periplast of slightly metabolic species (e.g., *E. spirogyra, E. acus*) is thick (500 Å in *E. acus*) and under the groove it can form little and large teeth (Fig. 1 B).
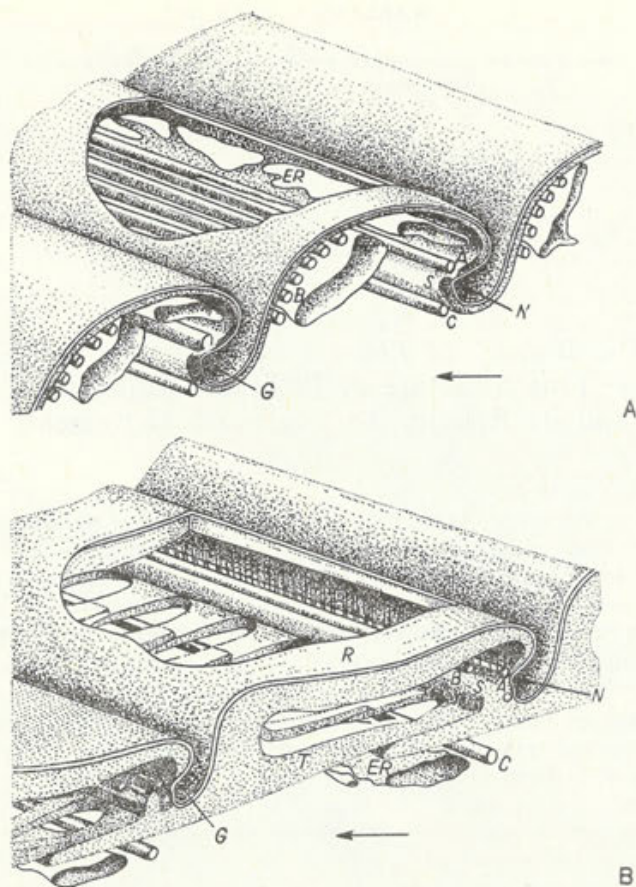
Fig. 1. Three-dimensional scheme of two patterns of the pellicular structures arrangement in *Euglena*. A — metabolic species: type *Distigma*, B — rigid species: type *Euglena acus*. R — ridge, S — shaft, G — groove, N — notch, ER — endoplasmic reticulum, T — tooth, A, B, C — microtubules, arrows show the anterior end of the cell (after Mignot 1965)

Periplast of especially metabolic species (e.g., *Distigma proteus*) is thin, and does not form teeth (Fig. 1 A).

Generally speaking, metabolic species have more numerous and more densely arranged strips than the rigid species. Gojdics (1953) in her monograph stated that all species of *Euglena* have strips arranged in an anticlockwise directions except *E. oxyuris* and perhaps two other species.

All authors are unanimous that the distinction of strips is not correlated with the flexibility of a cell. Strongly marked strips may be found in metabolic species (e.g., *E. granulata* and species of *Heteronema*), as well as in rigid ones (e.g., *E. tripteris*). On the other hand, there are metabolic species (e.g., *E. mutabilis*) as well as rigid ones (e.g., *E. acus*) which have a very delicate striation.

All living euglenoid cells are coated with a thin or wide layer of mucus (Leedale 1967, Barras and Stone 1968, Buetow 1968, Mignot 1966). The mucus appears to be ejected as thick threads that fuse perpendicularly in rows along the pellicular strips (Hollande 1942, Gojdics 1953, Pringsheim 1956). Sometimes, it accumulates with extraneous materials as warts which are very well visible in *E. spirogyra* (Leedale 1964, 1967, Echlin 1971).

The mucus is secreted from mucus bodies associated with pellicular complex. In *E. viridis* (de Haller 1959), *A. longa* (Lefort 1963) and *A. fennica* (Fize and Michel 1972) the mucus bodies within the cytoplasm have been observed.

Mucus can be involved in supplying the lubricant to the articulations of the pellicle during euglenoid movements (Leedale 1964) as well as in lubricating gliding movement (Günther 1927, Diskus 1956, Jarosch 1962). Besides, it can be involved in the formation of envelopes — cysts and palmelloid stages (Barras and Stone 1968, Buetow 1968).

In regard to the fact that, as it was demonstrated above, the pellicular complex plays important role in euglenoid movements.it became the subject of present studies in *Euglena ehrenbergii*.

## Material and Methods

### Material

The following two types of *Euglena ehrenbergii* were the subject of the studies: (1) *Euglena ehrenbergii* consisting of a large, elongate, rodlike grains of paramylon. It was collected from the ditch at the west region of Warsaw, and cultured in water taken from the same place. They were kept in a diffusive light at 20–22°C. (2) *Euglena ehrenbergii* which is characterized by small grains of paramylon. This culture was grown from stock kindly supplied by Dr Eilo Hildebrand Inst. of Neurobiology, UFA, Julich, West Germany. The medium was also prepared according to Dr Hildebrand's instructions. Specimens were cultivated in an Erlenmayer bottle. The bottom of the bottle was covered with a sand mixed with some grains of an artificial soil and a green pea. The flask was filled up with a tap water. The medium was sterilized in a steam pot. The sterilized culture medium was stabilized for no less than three days and then it was inoculated with an original culture. Cultures were kept in a diffusive daylight, at 20–22°C.

### Methods

Photographs of the body movements and the arrangement of strips (Pl. I 1, 2, 3, 4) were taken in Zeiss light microscope.

Preparation for scanning electron microscopy: Cells were fixed in glutaraldehyde (buffered with 0.04 M cacodylate at pH 7.3), then rinsed in buffer alone (0.02 M) and remained in 0⁻4°C overnight. The small drops of the solution with specimens were put on the aluminum stub which was previously covered with the thin layer of 2% agar. The whole stub was rapidly immersed in liquid nitrogen and then it was transferred to a microscopic column. The examination of the specimens was carried out with a JEOL scanning electron microscope, set at 30 KV bean accelerating voltage Stubs were viewed at an angle of 45°.

Preparation for transmission electron microscopy: All fixatives were buffered with 0.04 M cacodylate (pH 7.3). Cels were fixed in 7% glutaraldehyde for 1 h then rinsed in buffer alone for 1 h,

and postfixed in 2% buffered osmium tetroxide for 1 h. All this procedure was carried out at 0–4°C. After fixation the cells were dehydrated in alkohol series and after two changes of a propylene oxide of 30 min each, transferred to the mixture of a propylene oxide and Epon 812 (1 : 1) for 24 h. At last the specimens were embedded in Epon 812. Sections were cut with a glass or diamond knives on an LKB or Tesla ultramicrotom, and were stained with uranyl acetate and lead citrate. The material was examined in JEM 100B electron microscope.

## Results and Discussion

### Pellicular Complex

The periplast of *Euglena ehrenbergii* forms the pellicular strips. The 48 pellicular bands are arranged helically along the cell from left to the right. Plate I 1–4 shows them in light microscope and Pl. II 5–9 in scanning electron microscope. The arrangement of pellicular strips is changed depending on the body shape. The strips are laying near parallel to the long axis of the body in an elongated part of a cell, and perpendicular in cell region showing the shape of a flattened sphere (Pl. I 1–4, Pl. II 5–9). Strips, like in other euglenoid flagellates, bifurcate few times at the posterior end and enter the canal of the reservoir at the anterior one (Pl. III 10).

The strips are very distinctive. They are caved within the middle part of the strip's ridge (Pl. III 11, Pl. IV 12). The width of the strips from notch to notch can oscillate between 0.6 to 1.5 μm.

*Euglena ehrenbergii* is covered by a very thick layer (1–1.2 μm) of mucus. The mucus does not form warts. It occurs as uniform layer with a network of mucus threads passing through it (Pl. IV 13, 14).

Periplast of the strip's ridge (0.1–0.2 μm thick) passes down and forms the strip's groove and then, under the groove, it splits on the large and little tooth (Pl. V 15,16, Fig. 2). Periplast is not the continuous layer. Its continuity is cut in each strip within the pellicular notch (Pl. V 15, 16). In this area periplasts of the neighbouring strips are connected with a fan of 8–11 fibrils (Pl. V 17). These fibrils are about 0.07 μm long and separated by the space (in the height of the fan) of 0.03 μm. On the oblique (to the long axis of the cell) sections these fibrils are shown as a striated layer (Pl. V 18).

The thickness of the periplast which forms the base of the teeth (under the groove) is 0.1–0.2 μm. The little tooth (0.1–0.5 μm long) is extended under the original band while the large one (1.0–1.6 μm long ) is passing under the ridge of the next strip. The large and little tooth are partially overlapped. They are separated by the cytoplasm of 0.1–0.13 μm width.

The large tooth has not the continuous structure. It is cut on 0.05–0.08 μm pieces, and separated by the 0.03–0.06 μm space of the cytoplasm (Pl. V 18).
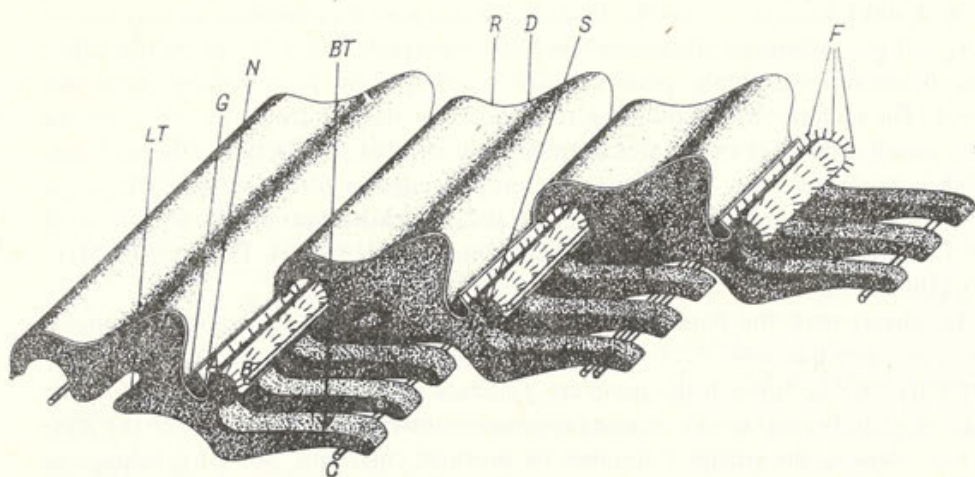
Fig. 2. Noncontractile structures of the pellicular complex of *Euglena ehrenbergii*. R — ridge, G — groove, N — notch, F — fibrils, A, B, C — microtubules, LT — little tooth, BT — large tooth, S — shaft, D — deppression

Microtubules are arranged parallel to each pellicular strip (Pl. V 15). One or two microtubules (A) connected by fibrillum, are lying near the notch, one is lying beneath the ridge (B) and one to three (C) are lying under the large teeth.

The endoplasmic reticulum with ribosomes is embedded in the space of the cytoplasm between teeth (Pl. V 15, 16, Pl. VI 19) reaching even the shaft of the strip (Pl. VI 20).

## Relation Between Euglenoid Movements and Ultrastructure

Examination of *Euglena ehrenbergii* in electron microscope allows to reveal the structure and architecture of the pellicular complex of this cell. As it appeared, the structure of *E. ehrenbergii* is similar to that one which is found in rigid species, for example in *E. spirogyra* (Leedale 1964, Leedale et al. 1965, Leedale 1966, 1967) *E. acus* or *Cyclidiopsis acus* (Mignot 1965, 1966). Although they have the similar scheme of the inner structure of the pellicular complex (Fig. 1 B, Fig. 2), they are distinctly different in regard to the degree of ability to euglenoid movement. In rigid species changes of the body shape are very limited, while in *E. ehrenbergii* they are very wide. *E. ehrenbergii* is able to wriggle, bulge, and assume the flattened sphere. On the plates VII, VIII, IX, the consecutive stages of contraction to the flattened sphere as well as relaxations, are demonstrated. The bulging (the body zone into which the cytoplasm is pushing) may begin to move along the cell from the anterior end (Pl. VIII 32–39), posterior end (Pl. IX 40–49) or even from both ends simultaneously (Pl. VII 21–31).

Chu (1946), studying *E. ehrenbergii* has found that its periplast is neither compact,

nor stiff and it is elastic in nature. Perhaps, in this way the appearing of very strong euglenoid movements in this species could be explained. However, no visible differences between, for example, periplast of *E. spirogyra* and *E. ehrenbergii* were observed. The number of microtubules (3–6) does not deviate from the other species having teeth either. It is visible, that even such complicated architecture of the periplast, which is moreover, very thick, does not prevent euglena from changing the shape of its body, as was postulated by Günther (1927), Hamburger (1911), Chadefaud (1937), Chu (1936), Pringsheim (1948, 1956), Leedale (1964, 1966, 1967), Mignot (1965, 1966).

Localization of the contractile elements responsible for euglenoid movements is still an open question. According to Chu (1946) centractile elements in *E. ehrenbergii* are located beneath the periplast. Changes of the body shape appear as the result of a fibrils contraction causing the compression of the periplast. When the pressure is released, the periplast resumes its previous condition. So, Chu belongs to the group of investigators who presume the presence of contractile elements within cytoplasm (Pringsheim 1948, 1956, Lozina-Lozinsky and Zaar 1963, Leedale et al. 1965, Leedale 1966, 1967). The structure and architecture of periplast would play the passive role, limiting and defining the degree of intensity of the euglenoid movements only.

Other authors suggest the presence of the contractile elements within pellicular complex of the euglenoid cell (Dangeard 1902, Günther 1927, Diskus 1956, Mignot 1966, Schwelitz et al. 1970, Mikołajczyk 1973). According to Mignot (1966) contractile fibrills (very often unnoticeable) join the teeth with the shaft. Schwelitz et al. (1970) studying the ultrastructure of the pellicular complex of *E. gracilis* uncovered the striated layer which spreads interiorly to the plasma mebrane, and which suggests highly organized contractile elements. Diskus (1956) confirmed, that the remaining of the cytoplasmic flow while the euglenoid movement is stopped, suggests the presence of elements responsible for euglenoid movements within the cortical part of the cell.

It seems that the rapid inhibition of euglenoid movements in *E. gracilis* evoked by 2.4-DNP is caused by its effect on the contractile elements within the pellicular complex of the cell (Mikołajczyk 1973).

The *Euglena ehrenbergii* ultrastructure of the pellicular complex as well as localization of the endoplasmic reticulum with ribosomes indicate the presence of the contractile elements between teeth (Pl. V 16, 19, 20). However, no structural elements showing similarity to the filaments found in other protozoan cells or in muscle cells could be found.

Institute of Experimental Biology and staff of the Laboratory of Physiology of Cell Movements for their help.

RÉSUMÉ

Le complèxe pélliculaire de l'*Euglena ehrenbergii* était étudié en microscopie photonique et éléctronique (système classique et scanning). Le corps est recouvert d'une couche de mucus très fine (1–1.2 μm). Au dessous on trouve la plasmalemme en forme de membrane tripartite typique. Le périplaste est discontinu. Il est interrompu dans chaque bande à l'endroit d'une encoche latérale au fond du sillon. A cet endroit les périplastes des bandes voisines sont réliés de 8–11 fibrilles disposées en éventail. Au dessous du sillon le périplaste se divise en deux en formant une petite et une grande dent. La grande dent est sémentée tous le 0.05–0.08 μm. Dans chaque bande on trouve 3–6 micro-tubules. Cette architécture du périplaste tellement compliquée n'empêche pas néanmoins la cellule d'effectuertoute une gamme des mouvements euglenoïdes. On suppose la présence des éléments contractiles dans le cytoplasme pénetrant entre les dents du complèxe pélliculaire.

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## EXPLANATION OF PLATES I–IX

Arrangement of the strips of *Euglena ehrenbergii* visible in the light microscope

1: Slightly contracted euglena
2: The higher magnification of the middle part of the body.
3: The stage of flattened sphere
4: The same cell in higher magnification

Scanning electron microscope of *Euglena ehrenbergii*

5–9: The shape of the body and arrangement of the strips
10: Entering the strips into the reservoir of a cell
11: Fragment of the strips
Fine structure of the pellicular complex of *Euglena ehrenbergii*
12: General view on the cross section of euglena
13, 14: Sections showing the mucus layer
15: Cross section of the pellicular complex showing microtubules
16: Cross section of the pellicular complex showing the other part of the cell
17: The fan of fibrils connecting the periplasts of the neighbouring strips
18: Section showing the striated structure of the large teeth, and the striated layer of the fibrils connecting the shaft with the pellicular ridge
19: Section of the cell showing the cytoplasm between teeth
20: Endoplasmic reticulum with ribosomes extended between teeth.

Consecutive stages of euglenoid movements leading to the flattened sphere and relaxations of the body of *Euglena ehrenbergii*

21–31: Bulgings begining to move alcng the cell from both ends simultaneously
32–39: Bulging begining to move from the anterior end
40–49: Bulging begining to move from the posterior end

Abbreviations used:

ML — mucus layer, MB — mucus body, Chl — chloroplast, N — notch, F — fibrils, A, B, C — microtubules, LT — little tooth, BT — large tooth, G — groove, R — ridge, ER — endoplasmic reticulum, RB — ribosomes, S — shaft, D — deppression of the ridge part of the strip, CT — cytoplasm between teeth.

PLATE I









E. Mikołajczyk

auctor phot.

PLATE II



E. Mikołajczyk

auctor phot.

PLATE III





E. Mikołajczyk

auctor phot.

PLATE IV



12

13

14

E. Mikołajczyk

PLATE V


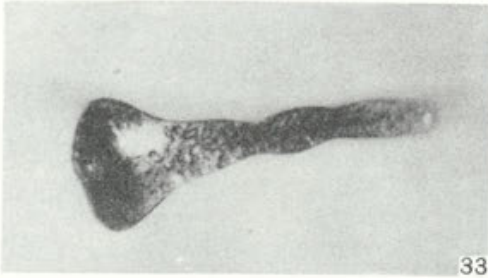






E. Mikołajczyk

PLATE VI
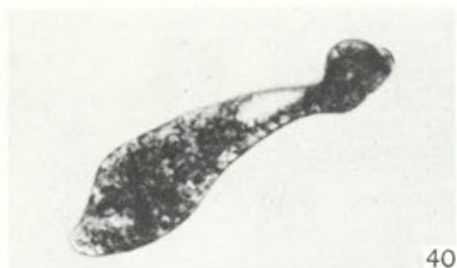


E. Mikołajczyk

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PLATE VII
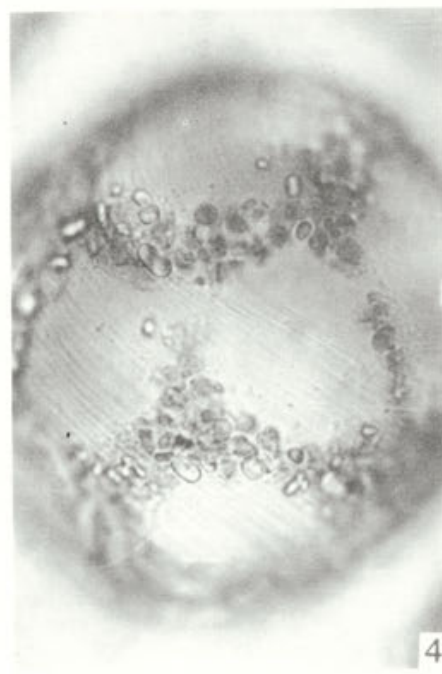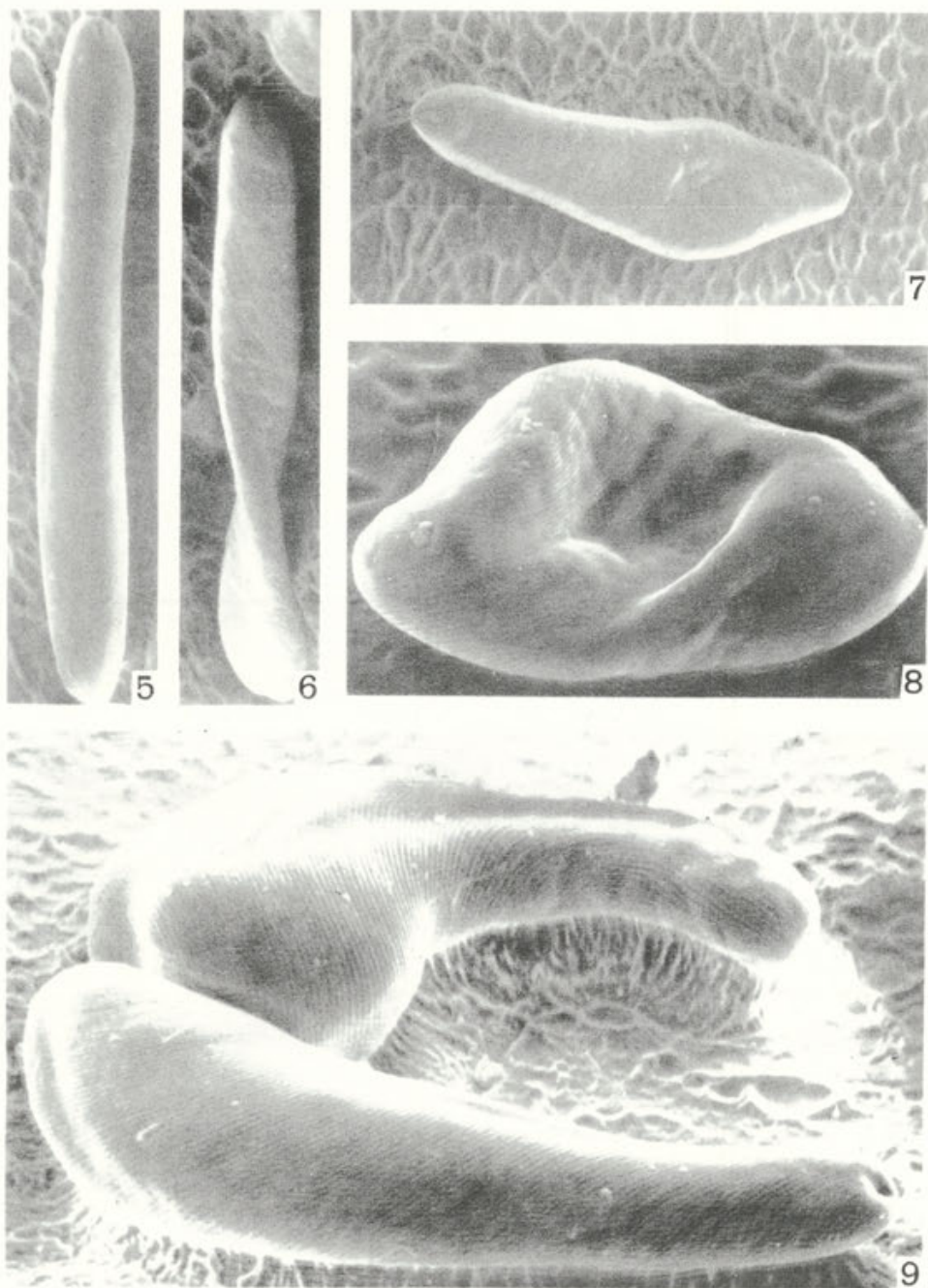


E. Mikołajczyk

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E. Mikołajczyk

PLATE IX



E. Mikolajczyk

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O. O. DIPEOLU[1]

# Studies on the Development of *Trypanosoma congolense* in Tsetse Flies (*Glossina* : *Diptera*) and the Factors Affecting it

*Synopsis.* During investigation into the development of *Trypanosoma congolense* in the tsetse flies, it was observed that about 50–60% of them could carry trypanosomes in the midgut up till 3 days after the infective feed. However, in only a few of them did the development establish. Infections became mature in the flies only after the trypanosomes had invaded the hypopharynx. Raising the temperature of maintenance of *Glossina morsitans* from 26°C to 31°C did not affect their susceptibility to *T. congolense*; at 20°C the development was not inhibited but only prolonged. The duration of development was longer in *G. austeni* than *G. morsitans*. There were also differences in the susceptibility of different strains of *T. congolense* to *G. morsitans*. The concentration of trypanosomes in the infective feed affected the initial infection rate but not the number of established infections. In the populations of *T. congolense* which had undergone repeated syringe passage through mice only few established infections became mature.

## Introduction

Since Kleine (1909 a, b) demonstrated that the tsetse flies were biological vectors of pathogenic trypanosomes, a large literature has accumulated on the relationships of *Glossina* and trypanosomes. However, most of these investigations were conducted with brucei group of trypanosomes to which the small laboratory animals are highly susceptible. The congolense group of trypanosomes, to which the laboratory animals are only moderately susceptible had received little attention. Between 1970 and 1972 comprehensive studies on the development of *T. congolense* in tsetse flies were undertaken in the laboratory. The results and observations of these studies are reported in this paper.

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# Material and Methods

### The Trypanosomes

Most of the experiments were conducted with derivatives of *Trypanosoma congolense* TREU 692; a second strain, *T. congolense* LUMP 92, was also used. TREU 692 was isolated by the Edinburgh Veterinary Expedition to East Africa in 1966 while LUMP 92 was isolated from a cow at Malakal, Upper Nile Province of the Sudan in 1969.

Each strain was firstly passaged cyclically through *G. morsitans*. The fly infected with each strain was then allowed to feed on and infect a mouse. A series of syringe passages were effected from mouse to mouse and the mice infected with the 2nd, 6th and 7th syringe passages of TREU 692 were killed at parasitaemia and stabilates made from their blood. These were designated 692-A, 692-B, and 692-C respectively. The populations of LUMP 92 which had been passaged twice and eleven times through mice were also made into stabilates and designated 92-D and 92-E respectively. All stabilates were preserved in liquid Nitrogen.

### The Flies

*Glossina morsitans* were used for most of the experiments. Another species, *G. austeni* were also used. The pupae of these species were obtained from the tsetse laboratory, Bristol (England) and from the colony of the Zoology Department, a subcolony of the Bristol stock. The experimental flies were maintained at 26°C and relative humidity of 65–70%. After sexing, they were placed in individual polystyrene cages of 1/2 in. diameter (Mews 1969). Unless otherwise stated, the first feed of the flies was the infective feed given within 36 h of eclosion. The following day, female flies were mated. Thereafter, flies were offered food on alternate days but those that did not feed within 10 min were removed and offered food daily until a meal was taken. The flies were maintained on mice, one mouse being allocated to each fly. The apparatus for restraining the mice was constructed as described by Cockings et. al. (1959). The flies maintained under these conditions were said to be subject to "standard treatment".

In the experiments where different factors were tested, the conditions were altered accordingly. When the effect of temperature was tested, the fly cages were placed in wide mouthed glass jars on perforated trays over a saturated solution of ptassium tartrate and the jars were kept in incubators. The lids of the jars were quickly replaced when flies were removed for feeding, so that the flies were exposed to a relative humidity of 75% for most of the time (Wiston and Bates 1960).

Throughout the period of investigation, the flies were given the infective feed by feeding them through chicken skin membranes on the stabilated populations of *T. congolense* after they had been diluted 1 : 100 with defibrinated ox blood. This method of artificial feeding as well as the method of dissecting the tsetse flies for the examination of trypansomes in the midgut had been described (Dipeolu 1974, Dipeolu and Adam 1974). Mice were screened for infection by examination of a wet preparation of tail blood. If no trypanosomes were seen after scrutiny of 50 microscopic fields at ×400 magnification, the animals were recorded as not infected. Mice were kept under observation or at least 6 weeks after the last feed taken by a fly.

# Results

### Flies under Standard Treatment

(1) Initial development in *Glossina* — groups of 10 *G. morsitans* were infected with 692-A. From the 3rd to the 12th day after the infective feed, one fly from each groups was killed each day and examined for trypanosomes. When present, their

Table 1

Infections in *G. morsitans* which had Fed on *T. congolense* 692-A

| Group No. of flies | Day of dissection | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | M | (M) | M | M | M | M | *M | M | F | F |
| 2 | (F) | (M) | M | F | *M | M | F | F | M | M |
| 3 | M | M | (M) | (F) | M | F | M | M | M | M |
| 4 | M | F | (F) | M | M | M | M | M | F | F |
| 5 | (M) | M | (M) | M | M | M | M | M | M | M |
| 6 | (F) | (M) | M | M | F | M | M | F | *F | M |
| 7 | (F) | M | M | F | F | F | F | F | M | F |
| 8 | M | (M) | (M) | M | M | M | F | M | F | *F |
| 9 | F | (M) | M | M | F | *F | M | M | M | F |
| 10 | *F | (F) | (F) | F | M | F | F | F | F | F |
| Total No. of flies with trypanosomes in midgut | 5 | 6 | 5 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |

Key: M = Male, F = Female, ( ) = "Infected" fly, i.e., trypanosomes restricted to posterior segment, * = "Established" infection, i.e., trypanosomes had migrated forward into the ectoparitrophic space of the middle and anterior segments.

position in the midgut was noted. As an example, the results of the first 10 groups (100 flies) are given in Table 1. About 50% of the flies dissected on each of days 3, 4 and 5 had trypanosomes in the midgut. As from the 6th day onwards, this proportion fell to a very low but almost constant number. It was observed that in flies killed between days 3 and 5, the trypanosomes were confined to the posterior part of the midgut, whereas in almost all those killed as from the 6th day the trypanosomes had spread forwards but were confined to the ectoperitrophic space. This pattern of development was repeated in all the groups of flies fed on 692-A. Consequently, flies with trypanosomes in the posterior segment of the midgut were referred to as "infected" while those in which the trypanosomes had migrated forward into the ectoperitrophic space of the middle and anterior segments of the midgut were referred to as having "established" infections. The final results of the initial tests, in which 670 *G. morsitans* were dissected (67 groups of 10 flies each) are shown in Fig. 1. 39 of the 67 flies (58%) dissected on day 3 were "infected". The proportion of infected flies decreased with time until about the 8th day when it became almost constant. Established infections were evident from as early as the 3rd day and the proportion became almost constant as from the 4th day.

(2) Cyclical development — 400 *G. morsitans* were fed on stabilates of 692-A. Since it was no longer necessary to dissect them daily, the flies were dissected in batches on days 3, 5, 7, 14 and 21 post infection. After day 7, the mice on which each fly had been feeding were examined thrice wekly for parasitaemia, any fly which infected its mouse was recorded as having a mature infection.
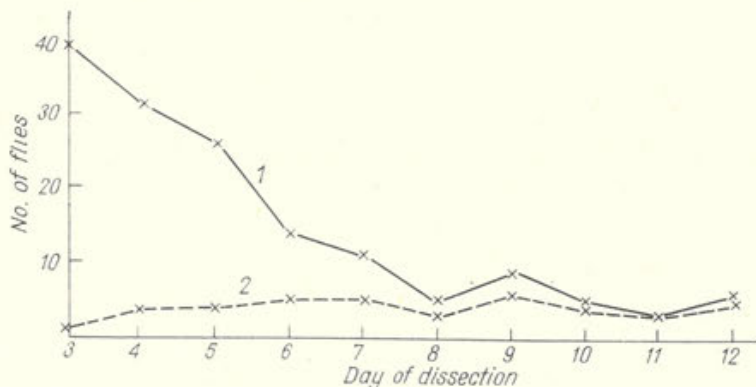
Fig. 1. Distribution of types of infections of *Trypanosoma congolense*, 692-A in 670 *Glossina morsitans*. ————— No. of flies out of 67 which are "infected". — — — No. of flies out of 67 with established infections

Table 2 shows that 64% of the flies killed on day 3 were infected and there was no established infection on this day. By the 5th day the proportion of infected flies had fallen to 40% and 10% of these infections had already established. As from the 7th day onwards, almost all the established infections had matured. A single fly in the 21 day group with a mature infection was detected by a probe test on the 14th day post infection. It was kept for 60 days during which time it fed on and infected 6 mice. The prepatent period in all the mice was the same — 8 days. By examination of the feeding records of other flies with mature infections, it was estimated that the development of this strain of *T. congolense* in *G. morsitans* took 7–10 days to be accomplished.

Modification of the Standard Treatment

In the following experiments the procedure was modified and the results were compared with those obtained by the standard treatment (Table 2). The significance of the difference was determined by calculating $X^2$ by means of a $2 \times 2$ contigency table. When the total numbers for comparison were less than 200, Yates correction was applied (Fisher 1941).

(1) Effect of temperature. After *G. morsitans* had taken their infective feed on 692-A, they were divided into two groups; one group was kept in an incubator at 31°C, the other at 20°C. The results of these tests are shown in Table 2. The pattern of infection in flies kept at 31°C the same as that of the flies kept under standard treatment (26°C) and the proportions of established and mature infections were also similar at both temperatures. By examination of the feeding records of the flies which infected mice, it was estimated that the duration of the development cycle at 31°C was 7–9 days. Hence, raising the temperature of fly maintenance from 26°C

Table 2

Infections of *T. congolense* in Tsetse Flies*: Comparison of Standard Treatments with Different Tests

| Treatments | Dissection on | | | | | | | | | | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | Day Three | | | Day Five | | | Day Seven | | | Day Fourteen | | | Day Twenty-one | | |
| | I | EI | MI | I | EI | MI | I | EI | MI | I | EI | MI | I | EI | MI |
| (1) Standard | (50) 64 | 0 | 0 | (50) 40 | 10 | 0 | (100) 14 | 9 | 7 | (100) 7 | 7 | 6 | (100) 7 | 6 | 6 |
| (2) Flies maintained at 31°C | (50) 60 | 0 | 0 | (50) 42 | 8 | 0 | (50) 14 | 10 | 6 | (50) 8 | 6 | 6 | (50) 6 | 6 | 6 |
| (3) Flies maintained at 20°C | (50) 68 | 0 | 0 | (50) 50 | 0 | 0 | (50) 10 | 2 | 0 | (50) 6 | 4 | 0 | (50) 4 | 4 | 0 |
| (4) *G. austeni* | (50) 36 | 0 | 0 | (50) 20 | 4 | 0 | (50) 18 | 6 | 0 | (50) 4 | 4 | 0 | (50) 4 | 4 | 0 |
| (5) Strain 92-D | (100) 18 | 0 | 0 | (100) 7 | 3 | 0 | (100) 10 | 5 | 0 | (100) 4 | 3 | 0 | (100) 4 | 2 | 2 |

( ) = Number of flies used for experiment, I = Infected per cent, EI = Established infection per cent, MI = Mature Infection per cent.
* Flies were *G. morsitans* except No. 4.

did not influence the rate of infection of this strain of *T. congolense* in *G. morsitans* and the duration of the development cycles was not affected.

The development of trypanosomes in those flies kept at 20°C was, however, delayed since it was not until day 7 that a few infections became established (Table 2). Moreover, none of the established infections had matured by day 21 possibly because insufficient time had been allowed for the trypanosomes to complete their development. Another 200 *G. morsitans* were therefore infected and kept in an incubator at 20°C. After 21 days, they were divided into two batches of 100 flies each. One batch was transferred to the fly room at the standard temperature of 26°C and kept there for a further 21 days; the other batch was retained in the incubator at 20°C also for another 21 days. Each fly was maintained on a single mouse. The results are shown in Table 3. At the end of the 42 days, 8 of the 100 flies transferred

Table 3

Infections of *T. congolense* in *G. morsitans* Maintained at 20°C

| Treatment | No. of flies | No. infected | No. of established infections | No. of mature infections |
|---|---|---|---|---|
| *G. morsitans* kept continuously at 20°C for 42 days | 100 | 8 | 8 | 6 |
| *G. morsitans* kept at 20°C for 21 days, then at 26°C for 21 days | 100 | 6 | 6 | 5 |

to 26°C possessed established infections and 6 of these were mature while 6 of those retained at 20°C possessed established infections and 5 of these were mature. Hence, at 20°C, the development of *T. congolense* in tsetse flies is not inhibited but only prolonged.

(2) Species of fly — To test whether 692-A would develop equally well in another species of tsetse flies, *G. austeni* was substituted for *G. morsitans* and kept under standard conditions. As shown in Table 2, there was no significant difference between *G. morsitans* and *G. austeni* in the proportion of mature infections on day 21 ($P < 0.20$). However, correlation between the number of established and mature infections was delayed until day 21 and examination of the feeding records of the 2 *G. austeni* which infected mice showed that the infections became mature 18 and 19 days post infection. Hence, the duration of the development cycle of 692-A in *G. austeni* (18–19 days) is longer than in *G. morsitans* (7–10 days).

(3) Strain of trypanosomes — 500 *G. morsitans* were fed on 92-D. The flies were kept under standard treatment and dissected in batches of 100 on days 3, 5, 7, 14 and 21 after the infective feed. The results are shown in Table 2. The infection rate of this strain was generally lower than that of 692-A. A fly of the 14 day group was

detected as mature by probing on the 14th day and was kept for 60 days during which time it fed on and infected 3 mice. The prepatent periods in the mice were 16, 17, and 19 days. This strain therefore differed from 692-A in:

   (i) generally lower infection rate in *G. morsitans*

   (ii) slower development in *G. morsitans*

   (iii) longer prepatent period in mice.

   (4) Concentration of trypanosomes in infective feed — The effect of feeding the flies on different numbers of trypanosomes is shown in Table 4. A ten fold increase

Table 4

infections in *G. morsitans* which had been Fed on Different Concentrations of *T. congolense* 692-A

| Day of dis- section | Dilution of stabilates | Concentration of trypanosomes in feed per ml. blood | Total flies used | Infected flies per cent | Established infections per cent |
|---|---|---|---|---|---|
| 3 | 1 : 10 | $4.4 \times 10^7$ | 20 | 100 | 0 |
|  | 1 : 100 | $4.4 \times 10^6$ | 50 | 64 | 0 |
| 5 | 1 : 10 | $4.4 \times 10^7$ | 20 | 45 | 5 |
|  | 1 : 100 | $4.4 \times 10^6$ | 50 | 40 | 10 |
| 7 | 1 : 10 | $4.4 \times 10^7$ | 27 | 16 | 8 |
|  | 1 : 100 | $4.4 \times 10^6$ | 100 | 14 | 7 |

in the number of trypanosomes significantly increased the proportion of flies infected at day 3 ($P < 0.05$) but by the 7th day the proportion of established infections in each group was similar.

   (5) Effect of syringe passage through laboratory animals: *G. morsitans* were fed on 692-A, 692-B and 692-C which had been passaged through mice twice, six and seven times respectively before preservation as stabilates. While all the established infections among the *G. morsitans* which fed on 692-A became mature, the proportions of established infection which became mature among *G. morsitans* which fed on 692-B and 692-C were very low (Table 5). When the experiments were repeated with another strain of *T. congolense* i.e., 92-D and 92-E, the only established infection among flies which fed on 92-E (11 passages) did not mature. Hence, it appears that repeated syringe passage of *T. congolense* through laboratory animals tend to decrease its infectivity to tsetse flies.

General Observations

Infection of hypopharynx: *T. congolense* completed its development cycle in a total of 44 *G. morsitans* and 2 *G. austeni* since all these flies infected mice. When these flies were dissected, all of them had numerous trypanosomes in the midgut, proventriculus, labrum-epipharynx and hypopharynx. It was observed that in those flies which had fed on populations of *T. congolense* which had been passaged several

had fed on 692-A and kept longer than seven days, almost all the established infections had become mature. In this investigation, the infection in a fly is recorded as mature only if it infected the mouse on which it had fed. In the course of the experiments, it became evident that the infection in a fly becomes mature only after the hypopharynx had been invaded by trypanosomes because all the 44 *G. morsitans* and 2 *G. austeni* which infected their mice had abundant trypanosomes in the hypopharynx. It appears, however, that trypanosomes of *T. congolense* which had been subjected to repeated syringe passages in the laboratory had lost the ability to invade the hypopharynx of the tsetse flies. This was observed among the flies which fed on 692-B, 692-C and 92-D. Although some of the flies carried established infections (Table 5) and trypanosomes were found in the midgut, proventriculus and labrum epipharynx, their hypopharynx were not invaded and they did not infect the mice on which they have fed. These results suggest that there is a trend towards a reduction in the proportion of mature infections in tsetse flies following repeated syringe passages of *T. congolense* in the laboratory. It is, however, of interest that this trend appears as early as the 6th and 7th artificial passages. In case of *T. brucei* up to $2^1/_2$ years (Duke 1923) and $5^1/_2$ years (Murgatroyd and Yorke 1937) of syringe passages were undertaken before this effect was manifested. This phenomenon had been recorded for *T. congolense* only by van Hoof et. al. (1937) They obtained scanty infections only in the midgut of *G palpalis* infected with a strain of *T. congolense* which had been passaged artificially 200 times in the laboratory.

This investigation shows that factors such as temperature at which the experimental flies are maintained, the species of tstse flies, the strain of *T. congolense* and their concentration in the infective feed influence either the initial rate of infection in the flies or the prepatent period in the mice or the duration of the development cycle in the tsetse. The observation that raising the temperature of fly maintenance from 26°C to 31°C did not affect the susceptibility of *T. congolense* to *G. morsitans* is directly opposite to what is known of *brucei* group of trypanosomes. Fairbain and Culwick (1950) had shown that the susceptibility of *T. rhodesiense* to *G. morsitans* can be increased if the flies are maintained at 28°C. Similarly, Dipeolu and Adam (1974) were able to obtain increased transmission rates with *T. brucei* when the temperature of maintenance of *G. morsitans* was increased from 26°C to 31°C. Since these could not be obtained for *T. congolense*, it is possible that the critical temperature for increased transmission rates of this species of trypanosomes is higher than 31°C. An attempt to maintain *G. morsitans* infected with *T. congolense* at 37°C was abandoned during this investigation because of the high mortality among the flies.

The results of this investigation cannot throw any light on the recent findings of Mshelbwala (1972) that the haemocoele of the tsetse flies could be invaded by all the development forms of the trypanosomes including the metacyclics. No attempt was made to look for trypanosomes outside the midgut, since by dissection at daily intervals, the sequence of the development of *T. congolense* could be progressively

and systematically followed from the time of the infective feed to the time when the infection became mature. Further studies on the role of the haemocoele are, however, desirable as they might throw some light on some unexplainable aspects of the development of trypanosomes in the tsetse flies.

ZUSSAMENFASSUNG

Während Üntersuchung an der Entwicklung der *Trypanosoma congolense* in Tsetse Fliegen wurde es beobachtet dass, 3 Tage nach Aufnehmen des trypanosomenhaltigen Blutes 50–60% der Fliegen die Trypanosomen im Verdaungskanal tragen konnen; aber die Zahl, in der die Entwicklung festgesetzt wird, niedrig war. Nur nach Eindringen der Trypanosomen in Hypopharynx der Fliege war die Infektion reif. Erhöhug der Erhaltungs-temperatur von 26°C zu 31°C beeinflussts die Empfanglichkeit der *G. morsitans* zu *T. congolense* nicht; aber um 20°C die Entwicklung wurde nur prolongiert und nicht gehemmt. Die Entwicklungsdauer war in *G. austeni* länger als in *G. morsitans*. Es waren auch Verschiedenheiten in der Empfanglichkeit von verschiedenen Linien von *T. congolense* zu *G. morsitans*. Die Konzentration der Trypanosomen im Blut beeinflusste das anfangliche Infektions-rate in *G. morsitans*; aber die Zahl der Fliegen in denen die Infektion festgesetzt war, war nicht beeinflusst. Nur eine niedrige Zahl der festgesetzten Infektion wurde reif in den Population der *T. congolense*, die durch Mäuse mehrmals im Labor passagiert worden sind.

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## CONTENTS