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ACTA PROTOZOOLOGICA
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Psammonobiotidae fam. nov. — une nouvelle famille de thécamoebiens (*Rhizopoda, Testacea*) du psammal supralittoral des mers

Psammonobiotidae fam. nov. — едно ново семейство черупчести ризоподи (*Rhizopoda, Testacea*) от супралиторалния псамал на моретата

Au cours des dernières années on a établi, à la suite des recherches sur les rhizopodes interstitiels du psammal supralittoral des mers, la présence d'une association thécamoebienne relativement riche dont les espèces composantes sont de distribution cosmopolite (Golemansky 1969, 1970 a, b, c, d, e, Valkanov 1970, Chardez 1972). En plus de certains rhizopodes thécamoebiens connus depuis longtemps, on a découvert aussi, dans les eaux souterraines littorales des plages des mers, plusieurs thécamoebiens inconnus jusqu'à présent, habitant exclusivement les especes interstitiels du psammal supralittoral. Ces thécamoebiens, considérés du point de vue écologique, sont des psammobiontes stricts (Golemansky 1973 a, b). Du point de vue morphologique, la plupart des thécamoebiens psammobiontes stricts montrent plusieurs particularités spécifiques qui les caractérisent et les distinguent nettement des autres thécamoebiens dulçaquicoles, mucicoles et terricoles.

Nos dernières études sur la taxonomie, la distribution et l'écologie des thécamoebiens du supralittoral des mers ont montré qu'une bonne partie de thécamoebiens psammobiontes stricts portent plusieurs caractères communs et proches du point de vue morphologique et écologique et qu'ils représentent en réalité un groupe de genres et d'espèces si bien délimité des autres thécamoebiens, que nous proposons les réunir dans une catégorie taxonomique nouvelle — la famille *Psammonobiotidae* fam. nov. du sous-ordre *Teastaceafilosa* de l'ordre *Testacea*. La nouvelle famille porte le nom du premier psammobionte typique du groupe des thécamoebiens que nous avons trouvé et décrit sous le nom de *Psammonobiotus communis* Gol., 1967 et qui a été plus tard retrouvé dans les eaux souterraines littorales de toutes les plages étudiées.

Psammonobiotidae fam. nov.

Genre type: *Psammonobiotus* Golemansky, 1967

Diagnose: Thèque chitinoide, transparente, bilatéralement symétrique, lisse ou recouverte d'éléments structuraux ovales, ellipsoïdaux ou de forme et de dimensions irrègulières. Section transversale circulaire ou dorso-ventralement comprimée. Le pseudostome, placé obliquement à l'axe longitudinale du corps, se trouve chez la plupart des espèces au centre d'un élargissement de la thèque en forme d'entonnoir.

Pseudopodes filopodes. Un seul noyau de type ovulaire. Psammobiontes stricts.

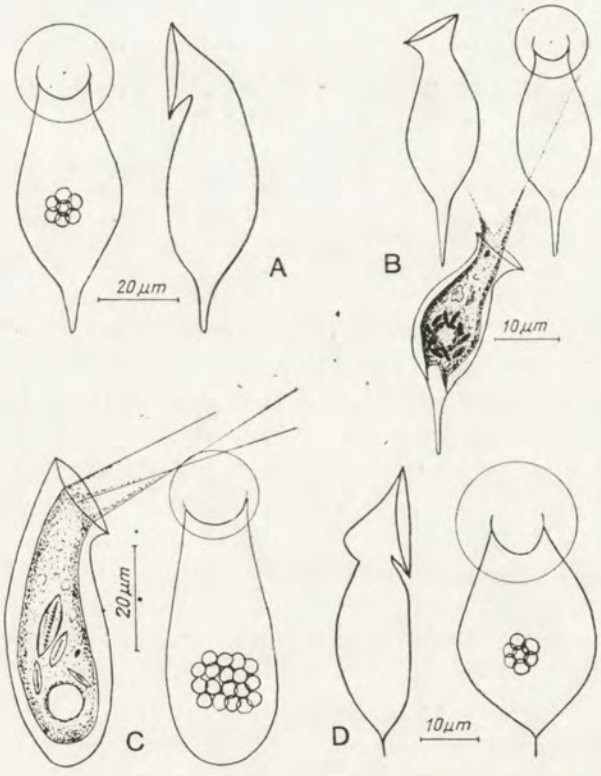

Fig. 1. A — *Pseudocorythion acutum* (Wailes) Valkanov, 1970, B — *Chardezia caudata* Gol., 1970, C — *Messemvriella filosa* Gol., 1972, *D* — *Pseudocorythion wailesi* Gol., 1971

Notes: Pour le moment la fam. *Psammonobiotidae* fam. nov. comprend 5 genres avec 11 espèces: *Psammonobiotus* Gol. (4 espèces), *Pseudocorythion* (Wailes) Valkanov (2 espèces), *Chardezia* Gol. (1 espèce), *Corythionella* Gol. (3 espèces) et *Messemvriella* Gol. (1 espèce). Toutes les espèces connues sont des psammobiontes obligeants de distribution cosmopolite.

Pour faciliter l'identification des geners de la fam. *Psammonobiotidae* fam. nov., connus jusqu'à présent, nous proposons ci-dessous un tableau taxonomique des genres, établie à la base des plus importants caractères morphologique des thèques. Les caractères taxonomiques de type général (type des pseudopodes, appareil nucléaire etc.), mentionnés dans la diagnose de la famille, n'y participent pas; on n'a

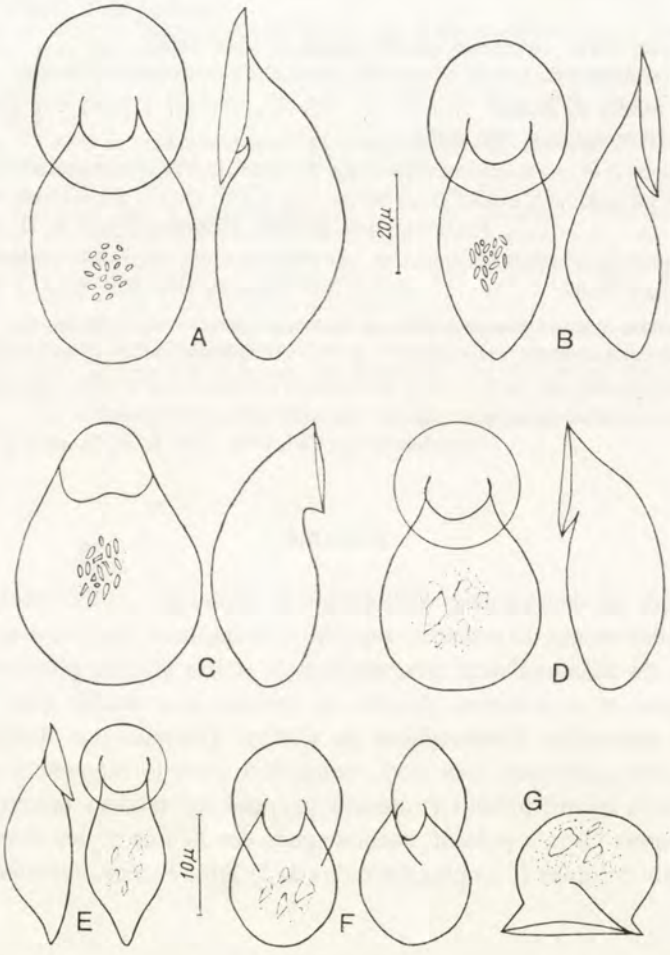

Fig. 2. A — *Corythionella pontica* Gol., 1970, B — *Corythionella minima* Gol., 1970, C — *Corythionella acolla* Gol., 1970, D — *Psammonobiotus communis* Gol., 1967, E — *Psammonobiotus linearis* Gol., 1970, F — *Psammonobiotus minutus* Gol., 1970, G — *Psammonobiotus balticus* Gol., 1972

utilisé que les caractères morphologiques des thèques, ce qui permet l'identification des genres à la seule présence des théques vides. Le tableau taxonomique est illustrée aussi de toutes les espèces connues des cinq genres, ce qui donne une idée visuelle plus précise sur les espèces et les genres de la fam. *Psammonobiotidae* fam. nov.

Tableau taxonomique des genres de la fam. *Psammonobiotidae* fam. nov.

1. Thèque chitinoïde, transparente, sans structure superficielle visible 2
— Thèque chitinoide, transparente, recouverte de plaques rondes, ovales, ellipsoïdales ou de forme et de dimensions irregulières 3

2. Pseudostome avec un élargissement en forme d'entonnoir; l'extrémité postérieure de la théque allongée en un appendice caudal creux et pointu . . . . . *Chardezia* Gol. (Fig. 1 B, Pl. II 10)

3. Thèque recouverte de plaques rondes, ovales ou ellipsoïdales 4
— Thèque recouverte de peu de plaques de forme et de dimensions différentes 7

4. Plaques rondes ou ovales 5
— Plaques ellipsoïdales et très petites 6

5. Pseudostome avec un élargissement en forme d'entonnoir; l'extrémité postérieure de la thèque se termine apar un appendice caudal creux ou par une épine; section transversale ovale ou ronde . . . . . *Pseudocorythion* (Wailes) Valkanov (Fig. 1 A, D, Pl. I 1, 2, 3, 4)
— Pseudostome avec un élargissement en forme d'entonnoir, l'extrémité postérieure arrondie, section transversale ronde . . . . . *Messemvriella* Gol. (Fig. 1 C, Pl. II 9)

6. Pseudostome avec un élargissement en forme d'entonnoir ou disposé sur un col court, l'extrémité postérieure arrondie ou lancéoleé . . . . . *Corythionella* Gol. (Fig. 2A, B, C, Pl. II 7, 8)

7. Pseudostome avec un élargissement en forme d'entonnoir ou couvert d'une visière, l'extremite postérieure arrondie ou se terminant par un court appendice caudal . . . . . *Psammonobiotus* Gol. (Fig. 2 D, E, F, G, Pl. I 5, 6) . . . . .

Résumé

A la suite de plusieurs années d'études sur la taxonomie, la morphologie et l'écologie des thécamoebiens du psammal supralittoral des mers, l'auteur a conclu qu'une bonne partie de thécamoebiens psammobiontes stricts portent plusieurs caractères morphologiques et écologiques proches et peuvent être reunis dans une famille nouvelle du sous-ordre *Testaceafilosa* de l'ordre *Testacea*. La nouvelle famille, nommée *Psammonobiotidae* fam. nov., comprend pour le moment 5 genres et 11 espèces. Dans le travail présent l'auteur a proposé un tableau taxonomique pour les genres connus jusqu'à présent, accompagnée des figures et des microphotos des espèces les plus typiques et les plus répandus de la fam. *Psammonobiotidae* fam. nov.

РЕЗЮМЕ

В резултат на своите многогодишни изследвания върху таксономията, морфологията и екологията на текамебите от супралиторалния псамал на моретата авторът е дошъл до заключение, че една значителна част от облигатните псамобионтни текамеби притежават редица близки морфологичин и екологичин белези и могат на бъдат обединени в отделно семейство от подразред *Testaceafilosa* на разред *Testacea*. Новото семейство, наречено *Psammonobiotidae* fam. nov., обединява за сега 5 рода и 11 вида. За известните до сега родове в работата е предложен ключ за определянето им, който е илюстриран и с рисунки на най-характерните и широко разпространени видове на сем. *Psammonobiotidae* fam. nov.

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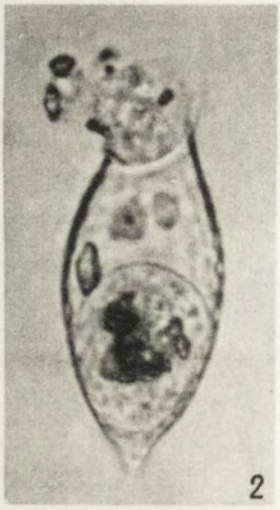
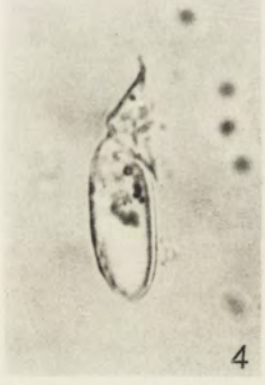
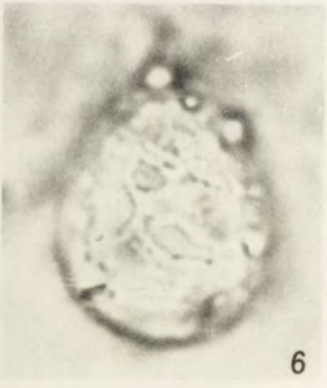
Received 10 July 1973

EXPLICATION DES PLANCHES I–II

1: *Pseudocorythion acutum* (Wailes) Valkanov, division ou copulation. ?1600×
2: Id, vue ventrale. 100×
3: Id, détail du revetement. 200×
4: *Pseudocorythion wailesi* Gol., 1971, vue latérale. 800×
5: *Psammonobiotud communis* Gol., 1967, vue ventrale. 1000×
6: Id, détail du revêtement. 1600×
7: *Corythionella pontica* Gol., 1970, vue latérale. 800×
8: Id, détail du revêtement. 1600×
9: *Messemvriella filosa*., 1972, vue laterale. 1200×
10: *Chardezia caudata* Gol., 1970, vue latérale. 100×







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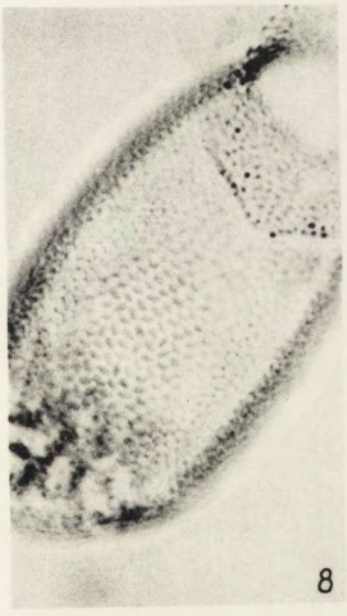

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Rosculus ithacus Hawes, 1963 (*Amoebida, Flabelluidae*) and the Amphizoic Tendency in Amoebae

Rosculus ithacus Hawes, 1963 (*Amoebida, Flabelluidae*), und die amphizoische Neigung unter Amöben

Until the recent discovery of primary amoebic meningo-encephalitis, most reports of free-living amoebae in endozoic situations involved organisms from the intestine of man or other animals, isolated either directly or, by cultivation from faecal samples, indirectly. Usually such organisms have been thought to be only in transit, probably as cysts, and not to feed or multiply endozoically. In most cases the amoebae were not suspected pathogens, though some were isolated from diarrhoeic stools of patients whose condition was attributed to other causes.

The discovery that amoebae belonging to one or more genera of normally free-living organisms are virulent invaders of the central nervous system of man and some laboratory mammals has directed attention to the possibility that more free-living species are facultatively parasitic (Page 1970 a) but has done little to change the customary assumption about the role of intestinal isolates. There is, in fact, no new reason for considering such intestinal strains and certain other endozoic isolates to be actual or potential pathogens, but the possibility that they carry on normal vegetative and reproductive activities within the metazoan body now deserves more careful consideration. Although it was recently found that a *Vahlkampfia* isolated from a turkey thrives at both room temperature and 37° (Page, in press), growth at the latter temperature is not necessarily indicative of pathogenicity (Griffin 1972). It does, however, indicate that temperature is no barrier to the multiplication of this bacteria-feeder in the intestine of a homoiotherm, while growth of the strain at 20–23° suggests that it is also free-living.

With a better knowledge of some genera, e.g., *Acanthamoeba*, it seems likely that some endozoic isolates reported long ago belong to free-living species, though in other cases the most that can be said is that they are members of characteristically free-living genera. In a recent case, an apparently pathogenic amoeba attacking *Callinectes sapidus* belonged to a genus otherwise composed of free-living species but seemed itself to be an obligate parasite (Sprague et al. 1969, Page 1970 b).

In contrast to the findings of "free-living" organisms in endozoic situations, Page (1971 a) reported the isolation of free-living strains of a species first described by Hogue (1914) as a non-pathogenic inhabitant of the oyster *Crassostrea virginica*.

This paper describes another such case, the isolation from two fresh-water habitats of strains apparently belonging to the species *Rosculus ithacus* Hawes, 1963, originally found in the rectum of the grass snake *Natrix natrix* (Hawes 1963). Isolation of this species from the free-living condition fulfils the prediction made by Hawes on the basis of the ease of cultivation of his isolate.

In addition to description and identification of the new isolates, the taxonomic position of *Rosculus* is discussed; the literature on endozoic occurrences of apparently free-living amoebae is briefly reviewed; and the general term *amphizoic* is proposed for protozoa which thrive in either an endozoic or a free-living (*exozoic*) situation, no matter which seems to be the primary habitat or whether the protozöon is actually parasitic on the host.

Materials and Methods

One clonal strain, designated 107, was isolated from the edge of the Old West River a few miles north of Cambridge; the second, 125, from a deep ditch behind King's College, Cambridge. Both were cultivated on non-nutrient agar (Page 1967 a) streaked with *Aerobacter aerogenes*. The fluid media in which cultivation was attempted were (1) mycological peptone (Oxoid mycological peptone 4% w/v in distilled water) and (2) proteose peptone yeast (Oxoid proteose peptone 1% w/v and Oxoid yeast extract 0.25% w/v in distilled water). Active amoebae were observed and measured as they moved on the under side of cover glasses, in hanging drops of amoeba saline (Page 1967 a). Mitotic figures and interphase nuclei were examined in Feulgen-fast green, Heidenhain's iron haematoxylin, and Kernechtrot preparations. The staining procedures (except haematoxylin) and induction of excystment were carried out essentially as described previously (Page 1967 a).

Numbers of measurements of each strain were: greatest dimension of active amoebae, 100; diameter of cysts, 100; diameter of floating forms, 25; nucleus and nucleolus, 25 each in trophic amoebae and cysts; locomotive rate, 10 amoebae; contractile vacuole, one period in each of 10 amoebae. All measurements were of living material except the 25 nuclei of strain 107, which were measured in a Kernechtrot preparation.

Results

Locomotive Form and Activity

The shape of these amoebae (Pl. I 1–4) varied from an elongate, limax-like one to one greatly expanded laterally with a hyaline zone extending its entire breadth. Intermediate forms were quite irregular or somewhat spatulate or fan-shaped, and amoebae with two hyaline zones, at opposite ends (Pl. I 3), were sometimes found. In most amoebae the hyaline zone, which might take up one-quarter or more of

a broad form, had an irregular edge; occasionally it was deeply divided (Pl. I 4). Any amoeba might be broad at one moment and elongated the next, so that measurements for length/breadth ratios were not possible, and only the greatest dimension of locomotive forms is given in Table 1, which summarises the measurements of these two strains and that of Hawes.

Table 1

Measurements of *Rosculus* Strains

Trophozoites	107	125	Hawes (1963)
Locomotive form µm			
Range	4.6–13.0	5.6–17.0	Commonly 14 µm
Mean	7.6	9.9	exceptionally 25 µm
Rounded (floating) µm			
Range	3.6–7.3	4.8–8.5	2.5–11.25
Mean	5.0	6.6	5.5
Locomotive rate µm/min	19–56	22–40	Not reported
Contractile vacuole			
Maximum size µm	1.5–2.0	1.9–2.8	None present
Mean period (sec)	18	21	
Nucleus, mean µm	2.4	2.3	1.9
Nucleolus, mean µm	1.2	1.1	0.8
Cysts			
Diameter µm			
Range	4.2–8.8	4.6–7.9	3.5–7
Mean	6.0	6.0	5.5
Nucleus, mean µm	2.3	2.0	Not reported

The actively advancing form was generally the broad one. In changing direction, one side of a very broad form might lead off in a new direction; if the amoeba continued in that direction, it expanded laterally at right angles to the new line of advance. In such changes of direction, the hyaline zone was often divided, and this was the origin of the forms which temporarily had hyaline zones at opposite ends. Sometimes a hyaline wave spread rapidly around part of the granular mass. Occasionally an amoeba gathered itself up into an irregular ball and then quickly resumed locomotion.

A few uroidal threads formed by adhesion were seen rarely.

Apparent attempts at cannibalism were common when two cells come together, and complete ingestion was observed. The process was very similar to that described for *Flabellula calkinsi* (Page 1971a). An amoeba containing another is pictured, from a fixed preparation, in Pl. I 5.

Since these strains possessed a contractile vacuole but that of Hawes (1963) did not, cultivation of strain 107 in fluid media resembling those of Hawes was attempted. (Hawes cultivated his strain on agar, in "weak peptone solution", and in a dilute horse-serum medium, in all cases feeding on bacteria). Amoebae with accompanying bacteria were transferred from a thriving day-old agar culture into tubes containing proteose peptone yeast, mycological peptone, and mycological peptone diluted 1:1 with amoeba saline (Page 1967 a). The peptone media are ordinarily used for axenic cultures of protozoa. Five days later rounded cells were found in all three tubes, as well as some active cells in the PPY and MP : AS tubes. Contractile vacuoles were functioning in the active cells. However, lasting cultures were not established in these media, in which bacterial growth was fairly heavy.

The floating forms (Pl. I 6) produced when amoebae were suspended in amoeba saline were rounded, with no radiate pseudopods, and soon put out a broad, flattish hyaline pseudopod and settled into the locomotive form.

Nucleus and Nuclear Division

The nucleus (Pl. II 8–10) is of the vesicular type common in amoebae, with a central nucleolus and a layer of presumptive chromosomal material between nuclear membrane and nucleolus. It was difficult to see in vivo, though with patience it could be discerned in a fair number of cells with phase contrast. Both nucleus and nucleolus were quite deformable. The diameters reported may err slightly on the large side because smaller nuclei were more difficult to distinguish. The interphase nucleus was usually completely Feulgen-negative, though the presumptive chromosomal material stained somewhat more darkly than did the nucleolus and in a few nuclei (perhaps early prophase) appeared slightly Feulgen-positive.

Reproduction was by binary fission accompanied by mitotic division (Pl. II 11–16) of the pattern common in many species of amoebae, i.e., with disintegration of the nucleolus followed by disappearance of the nuclear membrane. An undoubted metaphase plate was not found, perhaps being unrecognisable because of the small size of the nucleus. In anaphase the chromosome sets were only weakly Feulgen-positive. The stage shown in Pl. II 13 shows a spindle configuration resembling the "centrodesmose" of Hawes (1963). Mitotic figures are illustrated by photographs as more convincing than drawings in this case, though less clear.

Cysts

Strain 107 showed a greatly reduced capacity for encystment within a few months of isolation, but strain 125 was still producing abundant cysts more than a year after isolation. Encystment generally occurred in many cells within two days after inoculation of a fresh agar surface with trophic amoebae. The cysts (Pl. I 7) were oval or spherical, with a smooth inner wall about 0.3 μm thick and a thinner hyaline

outer coat. The oval shape tended to be more elongate in strain 125 than in 107. The cytoplasm contained several granules with a diameter ca. 0.3 μm which often obscured the nucleus. The latter was often eccentrically situated.

No excystments were actually observed until more than 48 h after inoculation of the excystment surface, and no empty cysts were seen. By the second day after inoculation, the walls of many cysts appeared to be softened (optically less dense). Cytoplasmic movement and contractile-vacuole activity were seen in advanced stages. The wall appeared eventually to be completely dissolved, since amoebae moved off leaving no empty cysts.

Discussion

Identification

The locomotive form and behaviour of these strains corresponded well with the description given by Hawes for his *Rosculus ithacus* from the grass snake. Any of the drawings in his Fig. 2 could have been made from these strains, and his description of the locomotive habit could be quoted verbatim for them. Nuclear structure is similar. As pointed out earlier, the reported nuclear sizes for strains 107 and 125 may be biassed toward the large side because nuclei were measured only when quite distinct. The nucleolus is relatively small, as in the Hawes strain, but his diagnosis may over-emphasise the proportionate diameters of nucleus and nucleolus. Allowing for differences in interpretation of some of Hawes's figures, the reports of mitosis seem consistent with a common pattern. The cysts are similar, though a higher proportion in strain 107 approximated a spherical form than Hawes noted. Similar cysts, both ovoid and spherical, are produced by other amoebae and flagellates.

The two points of difference which seem most significant in specific identification are the endozoic habitat of the Hawes strain and its lack of a contractile vacuole. No attempt has been made to infect grass snakes with strains 107 and 125, as Hawes did with his amoebae. A successful infection (though not without significance) would not establish the specific identity, since other species might also be introduced successfully, and it is conceivable that different strains of a species might differ in their ability to grow endozoically.

The absence of a contractile vacuole in the Hawes strain and its presence in these strains is a greater problem. All well-studied species of fresh-water and soil amoebae except *Pelomyxa palustris* (unique in several ways) have contractile vacuoles, and the media which Hawes used would seem also to require such a means of osmoregulation. However, the obviously careful observations of Hawes make it very unlikely that he overlooked a contractile vacuole.

The identity of strains 107 and 125 as members of the genus *Rosculus* seems certain, and the contractile-vacuole discrepancy does not seem at this time sufficient ground for doubting the specific identification as *R. ithacus*.

As far as I know, the Hawes strain is no longer in existence.

Taxonomic Position of the Genus

Hawes (1963) discussed the possible affinities of *Rosculus* with the parasitic genus *Endolimax*. Without discounting that suggestion, it should be noted that some of the characters which he presents as similarities are too generally distributed to serve as evidence in themselves; that we now have more information on locomotive behaviour and form in diverse amoebae; and that we now have more reason than did Hawes to seek relatives of such endozoic isolates among free-living amoebae as well as among parasitic groups.

A comparison of *Rosculus* with the marine amoeba *Flabellula calkinsi* (Page 1971 a) shows notable and obvious similarities in locomotive form and behaviour. One difference is, that, so far as I have observed, *F. calkinsi* does not show the rapid running of a hyaline border around the edge of the granular mass seen in *R. ithacus* ("sometimes rippling about the endoplasmic mass so as to skirt the whole length of the organism", as Hawes described it). A voracious cannibalism, though not in itself suitable as a taxonomic criterion, is another common characteristic of the two species. Cannibalism has been reported in other amoebae (Page 1967 b, 1971 b, in press, and other authors) but is particularly noticeable in these species. (I have also observed apparent cannibalistic attempts in *F. citata* but have not tried to determine whether ingestion actually occurs). *F. calkinsi*, though small, is larger than *R. ithacus*. It is suggested that these species are related but should be classified, as now, in the different genera. In some ways *F. calkinsi* is intermediate between *R. ithacus* and the much larger *F. citata*. The differences between *F. calkinsi* and *F. citata* include the frequent occurrence of uroidal filaments in the latter (Page 1971 a).

In proposing a union of the families *Mayorellidae* and *Paramoebidae* under the latter name, Page (1972) preferred to retain the genus *Flabellula* Schaeffer, 1926, traditionally a mayorellid, within the united family rather than place it in the separate family *Flabellulidae* Bovee, 1970, because it seemed inappropriate to classify it with *Vannella* Bovee, 1965, as Bovee (1970) did, if the former family *Mayorellidae* were to be divided. *Vannella* has a locomotive habit resembling that of *Thecamoeba* and *Platyamoeba* more than that of *Flabellula* (as the latter was emended by Bovee, 1965). (Hülsmann and Haberey 1973 have reported an investigation of amoeboid movement in *Vannella* sp., which they call *Hyalodiscus simplex*). However, the similarity between *Flabellula* and *Rosculus* and the lack in locomotive forms of both of the conical pseudopods characteristic of the *Paramoebidae* (= *Mayorellidae*) suggest the advisability of grouping them in a separate family, and it is here proposed that the family *Flabellulidae* Bovee, 1970, should include *Flabellula* and *Rosculus* but

not *Vannella*, which should be transferred to the *Thecamoebidae*. In *Flabellula* and *Rosculus* there is a more eruptive cytoplasmic streaming and locomotion than in the *Thecamoebidae*, including *Vannella*. For example, in *F. citata* cytoplasmic fluid may intrude into the hyaline zone at one point along its boundary with the granular cytoplasm and then spread laterally through the rest of the hyaline zone. In *Vannella* the cytoplasmic flow is more uniform across the entire breadth. The conical pseudopods occasionally formed singly at one side of the hyaline zone of *F. citata* differ from those of the paramoebids (= mayorellids) in being formed by a cleft in the hyaline zone rather than being pushed forward from the hyaline zone.

The mitotic pattern of *Rosculus*, as illustrated here, presents no obstacle to a close relationship with *Flabellula*, though it is of a type shared by many amoebae.

It should be emphasised that the genus *Rosculus* must not be used for any diverse tiny amoebae which appear difficult to classify; size alone is not a criterion. Nor does this suggested relationship with *Flabellula* rule out the possibility of an affinity with strictly parasitic genera, as hypothesised by Hawes.

The Amphizoic Habit

The list in Table 2 of amoebae which probably or certainly belong to free-living species but have been reported from endozoic habitats is intended to be suggestive rather than complete, and the list of references is likewise representative rather than complete (especially for those species implicated in amoebic meningo-encephalitis). The species are listed as originally reported, without regard to possible synonymies; the generic names are corrected where necessary and possible. The host list includes experimental infections. It must be recognised that some reports of intestinal occurrence may be erroneous because of the method of collecting a faecal sample. It is also quite possible that some reports actually are based only on transient cysts. Nevertheless, such findings continue; most contemporary workers are careful to guard against contamination; and it now appears likely that a number of "normally" free-living species are capable of thriving in the intestine of homoiotherms and in that organ and elsewhere in poikilotherms.

It is quite probable that endozoic is (as in the case of other micro-organisms) usually not equivalent to parasitic or pathogenic. In the case of amoebae, Griffin (1972) has shown that growth at 37° is not in itself indicative of virulence (*Hartmannella* and *Tetramitus*). It does, as previously mentioned, suggest an endozoic capability. Fulton (1970) was able to grow several strains of *Naegleria gruberi* and one of *Tetramitus rostratus* at 33° and, in the case of at least two strains of *N. gruberi*, up to 41°. The recent finding of Page (in press) for a strain of *Vahlkampfia* has already been mentioned.

It seems likely, therefore, that there may be more free-living amoebae which can colonise the vertebrate intestine (possibly by rectal invasion) than have been reported. For marine invertebrates, especially, the possibilities would appear even

Table 2

A Partial List of Amphizoic and Possibly Amphizoic Amoebae

Species	Host	Definitely free-living	References
Naegleria aerobia	man, mouse (CNS, p)	+	Culbertson et al. 1968, Singh and Das 1970, 1972
Acanthamoeba astronyxis	man (CNS, p)	+	Callicott et al. 1968
Flabellula calkinsi	oyster	+	Hogue 1914, Page 1971a
Acanthamoeba castellanii	mouse, guinea pig (CNS, p)	+	Červa 1967
Acanthamoeba culbertsoni	man, mouse (CNS, p)	+	Singh and Das 1970, 1972
Sappinia diploidea	elk, bison (ent)	+	Noble 1958
Acanthamoeba gallopavonis	turkey (ent)		Walker 1908
Naegleria fowleri	man (CNS, p)	+	Carter 1970, Anderson and Jamieson 1972, Willaert et al. 1973
Naegleria gruberi	man (ent)	+	Schardinger 1899
Acanthamoeba hyalina	*Blaberus craniifer*		Briscoe 1971
Acanthamoeba intestinalis	horse, pig, cat, turkey (ent)		Walker 1908
Rosculus ithacus	*Natrix natrix* (ent)	+	Hawes 1963
Vahlkampfia lacertae	*Lacerta agilis, L. muralis* (ent)		Hartmann and Prowazek 1907, Nägler 1909, Dobell 1914
Amoeba lobospinosa	man (ent, liver, p?)		Craig 1912
Entamoeba moshkovskii	not known	+	Neal 1953, Bovee and Wilson 1963
Amoeba (*Vahlkampfia*?) *ovis*	sheep (ent)	+	Schmidt 1913
Acanthamoeba polyphaga	crane (ent)	+	Kocan and Page, unpublished
Saccamoeba renacuajo	*Bufo* (ent)		Frenzel 1892
Amoeba reticularis	man (ent)	+	Celli and Fiocca 1894
Tetramitus rostratus	man (ent, urine)	+	Fulton 1970
Acanthamoeba rhysodes	mouse (CNS, p)	+	Singh and Das 1972
Amoeba spinosa	man, guinea pig, frog (ent)	+	Celli and Fiocca 1894
Hartmannella tahitiensis	*Crassostrea commercialis* (p?)		Cheng 1970
Hartmannella vermiformis?	crane (ent)	+	Kocan and Page, unpublished
Acanthamoeba sp.	bull (lung, p)	+	McConnell et al. 1968
Acanthamoeba sp.	crane (ent)		Kocan and Page, unpublished
Platyamoeba sp.	crane (ent)		Kocan and Page, unpublished
Vahlkampfia sp.	turkey (ent)		Page, in press
Vahlkampfia spp.	elk, bison, cattle, sheep, moose, marmoset (ent)		Noble 1958
Vahlkampfiid	crane (ent)		Kocan and Page, unpublished
Amoeboid protozoa	oysters (tissues and mantle fluids)		Sawyer 1968

Notes. Abbreviations: CNS — occurring in central nervous system (amoebic meningo-encephalitis); ent — isolated from intestine or faeces, p — pathogenic.

The amoebae from crane faeces were isolated by Dr. Richard M. Kocan and identified by FCP.

greater, both because of the greater similarity between internal and external environments and because of the increased possibility of introduction into the digestive tract or other organs of animals which feed by passing a constant stream of water through their bodies or at least their mantle cavities. The gills of fish are, of course, another possible site which has been investigated by some workers.

The term *amphizoic* is proposed to describe protozoa which not only occur but also feed and multiply well in both the free-living (*exozoic*) and endozoic conditions. Most such protozoa are probably basically free-living, but employment of the term amphizoic expresses a more comprehensive concept than does "facultatively endozoic" in that it allows for any which are only facultatively free-living or, whatever their own habitat, belong to normally endozoic groups (e. g., *Entamoeba moshkovskii*). Furthermore, though the less comprehensive term "facultatively endozoic" is a good one, it does imply that endozoic occurrence is rather infrequent, and careless usage is rather likely to corrupt it into "facultatively parasitic". There may actually be protozoa which, though more numerous in the exozoic habitat, are very common endozoically. Ectozoic commensals or parasites which also occur in the free-living condition are also comprehended under this term.

Although the primary concern of this discussion is with amphizoic amoebae, some other protozoa are amphizoic. In discussing *Tetrahymena*, for example, Corliss (1972) included in his categories both facultatively parasitic and facultatively free-living groups.

In many cases, any given strain or all strains of a given species can probably thrive in both habitats, and that is the intended primary significance of the term. It might not be incorrect to designate as amphizoic a species in which different strains are more tolerant of one habitat than the other, or even amphizoic genera, in which some species are free-living and others endozoic (*Paramoeba*?). However, the term should be used in an expanded sense only when the meaning is unmistakeable.

Since protozoa often described as "coprozoic" or "coprophilic" may overlap with amphizoic protozoa, a caution about use of those terms seems appropriate. These terms, often applied to amoebae isolated from faeces, may be misleading if it is not made clear whether the amoebae originated in the intestine or as contaminants after defecation. If the former is the case and the amoebae were active in the intestine before defecation, then coprozoic amoebae are also endozoic and amphizoic. Furthermore, to some readers the terms coprozoic and coprophilic may wrongly imply faecal contamination of fresh-water habitats in which such protozoa are found. It is likely that such bacterivorous amoebae thrive in any suitable habitat where adequate bacterial food is present and faeces simply offer an especially rich food source.

Amphizoic protozoa will undoubtedly continue to attract increasing interest for the light which they shed on the origin of parasitism, as well as for reasons of practical parasitology.

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Summary

Two strains of small amoebae from fresh water are identified as belonging to the species *Rosculus ithacus* Hawes, 1963, originally described from the grass snake *Natrix natrix*. Their locomotive behaviour, mitotic pattern, and cysts are described. Similarities between the genera *Rosculus* and *Flabellula* are discussed, and the genera are classified in the family *Flabellulidae* Bovee, 1970. *R. ithacus* is an example of the species of amoebae, apparently more numerous than formerly realised, which can thrive in both endozoic and free-living habitats. The term *amphizoic* is proposed for such protozoa, which may be either facultatively endozoic or facultatively free-living.

Zusammenfassung

Zwei Stämme kleiner Süsswasseramöben werden als der Art *Rosculus ithacus* Hawes, 1963, angehörig identifiziert, eine Art, die ursprünglich aus der Schlange *Natrix natrix* beschrieben worden ist. Ihr lokomotorisches Benehmen, ihr Teilungsmodus, und ihre Cysten werden beschrieben. Ähnlichkeiten zwischen den Gattungen *Rosculus* und *Flabellula* werden erörtert, und diese Gattungen werden in die Familie *Flabellulidae* Bovee, 1970 , eingeordnet. *R. ithacus* ist ein Beispiel jener Amöbenarten, wahrscheinlich zahlreicher als früher erkannt, die in den beiden Lagen, der endozoischen und der freilebenden, gedeihen können. Der Ausdruck "amphizoisch" wird für solche Protozoen vorgeschlagen, die entweder fakultativ endozoisch oder fakultativ freilebend sein können.

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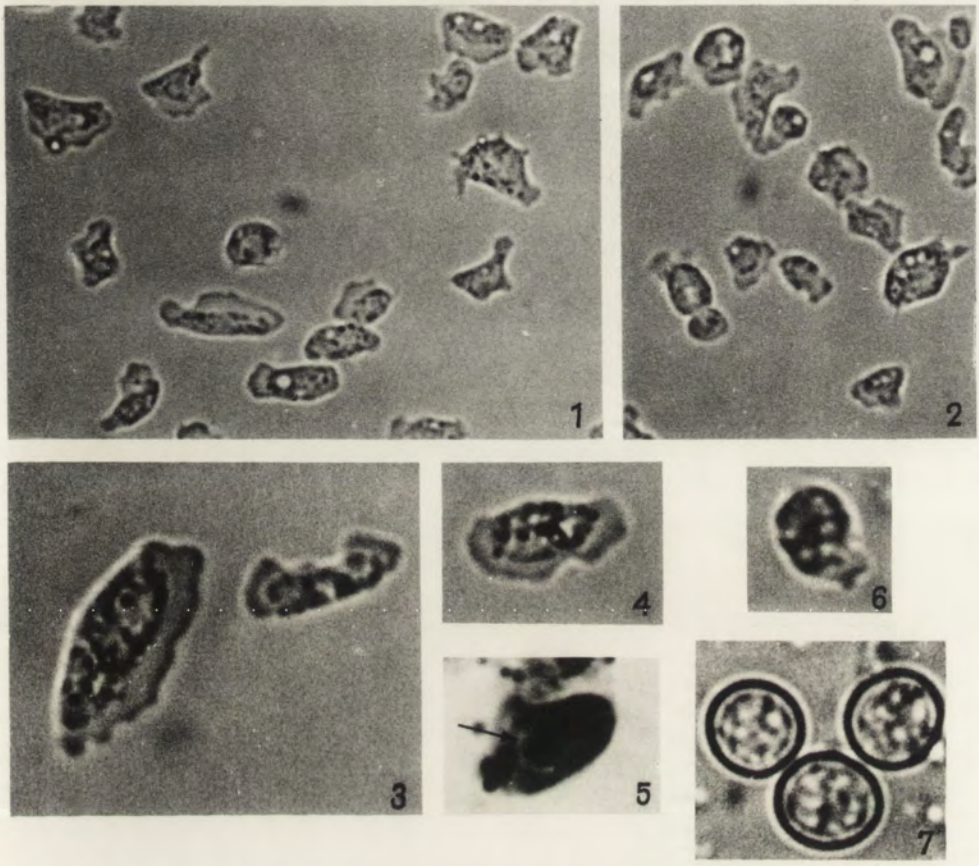
EXPLANATION OF PLATES I–II

1–4: Living locomotive forms of *Rosculus ithacus* (1 and 2, 2000×; 3 and 4, 3200×)
5: Amoeba which has ingested another (arrow), haematoxylin (5000×)
6: Floating form (3200×)
7: Cysts (4000×)
8–10: Amoebae with interphase nuclei (arrows), haematoxylin (8000×)
11: Probable prophase nucleus, haematoxylin (8000×)
12: Prophase, haematoxylin (8000×)
13–16: Division stages, Feulgen-fast green (8000×)



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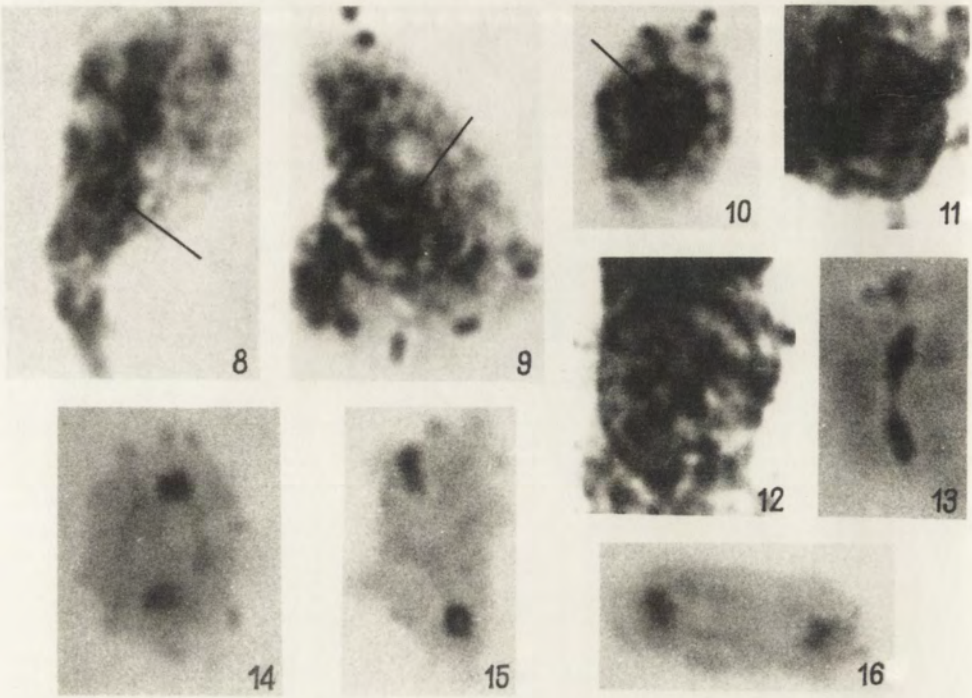

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New Features of the Tetrahymenid Cortex Revealed by Protargol Staining

Caractéristiques nouvelles du cortex tetrahyménien révélées par coloration au protargol

The cortex of *Tetrahymena* and related genera has been extensively studied by a variety of techniques for taxonomic purposes (much of this work has been reviewed by Corliss 1970 and 1971). Using a modified protargol technique (essentially that of Jerka-Dziadosz and Frankel 1969) we have found interesting features which seem to have escaped earlier investigators. These features can be seen in several published micrographs, as noted below, but they have not been mentioned in the papers they illustrate. We believe they are of taxonomic importance at several levels.

Methods

Briefly, the protargol method involves the following operations: Cells from axenic peptone or monoxenic cerophyl are concentrated by centrifugation and fixed in Bouin's fluid or a sublimate mixture. The fixative is washed out thoroughly and the cells are transferred to a drop of albumin-glycerol (proportions of egg white and glycerol may vary) which is then deposited by means of a fine micropipette on a coverslip which has been smeared with albumin and allowed to dry. The cells must be laid down in a layer of the proper thickness, such that the cells will be visible as raised bumps when the smear dries. When the smear has dried sufficiently, it is plunged into 70% ethanol, where it should remain for at least 10 min. The smear is then run down to water and next into 0.1% $KMnO_4$ until a strong golden-brown color develops, about 2 min, depending on the albumin. Next the smear is washed in water and soaked in 5% oxalic acid until the brown color disappears. The smear is washed thoroughly in water and placed in fresh 1% protargol at 45–65°C until the protargol solution turns milky, about 0.5 h. No "catalyst" is used. The smear is washed and developed as usual, then rinsed and placed in 5% sodium thiosulfate, after which it is dehydrated and mounted in a neutral resin. Cells which are properly stained do not require toning. Success depends largely on thorough washing and above all on the quality of the albumin, which should be the thin albumin from a very fresh egg.

Strains used in this study include various wild, domestic, amicronucleate, or micronucleate strains of *Tetrahymena pyriformis*, wild strains of *T. setifera*, *Glaucoma* spp., *Colpidium* spp., *T. rostrata*, strain L-FF, *T. patula*, and strain V_2, *T. vorax*. Axenic cultures of species other than *T. py-*

riformis were grown in a skim milk medium: 1% skim milk powder, 0.5% tryptone, 0.25% yeast extract, neutralized with NaOH before autoclaving.

Zeiss optics and Polaroid "type 55" film were used. Optimal resolution was obtained by filling the space between the slide and the front lens of the condenser with immersion oil.

Results

Our Pl. I 1 demonstrates that the anterior ends of most kineties (the first "parag tene", Ehret 1967) consist of double kinetosomes, the posterior one only beine ciliated. This feature is found in all *Tetrahymena* and *Colpidium* strains we havs examined (upwards of 150 separate strains) but in none of the *Glaucoma* strain- (more than 20 were examined). The double kinetosomes are also visible in a number of published micrographs, notably in Fig. 6 of Pitelka 1961. The excellent electron micrographs of Peck 1971, including some sagittal sections of the anterior pole of *G. chattoni*, confirm our observation that the structure is absent in *Glaucoma*.

We believe this feature constitutes a characteristic of the family *Tetrahymenidae*, in conjunction with the characteristics of the oral apparatus, and may aid in the correct placement of the genera recently transferred to the *Glaucomidae* by Corliss 1971. Most of these genera have not been studied rigorously. Unfortunately the region in question is not visible in the illustrations of Iftode et al. 1969 (*Turaniella vitrea*).

The double kinetosomes are visible with difficulty in Chatton-Lwoff preparations. They arise in the opisthe at the time of cytokinesis, and are found at the tips of kineties *5* to *n-2*, approximately.

Plate I 2 demonstrates a feature of stomatogenesis in *T. pyriformis* which has not been previously noted. Although Frankel 1964 remarks on the frequent occurrence of quartets of basal bodies in the assembly of the opisthe membranelles, most of our preparations show doublet patterns. Each membranelle is formed through the concatenation of these doublets, which may sometimes first assemble into quartets as they are integrated into the larger structure. Doublets are formed even among the "supernumerary" kinetosomes behind the third membranelle.

Of special interest is the fact that the third membranelle consists initially of approximately 13 doublets (seldom fewer and never more were found in our material, among third membranelle anlagen which were judged "complete"). The anlage of this membranelle is thus considerably longer than the mature structure (see below). Therefore a reduction must occur during development. If kinetosomes can be "reused", some of the extra length could ultimately provide the "third row" of the membranelle, although we believe instead that the "third row" arises by neoformation just as in the other two membranelles. In fact, cells such as those in Pl. I 3 and 4 seem to indicate that at least part of the reduction occurs after the third row of basal bodies is laid down. The mature membranelle consists of about 17 kinetosomes,

and most of the reduction involves the two rows which are deposited first. Both of the other membranelles may be reduced during development, but to a much lesser extent.

We would suggest from this finding that it may not be reasonable to attempt the theoretical derivation of other hymenostomes from *T. pyriformis* solely on the basis of the relative and subjectively estimated "complexity" of the mature buccal apparatus, when that structure results from a "simplification" or a reduction of a larger anlage. In fact, such derivations have been made without a complete understanding of some of the relevant details of the mature membranelles. From the protargol material it is possible to specify rather exactly the position of each kinetosome of the membranelles, although not all details will normally be seen in a single specimen. Details of the various species we have examined will be documented at a later date, but for the present it will suffice to indicate in a schematic (Fig. 1) the general

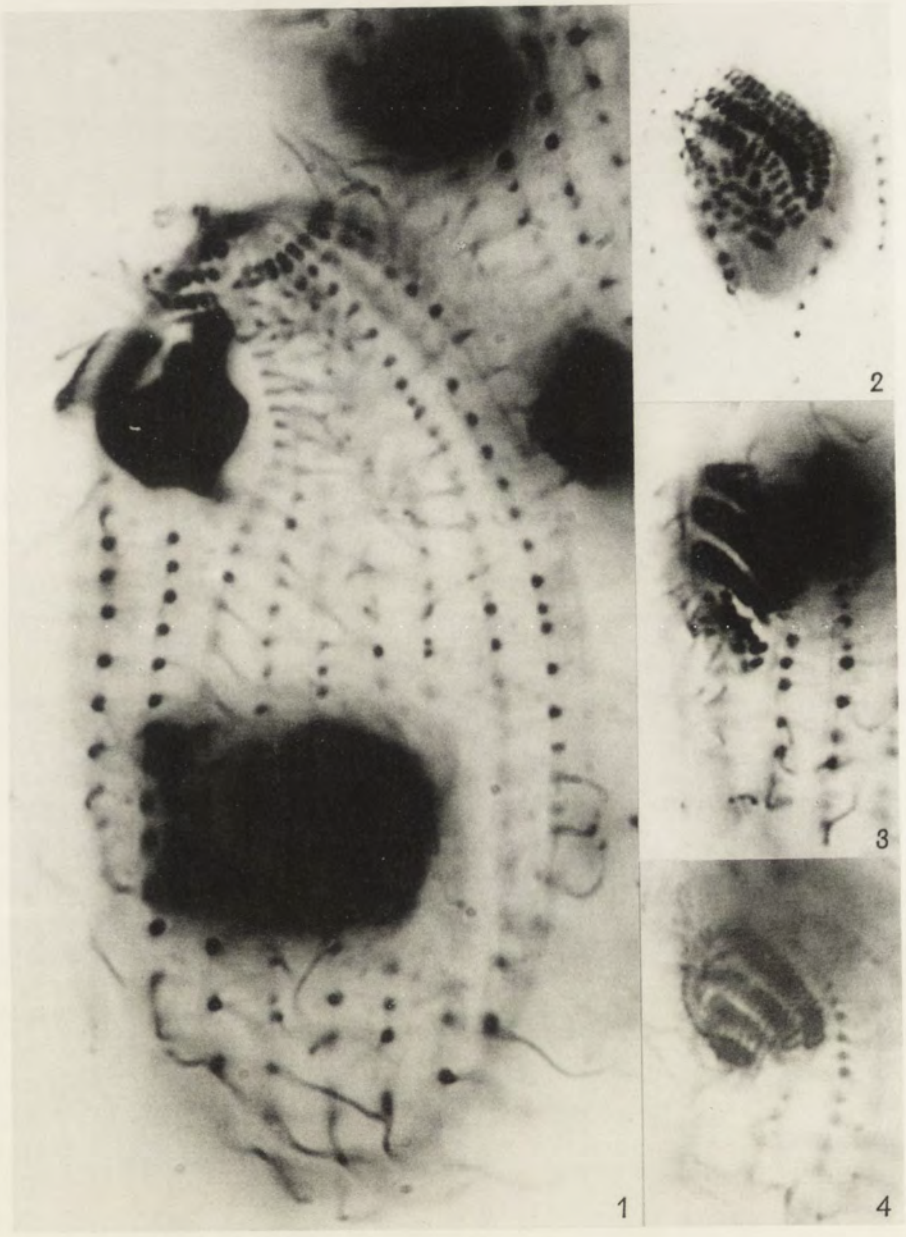
Fig. 1. Placement of basal bodies in the oral apparatus of *Tetrahymena pyriformis*. The positions indicated by open circles are variable between strains but usually not within strains

features of the detailed placement of the kinetosomes for *T. pyriformis*. This schematic is to be compared with the published electron micrographs (e.g., Plates II and V of Nilsson and Williams 1966 and Fig. 4 of Wolfe 1970) and contrasted with the other published drawings based on these same micrographs (as in Fig. 1 of Nilsson and Williams 1966). The curious irregularities seen in our Fig. 1 are perpetuated with great precision within individual strains and under a variety of culture conditions, although species may vary especially in the presence of basal bodies which connect the tips of the second and third membranelles (open circles in Fig. 1).

EXPLANATION OF PLATE I

Tetrahymena pyriformis, syngen 2 mt III, from axenic peptone, stained with protargol. 2500×

1: Crown of double kinetosomes on the anterior pole of the cell. Microtubular systems are visible behind the mouth
2: Morphogenesis of the opisthe membranelles. M1 consists of 19 files of basal bodies, some of them triple; M2 has 15 double files; M3 has 13 doublets
3: Completed membranellar anlagen. M3 has about 13 files of 3 kinetosomes each. The microtubular systems are visible
4: Slightly later stage in stomagenesis. The UM is almost entirely formed. M3 has about 10 triple files of kinetosomes



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Updating the Tetrahymenids. I. General Considerations and Application to the Genus *Colpidium*

Modernisant les Tetrahyméniens
I. Questions générales et application au genre *Colpidium*

Species Concepts and the Tetrahymenids

Corliss 1972 affects extreme confusion on the meaning and limits of the category, "species". Perhaps different strains of *Tetrahymena rostrata* are actually distinct species or perhaps *T. setifera* is only a variety of *T. pyriformis*. The resolution of this indecision depends mainly on what standards one sets in constituting a "species", for the information needed to resolve the issue in the tetrahymenids has been increasing at a rapid rate.

If "species" refers only to some arbitrary rubric which will allow curators to pin neat labels on every specimen, we provide undeserved comfort to those who, having identified a representative of such a "species", pretend they understand it. In point of fact, much of the physiological data on "*T. pyriformis*" applies only to the particular strains used for the experiments, because of the great quantitative and qualitative differences which separate the diverse forms so named (see Loefer et al. 1958, Holz et al. 1959, Borden et al. 1973 a, b, Nyberg 1971). Clearly "*T. pyriformis*" is not homogeneous. It consists of a large number of mutually exclusive micronucleate forms, of which a large number are still to be described, and a variety of amicronucleate strains which exhibit differences at least as great as those separating the sexual strains, with the exception that they lack mating types. We have recently demonstrated that amicronucleate strains of nearly identical phenotype can be isolated from widely separated locales (manuscript in preparation). Thus, amicronucleates are not only isolated relict populations. Further, there are evident phenotypic homologies among the described breeding groups, and no clear connection, by the same criteria, of these strains with the amicronucleate phenotypes presumably derived from various unknown micronucleate "*T. pyriformis*" (Borden et al. 1973 a, b).

Some have despaired of applying the "biological species concept" to ciliates

because of the difficulty in inducing conjugation for many forms. Others have made significant use of the biological species concept in settling on useful taxonomic criteria (Carter 1972) even when conjugation is difficult to control. And even the strongest critics of the species concept (e.g., Sokal and Crovello 1970) agree that the yardstick which should be applied is an empirical one, derived by consideration of those organisms for which the biological test of speciation can be applied in some degree. That is, differences of the magnitude separating known biological species should be used as a guide in erecting species designations for closely related but less thoroughly studied organisms. For organisms which are incapable of sexuality, the same yardstick may be conveniently applied, providing that a similar degree of discontinuity can be demonstrated. Taxonomy seeks natural discontinuities as a basis for classification.

The applicability of the biological (as opposed to the traditional arbitrary) species concept to "*T. pyriformis*" has recently been demonstrated by Borden et al. 1974. The distinct phenotypes discovered in that study have proven invaluable in assigning wild strains to breeding groups before mating behavior has been tested and often before the strain has matured. In other cases, some or all of the known breeding groups can be immediately eliminated, and unsuspected similarities may emerge between sets of unidentified strains. "*T. pyriformis*" is not a continuous spectrum, and, where most thoroughly studied, it can be decomposed into discrete objective species which are of wide distribution.

The studies of Nanney have demonstrated that all known cortical parameters do vary within breeding groups, and to such an extent that they are not well suited for taxonomic purposes when used alone (Nanney 1967). Further, the great stability of the cortex in *Tetrahymena* may make the analysis of individual clones misleading (Nanney 1966 a, 1966 b, 1968). However, a unique constellation of enzymatic phenotypes characterizes each of the known breeding groups in the *T. pyriformis* complex. Even strains which have novel variant alleles can be correctly assigned, when enough enzyme systems are examined and when zymograms are prepared under properly standardized conditions.

Much of this information has been available for many years. But it has never been incorporated into the taxonomic practice of those who have attempted to classify *Tetrahymena* and related genera. We do not advocate a complete abandonment of the current taxonomic structure, no matter how precarious, but we do want to initiate a reexamination of certain points which have been neglected or even consciously ignored where convenience or "tradition" was invoked, as if the promulgation of the traditions we ourselves have created should take precedence over evidence. Stability is desirable only when what is stabilized is correct to begin with. Otherwise it is merely stagnation. Reiteration creates dogma, not truth.

Some of the opinions we would examine were once the best possible, considering the available evidence. The discrepancy between experimental results and taxonomy which now confronts us is not to be "blamed" on anyone — it results from the joint

failure of experimental and taxonomic interests to communicate to each other the implications of their findings. The view that taxonomy is a "necessary evil" has been widespread among experimentalists to such an extent that comparative issues of some importance have been neglected while detailed responses of unknown or incorrectly identified organisms were studied instead (see the laconic warning of George 1973). Ultimately, all progress in biology depends on taxonomy. It is our principal assurance of continuity from one generation of scientists to the next and our only assurance that the organism studied today pertains to the one studied yesterday. It is always important to know in what organism a described phenomenon has occurred — otherwise there is little possiblity of linking this observation to the body of knowledge known as "biology". There are great heaps of such observations which lack labels. They are puzzle pieces without a place, and they are ultimately cast off to be replaced by new ones more adequately equipped.

The *Colpidium* Problem

The genus *Colpidium* has been afflicted by a surprising amount of indifference. In spite of the large number of names available for the very common strains which Jankowski 1967 calls "*C. campylum*", a satisfactory synonymy has not been published, and the only comparative data available seem to be the few sentences of Corliss' abstract of 1953, which has been quoted without elaboration from time to time (Czapik 1968). As this abstract predates the most important studies on the genetics and biology of the tetrahymenids, especially those pertaining to the stability of the cortex, it is necessary to reexamine the utility of the criteria originally offered by Corliss. In addition, problems relating to the original species descriptions have not been considered thoroughly.

The species of *Colpidium* which is (or are) most problematical would be *C. campylum* and the associated names of species, *C. truncatum*, *C. striatum*, and *C. colpidium*, which may or may not be synonymous. Over the past several years we have been collecting strains of the "*C. campylum*" group. Six of these have been domesticated in axenic media and are described in some detail below. Strains of other species and from other sources have been examined and will be mentioned as appropriate. We have attempted to assess the taxonomic utility of morphological and biochemical characteristics in these strains, with special attention to interclonal variation. Other papers in this series will deal with similar problems in related ciliates.

Materials and Methods

The strains used for this investigation (Table 1) have been collected over a period of several years and maintained in bacterized cerophyl medium at 15°C. Strains resembling *C. campylum* have been easily established in axenic medium by washing single cells through antibiotic peptone

Table 1

Strains of *Colpidium* Used in This Investigation

Strain Designation	Date	Location	Present Condition	Species
Snoqualmie R.	July 1970	Snoqualmie River, King County, Washington	extinct	*C. campylum*
UI-7148i	Sept. 1971	"Hidden Valley", Rocky Mountain National Park, Colorado, elev. 2000 m	peptone	*C. campylum*
UI-7196	Sept. 1971	Crystal Lake Park, Urbana, Illinois	peptone	*C. campylum*
UI-7205f	June 1972	Snowmelt stream, Mount Seymour Provincial Park, British Columbia, Canada, elev. 1300 m	peptone	*C. campylum*
UI-7212e	June 1972	Seattle, Washington	peptone	*C. campylum*
UI-7213a	June 1972	Isolates from one collection, Seattle, Washington	peptone	*C. campylum*
UI-7213e	June 1972		peptone	*C. campylum*
OS-1	about 1963	University of Washington, Seattle, Washington	cerophyl	*C. kleini*

(Phillips 1967) and then putting the washed cells into skim milk peptone (1% skim milk powder, 0.5% tryptone, 0.25% yeast extract) after 24 h. The cells will not divide in the presence of high concentrations of antibiotics. We have not been able to grow *C. colpoda* or *C. kleini* (Foissner 1969, 1970) in axenic culture.

Electrophoretic methods generally follow Shaw and Prasad 1970, with slight modifications as described by Borden et al. 1973 b, and available from us by request. Cytological staining methods have been described in an earlier paper (McCoy 1974 b).

Results

Electrophoretic Analysis of "*C. campylum*" Strains

Ten enzyme systems (Table 2) were well resolved by the starch gel electrophoresis method and suitable for interspecific comparisons. Enzyme systems not known to vary between species do not constitute a reasonable test of specific identity. All axenic *C. campylum* strains were electrophoresed on the same set of starch gels. A sample of inbred strain B, syngen 1, *T. pyriformis* was included on each gel. The *C. campylum* strains were identical for soluble forms of malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH1), two acid phosphatase bands (P-2), lactate dehydrogenase (LDH), and a butyryl esterase (E-2). At least one variant was found for tetrazolium oxidase (TO), mitochondrial isocitrate dehydrogenase (IDH2), hydroxybutyrate dehydrogenase (HBDH) one set of acid phosphatase bands (P-1), and one set of esterase bands (E-1) which were slightly activated by taurocholate and active on butyryl and proprionyl esters. Multiple variants were found for the esterases,

Table 2

Relative Mobilities of Enzyme Systems of *Colpidium campylum* as Measured by Starch Gel Electrophoresis

Strain	7196	7148i	7212e	7213a	7213e	7205f	
Source	Illinois	Colorado	Washington	Washington	Washington	British Columbia	
Enzyme System:							Reference Standard: *T. pyriformis*, syngen 1 (mobility in cm)
TO	0.74*	0.84	0.74*	0.74*	0.74*	0.74*	9.0
	0.61		0.61	0.61	0.61	0.61	
	0.29		0.29	0.29	0.29	0.29	
	0.16		0.16	0.16	0.16	0.16	
MDH (soluble)	0.67	0.67	0.67	0.67	0.67	0.67	1.5 (soluble form)
IDH1 (soluble)	0.75	0.75	0.75	0.75	0.75	0.75	10.0 (soluble form)
IDH2 (mitochondrial)	0.44	0.44	0.40	0.44	0.44	0.44	10.0 (soluble form)
HBDH	0.75	0.93	1.15	0.93	0.75	0.93	1.5
P-1	1.27	1.05	1.05	1.27	1.05	1.27	9.0 (fastest-moving well focused band)
	0.97			0.97		0.97	
P-2	0.67	0.67	0.67	0.67	0.67	0.67	9.0 (fastest-moving well focused band)
	0.83	0.83	0.83	0.83	0.83	0.83	
LDH	—2.22	—2.22	—2.22	—2.22	—2.22	—2.22	3.5
E-1	2.97*	2.56*	2.56*	2.92*	2.97*	2.92*	5.5 (relative to "E-3", common to all tetrahymenids)
	3.18	2.87	2.87	2.66	3.18	2.66	
		2.20	2.20				
			2.00*				
E-2	4.50	4.50	4.50	4.50	4.50	4.50	2.7 ($E\text{-}2^B$)

Mobilities are calculated relative to the fastest-moving band in the syngen 1 sample which was included on each starch gel.

* Principal band.

which tend to be hypervariable in species of the *T. pyriformis* complex. The non-specific esterase "E-3" which is of identical mobility in all *Tetrahymena* strains we have examined is identical also in "*C. campylum*", and was therefore excluded form the analysis.

Table 3

Difference Matrix for *C. campylum* Strains
Fraction of Enzyme Systems Differing between Strains

	7148i	7205f	7196	7212e	7213a	7213e
7148i	—	0.3	0.4	0.4	0.3	0.4
7205f		—	0.2	0.4	0.0	0.3
7196			—	0.4	0.2	0.1
7212e				—	0.4	0.3
7213a					—	0.3
7213e						—

A summary of pairwise comparisons is given in Table 3. It is seen that every strain shares at least 70% of its enzyme systems with one other strain, and no pair of strains differs by more than 40% if the enzyme systems. These samples are much more coherent than similar assortments of *T. setifera*, for instance (manuscript in preparation). Variation within syngens of *T. pyriformis* is sometimes of this magnitude (Borden et al. 1974 a). In addition, the high level of polymorphism of the esterases (4 distinguishable phenotypes in 6 strains) leads to an inflated estimate of interstrain divergence. In summary, the zymographic data offer no indication of species-level differences between the axenic strains.

Cortical Analysis of *C. campylum* Strains

Tables 4 and 5 summarize results from mass cultures, some of them repeated after an interval of several months. No two strains were identical. Moreover, several of the strains do not conform to any of the species diagnoses of Corliss 1953. Our observations extend the corticotypic range of "*C. campylum*"-like forms to 16–30. The placement of the contractile vacuole pore (CVP) varies slightly and the CVP may also be double, occurring on kineties 4 and 5 or 5 and 6. The frequency of CVP positions other than kinety 5 is usually well below 5%, and it would be necessary to count enormous numbers of cells to establish significant interstrain differences.

If the *Colpidium* cortex were extremely stable, accidental variations might be perpetuated over long periods of time and thus be mistaken for species-level differences. That is, genetically identical strains with epigenetic differences might appear distinct on a semi-permanent basis because of a high cortical and physiological inertia. To investigate this possibility we chose the method of subclonal analysis

Table 4

Summary of Cortical Data for *C. campylum* Strains

Strain Source	7196 Illinois	7148i Colorado	7212e Washington	7213a Washington	7213e Washington	7205f British Columbia	Snoqualmie R. Washington
Corticotype:							
Upper limit	24	20	23	28(30)	23	24	30
Lower limit	21	16	18	22	17	23	27
Mode	23	18	21–22	26	20–21	(24)*	28

* 20 fissions after clonal isolation in skim milk peptone.

Table 5

Distribution of Corticotypes in *C. campylum* Strains

Strain	Corticotype															N	Medium
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30		
7148i	0.19	0.24	0.42	0.14	0.01											74	C
7196						0.01	0.10	0.82	0.07							84	P
7205f								0.40	0.60							40	P
7212e						0.04	0.89	0.07								47	P
			0.01	0.04	0.16	0.44	0.35									74	C
7213a							0.02		0.02	0.14	0.75	0.03	0.02		0.02	62	P
							0.014	0.027	0.041	0.236	0.615	0.061	0.007			148	C
							0.01	0.01	0.08	0.23	0.59	0.08				111	C
7213e						0.03	0.73	0.21		0.03						29	P
		0.007	0.054	0.122	0.358	0.318	0.128	0.013								148	C
		0.007	0.034	0.196	0.277	0.358	0.128									148	C
Snoqual-mie R.												0.05	0.54	0.38	0.03	39	C

Media: C = cerophyl, P = skim milk peptone;

(Nanney 1966 a, b, 1968) for strains UI-7213a and UI-7213e, which were isolated from the same collection but which were distinguished by their enzymatic mobilities as well as their cortical characteristics.

For each of these two strains a mass culture in 50 ml of cerophyl was started with a 0.1 ml inoculum from a stationary phase skim milk peptone culture. At the same time, single cells were isolated into individual depressions of cerophyl to establish a number of subclones of the population in the peptone tube. These cells grew vigorously and were used to inoculate separate 50 ml flasks of cerophyl. Flask cultures were harvested at the end of log phase and in some cases a second flask was started with several drops from the first culture. Subclonal cultures are calculated to represent the 20-fission progeny of single cells, and the mass cultures should be about 10 fissions removed from the peptone tube. Second generation flask cultures represent about 26 fissions from the peptone tube for subclones and about 16 fissions for the mass cultures.

The results of the subclonal analysis are given in Table 6. It is seen that strain UI-7213a was unchanged between 10 and 16 fissions in mass culture. This strain is therefore approximately at equilibrium. Neither is there a noticeable change relative to cells directly from peptone (Table 5). The subclones show high uniformity and fall into two major classes. Subclones 1–8 contain almost entirely cells of corticotypes 25 and 26. In each case corticotype 26 predominates. It is clear that most of these subclones are derived from cells of corticotype 26, which is the modal type in the mass culture. Subclones 9-12 seem to have arisen from cells of low corticotypes, and show much higher variances than subclones 1–8. Clones with high variance thus correspond to the low corticotypes which have a high probability of gaining a kinety. Cells of low corticotype arise infrequently from cells of high corticotype and must frequently regain a row to give the observed population structure. The individual subclones are comparable in stability with clones of *T. pyriformis* (Nanney 1968).

Strain UI-7213e shows another pattern. Cells from peptone cultures were initially clustered around corticotype 22. Several months later, when the subclonal analysis was done, the first mass culture contained mainly corticotypes 20 and 21. However, the corresponding subclones are remarkably deficient in corticotype 20. Further, the second mass culture (approximately 16 fissions out of peptone) also shows a relative decrease in the frequency of corticotype 20, and a corresponding gain by corticotypes 19 and 21. It follows that corticotype 20 tends to accumulate in peptone but is not stable and is not replaced in cerophyl. The subclones which have the highest frequencies of this corticotype also have the highest variances. However, the distribution of corticotypic classes in the other subclones suggests that the population may drift toward corticotype 20 in spite of its instability. Yet most of the subclones show fairly low variances, and again the cortex seems in general about as stable as that of *T. pyriformis*. High stability means that individual clones should be allowed to undergo a very large number of fissions before any

Table 6

Distribution of Corticotypes in Subclones of Known Age

Age in Fissions		Corticotype												N	$\hat{\sigma}^2$
		17	18	19	20	21	22	23	24	25	26	27	28		
Strain 7213a															
Mass Culture:															
	10						0.014	0.027	0.041	0.236	0.615	0.061	0.007	148	0.808
	16						0.01	0.01	0.08	0.23	0.59	0.08		111	0.728
Subclones:															
1	20									0.03	0.97			37	0.0270
2	20									0.04	0.96			74	0.0394
3	20									0.07	0.93			74	0.0639
4	20									0.07	0.93			74	0.0639
5	20									0.09	0.91			47	0.0796
6	20									0.16	0.84			37	0.140
7	20									0.18	0.81	0.01		74	0.165
8	20									0.22	0.78			74	0.172
9	20								0.03	0.69	0.28			74	0.248
10	20							0.03	0.19	0.77	0.01			74	0.262
11	20							0.07	0.26	0.66	0.01			74	0.403
12	20						0.02	0.15	0.78	0.05				74	0.245
Strain 7213e															
Mass Culture:															
	10	0.007	0.054	0.122	0.358	0.318	0.128	0.013						148	1.227
	16	0.007	0.034	0.196	0.277	0.358	0.128							148	1.162
Subclones:															
1	20		0.15	0.85										41	0.128
2	20			0.97	0.03									74	0.0270
2	26		0.07	0.90	0.03									74	0.0942
3	20			0.58	0.39	0.03								74	0.305
4	20			0.01	0.38	0.61								74	0.271
5	20			0.145	0.496	0.333	0.026							117	0.529
6	20				0.08	0.92								74	0.076
7	20				0.01	0.99								74	0.014
8	20				0.01	0.96	0.03							83	0.036
9	20				0.04	0.92	0.04							51	0.080
10	20				0.04	0.93	0.03							74	0.068
11	20				0.11	0.85	0.04							76	0.142
12	20			0.03	0.07	0.89	0.01							74	0.180
13	20				0.07	0.85	0.08							74	0.150
14	20				0.02	0.24	0.74							74	0.227

judgement of the natural corticotypic range of the strain is made. And the observed ranges for these strains do not seem to be correlated with similar affinity measures based on the zymographic data. Thus there is no indication here, either, that the strains we have examined represent separable species. Instead, our results are compatible with the existence of genetic and epigenetic variability in the strains examined. Variation of this magnitude is known in several of the biological species of the *T. pyriformis* complex, and the ranges of cortical parameters for these species overlap (Nanney 1967).

We have also tried to estimate length, width, macronuclear position, and CVP position in these strains, and have found no repeatable distinguishing characteristics. The CVP may vary from 1/6 of body length from the posterior pole to a nearly equatorial position. It seems that the CVP is found approximately at a fixed distance from the posterior end, regardless of cell length. The same is true for macronuclear position: in very small (starved) cells the nucleus is found in the anterior third of the cell, next to the mouth. Cell length and width depend largely on the past and present nutritional history of the culture. Cells which have been starved for several weeks are short (down to 30 μm) and become very fat upon refeeding. Well fed cells may be up to 65 μm, and retain their length (but not their width) for several days after the food supply is exhausted.

Buccal Apparatus

Plate I 1 and 2 illustrate the detailed structure of the membranelles for "*C. campylum*". The peculiar structure resembling the constellation Ursa major, at the tip of M3, is like the corresponding structure in the genus *Tetrahymena* (Wolfe 1970, McCoy 1974 b). However, M3 is very much longer in *C. campylum* than in *T. pyriformis*, consisting of around 30 basal bodies. M2 is slightly larger than in *T. pyriformis* and its tip is arranged differently. M1 consists initially of about 21 double files of basal bodies, but later is reduced to a single row with the exception of the tip, which forms a closely packed brush of cilia.

The oral apparatus of *C. kleini* (Pl. I 3) differs from that of *C. campylum* in the size of M3, which is much more extensive, although its tip is again modified as in the other tetrahymenids, and also in the width of M1, which is double instead of single, again with a thickened tip. Plate I 4 shows a tract of fibrils associated with M3 which may be mistaken for an extra row of basal bodies. Because of the geometry of the buccal cavity, these fibrils are seen end-on when M3 is in focus. Similar fibrils may be seen in Pl. I 2 (*C. campylum*). The brief description of the buccal apparatus of *C. colpoda* given by Iftode et al. 1969 may be taken as an indication that *C. colpoda* is quite similar to *C. kleini* in this respect, although it is not possible to determine from their figures if M1 is double or triple.

Conjugation

Even the wizard Maupas admitted the induction of conjugation was difficult to control in his "*C. truncatum*" (1889). Our strains have been refractory with one exception (although we have not tested all combinations of stocks nor all plausible conditions for mating). Collection UI-7196 provided a variety of *Colpidium* and *Glaucoma* strains, one of which survives as an axenic culture. Some of the original isolates were mixed after exhausting their food supply (cerophyl), and out of thousands of cells, two pairs were found and isolated into cerophyl. These grew rapidly, and subclones were initiated in cerophyl. The sublines did not self, but a few pairs formed when all sublines were pooled. This time 11 pairs were recovered, but all died within 24 h. It is presumed that these facts are due to the occurrence of something like "genomic exclusion" (Allen 1967). And it is clear that *C. campylum* has mating types. Conjugation has been reported more frequently for *C. colpoda* and *C. kleini*, but we have not yet encountered conjugating strains.

Discussion

Taxonomy of *Colpidium*

Natural variability is not the only problem to be encountered when working with strains of *Colpidium*. A comparison of the information given by Kahl 1930–1935, Corliss 1953, Jankowski 1967, and Foissner 1969, 1970 can only lead to bewilderment until a systematic comparison of their data is made. An early attempt of this nature was undertaken by Furgason 1936 in his voluminous doctoral dissertation, but he had not then encountered the small Colpidiums, and he considered their illustrations (including the excellent figures of Gelei and Horváth 1931) to be pictures of *Tetrahymena*. This conclusion was perhaps inevitable until the "wet" silver methods came into general use. Furgason's survey of the literature on *Colpidium* begins in 1776, but it is not until 1885 that more species are added to the genus, by Stokes 1885, 1886.

The only differentia offered by Stokes which could serve to separate his *C. truncatum*, 1885, *C. striatum*, 1886, and *Tillina campyla*, 1886 (later to become *C. campylum*) are size and proportions. All subsequent descriptions have respected these measurements more or less, except for Corliss' *C. striatum* (1953) which is 75–95 μm in length, while the original was only 50 μm. From the rest of Corliss' description, it is clear that his *C. striatum* is Foissner's *C. kleini*, 1969 and that the latter name is the valid one. Foissner 1969 mentions the possibility of synonymy.

Corliss bases his identification of *C. truncatum* on the usage of Maupas 1889 who reports its behavior during conjugation and who offers some simple figures of it. However, Maupas never distinguished between *C. truncatum* and *C. campylum*

or *C. striatum*, apparently because he was unaware of these last two. He gives characters which distinguish *C. colpoda* from *C. truncatum*, but nothing further, in spite of his habitual precision, and in such terms that he is clearly meeting the expected criticism that his two strains of *Colpidium* are really only one species. In fact, the figures of Maupas resemble Stokes' drawing of *T. campyla* more closely than they do *C. truncatum*. Maupas reports his *C. truncatum* conjugates rarely (Corliss 1953 says "readily").

None of these facts actually allows the conclusions that Maupas knew of the three possible names for his organism, or that he had considered all of these names in deciding on "*C. truncatum*". Therefore there is no reason to identify the *C. truncatum* of Maupas with the *C. truncatum* of Stokes even if one admits the validity of the latter. *C. truncatum* was rejected by Bresslau (1922 – Furgason insists the data is 1923, the date printed on the bound volume, instead of 1922, the date found on the title page of this issue), who said of it, "Es ist also wahrscheinlich, daß *C. truncatum* Stokes gar nicht auf *Colpidium*, sondern vielmehr auf eine *Colpoda*-Art zu beziehen ist". (pp. 22–23). Bresslau believed that Stokes had described the second *Colpidium* (1886) as *Tillina campyla*. Accordingly, Bresslau changed the form of the specific name to *campylum*, which he thought apt, and which was perfectly legal providing that *T. campyla* were actually a *Colpidium*. Furgason misconstrued Bresslau's reference to the "zweite Colpidienart" to mean the nominal species, *C. truncatum* (which Bresslau considered spurious), instead of the real animal, the small *Colpidium*, and, not surprisingly, found Bresslau's taxonomic procedures very confusing.

Now, Stokes reports transverse fission in *C. truncatum*, eliminating for us both *Colpoda* and *Tillina*. Stokes also mentions a "large, conspicuous vibratile membrane" and shows this structure apparently positioned obliquely on the animal (his Pl. XV), which is from 42 to 52 μm in length. Even with phase-contrast microscopy, we do not consider *Colpidium* to have a "large and conspicuous" membranelle of any description. Furgason, too, noted (1936) that this "large and conspicuous" structure was fairly small in Stokes' drawing. The same "conspicuous" membrane is also mentioned, however, in the 1886 description of *C. striatum* and is similarly figured.

Neither the size range nor other details given for *C. truncatum* allow one to set its limits as to CVP position, number of meridians, etc., which would distinguish it from *C. campylum*, whether or not *C. truncatum* of Maupas is excluded from consideration after our arguments stated above. *C. truncatum* of Corliss is therefore also arbitrary.

C. striatum Stokes, whose size is given as 50 μm, seems to differ from *C. campylum* only in the fattness of the cells, and we consider it synonymous with the latter. But, as *C. striatum* appears to be identical to *C. truncatum* also, both names would fall as junior synonyms of *C. truncatum*. We consider the differentia offered by Stokes

to be absolutely inadequate to establish specific differences among these forms, especially in light of our own comparisons of wild strains which could fit any of his descriptions.

Schewiakoff 1893 gives no differentia except size which would allow the separation of his *Glaucoma colpidium* from the organisms described by Stokes. Further, *G. colpidium* cannot be transferred to the genus *Colpidium* without considering possible synonymy. Kahl attempted this and concluded from the studies of Gelei and Horváth 1931 and Bresslau 1922 that Schewiakoff's *G. colpidium* was a junior synonym of *C. campylum*. (Because of Kahl's statement of synonymy, the correct form of citation is *C. colpidium* Kahl, 1935). In contrast, *C. colpidium* of Corliss 1953 is much larger than Schewiakoff's animal (65–85 μm) compared to 60–67 μm in the original). It may be remembered that Schewiakoff did not recognize any of the small Colpidiums previously described, placing them in synonymy with *C. colpoda*. Therefore he may have simply created his own name for such organisms. It would not be the first time he had redescribed one of Stokes' animals. For instance, *Cyrtolophosis mucicola* Stokes, 1885 is listed by Schewiakoff as a synonym for his own *Balantiophorus minutus* Schewiakoff, 1889 (see McCoy 1974 a).

C. echini Russo, 1914 can be rejected outright and might belong with organisms of similar appearance and habitat in the *Philasterina* (Lynn and Berger 1972). *C. pannonicum* Gelei, 1932 is evidently none other than *Loxocephalus* (*Dexiotrichia*) with the caudal cilium missed. *C. glaucomaeforme* Gelei, 1935 is of course a "*T. pyriformis*".

The only one of these species which is definitely known is *C. campylum* of Bresslau and of Gelei and Horváth. By the law of priority the valid name would be *C. truncatum*. However, a consideration of "common usage" leads us to call for the retention of *C. campylum*, since *C. truncatum* has been mentioned only rarely, and generally only as an uncertain species. Kahl left the problem unresolved, and Bresslau thought the earlier name applied to a different genus. Furgason 1936 comes close to recognizing the synonymy in his doctoral dissertation, but while he at first considered all the small Colpidiums as a species of *Tetrahymena*, which he called *T. campyla*, the synonymy is stated only inferentially, and is not included in the 1940 paper. Since virtually all reviews and textbooks have accepted Bresslau's analysis, we believe "common usage" is properly invoked.

Thus, the present analysis of wild strains given above tends to support the biological conclusions of Bresslau 1922 and Jankowski 1967. Until it can be convincingly demonstrated by repeated collections and detailed analysis (not to be published only as abstracts!) that the small Colpidiums do not form a continuum in terms of morphological or other parameters, they must all be lumped together as *C. campylum*, just as the species complex *Paramecium aurelia* contains many true species (Sonneborn 1957) which can now be distinguished with comparative ease by their zymographic patterns (Tait 1970). When reliable means are presented for

the resolution of *C. campylum* into breeding groups (true biological species), the whole presumed complex might be responsibly subdivided and renamed. In view of the high degree of homogeneity shown by the zymograms, such a subdivision would have to be based only on the biological test of speciation, the breeding structure.

The International Code of Zoological Nomenclature provides for the designation of a published figure as "type", but it would be preferable to select a living strain so as to define the isozymic and physiological aspects of *C. campylum*. We therefore now designate strain UI-7196 as the type strain (neotype), on the provision that another strain does not prove to be more suitable by its credentials during the year following publication of this manuscript, and further propose to carry out the ritual for formalizing a neotype at the expiration of that time. Interested investigators are urged to communicate directly with us in the matter of selecting a possible alternate strain which will be available for future biologists. The designation of a neotype seems essential to prevent future confusion over the identity of *C. campylum* in what may yet prove to be an extremely cryptic species complex. We expect to be able to preserve the neotype strain in liquid nitrogen (Simon 1972 and personal communication).

The two larger forms of this genus have a much less complicated history. *C. colpoda* has been described in some detail, most recently (in passing) by Iftode et al. (1969), whose figures are probably the only published illustrations from protargol-stained specimens of *Colpidium* previous to ours. *C. kleini* Foissner, 1969 is evidently the same as *C. striatum* of Corliss 1953, which, as we have seen, is preoccupied as a synonym of *C. campylum*. The same organism is also found among Jankowski's illustrations of *C. colpoda*. While *C. colpoda* seems to be very distinct from *C. kleini*, some strains we have recently isolated seem to have intermediate numbers of kineties. The possibility of homogeneity or cryptic speciation remains, and could be tested best through the mating behavior.

Jankowski 1967 recommends a subdivision of the genus into two genera, *Colpidium* (including *C. colpoda* and presumably *C. kleini*) and *Dexiostoma* (including *C. campylum* only). We see no practical advantage in this arrangement, and the similarities between the included species are obviously enormously greater than the similarity of any of these species to other genera.

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Summary

Strains of the genus *Colpidium* were examined by starch gel electrophoresis and protargol staining. The results tend to confirm in part the conclusions of Jankowski 1967. While it appear to us that the valid name of the form usually called "*C. campylum* Bresslau, 1922" is *C. truncatum* Stokes, 1885, we recommend the retention of *C. campylum* as a *nomen conservandum*. There is no evidence of multiple species in the *C. campylum* group, and we recognize only three species: *C. colpoda*, *C. kleini*, and *C. campylum*.

RÉSUMÉ

Souches du genre *Colpidium* ont été soumises à l'électrophorèse en gel d'amidon et à la coloration au protargol. Les resultats tendent à confirmer les conclusions de Jankowski 1967. Quoiqu'il nous semble que le nom valide de l'espèce appelé le plus souvent "*C. campylum* Bresslau, 1922" est par contre *C. truncatum* Stokes, 1885, nous recommandons néamoins la conservation de *C. campylum* comme *nomen conservandum*. Il manque d'évidence des espèces multiples dans le groupe "*C. campylum*", et nous ne reconnaissons que trois espèces: *C. colpoda*, *C. kleini*, et *C. campylum*.

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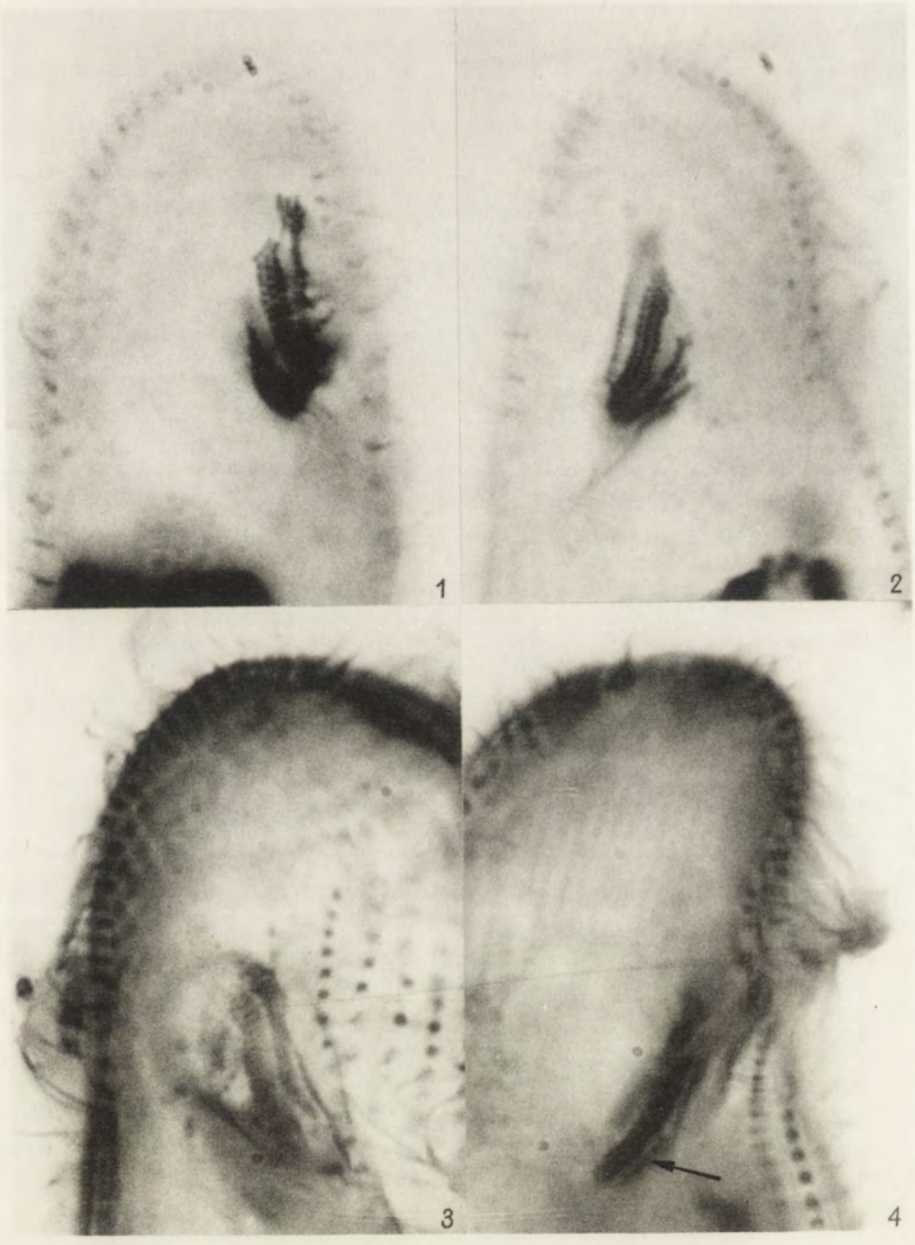
EXPLANATION OF PLATE I

Buccal apparatus of *Colpidium campylum*. Protargol, 2500×. Strain UI-7213e

1: Organization of the first and second membranelles, viewed from the dorsal side
2: Organization of the second and third membranelles, viewed from the ventral side. Fibrils lead from the base of the third membranelle into the cytoplasm

Buccal apparatus of *C. kleini*. Protargol, 2500×. Strain OS-1

3: Organization of the first and second membranelles, dorsal view. M1 consists of two rows of basal bodies over most of its length
4: Posterior and of M3, showing the tract of fibrils originating from the base of the membranelle (arrow)



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Further Studies on *Halteria*

Weitere Studien an *Halteria*

Considerable information has been published on the grosser structures of the oligotrich ciliates *Halteria grandinella* O. F. Müller, 1786 and *Halteria bifurcata* Tamar, 1968. However, many structural questions remain to be solved, especially in regard to the smaller organelles of the buccal cavity and the infraciliature. Also, little has been reported on some aspects of the life cycle, and there appeared to be only incomplete knowledge of the means by which the saltatory movement is performed. The present research was undertaken in the hope of shedding new light on some of these, and perhaps additional, problems.

Materials and Methods

H. bifurcata were obtained in water samples from the bayou to the north of the mill dam on Otter creek, in North Terre Haute, Indiana (lat. 39° 30.9′N, long. 87° 22.4′ W). *H. grandinella* were found in samples from the vicinity of the mill dam.

H. bifurcata and *H. grandinella* were cultured under constant illumination at 20°C in filtered bayou water to which a small quantity of skimmed milk powder was added at 4–5 day intervals. Later, *H. grandinella* were transferred to the first, best culture medium for *H. grandinella* (Tamar 1968), with excellent results.

The culture medium was prepared in an improved manner. To permit more accurate weighing, the salts were dissolved at 10 times the proper concentration in distilled water. The resulting milky flocculate disappeared when the pH was brought to 7. The solution was then stored in a refrigerator. Immediately before use a desired amount of the solution was diluted 10-fold (1:9) with distilled water, the proper quantity of skimmed milk powder was added, and the pH was again brought to 7.

Cultures in medium should be incubated between 23° and 29°C. If the inoculation fails to produce a large population within five days, excellent results may be obtained by adding an additional small quantity of skimmed milk powder.

The $FeCl_3 \times 6H_2O$ in the medium is probably significant in providing trace elements.

Behaviour in lakes of culture fluid was investigated with a stereoscopic microscope. Otherwise, live and deteriorating specimens were studied and measured by phase-contrast microscopy and phase photomicrography.

Results

Adoral Membranelles

H. bifurcata

The two divisions of each adoral membranelle (Tamar 1968) differ noticeably in width (Pl. I 2). In one immobile specimen the thinner portions of the adoral membranelles, at about their mid-lengths, varied between 0.6 μm and 1.0 μm in width. At the same levels the thicker portions varied between 1.4 μm and 2.7 μm in width.

In immobile fresh specimens the distal ends of both divisions are normally seen to be subdivided (Pl. I 2). While in 8 specimens the thinner portions were distally split into two parts, they were not observed to be subdivided in three specimens. In two other specimens the thinner portions consisted of 6 components of consecutive size, the 5 shorter ones curving off at increasingly distal levels. In 9 specimens the wider portion was seen to be subdivided distally into, variously, a minimum of 7–9 components.

The described terminal splitting of both divisions may cease to be visible even when the adoral membranelles initiate only slight motion. Perhaps the distal subdivisions close up on movement.

The observed terminal subdivisions appear to be natural. They do not resemble the membranelles' complete separation into their constituent cilia which occurs during deterioration.

H. grandinella

As in *H. bifurcata* (Tamar 1968), in *H. grandinella* phase contrast reveals a midline at the base of each adoral membranelle. However, in *H. grandinella* each adoral membranelle is not clearly demarcated into two parts, but basically forms a single unit. The last, beyond a proximal portion, then spreads into many distinct cilia groups. In a specimen one adoral membranelle separated into 13 parts.

An adoral membranelle which is smaller than the others is located close to the external opening of the buccal cavity. It is divided into two separate parts for most of its length, beyond a short undivided proximal portion, and beats particles, etc. toward the buccal cavity's entrance. In one specimen its length measured 5.5–6 μm, while in another it appeared to be over 7 μm long.

No search was made for this smaller membranelle in *H. bifurcata.*

Oral Membranelles

H. bifurcata and *H. grandinella*

The oral membranelles are each longitudinally bisected by a midline. They have graduated lengths, being longest anteriorly, toward the exterior opening of the buccal

cavity. They do not diminish in width toward their blunt distal ends, which appear to be split into a number of portions.

The striations previously observed in the area of the oral membranelles (Tamar 1968) are cross structures which seem to lie in or on, or compose, the oral membranelles. One row of such cross structures appears to run diagonally over each oral membranelle (Pl. I 3–5). The cross structures of each row are closest to the origin of their oral membranelle anteriorly. They extend posteriorly and inward at an angle, thus running diagonally away from the oral membranelle's base in an interior direction.

The cross structures reach a length of up to about 1 μm and attain a width of approx. 0.1–0.2 μm.

H. bifurcata

The cross structures seem to be thinner, or to some extent pointed, at their interior (posterior) ends.

There are six cross structures in the most interior row, over the innermost oral membranelle. The next, more anterior row has about 10–12 cross structures, and the following row approx. 14.

H. grandinella

The oral membranelles reach a width of about 1 μm. Those nearest to the buccal cavity's exterior opening reach a length of about 10 μm, while the innermost are closer to 5 μm in length.

In a number of specimens, when the other oral membranelles were fairly inactive, the innermost, smallest membranelle still vigorously beat inward, into the channel (cytopharynx) leading to a forming food vacuole.

Paroral Membranelles

H. bifurcata and *H. grandinella*

Numerous (20+?) paroral membranelles originate from the side of the buccal cavity more or less opposite to that giving origin to the oral membranelles. The part oral membranelles are directed toward the organism's interior, posterior, and righ-side. They extend from the buccal cavity through the cytostome and through a long section of the cytopharynx toward the forming food vacuole at the interior end of the cytopharynx.

In several preparations the paroral membranelles were carried to the outside of the specimen during its deterioration (Pl. I 6, 7). They then came to be located close to, and in front of, the distorted buccal cavity's new exterior opening.

The thin paroral membranelles are of considerable, but not uniform, length. Perhaps the most interior are longest. Groups of adjacent membranelles have similar lengths.

H. bifurcata

Some relocated, external paroral membranelles had estimated lengths of about 13 μm, while that of others reached up to approx. 20 μm.

Longer membranelles which continued their undulating movements externally often formed several curves toward their distal ends (Pl. I 7).

H. grandinella

The following approximate length measurements were obtained for different paroral membranelles: 4–5 μm, at least 5–6, 5.8, 6.7, 7.7, 9.6, the length of the buccal cavity (8–10 μm), 10.1, and several of at least 10 μm.

The paroral membranelles beat toward the cell interior (developing food vacuole), producing a flame-like, flickering effect. Each membranelle performs an undulating or whip-like motion; a wave travels down the membranelle. The paroral membranelles' activity was continuous and marked even when the oral membranelles were relatively inactive.

Paroral Kinetosomes

H. bifurcata and *H. grandinella*

The paroral membranelles originate from a somewhat curved row of paroral kinetosome structures (Pl. I 8, 9). This row lies along the posterior portion, and most of the length, of one side of the buccal cavity, approximately opposite to the oral membranelles. It does not extend to the buccal cavity's exterior opening.

H. bifurcata

An estimated 14–20 (perhaps more?) paroral kinetosome structures form a row with a linear (straightened) length of at least 5.7–6 μm.

H. grandinella

There are over 20 (probably only a few more) paroral kinetosome structures. All, or at least most, give rise to paroral membranelles.

The paroral kinetosome structures are 0.1–0.2 μm wide. They gradually decrease in length toward the interior. Their lengths fall between 0.27 and 0.7 μm.

The curved row of kinetosome structures occupied a space of about 7 μm in one specimen, and its linear length should be about 8–9.5 μm (8 specimens).

Bristle Kinetosomes

H. bifurcata

Each bristle arises from what looks like a separate kinetosome structure. The four kinetosome structures of one group of bristles are located within a single equatorial indentation of the cell surface.

Macronucleus — Interphase

H. bifurcata

The interphase macronucleus contains large chromatin granules and is typically elongated or irregularly sausage-shaped. It is constricted somewhere along its length, often near the middle. It was observed to shorten and round up during the deterioration of several specimens, and presumably the oval or rounded macronuclei seen in this research, and by Tamar 1968, had taken that shape due to deterioration.

Before significant deterioration, five macronuclei had the following approximate length and mid-width: 22×7.2 μm, 20.2×3.8 μm (was markedly thinner in the middle), 19.2×6.7 μm, 18.2×6.7 μm, 15.8×6.7 μm.

In most interphase macronuclei a spherical body larger than the normal chromatin granules is present. In one macronucleus there were two such bodies, of different sizes. The described bodies may be larger chromatin spherules or endosomes.

H. grandinella

This species differs from *H. bifurcata* in that its macronucleus is not clearly visible until after some cell deterioration and enlargement have occurred.

H. grandinella's interphase macronucleus consists of two subglobose portions separated by a constriction. The last often lies about 1/3 of the distance from one end, but may be located at other points, such as the middle (also see Tamar 1965).

In 14 relatively fresh specimens the macronucleus ranged from 13.9×8.6 μm to 22×7.7 μm. The average length was about 18.1 μm.

The presence of a larger spherical body in the macronucleus could not be established. In a few macronuclei there were one or two conglomerates of chromatin granules.

Macronucleus — Reproduction

H. bifurcata

Macronuclear reorganization (the formation of the chromatic reticulum, etc.), and therefore also macronuclear division, occur only after micronuclear division has taken place. In three examined specimens the macronucleus had the gross interphase appearance during micronuclear prophase, in five specimens during micronuclear anaphase, and in four specimens during micronuclear telophase after the micronuclei had divided. Only in one macronucleus did there seem to be larger chromatin masses during micronuclear telophase (the new micronuclei had separated, but contained chromosome threads).

Macronuclear reorganization was observed in a cell which still had no constriction, but in which the new adoral zone was well-developed.

H. grandinella

In two specimens the two micronuclei had completely separated and seemed to be in interphase, and the new adoral and oral zones were fairly large and showed activity. The macronucleus, however, still had the gross interphase appearance.

Micronucleus — Interphase

H. bifurcata

The spherical interphase micronucleus is dark gray under dark phase contrast. In it are visible a number of black lines, and often spots, of chromatin, and occasionally a small, dark mass.

Micronucleus — Reproduction

H. bifurcata

As reported previously (Tamar 1968), all of micronuclear mitosis precedes macronuclear division. However, when the micronucleus exhibits the chromosome network of prophase the new adoral zone is already in a posterior position. By micronuclear anaphase the new adoral zone is active and sometimes well-developed.

Three micronuclei in prophase measured about 6.2×4.9 μm, 6.1×4.8 μm and 5×5 μm, and four in anaphase were approx. 7.6×5 μm, 7.6×4.2 μm, 6.4×5.4 μm and 6.2×4.2 μm. Two telophase (?) micronuclei, still elongated and connected by a thin band, each were approx. 5×2.5 μm.

As in many ciliates (Grell 1968), the chromosomes become masked by some substance during micronuclear mitosis. Only a number of thicker long structures or bands can then be seen (Pl. II 10–12). Before these divided, as many as 8–9 such bands could be counted.

Contractile Vacuole

H. bifurcata and *H. grandinella*

In both species the contractile vacuole lies to the left of (and posterior to) the buccal cavity, assuming the buccal cavity to be ventral, after Gelei 1954, etc. This was confirmed for *H. grandinella* by the student Carl Conley.

The above results corroborate those obtained for *H. bifurcata* by Tamar 1968. The results given in abstract 117 (Tamar 1972) are erroneous.

H. bifurcata

At 24–26°C the systolic intervals in 10 fresh specimens from a bayou water culture were as short as 4–5 sec. However, among a series of such intervals there was an occasional one of 7.5 sec or even 10.5 sec.

H. grandinella

Carl Conley determined that at 24°C 15 specimens from mill dam water cultures had an average systolic interval of 5 sec, and 7 of 5.5 sec. A few specimens had longer average intervals (3 specs. — 6 sec; 3 specs. — 7 sec; 2 specs. — 8 sec).

Algae

H. bifurcata

In one culture kept under continuous illumination for four weeks at 20°C, the bayou water had a high concentration of free-living algae. The light yellow or greenish-yellow algal cells were mostly elliptical or sub-cylindrical and reproduced by transverse fission. They were possibly a *Nannochloris* species.

Some of the *H. bifurcata* contained as many as 100 of these algal cells (Pl. II 13, 14). The internal algae, indistinguishable from the free-living cells, did not lie in discernible food vacuoles, but apparently directly in the cytoplasm. None were partially digested.

Sixty cytoplasmic algal cells varied from 0.9×1.17 μm and 1.1×1.1 μm (the smallest) to 1.76×2.73 μm and 1.76×2.6 μm (the largest). Their average length was 2.0 μm and the average width 1.38 μm.

H. grandinella

Many free-living green algae were found in one constantly illuminated culture. These were mostly more or less elliptical (some were elongated, and a few round) and each contained a pyrenoid body.

The algal cells were also present in the cytoplasm of many specimens (Pl. II 15). Sixteen examined specimens had anywhere from 4 to 61 algae. None of the algal cells had been partly digested, and they could not be seen to lie in food vacuoles. One H. *grandinella* was observed to ingest such an alga.

The average length of 60 internal algae was about 5.4 μm and their average width about 3.5 μm. The smallest cells were 3.4×2.2 μm and 4.4×1.4 μm, and the largest 7.2×5.3 μm and 6.9×4.7 μm. The rounded pyrenoid bodies had an average diameter of 1.6–1.7 μm.

Thin, needle-like diatoms were also present in the culture medium. The examined *H. grandinella* with enclosed algae also contained varying numbers of the diatoms.

Macronuclear Bacteria

H. bifurcata

Two specimens from a bayou water culture had vibrio- or comma-shaped gray bodies in the macronucleus (Pl. II 16,17). These were less numerous in one specimen,

but completely filled the macronucleus of the other. The bodies had no (optically) visible internal structure and were presumably bacteria.

The typical length of 44 non-dividing bodies was about 1.15 μm, although length commonly varied from 1.0 to 1.3 μm. The most frequent maximum width was about 0.6 μm, but it often ranged between 0.5 and 0.65 μm. The outlined daughter cells of 8 transversely dividing such bodies varied in length from 0.65 to 1.1 μm; their widths were in the normal range.

Food Bacteria

H. bifurcata

In bayou water cultures containing skimmed milk powder, small, spherical objects, apparently cocci, moved about rapidly in the food vacuoles of several specimens. In other cases such objects were provisionally identified as diplococci, and in one specimen as perhaps vibrios. Thus in the described cultures, cocci, or perhaps vibrios, appear to be the chief food bacteria.

H. grandinella

In three specimens spherical objects, apparently cocci, moved about rapidly in food vacuoles. In one specimen the particles were of two distinct sizes, suggesting that a larger coccus was also being ingested.

Conjugation

H. bifurcata

A pair of conjugating specimens were observed to be fused along approximately the anterior halves of their cells. Their greatest combined width was about 47–48 μm. At the anterior end, beyond their adoral zones, the fused conjugants jointly gave rise to a small bulge (Pl. III 18). The last appeared to have an irregular surface, extended an estimated 3–5 μm beyond the peristomal constriction, and was some 5–10 μm wide near its tip. In some polar views each conjugant projected beyond the other on one side, as is the case in *H. grandinella* (Szabó 1934, Abb. 3).

At the time of observation each conjugant retained four groups of bristles (Pl. III 19) and about 11 adoral membranelles. The retained adoral membranelles of both conjugants continuously beat clockwise in synchrony. The conjugating pair performed occasional jumps, to which the otherwise immobile, outward-extended bristles may have contributed.

In another, deteriorating pair of conjugants the micronucleus of one specimen had divided, while that of the other had not. Each had a normal, interphase macronucleus.

Jumping

H. bifurcata

As coverglass preparations began to dry, specimens became immobilized. Many of these performed sudden, synchronous contractions of all their adoral membranelles and bristles, often at frequent intervals.

When the adoral membranelles were not contracted, the bristles projected outward from the cell in the usual manner. At the time the adoral membranelles were contracted, or pulled against the cell's anterior, all four bristles of each bristle group were thrown forward. The bristles then curved anteriorly, closely following the cell contour at least in their proximal portions. At this time distal portions of the bristles extended anteriorly beyond the adoral membranelles. Simultaneously with the subsequent relaxation of the adoral membranelles, the bristles were once again projected outward from the cell equator.

The described synchronous contractions can only be interpreted as abortive efforts by the immobilized specimens to perform saltatory movements. Thus jumping in *H. bifurcata* apparently involves the contraction in unison of the adoral zone and the bristles.

H. grandinella

Numerous immobilized *H. grandinella* also simultaneously contracted the adoral zone and all the bristles. The last then curved around the anterior part of the cell.

The contraction of all bristle groups was also observed during the jumping movements of a specimen still swimming freely under a coverglass. The jumping contractions of this specimen were of short duration. The bristles immediately returned to the extended position, as is presumably normal.

The jumping contractions of specimens under normal conditions are instantaneous. However, in immobilized, deteriorating specimens these contractions were often long-maintained. Then their adoral membranelles continued to vibrate while close to the anterior body in a drawn-out contracted state.

Following the jumping contractions of immobilized specimens, the distal ends of their bristles were curved forward as the bristles returned to, and reached, the extended position. Then the distal ends of the bristles straightened upon the more proximal bristle portions.

In M/100 KCl in distilled water, during the period of minimal spiraling activity following reversal (Tamar 1965, 1967), one spiraling specimen performed weak backward jerks, each accompanied by an only partial forward jerk of the bristles. Another specimen, which had stopped reversal without extending its bristles, did a few barely noticeable jerks, presumably by means of its adoral membranelles.

Reversal

H. grandinella

Upon adding an equal quantity of M/100 KCl in distilled water to a drop of culture fluid, the *H. grandinella* do rapid backward spiraling or reversal (Tamar 1965, 1967). During such reversal the bristles remain contracted and in the forward position (along the anterior body). As reversal slows or is terminated in M/100 KCl, the bristles are thrown out into the extended position. Typically the bristles are thrown into extension individually, but in some cases two or three bristles of a group were extended simultaneously.

In M/100 KCl many specimens went into faster reversal once more, after they had slowed, stopped, or even slowly spiraled forward, and after the bristles had been extended. The bristles then remained in the extended position during reversal.

In M/100 KCl directly following the original reversal the adoral membranelles no longer contract fully but only vibrate, and the bristles are extended out by the specimens and remain immobile. Now the organisms very slowly rotate backward. The strength of vibration of the adoral membranelles appears to increase with time, and this results in a more vigorous rotation. The above was determined at 150× and 600× with phase contrast.

Forward Spiraling

H. grandinella

During forward spiraling the bristles remain in the extended position. Due to the specimen's forward motion and the bristles' flexibility, the extended bristles are bent backwards, curving toward the forward spiraling specimen's posterior.

Behavior

H. bifurcata

An outstanding characteristic of *H. bifurcata* is its tendency to remain quiescent for periods of time. It is then almost immobile, although its adoral zone (and oral membranelles?) remain active, or it turns slowly.

Such often extended periods of relative immobility may be suddenly broken by a short jump, a long jump or dart which covers a considerable distance, or a long period of reversal (backward spiraling). Several of one of the enumerated movements, or different ones, may follow one another. The short jumps are usually of a turning or whirling nature, and often occur at intervals.

Reversal is initiated especially after a specimen contacts the substrait or the surface. Longer jumps or darts could result from a smaller protozoan, etc. colliding with a specimen.

H. grandinella

In contrast to *H. bifurcata*, *H. grandinella* is not seen suspended in a semi-immobile condition in the culture fluid. In its basic state *H. grandinella* performs forward spiraling. The last is typically slow, but frequently temporarily increases in rate. Forward spiraling becomes markedly faster when the culture fluid is slightly disturbed (see Tamar 1965).

Forward spiraling is normally frequently interrupted by fairly short, primarily backward, jumps.

H. bifurcata Variety

An aberrant stock of *H. bifurcata*, unique in its body shape and bristles, was found in a bayou water culture. The body is flattened anteriorly and terminates posteriorly in a variously-rounded point (Pl. III 20–23). In an anterior direction the narrowing beyond the equator is followed by a marked widening (the origin of the adoral membranelles), after which there is a further constriction leading to the flat anterior surface (Pl. III 21–23).

The mid-body lengths of 12 specimens were evenly distributed from 18.7 μm to at least 21.3 μm. The same specimens' maximum body widths (at the cell equator) varied evenly from 18.5 μm to at least 21.1 μm. In all except 2 specimens length exceeded width, but it did so by only 0.5–1.5 μm.

Bristle 1 of each group is typically not curved but straight and points sharply anteriorly, often at an angle of 35–40 degrees from the anterior-posterior axis. Bristle 4 is much shorter than the others (Pl. III 20), and is divided into two parts (or is double) throughout its length.

The length of bristles 1, 2 and probably 3 lies between 32 and 35 μm. Thus these bristles are unusually long, especially in view of the small body. In one observation the longer end branch of bifurcated bristle 2 measured very approximately 11.5 μm, while the shorter end branch was close to about 6.5 μm long. Bristle 4 has a typical total length of 13–14.5 μm. There may be a significant difference in the length of its two divisions.

There are 16 adoral membranelles, the normal number, but in the few examined specimens these appeared to not have the usual *H. bifurcata* structure. Most of 24 measured adoral membranelles had a straightened length between 12.5 and 14.5 μm.

An elongated macronucleus, paroral membranelles and oral-membranelle cross structures are present.

Since bristle 4 of the new stock is aberrantly short, the name *H. bifurcata* var. *brevis* is suggested.

Discussion

The earlier structural description of *H. bifurcata* (Tamar 1968), including the presence of only 7 oral membranelles, could be confirmed. However, it was now possible to determine that the macronucleus is elongated or irregularly sausage-shaped in active specimens and only becomes ovoid after deterioration begins. This is especially interesting because Szabó 1934, who used several fixatives, described the macronucleus of *H. grandinella* as sausage-shaped, whereas André 1912, Kahl 1935 and Tamar 1965 found it to be ovoid. Dragesco 1970 described it as oval (avalaire)-elongated. The macronucleus cannot be discerned in active *H. grandinella*.

The present observations of undigested algae and diatoms which appeared to lie directly in the cytoplasm of *Halteria* agree well with similar previous observations (Tamar 1968). One should be cautious in hypothesizing either a symbiotic or a nutritional role for such cells. Perhaps they are accidentally ingested and are only temporarily retained. The accidental ingestion of algae and diatoms is made more plausible in the present cases by the fact that the same cells abounded in the culture fluid.

The comma-shaped gray bodies found in the macronucleus of *H. bifurcata* specimens are comparable to the bacteria described from the macronucleus of *Paramecium aurelia* by Preer 1969. If the bodies seen in this research are closely related to Preer's bacteria, named alpha, the present results would support Preer's hypothesis that her short rods and crescents, and not the spiral form, are reproductive. Also, the fact that the *H. bifurcata* bodies divide transversely could then be of general taxonomic value. Although the present bodies totally filled a macronucleus, alpha's short rods and crescents are never as abundant in *P. aurelia's* macronucleus as is alpha's spiral form.

Conjugation in *Halteria* has previously been described only by Szabó 1934, who repeatedly observed it in *H. grandinella*. Conjugation in *H. bifurcata*, as viewed twice, appears to basically resemble the process in *H. grandinella*.

There have been two views of the means by which jumping is performed. Kent 1882 referred to *H. grandinella*'s bristles as "that central girdle of setae by which the animalcule accomplishes its leaping movements". Szabó 1934 also believed the bristles to bring about *H. grandinella*'s jumps. He erroneously stated that the bristles are bent toward the anterior body preceding a jump, and that they produce the jump through a very rapid backward movement. Fauré-Fremiet 1953 as well considered the saltatory movements of *H. grandinella* to result from the "brusque flexions" of the bristles, and Tamar 1967 echoed this view.

On the other hand, Kahl 1930 believed that the jumping of *Halteria* is carried out by means of the adoral membranelles, and that the bristles have an exclusively tactile function. He cited the closely-related genus *Strobilidium* Clap. et Lachm.,

which has no bristles, as also capable of jump-like or jerking movements. The author has seen different *Strombidium* species (oligotrichs with no bristles) respectively make backward or forward jump-like movements (unpublished observations).

The present observations establish the method by which jumping is performed in the investigated *Halteria* species, and partially resolve the disagreement between Kahl and the other named researchers. It is still possible that the highly-developed adoral membranelles supply most of the motive power of the jump, and that the bristles are primarily tactile (or sensitive to vibration — see Tamar 1968).

The saltatory movement of other *Halteria* most probably requires processes fundamentally identical to those described.

A marine *Mesodinium* species was also found to make its backward jumps partly by throwing its cirri forward (unpublished observations).

The noted inactivation of the bristles in M/100 KCl during minimal spiraling is in good agreement with the reported inhibitory effect of this solution on jumping (Tamar 1965, 1967).

The present research fills out our knowledge of *H. grandinella*'s reversal by establishing that during this movement the bristles remain in the contracted, forward position. This information also clarifies the relationship between the higher-threshold reversal and the low-threshold jumping. Above a stimulus threshold the bristles remain in the contracted jumping position and the adoral membranelles are repeatedly contracted toward the anterior, instead of just once, to produce a continued movement, reversal, rather than the single, isolated jumping motion. Thus jumping could be viewed as a short, reduced reversal, just as the avoidance reaction of other protozoa is a short reversal. However, a possible distinction remains — the forward movement of the bristles in jumping may supply a backward impetus which is lacking after the initial moment of reversal. This is suggested by the finding that while spiraling (by the adoral membranelles) is inhibited in M/300 KCl and M/500 KCl, jumping is not noticeably affected (Tamar 1965, 1967).

The maintained forward position of the bristles during reversal should greatly reduce any resistance by them to this movement.

The behavior of *H. bifurcata* does not differ greatly from that attributed to *Halteria minima* by Gelei 1954.

Abstract 117 (Tamar 1972) is premature in regard to the buccal cavity organelles, and the buccal cavity is not considered ventral in locating *H. bifurcata*'s contractile vacuole.

Note added in proof: Prior to publication, the following electron microscope study has come to my attention: Grain, J. 1972: Etude ultrastructurale d'*Halteria grandinella* O.F.M. (Cilie Oligotriche) et considérations phylogénétiques. Protistologica, 8, 179–197.

Summary

New information is supplied on the organelles of the buccal cavity in *Halteria bifurcata* and *Halteria grandinella* — the oral membranelles (and their cross structures), the paroral membranelles and the paroral kinetosomes. How the saltatory movement is performed and the position of the bristles during reversal are reported and discussed, and the relationship between jumping and reversal is elucidated. The adoral membranelles, bristle kinetosomes, macronucleus and micronucleus in interphase and reproduction, contractile vacuole and systolic intervals, internal algae, macronuclear and food bacteria, conjugation, behavior and a new *H. bifurcata* variety are also dealt with.

ZUSAMMENFASSUNG

Neue Befunde werden über die Mundspalt-Strukturen von *Halteria bifurcata* und *Halteria grandinella* gegeben — die Mundmembranellen (und ihre Kreuz-Strukturen), die Paroralmembranellen und die Paroralen Basalkörper. Wie der Sprung gemacht wird und die Position der Springborsten während dem Rückwärtsschwimmen werden mitgeteilt und diskutiert, und die Beziehung zwischen dem Springen und dem Rückwärtsschwimmen wird erläutert. Die Adoralmembranellen, Basalkörper der Springborsten, Makronucleus und Mikronucleus im Ruhestadium und in der Zellteilung, pulsierende Vakuole und Pulsationsfrequenzen, innere Algen, makronucleare und Ernährungs-Bakterien, Konjugation, Benehmen und eine neue *H. bifurcata* Varietät werden auch behandelt.

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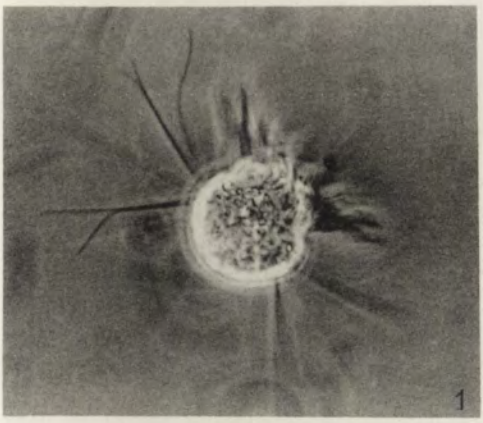
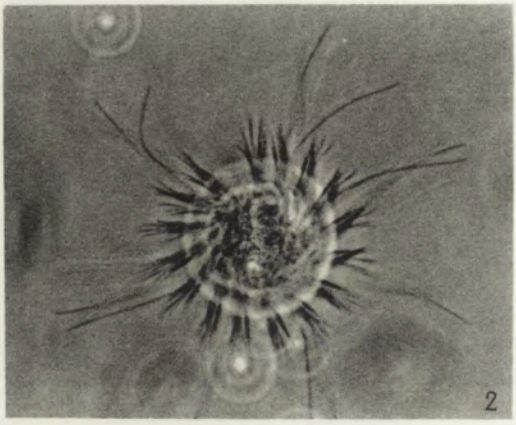
EXPLANATION OF PLATES I–III

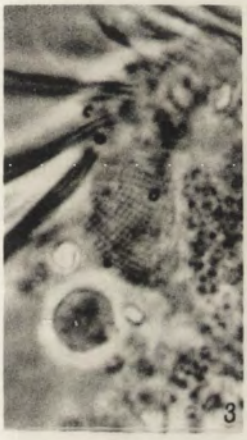
Halteria bifurcata Tamar — Figs. 1–3, 6, 7, 10–14, 16–23
Halteria grandinella Müller — Figs. 4, 5, 8, 9, 15

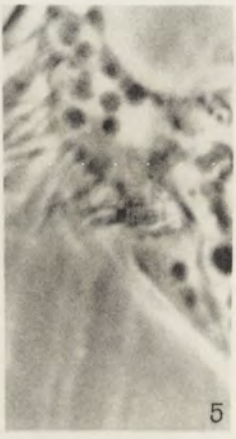
1: Side-view, showing adoral membranelles and bristles 1, 2 and 4 of a group
2: Polar view. The distal subdivisions of the two portions of each adoral membranelle are visible
3: Striations due to the oral membranelles and their cross structures. The proximal parts of adoral membranelles are nearby
4: The oral membranelle region. The cross structures of several oral membranelles are visible
5: View of the cross structures of several oral membranelles
6: Buccal cavity organelles carried to the exterior during deterioration. Oral membranelles (O), paroral kinetosome structures (K), paroral membranelles (P)
7: Oral membranelles (O) and still active, undulating paroral membranelles (P) carried to the outside
8: Row of paroral kinetosome structures
9: Row of paroral kinetosome structures
10: Earlier anaphase micronucleus
11: Later anaphase micronucleus
12: Later anaphase micronucleus
13: *Nannochloris* (?) algae in a specimen's cytoplasm
14: Internal *Nannochloris* (?) algae at higher magnification
15: Cytoplasmic algae with pyrenoid bodies
16: Crescent-shaped gray bodies filling the macronucleus
17: The gray bodies enlarged further. Some are dividing transversely
18: Side-view of conjugating pair. A single anterior bulge has formed beyond their adoral membranelles
19: Polar view of conjugants. Four bristle groups are visible in the top specimen
20: New stock. Bristles 4 are shortened. Note the wide, flattened anterior surface
21: New stock. Bristles 1 are straight and the widening at the adoral membranelles is visible on one side
22: New stock. The typical body form and a shortened bristle 4 can be seen
23: New stock. Bristle 2 and bristle 4 are in focus on one side

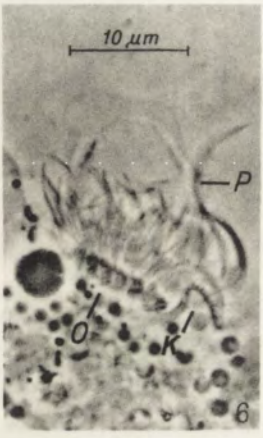
(All photomicrographs by dark phase contrast, Zeiss microflash (60 watt/sec). Figs. 1, 2, 13, 18–23 taken at 500×, all others at 1250× Kodak Tri-X 35 mm film, D-76 developer)

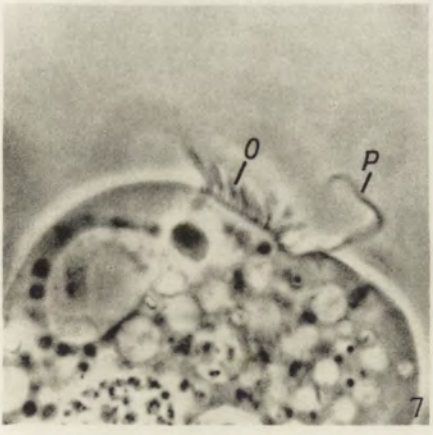


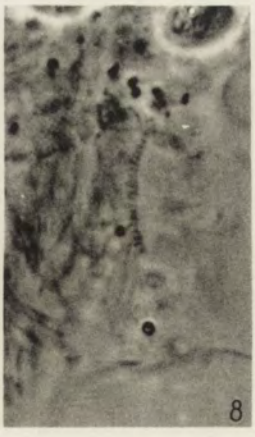


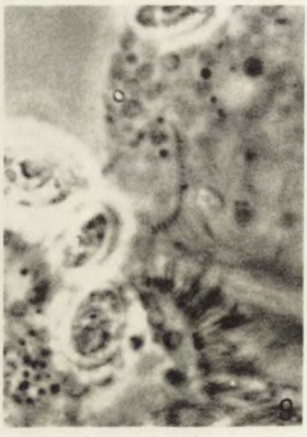




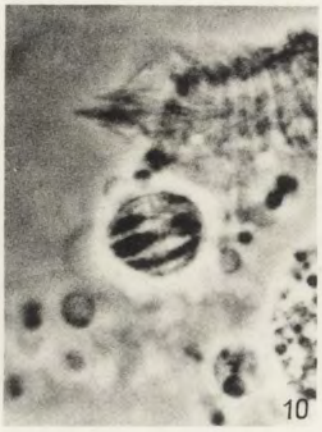


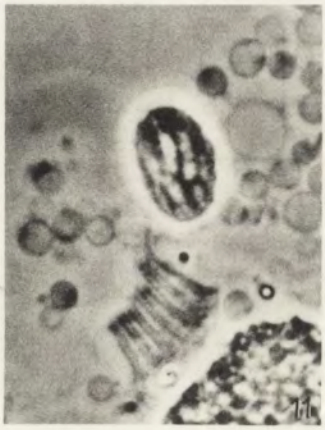


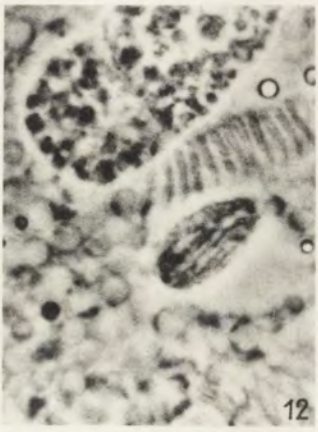


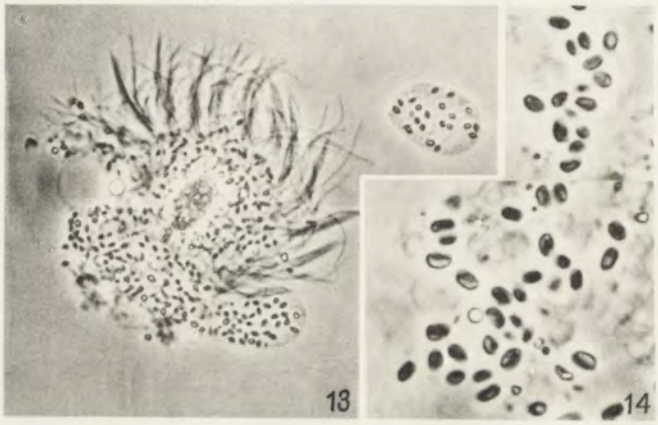


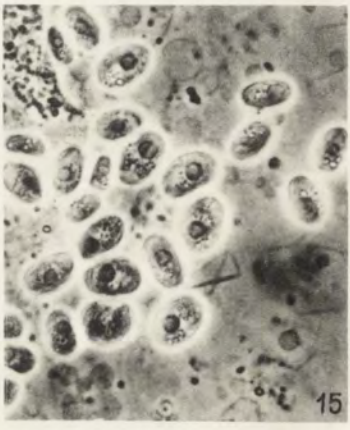
H. Tamar auctor phot.

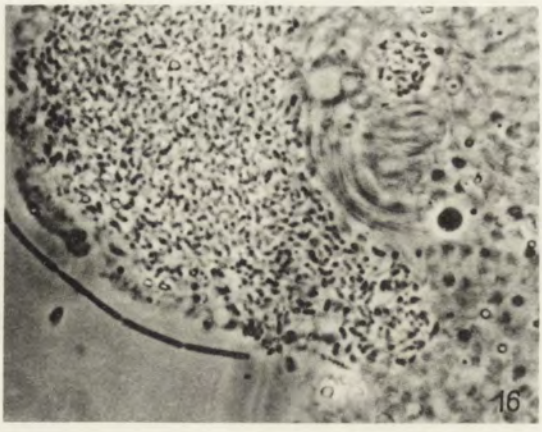


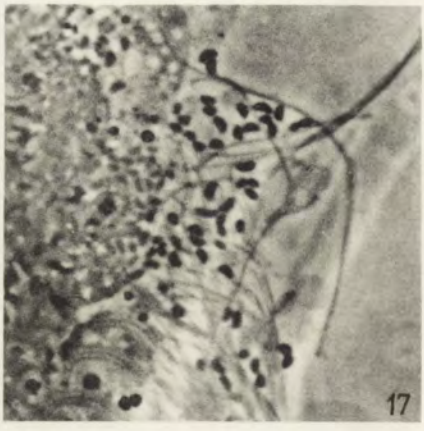












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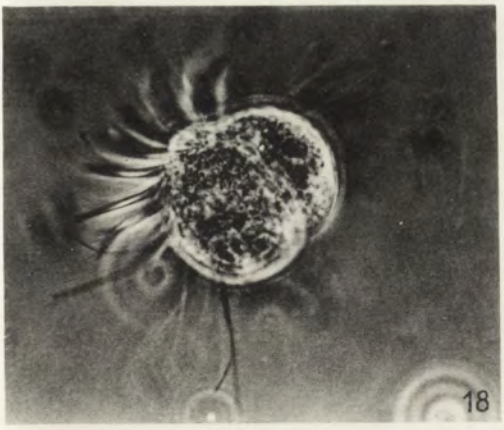
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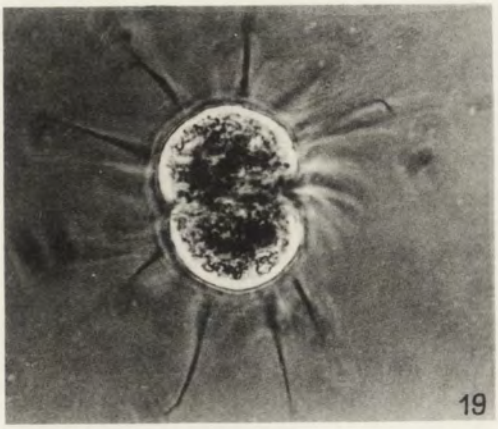
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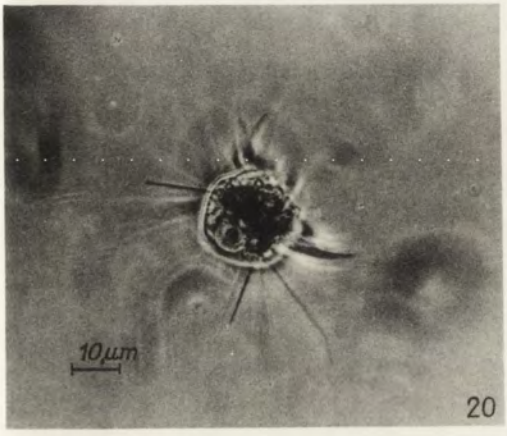

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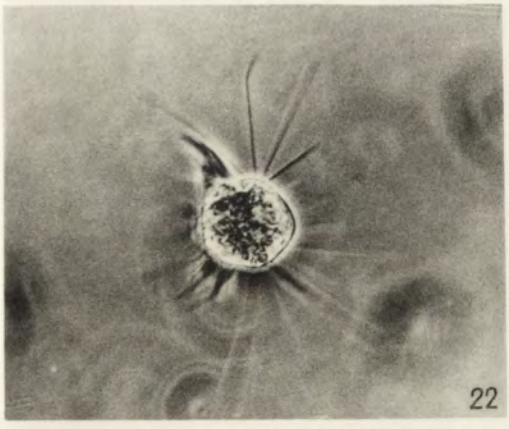
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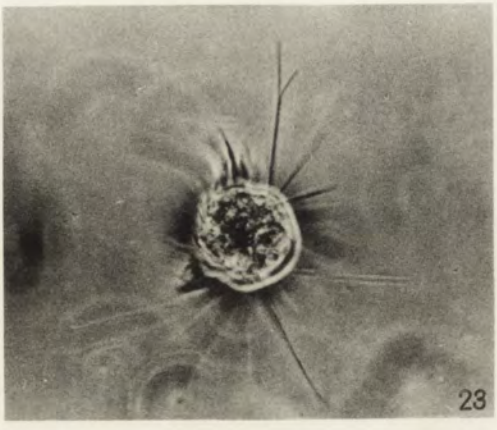
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The Systematics of the Genus *Anigsteinia* Isquith, 1968

Le système du genre *Anigsteinia* Isquith, 1968

In the past twenty years or so, there have been several taxonomical works dealing with the family *Spirostomatidae*. Most have dealt with the naming of new species or clarification of the definition of previously named species. Two genera have received most of the attention: *Blepharisma* has been the subject of several papers attempting to alleviate some of its tremendous intrageneric confusion (Suzuki 1954, Hirshfield et al. 1965, Dragesco 1970, and Hirshfield et al. 1973); *Spirostomum* has been the subject of a paper in which all of its species were clearly defined (Repak and Isquith 1973).

Within the family *Spirostomatidae*, the following are the established genera: *Spirostomum* Ehrenberg, 1838, *Blepharisma* Perty, 1849, *Gruberia* Kahl, 1932, *Parablepharisma* Kahl, 1932, *Phacodinium* Prowazek, 1900, *Protocruzia* de Faria, de Cunha et Pinto, 1915, *Pseudoblepharisma* Kahl, 1926, *Spirostomina* Gruber, 1884, and *Transitella* Gellert, 1950 (Corliss, 1961). Each of these genera is quite clearly defined, and with the probable exceptions of *Protocruzia* and *Spirostomina*, each possesses the characteristics of the family.

Hirshfield et al. (1965) gave a very precise definition to the genus *Blepharisma*, the major features of which are in Table 1.

Unfortunately, several species that clearly did not possess the generic characteristics were not excluded from it. If these species had been excluded, the remaining blepharismas would have formed an extremely homogeneous, distinct, grouping. The forms that were enigmatically retained were *B. clarissimum* Anigstein var. *arenicola* and *B.* (*Spirostomum*) *salinarum* (Florentin) Kahl. Most notable, these two elongate marine forms both possess lacunar contractile vacuole systems. *Blepharisma*, by definition, does not have such a system. Furthermore, these forms did not fit into any of the other genera of the family, even though they clearly are spirostomatids.

In 1968, Isquith suggested the establishment of the genus *Anigsteinia* to contain these two species, including several varieties, and the species *B. candidum* Yagiu

and Shigenaka, 1956, which had not been included in the Hirshfield et al. (1965) paper. Hirshfield et al. (1973) support the removal of these species from the genus *Blepharisma*.

General Description of the Genus

Anigsteinia is characterized by being a quite elongate spirostomatid, with a lacunar contractile vacuole system. On the left margin of the peristome there is a well-developed adoral zone of membranelles (AZM), and on the right margin a relatively inconspicuous undulating membrane is present. Slight body contraction (without body torsion) is apparent in some species. The somatic ciliature of *Anigsteinia* is basically the same as that found in other spirostomatids. The ciliary rows run the length of the body. The rows on the left side of the body, in the region of the oral groove, turn towards and terminate at the AZM. The somatic rows on the right side of the organism run straight to the anterior tip of the organisms. The genus was originally established by Isquith in 1968.

In essence, this genus is distinquished from *Blepharisma* by the possession of the collecting canal for the contractile vacuole, a more elongate body, a less conspicuous undulating membrane, and somatic contractility in some species. It is distinguished

Table 1

Characteristics of *Anigsteinia Spirostomum Blepharisma* and the Species of *Anigsteinia*

Organism	Body Length	Length:Width Ratio	Contractile				Macronucleus
			with Torsion	without Torsion	Dorsal Canal	UM	
Blepharisma	30–700 μm	3–5 : 1	—	—	—	+	compact, filiform, or noded
Spirostomum	150 μm–4 mm	6–18 : 1	+	—	+	—	compact, filiform, or noded
Anigsteinia	160–1000 μm	7–20 : 1	—	±	+	+	noded or fragmented
A. salinara	160–360 μm	3 : 1	—	—	+	+	50–75 fragments of 2.5–5.0 μm each
A. candida	330–520 μm	10 : 1	?	?	+	+	181–287 fragments of 2.1–5.6 μm each
A. clarissima	160–380 μm	10 : 1	—	+	+	+	44 nodes
A. clarissima var. *arenicola*	200–450 μm	7–10 : 1	?	?	+	+	30–50 nodes
A. longissima	500–1000 μm	12–20 : 1	?	?	+	+	30 nodes
A. oligonucleata	320–400 μm	6 : 1	—	—	+	?	8–12 nodes

from *Spirostomum* by the lack of body torsion during contraction, and the presence of an undulating membrane. A comparison of the three genera is provided in Table 1.

Phylogenetically, *Anigsteinia* appears to fit between the other two genera. The closeness of theses three is well illustrated by *Anigsteinia salinara*. It was originally named *Spirostomum salinarum* by Florentin in 1899, but it was transferred to *Blepharisma* by Kahl in 1928. Since the two genera are quite distinct, if *A. salinara* were not an intermediate between the two, such confusion would not have been possible. The creation of the intermediate genus, *Anigsteinia*, should eliminate any further taxonomical confusion as to where Florentin's organism belongs as well as several closely related species.

Table 2

Key to the Genus *Anigsteinia*

1.	Macronucleus consists of about 10 large nodes	*A. oligonucleata*
1′.	Macronucleus in more than 25 parts	2.
2.	Macronucleus consists of 200–300 fragments	*A. candida.*
2′.	Macronucleus consists of fewer than 100 pieces	3.
3.	Macronucleus scattered (50–75 pieces)	*A. salinara*
3′.	Macronuclear nodes in chain-like arrangement	4.
4.	Body length less than 500 μm	*A. clarissima*
4′.	Body length more than 500 μm; organism worm-like	*A. longissima.*

All of the species now included in the genus are marine. Also, they all have either moniliform (noded) or fragmented macronuclei. See Tables 1 and 2.

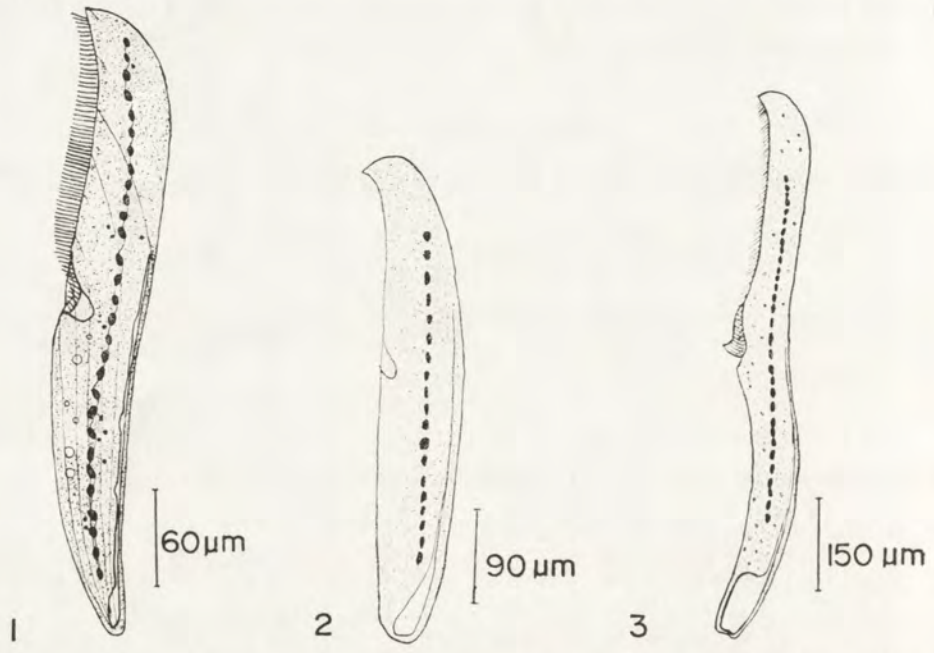
1. *Anigsteinia clarissima* (Anigstein) Isquith, 1968

Synonymy: *Blepharisma clarissimum* Anigstein, 1912
B. clarissimum forma *arenicola* Kahl, 1932
B. clarissimum var. *arenicola* described by Dragesco in 1960
Non: *B. clarrisimum* var. *longissimum* Kahl, 1932
B. clarissimum described by Dragesco in 1966.

This species contains all the generic characteristics and seems to be the most frequently encountered form, therefore, it is designated as type species for the genus. Its size range is 160–450 μm and has a length to width ratio of 7–10:1. The body is generally laterally compressed and slightly contractile. The peristome extends from slightly less to one-half the total body length. The undulating membrane is inconspicuous, and the lacunar contractile vacuole system is rather small.

In the form described by Anigstein (1912), the macronucleus consists of about 44 nodes. Kahl (1932) described about 30 nodes, and in Dragesco's (1960) description there were more than 50. Dragesco (1960) considered his form to correspond to Kahl's description, in spite of the macronuclear nodal number differences. In this we agree with Dr. Dragesco. Both Kahl (1932) and Dragesco (1960) depicted interkinetic protrichocysts in this colorless species.

When Kahl originally described the *arenicola* form, it was described as having a length: width ratio of 7–10:1, whereas *B. clarissimum* of Anigstein (1912) was 10:1. Also, the *arenicola* form had a slight anterior beak not present in the rest of the species. These distinctions are not considered sufficiently significant to consider the establishment of more than a single species, *Anigsteinia clarissima* to contain them. Figure 1 represents Anigstein's (1912) *B. clarissimum*, and Fig. 2 is the *arenicola* form of Kahl (1932).



Figs: 1. *Anigsteinia clarissima* after Anigstein 1912. 2. *A. clarissima* var. *arenicola* after Kahl 1932. 3. *A. longissima* after Kahl 1932

2. *Anigsteinia longissima* (Kahl)

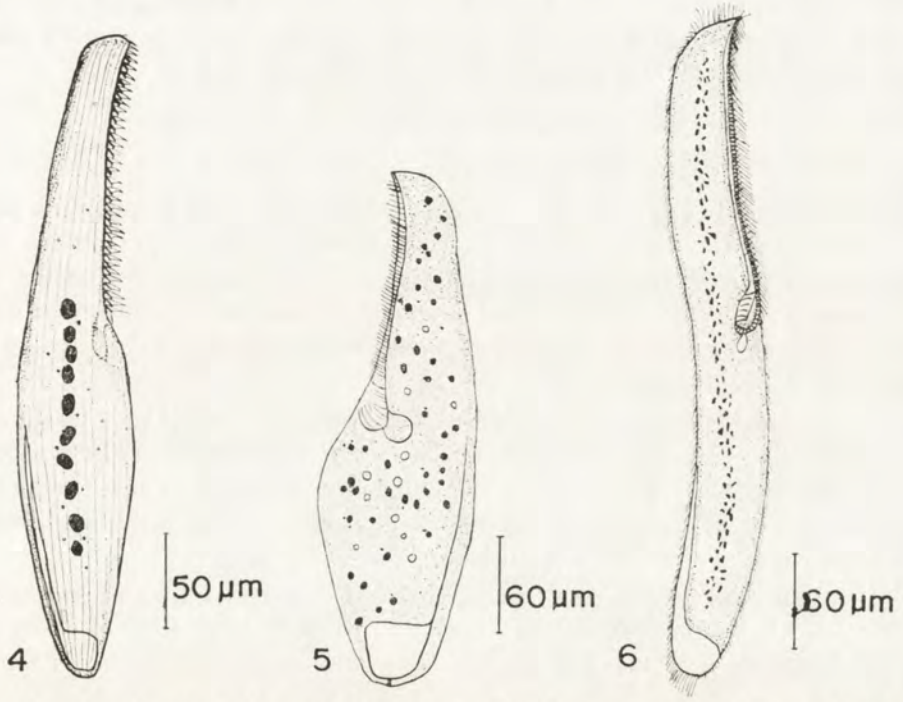
Synonymy: *B. clarissimum* var. *longissimum* Kahl, 1932
Non: *B. clarissimum* Anigstein, 1912
B. clarissimum var. *arenicola* Kahl, 1932
B. clarissimum described by Dragesco in 1960.

This large, thin organism ranges in size from 500–1000 µm. It is quite thin, with a length:width ratio of 12–12:1. The contractile vacuole proper, is invisible or small, rounded and terminal, a lacunar system is present. The peristomal length is approximately two-fifths of the body length. A small undulating membrane is present. The macronucleus consists of about 30 nodes. The species inhabits waters of 3–9% salt. See Fig. 3.

3. *A. oligonucleata* sp. nov.

Synonymy: *B. clarissimum* described by Dragesco in 1966.

This 320–400 μm organisms has a slight anterior beak. Its peristome extends about one-half the body length. The moniliform macronucleus consists of 8–12 nodes and there are 7 or 8 micronuclei. The contractile vacuole is fed by a long collecting canal. The body is non-contractile. See Fig. 4.



Figs: 4. *Anigsteinia oligonucleata* after Dragesco 1966. 5. *A. salinara* after Florentin 1889. 6. *A. candida* after Yagiu and Shigenaka 1956

4. *Anigsteinia salinara* (Florentin) Isquith, 1968

Synonymy: *Spirostomum salinarum* Florentin, 1899
Blepharisma salinarum Kahl, 1928

Non: *B. salinarum* described by Dragesco in 1963. This form does not seem to be a member of *Anigsteinia*.

Florentin (1899) described this form from extremely saline waters (65 g/l). It ranges in size from 160–360 μm. The organism is colorless to faintly yellow, non-contractile, and rigid. The somatic cilia are short and numerous. The peristome extends about one-half the body length, starting at an anterior beak. There is a well-developed AZM; the undulating membrane extends about one-fifth the peristomal length. The cytopharynx is quite short.

The macronucleus consists of 50–75 spherical fragments of 2.5–5.0 μm diameter each; Florentin (1899) was also able to distinguish micronuclei. The collecting canal feeding the contractile is quite obvious. The mid-region of the organism tends to appear quite swollen.

In 1928, Kahl transferred this organism from *Spirostomum* to *Blepharisma*, because it had an undulating membrane and was non-contractile. He felt that Florentin had placed it in the former species, since that author had not observed the undulating membrane. Kahl (1928) further questioned Florentin's placement of the organisms, since it was non-contractile. The form occurs in strongly saline waters and also weakly brackish ones (Kahl 1928). See Fig. 5.

5. *A. candida* (Yagiu et Shigenaka, 1956)

Synonymy: *Blepharisma candidum* Yagiu et Shigenaka, 1956.

The size range of this colorless organism is 330–520 μm; the length:width ratio is 10:1. It is flattened and very flexible. The body curves slightly to the right with the anterior end tapering to a beak-like projection. The ciliary rows on the left side start at the peristomal margin and run obliquely posteriorly; those on the right surface start at the anterior end and run parallel to the long axis of the body. There are 22–27 somatic ciliary rows.

The peristome starts at the anterior beak and runs one-half the body length and connects with a pocket-like oral aperture. The undulating membrane of this species is quite inconspicuous, being located only in the immediate vicinity of the oral aperture, and does not extend along the peristomal margin. During predivision stages, an entire v-shaped area can be clearly seen.

The macronucleus consists of small, spherical or ellipsoidal beads, ranging in size from 2.1–5.6 μm. In the fifteen organisms studied, the number of beads per organism ranged from 181–287. When stained with hemotoxylin, the beads appear to be compact. During vegetative division, two or three macronuclear beads aggregate and, after a period of three hours, coalesce into ten to twenty large masses. In cytokinesis, the masses elongate and split. At this same time, micronuclear division also occurs. The contractile vacuole of this species displays a definite lacunar canal (Yagiu and Shigenaka 1956). See Fig. 6.

Discussion

Based upon the morphological descriptions of the species described in this paper, *Anigsteinia* is considered to be a distinct genus. The removal of these species from the genus *Blepharisma*, also strengthens the delineation of that genus, since it now contains a much more homogeneous collection of species. Since the genus has been only relatively rarely collected, it did not seem wise to impose strict species definitions

at this point. It is quite probable that, as more members of the genus are found, they would not fit into existing very strict delineations, and we would have to reconcile the problem by the establishment of many species, much as was done in the genus *Blepharisma*. Of course, as more members of the genus are found, if there are no intergrades, it might then be justifiable to tighten up the species definitions.

Although all members of the genus so far described are marine and have noded or fragmented nuclei, there is no reason anticipate that all members of the genus will have these characteristics, and therefore, they have not been included in the generic definition.

If anyone has a culture, or slides, of organisms that are now included in the genus *Anigsteinia*, or which might be included in the genus, it would be appreciated if they would be sent to Dr. I. R. Isquith at Fairleigh Dickinson University, Teaneck, N. J., U.S.A.

Summary

Anigsteinia, a number of the heterotrichous family *Spirostomatidae*, is described. This elongate genus is intermediate between *Blepharisma* and *Spirostomum*, possessing a contractile vacuole like that of *Spirostomum*, but also an inconspicuous undulating membrane. Also, slight contractility, without body torsion, is present in some species. All species presently described are marine and have noded or fragmented macronuclei. A guide to the members of *Anigsteinia* is provided.

RESUMÉ

On decrit *Anigsteinia* de la famille *Spirostomatidae*. Ce genre allonge semble etre intermediaire entre *Blepharisma* et *Spirostomum*, et possede une vacuole pulsatile qui est contractile comme celui de *Spirostomum*, mais aussi une membrane ondulante. On trouve pareillement dans quelques especes une contractilite menue, sans torsion du corps. Toutes les especes actuellement decrites sont marines et ont des macronoyaux nodulaires ou fragmentes. On fournit un guide des membres de *Anigsteinia*.

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Morphologie und Taxonomie einiger Ciliaten eines kanadischen Prärieboden

Morphology and Taxonomy of some Ciliates of a Canadian Prairie Soil

Als ein kanadischer Beitrag zum Internationalen Biologischen Programm (IBP) werden seit 1967 umfassende ökologische Untersuchungen am Graslandökosystem der Prärie im südlichen Saskatchewan durchgeführt ("Matador Project"). Im Rahmen dieses Unternehmens führte Bick (1972) Untersuchungen an Protozoen in Prärieböden durch, wobei er im feuchten O_L- und O_F-Horizont, also in den obersten Bodenschichten, die ein hoher Gehalt an organischer Substanz kennzeichnet, einen verhältnismässig starken Ciliatenbesatz nachweisen konnte.

Einige interessante Ciliatenarten wurden einer eingehenden morphologisch-taxonomischen Untersuchung unterzogen.

Material und Methoden

Die Proben wurden auf natürlichem Grasland der Sceptre Association der Braunen Graslandböden (Chernozemic brown soils; kastanienbraune Böden der Trockensteppen) gewonnen. Die Textur des Bodens ist lehmig; im Sommer wird bei Trockenheit der Boden extrem hart und zeigt tiefe Trockenrisse. Das Probenmaterial wurde am 16. Juni 1971 mit einem hydraulisch betriebenen Bohrgerät aus dem Bereich 0–10 cm Tiefe entnommen.

Die Erdproben wurden nach Lufttrocknung in Plastiksäckchen verpackt und so nach Bonn überführt, wo sie bearbeitet wurden.

Nach Aufschwemmen der Bodenproben und Excystierung der Ciliaten wurden einzelne Arten isoliert und in kleinen Petrischalen zur Vermehrung gebracht. Als Kulturmedium diente ein dekantierter, abgekochter Brennesselaufguss, dem ein Reiskorn und wenig getrocknetes Eigelb zugesetzt wurde.

Die Dauerpräparate wurden nach der Protargolmethode (Dragesco 1962, Tuffrau 1967) hergestellt. Der in den Zeichnungen eingezeichnete Masstab entspricht in allen Fällen einer Länge von 10 µm. Systematik nach Corliss (1961).

Folgende Abkürzungen fanden Verwendung:

AZM = adorale Membranellenzone (adoral zone of membranelles)
CC = Caudalcirren (caudal cirri)
CV = kontraktile Vakuole (contractile vacuole)

D = Dorsalborsten (dorsal cilia)
K = Kinetosom (kinetosome)
Ma = Makronucleus (macronucleus)
Mi = Mikronucleus (micronucleus)
T = Trichocyste, Trichite (trichocyst, trichite)
UM = undulierende Membran (undulating membrane).

Ergebnisse

Unterklasse: *Holotrichia*

Ordnung: *Gymnostomatida*

Unterordnung: *Rhabdophorina*

Familie: *Enchelyidae*

Lacrymaria cohni Kent (Abb. 1)

Länge: 70–90 µm, schlank (etwa 6 : 1), im hinteren Drittel ein wenig verdickt und nach hinten leicht konisch zulaufend. Der Hals ist nicht stark verschmälert, jedoch deutlich kontraktil, ähnlich wie bei anderen *Lacrymaria*-Arten; an der Spitze sitzt ein ca 10 µm langes Kopfstück. Der mit Trichiten ausgestattete Schlund erscheint als Reuse. In allen beobachteten Fällen war der Makronucleus auffallend fragmentiert; er setzt sich meist aus 8 mehr oder weniger runden Brocken zusammen, während der Mikronucleus entweder in Einzahl vorhanden ist oder nicht zu erkennen war. Die kontraktile Vakuole liegt terminal.

Etwa 12 Kineten laufen meridional über den Zelleib. Die Cilien sind ca 12 µm lang. Bemerkenswert ist, dass die Kinetosomen in der Halsregion paarweise, sonst aber einzeln stehen. Etwa 12 sehr dicht stehende Reihen von Cilien überziehen den Kopf schräg von links oben nach rechts unten. Jede dieser Reihen setzt sich aus 11–13 Cilien zusammen.

Die Bewegung des Ciliats ist im Substrat bohrend, im freien Wasser schnelle Rotation um die Längsachse. Über die Ernährungsweise konnten keine Beobachtungen gemacht werden.

Die Morphologie des Ciliats entspricht zum grossen Teil der Beschreibung von Kahl (1930). Die äussere Körpergestalt, die Grösse und der vielkernige Makronucleus waren in erster Linie für die Artdiagnose massgebend.

Familie: *Spathidiidae*

Spathidium atypicum n. sp. (Abb. 2)

Länge: 100–150 µm, Gestalt konstant, sehr schlank (9 : 1). Der verhältnismässig kurze Mundwulst ist etwa 10–12 µm lang und mit Trichocysten besetzt. Dahinter verjüngt sich der Körper halsartig. In der etwas verdickten Mitte liegt der für *Spa-*

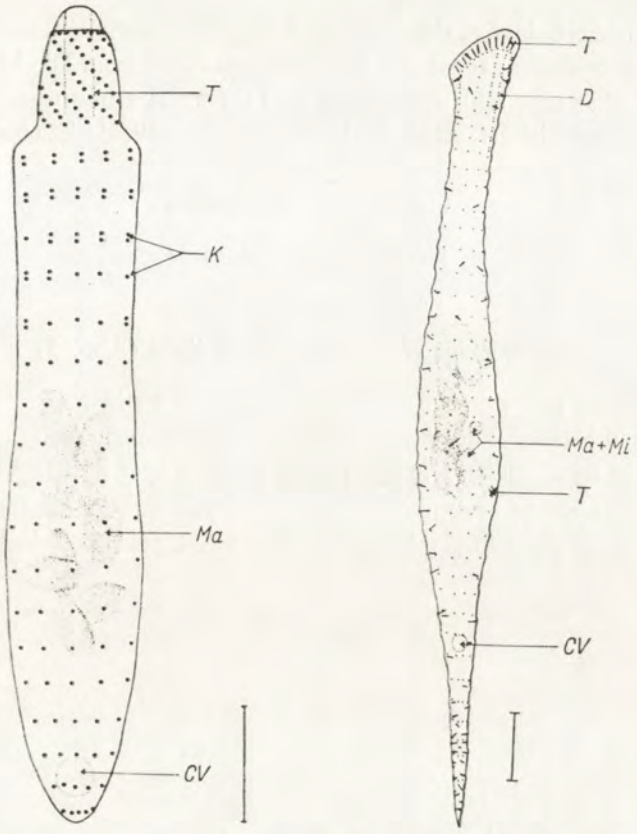

Abb. 1. *Lacrymaria cohni* Kent. Infraciliatur nach Protargolpräparaten
Abb. 2. *Spathidium atypicum* n. sp. Infraciliatur nach Protargolpräparaten

thidium-Arten typische, langgestreckte, gewundene Makronucleus mit dem einzelnen Mikronucleus. Zum caudalen Pol hin läuft der Zelleib spitz zu. Bei einigen Formen war ein fast körperlanger Schwanz ausgebildet. Die kontraktile Vakuole ist nicht ganz terminal, sondern ein wenig zur Mitte hin gelegen. Auffallend ist die leicht gerippte Pellicula. Sie war sehr deutlich sichtbar, wenn sich die Ciliaten durch die Fixierung zusammenzogen.

Etwa 8 Kineten sind vorhanden, deren Cilien etwa 8 µm lang sind. Hinter dem Mundwulst sind die Wimpern dicht zusammengerückt. Die ca 2 µm langen Trichocysten liegen nicht nur im Mundwulst, sondern sind überall unter der Pellicula zu finden. Oft treten sie im Schwanzabschnitt gehäuft auf. Die dreireihige Dorsalbürste setzt sich aus Borsten zusammen, die im Protargolpräparat immer je 2 Kinetosomen aufweisen.

Diese *Spathidium*-Art ernährt sich räuberisch von Ciliaten und Amöben. Im Substrat bewegt sie sich kriechend, im freien Wasser mässig schnell drehend, wobei die Ciliaten mit dem Halsabschnitt rotieren.

Die Möglichkeit, dass es sich um eine Variation innerhalb einer bekannten Art der Gattung *Spathidium* handelt, ist sicherlich auszuschliessen. Die hier beschriebene Art ist durch ihre sehr schlanke Gestalt und ihr meist schwanzartig ausgezogenes Hinterende gekennzeichnet. Auch die leicht gerippte Pellicula ist eine Besonderheit.

Unterklasse: *Spirotrichia*

Ordnung: *Heterotrichida*

Familie: *Gyrocorythidae*

Bryometopus cf. *pseudochilodon* Kahl (Abb. 3)

Grösse: 40–60 µm, dorsoventral abgeflacht, von ovoider Gestalt. Der Macronucleus ist rund bis oval, mit runden oder länglich ovalem Binnenkörper. Ein Micronucleus liegt in seiner Nähe. Die kontraktile Vakuole liegt im letzten Körperviertel.

Das Peristomfeld ist breit und mit einer Ektoplasmalippe ausgestattet. Auf der Ventralseite zieht die adorale Membranellenzone schräg von links nach rechts,

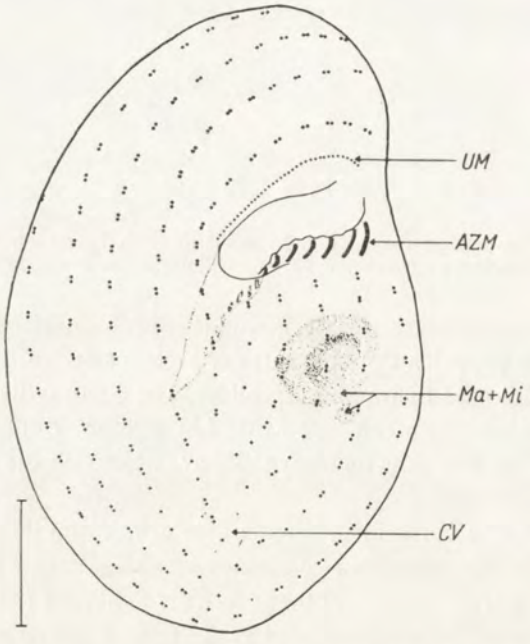

Abb. 3. *Bryometopus* cf. *pseudochilodon* Kahl. Infraciliatur der Ventralseite

verläuft etwa in der Körpermitte in das Zellinnere und endet vor dem letzten Drittel. Auf der Peristomlippe findet man eine sehr zarte, langgestreckte undulierende Membran.

Dorsal wie ventral weist der Ciliat je 8–10 Kineten auf. Ventral stehen in ihnen die etwa 8 µm langen Cilien doppelt, wobei zwei miteinander verklebte Einzelcilien ein Cilienpaar bilden. Auf der Dorsalseite stehen die Wimpern einzeln.

Der Ciliat bewegt sich langsam gleitend. Als Bakterienfresser vermehrte er sich besonders gut in fauligen Kulturen.

Kahl (1932) beschrieb als erster diese Ciliatenart. Doch weisen er und später Matthes und Wenzel (1966) ausdrücklich darauf hin, dass eine undulierende Membran nicht vorhanden sei. Andere Autoren (Wenzel 1933, Gellért 1955) fanden Formen, die mit der von Kahl beschriebenen übereinstimmen und somit ebenfalls ohne undulierende Membran ausgestattet sein dürften. Dieses Organell war bei der hier beschriebenen Form in Protargolpräparat deutlich sichtbar. Obwohl dieser Ciliat möglicherweise zu einer neuen *Bryometopus* Art mit undulierender Membran gehört, sei er vorläufig zu *B. pseudochilodon* Kahl gestellt, da es sich vielleicht nach weiteren genauen Untersuchungen der Infraciliatur herausstellen wird, dass diese Formen identisch sind.

Ordnung: *Hypotrichida*

Familie: *Urostylidae*

Amphisiella raptans n. sp. (Abb. 4)

Länge: 300–500 µm. Der langgestrecke, weiche und biegsame Zelleib verjüngt sich dem Hinterende zu. Dabei erfährt die hintere Körperhälfte eine halbe Drehung um die Längsachse. Das letzte Fünftel des Ciliats ist fast durchscheinend hell, während der übrige Körper trübe granuliert erscheint. Man findet 8 ovale Makronuclei und 4 Mikronuclei vor. Die kontraktile Vakuole liegt im ersten Körperdrittel nach links verschoben und bildet dorsal eine deutliche bläschenförmige Erhebung. Die adorale Zone nimmt ein Fünftel der Gesamtlänge des Tieres ein. Sie setzt sich aus etwa 33 Membranellen zusammen. Das Peristom ist weit geöffnet, die Peristomlippe krümmt sich nach vorn und nähert sich der Zone. Auf dem Frontalfeld stehen 7 kräftige Cirren: vorn 3 schräg hintereinander, einer an der Peristomlippe und davon rechts gelegen die restlichen 3. Die Marginalreihen, die aus etwa 80 Cirren bestehen, sind hinten geschlossen. Eine einzige Ventralreihe entspringt frontal rechts hinter dem Ende des Strudelorganells und verläuft ventral bis zu den Transversalcirren, von denen 3 vorhanden sind. Sie sind nicht auffallend länger als die Cirren der Marginalreihen, etwa 10–12 µm. Die Dorsalborsten sind 4–5 µm hoch.

Der Ciliat ist eine neue, untypische Art von *Amphisiella*. Abweichend vom Grundtyp sind die sehr schlanke, konische Körpergestalt und das Vorhandensein von nur 3 Transversalcirren. Doch da insgesamt 2 Marginalreihen, nur 1 Ventralreihe plus Frontal- und Transversalcirren vorhanden sind, ist nach Borror (1972) der Ciliat in die Gattung *Amphisiella* einzureihen.

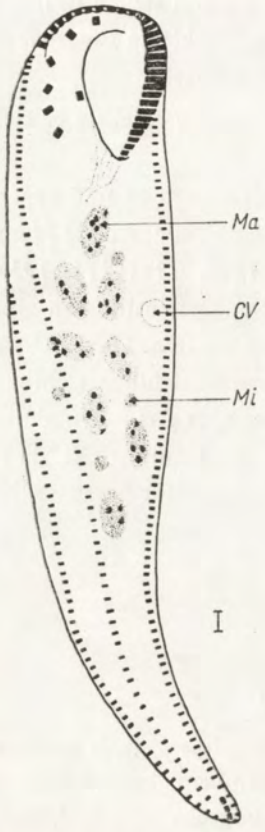

Abb. 4. *Amphisiella raptans* n. sp. Ventralseite

Das Tier ist ein sehr gefrässiger Räuber: in eine Kultur von *Colpoda steini* Maupas ausgesetzt, verschlang ein Individuum innerhalb einer Minute 12 dieser Ciliaten, die durchschnittlich etwa 30 µm gross waren. Auch Amöben dienen als Nahrung. Die Bewegung ist rasch umhersuchend, scheinbar aggressiv.

Familie: *Holostichidae*

Holosticha tetracirrata n. sp. (Abb. 5)

Länge: 120–160 µm, meist 130 µm. Länge und Breite stehen im Verhältnis 4 : 1 Der Zellkörper ist schlank ellipsoid, dabei meist nach rechts neigend. Er ist biegsam und trübe granuliert. Die adorale Membranellenzone nimmt das erste Körperviertel ein. Kurz vor der Mitte liegt auf der linken Seite die kontraktile Vakuole. Bemerkenswert ist, dass der Makronucleus in viele granulierte Teile zerlegt ist (meist um 30, oft aber auch mehr). Vor der Zellteilung verschmelzen diese zu einem einheitlichen Kern.

Der Ciliat weist neben den obligatorischen 2 Ventralreihen und den Transversal-

cirren auf dem Frontalfeld 3 deutliche Frontalcirren auf und gehört daher zur Gattung *Holosticha*. Neben diesen Frontalcirren steht ein einzelner Buccalcirrus an der ca 18 µm langen Peristomlippe, die von einer 5 µm hohen undulierenden Membran begleitet wird. Aus etwa 24 Membranellen setzt sich die adorale Zone zusammen. Der Cytopharynx ist auffallend lang: er verläuft schräg von links nach rechts bis über die Körpermitte hinaus.

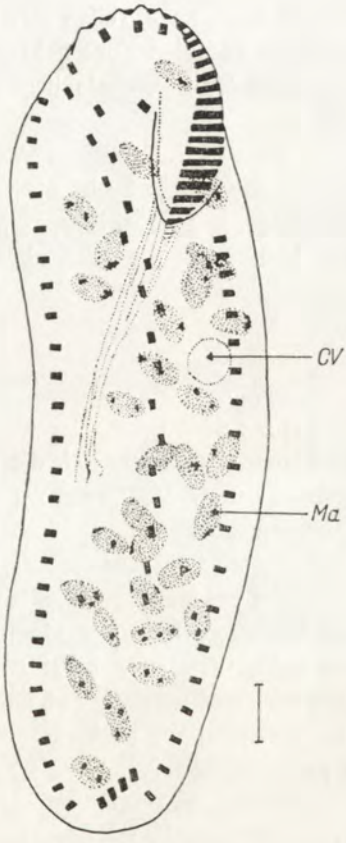

Abb. 5. *Holosticha tetracirrata* n. sp. Ventralseite

Die beiden Marginalreihen laufen hinten nicht zusammen. Die linke setzt sich aus etwa 20, die rechte aus etwa 27 Cirren zusammen, die 10 µm lang sind. Im Frontalfeld beginnen — die einzelnen Cirren leicht gegeneinander versetzt — die beiden Ventralreihen. Sie ziehen in einer ventralen Rinne durch die Körpermitte, wo sie derart genähert sind, dass sie fast zu einer einzigen Ventralreihe verschmelzen. Sie enden schon vor dem letzten Körperviertel.

Die etwa 10 µm langen Transversalcirren stehen zu viert in seiner Schrägreihe (daher die Artbezeichung *tetracirrata*). Seltene Varianten besitzen nur 3 Transversalcirren. Die Dorsalborsten sind 3 µm lang.

In Kultur war der Ciliat sehr gut zur Massenentfaltung zu bringen. Jedoch traten manchmal Krüppelformen auf, was wahrscheinlich auf ungeeignete Nahrung zurückzuführen war. *Holosticha tetracirrata* ernährt sich von Algen, Diatomeen und Bakterien.

Diese neue Art von *Holosticha* grenzt sich von ähnlichen deutlich ab: Gellért (1955) fand im Humus unter Moosrasen eine *Holosticha muscicola*, die der hier beschriebenen Form hinsichtlich der Ciliatur ähnlich ist. Doch ihr Makronucleus besteht nur aus 2 Teilen mit 1 Mikronucleus, und ihre Peristomlippe ist nach links zur adoralen Zone gekrümmt. Deswegen und aufgrund anderer geringfügiger abweichender Merkmale liegt hier sicherlich keine Modifikation der genannten Art vor.

Auch von *Holosticha manca* var. *plurinucleata* Gellért unterscheidet sich der vorliegende Ciliat: So ist die von Gellért (1954) gefundene Form mit 80 µm Gesamtlänge, nur 22 Makronuclei, 6 Transversalcirren und anderen weniger bedeutenden Merkmalen erheblich abweichend.

Familie: *Oxytrichidae*

Trachelostyla canadensis n. sp. (Abb. 6)

Länge: 63–80 µm. Der Ciliat ist von ovoider bis länglich elliptischer Gestalt. Er besitzt 2 Makronuclei, die unregelmässig gebaut und etwa im Verhältnis 2 : 1 gestreckt sind. 2 Mikronuclei sind vorhanden. Die kontraktile Vakuole ist nicht ganz zentral gelegen, sondern ein wenig nach links gerückt.

Meist wird die gesamte erste Hälfte des Zelleibs von der adoralen Zone eingenommen. Nur bei länger gestreckten Formen ist sie ein wenig kürzer. Sie beginnt frontal mit besonders kräftigen Membranellen (ca 16 µm lang), verläuft lange parallel zur linken Seite und biegt erst in ihrem letzten Viertel schräg zum Cytostom. Das Peristom ist stark zur linken Seite hin verlagert ("*Gonostomum*" -Charakter). Die Peristomlippe ist verhältnismässig kurz: ihre Länge liegt zwischen 7 µm und 9 µm.

Auf dem Frontalfeld findet man gewöhnlich 10 verstärkte Cirren: ausser 3 Frontalgriffeln, die mit einer Länge von 12 µm die Länge der 4 ersten Membranellen der adoralen Zone nicht erreichen, sind 1 Buccalcirrus, eine Zweiergruppe von Cirren zwischen der rechten Marginalreihe und den 3 Frontalgriffeln und ausserdem meist 4 Cirren zwischen dieser Zweiergruppe und dem Buccalcirrus vorhanden. Variationen treten nur bei den letztgenannten 4 Cirren auf, von denen manchmal einer fehlen kann.

Ventralcirren sind nicht vorhanden. Die Marginalreihen laufen hinten zusammen. Es wurden auf der linken Seite 13–14, auf der rechten 21–23 Marginalcirren gezählt, die je 9 µm lang sind. Zwischen ihnen stehen 3 Caudalcirren. Nur 2 ca 10 µm lange Transversalcirren sind vorhanden. Sie überragen den caudalen Pol zu drei Vierteln ihrer Länge. Die 3 µm hohen Dorsalborsten stehen in 4 Längsreihen.

Der Ciliat trat in jedem Erdaufguss recht häufig auf und ist ein charakteristischer Bewohner dieses Biotops. Er ist ein Bakterienfresser und liess sich relativ lange in Kultur halten. Die Bewegung ist mässig schnell und wird ruckhaft durch die verhältnismässig langen Pausen für die Nahrungsaufnahme unterbrochen. Schwimmend dreht sich der Ciliat lebhaft um seine Körperachse.

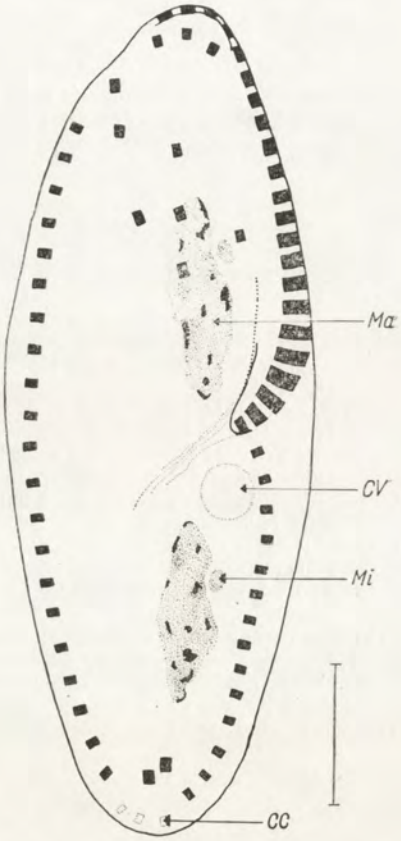

Abb. 6. *Trachelostyla canadensis* n. sp. Ventralseite

Da der Ciliat durch das Fehlen der postoralen Ventralcirren und den Besitz mehrerer Frontalcirren gekennzeichnet ist, ist er zur Gattung *Trachelostyla* zu stellen. Allerdings unterscheidet er sich von anderen Arten dadurch, dass ihm sämtliche Ventralcirren fehlen und er nur 2 Transversalcirren aufweist. Jedoch sind innerhalb dieser Gattung grosse Unterschiede in Körperform und Ciliatur zu finden (Borror 1972).

Zusammenfassung

Spathidium atypicum n.sp., *Amphisiella raptans* n. sp., *Holosticha tetracirrata* n. sp., *Trachelostyla canadensis* n. sp., *Lacrymaria cohni* Kent und *Bryometopus*

pseudochilodon Kahl sind neue bzw. wenig bekannte Ciliatenarten eines Prärieboden aus dem südlichen Saskatchewan (Kanada). Ihre Infraciliatur wird anhand von Protargolpräparaten in der vorliegenden Arbeit dargestellt.

SUMMARY

Spathidium atypicum n. sp., *Amphisiella raptans* n. sp., *Holosticha tetracirrata* n. sp., *Trachelostyla canadensis* n. sp., *Lacrymaria cohni* Kent and *Bryometopus pseudochilodon* Kahl are new or relatively unknown species of ciliates from the Prairie soil of Southern Saskatchewan (Canada). Their infraciliature was investigated by the means of the Protargol method and is described in this work.

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Некоторые микроспоридий (*Microsporidia, Nosematidae*) из колюшек *Pungitius pungitius* и *Gasterosteus aculeatus* Финского Залива

Some Microsporidians (*Microsporidia, Nosematidae*) from Sticklebacks *Pungitius pungitius* and *Gasterosteus aculeatus* of the Finnish Bay

По сводкам и работам, опубликованным в последние годы известно около 35 видов микроспоридий, паразитирующих в пресноводных и эвригалинных рыбах (Putz et al. 1964, Putz and McLaughlin 1970, Lom and Weiser 1969, Газимагомедов и Исси, 1970). В свое время большое количество исследований было опубликовано по микроспоридии *Glugea anomala*, заражающей — трехиглую и девятииглую колюшек. Монье (Moniez 1887), Телоан (Thelohan 1895), Штемпель (Stempell 1904), Вайсенберг (Weissenberg 1913) и Дебезье (Debaisieux 1920) приводят в своих работах описание этого вида. Вайсенберг (Weissenberg 1968) провел успешное заражение личинок колюшки спорами *Glugea anomala* и детально изучил патологические процессы, происходящие в инвазированных паразитом клетках. Спрэг и Верник (Sprague and Vernick 1968) описали из четыреиглой колюшки *Apeltes quadracus* микроспоридию *Glugea weissenbergi*, которая отличается от *G. anomala* только большими размерами спор.

Исходя из значительного списка работ по *G. anomala* можно предположить, что этот вид исследован довольно подробно. Поэтому, приступая к работе с колюшками Финского Залива, мы ставили перед собой задачу лишь выяснить сезонность и влияние пола хозяина на зараженность этих рыб микроспоридией *G. anomala*. В ходе нашего исследования в девятииглой и трехиглой колюшках было обнаружено 3 вида микроспоридий, которые существенно различались между собой по целому ряду признаков. Два вида паразитов массовые, и мы имели возможность произвести их детальное изучение. Третий вид был встречен всего лишь один раз в трехиглой колюшке *Gasterosteus aculeatus*.

Материал и методика

Колюшек брали из уловов рыболовецкой бригады, база которой расположена на берегу Финского Залива, неподалеку от станции Старый Петергоф. С начала мая по конец октября было исследовано по 350 экземпляров девятииглой (*Pungitius pungitius*) и трехиглой (*Gasterosteus aculeatus*) колюшек. Общея длина девятииглых колюшек колебалась в пределах 3.7–7.1 cm, а трехиглых колюшек — 3.8–7.3 cm. Возраст рыб не определяли. Из цист и икринок, содержащих споры и другие стадии развития микроспоридий, готовили мазки, которые фиксировали в метаноле с последующей окраской их по Гимза-Романовскому или в жидкости Буэна с дальнейшей окраской железным гематоксилином по Гейденгайну. Для выявления слизистых капсул у спор микроспоридий в водную суспензию последних добавляли тушь. Измерение спор было проведено окуляр-микрометром, измерялось по 50 спор каждого вида микроспоридий. Из яичников, содержащих пораженные микроспоридиями икринки, были сделаны гистологические срезы, которые крашивали железным гематоксилином или по Гимза-Романовскому. Изучение свежих спор и их фотографирование проводили на водных препаратах, изготовленных по методике Лома и Вайзера (Lom and Weiser 1969). На предметное стекло наносили тончайший слой 1.5% агара. Затем на агар помещали капельку водной суспензии спор, которую накрывали покровным стеклом. Края стекла для предотвращения высыхания воды обмазывали расплавленным парафином. Таким образом устранялось нежелательное для наблюдения колебание спор и воде. Фотографирование спор было выполнено на фотоустановке ФМН-3 фирмы ЛОМО на кафедре зоологии беспозвоночных Ленинградского Универзитета.

Результаты

Glugea anomala (Moniez, 1887) Gurley, 1893

К этому виду мы относим микроспоридию, которую встречали только на девятииглой колюшке. Характерные белые цисты 1–4 mm в диаметре располагались чаще всего в подкожной соединительной ткани тела рыб и на внутренней стороне их жаберных крышек. Реже цисты лежали в глубоких тканиях туловища, вызывая образование опухолевидных визвышений на теле, либо на париетальном листке мезентерия. Было отмечено несколько случаев поражения роговицы глаза колюшек, и лишь один раз наблюдали большую цисту, лежащую на печени рыбы. Споры овальной формы (Табл. I 1) и размером 4.6 (3.5–5.1)×2.3 (2.1–2.6) μm чаще всего лежали поодиночке. Наши данные полностью согласуются с наблюдениями Лома и Вайзера (Lom and Weiser 1969).

Glugea gasterostei sp. n.

Из 350 исследованных *Gasterosteus aculeatus* лишь одна колюшка, размером 6.8 cm (♀) была заражена этой микроспоридией. В задней половиние полости тела рыбы на висцеральном листке мезентерия располагалась крупная желтовато-белого цвета циста размером 3×4 μm. Весьма плотное содер-

жимое этой цисты состояло из огромного количества спор паразита. Так как мы одновременно имели дело с *G. anomala* из девятииглой колюшки, то различие в размерах и форме спор этих двух видов микроспоридий сразу было замечено. Приводится описание лишь спор микроспоридии *G. gasterostei*, sp. n. так как других стадий развития паразита в мазках не наблюдали.

Свежие споры размером 5.6 (4.9–6.0)×2.6 (2.1–2.8) μm илеми удлиненно-эллипсоидную форму (Табл. I 2). Наиболее широкая часть споры приходилась на ее середину, в то время как концы выглядели очень сходно. Большая задняя вакуоль занимала половину, а иногда и две трети размера споры. Споры окрашивались по Гимза-Романовскому весьма разнообразно. Они имели скорее всего одно ядро, расположенное либо на заднем конце, либо в центре споры. После фиксации в жидкости Буэна и окраски железным гематоксилином споры приобретали размер 4.1 (3.8–4.4)×1.9 (1.7–2.1) μm и также имели одно ядро. В водных препаратах многие споры выбрасывали очень тонкую, малозаметную нить, максимальная длина которой доходила до 160 μm. Слизистой капсулы вокруг спор в туши не наблюдали.

Хотя большинство спор лежало в водных препаратах поодиночке, мы, следуя общепринятой традиции относить все виды, образующие крупные соединительнотканные цисты к роду *Glugea*, поместили в него и данный вид микроспоридии. Видовое название паразита дается по его хозяину — трехиглой колюшке *Gasterosteus aculeatus*.

Thelohania baueri sp. n.

Этот вид поражает икринки девятииглой колюшки *Pungitius pungitius* и трехиглой — *Gasterosteus aculeatus*. Как правило, в пораженном яичнике находили несколько крупных зараженных икринок, которые, благодаря своему характерному белому цвету, легко отличались от желтовато-оранжевых здоровых. Процент пораженных икринок был различным. Изучение продольных гистологических срезов гонад показало, что микроспоридии поражали до 15% яиц от общего их числа. Споры и другие стадии развития паразита локализовались только в желтке икринок, причен были поражены ооциты разных размеров. Наблюдались, как полностью заполненные паразитами икринки, так и яйца, внутри которых лежали лишь небольшие группы спор и панспоробластов. В последнем случае они лежали непосредственно под оболочкой икринки. В некоторых случаях в икринках споры паразита лежали группами внутри округлых светлых зон. Эти зоны можно рассматривать либо как спорогональные вакуоли, либо просто как пустые пространства на месте жировых капель. Также как Вайзер (Weiser 1949) и Соммерфельт и Уорнер (Summerfelt and Warner 1970), мы видели атрезию некоторых зараженных микроспоридиями икринок, которые бйли окружены и заполнены клетками соединительной ткани. Интересно отметить, что на гистологических срезах, окрашенных по Гимза-Романовскому, скопления спор па-

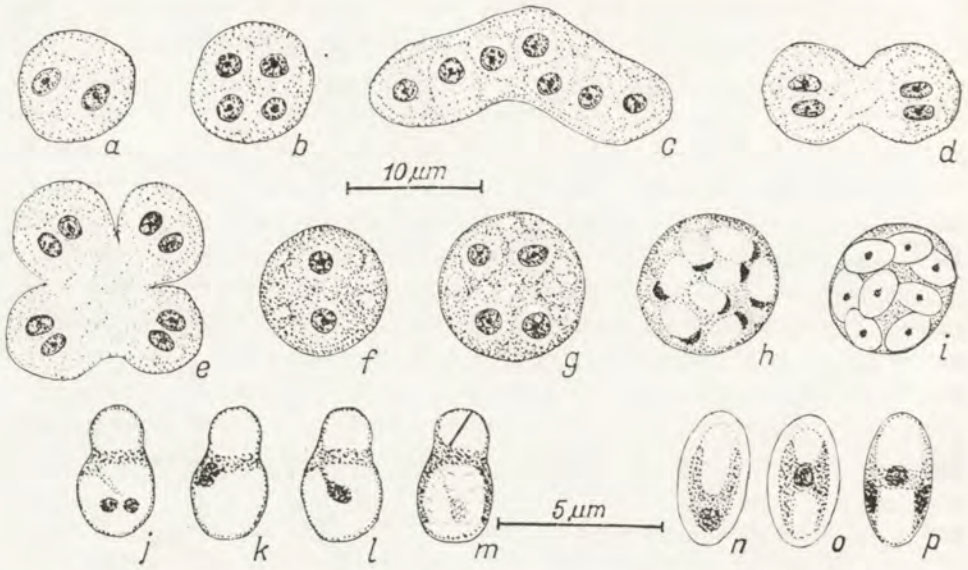

Рис. 1. Некоторые стадии шизогонии и спорогонии микроспоридии *Thelohania baueri* sp. n. (a–m) и споры микроспоридии *Glugea gasterostei* sp. n. (n–p). a–e — шизонты, f–g — споронты, h — обособление споробластов, i — панспоробласт, j–p — зрелые споры (j–l и n–o — окрашенные по Гимза-Романовскому, m и p — железным гематоксилином по Гейденгайну)

Fig. 1. Some stages of schizogony and sporogony of microsporidian *Thelohania baueri* sp. n. (a–m) and pores of microsporidian *Glugea gasterostei* sp. n. (n–p). a–e — schizonts, f–g — sporonts, h — formation of sporoblasts, i — pansporoblast, j–p — ripe spores (j–l and n–o stained on Giemsa-Romanovsky, m and p — stained with Heidenhain's hematoxylin)

разита приобретали синий цвет, благодаря чему четко выделялись на красновато-фиолетовом фоне желтка икринок.

На мазках, изготовленных из гонад *Pungitius pungitius*, удалось проследить некоторые стадии шизогонии и спорогонии паразита (Рис. 1 a–m). Лентовидные шизонты содержали по 4–7 ядер и имели средний размер 21×8 µm. Реже встречались округлые 4–8-ядерные плазмодии. Цитоплазма клеток паразита на этой стадии слабо окрашена в бледно-голубой цвет. Двухядерные споронты округлой формы и диаметром 6–8 µm были следующей наблюдаемой нами стадией. Споронты, содержащие 4 ядра, имели более интенсивно окрашенную цитоплазму по сравнению с предшествующими стадиями. В темносиней цитоплазме восьмиядерных споронтов уже различались светлые участки, соответствующие будущим споробластам. Восьмиспоровые панспоробласты, в которых процесс спорогонии уже завершился, были диаметром около 10 µm. Споры в таких панспоробластах выглядели как светлые овальные вакуоли, окруженные темносиним ободком.

Свежие споры типичной грушевидной формы (Табл. I 3) имели большую заднюю вакуолю. Она занимала обычно половину и лишь иногда две трети длины споры. Споры в зависимости от их размера можно условно подразде-

лить на две группы, а именно: нормальные споры длиною 4.5–6.0 μm и макроспоры — 6.0–7.3 μm. Подавляющее число спор относилось к первой группе и имело средний размер 5.4×2.7 μm. Макроспоры были более характерны для *Gasterosteus aculeatus*, нежели для *Pungitius pungitius*. Длина полярной нити, которая выбрасывалась в воде, составляла 200–220 μm. В препаратах с тушью слизистых капсул вокруг спор отмечено не было. В мазках, окрашенных по Гимза-Романовскому, наблюдались споры как с одним, так и с двумя ядрами. У микроспоридий из трехиглой и девятиглой колюшек имеются некоторые различия, а именно: разное количество спор в панспоробластах (Табл. 1), а также большее количество межспоровой цитоплазмы в панспо-

Таблица 1

Table 1

Соотношение панспоробластов *Thelohania baueri* sp. n., содержащих различное число спор, в зависимости от вида колюшек

Frequence of Pansporoblasts of *Thelohania baueri* sp. n. with a Various Number of Spores in Connection with Stickleback Species

Хозяин Host	Число спор в панспоробласте Number of Spores in Pansporoblast					
	4	6	88	9–12	16	>16
P. pungitius	9	9	70	8	4	—
G. aculeatus	—	4	15	58	18	4

робластах микроспоридий из *Gasterosteus aculeatus* по сравнению с таковыми из *Pungitius pungitius*.

Анализ экстенсивности заражения обеих колюшек микроспоридией *Thelohania baueri* sp. n. показал, что вид *Pungitius pungitius* является основным хозяином для этого паразита (Табл. 2).

Таблица 2

Table 2

Зараженность самок двух видов колюшек *Thelohania baueri* sp. n. в 1972 г.

Infection of Females of Two Species of Stickleback with *Thelohania baueri* sp. n. during 1972

Хозяин Host	Число исследованных рыб Number of fishes examined	Процент заражения по месяцам Frequence (%) of infection per month					Общий % заражен. Total infection %
		май May	июнь June	август August.	сентябрь Sept.	октябрь October	
P. pungitius	223	14	9	данных нет no data	данных нет no data	15.4	12.5
G. aculeatus	254	2.3	2.6	4.4	6.6	2.3	3.5

Таблица 3

Table 3

Сравнительные данные по микроспоридиям, паразитирующим в икре рыб

Comparative Data on Microsporidians invading Fish Eggs

Вид микроспоридии Species of microsporidian	Хозяин и место обнаружения Host and distribution	Локализация Localization	Размер спор в μm Dimension of spore in μm	Форма спор Shape of spore	Число спор в панспоробластах Number of spores in pansporoblast	Полярная нить в μm Polar filament in μm
Cocconema šulci (Rašin, 1949)	*Acipenser ruthenus* Чехословакия Czechoslovakia *A. güldenstädti* и and *A. baeri* СССР USSR	икринки eggs	свежие fresh 2.5	круглые round	большое numerous	120
Plistophora elegans (Auerbach, 1910 a)	*Abramis brama* *Leuciscus cephalus* ФРГ GFR	соед. ткань яичника и икринки connect. tissue of ovarium and eggs	10×4	длинные и узкие long and narrow	разное большое число various and numerous	—
Plistophora mirandella (Vaney et Conte, 1901)	*Alburnus mirandella* Франция France	соед. ткань яичника и икринки connect. tissue of ovarium and eggs	12×6 7.5×4	овоидные ovoidal	большое numerous	очень длинная very long

Plistophora oolytica (Weiser, 1949)	*Esox lucius* *Leuciscus cephalus* Чехословакия Czechoslovakia	икринки eggs	8.4×4.2, 5.5×3.5, 3.5×1.5 (*L. cephalus*) 7×3, 5–3; 5×3; 3×1.5 (*E. lucius*)	овальная или грушевидная oval or pyriform	—	—
Plistophora ovariae (Summerfelt, 1964)	*Netemigonus crysaleucas* США USA	икринки, иногда споры в печени и почках eggs, sometimes spores are found in liver or kidney	свежие fresh 8.4×4.2 окраш. stained 6.4–6.6×3.3–3.4	овальные или эллипсоидные oval or ellopsoidal	8, 12, 16 наиболее часто most frequent 12	110–160
Thelohania ovicola (Auerbach, 1910)	*Coregonus exiguus bondella* Швейцария Switzerland	икринки eggs	6–8×4–6	яйцевидная или овальная egg-shaped or oval	не указывается no data (на Рис. 8) (on Fig. 8)	25–30
Thelohania baueri sp. n.	*Pungitius pungitius*, *Gasterosteus aculeatus* СССР USSR	икринки eggs	свежие fresh 5.4×2.7 реже seldom 6–7, 3×3.2–3.5 окраш. stained 4.1–2.2	грушевидная pyriform	4, 6, 8, 10, 12, 16 и редко больше and sometimes more наиболее часто most frequent 8, 12	200–220

[1] Описание вида сделано только по гистологическим срезам.
Description of species has been made using histological sections only.

Работа по определению влияния возраста хозяина на зараженность их микроспоридиями не проводилась из-за трудностей, связанных с установлением возраста у этих рыб. Однако изучение размеров зараженных и незараженных колюшек показало, что рыбы более крупных размеров чаще заражены микроспоридиями. Так средняя общая длина у инвазированных самок трехиглых колюшек была 6.75 cm, в то время как у незараженных — 6.45 cm. Для девятииглой колюшки эти цифры были соответственно равны 5.47 и 5.22 cm.

Описываемый здесь вид помещен в род *Thelohania*, так как при развитии в основном хозяине преобладающее число спор в панспоробластах было 8. Видовое название этой микроспоридии дается в честь проф. О. Н. Бауера.

Обсуждение

Микроспоридии, паразитирующие в рыбах, изучены еще крайне недостаточно. Из имеющейся по микроспоридиям рыб литературы подавляющее большинство работ посвящено описанию новых видов, в то время как работ по биологии паразитов немного. Практически полное отсутствие экспериментальных исследований в этой области привело к тому, что еще мало данных известно о путях заражения рыб микроспоридиями, о специфичности этих паразитов, их патогенности и т. д. Основными критериями, которыми пользуются при описании новых видов, являются их способность развиваться в определенных тканях или органах одного или нескольких хозяев, а также размеры и форма спор. По этим признакам *Thelohania baueri* не может быть идентифицирована ни с одним из ранее описанных видов микроспоридий из икринок рыб (Табл. 3). Вопрос относительно микроспоридий из соединительной ткани колюшек намного сложнее. Видовая самостоятельность *G. anomala* и *G. gasterostei*, судя по разнице в размерах и форме спор, не вызывает сомнений. В то же время, исходя из описания спор *G. anomala* в ранних работах, можно предположить, что исследователи имели дело с несколькими разными видами, образующими у колюшек крупные соединительнотканные цисты. Так, размеры спор *Nosema* (*Glugea*) *anomala* по Монье (Moniez 1887) составляют 3.5×1.5 μm, по Телоану (Thelohan 1895) — 4.0–4.5×3 μm, по Штемпелю (Stempell 1904) — 6×2 μm (что примерно соответствует размерам спор *G. gasterostei* — 5.6×2.6), а по Вайсенбергу (Weissenberg, 1913) — 3.5×2.3 μm[1]. Положение еще более усложняется если учесть, что Штемпель и Вайсенберг имели дело с микроспоридиями только из одного хозяина — трехиглой колюшки и поэтому вариабильность в размерах спор нельзя отнести за счет развития паразита в разных видах хозяев. Телоан же обнаружил

[1] Сходные размеры спор (3.5×2.5 μm) микроспоридии *G. anomala* из трехиглой колюшки приводятся в работе Арм (Arme 1972).

паразитов со спорами одного размера как в трехиглых так и девятьюиглых колюшках.

По нашим данным разные микроспоридии из колюшек Финского залива были строго приурочены к определенным видам рыб, т.е., *G. anomala* к колюшке *Pungitius pungitius*, а *G. gasterostei* — к *Gasterosteus aculeatus*.

Противоречия в описании микроспоридии *G. anomala* в работах различных авторов можно объяснить, видимо, долго существовавшей традицией относить микроспоридий из одного хозяина к одному виду. Выходом из создавшегося положения должно быть, по нашему мнению, более внимательное отношение к размерам, форме и строению спор микроспоридий из колюшек разных мест обитания, так как не исключено существование у них другух видов паразитов. Чтобы окончательно подтвердить их видовую самостоятельность или идентичность необходимо проведение экспериментальных исследований по перектестному заражению колюшек этими видами микроспоридий. До тех пор пока такие исследования не будут проведены, мы предлагаем считать описанный в настоящей работе вид *Glugea gasterostei* как новый и сохранить за видом, паразитирующим в девятьюиглых колюшках, название *Glugea anomala* Moniez, 1887.

Резюме

Три вида микроспоридий было обнаружено в колюшках из Финского залива. За видом, инвазирующим главным образом подкожную соединительную ткань *Pungitius pungitius*, сохранено название *Glugea anomala* Moniez, 1887. Споры *Glugea gasterostei* sp. n. имеют более крупные размеры и другую форму в сравнении со спорами *G. anomala*. Третий вид, *Thelohania baueri* sp. n. заражает икринки *Pungitius pungitius* и реже *Gasterosteus aculeatus*. Наряду с грушивидными спорами этого вида наблюдали некоторые стадии шизогонии и спорогонии.

SUMMARY

Three microsporidian species were found in sticlebacks from Finnish Bay.

Glugea anomala (Moniez, 1887) Surley, 1893 — white cysts measuring 1–4 mm in the diameter most frequently situated in subcutaneous connective tissue. Oval spores measured 4.6 (3.5–5.1) ×2.3 (2.1–2.6) μm. Host — *Pungitius pungitius*.

Glugea gasterostei sp. n. — yellowish-white cysts measuring 3×4 mm were situated in the merentery, in the posterior part of the body cavity. Elongate-oval spores measured 5.6 (4.9–6.0) x 2.6 (2.1–2.8) μm. Big posterior vacuole occupied a half or even two third of the spore inside. After fixation in Boin's fluid the spores measured 4.1 (3.8–4.4)×1.9 (1.7–2.1) μm. Mucous enveloppe surrounding spores was not observed. *Glugea gasterostei* sp. n. was found only one in a female of *Gasterosteus aculeatus*.

Thelohania baueri sp. n. — a parasite of eggs of *Gasterosteus aculeatus* and *Pungitius pungitius*.

Pear-shaped spores with large posterior vacuole formed two dimensional groups: smaller spores measured 4.5–6.0 μm in length (mean dimensions 5.4×2.7 μm), and greater ones 6.0–7.9 μm long. Greater spores were more frequent in *Gasterosteus aculeatus*. Polar thread was 200–220 μm long.

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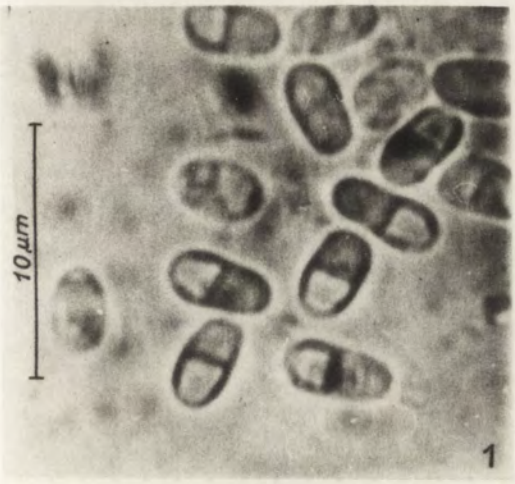
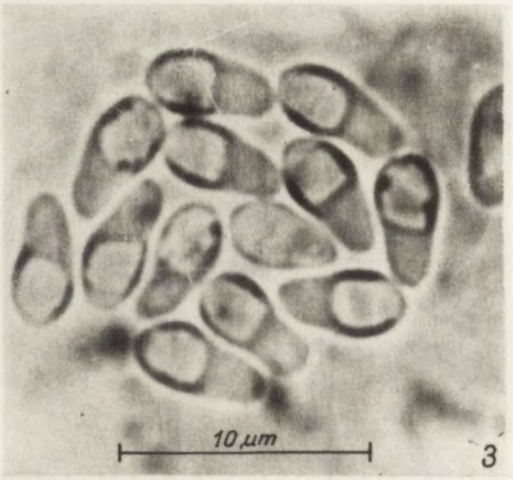
ПОДПИСЬ К ТАБЛИЦЫ I

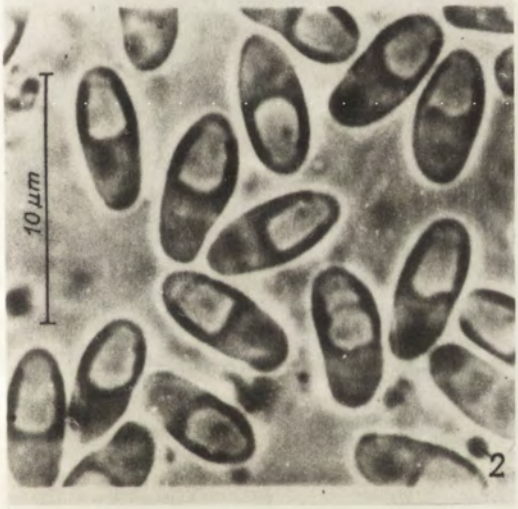
1-3: Живые споры. 1 — *Glugea anomala*, 2 — *G. gasterostei* sp. n. 3 — *Thelohania baueri* sp. n. — при одном и том же увеличении. 4 — Икринка колюшки *Pungitius pungitius* зараженная микроспоридей *Th. baueri* sp. n. (в центре) в проходящем свете

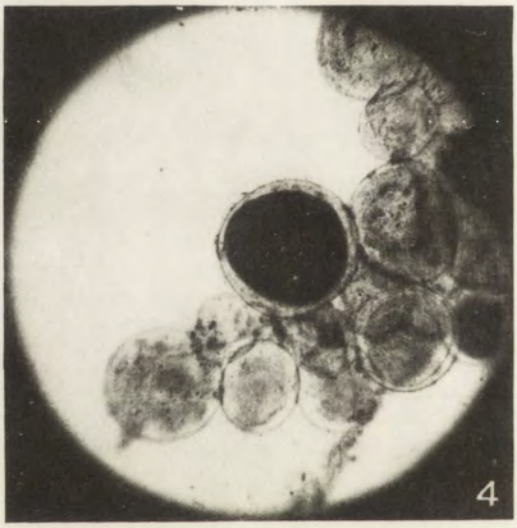
EXPLANATION OF PLATE I

1-3: Fresh spores. 1 — *Glugea anomala*, 2 — *G. gasterostei* sp. n., 3 — *Thelohania baueri* sp. n. — using the same magnification. 4 — The egg of *Pungitius pungitius* affected by *Th. baueri* sp. n. (in the middle) as seen in transmitted light









V. N. Voronin auctor phot.

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Zoological Survey of India, 8 Lindsay Street, Calcutta 16, India

A. K. MANDAL and K. N. NAIR

Observation on *Eimeria wassilewskyi* Rastegaieff (*Protozoa* : *Eimeriidae*) from *Axis axis* (Erxleben) in Andaman Island, India

Les observations sur *Eimeria wassilewskyi* Rastegaieff (*Protozoa* : *Eimeriidae*) de *Axis axis* (Erxleben) d'île d'Andaman, Inde

Rastegaieff (1930) described *Eimeria wassilewskyi* based on the observations of unsporulated oocysts isolated from the Spotted deer, *Axis axis* (Erxleben), obtained from India and kept as an inmate at Leningrad Zoo, Soviet Union. Pellerdy (1963) refers *E. wassilewskyi* recorded from *Cervus elaphus* Linnaeus (Red deer) and *Cervus nippon hortulorum* Swinhoe (Sika deer or Japanese deer) from different parts of the world. In the introductory note of Coccidia and Coccidiosis, Pellerdy (1965) felt the necessity of close investigation of the coccidian species occurring in members of the family *Cervidae* for determining the exact identity of the parasites. Recently Bhatia (1968) described a new species, *E. cheetali* and an undetermined species of *Eimeria* from *Axis axis* of Lucknow Zoo and also found the former in *Antelope cervicapra* (Linn.). Pande et al. (1970) also recorded *E. cheetali* from *Axis axis* of the same Zoo. However, in the monographic treatise of the coccidian parasites of ruminants Levine and Ivens (1970) remarked on the necessity of future study for establishing the validity of *E. wassilewskyi*.

It is evident from the foregoing account that *E. wassilewskyi* enjoys a wider distribution than the other species of *Eimeria* reported from the members of the family *Cervidae*. Inspite of the repeated records, no attempt is seen to be made to describe *E. wassilewskyi* Rastegaieff in detail. In a recent faunistic Survey (July–August, 1972) of the Andaman Islands, India, the authors had the opportunity to examine the Spotted deer (*Axis axis*) which are believed to have been introduced to the Bay islands from the Indian main land.

Material and Methods

Of the two live hosts and several faecal pellets examined, one live stag from Solbay, Wright Myo, Andaman Islands, India, proved positive infection and yielded oocysts of *E. wassilewskyi* in fairly

large numbers. These oocysts are in various stages of development. The oocysts were kept in 2.5% potassium dichromate solution for further observations and those are offered in this communication.

Description of *E. wassilewskyi* Rastegaieff

Oocyst egg-shaped (Fig. 1), measuring 17.5 μm to 19.5 μm in length with a mean of 18.5 μm and 13.5 μm to 15.5 μm in width with a mean of 14.25 μm. The shape index is 1.3. It is provided with double wall of 0.75–1.00 μm in thickness, the outer being thinner than the inner one and of yellowish in colour. The cytoplasmic mass is almost spherical, situated at the middle and appears like refractile globules

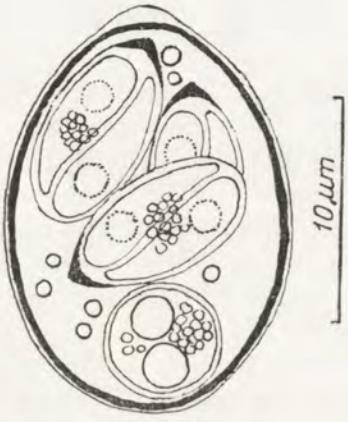

Fig. 1. *Eimeria wassilewskyi* Rastegaieff, oocyst

of 7.5–8.5 μm in diameter. A micropyle is seen at the anterior end of the oocyst measuring 3.5–4.5 μm. In the course of development, residual mass is seen as a spherical body measuring 3.75 μm in diameter and ultimately lies dispersed as 4–8 refractile bodies. The sporocyst is broadly elliptical in shape with tapering anterior end having a clear shiny area but without any distinct knob. The sporocyst measures 8.5 μm to 10.5 μm in length with a mean of 9.00 μm and 3.5 to 5.5 μm in width with a mean of 4.5 μm. The sporocystic residuum is present as globular concentrated central mass with a few scattered granules. The sporozoites are elongated, tapering at anterior end, almost blunt at the posterior. They lie with their broader ends apposed to the narrower ends of the other within the sporocyst. Each sporozoite measures 7.00–8.00 μm in length with a mean of 7.5 μm and 2.00-3.00 μm in width at its broader end with a mean of 2.5 μm. A clear hyaline refractile area is present at the broader end of the sporozoite, but the position of the nucleus is not clear. The sporulation time is 2–3 days at room temperature (30°C).

Discussion

The similarities in shape and size of the oocyst described above with *Eimeria wassilewskyi* Rastegaieff (1930) from *Axis axis* are taken into consideration for treating both to represent a single species.

The description of *E. wassilewskyi* given by Levine and Ivens (1970) is visibly discrepant from the original description (in oocystic size) of the species by Rastegaieff (1930) particularly from Table 1 of p. 393 and Table 3 of p. 399. It is apparent that Levine and Ivens (1970) erroneously ascribed *E. wassilewskyi* Rastegaieff, 1930 as having oocysts with higher dimension (28.8 × 27.9 μm). The oocysts with lesser dimensions (18.0 × 14.4 μm) represent the species *E. wassilewskyi*. Landram and Honess (1955) treated *E. hegneri* Rastegaieff, 1930 (Host *Cervus canadensis*) as conspecific with *E. wassilewskyi* Rastegaieff, 1930. However, due to the inadequate description of *E. hegneri* it is difficult to consider this species as a distinct one from *E. wassilewskyi* at present.

Acknowledgements

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Summary

Observations on the sporulation and sporulated oocysts of *Eimeria wassilewskyi* Rastegaieff, 1930 collected from the Spotted deer, *Axis axis* (Erxleben) at Solbay, Wright Myo, Andaman Islands, India are recorded.

RÉSUME

Le travail present les observations sur le sporulation et des oocyste sporulés d'*Eimeria wassilewskyi* Rastegaieff, 1930 recoltés de cérf *Axis axis* (Erxleben) provenante de Solbay, Wright Myo, d'île d'Andaman.

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Cytochemical Study of Diskinetoplastic Forms of *Trypanosoma equiperdum* and *Trypanosoma evansi*

Цитохимическое изучение дискинетопластных форм *Trypanosoma equiperdum* и *Trypanosoma evansi*

In the course of the last years a new wave of interest is observed to the known phenomenon (Werbitzki 1910, Kudicke 1911) of the loss of one organelle in the *Trypanosoma* cell — the kinetoplast and to the appearance of the so called akinetoplastic form as a result. It has been found rather recently that not the entire kinetoplast disappears after this process but only the DNA contained in it. The new arising forms had been called the diskinetoplastic (DK) and the whole phenomenon — diskinetoplastia.

The return to this interesting phenomenon on a level of investigation allowed to elucidate considerably which changes in the cell are accompanying the loss of the kinetoplastic DNA. The biochemical methods showed that besides the selective suppression of the kinetoplast DNA replication, the diskinetoplastic agents evoke: the inhibition of synthesis of the kinetoplastic RNA and the general mitochondrial cell activity. Simultaneously the activity of glycolytic enzymes becomes stimulated (Kussel et al. 1967, Steinert and Van Assel 1967, Steinert et al. 1969, Bayne et al. 1969, Hill and Anderson 1969, Simpson 1968, Renger and Wolstenholme 1970, 1971).

Those biochemical changes involve morphological changes in the cell. On the level of light microscopy those changes are manifested by the loss of the kinetoplast, and on the electron microscopical level by the loss or desintegration of kinetoplastic DNA as well as by the reduction and impairment of the mitochondrial system.

Nevertheless two uneven groups may be distinguished on the basis of their capacity to exist in the diskinetoplastic state. In the majority of species the absence of kinetoplastic DNA involves the loss of reproduction and transformation capacity as well as impairment of motion. It makes also impossible the formation of subcultures and leads finally to the death of the cell. An exception present the trypanosomes of the group brucei in the blood of vertebrate hosts: they remain viable under the above conditions i.e., they are able to produce real diskinetoplastic forms.

The above new data concerning the biochemical changes accompanying diskinetoplastia have been gained mostly on the species not capable for true diskinetoplastia i.e., in the cases of spurious diskinetoplastia. The lethality of those diskinetoplastic forms involves a difficulty in discriminating the general pathological changes in them from the real consequences of the lack of kinetoplast itself.

Unfortunately the literature provides no data concerning the changes evoked by the true diskinetoplastia in the brucei group. However, just those cases present the most interesting point in this subject. They not only indicate the different qualitative state of the cell but also a different role of kinetoplast in its metabolism. They also indicate changes accompanying diskinetoplastia sensu stricto without accumulation of general pathological changes.

The aim of the present study has been to analyse the changes occurring in the cells of *T. equiperdum* and *T. evansi* in the course of their transformation into the diskinetoplastic state after the action of acriflavin and the cytochemical peculiarities of the diskinetoplastic forms.

Material and Methods

The objects of our study were the blood froms of *Trypanosoma equiperdum* and *T. evansi*. The strains studied were cultivated in albino mice by means of regular subcutaneous transplantation at 4–5 days intervals. The diskinetoplastic forms of *T. equiperdum* and *T. evansi* were obtained by means of subcutaneous injection of acriflavin on the level of infection (on the 3rd-4th day after infection). The amount of injected acriflavin was 0.01 mg/g. of the mouse weight.

After the injection of acriflavin the blood samples were taken every day till the moment of the animal death. Samples were examined with regard to the dynamics of the parasite numerosity in blood and the process of accumulation of diskinetoplastic forms. On the smears stained according to Romanowski-Giemsa the mean number of trypanosomes was counted for the vision field of microscope (7×9) and the percentage of diskinetoplastic forms established for each 300 cells examined. Cells were considered as diskinetoplastic when their kinetoplast failed to stain at all.

For cytochemical analyses two points were selected: (1) 24 hours after the application of acriflavin i.e., at the moment of the mass death of trypanosomes and the onset of formation of diskinetoplastic forms, (2) 96 hours after the application of acriflavin i.e., the time of appearing large numbers of true diskinetoplastic forms. At these points the samples were taken for cytochemical comparison of the altered acriflavin-treatedf orms with the normal ones. The results of the study of normal individuals of *T. equiperdum* and *T. evansi* had been reported in our previous publications (Wartoń and Kallinikova 1971, Kallinikova and Wartoń 1972, Wartoń and Kallinikova 1973). The normal and the diskinetoplastic forms of each species have been compared utilizing the same cytochemical indicators: the content and topography of nucleic acids (DNA using the Feulgen reaction, RNA according to Brachet) proteins according Mazia, histones (10 B Amido black staining according to Alfert and Geschwind), SH-containing proteins (Barnett and Seligman), Janus green staining determination of cytochrome oxidase activity according to Moog, NAD-H and NADP-H dehydrogenases (Nachlass), peroxidase (benzine method).

Results

Soon after the introduction of acriflavin, the number of *Trypanosoma equiperdum* and *T. evansi* individuals in the blood of mice began to fall and after 48 h the parasites were practically absent (Pl. I). However, even 24 h later single individuals appeared again and 96 h after the application of acriflavin their number became high and continued to increase. Finally, 6–7 days later the mice died of the infection.

This parasitemia was accompanied by the appearance of the diskinetoplastic forms. Normally, prior to the application of acriflavin, the number of those forms remained on a rather low level and constituted up to 2.4% in *T. equiperdum* and 6.99% in *T. evansi*. 24 hours after application of acriflavin the number of diskinetoplastic forms increased considerably and continued to rise steadily independently of the fluctuations in the general population of trypanosomes. It reached a maximum (91% for *T. equiperdum* and 87% for *T. evansi*) after 96 h and remained at this high level till the death of the mice.

In this way, two fundamental moments are observed in the dynamics of the trypanosome density after the introduction of acriflavin:

(1) 24 hours after the introduction of the compound: the moment of a mass destruction of trypanosome and appearing of the first diskinetoplastic forms.

(2) 96 hours after the treatment — the moment of appearing of a high number of viable diskinetoplastic forms.

Just these moments were chosen by us for the cytochemical analysis of the trypanosomes.

As it has been indicated above (Kallinikova and Wartoń 1972, Wartoń and Kallinikova 1973), both species studied showed very similar cytochemical characteristics in the normal form. After the action of acriflavin, the changes in the cells of both species proved to be essentially the same.

24 hours after the introduction of acriflavin, the Feulgen reaction failed to reveal DNA in the kinetoplast and was much less intense in the nucleus of the majority of the remaining cells of *T. evansi* and *T. equiperdum*.

At this point the cells are characterized by a much lowered content of RNA. The staining with pyronin is pale and diffuse. Against this background the brown grains of volutine are distinctly visible, mainly in the anterior end of the cell — rather rarely around the kinetoplast which in those conditions is either not stained or not visible at all against the bright-red background of the cytoplasm. Methylgreen stains only the nucleus.

The intensity of the general protein staining is also much lowered. The cytoplasm is very pale after staining. Neither kinetoplast nor nucleus are discernable against this background. After hydrolysis, staining becomes somewhat more intense which indicates that a part of the proteins remains still bound to nucleic acids after the application of acriflavin. After hydrolysis, however, none of the organelles become differentiated against the background of the cell.

In the material prepared for demonstrating the SH-containing proteins, trypanosomes were too few for an evaluation of the changes in concentration of those proteins. However, a decrease of the intensity of the reaction was observed.

In the few remaining cells the presence of histones could not be demonstrated even in the nucleus.

Much fewer granules stain with Janus green in comparison with normal cells. They also become brown more slowly than prior to the introduction of acriflavin. Nevertheless the granules situated on the position of kinetoplast still appear first.

The activity of cytochrome oxidase being not very high in the normal cells, becomes still less conspicuous 24 h after application of acriflavin (Pl. II 4, III 11). The indophenolblue grains are not only more scarce but they stain more slowly than in the normal cells. In all the cells some granules occur in an undefined localization.

The reaction for NAD-H dehydrogenase remains positive but slightly less intense than in normal cells (Pl. II 5, III 14). Sometimes 2–3 big clumps are formed instead of small granules which remain also present.

The NADP-H dehydrogenase activity is much more lowered and often is limited to one big granule and — rather rarely — to 2–3 small granules situated outside kinetoplast area (Pl. II 8, III 17). Some cells occur which are deprived of this enzyme activity.

The peroxidase reaction remains negative as in the normal cells even after injection of acriflavin.

At the period of a new invasion of trypanosomes in the blood i.e., 96 h after the injection of acriflavin, the loss of a positive DNA reaction in the kinetoplast becomes evident in nearly all the cells. In the nucleus the DNA reaction remains being, however, sometimes less intense than under normal conditions. This is evident from both the Feulgen reaction and the Methylgreen staining.

For the numerous cells of *T. equiperdum* and *T. evansi* present at his moment the nearly normal picture of RNA is characteristic. It deviates from the norm by a higher number of volutine granules placed mostly at the end of the cell opposite the kinetoplast (Pl. I). Those granules are diffuse, without distinct outlines. One of them — the biggest — is often situated at the end of the kinetoplast.

In harmony with the RNA picture the normalization of the cytochemical picture of the general proteins takes place in both trypanosoma species (Pl. I). Sometimes the protein staining seems even to be more intense than normal. After hydrolysis, staining becomes more bright and homogenous. In contrast to the normal results, the nucleus fails to appear against the background of the cell even after hydrolysis. Staining of volutine granules becomes metachromatic.

The reaction of SH-containing proteins does not deviate from that of the normal cells. It is, however, sometimes weaker, especially in the flagellum (Pl. I).

Histones still fail to appear in the nucleus, even 96 h after the introduction of acriflavin when cells are viable although being diskinetoplastic (Pl. I). The kineto-

plast either does not differentiate against the background of the cell or its place is only marked by a certain condensation of stain.

In both *Trypanosoma* species the staining with Janus green is much less intense when compared not only with the norm but also with the results studied in the first 24 h of acriflavin action upon trypanosomes. Before all, the number of granules which oxidize this dye is much reduced. Besides, the sequence in staining is not observed, as it was in the case of normal cells (first — kinetoplast and the zone around it, and afterwards the remaining regions of the cell). In the diskinetoplastic cells, the granules which remained, stain simultaneously. Many cells fail to oxidize Janus green.

The picture of demonstrating the cytochrome oxidase activity of trypanosomes in the course of 96 h after injection of acriflavin is not uniform (Pl. II 3, III 12). In the majority of cells it is below the norm as well as below its level in the experimental trypanosomes studied 24 h after the application of the compound. Nevertheless cells occur in which the activity of the enzyme is not only reduced but — in the contrary — raised over the norm.

In the majority of cases the reaction for NAD-H-dehydrogenase remains positive in both species studied (Pl. II 6, III 15), being, however, still more distinctly lowered as to the norm than it was 24 h after the action of acriflavin. Now it is represented by tiny grains only.

Simultaneously, the reaction for NADP-H-dehydrogenase is negative in the majority of cells of both species. In the few cases when the positive reaction remains, it is considerably suppressed when compared as well with the norm as with the picture of the enzyme activity after the 24 h long action of acriflavin being represented by 1–4 granules only (Pl. II 9, III 18).

The peroxidase reaction continues to be negative.

Discussion

The studies presented above indicate that after the action of acriflavin distinct changes occur in the population density of *T. equiperdum* and *T. evansi* in the blood of mouse, in the morphology of the parasites, and in their metabolism. Those changes have their dynamics.

At the first stage, within the initial 24 h of the acriflavin action, a mass death of trypanosomes takes place in conformity with the symptoms of the toxic action of the agent — DNA fails to be manifested in the kinetoplast, being also less intensely stained in the nucleus. The reaction for: RNA, general proteins, SH-bound proteins and histones are considerably below normal levels.

The mitochondrial activity of the cell (staining with Janus green B, cytochromoxidase, diaphorases) are considerably suppressed. The cells of a similar cytochem-

ical characteristic as described above cannot remain viable for a long time. Those metabolic changes together with the fact of destruction of the most fundamental part of the trypanosome should be looked upon as a manifestation of a general toxic action of the applied amounts of acriflavin.

Simultaneously, at this phase of acriflavin action, the number of diskinetoplastic forms starts to increase, DNA ceases to appear in the kinetoplast i.e., the transit to diskinetoplastia sets on. Consequently the first days of acriflavin action present a complicated picture of the general toxic effect and of diskinetoplastia itself. The cytochemical data indicate that although the "hot point" of the compound action is the kinetoplastic DNA, the action of acriflavin spreads over the whole cell, being mostly reflected in the nucleus since its histones fail to appear and the DNA reaction is less intense.

The dynamic study of the acriflavin action permitted to discriminate the general toxicologic effect from the changes connected with the diskinetoplastia itself.

Diskinetoplastia in its pure form is presented in trypanosomes appearing again in the blood of mice after the 96 h long action of acriflavin. At this time, about 90% of cells are deprived of the kinetoplast visible under light conditions, being, however, fully viable: they move actively, multiply and bring about the death of the host. Those forms differ by their cytochemical characteristics from the normal trypanosomes of the cells at the moment of the toxic effect. Their characteristic is not only the disappearance of the kinetoplastic DNA but also the normalization i.e., restitution of the cytochemical reactions for RNA and proteins up to the normal level which is in conformity with the viability of diskinetoplastic forms and with preservation of their fundamental life functions. In spite of this, the mitochondrial activity of those forms is still more suppressed than it was at the moment of the toxic action of acriflavin. Many cells fail to oxidize Janus green at all and to reveal the activity of flavine enzymes. The activity of cytochrome oxidase is more complicated. Despite its suppression in the majority of cells — it seems even to rise in a part of them. This has been observed by some other authors (Thirion and Kupersztejn 1968).

The above results may be explained by two possibilities: either the raised activity of the enzyme is due to the few trypanosomes which preserved their kinetoplast, or the cytochrome oxidase is not connected with the activity of the kinetoplast in the species studied.

As it has been mentioned above, little is known about the true diskinetoplastic forms of the *brucei* group of trypanosomes although the study of diskinetoplastia had been initiated in this group. Despite the preservation of their fundamental vital functions and the loss of the kinetoplastic DNA, the suppression of their oxidative activity has been shown biochemically (Vickerman 1965).

The cytochemical study of the diskinetoplastic forms of *T. evansi* and *T. equiperdum* have also shown the impairment of the kinetoplast, the loss of kinetoplastic DNA — even in the *brucei* group reflects on the cellular metabolism and most

important, on the oxidative activity. Consequently, despite the reduction of the "kinetoplast–mitochondrion" system in the *brucei* group and the loss of the "key-position" by kinetoplasts in the metabolism of those species (Kallinikova and Wartoń 1972, 1973), its role in cell metabolism remains actual a considerable degree.

The cytochemistry of the diskinetoplastic forms under study indicates that diskinetoplastia sensu stricto — besides the well known inability of kinetoplast to stain for DNA — is characterized by suppression of the respiratory activity, by a loss (up to a certain degree) of activity of the flavine enzymes and by a fully normal cytochemical picture of RNA and proteins. The normalization of the RNA reaction is also relative: the number of "volutine" granules is much increased in the diskinetoplastic forms. It should be also mentioned that in these forms the nucleus deviates from the norm in its cytochemical characteristic. Its DNA content seems to be reduced and the presence histones cannot be demonstrated.

The absence of peroxidase activity in those forms indicates that, in spite of the alteration of the respiratory turnover, the reserve way of oxidation with the share of H_2O_2 is omitted.

It is known that — despite their viability — the diskinetoplastic forms of the *brucei* group loose one of their biological characters i.e., the capacity to continue their cycle in the invertebrate host. Presumably this unique biological "defect" is associated with their metabolic peculiarities revealed cytochemically.

Summary

Appearing of diskinetoplastic forms of *T. equiperdum* and *T. evansi* after the action of acriflavin is accompanied at the first stage by a general toxic effect. 24 h after introduction of acriflavin a complex picture is observed of this non-specific effect and of the onset of appearing of diskinetoplastic forms.

The general toxic action is manifested as a sharp suppression of the RNA and protein reactions and a much less distinct inhibition of the respiratory enzymes. The diskinetoplastic forms appear in large numbers, 96 h after the introduction of acriflavin. They differ by their cytochemical characteristics from the normal trypanosomes and from the cells at the moment of the general toxic effect. The loss of kinetoplastic DNA is characteristic for them as well as a sharp suppression of the respiratory activity, in particular the activity of flavin enzymes and abundance of volutine, some deviations from the norm in the cytochemistry of nucleus. The picture of RNA and proteins remain generally normal.

The metabolic peculiarities of diskinetoplastic forms of the *brucei* group trypanosomes — as found in this study — may explain their inability to continue their cycle in an invertebrate host.

РЕЗЮМЕ

Возникновение дискинетопластных форм *T. equiperdum* и *T. evansi* под влиянием акрифлавина сопровождается на первых этапах общетоксическим эффектом. В первые сутки после введения препарата наблюдается сложная картина этого неспецифического эффекта и начала возникновения дискинетопластных форм. Общее токсическое действие выражается в резком подавлении реакции на РНК, белки и менее значительнон ингибировании дыхательных ферментов. Сами дискинетопластные формы *T. equiperdum* и *T. evansi* появляются в массовом количестве через 96 часов после введения акрифлавина и отличаются по своей цитохимической характеристике как от нормальных трипаносом, так и от клеток в момент общетоксического эффекта. Для них характерна потеря кинетопластной ДНК, резкое подавление дыхательной активности, в частности активности флавиновых ферментов, обилие волютина, некоторое отклонение от нормы в цитохимии ядра и в общим нормальная картина РНК и белков.

Найденные метаболические особенности дискинетопластных форм трипаносом группы *brucei* могут объяснить их неспособность продолжать цикл в беспозвоночном хозяине.

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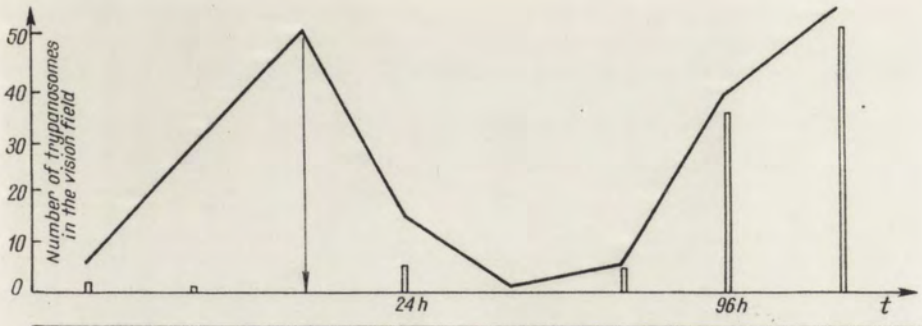
EXPLANATION OF PLATES I–III

I. Action of acryflavin on the number of trypanosomes and on the cytochemical characteristic of the trypanosome cell

II. *Trypanosoma equiperdum* — action of acryflavin on the activity of respiratory enzymes
Cytochrome oxidase reaction: norm (1), after 24 h (2), after 96 h (3).
NADH-dehydrogenase reaction: norm (4), after 34 h (5), after 96 h (6). NADPH-dehydrogenase reaction: norm (7), after 24 h (8) after 96 h (9)

III. *Trypanosoma evansi* — action of acryflavin on the activity of respiratory enzymes
Cytochrome oxidase reaction : norm (10), after 24 h (11), after 96 h (12). NADH-dehydrogenase reaction: norm (13), after 24 h (14), after 96 h (15). NADPH-dehydrogenase reaction: norm (16), after 24 h (17), after 96 h (18)



Cytochemical reactions	Norm	Effect of acryflavine			
		after 24 h (diskinetoplastia + toxicologic effect)		after 96 h (diskinetoplastia)	
		T. equiperdum	T. evansi	T. equiperdum	T. evansi
DNA					
RNA					
General proteins					
Histones					
Stt-containing proteins					
Janus green					
Cytochrome oxydase					
NAD-H dehydrogenase					
NADP-H dehydrogenase					

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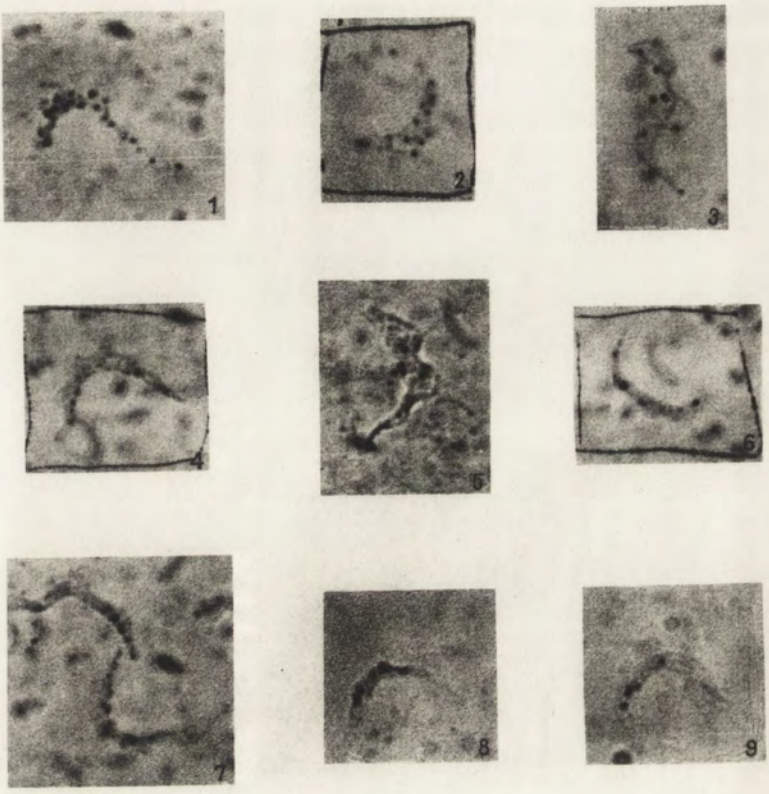

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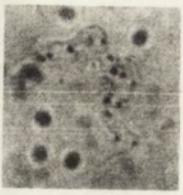
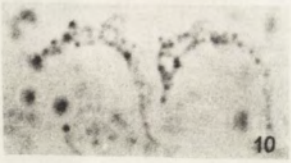
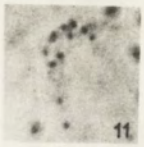
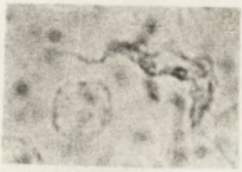
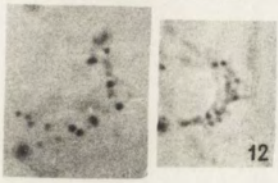
PLATE III

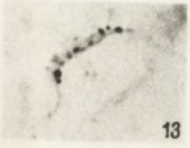


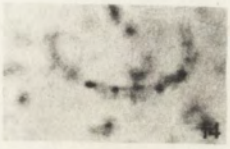


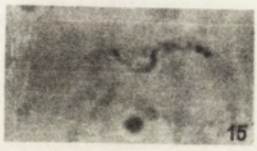


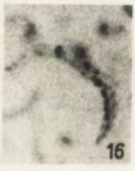


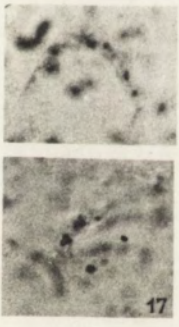


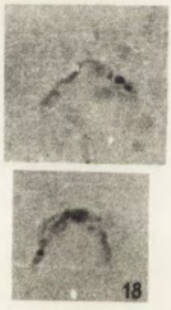












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Tome VIII

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Fascicule 4

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