PL ISSN 0065–1583

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

VOLUME 22

Number 2

W A R S Z A W A 1 9 8 3

POLISH ACADEMY OF SCIENCES
NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY

ACTA PROTOZOOLOGICA

International Journal of Protozoology

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ACTA PROTOZOOLOGICA appears quarterly. The indexes of the previous volume appear in No. 1 of the next volume.

Indexed in Current Contents and in Protozoological Abstracts.

ACTA PROTOZOOLOGICA
Vol. 22, No. 2, pp. 97–126 (1983)

The suborder *Urostylina* Jankowski (*Ciliophora, Hypotrichida*): Morphology, Systematics and Identification of Species

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Received on 15 March 1982 *and revised on* 28 November 1982

Synopsis. We analyze objectively the variability of characters of internal anatomy and ciliary structure applicable to species identification in urostyline hypotrichs. We conclude that cell shape, especially length/width ratio, habitat, number and arrangement of nuclei, number and distribution of cirri developing from malar and paramalar streaks (defined in text), number and distribution of midventral cirri, and number of rows of right marginal cirri are particularly useful in species identification. By contrast, certain time-honored characters such as cell length, number of rows of left marginal cirri, number of transverse cirri, and characters of endoplasmic inclusions including food vacuoles are probably not safely applicable to species identification. We describe *Bakuella variabilis* sp. n., *Holosticha estuarii* sp. n., and *H. polystylata* sp. n. We erect *Pseudokeronopsis* gen. nov. to contain species formerly assigned to *Keronopsis*. The paper includes a binary key for identification of all species and genera in the suborder, followed by notes on nomenclature and a listing of synonymy of all species.

Present understanding of ciliates of the order *Hypotrichida* reflects three centuries of progress. Antoni van Leeuwenhoek's 18th letter to the Royal Society of London, read before them in February, 1677, contained the first description of hypotrichs. In 1786, Müller still placed most of them in two genera. During the following century, many microscopists began publishing on these easily recognizable free-living protozoa, of which the impressive monographs by Ehrenberg (1838 and earlier works) and Stein (1859) remain particularly valuable. The second century of "hypotrichology" came to a climax with the nearly simultaneous appearance of the third major section of Bütschli's treatise on the protozoa (1887—1889) and Stokes' listing the freshwater infusoria of the United States of America (1888). These volumes, along with Kent's manual (1881) had to suffice for 50 years for identification of hypotrichs.

The encyclopedic publications of Kahl (especially 1932, 1935) on the free-living ciliates of the world exclusive of the tintinnids still provide the main means of identification of hypotrichs.

In 1961, Fauré-Fremiet erected two suborders and arranged hypotrichs into families on the basis of the number and arrangement of the ventral ciliature during interphase. In the same year Corliss gave a diagnosis of the order and clarified some nomenclatural problems by listing junior synonyms of the genera he recognized.

Borror's 1972 revision of the order arranged genera into 6 families on the basis of morphological characters and hypotrich biology as understood at that time, diagnosed all families and genera, and listed synonymies for each of 310 species.

The past decade has seen major advances in biology in general, and knowledge of hypotrich cytology, morphogenesis, chemistry, genetics, and microdistribution has increased in an expected manner. At least 30 new species of urostylines have been described since Borror's 1972 revision.

Our understanding of ultrastructural homologies among ciliary structures has expanded via scanning and transmission electron microscopy (see especially papers by Grimes, Hammersmith, Jerka-Dziadosz, and Walker) such that published information is now available on the fine structure of species in at least 17 genera. As discussed earlier (Borror 1979), descriptions of cortical morphogenesis now exist in a much wider range of genera than was the case in 1972. Recently Swanton et al. (1980) indicated intergeneric and interpopulational differences at the molecular level of possible significance to hypotrich evolution and speciation.

Continuing investigation of hereditary systems in protozoa allows further understanding of the interplay of genetics and environmental factors in influencing characters of systematic importance among hypotrichs (see especially works by Frankel 1972 and Génermont 1976). At the same time, a greater appreciation of intrapopulation variability in hypotrich structure (e.g., Gates 1979, Tuffrau 1977) necessitates reconsideration of many previous decisions regarding species separation in ciliates. Genetically isolated groups of populations in nature represent a complex spatial arrangement, and may not always be separable on the basis of classical morphological criteria, while at the same time morphologically different, but presumably genetically related, forms may have unexplained differences in distribution (Borror 1980, Bamforth 1981).

During the last 9 years many taxonomic changes have been suggested for hypotrichs. In 1974, Stiller published a lengthy treatise on hypotrichs summarizing previous work and suggesting some nomenclatural changes. In 1977 Corliss outlined a classification of hypotrichs involving 11 families: this was expanded into a listing of assigned genera in 1979.

In 1979, on the basis in part of morphogenetic characters, Borror redefined the *Urostylidae* to contain only the genera *Urostyla* (type), *Bakuella*, *Holosticha*, *Keronopsis*, *Pseudourostyla* and *Uroleptus*. He removed *Amphisiella*, *Paraurostyla*, *Kahliella* and *Paraholosticha* from the *Urostylidae* on morphogenetic grounds. *Balladyna*, *Banyulsella*, *Lacazea*, *Kerona*, and *Epiclintes* were considered of uncertain systematic position. Since then, Wicklow (1979) has shown that neither *Kerona* nor *Epiclintes* resemble urostylids morphogenetically.

In that same year, Tuffrau described a new family, *Kahliellidae*, and presented a proposed classification of hypotrichs he considered to be in the suborder *Stichotrichina* Fauré-Fremiet, 1961. Simultaneously, Jankowski (1979) divided urostylids among 2 superfamilies and 7 families (3 new). In 1981, Wicklow provided a critical review of Jankowski's classification. In 1980, Jankowski erected the name *Keronina* as a replacement name for the suborder *Urostylina* Jankowski 1979. Both Tuffrau and Jankowski documented their changes by reference to previous publications, offering little new experimental or observational evidence of natural relationships.

In 1981, Wicklow emphasized morphogenetic differences among these genera, and described a new but closely related genus *Thigmokeronopsis*. He divided urostyline genera into three families and two superfamilies. He recognized the subordinal name *Urostylina* Jankowski 1979, and defined the suborder to correspond to Borror's 1979 definition of the family *Urostylidae*.

With some reluctance we must now consider a topic of considerable nomenclatural importance. To begin, we must refer particularly to Hemberger and Wilbert's recent paper (1982) relative to the status of *Keronopsis* Penard 1922. Although technically this is the sort of nomenclatural detail to be included in a later section of this paper, its full presentation here, in the introduction, will hopefully reduce some of the confusion that might otherwise result from the issues involved.

Penard (1922) erected the generic name *Keronopsis* for one new species (*helluo*). Kahl (1932) considered it the type species of a subgenus of *Holosticha* that later (Borror 1972) attained full generic status as recently redefined (Borror 1979).

Hemberger and Wilbert (1982) reexamined Penard's description, pointing out that the ventral rows of cirri in *K. helluo* are more widely spaced than the urostyline pattern. Further, they noted that *K. helluo* divides only within a cyst membrane.

Penard drew a compressed specimen with skewed rows of cirri and a distended contractile vacuole. Nonetheless, a row of malar cirri (as defined below) is obvious as well as cirri at the anterior end of the frontal field interpretable as being of the same alignment as in *Paraholosticha* and *Kerona*.

We agree with Hemberger and Wilbert that all these characters define species previously included in *Paraholosticha*. We agree further that these characters are of diagnostic importance at the generic level. Since *Keronopsis* Penard (1922) predates *Paraholosticha* Kahl (1932), then the latter must fall as a junior synonym, and *Keronopsis* becomes the correct generic placement for most, if not all, species formerly in *Paraholosticha*. By such interpretation, *Keronopsis* Penard is a genus of nonurostyline hypotrichs closely related to *Kerona* in the *Keronidae*, as concluded by Hemberger and Wilbert 1982.

Unfortunately, this creates a nomenclatural problem. Although Hemberger and Wilbert suggested that species formerly in *Keronopsis* be shifted to *Holosticha*, we believe that they are distinct from *Holosticha* at the generic and indeed the familial level. We thus erect *Pseudokeronopsis* gen. nov. with *Pseudokeronopsis rubra*

(Ehrenberg, 1838) as type by original designation to contain most if not all the species other than *K. helluo* listed by Borror (1972) under *Keronopsis* (see key and species list).

Over the past decade we've examined hypotrichs from over 30 genera, including division processes in over 20 of these. Since 1971 we have isolated urostylines from collections representing freshwater, terrestrial, and marine habitats. These investigations, along with published information, have permitted sorting out genera validly contained in the suborder (Borror 1979, Wicklow 1981). Repeated isolations from nature have further allowed observation of the variety of morphology within populations and understanding of the relative value of characters we've used previously for species identification.

The following presents an objective analysis of variability of characters of internal anatomy and ciliary structure applicable to species identification in urostylines. Subsequent development of a valid set of diagnostic characteristics allows description of three new species and construction of a key to urostyline species. These are augmented by listing of synonymies and discussion of nomenclatural issues.

Materials and Methods

During the last decade we isolated urostylines from about 60 original field collections, representing such habitats as *Sphagnum* bogs, freshwater marshes, roadside ditches, soil, lichens and mosses from rocks and trees, tidal marsh sediment, marine sand and gravel, intertidal rock pools, and seawater. We examined most collections preliminarily within a day of collection, isolating for further culture hypotrichs encountered. We maintained cultures at 20° C. A variety of additives including rice grains, split peas, and Cerophyl provided fungal and bacterial nutrients for bacterivorous hypotrichs. Diatom cultures or a *Tetrahymena* species served as a source of nutrition for algivores and carnivores, respectively. Additionally, many stocks grew in unpurified aliquots of the original collection.

We employed a variety of cytological methods, including a nigrosin method (Borror and Evans 1978) and a protargol technique for surface structures, and Feulgen nucleal reaction, hematoxylin, and acetocarmine techniques for nuclei. Preparations for SEM followed techniques in Wicklow 1981.

Line drawings to show anatomical features, new species described, and species for which few accurate published illustrations occur, were executed with ink on scratchboard. Type material of new species is in the slide collection of the senior author. This paper is contribution No. 133 of the Jackson Estuarine Laboratory of the University of New Hampshire.

Results

The following paragraphs outline external and internal morphological characteristics and ecological relationships that apply in species identification of urostylines, as well as descriptions of three new species.

Internal Features

Nuclei

Macronuclei usually conform to one of three general configurations, multiple, moniliform, or double.

Multiple macronuclei occur in sufficient number (usually in excess of 16 units) that they occur irregularly through the cytoplasm. In the simplest configuration, they're distributed in an irregular helix along a U-shaped sequence or in what appears to be at least two linear groupings. Adjacent fragments suggest they still maintain nuclear membrane continuity. As fragments increase beyond 64, patterns are less discernable. Such configuration is the most widespread (in all genera but *Uroleptus*); it co-occurs more than other nuclear configurations with multiplicity of frontal cirri and variability in cirral patterns, thus may be a primitive condition.

Moniliform macronuclei occur in an even or raggedly linear group of 8–16 units aligned more or less in a single series. Individual variants in some populations occasionally only have four fragments.

Double macronuclei occur in some species of all genera except *Pseudourostyla* and *Thigmokeronopsis*, and in all species of *Uroleptus*. The two fragments are aligned in the left cytoplasm. Occasionally, populations of hypotrichs in all other ways resembling known species have 4 macronuclei instead of 2.

Prior to fission, the macronucleus condenses into a single entity. By cytokinesis, the macronucleus has begun to divide such that each daughter cell has at least two subunits. In species with moniliform or multiple patterns, it takes a period of time, post-fission, to attain the maximum number of macronuclear units, which will also vary as a function of generation time and cell size.

The number of micronuclei is not well-quantified. In most cases urostylines have 2–4 micronuclei, either scattered along the multiple or moniliform series, or closely adjacent to each of the two macronuclear units. As the exception, three species of *Holosticha* have one relatively large micronucleus situated between the two macronuclear units.

Cytoplasmic features

Pigmented cortical granules occur in many species. Patterns of granule grouping, distribution, size, and shape don't vary much within populations. Similar granulation patterns reappear in populations from different locations in nature. Density of granules and the intensity of pigmentation of individual granules vary and probably have a dietary basis.

Endoplasmic inclusions occur in some urostylines. For instance *Holosticha milnei* Kahl, 1932 (page 582, Fig. 108) supposedly has spherical refractile bodies, often at either end of the cell. We have seen similar bodies in members of populations of *Holosticha diademata*. The distribution and number of such bodies are

not constant within a population, since they're produced out of synchrony with cell division.

Zoochlorellae may occur in the cytoplasm. The complete identity otherwise in both internal and external (including ciliary) structures of pairs of green and non-green populations suggests that simple possession of zoochlorellae should not suffice for distinguishing species.

External Features other than Ciliation

Shape

Specializations of shape such as the "tail" of *Uroleptus* or the cephalization of *Holosticha discocephalus* are easily observable. More subtle differences in form are less easily quantified. Fixation artifacts may alter shape of stained specimens; coverslip pressure easily distorts hypotrichs. Finally, natural variation occurs within populations, especially in reorganizing or aged cultures.

Length/width ratios show little variation within populations, or between populations recovered from widespread localities identified by independent means, except for what is to be expected in prefission or reorganizing individuals or in nutritional giants. For instance, the L/W ratio in *Holosticha violacea* is greater than 6/1, in *H. vernalis* about 4/1, and in *H. mystacea* about 2/1.

Buccal cavity length varies considerably within a population, and presents innate difficulties in making a precise measurement because of the complex assymetrical three-dimensional structure of the buccal region.

Cell length is often the easiest and the first measurement to take, thus there is considerable evidence that length varies within a culture as a function of nutritional state and culture age. Often one isolates populations identical in all respects except for differences in cell length. This may reflect growth temperature, diet, generation time, and other known environmental variables not necessarily linked to genetic stability.

Suppleness is displayed to some degree by all urostylids, such that they bend laterally while feeding unhampered on the substratum. This is a difficult character to judge, but has been employed to separate hypotrichs of other families (e.g., the stiff *Stylonychia* from the usually supple *Oxytricha*).

Characters of the Ciliature

The following terminology follows definitions in Borror (1979) except as herein redefined (Fig. 1).

Urostylines bear buccal, frontal, and somatic ciliature that are usually distinct ontogenetically and spatially (Borror 1979). Buccal ciliature includes the AZM, endoral membrane, paroral membrane, and paroral cirrus (Fig. 1). Frontal cilia-

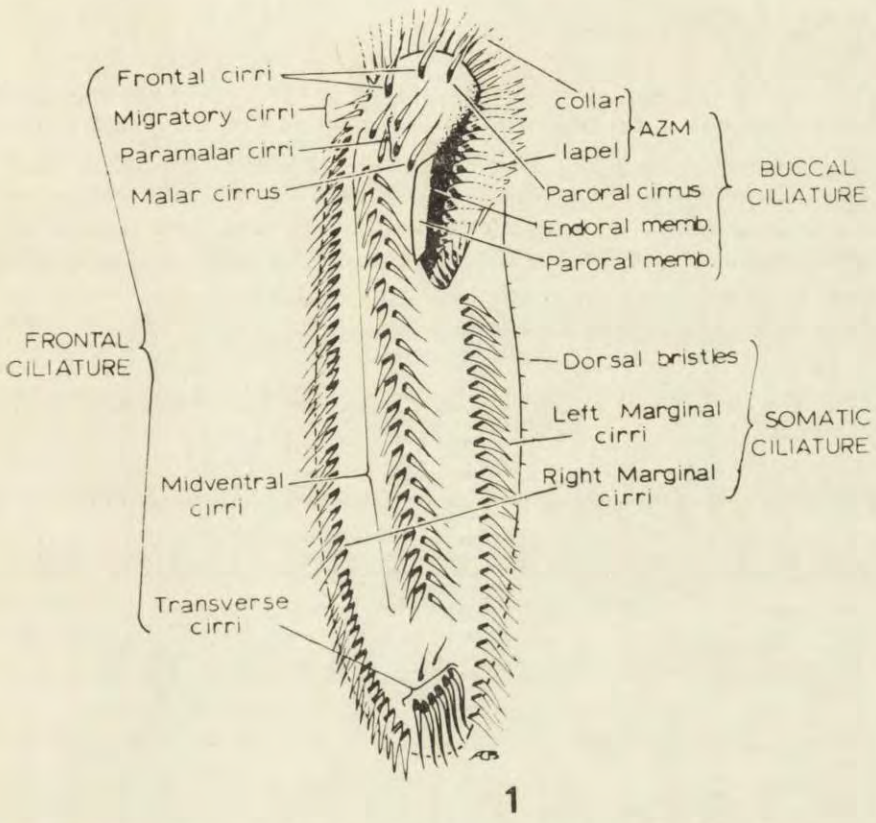


Fig. 1. Diagrammatic representation of ciliary organelles of a generalized urostyline hypotrich, ventral aspect, depicting the terms used in this paper to describe various parts of the frontal, buccal, and somatic ciliature. Figs. 2–23 represent the ventral aspect of various urostyline hypotrichs, showing ciliary organelles outlined in black, ciliary bases black, endoral membranes (where visible from this aspect) as white lines against a black background, and macronuclear nodes outlined in dash lines. Brackets indicate lengths of 20 μm. All are executed with ink on scratchboard

ture includes a longitudinal series of midventral cirri (usually in a double or "zig-zag" group near the ventral midline), transverse cirri posterior to the midventral cirri, and additional cirri on the anterior ventral surface described in detail in the following section (Fig. 1). Somatic ciliature includes dorsal bristles and right and left marginal cirri. We employ "somatic" here in a ciliatological sense of being the opposite of "buccal" (Borror 1979), rather than as the embryological opposite of "generative".

The following paragraphs discuss the sorts of variability in each ciliary type that occur within and among populations of urostylines. Note that generally the larger the cell, the larger the cirri (the greater the number of kinetosomes per cirrus) within a population (Bąkowska 1981).

Buccal Ciliature

AZM. Although some descriptions at the level of the light microscope allege differences between the collar membranelles and those of the left lateroventral side of the buccal cavity (lapel), such differences may reflect merely the stage of the life cycle. Under certain circumstances the cell may regenerate the lapel giving the AZM a split appearance. There are, to be sure, differences in the number and extent of rows, and length, of cilia in the collar and lapel membranelles as well as differences in the total number of membranelles in the AZM.

Other Buccal Ciliature. It has only been with application of electron microscopy that a general understanding of the three-dimensional array and morphogenesis of the "undulating membrane" has been reached (Jerka-Dziadosz 1981). The endoral membrane is a single file of cilia in the buccal cavity. Each of these cilia beats for about half its length in close contact with the roof of the buccal cavity. The posterior end of the paroral membrane usually terminates anterior to the posterior end of the buccal cavity. In some populations, the anterior end of the paroral membrane continues to the left across the anterior part of the buccal cavity, forming a hook-like extension superficially similar to the sort of specialization in some members of the genus *Oxytricha*. The paroral cirrus (or cirri) differentiates from the anterior terminus of the developing undulating membrane.

Frontal Ciliature

Frontal ciliature shares a common morphogenetic origin from a field of cilia differentiating on the ventral surface adjacent to the parental paroral membrane, or, in the opisthe, to the right of the opisthe oral primordium. In urostylines, frontal ciliature develops from a series of more than five streaks. The streaks are oblique to the long axis of the cell, except, for the 2–3 nearest the paroral membrane that are more longitudinal. Each streak usually differentiates into only about 3–4 cirri. Definitive arrangement of cirri occurs through combined partial resorption of some cirri, migration, differential hypertrophy, and rotation of elements of streaks.

The streak closest to the paroral membrane gives rise to a frontal cirrus at the extreme anterior end of the ventral surface of the cell, and one or more malar cirri adjacent to the paroral membrane. The next mediad streak gives rise to one or more frontal cirri and, in a few cases, to a short row of paramalar cirri in a longitudinal group between the malar cirrus and the midventral cirri.

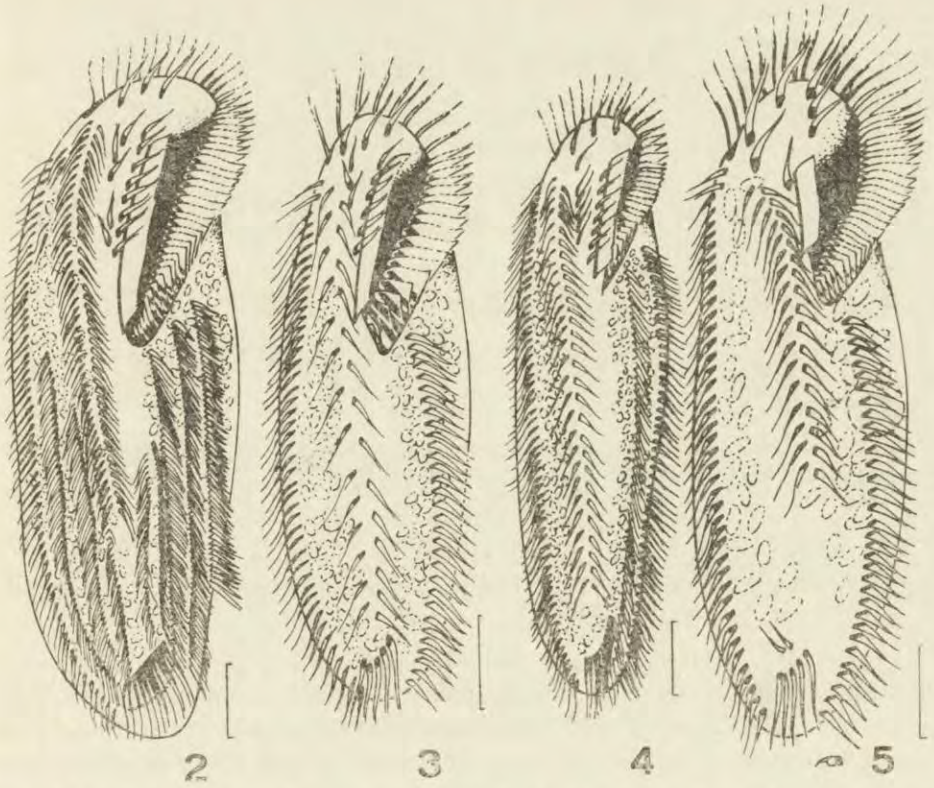
Most of the remaining oblique streaks differentiate into two cirri each, such that a zig-zag lingitudinal sequence develops: the midventral cirri. The two anteriormost (rightmost) migratory cirri developing in the posteriormost streak shift anteriorly to a position lateral of the anteriormost midventral cirri (Hill 1980). The posteriormost (leftmost) cirri in many of the streaks develop into transverse cirri.

Variation in Frontal Ciliature

Frontal cirri In *Urostyla*, *Holosticha*, *Uroleptus*, and *Bakuella*, the anteriormost frontal cirri are hypertrophied and clearly differentiated from midventral cirri (Figs. 2, 3, 6, 7, 27). The right end of the AZM extends only slightly posteriad of the frontal cirri. Except in *Uroleptus*, members of these genera sometimes possess supernumerary malar and paramalar cirri.

By contrast, in *Pseudokeronopsis*, *Pseudourostyla*, and *Thigmokeronopsis*, there is little differentiation between frontal and midventral cirri (Figs. 8, 9, 20–23, 26, 28). The right end of the AZM tends to be more prolonged posteriorly than in the preceeding four genera. Supernumerary malar and paramalar cirri generally are absent. Thus one can distinguish a "*Urostyla*-pattern" and "*Pseudokeronopsis*-pattern" (see Fig. 4 in Borror 1979).

Malar cirri. As a rule, urostylines (and hypotrichs in general) have a single malar cirrus, but it may be absent in some members of some populations. Some species, for example *Thigmokeronopsis jahodai*, have two malar cirri (Fig. 9). In



Figs. 2–5. 2 — *Bakuella variabilis* sp. n., 3 — *Holosticha estuarii* sp. n., 4 — *Holosticha polystylata* sp. n., 5 — *Holosticha sylvatica* Foissner, 1982

a few species with the "*Urostyla*-pattern" of frontal ciliature such as *Holosticha estuarii* (Fig. 3), there may be a series of malar cirri that are variable in number within and among populations (Table 1).

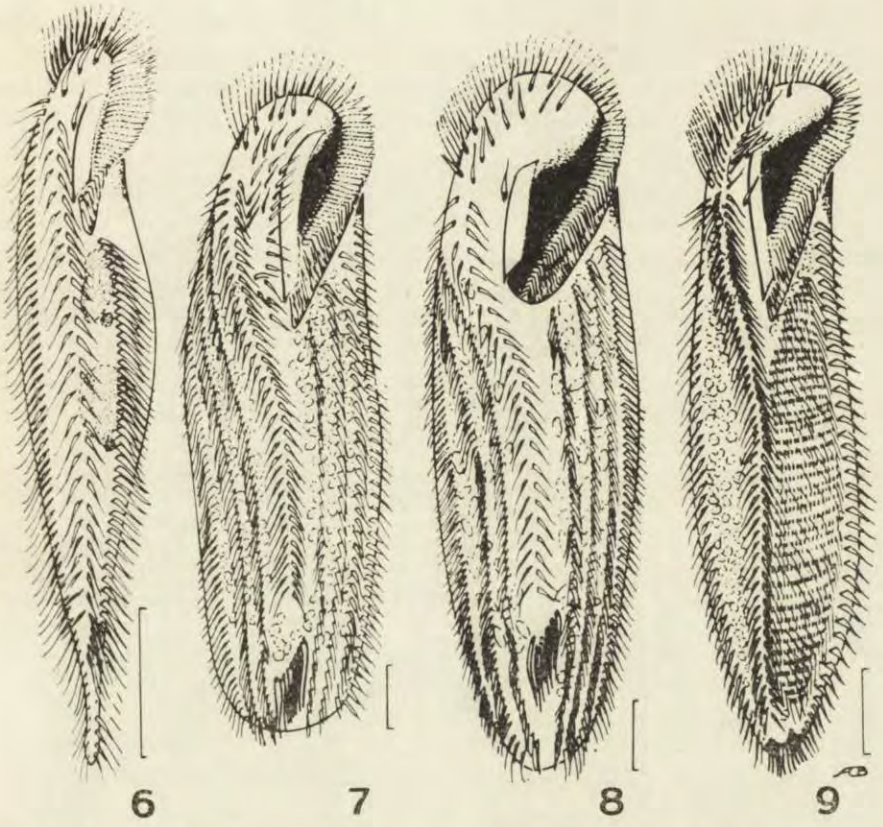
Table 1

Variability of ciliature in populations of species of *Holosticha*

Species	Transverse cirri	Pairs of midventral cirri	Frontal and paramalar cirri	Number of				
				Frontal and malar cirri	Paroral cirri	Rows of right marg. cirri	Rows of left marg. cirri	Sample size
H. estuarii			1+3	1+7	1	1	1	1
			1	1+6	1	1	1	1
			1+1	1+8	1	1	1–2	1
			1+3	1+6	1	1	1	1
H. intermedia	10–12	19–21	1	1+1	1	1	1	4
H. multistylata	8–11	14–21	2	1+(2–5)	1	1	1	17
	6–11	9–26	2	1+(2–3)	1	1	1	19
	10–12	25	2	1+3	1	1	1	4
	7–9	11–20	2+(0–3)	1+(1–4)	1	1	1	18
	8	14–18	2	1+(1–3)	1	1	1	6
	8–11	11–15	2	1+(3–4)	1	1	1	7
	7–10	20–27	2	1+(4–7)	1	1	1–2	11
	8–10	24–28	2	1+(6–10)	1	1	1	13
H. sphagni	4–5	11–20	1	1+(1–2)	1	1	1	8
	2–7	7–10	1	1+(1–2)	1	1	1	12
H. sylvatica	9–11	17–19	2	1+1	4–5	1	1–2	10
	9–11	12–19	2	1+1	3–6	1	1	19
	6–9	14–19	2	1+1	3–5	1	1	30
	6–8	13–19	2	1+1	4–6	1	1	30
	7–8	15–17	2	1+1	4	1	1	4
H. polystylata	10–11	30–45	1+(5–8)	1+(6–9)	1	1	1–2 1/2	10

Paramalar cirri. In the few urostylines in which such cirri occur, they're variable in number (Table 1). Their presence in some members of a population may be diagnostic (Figs. 2–4).

Midventral cirri. In *Bakuella*, *Thigmokeronopsis*, *Pseudokeronopsis* and occasional variants of some *Holosticha* species, there are exceptions to the general pattern of differentiation of ciliary structures from the streaks of the frontal ciliature. In *Bakuella* (Fig. 2), some of the frontal streaks proliferate three or more cirri that form oblique or longitudinal series. In *Thigmokeronopsis* (Fig. 9), part of each frontal streak forms the thigmotactic cirri. Within some populations of *Holosticha*



Figs. 6–9. 6 — *Uroleptus musculus* (Müller, 1773), 7 — *Urostyla grandis* Ehrenberg, 1830, 8 — *Pseudourostyla cristata* (Jerka-Dziadosz, 1964), 9 — *Thigmokeronopsis jahodai* Wicklow, 1981

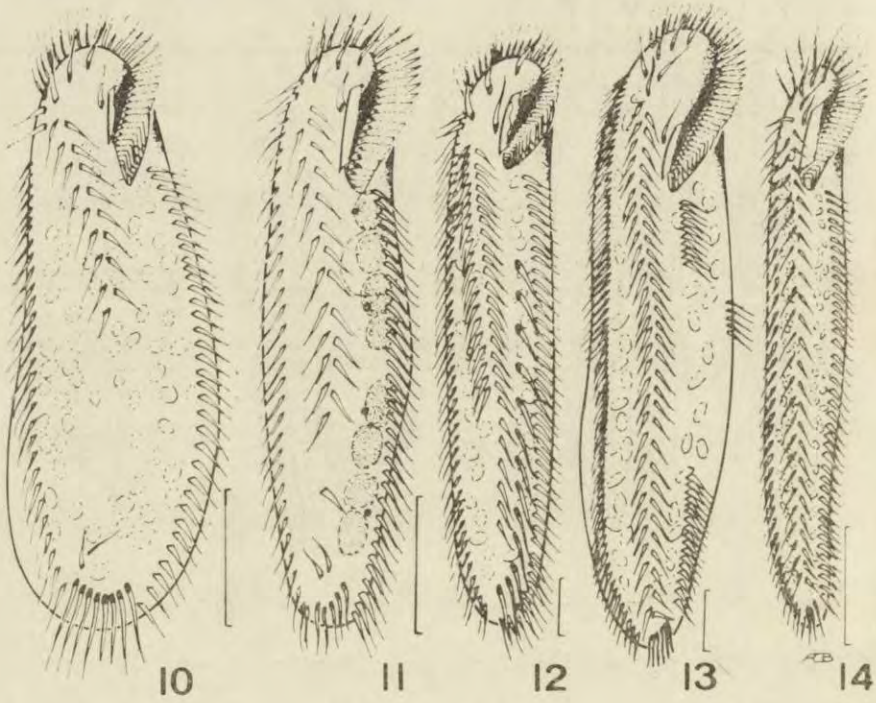
multistylata (Fig. 16), the streaks also may form more than two cirri, such that the midventral cirri have an unusually complex arrangement (Fig. 25).

There also is a difference among groups of populations of *Holosticha* in the distance between the posterior end of the series of midventral cirri and the transverse cirri. In most *Holosticha* species, there is little gap present. In *H. scutellum* and *H. sphagni* however, the midventral series of cirri extends only about 1/2 cell length, leaving a conspicuous gap anterior to the transverse cirri (Figs. 10, 11).

Migratory cirri. There are usually about two migratory cirri just posterior to the right end of the AZM, anterior and ventral of the anterior terminus of the row of right marginal cirri, and lateral of the midventral cirri. We have documented presence of such cirri (from SEM and cytological preparations, as well as in life) in all urostyline genera so far examined.

Transverse cirri. The number of transverse cirri — either within or among populations — is variable. For example, in *Holosticha multistylata* (Fig. 16), they vary from 6–11 (Table 1). Within some populations of *H. sphagni* (Fig. 11), they

vary from 2–7. In some *Pseudokeronopsis decolor* and *P. rubra* (Figs. 21, 22) populations, they may be reduced to a single cirrus or be absent entirely. They're of variable occurrence in *Uroleptus musculus* (Fig. 6). Indeed, within one dividing cell of *U. musculus*, new transverse cirri may appear in the proter but not in the opisthe or the original cell.



Figs. 10–14. 10 — *Holosticha scutellum* (Cohn, 1866), 11 — *Holosticha sphagni* (Grolière, 1975), 12 — *Holosticha lacazei* Maupas, 1883, 13 — *Holosticha velox* (Quennerstedt, 1869), 14 — *Holosticha violacea* Kahl, 1928

There is no evidence that transverse cirri arise in any other way than as cirri at the posterior ends of the posteriormost oblique streaks. This situation contrasts to *Paraurostyla*, where transverse cirri arise at the posterior ends of longitudinal streaks.

Somatic Ciliature

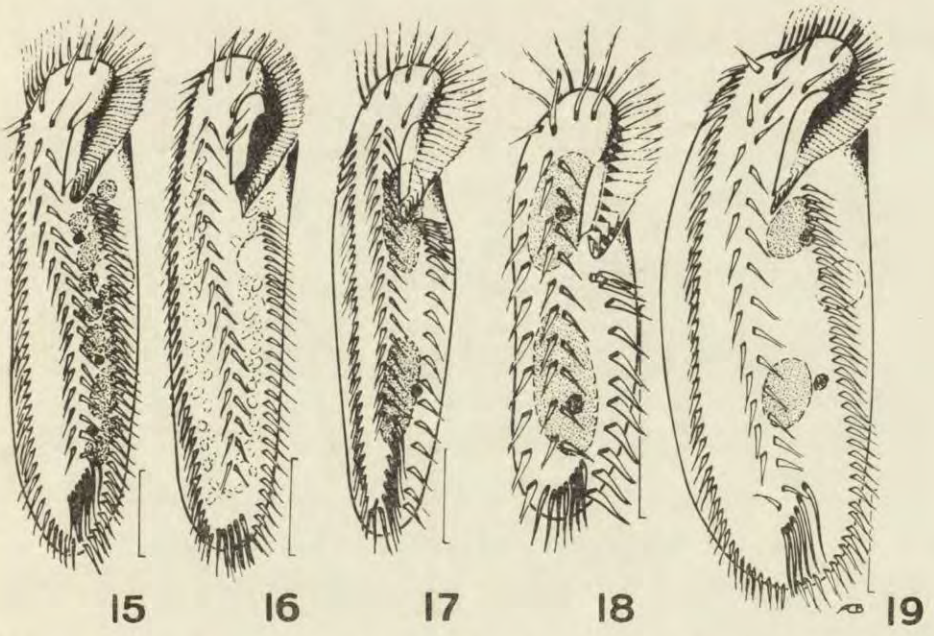
Marginal cirri (Table 1). There is little variation in the number of rows of right marginal cirri within or among populations except in *Urostyla* and *Pseudourostyla*, in which several (variable in number) rows occur. In all other genera, there is only a single row of right marginal cirri.

Left marginal cirri are more variable. In many populations of *Holosticha* and

Pseudokeronopsis, one finds cells with partial or complete additional rows of left marginal cirri. *Bakuella variabilis* (Fig. 2) is also plastic in this character.

In most genera, each row of marginal cirri is self-replicating, but in *Pseudourostyla cristata* (Fig. 8), both right and left marginal cirri arise from ventral primordia of several streaks each. Such a difference in ontogeny of marginal cirri in *Pseudourostyla* suggested to Wicklow (1981) that this genus be separated from other urostylines at the level of superfamily.

The anterior terminus of the row(s) of left marginal cirri is nearly straight. In *Holosticha kessleri* and *H. diademata*, however, there is an inward displacement of the anteriormost several cirri that occurs late in morphogenesis, does not represent retention of parental cirri, formation of cirri from additional streaks, or any particularly unusual morphogenetic process (Figs. 17, 18).

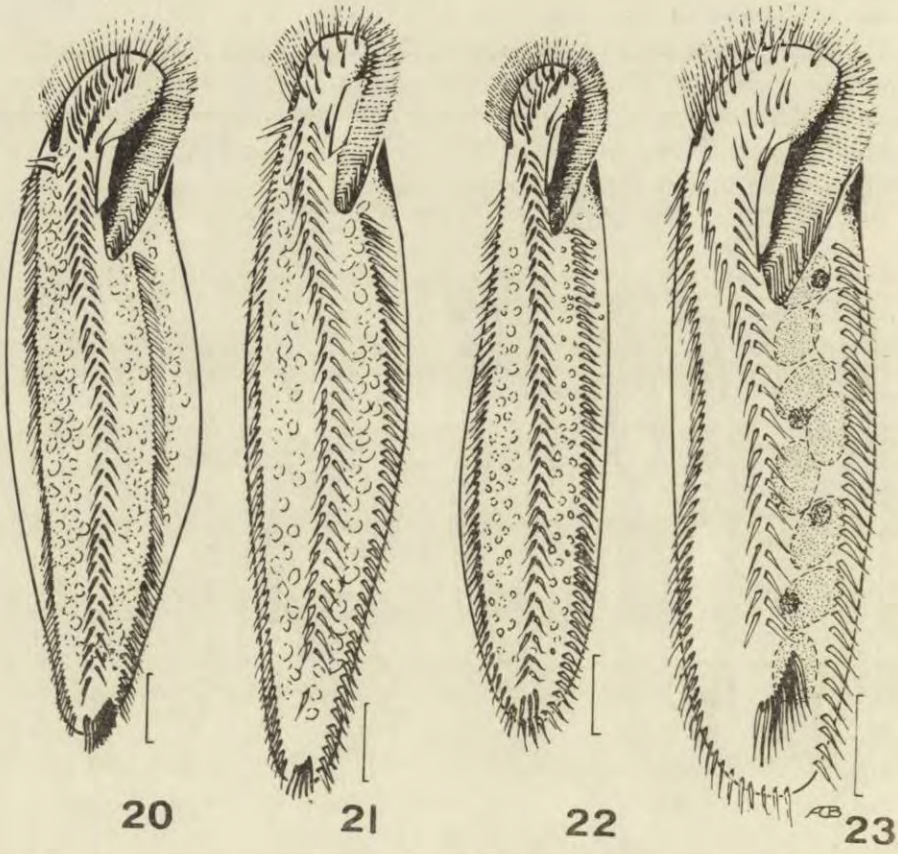


Figs. 15–19. 15 — *Holosticha intermedia* (Bergh, 1889), 16 — *Holosticha multistylata* Kahl, 1928, 17 — *Holosticha kessleri* Wrześniowski, 1877, 18 — *Holosticha diademata* (Rees, 1883), 19 — *Holosticha camerounensis* Dragesco, 1970

In genera with multiple rows of right marginal cirri, there may be some initial difficulty in one's noticing the separate character of the midventral cirri. However, the two cirri of each of the oblique sets of two in the midventral series are set at slightly different angles from one another, and have different fiber bundles associated with them (Wicklow 1981).

In two psammolittoral urostylines, *Holosticha lacazei* (Fig. 12) and *H. alveolata*,

there is evidence that at least during regenerative morphogenesis, right marginal cirri arise not from streaks within the original marginal row but from an additional anterior cirrus from many of the oblique streaks of the frontal field. With these exceptions however, right marginal cirri represent somatic ciliature and the midventral cirri represent frontal ciliature.



Figs. 20–23. 20 — *Pseudokeronopsis pulchra* (Kahl, 1932), 21 — *Pseudokeronopsis rubra* (Ehrenberg, 1838), 22 — *Pseudokeronopsis decolor* (Wallengren, 1900), 23 — *Pseudokeronopsis similis* (Stokes, 1886)

Dorsal bristles. The number of rows of dorsal bristles in urostylines has been little quantified, and there is little information in their development. In view of the known simple variation among populations of ciliates in kinety number as a function of a variety of environmental and genetic mechanisms, one probably should not attempt to make taxonomic distinctions on such a characteristic as number of rows of dorsal bristles. Apparently, caudal cirri (developing from the posterior ends of some rows of dorsal bristles) are rare or absent in urostylines.

Ecological Characteristics

Although many protozoa are euryhaline, there is little evidence that marine urostylines ever occur in freshwater, or the reverse. Most genera are represented in both habitats (except *Thigmokeronopsis* — marine, and *Uroleptus* — freshwater), but morphologically identical populations usually are not found from both freshwater and marine habitats.

It is likely that a number of the smaller (under 75 μm) urostylines are strict bacterivores, or at least feed nearly solely upon colorless microorganisms, while the largest are polyphagic. Populations in culture successfully grow on a variety of nutritional sources, including colorless flagellates, algae, or bacteria. Within a polymorphic population, some of the largest cells may be cannibalistic, the medium-sized ones carnivores, and the smallest bacterivores.

Features of Taxonomic Value

Certain time-honored characters such as cell length, number of rows of left marginal cirri, number of transverse cirri, and characters of endoplasmic inclusions including food vacuoles are probably not safely applicable to species identification. Such a conclusion suggests taxonomic realignment of many nominal species. On the other hand, one can develop a series of characteristics for determining species that reflects important differences among populations and phylogenetic relationships. Such characteristics allow construction of a key to species and form a logic for redefining each recognizeable species.

Features that are particularly useful in species identification include shape of the cell, especially length/width ratio, habitat (freshwater or marine), number and arrangement of macronuclear units, number and distribution of malar, paramalar, and midventral cirri, and the number of rows of right marginal cirri. These conclusions allow recognition of three heretofore undescribed species.

Bakuella variabilis sp. n.

Length about 225–240 μm. Shape, from ventral aspect, as in Fig. 2. Buccal cavity about 1/3 cell length. Two-5 rows of left marginal cirri, and one row of right marginal cirri. One paroral cirrus present. Frontal cirri include 8–10 malar cirri and 2–3 paramalar cirri. Midventral cirri in typical urostyline zig-zag grouping except posteriorly, where they occur in about 3 short longitudinal rows. About 12 transverse cirri present. Migratory cirri occur between midventral and right marginal cirri.

Cortex heavily granulated over entire surface, with groups of 2–4 granules per cirrus near each cirrus base. Additional dense groups of granules lie in spaces between rows of cirri. On dorsal surface, granules in oblique rows, 3–10 granules per row.

The ciliate is flexible, and opaque. It feeds while crawling very slowly forward

(about 1 body length/4 s) either straight or in a slight left-hand curve. In reversing, the posterior end bends to the right, and the body turns to the right. It swims only when strongly stimulated, or mechanically lifted from the substratum. Then it moves in a slow wide counterclockwise helix.

The contractile vacuole pore lies in the left mid-dorsal cytoplasm, and empties dorsally. Food vacuoles contain remains of flagellates.

The macronucleus is divided into many (over 100) small fragments that are scattered throughout the cell.

This species has subordinal characters of the midventral cirri, and the generic characters of one row of right marginal cirri, one or more rows of left marginal cirri, and having some of the midventral cirri, especially in the posterior part of the cell, arranged in short oblique rows of more than 2 cirri each. It differs from *B. crenata* Agamaliev and Alekperov, 1976 in having a multiple type of macronucleus (as well as many features of ciliation), and from *B. marina* A. et A., 1976 at least in habitat and the number of malar and paramalar cirri. Type locality: A temporary pool in a flooded agricultural field in Lee, New Hampshire, U.S.A. (70°58′ W. Long., 43°8′ N. Lat.). The name variabilis emphasizes its diversity of cirral arrangement within a population.

Holosticha estuarii sp. n.

Length 140–248 μm, shape generally as in Fig. 3. Variability of ciliary structures as in Table 1. Usually 1–2 rows of left marginal cirri, 1 row of right marginal cirri, 8–15 transverse cirri, 6–8 malar cirri, and 0–3 paramalar cirri. Macronucleus in numerous fragments. Cortex heavily granulated with tiny yellow cortical granules generally in areas between ciliary organelles, as well as in groups at bases of cirri. Granules in cortex generally in short rows, except in rosettes about bases of dorsal bristles in some populations.

This hypotrich possesses typical urostyline midventral cirri, one row of right marginal cirri, and about 3 frontal cirri clearly differentiated from the midventral cirri, characteristics shared with other *Holosticha* species. Except for the following (also new) species, this is the only member of the genus with two or more paramalar cirri. A row of several malar cirri, highly pigmented cortex, and marine habitat also aid in distinguishing this species.

Members of this species have been isolated from a tidal marsh at Durham, New Hampshire, U.S.A., the type locality (70°52′ W. Long., 43°6′ N. Lat.) as well as tidal marshes in Rye, New Hampshire, and Huntington, New York. They were isolated in each instance from algal mats in panne ponds, hence the specific name estuarii.

Holosticha polystylata sp. n.

Length 160–200 μm, shape as in Fig. 4. Variability of ciliary structures as in Table 1. One-3 rows of left marginal cirri, one row of right marginal cirri. Macro-

nucleus in about 125 fragments. Ciliates appear light pinkish red in transmitted light. Spherical golden granules in surface cortex, each about 0.75 μm diam., occurring as individual granules or in longitudinal groups of 2–3 in intermeridional areas, but not in obvious rows or in clumps near cirri. Additional numerous tiny (less than 0.5 μm diam.) granules just beneath cortex, singly or in longitudinal rows of 2–5 granules.

We isolated this ciliate from alkaline marl sediments adjacent to Mud Pond, Lloyd-Cornell Reservation, McLean, New York, U.S.A. (type locality: 68°23′ W. Long., 42°32′ N. Lat.). We've isolated it also from a freshwater aquarium in Durham, New Hampshire. Jerka-Dziadosz isolated it from a similar source in Poland (Jerka-Dziadosz and Janus 1972).

The ciliate is immediately recognizeable by its color, as well as the numerous paramalar cirri that are suggested by the name polystylata.

Key to Genera and Species of *Urostylina*

1. Frontal cirri usually hypertrophied, and distinct from midventral cirri. Some species with some of frontal cirri in short longitudinal rows; others with only about 4–5 frontal cirri. AZM not prolonged to the cell's right posterior to the hypertrophied frontal cirri . . 2
1′. Frontal and midventral cirri not distinct, anteriormost cirri little hypertrophied. AZM prolonged posteriorly to the cell's right of the frontal cirri 35
2(1). Two or more complete rows of right marginal cirri. *Urostyla* Ehrenberg, 1838 . . . 3
2′. Only one complete row of right marginal cirri 8
3(2). Macronucleus divided into 8 or more relatively small fragments, and difficult to observe in life 4
3′. Macronucleus in 2–4 relatively easily observed segments 5
4(3). Freshwater ciliates. *Urostyla grandis* Ehrenberg, 1830 (Fig. 7).
4′. Marine ciliates. *U. marina* Kahl, 1932.
5(3′). Freshwater ciliates. *U. multipes* (Claparède et Lachmann, 1858).
5′. Marine ciliates 6
6(5′). Three or more rows of left marginal cirri. *Urostyla gracilis* Entz, 1884.
6′. Only 1 or 2 rows of left marginal cirri 7
7(6′). Cortex with pigment granules. *U. concha* Entz, 1884.
7′. Cortex not pigmented. *U. dispar* Kahl, 1932.
8(2′). Some of midventral cirri, especially in the posterior half of the cell, arranged in short oblique rows of more than 2 cirri each.
Bakuella Agamaliev et Alekperov, 1976 9
8′. Midventral cirri in typical zigzag series, usually two cirri per original streak 12
9(8). Macronucleus divided into many relatively small fragments that are difficult to observe in life 10
9′. Macronucleus in two segments. *Bakuella crenata* Agamaliev et Alekperov, 1976.
10(9′). Marine ciliates with only one row of left marginal cirri. Only three or fewer malar cirri present 11
10′. Freshwater ciliates with 2–5 rows of left marginal cirri, and about 8 malar cirri. *Bakuella variabilis* sp. n. (Fig. 2).
11(10). Three malar cirri present. Posteriormost several groups of oblique rows of midventral cirri with about 5–8 cirri per row. *B. marina* A. et A., 1976.

11′. No malar cirri present. Posteriormost several oblique groups of midventral cirri with only about 3 cirri each. *B. agamalievi* nom. nov. (See nomenclatural notes in following section).

12(8′). Posterior half of ciliate distinctly narrowed into a tail. Transverse cirri (often difficult to see) in a J-shaped row, reduced, or absent. Freshwater ciliates with two macronuclei, three frontal cirri, and one malar cirrus. *Uroleptus* Ehrenberg, 1831 13

12′. Cell variously oval to elongate, but without a tail as described above. Both freshwater and marine ciliates with various arrangements of frontal cirri and macronuclei. *Holosticha* Wrześniowski, 1877 14

13(12). Tail extremely elongate and narrow, nearly as long as the rest of the cell. Cell narrow, buccal cavity only about 1/8 cell length. *Uroleptus lamella* Ehrenberg, 1831.

13′. Tail less extreme, only about 1/3 cell length, or shorter. Buccal cavity about 1/4–1/5 cell length. *Uroleptus musculus* (Müller, 1773) (Fig. 6).

14(12′). Macronucleus often difficult to observe in life, divided into many (usually over 30) fragments, often 5 μm in diameter or less, and either scattered throughout the cell, or in at least two linear groupings 15

14′. Macronucleus in 15 or fewer fragments, usually more or less in a single longitudinal or raggedly C-shaped grouping 25

15(14). Usually two or more paramalar cirri developed from the second frontal streak mediad from the paroral membrane, lying between malar cirri and the anteriormost midventral cirri. Also usually a row of 3 or more malar cirri. Cortex highly pigmented 16

15′. Paramalar cirri 0–1, whether or not cell is pigmented. Usually the pigment, and the paramalar cirri, are absent 17

16(15). Bright red freshwater or bog ciliates with a row of paramalar cirri. *Holosticha polystylata* sp. n. (Fig. 4).

16′. Yellow-green estuarine ciliates with usually no more than 2 or 3 paramalar cirri. *Holosticha estuarii* sp. n. (Fig. 3).

17(15′). Ciliates from marine sands, with *Discocephalus*-like "cephalization" at anterior end. *Holosticha discocephalus* Kahl, 1932.

17′. Anterior end without the neck-like constriction. Habitat various 18

18(17′). Three-6 paroral cirri present. *Holosticha sylvatica* Foissner, 1982. (Fig. 5).

18′. Only one paroral cirrus present 19

19(18′). Midventral cirri extend to transverse cirri with no obvious gap 20

19′. An unciliated gap between the foreshortened series of midventral cirri and the transverse cirri, except that two small cirri may lie just anterior to the transverse cirri. Supple ciliates with colorless cortex, from freshwater and marine habitats. Buccal cavity narrow, paroral membrane relatively short. *Holosticha scutellum* (Cohn, 1866) (Fig. 10). *H. adami* Foissner, 1982 also keys out here.

20(19). Ciliates from mosses or freshwater, usually with a row of two or more malar cirri, and strings of obvious greenish granules in the cortex generally in addition to granules at bases of cirri. Usually 6–12 transverse cirri. *Holosticha multistylata* Kahl, 1928 (Figs. 16, 24, 25).

20′. Ciliates from various habitats with 0–2 malar cirri. Granulation various. Usually 3–8 transverse cirri 21

21(20′). Transverse cirri large, obvious, often 15 or more, in a J-shaped group extending forward nearly to cell equator or beyond 22

21′. Transverse cirri smaller, inconspicuous, usually 6 or fewer, set very close to the posterior end of the cell 23

22(21). Right half of midventral cirri in groove, beating medially. Row of right marginal cirri ends anterior to transverse cirri. *Holosticha alveolata* Kahl, 1932.

22′. Right half of midventral cirri not so oriented. Right marginal cirri extend posteriorly beyond the transverse cirri. *Holosticha lacazei* (Maupas, 1883) (Fig. 12).

23(21′). Pigmented (brownish-yellow) granules at some of cirrus bases. Marine ciliates. *Holosticha velox* (Quennerstedt, 1869) nov. comb. (Fig. 13). (See nomenclatural notes.)

23′. Cortex clear, with no such pigmented granules at bases of cirri 24

24(23′). Freshwater ciliates with about a 4/1 length/width ratio. *Holosticha vernalis* Stokes, 1887.

24′. Elongate freshwater or marine band-shaped supple ciliates with greater than a 6/1 length/width ratio. *Holosticha violacea* Kahl, 1928 (Fig. 14).

25(14′). Macronuclei 4–16, usually 8–16, in an even or raggedly moniliform series or in an irregular C- or U-shaped group. Occasional individuals in some populations with 4 macronuclei 26

25′. Only 2 macronuclei usually present (although occasional individuals with 4) 28

26(25). Plump oval ciliates with a length/width ratio of about 2/1. Buccal cavity about half cell length. *Holosticha mystacea* (Stein, 1859).

26′. More elongate ciliates with a length/width ratio of 3/1 or greater, and a buccal cavity no more than 1/3 cell length 27

27(26′). Ciliates with a nearly complete row of midventral cirri. *Holosticha intermedia* (Bergh, 1889) (Fig. 15).

27′. Ciliates with a row of midventral cirri only about half cell length. *Holosticha sphagni* nov. comb. (Fig. 11). (See nomenclatural notes.) *H. sigmoidea* Foissner, 1982, with more cortical granulation, keys out here also.

28(25′). Micronuclei (2 or more) very small, inconspicuous, about 5 μm in diameter, and variously adjacent to each macronuclear fragment 29

28′. One large conspicuous micronucleus lying between the two macronuclear fragments 33

29(28). Anterior several left marginal cirri set mediad of rest of group, forming a hook-like grouping 30

29′. Anteriormost left marginal cirri in line with the rest of the group 31

30(29). Large (about 125 μm) spindle-shaped ciliates with narrowed posterior half with slightly concave sides. Usually 25 or more left marginal cirri. *Holosticha kessleri* Wrz., 1877 (Fig. 17).

30′. Smaller (45–117 μm) ciliates, more oval in outline, with only about 15 left marginal cirri, *H. diademata* (Rees, 1883) (Fig. 18).

31(29′). Large conspicuous buccal cavity 1/3–1/2 cell length, often with hypertrophied paroral membrane. *Holosticha camerounensis* Dragesco, 1970 (Fig. 19).

31′. Buccal cavity narrow, with less conspicuous paroral membrane 32

32(31′). At least three large frontal cirri. *Holosticha gibba* (Stein, 1859).

32′. Only two large frontal cirri. *Holosticha dragescoi* nom. nov. (See nomenclatural notes).

33(28′). Midventral cirri extend to transverse cirri with no large gap 34

33′. Midventral series of cirri extends only about 1/2 cell length. *Holosticha muscicola* Gellért, 1956.

34(33). Large (about 200 μm) ciliate, with transverse cirri set at about the last 3/4 of cell, not overlapping posterior end. *Holosticha navicularum* Kahl, 1932.

34′. Smaller (about 100 μm) ciliate, with transverse cirri greatly overlapping posterior end. *H. brevis* Kahl, 1932.

35(1′). Two or more, often 4–7, rows of left marginal and several rows of right marginal cirri that develop from ventral primordia. Ciliates from mosses and freshwater habitats, with multiple macronuclei. *Pseudourostyla cristata* (Jerka-Dziadosz, 1964) (Fig. 8).

35′. Usually only one row of right marginal cirri and 1–2 (usually only 1) row of left marginal cirri. Includes both freshwater and marine ciliates 36

36(35′). Dense thigmotactic field of cirri between midventral and left marginal cirri. *Thigmokeronopsis jahodai* Wicklow, 1981 (Fig. 9).

36′. No such field present. *Pseudokeronopsis* gen. nov., for species mostly in *Keronopsis* formerly; see introduction and nomenclatural notes below 37

37(36′). Macronucleus often difficult to observe in life, divided into 4 or more (usually 10 or more) fragments, either in a moniliform series or scattered throughout the cell 38
37′. Only 2 relatively obvious large macronuclei 45
38(37). Ciliates with 32 or more macronuclei, usually scattered throughout the cell. Usually from marine or estuarine habitats 39
38′. Freshwater or moss ciliates with macronuclei in a moniliform series of usually 10–16 fragments 44
39(38). Ciliates obviously yellow-red pigmented at 40X magnification 40
39′. Ciliates colorless or brownish under low magnification, even if pigment granules may be evident at higher magnification 43
40(39). Dorsal cortical pigment in short crossrows of fine red granules rather than rosettes. *Pseudokeronopsis multinucleata* (Maupas, 1883).
40′. Dorsal cortical pigment in rosettes of granules grouped around bases of dorsal cilia . . . 41
41(40′). Dorsal rosettes narrowly set. *Pseudokeronopsis flavicans* (Kahl, 1932).
41′. Dorsal rosettes more widely spaced 42
42(41′). Cell relatively wide and "baggy". Rows of ventral granules visible at low magnification, because the dark red granules around the bases of the cirri are as big as other granules in the general cortex. Transverse cirri numerous (8–14). *Pseudokeronopsis pulchra* (Kahl, 1932) (Fig. 20).
42′. Cell narrower. Rows of ventral granules around bases of cirri only visible at higher magnification, because the granules there are smaller than the granules otherwise in the ventral cortex. Transverse cirri reduced to 6 or fewer, or absent. *Pseudokeronopsis rubra* (Ehrenberg, 1838) (Fig. 21).
43(39′). Cortex with sparse yellow granules. Buccal cavity about 1/3 body length. Cytoplasm sometimes contains sand grains. *Pseudokeronopsis ovalis* (Kahl, 1932).
43′. Cortex colorless. Buccal cavity about 1/4 body length. *Pseudokeronopsis decolor* (Wallengren, 1900) (Fig. 22).
44(38′). Contractile vacuole in the posterior 1/4 of cell. *Pseudokeronopsis retrovacuolata* (Tucolesco, 1962).
44′. Contractile vacuole empties dorsally near midpoint of cell, just posterior to the buccal cavity. *Pseudokeronopsis similis* (Stokes, 1886) (Fig. 23).
45(37′). Cortex with large yellow granules. Paroral membrane with hypertrophied hook-like anterior extension. *Pseudokeronopsis spectabilis* (Kahl, 1932).
45′. Cortex colorless. Buccal cavity with normal paroral membrane 46
46(45′). Lance-shaped marine ciliates with about 5 transverse cirri. *Pseudokeronopsis pernix* (Wrześniowski, 1877).
46′. Oval ciliates from freshwater with reduced or absent transverse cirri. *Pseudokeronopsis ovata* (Horváth, 1933) nov. comb. (See nomenclatural notes in following section).

Notes on Nomenclature

Arrival at a valid set of criteria for distinguishing species not only allows construction of a key to species, but also requires updating of lists of synonymies. Justification for amendments, nomina nova, and new combinations is also warranted.

The definition of the suborder *Urostylina* Jankowski, 1979. Erection of the new genus *Thigmokeronopsis* by Wicklow (1981) as well as our additional investigation of modes of morphogenesis and fine structure in urostylids and other hypotrichs suggests changes over definitions previously published (Borror 1979). Specifically,

the system of divisions of the *Urostylina* Jankowski, 1979 and family diagnoses suggested by Wicklow (1981) are followed here.

We are expanding the family description of the *Urostylidae* appearing in Borror (1979 b) to apply to the suborder. It thus encompasses those hypotrichs with somatic ciliature including rows of dorsal bristles and one or more rows of right and left marginal cirri and frontal ciliature with variously arranged frontal, midventral, migratory, and transverse cirri (the latter sometimes reduced, inconspicuous, or absent). Such frontal and midventral cirri differentiate during prefission morphogenesis from a longitudinal field of more than five oblique ciliary streaks.

Urostyla

A number of species, originally described in *Urostyla*, are now considered members of *Paraurostyla* Borror, 1972, including *weissei* Stein, 1859, *flavicans* Wrześniowski, 1869, *vernalis* Stokes, 1894, *paragrandis* Wang, 1930, *polymicronucleata* Merriman, 1937, *coei* Turner, 1939, *hologama* Heckmann, 1965, *latissima* Dragesco, 1970, and *naumanni* Lepşi, 1935. In 1972, Borror placed *Urostyla intermedia* Bergh, 1889 into *Holosticha*, and *Urostyla cristata* Jerka-Dziadosz, 1964, and *U. muscorum* Kahl, 1932 into *Pseudourostyla*. *Urostyla urostyla* (Clap. et Lach., 1858) Kahl (1932) was listed in *Pseudourostyla* in error by Borror, 1972. Based on arrangement of frontal cirri and nuclear configuration, it should be in the genus *Urostyla*.

Borror placed *Eschaneustyla brachytona* Stokes, 1886 in *Urostyla* in 1972. Since observing it in interphase and in morphogenesis, we're convinced it is not a urostyline. Borror considered *Hemicycliostyla marina* Kahl, 1932 and *Urostyla rubra* Andrussowa, 1886 both as members of *Urostyla* in 1972, but they should be of questionable taxonomic placement pending rediscovery.

Bakuella

In 1972, Agamelicv described a species identified by him as *Holosticha manca*. Irregularities of arrangement of cirri of the midventral group suggest that it is a multinucleate marine member of *Bakuella*. It lacks the malar cirri of *B. marina*, and has a foreshortened series of midventral cirri. We suggest the name *Bakuella agamalievi* nom. nov. Definitive placement of *Paraurostyla pulchra* Buitkamp, 1977 should await information on morphogenesis, but it may have affinities to *Bakuella variabilis*.

Uroleptus

In 1972, Borror considered members of Kahl's subgenus *Paruroleptus* to belong properly in *Uroleptus*, since transverse cirri had often been overlooked by previous workers — a fact noted by Kahl in his original description of the subgenus *Paruroleptus*. In 1979, Borror noted that *Uroleptus* is a poorly understood genus, plagued by an astonishingly complex nomenclature, and dozens of super-

ficially described nominal species. The number of transverse cirri is extremely variable within a clone. *Uroleptus kahli* Grolière, 1975 and *U. kahli* Buitkamp, 1977 should be excluded from the *Urostylina* since they're not documented as having the characteristics of the suborder. These *Cladotricha*-like forms, because of their misleading simplicity and likely polyphyletic origin, should be considered of uncertain taxonomic position pending rediscovery, and conformation of division process and cirral homologies with more typical hypotrichs.

Uroleptus muscorum Kahl, 1932 in Fernández-Leborans, 1981 and *U. elongatus* Fernández-Leborans, 1981 probably should be assigned to the genus *Strongylidium*. There is no evidence of their possessing midventral cirri and other urostyline characteristics. *Paruroleptus strenuus* Dingfelder, 1962 likewise does not have urostyline characteristics, and probably is an *Onychodromus*. In 1972, Borror placed *Uroleptus humicola* Gellért, 1956 in synonymy with *Holosticha multistylata*; it more than likely is a *Uroleptoides*. *Uroleptus packi* Calkins in Pack, 1919, *U. halseyi* Calkins, 1929, *U. matthesi* Wenzel, 1953, *U. mobilis* Engelmann, 1862, and *U. natronophilus* Dietz, 1965 are probably not urostylines; they more closely resemble *Cladotricha koltzowii*.

Holosticha

Borror placed *Pleurotricha macrostoma* Dragesco, 1970 in *Holosticha* in 1972. As it shows no characteristics of the suborder, it is excluded in this revision, and probably is best considered as a true *Pleurotricha*. *Holosticha setifera* Kahl, 1932 (suggested as a replacement name for *H. obliqua* Kahl, 1928) has several oxytrichid characteristics, including caudal cirri and un-urostyline-like arrangement of frontal cirri, suggesting that it be excluded from the *Urostylina*. It may be related to *Gastrostyla*.

Balladyna similis Kahl, 1932, placed in *Holosticha* by Borror, 1972 and in *Balladynella* by Stiller, 1974, also does not show evidence of possessing urostyline characteristics, hence is excluded from this revision.

In 1972 Borror shifted generic placement of a number of species originally described in *Holosticha*. These included transfer of *annulata* Kahl, 1928, to *Amphisiella*; *fossicola* Kahl, 1932 and *hymenophora* Stokes, 1886 to *Paraurostyla*; *monilata* Kahl, 1928, *multinucleata* Maupas, 1883, *decolor* Wallengren, 1900, *globulifera* Kahl, 1932, *retrovacuolata* Tucolesco, 1962, *flava* deMorgan, 1926, and *similis* Stokes, 1886 to *Keronopsis* (now *Pseudokeronopsis*); *aquariumdulcium* Bürger, 1905 to *Trichotaxis*; *musculus* Kahl, 1932, *simplex* Kahl, 1932, *caudata* Stokes, 1886, *caudatus* (Stokes, 1886), *lacteus* Kahl, 1932, and *magnificus* Kahl, 1932 to *Uroleptus*; *coronata* Gourret et Roeser, 1888 to *Gastrostyla*, and *dubium* Gelei, 1954 and *mononucleata* Gelei, 1954 to *Pleurotricha*. Borror considered then that *Holosticha fontinalis* Lepşi, 1926 and *H. longiseta* Lepşi, 1951 were not valid species.

Neither *H. manca* var. *mononucleata* Gelei, 1955 nor *H. rostrata* var. *vesiculata* Vuxanovici, 1963 in Stiller, 1974 were figured, hence are nomina nuda.

Pseudourostyla

P. urostyla and *P. muscorum*, that Borror placed in this genus in 1972, probably are *Urostyla* species based on arrangement of frontal cirri.

Trichotaxis

This genus was separated from *Holosticha* formerly on the basis of a ciliate having two rows of left marginal cirri. Since this character is so variable (see for example populations tabulated in Table 1), we no longer consider this a valid taxon. Its species are distributed as follows:

Table 2
Most Probable Identity of *Trichotaxis* Species

as listed as members of the genus *Trichotaxis* by Borrow, 1972	most probable identity
stagnatilis Stokes, 1981 (type)	*Pseudokeronopsis similis*
caudata (Ehrenberg, 1838)	*Uroleptus musculus*
crassa (Clap. et Lach., 1858)	*Pseudokeronopsis rubra*
euplotes (Dragesco, 1960)	*Balladyna*?
multinucleatus Burkovsky, 1970	*Paraurostyla*?
rubentis Sarmiento et Guerra, 1960	*Holosticha camerounensis*?
veiox (Quenn., 1869)	*Holosticha velox*
villaensis Sarmiento et Guerra, 1960	*Holosticha*?

Uroleptopsis

Uroleptopsis includes species separable from *Pseudokeronopsis* only by apparent absence of transverse cirri. Since such structures are of variable occurrence (or absence) in some clones of marine *Pseudokeronopsis*, continued recognition of *Uroleptopsis* as a valid taxon is not warranted, and its species can be distributed as follows:

Table 3
Most Probable Identity of *Uroleptopsis* Species

as listed as members of the genus *Uroleptosis* by Borror, 1972	most probable identity
citrina Kahl, 1932 (type)	*Pseudokeronopsis rubra*
ovata (Horváth, 1933)	*Pseudokeronopsis ovata*
roscoviana (Maupas, 1883)	*P. multinucleata*
viridis (Perej., 1886)	*P. decolor*

Pseudokeronopsis

Several species originally described in *Keronopsis* have been placed in *Holosticha*, including *K. gracilis* Kahl, 1932, *thononensis* Dragesco, 1966, *sphagni* Grolière,

1975, *arenicola* Dragesco, 1963, *rubra* in Jerka-Dziadosz and Janus, 1972, *longicirrata* Gelei et Szabados, 1950, and *macrostoma* Reuter, 1963. *Keronopsis wetzeli* Wenzel, 1953 belongs in *Paraholosticha*.

Nomenclatural innovations required

(1) *Bakuella agamalievi* nom. nov., for *Holosticha manca* in Agamaliev, 1972.

(2) *Holosticha dragescoi* nom. nov., for *Keronopsis arenicola* Dragesco, 1963. Transfer of Dragesco's species to *Holosticha* requires a new name since the name *arenicola* is preoccupied.

(3) *Holosticha sphangi* (Groliére, 1975) nov. comb. because of transfer from *Keronopsis*.

(4) *Holosticha velox* (Quennerstedt, 1869) nov. comb. because of transfer from *Trichotaxis*.

(5) There is an extensive series of new combinations required for species originally described in *Keronopsis* but now placed in *Pseudokeronopsis* (see discussion in introduction). Among these additionally is *Pseudokeronopsis ovata* (Horváth, 1933), originally described in *Paraholosticha*, and transferred by Borror (1972) to *Uroleptopsis*.

Synonymy of Species of *Urostylina*

The nomenclatural situation reflected by the revisions discussed above is summarized as follows:

Urostylina Jankowski, 1979
1. *Urostyloidea* Bütschli, 1889
   1. *Urostylidae* Bütschli, 1889
      1. *Urostylinae* Bütschli, 1889
         1. *Urostyla* Ehrenberg, 1838 (*U. grandis* type by monotypy)
            1. *U. grandis* Ehrenberg, 1830
               Oxytricha fusca Perty, 1852
               Urostyla trichogaster Stokes, 1885
               Hemicycliostyla sphagni Stokes, 1886
               H. trichota Stokes, 1886
               Urostyla caudata Stokes, 1886
               U. gigas Stokes, 1886
               U. muscorum Kahl, 1932
               Pseudourostyla muscorum (Kahl, 1932) Borror, 1972
2. *U. concha* Entz, 1884
3. *U. gracilis* Entz, 1884
               U. limboonkengi Wang et Nie, 1932
               U. pseudomuscorum Wang, 1940
4. *U. marina* Kahl, 1932
               Paraurostyla marina (Kahl, 1932) Borror, 1972
               Keronopsis gracilis Dragesco, 1965
5. *U. multipes* (Claparède et Lachmann, 1858) Kahl, 1932
               Oxytricha multipes Clap. et Lach., 1858
               O. urostyla Clap. et Lach., 1858

6. *U. dispar* Kahl, 1932
   Paraurostyla dispar (Kahl, 1932) Borror, 1972

2. *Holostichinae* Fauré-Fremiet, 1961
   1. *Holosticha* Wrześniowski, 1877 (*H. kessleri* type by monotypy)
      1. *H. kessleri* Wrześniowski, 1877
         Oxytricha kessleri Wrz., 1877
         H. kessleri var. *aquae-dulcis* Buchar, 1957
      2. *Holosticha alveolata* Kahl, 1932
      3. *H. brevis* Kahl, 1932
         Keronopsis longicirrata Gelei et Szabados, 1950
         H. rostrata Vuxanovici, 1963
      4. *H. camerounensis* Dragesco, 1970
         H. contractilis Dragesco, 1970
      5. *H. diademata* (Rees, 1883) Kahl, 1932
         Amphisia diademata Rees, 1883
         Holosticha thiophaga Kahl, 1928
         H. milnei Kahl, 1932
         H. simplicis Wang et Nie, 1932
         H. teredorum Tucolesco, 1962
         H. coronata Vuxanovici, 1963
      6. *H. discocephalus* Kahl, 1932
      7. *H. dragescoi* nom. nov.
         Keronopsis arenicola Dragesco, 1963
      8. *H. estuarii* sp. n.
         Trichotaxis pulchra Borror, 1972
      9. *H. gibba* (Stein, 1859) Kahl, 1932
         Oxytricha gibba Stein, 1859
         O. wrześniowskii Mereschkowsky, 1878
         Holosticha punctata Rees, 1883
         H. arenicola Kahl, 1932
         H. viridis Kahl, 1932
         H. algivora Kahl, 1932
         H. rhomboedrica Vuxanovici, 1963
      10. *H. intermedia* (Bergh, 1889) Kahl, 1932
         Urostyla intermedia Bergh, 1889
         Keronopsis monilata "variant" in Kahl, 1932
         Holosticha extensa Kahl, 1932
         H. vesiculata Vuxanovici, 1963
         H. tenuiformis Vux., 1963
         Keronopsis monilata in Dragesco, 1966, Dragesco, 1970, and Grolière, 1975
         Holosticha lacazei in Pätsch, 1974
         H. randani Grolière, 1975
         H. distyla Buitkamp, 1977
      11. *H. lacazei* Maupas, 1883
      12. *H. multistylata* Kahl, 1928
         H. (*K.*) *muscorum* Kahl, 1932
         Keronopsis macrostoma Reuter, 1963
         K. muscorum in Grolière, 1975
      13. *H. muscicola* Gellért, 1956
      14. *H. mystacea* (Stein, 1859) Kahl, 1932

15. *H. navicularum* Kahl, 1932
16. *H. polystylata* sp. n.
    Keronopsis rubra in Jerka-Dziadosz et Janus, 1972
17. *H. scutellum* (Cohn, 1866) Kahl, 1932
    Oxytricha scutellum Cohn, 1866
    Holosticha manca Kahl, 1932
    H. manca var. *plurinucleata* Gellért, 1956
    H. interrupta Dragesco, 1966
    H. tetracirrata Buitkamp et Wilbert, 1974
18. *H. sphagni* (Grolière, 1975) nov. comb.
    Keronopsis sphagni Grolière, 1975
19. *H. sylvatica* Foissner, 1982
20. *H. velox* (Quennerstedt, 1869) nov. comb.
    Oxytricha velox Quenn., 1869
    Trichotaxis velox (Quenn., 1869) Kahl, 1932
    Keronopsis gracilis Kahl, 1932
    Holosticha sp. in Kahl, 1932, p. 575
21. *H. vernalis* Stokes, 1887
    Keronopsis thononensis Dragesco, 1966
22. *H. violacea* Kahl, 1928
    H. grisea Kahl, 1932
    H. gracilis Vuxanovici, 1963

2. *Bakuella* Agamaliev et Alekperov, 1976
(*B. marina* type by orig. descr.)
1. *B. marina* Agamaliev et Alekperov, 1976
2. *B. crenata* A. et A., 1976
3. *B. agamalievi* nom. nov.
   Holosticha manca in Agamaliev, 1972
4. *B. variabilis* sp. n.

3. *Uroleptus* Ehrenberg, 1831
(*U. musculus* type by subseq. designation)
1. *U. musculus* (Müller, 1773) Ehrenberg, 1831
   Trichoda musculus Müller, 1773
   T. piscis Müller, 1786
   Uroleptus hospes Ehrenberg, 1831
   U. piscis (Müller, 1786) Ehrenberg, 1831
   Oxytricha caudatus Ehrenberg, 1838
   Uroleptus violaceus Stein, 1859
   Amphisia piscis Kowalewski, 1882
   Uroleptus limnetis Stokes, 1885
   Platytrichotus opisthobolus Stokes, 1886
   Holosticha caudata Stokes, 1886
   Uroleptus longicaudatus Stokes, 1886
   U. dispar Stokes, 1886
   U. sphagni Stokes, 1886
   Holosticha (*Paruroleptus*) *musculus* (Müller, 1773) Kahl, 1932
   H. (*P.*) *simplex* Kahl, 1932
   H. (*P.*) *caudatus* (Stokes, 1886) Kahl, 1932
   H. (*P.*) *lacteus* Kahl, 1932
   H. (*P.*) *piscis* (Müller, 1786) Kahl, 1932

H. (*P.*) *magnificus* Kahl, 1932
Uroleptus caudatus (Stokes, 1886) Kahl, 1932
Paruroleptus novitas Horváth, 1933
Uroleptus setiformis Bary, 1950
Paruroleptus lepisma Wenzel, 1953
P. ophryoglena Gelei, 1954
Uroleptus poianae Lepsi, 1957
Paruroleptus viridis Vuxanovici, 1963
P. gibbosus Vux., 1963
P. pectinatus Vux., 1963
Uroleptus dubius Vux., 1963
U. lacteus (Kahl, 1932) Borror, 1972
U. novitas (Horváth, 1933) Borror, 1972
Trichotaxis caudata (Ehrenberg, 1838) Borror, 1972
Paruroleptus caudatus in Grolière, 1975
Holosticha (*Paruroleptus*) *musculus* in Martin et Fedriani, 1978

2. *U. lamella* Ehrenberg, 1831
Oxytricha lamella Perty, 1852
Uroleptus rattulus Stein, 1859

2. *Pseudokeronopsidae* fam. nov.
1. *Pseudokeronopsinae* subfam. nov.
1. *Pseudokeronopsis* gen. nov. (*P. rubra* (Ehrenberg, 1838) type by original designation)
1. *P. rubra* (Ehrenberg, 1838) nov. comb.
Oxytricha rubra Ehrb., 1838
O. protensa Perty, 1852
O. crassa Claparède et Lachmann, 1858
O. flava Cohn, 1866
Holosticha flavorubra Entz, 1884
H. flava deMorgan, 1926
H. (*Keronopsis*) *rubra* (Ehrb., 1838) Kahl, 1932
H. (*Trichotaxis*) *crassa* (Clap. et Lach., 1858) Kahl, 1932
Uroleptosis citrina Kahl, 1932
Trichotaxis crassa (Clap. et Lach., 1858) Borror, 1972
Keronopsis rubra (Ehrb., 1838) Borror, 1972
2. *P. decolor* (Wallengren, 1900) nov. comb.
Holosticha decolor Wallengren, 1900
H. (*Keronopsis*) *decolor* (Wall., 1900) Kahl, 1932
K. globulifera Kahl, 1932
Uroleptopsis viridis (Perejaslawzewa, 1886) Kahl, 1932
K. tannaensis Shigematsu, 1953
K. decolor (Wall., 1900) Borror, 1972
3. *P. flavicans* (Kahl, 1932) nov. comb.
K. flavicans Kahl, 1932
4. *P. multinucleata* (Maupas, 1883) nov. comb.
Holosticha multinucleata Maupas, 1883
H. (*Keronopsis*) *multinucleata* (Maupas, 1883) Kahl, 1932
Uroleptus roscoviana Maupas, 1883
Uroleptopsis roscoviana (Maupas, 1883) Kahl, 1932
K. multinucleata (Maupas, 1883) Borror, 1972
5. *P. multiplex* (Ozaki et Yagiu, 1941) nov. comb.

Keronopsis multiplex Ozaki et Yagiu, 1941

6. *P. ovalis* (Kahl, 1932) nov. comb.
   Holosticha (*Keronopsis*) *ovalis* Kahl, 1932
   Keronopsis arenivorus Dragesco, 1954
   K. ovalis (Kahl, 1932) Borror, 1972
7. *P. ovata* (Horváth, 1933) nov. comb.
   Paraholosticha ovata Horváth, 1933
   Uroleptopsis ovata (Horváth, 1933) Borror, 1972
8. *P. pernix* (Wrześniowski, 1877) nov. comb.
   Oxytricha pernix Wrz., 1877
   Holosticha (*Keronopsis*) *pernix* (Wrz., 1877) Kahl, 1932
   Keronopsis pernix (Wrz., 1877) Borror, 1972
9. *P. pulchra* (Kahl, 1932) nov. comb.
   Holosticha (*Keronopsis*) *pulchra* Kahl, 1932
   Keronopsis pulchra Kahl, 1932 in Borror, 1972
10. *P. retrovacuolata* (Tucolesco, 1962) nov. comb.
    Keronopsis retrovacuolata Tucolesco, 1962
11. *P. similis* (Stokes, 1886) nov. comb.
    Holosticha similis Stokes, 1886
    Trichotaxis stagnatilis Stokes, 1891
    Holosticha aquariumdulcium Bürger, 1905
    H. (*Keronopsis*) *monilata* Kahl, 1928
    H. (*K.*) *similis* (Stokes, 1886) Kahl, 1932
    Keronopsis clavata Vuxanovici, 1963
    Keronopsis similis (Stokes, 1886) Borror, 1972
12. *P. spectabilis* (Kahl, 1932) nov. comb.
    Holosticha (*Keronopsis*) *spectabilis* Kahl, 1932
    Kahliela leptocirra Tucolesco, 1962
    Keronopsis spectabilis (Kahl, 1932) Borror, 1972

2. *Thigmokeronopsinae* Wicklow, 1981
   1. *Thigmokeronopsis* Wicklow, 1981 (*T. jahohai* type by monotypy)
      1. *T. jahodai* Wicklow, 1981

2. *Pseudourostyloidea* Jankowski, 1979
   1. *Pseudourostylidae* Jankowski, 1979
      1. *Pseudourostyla* Borror, 1972 (*P. cristata* type by orig. design.)
         1. *P. cristata* (Jerka-Dziadosz, 1964) Borror, 1972
            Urostyla cristata Jerka-Dziadosz, 1964
            Pseudourostyla levis Takahashi, 1973

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EXPLANATION OF PLATE I

24: *Holosticha multistylata*, scanning electron micrograph (SEM), ventral aspect, showing marginal cirri (Mc), midventral cirri (Mv), and transverse cirri (Tv). 630×
25: *H. multistylata*, SEM, ventral aspect depicting supernumerary midventral cirri (Mv) and left marginal cirri (labelled 1–6). Transverse cirri (Tv) are also evident. 550×
26: *Pseudokeronopsis ovalis*, ventral aspect, SEM, showing marginal, midventral, and transverse cirri 750×
27: *H. multistylata*, anterior end of ventral surface, showing hypertrophy of anterior frontal cirri (Fc) compared with remaining midventral cirri (Mv); malar cirri (Ma), a paroral membrane (Pm) and endoral membrane (Em) are evident. Membranelles end at the anterior of the cell. 750×
28: *P. ovalis*: anterior part of ventral surface, demonstrating size uniformity of midventral cirri (Mv); one malar cirrus (Ma) lies beside the paroral membrane (Pm). The membranelles extend past the anterior of the cell to end along the cell's right side next to the migratory cirri (Mg). 1500×

A. C. Borror et al. auctores phot.

ACTA PROTOZOOLOGICA
Vol. 22, No. 2, pp. 127–138 (1983)

Morphologische und diskriminanzanalytische Trennung von *Colpoda aspera* Kahl, 1926 und *Colpoda elliotti* Bradbury et Outka, 1967 (*Ciliophora*: *Colpodidae*)

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Received on 22 November 1982

Synopsis. In Rohkulturen von *Colpoda elliotti* Bradbury et Outka, 1967 traten Individuen auf, die *C. aspera* Kahl, 1926 stark ähnelten. Daher wurden an 1 Population von *C. aspera* und 2 Populationen von *C. elliotti* morphologische und morphometrische Untersuchungen durchgeführt. Für die Diskriminanzanalyse wählten wir eine Irrtumswahrscheinlichkeit von alpha = 0.001 vor. *Colpoda aspera* unterscheidet sich signifikant von den beiden *C. elliotti*-Populationen, die morphologisch und morphometrisch nicht trennbar sind. Um Individuen der Gruppe *C. elliotti/aspera* mit der Diskriminanzanalyse zu trennen, sind 4 Merkmale notwendig: Distanz vom anterioren Körperende bis zum Makronucleus, Anzahl der Basalkörperpaare der 3. Somakinete rechts des Oralapparates, Länge und Breite der linken Polykinete. Morphologisch unterscheiden sich die Individuen dieser Gruppe nur durch die schwierig erkennbaren und ziemlich variablen Merkmale „Struktur der Pellicula und des Makronucleus". Obwohl unsere Untersuchungen keinen eindeutigen Nachweis für die Selbständigkeit dieser Species liefern, befürworten wir ihre Aufrechterhaltung, da die morphometrische Trennung mit grosser Sicherheit erfolgen kann und im Freiland bisher keine ausgeprägten Übergangsformen beobachtet wurden. Wir anerkennen 4 kleine *Colpoda*-Arten: *Paracolpoda steinii* (Maupas, 1883), *Colpoda aspera* Kahl, 1926, *C. edaphoni* Foissner, 1980 und *C. elliotti* Bradbury et Outka, 1967.

In neuerer Zeit werden zunehmend morphometrische Methoden zur Beschreibung und Abgrenzung von Ciliaten-Arten eingesetzt (Gates und Berger 1974, Gates 1978, Berger und Hatzidimitriou 1978, Kazubski 1979, 1980, Bernatzky et al. 1981, Foissner 1982). Wenn man eine Art auch mathematisch nicht beweisen kann, so steht doch ausser Frage, dass quantifizierende Methoden eine wertvolle Ergänzung zu den klassischen morphologischen Kriterien sind, die allein oft nicht ausreichen, um eine Art so zu beschreiben, dass sie sicher determinierbar wird (Berger 1978, Foissner 1982).

Die vorliegende Studie wurde durch die Beobachtung angeregt, dass in gut ernährten Kulturen von *Colpoda elliotti* Bradbury und Outka, 1967 Formen auftraten, die *Colpoda aspera* Kahl, 1926 ähnelten. Da diese Species auch in vielen anderen Merkmalen weitgehend übereinstimmen, lag der Verdacht nahe, dass sie synonym sein könnten. Um dies zu prüfen, führten wir neben den normalen morphologischen und statistischen Untersuchungen eine Diskriminanzanalyse durch.

Material und Methoden

Untersuchungsmaterial: *Colpoda elliotti* Population I konnten wir an Hand von Originalpräparaten studieren, die uns Frau Dr. Phyllis C. Bradbury (North Carolina State University, Raleigh, NC, U.S.A.) freundlicherweise zur Verfügung stellte. Population II entdeckten wir in der oberen Bodenschicht (0–5 cm) eines intensiv bewirtschafteten Feldes im Tullnerfeld (Niederösterreich). Die Tiere traten 5 Tage nach Wassersättigung der luftgetrockneten Bodenprobe mit geringer Abundanz auf. Zur Kultur wurde ein Teil des Wassers aus der Bodenprobe gepresst und mit etwas getrocknetem Eigelb versetzt. Die Tiere vermehrten sich in diesem Medium nur mäßig gut. Nach etwa 2 Wochen ging die Kultur zu Grunde.

Colpoda aspera fanden wir in der oberen Bodenschicht (0–5 cm) eines durch häusliche Abwässer eutrophierten Schneetälchens dicht unterhalb des Hotels Wallackhaus an der Großglockner-Hochalpenstraße (Foissner 1981). Die Tiere traten 5 Tage nach Wassersättigung der luftgetrockneten Bodenprobe so zahlreich auf, dass eine Kultur nicht nötig war. Weitere Populationen, die allerdings nur morphologisch untersucht wurden, fanden wir in Böden des Tullnerfeldes (Niederösterreich) und der Haitzing-Alm bei Bad Hofgastein (Salzburg).

Morphologische Methoden: Beide Populationen von *C. elliotti* wurden mit der bei Corliss (1953) beschriebenen nassen Silberimprägnationsmethode nach Chatton–Lwoff imprägniert. Von Population II wurden ausserdem Protargolimprägnationen und trockene Versilberungen nach den bei Foissner (1976, 1982) angeführten Methoden angefertigt. *Colpoda aspera* imprägnierten wir mit der trockenen Versilberungsmethode nach Foissner (1976), mit Protargol (Foissner 1982) und mit einer modifizierten Silbercarbonatmethode nach Fernández-Galiano (1976), die zu Dauerpräparaten führt (Foissner, unveröff.). Alle Populationen wurden einer sorgfältigen Lebendbeobachtung (Durchlicht, Phasenkontrast) unterzogen.

Biometrie und graphische Darstellung: Zu den Messungen verwendeten wir ein Okularmikrometer. Bei Beobachtung mit dem Ölimmersionsobjektiv entsprach 1 Teilstrich 1.4 µm. Zwischenwerte wurden geschätzt. Alle Zeichnungen sind sehr leicht schematisiert. Mit Ausnahme der auf *in vivo* Beobachtungen basierenden wurden sie mit einem Zeichenapparat der Firma Reichert angefertigt.

Mathematische Methoden: Entsprechend einem Vorschlag von Berger (1978) erfolgte die biometrische Charakterisierung mit folgenden Parametern: arithmetisches Mittel ($\bar{x}$), Median (M), Standardabweichung (s), Standardfehler des Mittelwertes ($s_{\bar{x}}$), Variationskoeffizient (V_r), Extremwerte. Die Formeln für diese Berechnungen, die mit einem TI-58 Kleincomputer von Texas Instruments durchgeführt wurden, können einschlägigen Lehrbüchern über Biostatistik entnommen werden (z. B. Cavalli-Sforza 1974).

Klassisch wird die Frage, ob sich an Hand der gemessenen Parameter die 3 Populationen von *Colpoda* statistisch gesichert unterscheiden, mit der Varianzanalyse beantwortet. Da die Varianzen der verschiedenen Messdaten stark variieren, wäre ein entsprechendes parameterfreies Verfahren, zum Beispiel der H-Test nach Kruskal und Wallis anzuwenden.

Wir haben einen anderen Weg eingeschlagen, der uns für den Systematiker interessanter erscheint, weil er mehr Informationen liefert. Voraussetzung ist, das die Messdaten jedes Merkmals als angenähert normal verteilt angesehen werden können. Die Daten wurden mit dem Test nach David et al. (1954) überprüft. Danach besteht kein Grund, sie als nicht normal verteilt anzusehen. Fehlende Einzelwerte wurden durch den Mittelwert der vorhandenen ersetzt.

Können Gesamtheiten, hier die Populationen von *Colpoda*, nicht durch ein (quantitatives) Merkmal sicher getrennt werden, so kann dies doch durch die Verwendung mehrerer Merkmale möglich sein. Mit der Diskriminanzanalyse kann entschieden werden, wie und mit welcher Irrtumswahrscheinlichkeit dies erreichbar ist. Die mathematischen Grundlagen stammen von Fisher (1936, 1938). Einführungen finden sich u. a. bei Weber (1980), leider mit Rechenfehlern bei den Beispielen, und Bauer (1954). Eine auch für den mathematisch wenig geübten Leser geeignete Darstellung gibt Bühler (1964). An Hand des vorliegenden Problems wurde ein Programm in hpl für den Kleincomputer HP 9825 S erarbeitet, das Interessenten zur Verfügung steht. Über eine dabei verwendete Verbesserung der Trennungskriterien soll and anderer Stelle berichtet werden.

Die Diskriminanzanalyse fasst mehrere Merkmale zu einem Komplexmerkmal so zusammen, dass der Unterschied zwischen den Gesamtheiten möglichst groß wird. Dazu werden die Merkmale $x_1, x_2, \ldots, x_n$ mit den Gewichtungsfaktoren $b_1, b_2, \ldots, b_n$ multipliziert und dann addiert. So entstehen die Komplexmerkmale:

$$X_A = b_1 x_{A1} + b_2 x_{A2} + \ldots b_n x_{An}$$

und

$$X_B = b_1 x_{B1} + b_2 x_{B2} + \ldots b_n x_{Bn}$$

Die Faktoren $b_1, b_2, \ldots b_n$ werden mit Hilfe der Diskriminanzanalyse so berechnet, dass d_x/S_x = Maximum wird. d_x ist die Mittelwertsdifferenz der Komplexmerkmale A und B, S_x ist die dazugehörige Standardabweichung. Bei möglichst kleiner Standardabweichung ist die Trennung der Populationen am besten. Ein Scheidewert K wird so bestimmt, daß die beiden Verteilungen A und B symmetrisch bezüglich ihrer Flächen getrennt werden. Liegt nun später ein einzelnes (es genügt eins!) Individuum vor, so muss nur das Komplexmerkmal bestimmt werden. Ist der erhaltene Wert kleiner K, so gehört das fragliche Individuum zur Population A, sonst zu B. K besitzt, da er aus einer endlichen Zahl von Messungen gewonnen wurde, einen Fehler. Dieser gibt an, mit welcher Unsicherheit die Trennung behaftet ist. Er gestattet auch, den Bereich zu berechnen, in dem das Komplexmerkmal keine sichere Trennung erlaubt.

Zur Prüfung der vorliegenden Populationen wählten wir eine Irrtumswahrscheinlichkeit vor. Sie gibt an, wie groß die Unsicherheit der Trennung von 2 Populationen maximal sein soll. Wir haben alpha = 0.001 benutzt, d.h. bei 1000 späteren Bestimmungen soll im Durchschnitt maximal 1 unentscheidbarer Fall vorkommen. Es bleibt natürlich ein Akt der Willkür, ab welchem alpha man von einer wirklichen Trennung sprechen kann. Der von uns gewählte Bereich erscheint uns für die Praxis am vernüftigsten, da verhältnismäßig große Unterschiede zwischen 2 Taxa vorhanden sein müssen, damit sie getrennt werden.

Ergebnisse

Morphologische Beobachtungen

Colpoda elliotti Population I: Die morphologische Beschreibung dieser Population führten Bradbury und Outka (1967) durch. Die biometrische Charakteristik findet sich in Tab. 1.

Tabelle 1

Biometrische Charakteristik von *Colpoda elliotti*. Obere Zeile: Population I von Marin County, Kalifornien. Untere Zeile: Population II vom Tullnerfeld, Österreich. Bei Population I basieren alle Werte auf Chatton-Lwoff versilberten Individuen. Bei Population II basieren die Merkmale 4–6 auf protargolimprägnierten, die übrigen auf Chatton-Lwoff versilberten Individuen. M — Median, n — Stichprobenumfang, s — Standardabweichung, $s_{\bar{x}}$ — Standardfehler, V_r — Variationskoeffizient, $\bar{x}$ — arithmetisches Mittel

Merkmal	$\bar{x}$	M	s	$s_{\bar{x}}$	V_r	Extremwerte	n
(1) Länge in µm	26.4	27.0	2.7	0.6	10.4	21–31	25
	24.6	24.0	1.6	0.3	6.5	21–28	25
(2) Grösste Körperbreite in µm	16,3	16.0	2.2	0.4	13.8	13–22	25
	15.5	15.0	1.3	0.3	8.6	13–18	25
(3) Anzahl der Somakineten	11.6	12.0	0.6	0.1	5.0	11–13	25
	12.1	12.0	0.5	0.1	4.3	11–13	25
(4) Länge des Makronucleus in µm	4.8	4.6	0.5	0.1	11.1	4–6	25
	4.5	4.2	0.6	0.1	13.5	4–6.5	25
(5) Breite des Makronucleus in µm	4.4	4.2	0.5	0.1	12.6	3.2–5.8	25
	4.2	4.2	0.5	0.1	12.8	3.2–5.5	25
(6) Distanz vom anterioren Körperende	13.5	13.5	1.2	0.2	8.6	11–16	25
bis zum Makronucleus in µm	9.9	10.0	1.4	0.3	13.7	8–13	25
(7) Anzahl der Basalkörperpaare der 3.	8.4	8.0	0.8	0.2	9.2	7–10	24
Kinete rechts des Oralapparates	8.1	8.0	0.7	0.1	8.2	7–9	25
(8) Länge der linken Polykinete in µm	5.7	5.6	0.2	0.03	2.9	5.6–6.2	25
	5.4	5.6	0.4	0.1	7.4	4.5–5.6	25
(9) Breite der linken Polykinete in µm	1.7	1.6	0.2	0.03	9.2	1.5–2.0	25
	1.6	1.6	0.1	0.02	6.4	1.4–1.8	25
(10) Länge der rechten Polykinete in µm	4.4	4.2	0.3	0.07	7.6	4.0–5.3	25
	4.2	4.2	0.1	0.02	2.5	4.0–4.4	25
(11) Distanz vom anterioren Körperende bis	5.3	5.6	0.5	0.1	9.6	4.2–6.2	25
zum Beginn des Oralapparates in µm	4.8	4.7	0.6	0.1	12.8	4.2–5.6	25
(12) Distanz vom anterioren Körperende bis	10.9	11.2	0.7	0.1	6.2	9.5–12.5	225
zum Ende des Oralapparates in µm	10.0	9.8	0.5	0.1	5.5	9–11.2	25
(13) Anzahl der Silberlinien zwischen der 2.	14.8	15.0	1.4	0.3	9.2	13–17	18
und 3. Kinete rechts des Oralapparates	15.0	15.0	1.3	0.3	8.7	13–17	25
(14) Distanz zwischen der 2. und 3. Kinete	3.3	3.1	0.4	0.1	13.6	2.5–4.5	25
rechts des Oralapparates (postoral) in µm	2.8	2.8	0.4	0.07	12.7	2.0–3.5	25
(15) Anzahl der postoralen Kineten	2.0	2.0	0.0	0.0	0.0	2–2	25
	2.0	2.0	0.0	0.0	0.0	2–2	25
(16) Anzahl der Kineten des Kiels (rechts)	4.5	4.0	0.5	0.1	11.4	4–5	25
	4.7	5.0	0.5	0.1	10.2	4–5	25
(17) Anzahl der Kineten des Kiels (links)	5.0	5.0	0.2	0.04	4.0	5–6	25
	5.0	5.0	0.0	0.0	0.0	5–5	25

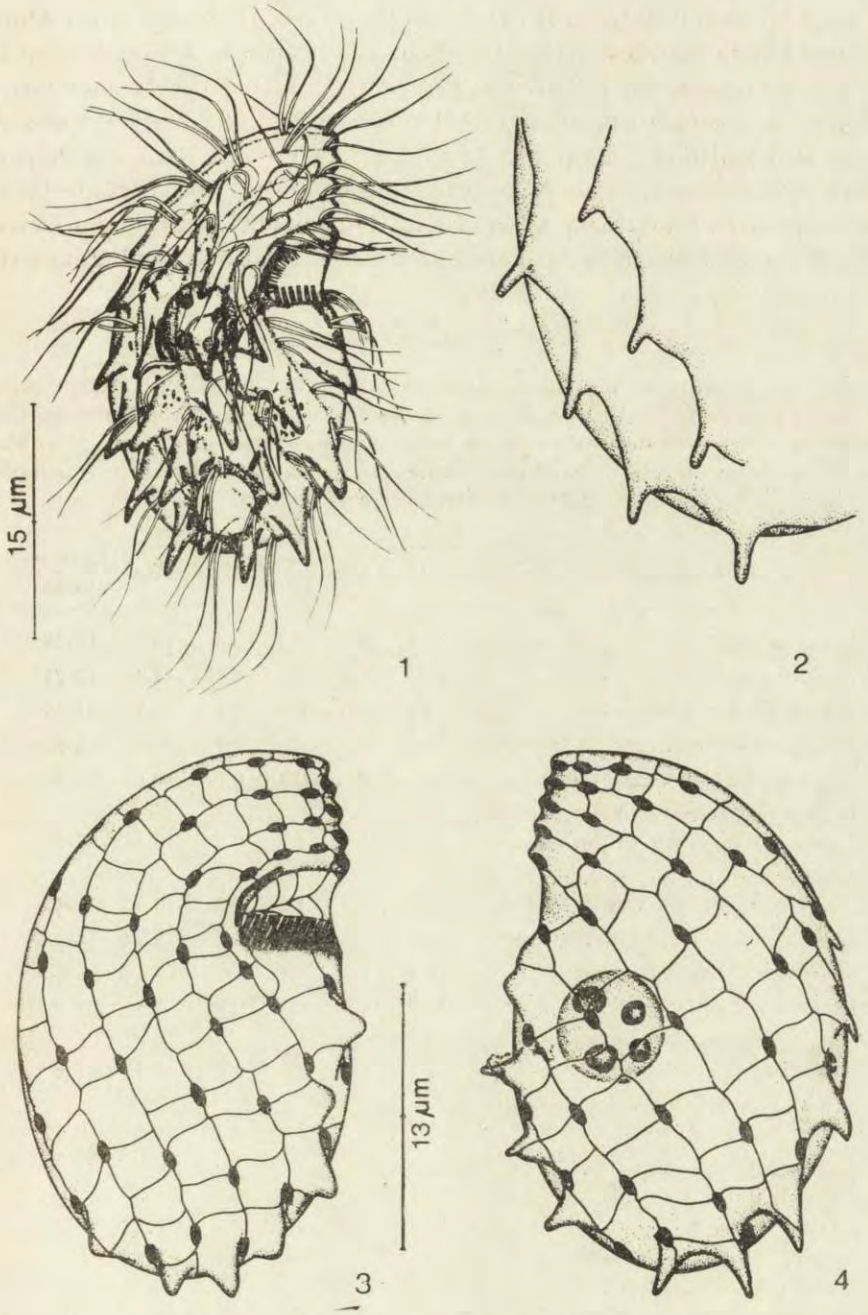

Abb. 1–4. *Colpoda elliotti* Population II. 1 — Rechts laterale Ansicht eines normal ernährten Individuums nach Lebendbeobachtungen (Freilandmaterial). 2 — Detail der Pellicula eines stark ernährten Individuums nach Lebendbeobachtungen (Kulturmaterial). 3, 4 — Infraciliatur und Silberliniensystem der rechten und linken Seite nach Chatton-Lwoff Silberimprägnation (Kulturmaterial)

Colpoda elliotti Population II (Abb. 1–4, 11–13, Tab. 1): Stimmt in der Morphologie und Morphometrie weitgehend mit Population I überein. Wir beschreiben daher nur Beobachtungen, die bei Bradbury und Outka (1967) nicht angeführt sind oder die von den ihren abweichen. Größe *in vivo* etwa 25–35×13–18 µm, also etwas grösser als Population I, die von 15–28×7.5–17 µm variiert. Nach den Präparaten ist das Verhältnis — vielleicht präparativ bedingt — allerdings umgekehrt (Tab. 1). Farblos, etwa 2 : 1 abgeflacht. Cilien in den vorderen fünf Sechsteln des Tieres ungefähr 9 µm, im hinteren Sechstel etwa 12 µm lang. Bradbury und Outka (1967)

Tabelle 2

Biometrische Charakteristik von *Colpoda aspera* (Population von den Hohen Tauern, Österreich). Alle Daten basieren auf Individuen, die mit der Versilberungsmethode von Fernández-Galiano imprägniert wurden. Nur Merkmal 13 stammt von trocken versilberten Exemplaren. M — Median, n — Stichprobenumfang, s — Standardabweichung, $s_{\bar{x}}$ — Standardfehler, V_r — Variationskoeffizient, $\bar{x}$ — arithmetisches Mittel

Merkmal	$\bar{x}$	M	s	$s_{\bar{x}}$	V_r	Extremwerte	n
(1) Länge in µm	23.0	24.0	2.4	0.5	10.5	16–28	25
(2) Grösste Körperbreite in µm	15.9	16.0	2.4	0.5	14.8	12–21	25
(3) Anzahl der Somakineten	12.1	12.0	0.4	0.1	3.3	11–13	25
(4) Länge des Makronucleus in µm	4.9	4.9	0.6	0.1	12.9	4–6.2	25
(5) Breite des Makronucleus in µm	4.3	4.2	0.4	0.1	10.1	3.6–5.6	25
(6) Distanz vom anterioren Körperende bis zum Makronucleus in µm	15.3	15.0	2.1	0.4	13.4	12–21	25
(7) Anzahl der Basalkörperpaare der 3. Kinete rechts des Oralapparates	10.9	11.0	1.2	0.2	10.9	9–13	25
(8) Länge der linken Polykinete in µm	4.3	4.2	0.4	0.1	8.9	4–5.3	25
(9) Breite der linken Polykinete in µm	1.3	1.3	0.1	0.02	9.4	1.1–1.4	25
(10) Länge der rechten Polykinete in µm	4.2	4.2	0.3	0.06	7.3	3.8–5.5	25
(11) Distanz vom anterioren Körperende bis zum Beginn des Oralapparates in µm	4.7	4.8	0.8	0.2	17.5	3.1–6.5	25
(12) Distanz vom anterioren Körperende bis zum Ende des Oralapparates in µm	10.3	10.5	1.0	0.2	9.4	8–12	25
(13) Anzahl der Silberlinien zwischen der 2. und 3. Kinete rechts des Oralapparates	17.9	18.0	2.7	0.5	15.3	14–24	25
(14) Distanz zwischen der 2. und 3. Kinete rechts des Oralapparates (postoral) in µm	2.8	2.8	0.5	0.1	18.1	2–4	25
(15) Anzahl der postoralen Kineten	2.0	2.0	0.2	0.04	10.2	1–2	25
(16) Anzahl der Kineten des Kiels (rechts)	5.0	5.0	0.2	0.04	4.0	4–5	25
(17) Anzahl der Kineten des Kiels (links)	5.2	5.0	0.4	0.1	7.3	5–6	25

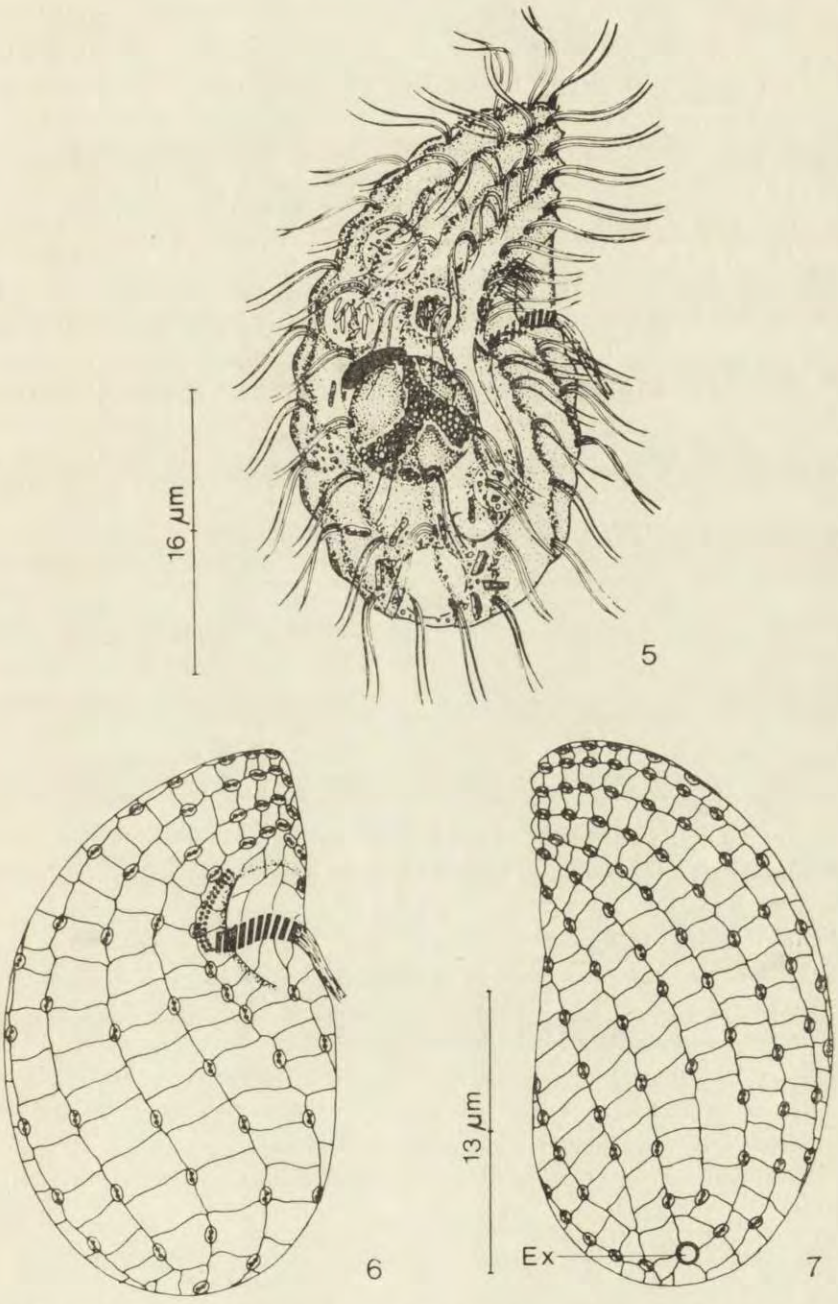

Abb. 5–7. *Colpoda aspera* (Freilandmaterial). 5 — Rechts laterale Ansicht nach Lebendbeobachtungen. Infraciliatur zum Teil nach Protargolimprägnation. 6, 7 — Rechts und links laterale Ansicht nach Protargolimprägnation (Infraciliatur, Körperform) und trockener Silberimprägnation (Silberliniensystem). Ex — Exkretionsporus der kontraktilen Vakuole. Leicht verändert nach Foissner (1980)

zeichnen die Cilien nur etwa 4–5 µm lang. Da sie häufig viel zu kurz gezeichnet werden, messen wir diesem Unterschied wenig Bedeutung bei. Nahrungsvakuolen nur 2–4 µm gross, die kleineren häufig ohne partikulären Inhalt. Kiel meist gerade, selten nach rechts zurückweichend. Lage des Makronucleus wenig konstant, teils links, teils rechts der Medianen. Form und Grösse der auffallenden Pelliculavorsprünge sehr verschieden, bei stark ernährten Individuen sind sie kaum erkennbar. Bei den im Freiland beobachteten Exemplaren waren sie aber fast immer sehr auffallend (Abb. 1). In den Graten dieser Vorsprünge, die sich mit Protargol meist intensiv imprägnieren (Abb. 9), liegen viele winzige Granula (Protrichocysten? Mitochondrien?). Erste Somakinete rechts des Oralapparates dicht unterhalb desselben mit 3–4 ziemlich dicht hintereinander angeordneten Basalkörpern, was auch aus der Abb. 3 von Bradbury und Outka (1967) ersichtlich und bei *C. aspera* noch ausgeprägter ist (Abb. 12, 15). Postoral 2 Kineten, eine dritte endet dicht vor dem Kiel und könnte daher ebenfalls noch als postoral eingestuft werden. Die linke Polykinete besteht aus etwa 15 „Membranellen". Die langen Pharynxfibrillen sind nur nach Protargolimprägnation erkennbar (Abb. 1).

Colpoda aspera (Abb. 5–7, 14–16, Tab. 2): Die morphologische Beschreibung dieser Population findet sich bei Foissner (1980), die biometrische Charakterisierung in Tab. 2. Untersuchungen an anderen Populationen führten zu keinen wesentlichen neuen Erkenntnissen. Die lappenartigen Pelliculavorsprünge sind je nach Ernährungszustand und Population sehr variabel, häufig sind sie kaum erkennbar.

Diskriminanzanalyse

Für die Numerierung der Merkmale siehe Tabelle 1 und 2. Prüft man *C. elliotti* Population I gegen *C. aspera*, so liefern die Berechnungen: $X = x_7 - 3.134x_8 - 6.435x_9$.

C. elliotti Population I	*C. aspera*
$m = -20.184$	$m = -10.870$
$s = 0.848$	$s = 1.505$

$K = -16.826$; alpha = 0.00028; Unsicherheitsbereich von − 16.832 bis − 16.823. Es sind also 3 Merkmale notwendig, um die geforderte Sicherheit zu erreichen. Bei 10 000 Bestimmungen werden durchschnittlich 2.8 Fälle auftreten, die in den Unsicherheitsbereich fallen und daher nicht entscheidbar sind.

Bei der Prüfung von *C. elliotti* Population II gegen *C. aspera* ergibt sich: $X = x_1 - 1.893x_6 - 4.254x_7 + 47.912x_9 - 3.175x_{12} - 1.007x_{13}$.

C. elliotti Population II	*C. aspera*
$m = +2.090$	$m = -42.074$
$s = 6.520$	$s = 5.734$

$K = -21.408$; alpha = 0.00068; Unsicherheitsbereich von −21.414 bis −21.400. Man benötigt demnach 6 Merkmale, um eine Sicherheit von alpha 0.001 zu erreichen. Bei 10 000 Bestimmungen werden durchschnittlich 6.8 Fälle auftreten, die in den

Unsicherheitsbereich fallen und daher nicht entscheidbar sind. *Colpoda elliotti* Population II ähnelt daher *C. aspera* mehr als *C. elliotti* Population I.

Prüft man *C. elliotti* Population I gegen *C. elliotti* Population II, so stehen nur 16 Parameter zur Verfügung, da im Merkmal 15 keine Differenzen auftreten. Aber selbst mit 16 Merkmalen wird das geforderte alpha < 0.001 nicht erreicht, sondern nur 0.0113. Es würden also mehr als 1 nicht entscheidbarer Fall pro 100 Individuen vorkommen.

Betrachtet man die beiden Populationen von *C. elliotti* als gleich, kann man sie zu einer Gesamtheit vereinigen und diese gegen *C. aspera* prüfen. Die Berechnungen liefern:

$X = x_6 + 5.157\ x_7 - 10.404\ x_8 - 32.101\ x_9$.

C. elliotti Population I+II	*C. aspera*
$m = -56.09$	$m = -14.464$
$s = 5.703$	$s = 6.614$

$K = -36.817$; alpha = 0.00071; Unsicherheitsbereich von -38.187 bis -36.815. Es sind also 4 Merkmale notwendig, um die geforderte Sicherheit zu erreichen. Bei 10 000 Bestimmungen werden durchschnittlich 7.1 Fälle auftreten, die in den Unsicherheitsbereich fallen und daher nicht entscheidbar sind.

Diskussion

Wir haben versucht, die 4 kleinen *Colpoda*-Arten nach morphologischen Kriterien zu trennen (Tab. 3). *Colpoda steinii* Maupas, 1883 stellen wir wegen der ausgeprägten Caudalcilien vorläufig in die Gattung *Paracolpoda* Lynn, 1975. Die Endglieder dieser Reihe, *P. steinii* und *C. elliotti*, sind nach unserer Erfahrung bei sorgfältiger Beobachtung ohne Schwierigkeiten zu determinieren. *Colpoda aspera* und *C. edaphoni* sind dagegen sowohl untereinander als auch von den anderen Arten nicht leicht zu unterscheiden, da die Form und Grösse der lappen- und zahnartigen Vorsprünge der Pellicula sowohl nach unseren als auch nach den Untersuchungen von Bradbury und Outka (1967) sehr variabel und vom Ernährungszustand der Tiere abhängig sind.

Wir haben in Tab. 3 bewußt keine morphometrischen Charakteristika aufgenommen, da sich nach den bisher vorliegenden Befunden diese Arten biometrisch so stark ähneln, dass sie ohne höhere statistische Verfahren, wie Diskriminanz- und Multivarianzanalyse nicht sicher trennbar sind. Abgesehen davon, dass dazu nur wenige verlässliche Daten bekannt sind, wäre es bei dem hohen Zeitaufwand, den solche Untersuchungen erfordern, unrealistisch an Ökologen und Faunistiker mit der Forderung heranzutreten, ihre Determination danach aufzubauen.

Mit der Diskriminanzanalyse unterscheiden sich *C. aspera* und *C. elliotti* weitaus stärker als die 2 *C. elliotti*-Populationem. Im Freiland bereitet ihre Unterscheidung auch kaum Schwierigkeiten. In den von uns untersuchten Rohkulturen traten je-

Tabelle 3

Differentialdiagnose der kleinen *Colpoda*-Arten nach morphologischen Merkmalen. Zusammengestellt nach Angaben von Kahl (1926), Burt (1940), Bradbury und Outka (1967), Foissner (1980) und den vorliegenden Untersuchungen

Merkmal	*Paracolpoda steinii* (Maupas, 1883)	*Colpoda aspera* Kahl, 1926	*Colpoda edaphoni* Foissner, 1980	*Colpoda elliotti* Bradbury und Outka, 1967
Pellicula	glatt	mit meist wenig ausgeprägten lappen- oder zahnartigen Vorsprüngen	mit meist stark ausgeprägten lappenartigen Vorsprüngen	mit meist stark ausgeprägten zahnartigen Vorsprüngen
Struktur des Makronucleus	1 sehr großer, zentraler Nucleolus	Nucleolen meist bandartig, selten mehrere große, runde	viele sehr kleine Nucleolen	mehrere große Nucleolen
"Bart"	vorhanden	vorhanden	fehlt	vorhanden
Caudalcilien	2–3 stark verlängerte	fehlen	fehlen	mehrere leicht verlängerte
Somatische Infraciliatur	im posterioren Drittel stark reduziert	im posterioren Drittel wenig bis nicht reduziert	im posterioren Drittel wenig bis nicht reduziert	im posterioren Drittel wenig bis nicht reduziert

doch morphologische Übergangsformen auf, die aber eine biometrische bzw. diskriminanzanalytische Trennung nicht verhinderten. Das weist darauf hin, daß es sich nicht nur um Ökotypen einer einzigen Art handelt. Die Diskriminanzanalyse liefert uns für die Determination dieser Arten folgenden Bestimmungsschlüssel: „Für ein unbekanntes Individuum der Gruppe *C. elliotti/aspera* miss die Merkmale 6, 7, 8, 9 und errechne das Komplexmerkmal nach der oben genannten Formel. Ist das Resultat < -36.817 handelt es sich um *C. elliotti*, sonst um *C. aspera*. K-Werte zwischen -38.187 und -36.815 sind nicht entscheidbar".

Nur das Merkmal 7 ist ein zählbarer Parameter und somit von der Präparation unabhängig. Für die Merkmale 6, 8, 9 muss man dagegen annehmen, dass sie durch die Präparation mehr oder minder stark beeinflußt werden. Das beeinträchtigt die Aussagekraft unserer Untersuchungen, die mit verschiedenen Methoden durchgeführt worden sind, vielleicht nicht unerheblich. Es ist aber hervorzuheben, dass ein so festes und starres Organell wie die linke Polykinete bei der Präparation vermutlich keine wesentlichen Veränderungen erleidet.

Ausser den in Tab. 3 angeführten Species sind in neuerer Zeit noch 2 weitere kleine *Colpoda*-Arten beschrieben worden: *C. oblonga* Dragesco, 1972 und *C. acuta* Buitkamp, 1977. Da ihr Silberliniensystem nicht bekannt und ihre Infraciliatur

atypisch ist, kann die Gattungszugehörigkeit nicht als gesichert gelten. *Colpoda oblonga* könnte eine Grossglockneridae sein, *C. acuta* dürfte zu oder in die Nähe der Kreyellidae zu stellen sein (Foissner 1979, 1980, Jankowski 1980).

DANKSAGUNG

Herrn Helmut Berger danken wir für die graphische Ausgestaltung der Abbildungen von *C. elliotti*, Frau Karin Anrather für die fototechnische Assistenz. Dem Bundesministerium für Gesundheit und Umweltschutz, dem MaB-6 Programm der Österreichischen Akademie der Wissenschaften und dem Forschungsinstitut Gastein-Tauernregion sind wir für finanzielle Unterstützung zu Dank verpflichtet.

SUMMARY

Individuals of *Colpoda elliotti* Bradbury and Outka, 1967 strongly resembling *Colpoda aspera* Kahl, 1926 occurred in enrichment cultures. Thus, morphological and morphometrical investigations were performed on 1 population of *C. aspera* and 2 populations of *C. elliotti*. For discriminant analysis an error probability of alpha = 0.001 was choosen. *Colpoda aspera* differs significantly from the two populations of *C. elliotti* which are morphologically and biometrically inseparable. Four characters are necessary to separate individuals of the *C. elliotti/aspera* group by discriminant analysis: distance from anterior of specimen to the macronucleus, number of basal body pairs of the 3rd somatic kinety right to the oral apparatus, length and width of the left polykinety. Although our investigations do not show an unequivocal proof for the independence of these two species, we support their maintenance because they can be morphometrically separated confidently and hitherto pronounced transitions forms could be not observed in field samples. We recognize 4 small species of *Colpoda*: *Paracolpoda steinii* (Maupas, 1883), *Colpoda aspera* Kahl, 1926, *C. edaphoni* Foissner, 1980, and *C. elliotti* Bradbury and Outka, 1967.

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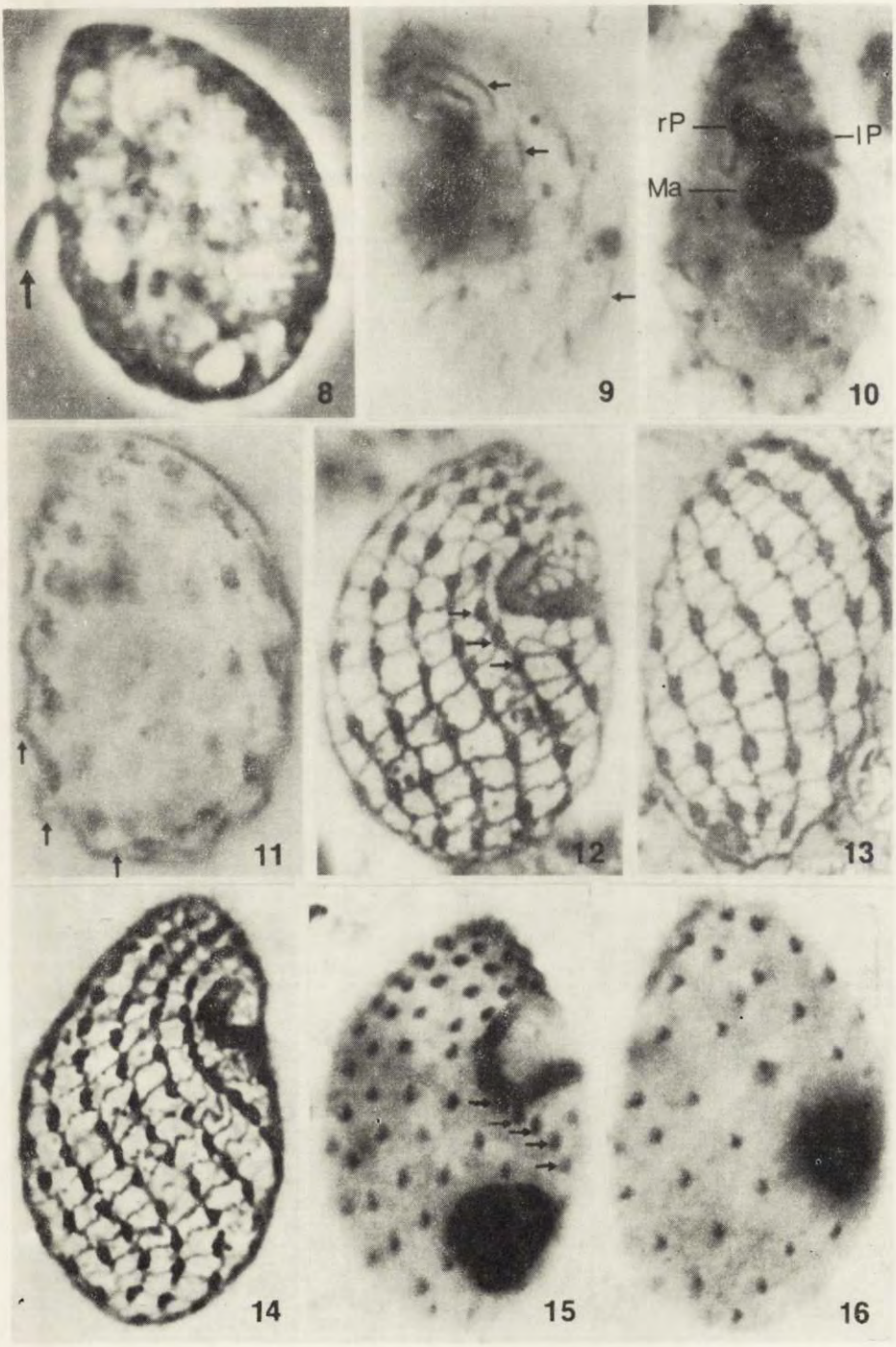
LEGENDE ZUR TAFEL I

8–13: *Colpoda elliotti* Population II (Kulturmaterial). 8 — Lebendaufnahme im Phasenkontrast. Der Pfeil weist auf den „Bart", die Cilien der linken Polykinete. 9 — Infraciliatur der linken Seite nach Protargolimprägnation. Die Pfeile weisen auf die kräftig imprägnierten Grate der Vorsprünge der Pellicula. 10 — Infraciliatur der Ventralseite nach Protargolimprägnation. Rechts im Bild sind die lappenartigen Vorsprünge der Pellicula gut erkennbar. Ma — Makronucleus, lP — linke Polykinete, rP — rechte Polykinete. 11 — Infraciliatur der linken Seite nach Chatton–Lwoff Silberimprägnation. Die Pfeile weisen auf zahnartige Vorsprünge der Pellicula. 12, 13 — Infraciliatur und Silberliniensystem der rechten und linken Seite nach trockener Silberimprägnation. Die Pfeile in Abb. 12 weisen auf 3 dicht hintereinander angeordnete Basalkörperpaare
14–16: *Colpoda aspera* (Freilandmaterial). 14 — Infraciliatur und Silberliniensystem der rechten Seite nach trockener Silberimprägnation. 15, 16 — Infraciliatur der linken und rechten Seite nach Silbercarbonatimprägnation. Die Pfeile in Abb. 15 weisen auf 5 dicht hintereinander angeordnete Basalkörperpaare



W. Foissner et G. Schubert auctores phot.

ACTA PROTOZOOLOGICA
Vol. 22, No. 2, pp. 139–156 (1983)

Cell Shape, Growth Rate and Cortical Pattern Aberrations in an Abnormal Strain of the Hypotrich Ciliate *Paraurostyla weissei*

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Received on 18 November 1982

Synopsis. Naturally occurring variant of the hypotrich ciliate *Paraurostyla weissei* with several phenotypic defects is described. These abnormalities include: modified shape, increased frequency of cortical reorganizations, slower growth rate and formation of monsters. Modifications in the ciliary pattern involve an increased number of the left marginal cirri and an increased number of rows of the marginal cirri. Less frequently occurs a formation of additional dorsal structures on the ventral surface and lack of some ventral structures. Rarely inversion of the left marginal cirri occurs. All these aberrations appear to be a pleiotropic effects of a recessive allele found in a natural population.

The ciliated protozoa are widely utilized in studies concerning the control of pattern determination. As Grimes (1982) recently pointed out, there are three main reasons for this: first, ciliates possess highly ordered, polarized, and asymmetric arrays of cilia, whose origin and development, can be easily followed by light and electron microscopy, second, they are sufficiently large to perform microsurgical operations, and third, they enable the investigator to perform classical Mendelian genetics.

A hypotrich ciliate *Paraurostyla weissei* can be studied using all three approaches. This ciliate has been used as an object for numerous investigations including pattern formation (Jerka-Dziadosz and Frankel 1969, Jerka-Dziadosz, 1974, 1980, 1981 a, b, 1982 a), size dependent regulation of the cortical structures (Jerka-Dziadosz 1976, 1977, Bąkowska 1980, 1981 Bąkowska and Jerka-Dziadosz 1980) and many others.

In this paper, we present a study of a naturally occurring variant of *P. weissei* which shows, in addition to abnormalities in some metabolic functions, several

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abnormalities in the cortical pattern. These include supernumerary rows of left marginal cirri, formation of additional dorsal bristles on the ventral surface, and spontaneous reversal of the polarity of the left marginal cirri. The genetic basis of this variant is not yet firmly etsablished, but the phenotypic aberration in the ciliary pattern seems to be a secondary pleiotropic effect of changes in cell proportions and decrease of the growth rate.

Material and Methods

The organisms used in this study were strains Z-6, Z-8 and G of a hypotrich ciliate *Paraurostyla weissei*. Strains Z-6 and Z-8 were initiated from zygocysts isolated after conjugation in a sample of ciliates collected in a pond in Zaborów, near Warsaw in September 1974. Strain G was obtained from a single cell of a sample collected in a pond near Gdańsk in August 1980. All other lines were progeny lines initiated from isolated conjugating pairs or zygocysts (Table 1). The aberrant lines EE-21 and B-18 were kindly supplied by Miss B. Dubielecka.

The ciliates were cultivated in Petri dishes in Pringsheim solution, using the fresh-water flagellate *Chlorogonium* sp. as a food source (Heckmann 1963). To promote rapid growth, the culture dishes were decanted every day and refilled with fresh food mixed 1 : 1 with culture medium. Once a week each culture was transferred to clean dish in order to avoid detritus adhering to the bottom of the dish.

In order to induce conjugation, culture of cells expressing complementary mating types were mixed and some food was added. Usually massive conjugation was observed the next day. In *Paraurostyla weissei* total, isogamontic conjugation involving complete fusion of two cells takes place (Jerka-Dziadosz and Janus 1975). After fusion and fertilization, a zygocyst devoid of all ciliature is formed. A single exconjugant emerges from the zygocyst several days after pair formation.

Conjugating pairs or zygocysts were isolated into depression slides, where the progeny clones were initiated from a single exconjugant. Progeny clones were cultivated on depression slides in moist chamber for several days, then they were transferred either to test tubes or to small Petri dishes. Observations were performed in the laboratory, in which temperatures were maintained most of the time at 18 to 21°C. A few series of observations were conducted in incubators held at 26°C.

For light microscope observations, samples of cultures were fixed in Bouin's fixative and stained with Protargol using a protocol described earlier (Jerka-Dziadosz 1972). For electron microscopy the ciliates were prepared as described previously (Jerka-Dziadosz 1980).

Statistical analysis included analysis of variance, correlation and regression analysis. These are rutine methods and descriptions can be found in statistical textbooks. Calculations were performed by the Computing Laboratory of the Nencki Institute.

Results

Morphology of Normal Cells of *P. weissei*

A typical cell of *Paraurostyla weissei* from clones Z-6, Z-8 and G are represented on Fig. 1 and Pl. I 1. The ciliates have an elongated elipsoidal shape and they are flattened dorso-ventrally, with a flat ventral surface and a slightly convex dorsal

surface. In well-fed cultures the length of cells ranges from 145 to 205 μm (Jerka-Dziadosz 1976).

On the ventral surface an oral apparatus and rows of locomotory cirri are situated. The oral ciliature consists of an adoral zone of membranelles (AZM) which bounds the peristome on the left side and the anterior margin of the cell. The right side of the peristome is flanked by inner (IPM) and outer (OPM) paroral membranelles (Jerka-Dziadosz 1981 a, b). Seven or eight frontal cirri are situated posterior to the distal portion of the AZM in two transverse rows. The internal architecture and size-dependent regulation of those structures was described by Jerka-Dziadosz (1980), and Bąkowska (1981).

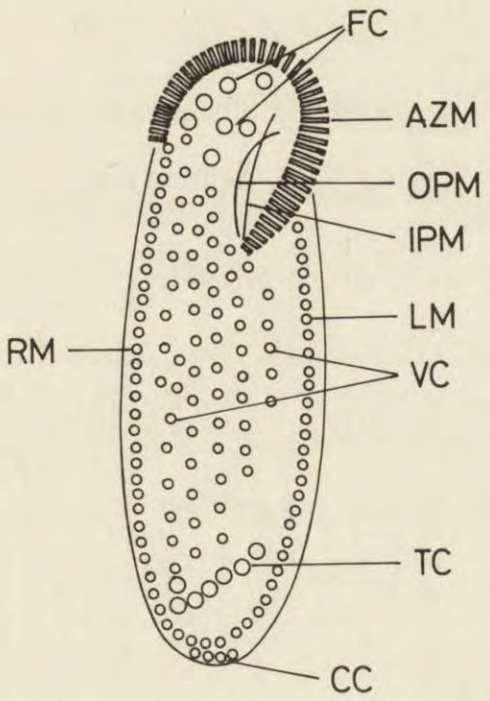

Fig. 1. Cortical anatomy of the ventral surface of *Paraurostyla weissei*. Abbreviations: AZM — adoral zone of membranelles, FC — frontal cirri, IPM — inner paroral membranelle, OPM — outer paroral membranelle, LM — left marginal cirri, RM — right marginal cirri, VC — ventral rows of cirri, TC — transverse cirri, CC — caudal cirri. The anterior of the cell is up

The middle of the ventral surface is occupied by four to five (occasionally six) longitudinal rows of ventral cirri. This variability is mainly caused by the fact that the rightmost ventral row is made up of two segments which sometimes do not join each other, but overlap instead, resulting in two rows.

Posteriorly to the ventral cirri is located an oblique row consisting of seven to nine transverse cirri. A single row of marginal cirri is located at both right and left

margins of the ventral side. Cells of normal size possess about fifty cirri on the left margin (Jerka-Dziadosz 1976).

On the posterior tip of cell of *P. weissei* a small group of caudal (CC) cirri (originating at the posterior termini of dorsal bristle primordia) are present. The dorsal surface is covered by five or six rows of dorsal bristle units (Jerka-Dziadosz 1982 a).

Origin and Characterization of the Abnormal Strains

The abnormal multi-left-marginal (*mlm*) phenotype which is the subject of this paper appeared among backross progeny of a cross between two clones of *P. weissei*, Z-6 and Z-8 (Table 1).

Table 1

Properties of strains of *P. weissei*

No	Strain designation	Place and date of isolation	Parents	Phenotype
(1)	Z-6	Zaborów IX. 1974	?	wild type
(2)	Z-8	Zaborów IX. 1974	?	wild type
(3)	Z-68	Cross in laboratory III. 1975	Z-6×Z-8	wild type
(4)	G	Gdańsk VIII. 1980	?	wild type
(5)	A-505	Cross in laboratory XI. 1977	Z-6×Z-68	multi-left-marginal
(6)	C-6	Cross in laboratory V. 1979	Z-6×Z-68	multi-left-marginal
(7)	C-12	Cross in laboratory V. 1979	Z-6×Z-68	wild type
(8)	E	Cross in laboratory X. 1980	Z-6×G	wild type
(9)	EE-21	Cross in laboratory IX. 1981	E×E*	multi-left-marginal
(10)	B-18	Cross in laboratory X. 1981	E-4×E-5′	multi-left-marginal
(11)	B-37	Cross in laboratory X. 1981	E-4×E-5′	wild type

* The exact parental lines are not known. Conjugating pairs were isolated from a mixture of all progeny E lines obtained from cross C-6×G.

Four abnormal lines showing the same phenotypic aberrations were obtained independently. These were clones A-505, C-6, EE-21 and B-18. Clones A-505 and C-6 are progeny lines isolated after a back cross between Z-68 and Z-6 (Z-68 is a progeny line from a cross of Z-6 and Z-8). EE-21 and B-18 are progeny lines of crosses between F1 lines of a cross between C-6 and wild type line G.

From the observations performed so far it appears that the abnormal phenotype may be caused by an recessive allele at a single gene locus. Detailed genetic analysis is under way (Dubielecka and Jerka-Dziadosz, unpublished) and will be published separately.

Under the dissecting microscope the abnormal lines can easily be distinguished by several features. These include: a low density of culture, presence of many reorganizing cells, presence of irregularly shaped monsters and rounded up cells. Frequently the left side of the cilitaes appears humped.

The abnormal phenotype was first observed in exconjugant clones 2–4 weeks after the zygocyst excystment. In some abnormal lines the slow growth rate is observed from the first division, in others the "abnormal" culture for several weeks was indistinguishable from normal sister culture (e.g., C-6).

Culture Growth, Cell Cycle and Reorganization

As it was already mentioned, the cultures of the aberrant clones grow much more slowly than the wild type sister clones, and they never reach the same density as the wild type cultures. In order to find out the difference in doubling time between wild type and mutated clones, the following experiments were performed. Twenty one cells of clone C-12 (wild type) were isolated into three-spot depression slides, one cell in each depression. The culture medium and *Chlorogonium* was added up to the volume of 0.5 ml. The number of cells in each depression was counted every 24 h during 5 days. The mean number of cells per depression was calculated and the growth curve is presented on Fig. 2. The doubling time for C-12 ranges between 12–18 h at room temperature (20° C). In parallel with this experiment, 21 single cells of the aberrant clone C-6 were isolated and kept in the same conditions as the control cells and the number of cells in each depression was noted every 24 h. The growth curve for C-6 line is presented on Fig. 2. It is evident that the doubling time of C-6 culture is at least twice as long as that of the control C-12 cells.

This experiment was repeated with other sister clones B-37 (wild type) and B-18 (multi-left-marginal). The results were very similar to those of the previous experiment (Fig. 2). Again, as in the previous experiment, the doubling time of the aberrant B-18 clone was at least twice as long as that of the wild type B-37 clone.

On Fig. 2 two additional curves are shown. The broken line with filled triangles indicates the mean number of the slowest-growing B-37 cells from six depressions in which the number of cells after the 5th day was less than 60. The broken line with open triangles represents the mean number of the fastest growing B-18 cells from 8 depressions in which three were 4 to 5 cells at the end of the experiment. As can be seen, the difference between the slowest growing control cells and fastest growing aberrant clones is still very remarkable.

Observations of multi-left-marginal B-18 cells revealed that in the B-18 group in 6 depressions at the 5th day of experiment the number of cells was lower than the

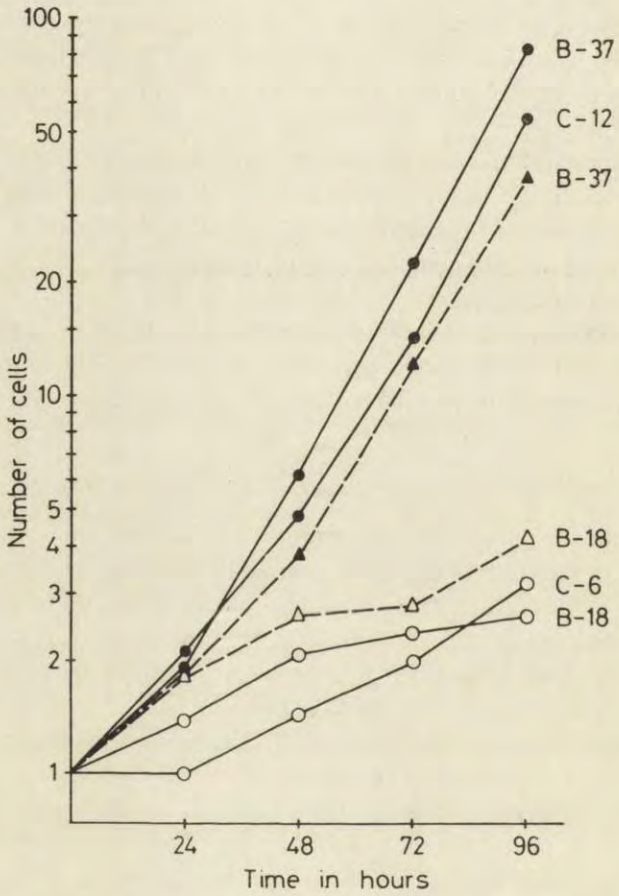

Fig. 2. The growth curves of wild type strain C-12 and B-37 (filled circles and triangles) and *mlm* strains C-6 and B-18 (open circles and triangles). The number of cells is shown on a logarithmic scale. Each curve represents an average of 21 to 29 replicates

previous day. Rounded up or dead cells were seen in some of those depressions. This indicates that the slowing down of the doubling time in multi-left-marginal clones results not only from an increase of the individual generation times, other factors such as cell death and reorganization may also be involved.

Observations of protargol preparations made from mass well-fed growing cultures of the B-18 line indicate that about 25% of a population may be removed from the normal division cycle and undergo a physiological reorganization or produce non-growing monsters. The presence or absence of the replication band in the macronuclei and of the appropriate primordia of ciliature seen on protargol stained preparations enables one to identify the stage of the cell in its cell cycle. 263 random cells from the B-18 line and 138 random cells from the C-12 (wild type) line were tallied according to stage in the division cycle. The results are shown as

a histogram on Fig. 3. In a control, growing population there were no reorganizing cells and monsters, and the percentage of cells in G-1 and S and D phases were relatively higher than in the aberrant clone. In a population of the aberrant B-18 clone, about 12.5% of the cells were rounded up monsters (Mb) with evident deformations in shape, usually without functioning mouthparts. About 13% of the

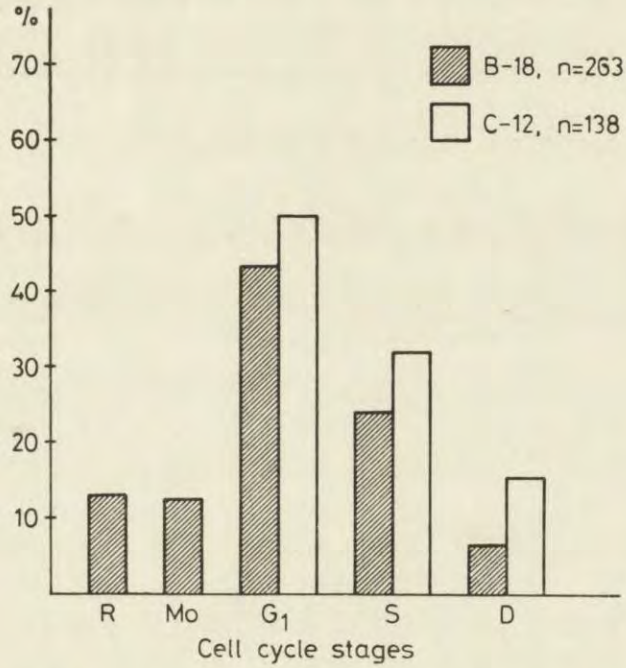

Fig. 3. Distribution of cells in different stages of the cell cycle in protargol stained preparations from well fed cultures of the control C-12 strain and the *mlm* B-18 strain

studied B-18 population were cells in different stages of physiological reorganization (R), replacing its ciliature by a new set. Most probably only a portion of those cells produce relatively normal ciliature and therefore yield cells able to grow and divide, whereas the rest produce cells with defective mouth-parts, this causing further reorganization monster formation.

In conclusion, it can be said that the growth rate of the aberrant multi-left-margina clones is two times slower than that of the normal wild-type clones and that the increase in doubling time is supplemented by an increased frequency of reorganization, monster formation and cell death.

It should also be mentioned that the life span of the aberrant clones is much shorter than that of the wild type clones. For instance the control line Z-6 isolated

from a zygocyst 8 years ago is still viable and able to conjugate, whereas both aberrant lines A 505 and C-6 lived no longer than 12 months, and line EE-21 lived only 3 months. Culturing the aberrant lines require special care.

Defects in the Cortical Pattern

In protargol stained preparations, the most outstanding feature of the aberrant clones is the presence of 2 or 3 rows of cirri at the left margin in most of the morphostatic cells (Pl. I. 2–4). In all studied abnormal clones, over 90% of the cells possess supernumerary rows of the left marginal cirri (Table 2). The rows were enumerated in such a way that the row closest to the ventral cirri is called LM-1, and subsequent rows to the left margin as LM-2, LM-3 etc.

Table 2

Percentage of cell with one, two and three left marginal rows in multi-left-marginal strains of *P. weissei*

Strain designation	Parents and date of conjugation	Date of preparation	%			n
			1 LM	2 LM	3 LM	
A-505	Z-6 × Z-68 16 XI 1977	13 V 1978	10.5	47.3	42.1	19
C-6	Z-6 × Z-68 11 V 1979	4 VI 1979	100	—	—	22
		2 X 1979	26	42	31	50
		7 XI 1979	24.5	41.5	33.9	53
		14 XII 1979	25.1	39.2	35.7	56
EE-21	E × E	18 XI 1981	5.5	63.8	30.5	36
B-18	14 X 1981 E-4 × E-5′ 31 X 1981	19 II 1982	4.5	47.5	47	44

Preparations of exconjugant cultures of line C-6 (Table 2) revealed that in a culture fixed about 20 days after the first cortical reorganization in an exconjugant, the cortical pattern shows no deviation from the normal pattern. Preparations made during several successive months showed that once the abnormality is expressed, the percentage of cells with 1 LM, 2 LM's and 3 LM's is relatively stable. In line C-6 the percentage of cells with 1 LM stays around 25%, whereas in the remaining abnormal lines it is much lower (about 5% in EE-21 and B-18, about 10% in A-505). The percentage of cells with two and three LM's is rather similar in all abnormal lines with a slight tendency for cells with 2 LM's to be more frequent. Morphostatic cells with more than 3 rows are rare and usually show other accompanying abnormalities.

The pattern of distribution of cirri is variable. Short segments of LM rows are seen in the anterior as well as posterior parts of the cell (Pl. I. 3, 4). The number of cirri in a particular row range from 4–48.

The increase in the number of the left marginal cirri is not the only defect in ciliary pattern in the *mlm* variant of *Paraurostyla weissei*. Observations of protargol stained preparations revealed that other abnormalities often coexist. These include an increase in the number of dorsal bristles and a deficiency in ventro-transversal cirri. Extra dorsal bristles are seen on the left dorsal side (neighbouring with the left marginal cirri) where they are arranged into one or two extra longitudinal rows.

Dorsal bristle units were also frequently seen at the anterior part of the left marginal row of cirri (Pl. II 5) and sometimes a complete dorsal row was present between two rows of the left marginal cirri. On a preparation of the aberrant B-18 line about 19% out of 77 cells possessed dorsal bristle units located on the ventral surface in addition to apparently normal set present on the dorsal surface. All of those cells possessed more than one row of left marginal cirri.

Another abnormality in all aberrant clones studied so far was a deficiency in the fronto-ventro-transversal complex of cirri (Pl. II 6). In such cells, there are usually only two rows of ventral cirri and only 2–3 transverse cirri. On a preparation of the aberrant line B-18, about 11.6% out of 77 cells showed a deficiency in ventral and transverse cirri. They all possessed additional marginal rows on the left side. Cells with both deficient ventral cirri and additional dorsal bristles are rare, and usually are those which enter the prelethal reorganization pathway.

Another defect observed in multi-left-marginal clones is that in macronuclear shape. Occasionally, instead of two egg-shaped macronuclei the cells possess one elongated Ma (Pl. II 5). In such macronuclei, the replication bands start at both ends and meet in the middle of the nucleus. The number of micronuclei varies between 2–8.

Finally, the last remarkable morphological aberration in the multi-left-marginal clones was a rare instance of spontaneous inversion of some of the left marginal cirri (Pl. II 8, Pl. III 9). In normal cells of *P. weissei*, all marginal and ventral cirri possess similar and characteristic ultrastructure (Jerka-Dziadosz 1980, Bąkowska 1980). In cells of normal size, the left marginal cirri are composed of 3–4 transverse rows of ciliated basal bodies (Pl. II 10) connected to one another at three levels by amorphous connectives which also surround the kinetosomes in a form of a cirral basket (cb). In protargol stained preparations the whole structure appears in the light microscope as a dark rhomboid plate. Each marginal cirrus is accompanied by three large microtubular fibers, also seen on protargol stained cells (Pl. III 10). Those are: (1) the anterior longitudinal fiber (AL) originating at the anterior left margin of each cirrus and running anteriorly, (2) posterior longitudinal fiber (PL), shorter than AL, originating at the right side of the cirrus and running posteriorly. The third and most characteristic fiber is the so called small subectoplasmic fiber

SSR, which originates at the posterior right side of the cirral basket and runs obliquely posterior. This fiber is very prominent and readily visible in ventral and right marginal cirri on Fig. 2.

Due to the characteristic positions of AL and SSR fibers it is possible to distinguish normal and inverted rows of cirri. One such cell with inverted LM segments is seen on (Pl. III 9). The segment of LM located immediately posterior from the AZM band shows normal orientation. Both AL and SSR are seen on the left side of the cirral row. The LM segments (indicated by asterisks) seen in the middle and posterior part of the cell are inverted antero-posteriorly. Both AL and SSR fibers are seen on the right side of the rows, the AL fiber is directed posteriorly and the SSR fiber runs toward the anterior-right of the cell. The left, most posterior row of marginal cirri in that cell shows normal orientation in its posterior part (out of focus), then it makes a U-turn at the place indicated by an arrow. The right anterior part of this row shows inverted orientation. Whether this indicates an intermediate step in the process of transformation of normal-to-inverted orientation or the opposite cannot be resolved.

Inverted segments of cirral rows and bent LM rows were observed in only few cells, and only on the left margin of the cell.

Morphometric Comparison of the Cortical Pattern in Normal and Multi-left-marginal-lines

In order to find out the significance of the morphological differences between normal and aberrant lines two sister clones of *P. weissei* were subjected to a morphometric analysis. Phenotypically aberrant C-6 line and the wild type C-12 line were grown in conditions as nearly identical as possible. Samples were fixed at known intervals in the clonal life, and data were collected from protargol stained preparations on the cell's length and width, and on the number of adoral membranelles and left marginal cirri. The data were then subjected to statistical analysis of variance and analysis of correlation and regression. For comparison of differences between pairs of data the Duncan test was used. The results are presented in Table 3 and Fig. 4.

From the first glance at Table 3, it appears that all C-6 cells are shorter than the wild type C-12 cells. This difference is statistically significant. This shortening of the long axis of the cell is expressed earlier than the multiplication of the rows of left marginal cirri. The width of the aberrant cells is not significantly different from that of C-12 cells and is similar in all aberrant cells. The length and width are significantly correlated in all studied groups except in C-6 cells with 3 LM rows (Table 4).

The number of adoral membranelles in control C-12 cells is significantly higher than in all aberrant cells.

Comparison of the number of left marginal cirri in the studied groups of cells indicates that the number of LM in wild type control cells (C-12) is significantly

Table 3

Morphometric data of control wild-type and multi-left-marginal strains of *P. weissei*

Symbol	Strain designation	*n*	Length micrometers	Width micrometers	Number of AZM	LM-1	LM-2	LM-3	Total LM
K	C-12	20	162–243 (204.2)	54–84 (62.6)	54–69 (62.6)	41–55 (49.7)			41–55 (49.7)
I	C-6	20	111–185 (149.6)	34–84 (65.1)	40–59 (50.5)	35–45 (40.1)			35–45 (40.1)
II	C-6	30	126–198 (164.2)	42–78 (59.2)	45–63 (54.3)	31–48 (40.9)			31–48 (40.9)
III	C-6	30	123–195 (158.1)	45–84 (65.2)	41–67 (53.1)	6–43 (31.9)	8–45 (28.6)		43–86 (60.5)
IV	C-6	30	129–189 (162.2)	48–84 (64.3)	36–61 (52.4)	4–48 (20.8)	6–41 (24.8)	2–41 (20.4)	39–95 (66.1)

Numbers in parentheses indicate the mean value.

The data concerning the wild type C-12 clone were collected from preparations made 6.5 months after conjugation, as were the data of groups II-IV concerning the aberrant clone C-6. The data concerning group I of clone C-6 with only one LM row, were collected from preparations made 3 weeks after isolation of the conjugating pair. Groups II, III and IV represent data from three subpopulations of the same preparation.

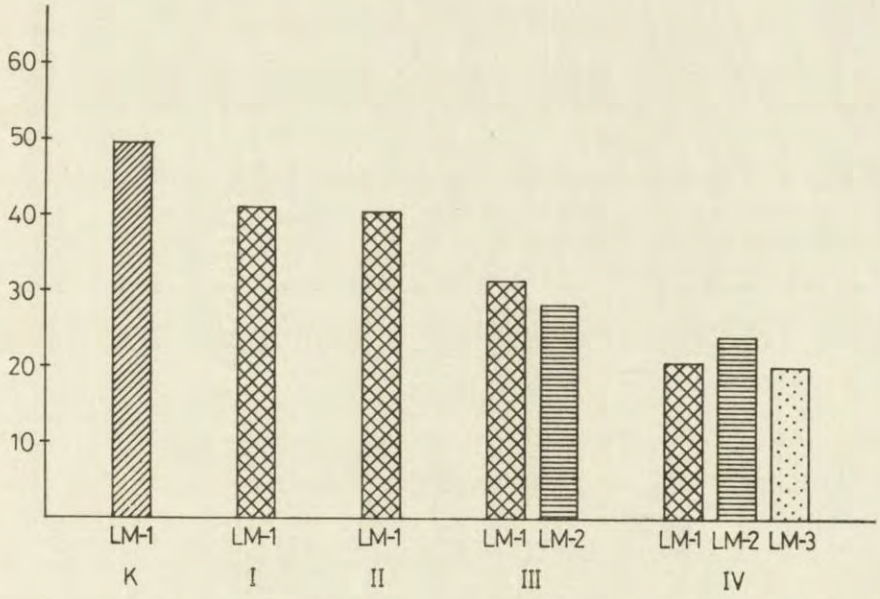

Fig. 4. Histogram showing the number of cirri in the left marginal cirral rows. Compare with Table 2

Table 4

Correlation coefficients (r) and probability of error (P) for morphometric parameters in control and multi-left-marginal lines of *P. weissei*

No	Pairs of variables	Strain designation and symbol									
		C-12 K		C-6 I		C-6 II		C-6 III		C-6 IV	
		r	P	r	P	r	P	r	P	r	P
(1)	L/W	0.57	xx	0.73	xxxx	0.70	xxxx	0.56	xxxx	N.S.	
(2)	L/AZM	N.S.		0.78	xxxx	0.71	xxxx	0.54	xxxx	0.62	xxxx
(3)	L/LM	N.S.		0.73	xx	0.62	xxxx	N.S.		N.S.	
(4)	AZM/total LM	0.60	xxx	0.63	xxx	0.55	xxx	0.51	xxx	0.42	x

N.S. – non significant, x – $P < 0.05$, xx – $P < 0.01$, xxx – $P < 0.005$, xxxx – $P < 0.001$.

different from the total number of LM in C-6 subpopulations regardless of the number of LM rows in the aberrant cells. In C-6 subpopulations with only one LM row the number of LM cirri is lower than in wild type cells by about 9 cirri. In subpopulations with 2 or 3 LM rows the total number of left marginal cirri increased on the average, by about 30% (from about 41 to about 66) without a significant correlated increase of other parameters such as the cell's length or width. In the remaining 25% of cells which possess only one LM row, the number of left marginal cirri is similar to that in young clones where multi-left marginal rows are not expressed.

The total number of the left marginal cirri in cells with 2 rows of LM is not significantly different from the total number of LM cirri in cells with 3 rows of LM at the 1% probability level, but is significantly different from wild type C-12 control cells and is significantly higher than in C-6 cells with only 1 LM row. The number of LM cirri in LM-1 is significantly different in subpopulations with 2 or 3 LM rows, whereas the number of cirri in LM-2 is similar in both groups (Fig. 4).

Analysis of variance of the number of cirri in LM-1 and LM-2 in subpopulations with two marginal rows (group III) indicated that there is no significant difference between them. That means that there is no constant tendency of, for instance, one row to be shorter and the other to be longer. A similar situation exists in the subpopulation with three left marginal cirri rows (group IV). This may indicate that the aberrant cell positions randomly the primordia (Pl. II 7) of marginal cirri. Study on development will clarify this question.

Discussion

The results of this study show that a particular strain of the ciliate *Paraurostyla weissei* possesses a number of structural abnormalities concerning the cortical pattern of ciliature, and defects in some cell functions. These abnormalities include: (1) modified shape, (2) increased frequency of cortical reorganizations, slower growth rate and monster formation, (3) increased variability in the number of ciliary structures, (4) an increased number of the left marginal cirri and an increased number of rows of marginal cirri, (5) an increased number of dorsal bristles and misplacement of extra dorsal units onto the ventral surface, (6) lack of some ventral structures, (7) spontaneous inversion of left marginal cirri.

As concerns the cell's shape, it has been shown in the Result section, that the mean length of the aberrant strain C-6 is about 75% of that of the normal cells. The diminution in length is the first phenotypic change observed when the aberrant clones develop following conjugation.

The multiplication of the LM cirri is somehow related to the increase and mispositioning of dorsal bristles and failures in production (or resorption) of some newly formed fronto-ventro-transverse cirri. In cells with only one LM row defects in the ciliature were not observed.

Antero-posterior inversion of some marginal cirri is very rare. The origin of the inversion is unknown, and how this inversion is related to other defects is also mysterious.

As to the increased frequency of cortical reorganizations and monster formation, it can be safely concluded that they are related to the abnormal ciliary pattern and/or the abnormal size. Defects in cortical structures caused by microsurgery inevitably induce cortical reorganizations (Jerka-Dziadosz and Frankel 1969) and attainment of a critical size concomitantly with proper intracortical proportions before a cell can divide was postulated by DeTerra (1974). If critical size was not attained the cells reorganize instead. Failures in formation of functioning mouthparts frequently lead to repetitive cortical reorganizations and cell death. Monster formation is usually related to abortive morphogenesis. The cause of the slower growth rate and how it is related to the abnormal size and ciliary pattern are at present unknown. In other cortical mutants, thus far described, defects in ciliary pattern are not accompanied by changes in the growth rate (Frankel 1973, Jerka-Dziadosz and Frankel 1979, Kaczanowski 1976). On the other hand in conical mutant of *Tetrahymena* changes in cell shape yielding unequal division products are concomittant with slower population growth, but the ciliary pattern appears normal (Doerder et al. 1975, Schäfer and Cleffmann 1982). In *Stentor* such factors as macronuclear shaping, division and DNA synthesis are sensitive to the state of cortex (DeTerra 1978).

Although the causal relationships between the above described different phenotypic defects in *P. weissei* are not known, their genetic basis seems to be common. The detailed genetic analysis is in progress. From the data available (Table 1, Dubielecka, unpublished) it seems very probable that the abnormal phenotype is caused by a recessive allele, which was present in the nucleus of the heterozygotic strain Z-6 and appeared as a recessive homozygotic segregant in backcross progeny of heterozygotic descendant of line Z-68.

The effect of this genetic variant on cortical pattern is very indirect. The cortical pattern aberrations appear some time after the changes in cell proportions and the depression of growth rate. It appears as if these cortical changes are indirect pleiotropic consequences of other disturbances.

Since in *Paraurostyla weissei* the whole ciliature (except the anterior AZM) is formed in each divisional morphogenesis and changes in cortical pattern are limited to periods of morphogenetic activity (Jerka-Dziadosz and Golińska 1977), the abnormality found in the *mlm* variant results from impaired developmental events which cause a high degree of instability in vegetative perpetuation of the preexisting pattern.

Two aspects of control of pattern determination have been distinguished as a result of morphometric analysis of cell parameters and cortical pattern of *P. weissei* as well as the analysis of the differentiation of ciliary structures. First, there is a long-range positional system responsible for positioning of primordia (Jerka-

Dziadosz 1974) and size dependent regulation of the number of forming structures (Jerka-Dziadosz 1976, 1977, Bąkowska 1980, 1981, Bąkowska and Jerka-Dziadosz 1980). The interdependence of the number of particular structures in cells can be used as an indication of the operation of the long-range positioning system. By extensive morphometric analysis of wild-type strains of *P. weissei* it has previously been documented that such aspects of the cortical pattern as the number of AZM membranelles and the total number of the left marginal cirri are both highly correlated with the cell size (Jerka-Dziadosz 1976). Moreover, the number of AZM membranelles and the number of LM are also related in a constant and characteristic way ($r = 0.84$, Bąkowska 1980). In the aberrant multi-left-marginal strains the correlation coefficients for the two pairs of data are lower (Table 4) the relation nevertheless exists in all subpopulations of the variant.

From all of these observations it can tentatively be concluded that the system responsible for the regulation of the number of forming elements of the cortical pattern is only slightly modified in the *mlm* variant.

The second aspect of the pattern differentiation seemingly affected in *mlm* variant is the mode of involvement of performed structures in pattern determination. It is already well documented that during morphogenesis in the wild type strains of *P. weissei* the performed ciliature contributes extensively and in predictable manner to the elaboration of primordial ciliary structures. This is particularly evident in the case of marginal cirri (Jerka-Dziadosz 1974, 1980). The marginal cirri (like kineties) are believed to reproduce cytotactically (Aufderheide et al. 1980) which means that the number of forming rows and their location and polarity follow that of the parental cell (Grimes 1976, Grimes and L'Hernault 1979). The variability of pattern of the left marginal cirri (Pl. I), the displacement of dorsal bristles (Pl. II) and the inversion of polarity (Pl. III) of marginal structures, all point to the conclusion that the cellular processes underlying cytotactic phenomena are seriously impaired in the *mlm* variant of *P. weissei*.

There is another interesting aspect of the destabilization of the number (and polarity) of marginal cirri. In a recenty described mirror-image doublet cells of *P. weissei*, variability in the number of the left marginal cirri in the symmetry reversed component and occasionally also reversal of some of those cirri was observed (Jerka-Dziadosz 1982 b). These doublets arised as a result of failures of separation of sister cells during division and subsequent rearrangement of the cortical structures in the right component of the doublet into a mirror-image of the normal left component. Interestingly, in the population of mirror-image doublets the frequency of cells with one, two and three LM rows is similar to that found in *mlm* variants (Jerka-Dziadosz 1982), (in the right, standard symmetry component of the doublet there is always only one row of the left marginal cirri). The inverted segments of marginal cirri seem to be more frequent than in the *mlm* variant. The doublet was isolated from the wild type G strain of *P. weissei* in which

single cells never show any kind of abnormalities in marginal or other cirri. Strain G is a wild type dominant homozygote with respect to the *mlm* allele (Jerka-Dziadosz,unpublished).

From the above considerations it can be concluded that destabilization of intracellular regulation of ciliary structures could be a final effect of some kind o-genetically determined defects, as well as a result of impaired intracortical interactions caused by modified juxtapositions of some cortical regions. That cytogeof metrical dislocation may have similar effect on the pattern as gene mutation is known in other ciliates as well (e.g., phenocopies of *janus* mutant in *Tetrahymena*, Nelson, unpublished observation, Frankel 1983).

The perturbations of developmental process by mutant genes or other means can help to recognize the functional relationship that create normal pathways that lead to the genesis of the normal pattern. Detailed study on development of the aberrant pattern in multi-left-marginal variant and in mirror-image doublet cells may shed new light on the nature of the developmental system.

A final remark concerns comparative considerations. As it was already mentioned a genetically based abnormality in cortical pattern was described in *Euplotes minuta* by Frankel (1973). There are some reports in the literature concerning both: variability in the number of marginal cirri in some hypotrich ciliates (Borror 1980) and multiplication of left marginal cirri in particular strain of one species (Walker and Grim 1973). This indicates that mutations causing instability of the number of cortical structures are probably quite frequent in the nature.

ACKNOWLEDGEMENTS

The authors wish to express their sincere thanks to Drs J. Bąkowska, J. Frankel, K. Golińska and E. M. Nelsen for critical reading the manuscript, discussion and helpful comments. Expert technical assistance of Mrs. K. Muszyńska and Mrs. L. Wiernicka is kindly acknowledged. This research was supported by grants MR.II. from Polish Academy of Sciences.

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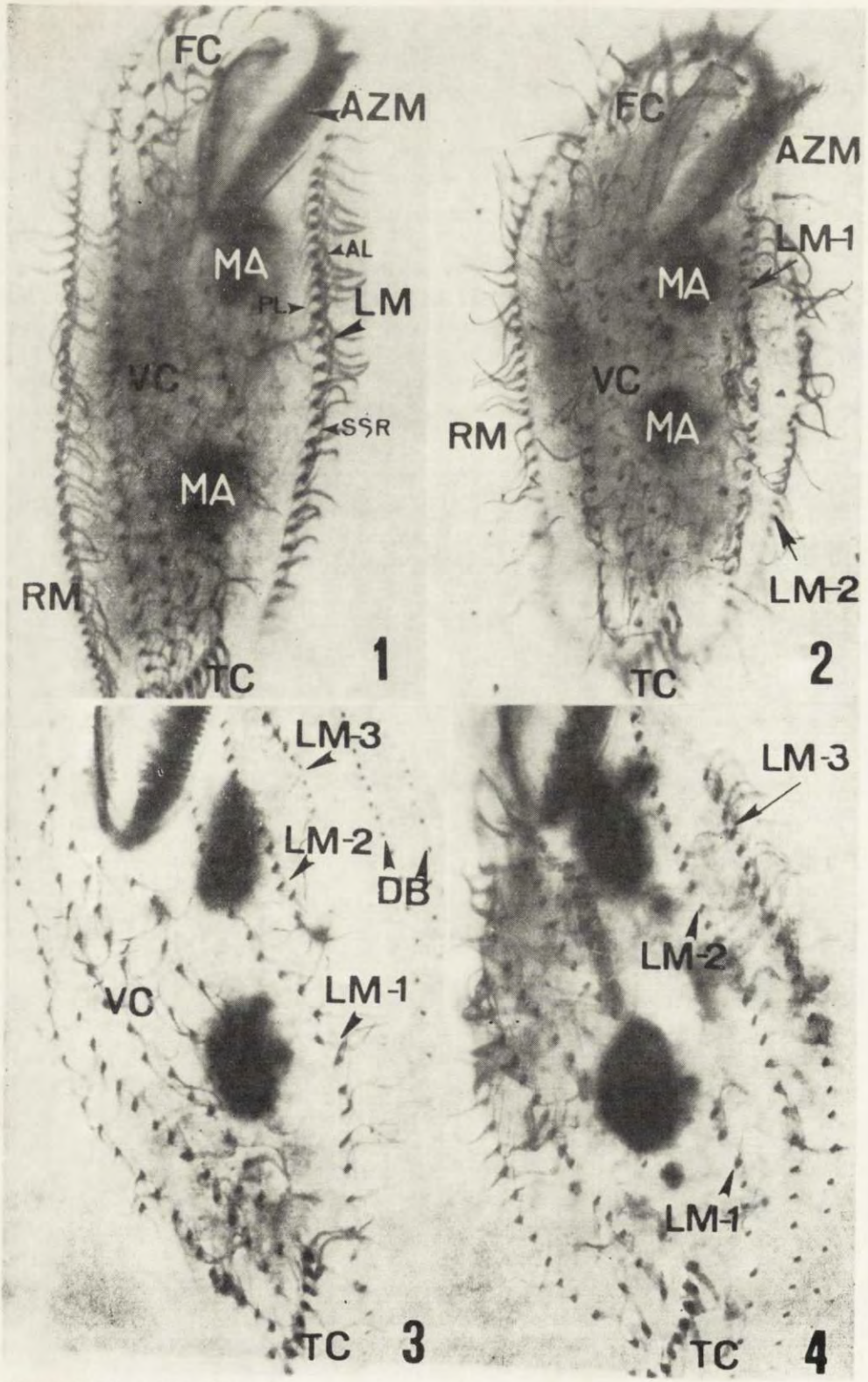
EXPLANATION OF PLATES I–III

Pl. I. Protargol stained cells of normal and aberrant cells of *P. weissei*. Anterior of the ciliate is up. Abbreviations as on Fig. 1

1: The ventral surface of the normal strain G cell of *P. weissei* AL — anterior longitudinal fiber, SSR — small subectoplasmic fiber (compare with phot. 10) Ma — macronuclei
2–4: The ventral surface of cells from the aberrant strain C-6. 3 — The ciliate possesses two marginal rows on its left side (LM-1 and LM-2) 4 — The ciliate possesses three marginal rows on its left side. LM-3 is the shortest row. Adjacent to LM cirri there are dorsal bristle rows (DB). 4 — The ciliate possesses three marginal rows on its left side, LM-2 is the longest row
Pl. II. The ventral surface of cells from the aberrant strain B-18 (Protargol preparation)

5: The dorsal bristle units seen at the anterior termini of left marginal cirri
6: An aberrant cell having three rows of left marginal cirri and a decreased number of ventral and transverse cirri.
7: A dividing cell where proter has three rows of LM primordia (LMp) the opisthe four rows of LM primordia
8: Ventral surface of an aberrant cell with the left marginal cirri bent (arrowheads)
Plate III

9: A left side of protargol stained cell from strain B-18. Inverted segments of left marginal cirri are indicated by an asterisk. The anterior-most segment shows normal orientation. The AL — fiber run anterior-left, the SSR fibers posterior left. In the inverted segments the AL and SSR fiber are on the opposite side. The double heavy arrows indicate the place where the inverted row makes an U-turn
10: An electron-microscope microphotograph of two marginal cirri from wild type C-12 strain. Note the position and orientation of the AL and SSR fibers, cb — cirral basket, c — cilia of the cirrus



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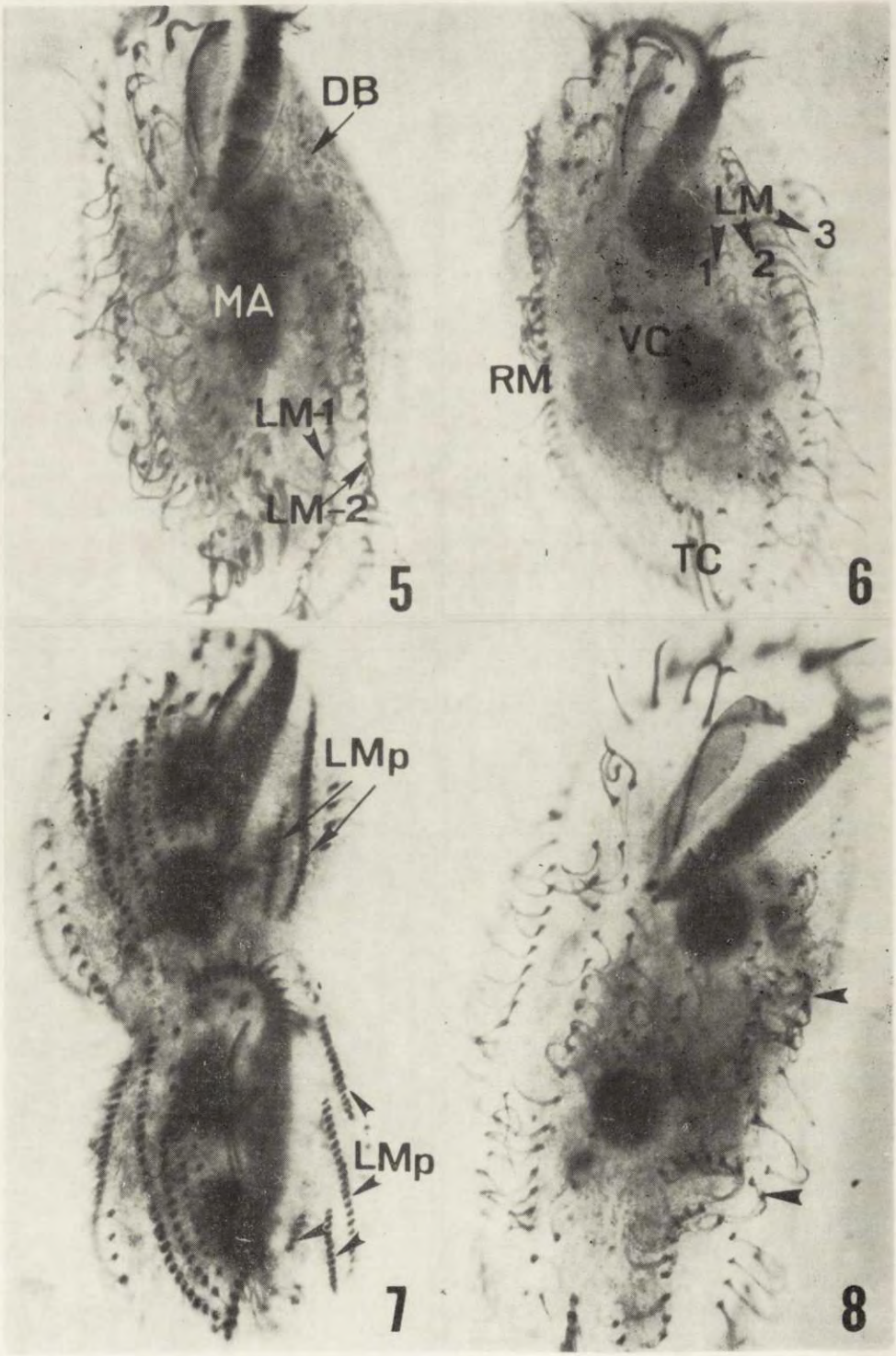

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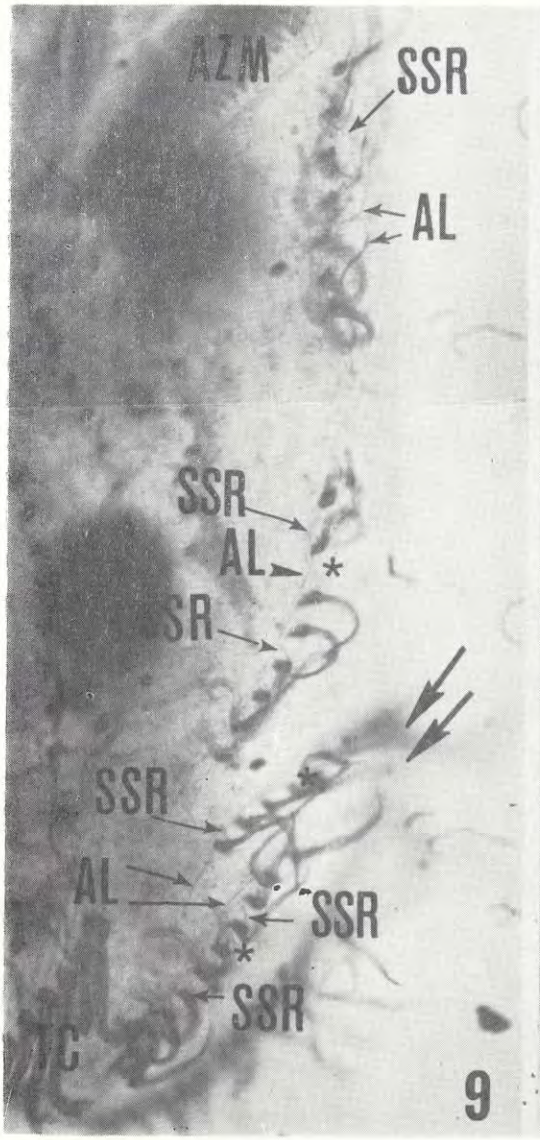

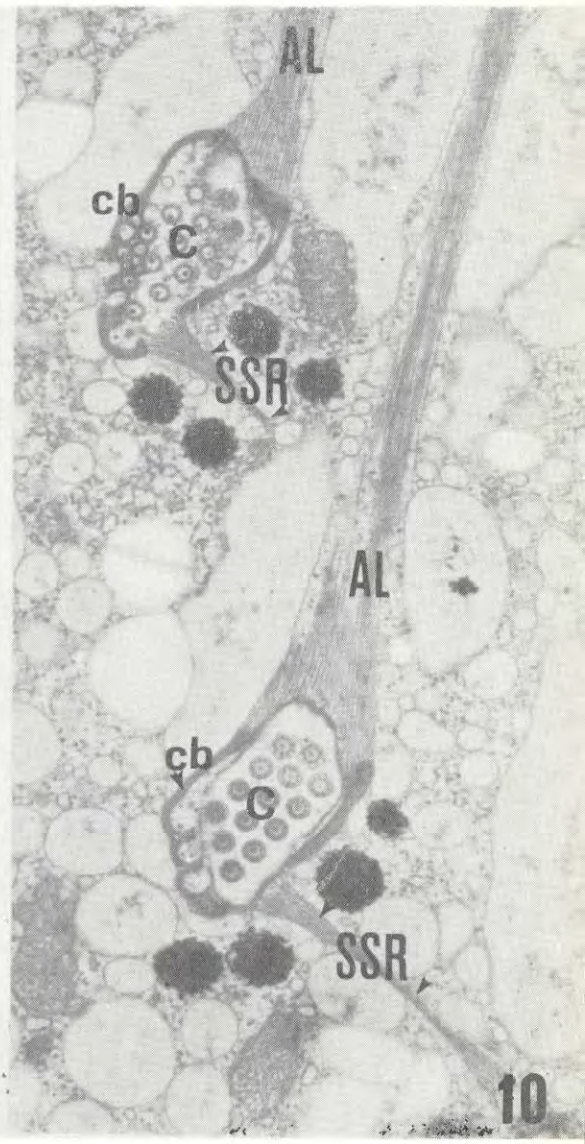

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ACTA PROTOZOOLOGICA
Vol. 22, No. 2, pp. 157–167 (1983)

Kinetics of Ingestion and Egestion of Food Vacuoles During Cell Cycle of *Chilodonella steini*

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Received on 4 November 1982

Synopsis. Chilodonella steini is a stenophagic ciliate feeding of diatomes which are ingested into separate food vacuoles. In experiments on the rate of ingestion and egestion of food vacuoles it offers an opportunity of easy scoring of diatome shells within individual specimens. Through combining data on the number of ingested diatomes and on the rate of their defecation it was estimated that at least about 100 food vacuoles are required for one cell cycle round.

About 70% of this amount may be ingested during the first 3 h of feeding by cells 12 h prestarved. Most of these diatomes were defecated during the next three to five hours. However, most of the prestarved cells required as long as 15 h of continuous feeding to achieve cell division. It is suggested that in prestarved cells the recycling of membrane material for food vacuole formation was decreased, concurrently with the substantial excess delay of duration of the cell cycle.

An essential cell cycle problem is its nutrient limitation. The problem is very complex (Holz 1973, Edelson and Cohn 1978, Rasmussen 1976, Nilsson 1976, 1979, Legner 1980) and in this report attention is confined to the study of the rate of food vacuole formation and egestion in relation to the achievement of cell division in uniformly prestarved and then again refed ciliate *Chiodonella steini* cells. Among other internal and external regulating factors, the uptake of the particulate nutrient depends upon a pool of membrane material within the cell phagoplasm (Fauré-Fremiet 1961) available for food vacuole formation. Numerous authors reported that the membrane involved in food vacuole formation is stored within the ciliate cytoplasm and may be recycled after egestion of former food vacuoles (Allen 1974, 1978, Allen and Wolf 1974, 1979, Nilsson 1979, Allen and Fok 1980, Kaczanowska and Garlińska 1981).

Chilodonella steini (*Kinetofragmophora*, *Chlamydodontidae*) is a strictly herbivorous and stenophagic organism (Radzikowski and Gołembiewska 1977). It ingests exclusively living diatomes, but not other particulate materials. It was

also observed (Moraczewski and Kaczanowska, unpublished) that every food vacuole (except defecative ones) contains only one single diatome. We took advantage of easy scoring of shells of diatomes within individual ciliate cells for evaluation of the rate of food vacuole formation and egestion.

In the present paper we are dealing with following specific questions:

(1) How many food vaculoes are formed during one cell cycle of 12 h prestarved and the refed *Chilodonella* cells

(2) To what extent the kinetics of food vacuole formation and egestion regulate the duration of the cell cycle in this experimental condition.

From our data it is estimated that at least about 100 food vacuoles are formed during one cell cycle of starved and then refed *Chilodonella* cells. About 70% of the pool of membrane required for the formation of 100 food vacuoles is available during the first 3 h of feeding of prestarved cells. The maximal rate of egestion is observed after about 2–5 h since the start of feeding of prestarved cells. This retrieved membrane material may be re-used to meet the minimal food vacuoles requirement of cells in the short duration of cell cycle. However, many cells required a much longer time of feeding to achieve the whole cell-cycle round. It is suggested that the prestarved cells, although virtually deprived of food vacuoles, do not use all the retrieved membrane material for subsequent food vacuole formation. An appearance of autophagic vacuoles in prestarved cells (Moraczewski and Kaczanowska 1981) may affect the efficiency of recycling the food vacuole membrane material in prestarved *Chilodonella steini* cells and may introduce an excess delay of duration of cell cycle.

Material and Methods

The clone of *Chilodonella steini* used in this study was derived from the second generation of intraclonal conjugation as described by Kaczanowski et al. 1980. This clone was in its immaturity period. Cultures of *Ch. steini* were routinely maintained and fed every second day according to procedure described by Radzikowski and Gołembiewska (1977). Cells were used for experiments about 10 h after feeding. All experiments started with the isolation of individual cells to plain autoclaved spring water for 12 h of starvation.

Refeeding proceeded as follows: a dense suspension of one strain of diatomes *Navicula* sp. (grown on agar with inorganic salts and kept in a constant light, as described by Radzikowski and Gołembiewska 1977) was equilibrated to about 0.3 ml of about 10^6 diatomes/ml and added to about 0.7 ml of spring water with 12 h starved cells.

The sizes of diatomes were fairly uniform and they averaged to: length 23.8 ± 0.6 μm, width 6.4 ± 0.6 μm and thickness 2.2 ± 0.6 μm ($n = 30$). A newly formed food vacuole of *Ch. steini* usually contains only one single diatome (Moraczewski and Kaczanowska, unpublished). In the late phase of digestion some phagolysosomes apparently fuse to form one big "defecative vacuole" containing the remnants of 2–6 diatomes. This vacuole is voided outside through a posteriorly located cytoproctal slit. Thus the total number of diatome shalls counted within the cytoplasm of *Ch. stieni* roughly corresponds to the number of formed food vacuoles, but leads to some overestimation of their actual number. This error (because of a variable number of food vacuoles con-

tributing to a defecative vacuole formation) did not exceed 2–6 vacuoles. The error is somewhat reduced by unusual and casual instances when the stimulation of the cytostome with a diatome of a feeding *Chilodonella* cell was not followed by its intake and an empty food vacuole was formed (Moraczewski and Kaczanowski, unupublished). These errors of estimation, however, are uniformly included in all our data.

The refed cell, as indicated in Results, was individually isolated and washed out in plain spring water to remove remains and not ingested diatomes and then placed on a slide. The cell was fixed for 3 min with a Carnoy fixative and mounted in 10% glicerol. The slide was covered with a cover slip and gently pressed to spread out the cytoplasmic content of the cell to count the total number of diatome shells. About 30 cells were used for every assay. Details of the feeding regime in every experiment are reported in Results. The cell division rate and the doubling time of *Ch. steini* were derived from experiments which ran concurrently with those on the rate of ingestion/egestion cycle.

Experiments presented in Fig. 2 and 3 a and b were made in three independent replicas, but only two are graphed for the clarity of drawings. Statistical calculations followed methods described by Sokal and Rohlf (1969).

Results

Starvation and Feeding of *Chilodonella steini*. General Observations

Well fed *Ch. steini* cells may contain up to 70–80 swallowed diatomes. These cells vigorously proliferated and revealed about 12 h mean generation time. However a minimal generation time of 7–8 h was also recorded among such cells. Dividing cells from well-fed cultures were completely stuffed with diatomes, with the exception of the preoral area and of the narrow edge of the ventral field. During cytokinesis these ingested diatomes were partitioned between the offspring and the defecative vacuole fell to the posterior daughter cell. In a few instances it was established that this defecative vacuole did not discharge its content during cytokinesis, and was voided by posterior daughter cell a few minutes after offspring separation.

Cells starved for 12 h contained only very few diatomes (on the average about 4 diatomes per cell). These diatomes were randomly located within *Chilodonella* cytoplasm. No dividers occurred among these starved cells, though among 6 h starved cells some dividers may occur. Some of these dividers were totally deprived of diatomes, as well as some nondividing cells. These transparent cells, cleared off of diatomes, were used to test their ability to feed with a new portion of diatomes. Virtually all nondividing cells fed, while dividing cells remained without diatomes. It appeared that these cells were not able to feed until they divided.

Thus it is concluded that dividing cells are unable either to feed, or to discharge defecative vacuole during divisional morphogenesis. Hence a temporal arrest of both these functions is inscribed in the cell cycle physiology of *Ch. steini*.

Starved cells swim freely in water. After feeding, the cells begin to creep on the dish bottom. They are able to swallow up to 5 diatomes per minute. These diatomes are separately ingested and rapidly passed to the main body trunk. The increase of the mean total number of diatomes/cell during subsequent hours of feeding of

prestarved cells is graphed in Fig. 1. Cells of variable interfission ages, randomly picked up for 12 h of starvation reacted uniformly to feeding (small *sd* of sequent samples). The mean total number of diatomes/cell increased rapidly during the

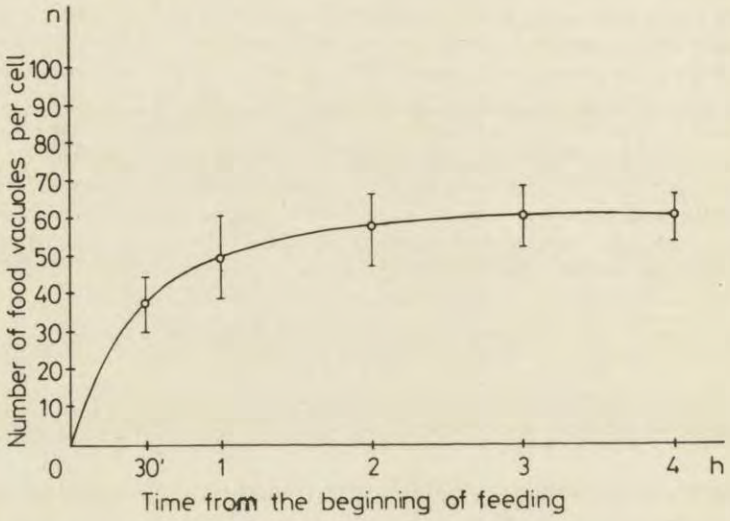

Fig. 1. Total number of diatomes/cell in *Chilodonella steini* 12 h starved cell during subsequent hours of refeeding. Every point of the curve averages data of five separate replicas of this experiment

first hour of feeding and this number leveled after 3–4 h of feeding. During 4 h no dividers appeared. The size of cells became fairly uniform during 12 h of starvation. However, some variability of dimensions appeared after 4 h of feeding, which gave rise to the question whether the ability to form food vacuole becomes a function of size.

Correlation of Cell Size with the Total Number of Swallowed Diatomes

To test the variability of cell dimensions after 4 h of feeding and possible correlation between cell size and the number of ingested diatomes, the following experiment was performed: randomly chosen cells were routinely starved for 12 h and then refed for 4 h. At the end of this experiment individual cells were washed out, fixed and mounted in glicerol (as described in Material and Methods), but they were not pressed. Every specimen was measured with a micrometer. Next, the same specimen was gently crushed to count the total number of ingested food vacuoles. For $n = 40$ cells the length of cells varied from 90 to 170 μm, width from 53 to 105 μm.

Correlation coefficient between the number of ingested diatomes and estimated cell surface (assuming that this surface equals the sum of surfaces of two elipses of diameters of total length and total width) was positive. It corresponds to $r = 0.688$ at $p = 0.01$.

Thus among other controlling factors it is expected that some positive feedback control exists between cell size and its capacity to form food vacuoles.

Rate of Egestion of Diatomes in 12 h Prestarved and then Refed *Ch. steini* Cells of Variable Interfission Ages

It was shown that the total number of ingested diatomes/cell may depend on time and rate of feeding, dimensions of cells and their phase (morphostatic or morphogenetic) within cell cycle. However, this number depends also upon the kinetics of defecation of ingested diatomes. The rate of diatome egestion was tested in prestarved cells which were refed for 1 h or for 4 h. All experiments started when the fed cells were washed out and replaced in plain spring water. The total number of diatomes/cell was counted during subsequent hours of starvation. The results of these experiments are presented in Fig. 2.

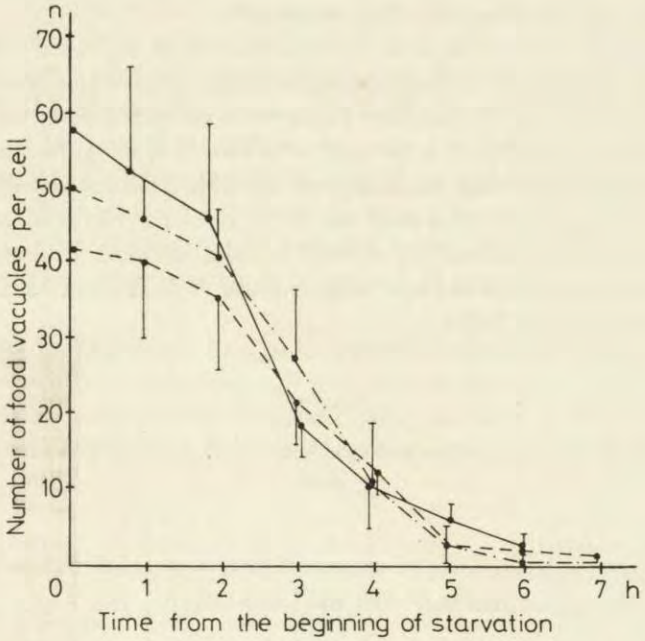

Fig. 2. Total number of diatomes/cell in *Childonella steini* cells starved for 12 h and then refed with a pulse of feeding followed with again starvation. Rate of loss of diatomes/cell is tested after 4h of pulse feeding (solid lines), and after 1 h of feeding (dashed lines)

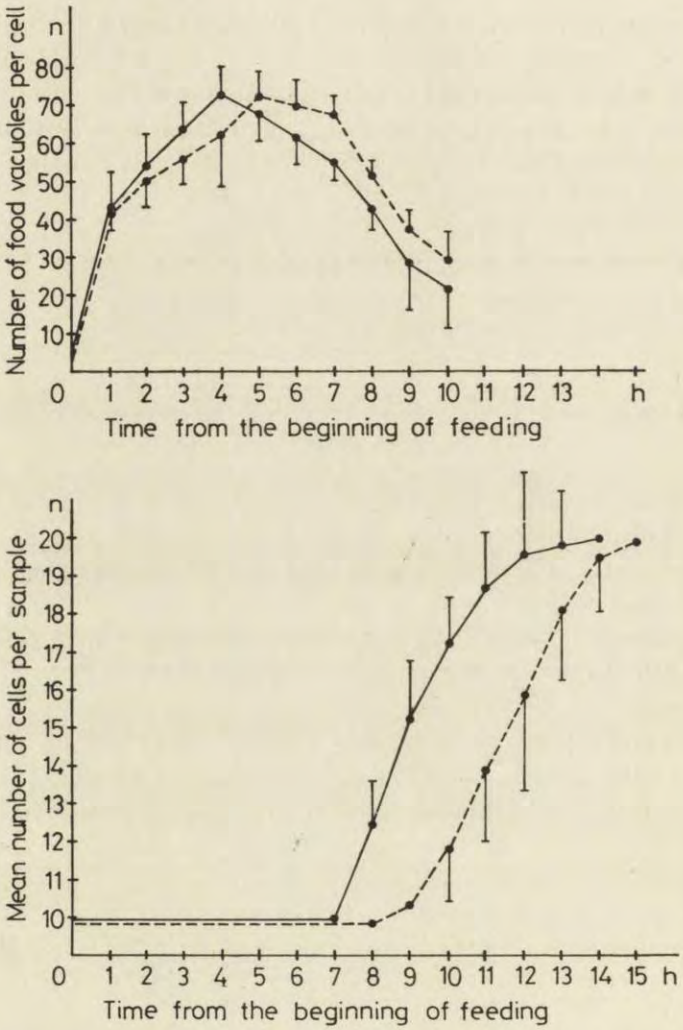

Fig. 3. Combined experiments on the total number of diatomes/cell (Fig. 3 a) and on the rate of proliferation (Fig. 3 b) of cells of the same interfission age (early postdividers) starved for 12 h during subsequent hours of continuous feeding. Corresponding data marked in Fig. 3 a and b either in solid line, or in dashed line

defecated food vacuoles during this cycle. Such data may be deduced from experiments on the kinetics of egestion of food vacuoles after feeding. The kinetics seemed to be similar in all tested conditions, although it was modified in respect of the initial number of diatomes/cell and/or the period of previous feeding.

Two separate calculations of the total number of food vacuoles made during one cell cycle of *Ch. steini* were based on the data from one experiment presented in Fig. 3 a and b (dashed lines) and complemented with two different sets of data on the rate of egestion respectively after 1 and 3 h of feeding. It occurred that the

total number of food vacuoles after 1 h of feeding was equal both in experiment 3 a (dashed line) and in respective experiment presented in Fig. 2. In both experiments this number averaged 43 diatomes/cell. The second set of data on the rate of egestion was directly derived from a control experiment on the rate of egestion after 3 h of feeding, carried out concurrently with experiment 3 a (dashed line). All these data and the two estimations are presented in Table 2. Both variants of estimation fitted together. Thus it was estimated that about 100 food vacuoles are formed in one 12 h prestarved *Ch. steini* cell which divides after about 8 h of constant feeding.

Table 2

The rate of increase and decrease of mean of diatomes/cell in *Chilodonella steini* during sequent hours of experiments respectively on feeding and defecation, and estimations of the total number of ingested food vacuoles during one cell cycle of 12 h starved and then refed cells

Timing from isolation of postidividers	12–13	13–14	14–15	15–16	16–17	17–18	18–19	19–20
Timing from the beginning of feeding	0–1	1–2	2–3	3–4	4–5	5–6	6–7	7–8
Increase of the total number of diatomes/ /cell Fig. 3a	43.6	6.9	7.5	4.5	8.5	–3	–20	–10
Decrease of the number of diatomes/cell								
(a) after 1 h of feeding	—	2.5	4.2	14.2	13.1	7	1	0
(b) after 3 h of feeding	—	—	—	7.2	7.8	25	6	6
Estimated number of ingested food vacuoles								
(a) 109	43.6	9.4	11.7	18.7	21.6	4	—	—
(b) 103 (2–4)	—	—	58	11.7	16.3	22	—	—

Two estimations are made by combining data on the total number of diatomes/cell as presented in Fig. 3 a (dashed line) with data on the rate of egestion of refed cells either (a) during 1 h (data presented in Fig. 2 the lowest curve), or (b) during 3 h of feeding (data not shown).

Total number of ingested food vacuoles = total number of diatomes/cell found after respective (a) 1 h and (b) 3 h of feeding + sum of food vacuoles ingested in subsequent hours of experiments — about 2 to 4 diatome shells kept during starvation.

Assuming an eliptic shape of every diatome, its mean total surface corresponds to about 405 μm^2. During one cell cycle about 100 diatomes are ingested, which corresponds to a turn-over of about 40 500 μm^2 of membrane to wrap them (not counting possible empty food vacuoles). This value is surely not available at time within the cytoplasm since maximum 70–80 vacuoles may be found in a cell. Thus this pool of membrane does not exceed about 30 00–32 400 μm^2.

This comparison makes evident that the re-cycling of the membrane or its new synthesis is needed to accomplish one cell cycle.

ACTA PROTOZOOLOGICA
Vol. 22, No. 2, pp. 169–173 (1983)

Stabilizing Effect of Cholesterol on Changes in Membrane Permeability Induced in *Paramecium octaurelia* by Lysolecithin and Valinomycin

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Received on 18 November 1982

Synopsis. The effect of the incorporation of free cholesterol by cells of *Paramecium octaurerlia* (299 s) on the physiological state of the cell was investigated. The stabilizing role of cholesterol in cell membranes, described by many authors, was also confirmed by its effect on protozoans. Ciliates grown in medium enriched in cholesterol were less susceptible to the cell damaging activity of valinomycin or lysolecithin than control cells. Cells treated with the sterol during growth showed strongly reduced release of potassium ions in the presence of valinomycin or lysolecithin.

Cholesterol in membranes carries out one of two different functions, depending on the type of membrane into which it is incorporated. In highly ordered synthetic membranes composed of lipids with saturated fatty acid chains, the presence of cholesterol enhances the fluidity of the membrane. Also below the temperature of lipid phase transition point the sterol increases the permeability of the membrane for water (de Gier et al. 1969).

The functional effect of cholesterol on the orientation, configuration, rotatory and linear diffusion of lipid molecules in biological membranes composed of a mixture of various phospholipids with different degrees of saturation of fatty acid chains is quite evident. The presence of the sterol in biological membranes results in altered fluidity and viscosity of the lipid bilayer and impeded rotatory and linear movement of lipid molecules within the bilayer.

The enrichment or impoverishment of membranes in cholesterol modifies the permeability of membranes of mammalian erythrocytes for small molecules of non-electrolytes and anions (Deuticke and Ruska 1976, 1978, Grunze and Deuticke 1974). Experiments with artificial membranes synthesized from phosphatydylcholine molecules revealed a positive correlation between the ability of cholesterol to reduce diffusion and ability of the sterol to condensate phosphatydyl molecules at the water-air interphase (Demel et al. 1972).

Changes in the permeability of membranes for cations have also been observed in the case of both passive and active transport (Cooper et al. 1975, Kroes and Ostwald 1971, Drabikowski et al. 1972, Poznansky et al. 1973, Wiley and Cooper 1975).

Cholesterol as a component of plasma membrane is indispensable for the life of animal cells. Consequently, much attention has been devoted to the role of cholesterol in the membranes of these cells. Much less, however, is known about the role of the cholesterol in membrane of ciliates (Conner et al. 1982, Szydłowska-Fabczak 1981) particularly in *Paramecium octaurelia*, which membranes contain sterols as stigmasterol or sitosterol. The results presented in this paper deal with the role of cholesterol in changes of permeability of the membrane of *Paramecium octaurelia*. The results of studies on the release of potassium ions in the presence of valinomycin or lysolecithin are an index of changes in membrane permeability, similarly as in the case of animal cells.

Materials and Methods

The organism studied was *Paramecium octaurelia* strain 299 s. The cells were cultivated by the method of Soldo and Wagtendonk and (1966, 1969) in axenic medium supplemented with 5 μg/ml of stigmasterol. Two types of growth medium were prepared: "S" – a standard medium containing 5 μg/ml of stigmasterol and "CH" – a standard medium supplemented with 2 μg/ml cholesterol. The pH the growth medium was 6.9–7.1.

The cells were grown in 100 ml Roux bottles for 6 days. After this time the cultures were washed with a solution containing: 1.5 mmol/l choline chloride + 1 mmol/l $CaCl_2$ + 0.3 mmol/l Tris-HCl (pH 7.3). Washing procedure was carried out three times at 800 g in MSE centrifuge.

The release of potassium ions was induced by valinomycin – a specific ionophore for these cations – and lysolecithin – a detergent which solubilizes the cell membrane. The latter compound is found *in vivo* and is formed within the cells throughout the enzymatic treatment of lecithin (Shinozawa et al. 1979).

Changes in the permeability of the membranes of *Paramecium octaurelia* for potassium ions were determined with the use of an electrode selective for these ions. The determinations were made in two steps: at first the initial concentration of potassium ions in the cell suspension was determined. One ml of ciliates with density 2×10^6 cells/ml was placed in a small vessel and the concentration of potassium ions was measured with a K^+-selective electrode connected via pre-amplifier type MPA-6 to recorder type KP-680. In the second step the same sample was supplemented with valinomycin or lysolecithin in ethanol to give a final concentration of these compounds of 5 μg/ml. The final concentration of ethanol in external solutions was less than 0.2% (v/v). The presence of these compounds in the external environment caused the passive release of potassium ions from the cell.

Measurements of cell membrane permeability were accompanied by determination of the survival of the cells in solution of valinomycin or lysolecithin at concentration of 5 and 10 μg/ml. The growth cycle and preparation of the cells for the measurements were as above. A known volume of the suspension was incubated in valinomycin or lysolecithin solution. After 3, 7, 15 and 30 min of incubation the per cent of cells not showing any physiological manifestations of life was determined. The studies embraced cells grown in standard "S" medium and in medium enriched in cholesterol ("CH" medium).

Results and Discussion

The use of K^+-selective electrode enabled rapid and direct measurement of changes in the concentration of K^+ ions in the external environment (Fig. 1 and 2).

The use of valinomycin in final concentration 5 μg/ml (Fig. 1) induced the release of potassium ions from the cells in agreement with the concentration gradient for this ion. Curve "CH" in Fig. 1 represents the concentration of potassium ions in the environment after treatment with the ionophore. When the curve is compared to curve "S", which illustrates similar changes in potassium ion concentration in the environment of cells grown in standard medium, it is evident that cholesterol in the membrane of ciliates inhibited the free release of K^+ ions from the cell (Table 1). A slightly weaker effect, was observed when lysolecithin was used to induce the release of potassium ions by the cells (Fig. 2). When the ciliates were tre-

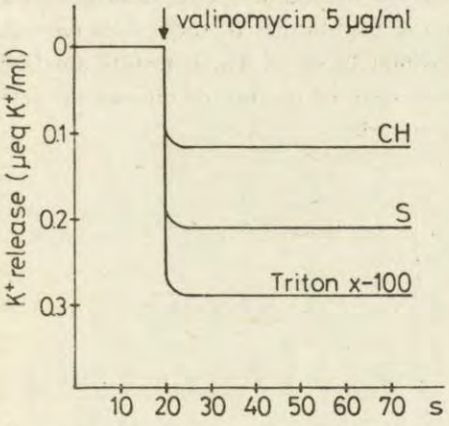

Fig. 1. Kinetics of K^+ release from *Paramecium octaurelia* (2×10^6 cells/ml) treated with valinomycin (5 μg/ml) and inhibition by cholesterol. "CH" — ciliates cultivated in medium with cholesterol. "S" — ciliates cultivated in control medium

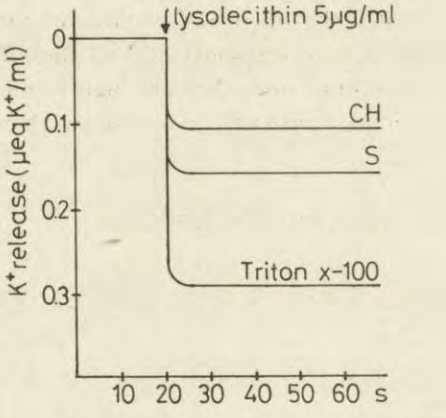

Fig. 2. Kinetics of release of potassium ions from *Paramecium octaurelia* (2×10^6 cells/ml) treated with lysolecithin (5 μg/ml) and inhibition of this effect by cholesterol

Table 1

K^+ release (in μeq/ml) from cells cultivated in different media "S", "CH" after lysolecithin or valinomycin treatment

Medium	Lysolecithin 5 μg/ml	Valinomycin 5 μg/ml	Triton X-100 5%
CH	0.10	0.12	0.29
S	0.16	0.21	0.29

ated with 5 μg/ml valinomycin the presence of cholesterol reduced the release of potassium ions by about 43% when compared to the controls whereas lysolecithin reduced this release by approximately 37%.

Total potassium ions content in the ciliates was determined with the use of Triton X-100. The concentration of potassium ions released by this detergent due to its solubilizing effect was 0.29 μg/ml being similar for cells grown in both "S" and "CH" medium.

The data obtained in this series of experiments are qualitatively and quantitatively comparable with those described by Shinozawa et al. (1979) for erythrocytes. If we consider the size of the studied protozoans and known concentration of potassium ions inside these cells on the one hand and the size of erythrocytes and potassium ions content within red blood cells on the other hand — then the values obtained in this study closely approximate those described above. Similar qualitative results have also been described by Kroes and Ostwald (1971) in studies on the permeability of guinea pig erythrocyte membranes. This author demonstrated that increased cholesterol content in the membranes of these cells reduced the passive transport of Na^+ ions by approximately 40%. These results are very interesting since they are highly compatible in spite of the use of different experimental approach and different biological material.

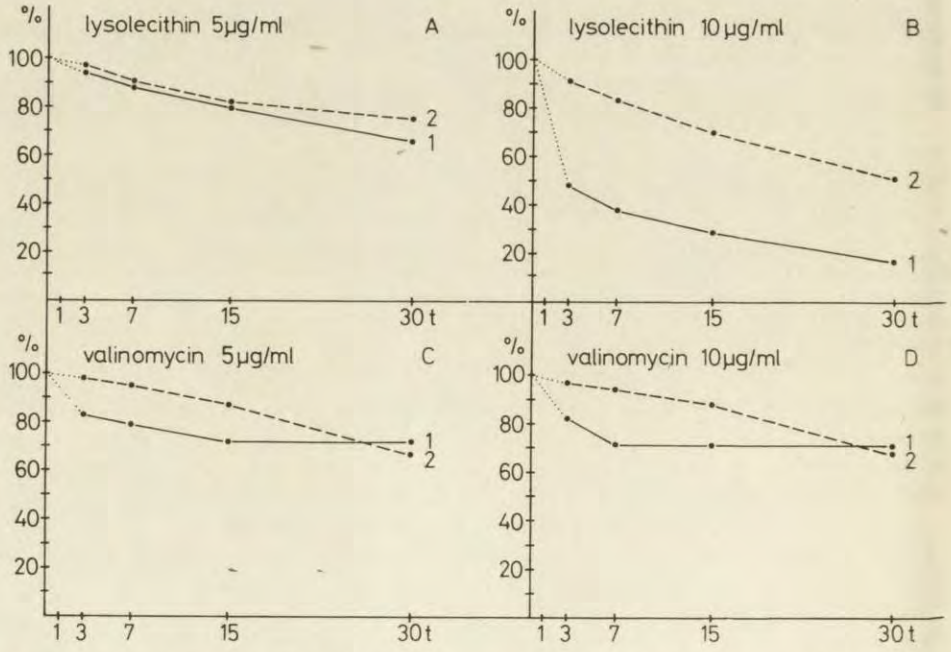

Fig. 3. Effects of lysolecithin (A, B) and valinomycin (C, D) on the survival rate of *Paramecium* exposed to standard culture medium (medium "S") and to culture medium enriched with cholesterol ("CH"). 1 — paramecia incubated in medium "S". 2 — paramecia incubated in medium "CH"

In the case of experiments with *Paramecium octaurelia* it should be emphasized that a similar inhibitory effect of cholesterol was observed regardless of the use of two agents which differ in the way they induce the release of K^+ ions.

Determination of the survival of ciliates incubated in medium containing 5 or 10 μg/ml of valinomycin or lysolecithin demonstrated the protective effect of cholesterol (Fig. 3 a–d). This was particularly evident in the case of higher lysolecithin concentration (10 μg/ml) (Fig. 3 b) since ciliates cultivated in the presence of cholesterol were more resistant to the lytic action of lysolecithin than protozoans in the control culture. After 30 min of incubation in this detergent, approximately 30% more cells lyse in standard medium containing 10 μg/ml lysolecithin than in medium supplemented with cholesterol. Control experiments proved that the employed concentration of ethanol did not cause lethal damage of the ciliate.

The stiffening of the membrane by cholesterol, reduced fluidity of the lipid bilayer and stabilizing effect of this sterol have been confirmed by Shinozawa et al. (1979) who found that increased concentration of the sterol in the membrane of erythrocytes resulted in reudced hemolysis of the cells.

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ACTA PROTOZOOLOGICA
Vol. 22, No. 2, pp. 175–181 (1983)

Measurements of Intracellular K^+-Activity in *Stentor coeruleus* with the Use of an Ion-selective Microelectrode

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Received on 18 November 1982

Synopsis. A K^+-selective microelectrode was used to measure free intracellular potassium of *Stentor coeruleus*. In the external medium with K^+-concentration of 1.3 mM, the intracellular K^+ activity was between 14.1 and 16.4 mM, averaging 15.2 ∓ 0.8 mM. The membrane potential difference measured simultaneously in the same external solution was -50.8 ∓ 2.5 mV. The potassium equilibrium potential calculated from intra- and extracellular potassium activities is -65.0 mV. This value is more negative than the membrane potential difference in resting conditions, indicating that the electrochemical potassium distribution across the cell membrane of *Stentor* is far from being in equilibrium.

The discovery of ion exchangers with high potassium selectivity made possible their wide use for making in a simple way the K^+-selective microelectrode suitable for continuous measurements of intracellular activity *in vivo*. At present measurements of this type are routinely performed on various cells including muscle, nerve and epithelial (Brown and Owen 1979, Nicolson 1980, Thomas 1978, Thomas and Moody 1980, Tsien 1980, Walker 1971).

It is generally known that neurons or muscle cells maintain a high intracellular K concentration due to specific K/Na pump. The existence of such or similar mechanism in protozoan cells has been suggested, however, not definitively proved. It was ascertained that like in cells of higher organisms, the concentration of K in cytoplasm of some protozoans is much higher than the K concentration in the environment (Table 1). It has been also demonstrated that the protozoan cells incubated in a external solutions with increased Na concentration, have the intracellular level of Na ions much lower than extracellular one, suggesting the existence of a sodium extrusion from the cell to the external medium. Data concerning this process are, however, incomplite and contradictory. In cells of *Tetrahymena* and *Acanthamoeba* the K and Na levels in the cytoplasm are not changed by the appli-

Table 1

Cytoplasmic K and Na concentrations in some fresh water ciliates and amoebae

Organism	Concentration	Method of determination	References
Spirostomum	K—7 meq/l of Na—1 cell water	equilibrium with isotopic tracer	Carter (1957)
Tetrahymena	K—31.7 meq/l Na—12.7 of cells	elemental analysis	Dunham and Child (1961)
Blepharisma	K—71.0 meq/l Na—3.2	flame photometry	Hilden (1970)
Stentor	K—12.5 meq/l Na— 0.1	ultramicroflame photometry	Wood (1973)
	K—20.3 meq/l Na — —	ion-selective microelectrode	Fabczak, present report
Acanthamoeba	K — 26.9 meq/l Na — 14.3 of cells	elemental analysis	Klein (1954)
Amoeba proteus	K — 24.8 meq/l Na — 1.1	elemental analysis	Prush and Dunham (1972)
Chaos chaos	K — 28.3 meq/l Na — 0.3	flame photometry	Bruce and Marshall (1965)
	K — — meq/l Na — 0.5	equilibration with isotopic tracer	Chapman-Andresen and Dick (1962)
Pelomyxa carolinensis	K — 34.5 meq/l Na — —	flame photometry	Riddle (1962)

cation of an ouabain, the well known inhibitor of K/Na pump, whereas in ciliate of *Blepharisma* the sensitivity of the cytoplasmic Na level to the action of ouabain was unequivocally established (Andrus and Giese 1963, Conner 1967, Hilden 1970, Klein 1964).

The present study was undertaken to investigate the applicability of using of ion-selective microelectrode for direct and continuous determination of the intracellular K ion activity in the protozoan cells of *Stentor* and to obtain preliminary data for elucidation of the mechanism of ion regulation in these cells.

Materials and Methods

(a) The cells. *Stentor coeruleus* was cultured in glass containers in semi-darkness at room temperature. The ion composition of the culture medium was as follows (in mM): NaCl 1, KCl 1.3, $CaCl_2$ 1, $MgCl_2$ 1, Tris-HCl 5 at pH from 7.4 to 7.6. The food source for the protozoans were axenic

cultures of *Tetrahymena pyriformis*. The cells for experiments were transferred preliminary to the fresh culture solution without the nutritional component for few hours, and after following change of the culture solution, the cells were cooled down to the temperature about 8°C by a feedback Peltier device (Fig. 2).

(b) The microelectrode. The conventional and ion-selective microelectrodes were pulled from aluminosilicate glass capillaries (W-P Instruments) on a vertical puller. The capillaries were fiilled with a solution of 0.1 M KCl or 0.1 M NaCl and then bevelled to an angle of 45° (Ogden and Citron 1978). Each micropipette was bevelled until an electric resistance of 3×10^7 ohms was reached, what corresponds to the electrode tip of about 1 μm. The microelectrode containing solution of 0.1 M NaCl was used as a reference electrode in the ion-selective measuring system and for measurements of internal cell membrane potential as well (Djamgoz and Laming 1981, Thomas 1978, Walker 1971). An identical micropipette as Na-filled one but containing solution of 0.1 M KCl was use for making of the K^+-selective microelectrode. The micropipette was filled with

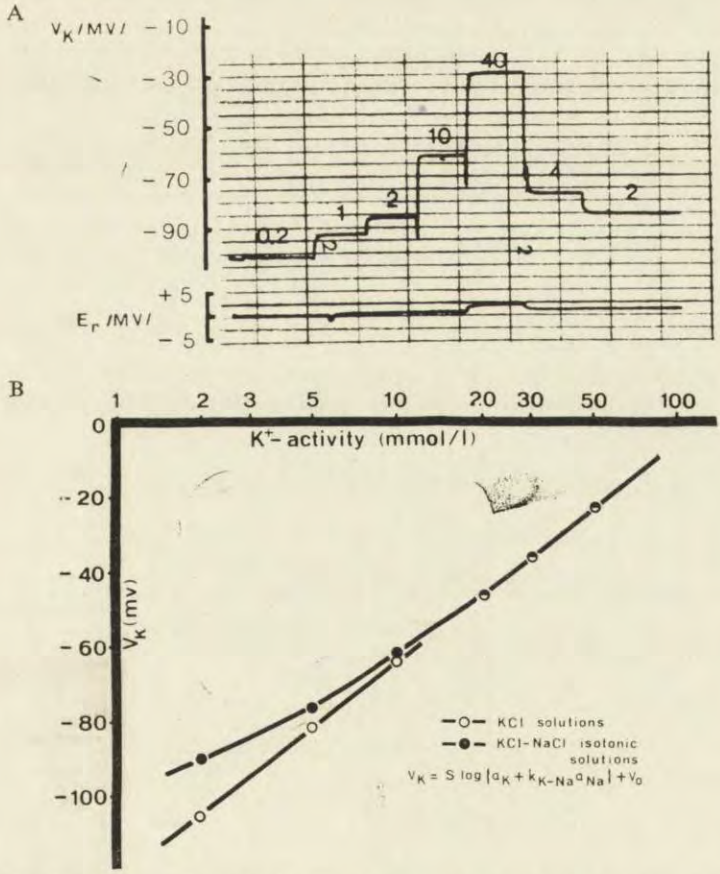

Fig. 1. Calibration traces and graphs for K^+-selective microelectrode. (A). The voltage responses of both the NaCl-filled microelectrode (E_r) and that for K^+-selective microelectrode (V_K) were monitored simultaneously. The calibration solutions contained 0.2, 1, 2, 4, 10, 40 mM of KCl concentration. (B) The voltage readings of V_K from (A) plotted against the logarithms of the K activities (a_K^i). The calibration points can be fitted by the Eq. 1 to within ± 1 mV assuming

$$k_{K\text{-}Na} = 1.4\times10^{-2}$$

a 2% solution (v/v) of dimethylchlorosilane in carbon tetrachloride by suction up to a height of 200–500 μm from the tip of electrode. After siliconizing the K-exchanger fluid (Corning No. 477317) was introduced to the appropriate height of the hydrophobic inner coating. An electrical resistance of the K^+-selective microelectrode prepared in this way was approx. 10^9 ohms. Each K^+-selective microelectrode was calibrated before and after experiment in different KCl concentrations (Fig. 1). Solution of NaCl was used for simulation of the ionic strength of the external solution and for determination of interference of Na ions by the Nikolsky equation:

$$V_K = V_o + S \log(a_K + k_{K-Na} a_{Na}) \quad (1)$$

where V_K is a microelectrode measured potential; V_o is a constant of a microelectrode; S is an empirical slope obtained from the electrode calibration ($\Delta V_K / \Delta \log a_K$); a_K and a_{Na} are K and Na ion activities, respectively; k_{K-Na} is the selectivity coefficient of a microelectrode. The electrical response of a microelectrode was linear in the range of 1–100 mM KCl solutions and the slopes (S) were between 52 and 60 mV per 10-fold change in K concentration at room temperature. V_o was obtained from the linear calibration curve. However, the potential of microelectrode in solutions of KCl/NaCl was not linear and the slope ($\Delta V_K / \Delta \log a_K$) is less than the corresponding one in pure KCl solutions, especially in the concentration range below 5 mM KCl (Fig. 1 B). The observed response time of the K^+-selective microelectrode to a stable potential was normally between 50 and 80 ms in the mixture solution. The selectivity coefficient (k_{K-Na}) in Eq. 1 was determined by using a least-squares linear regression method and was similar to those described elsewhere (Djamgoz and Laming 1981, Lux 1971, Oehme et al. 1976, Walker 1971, Walker and Brown 1977). When the K^+-selective microelectrode penetrates a cell, cell membrane potential difference (E_m) measured with a conventional reference microelectrode should be substracted from the K^+-selective microelectrode potential (V_K). Thus, Eq. 1 gives the following equation:

$$\Delta V_K = V_K - E_m = V_o + S \log(a_K^i + k_{K-Na} a_{Na}^i) \quad (2)$$

where a_K^i and a_{Na}^i are the intracellular K and Na ion activities, respectively.

(c) The electronics. Figure 2 B shows the arrangement for measurements of ΔV_K and E_m. A K^+-selective microelectrode (E_2) and a conventional microelectrode (E_1) were connected

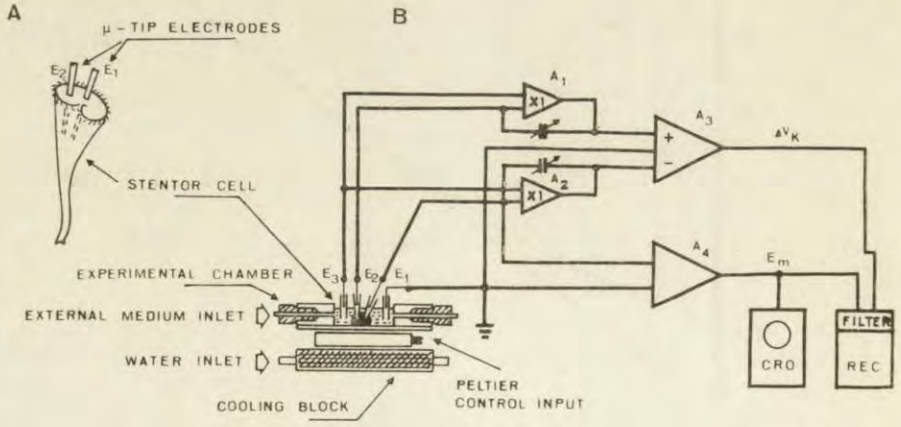

Fig. 2. Block diagram of the electrical measurements and experimental design of micropuncture study using a single-barrelled K^+-selective microelectrode. (A) Relative placement of NaCl-filled conventional (E_1) and K^+-selective (E_2) microelectrodes in cell of *Stentor*. (B) The outputs of both microelectrodes (E_1 and E_2) are connected to a high imput impedance (approx. 10^{15} ohms) differential amplifier (A_3) and to conventional preamplifier (A_4). (For details see text)

to A_3, a differential amplifier (Electrometer, model 219, Unitra). The conventional microelectrode (E_1) output was connected also to A_4, a high imput impedance preamplifier (Transidyne General, model MPA-6). Both amplifier outputs (ΔV_K and E_m) were connected to a pen recorder (Model 360, Medipan). These outputs were also connected to the digital voltmeters (Model V-625, Meratronic) to read precise voltage (voltmeters are not shown in Fig. 2 B). The fast signals of E_m were displayed on an oscilloscope (Osc., model 5103N, Tektronix). The intracellular K ion activities (a_K^i) were calculated by Eq. 2. The value of 0.16 mM was used for the intracellular Na ion activity (a_{Na}^i) in *Stentor* (Wood 1973). The relative contribution to the measured V_K by the intracellular Na ion activity can be negligible.

Results

Measurements of the transmembrane potential difference (E_m) and "potassium" potential difference (ΔV_K) were performed on prepared cell of *Stentor* at temperature of about 8 °C in a designed teflon chamber. Figures 2 A and 3 illustrate the method of measurement of intracellular K ion activity by the K^+-selective measuring system. Both recording microelectrodes were inserted simultaneously into the cell, usually in the frontal region of the cell by means of a mechanical micromanipulator (Fig. 2 A). Figure 3 B represents the membrane potential difference (E_m)

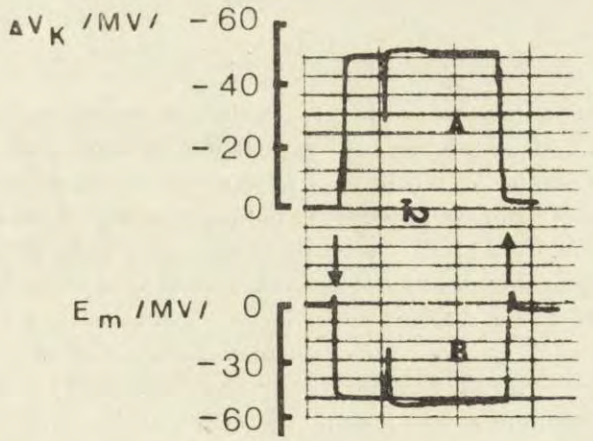

Fig. 3. The electrical responses of conventional (B) and K^+-selective (A) microelectrodes during puncture of *Stentor* cell. Arrows mark the microelectrode puncture (↓) and withdrawal (↑)

of the conventional (reference) microelectrode (E_1 in Fig. 2 B) with respect to the grounded electrode (E_3 in Fig. 2 B). The potential shown in Fig. 3 A represents the difference between the K^+-selective microelectrode potential and the conventional microelectrode potential ($\Delta V_K = V_K - E_m$). The correct penetration of both microelectrodes to the cell cytoplasm, was controlled by passing of current over the cell membrane with current injecting microelectrode (not shown in Fig. 2) and measuring the following change of the value of E_m.

The results of the measurements are presented in Table 2 as the mean values of cell membrane potential difference (E_m) and cytoplasm K activity (a_K^i). The intracellular K ion activity of 15.8±0.8 mM ($n = 13.8$) was obtained from 13 measurements on 8 cells of *Stentor*. The mean value of the resting membrane potential difference was equal −50.8∓2.5 mV ($n = 17.8$). In these measurements the K concentration of bathing solution was 1.3 mM. If one take it the K ion activity coefficient (γ_K) is equal to 0.75 (Robinson and Stokes 1959, Thomas 1978), the measured level of intracellular potassium activity is equivalent to a concentration of 20.3∓2.3 mM K^+ (Table 2), assuming the same K ion activity coefficient for the physiological saline and the cell cytoplasm.

Table 2

Membrane potential (E_m), cytoplasmic K activity (a_K^i), concentration (c_K^i), coefficient of ionic K activity (γ_K), and electrochemical equilibrium potential (E_m) in *Stentor*

E_m (mV)	a_K^i (mM)	c_K^i (mM)	γ_K	E_m' (mV)
−50.8∓2.5	15.2∓1.8	20.3∓2.3	0.75	−65.0

Discussion

Regulation of the ion movement between the cell interior and the cell environment is of an essential importance for maintenance of normal life processes in all cells. There is a number of biochemical methods for investigation of the cell ion regulation, however, many of methods are difficult to apply on account of the specificity of the studied cell. The greatest disadvantage of flame photometry or isotopic methods is the impossibility of practical usage of these methods *in vivo*. The direct analysis of an ion activity within the cell cytoplasm is probably the most important feature of an ion-selective microelectrode technique over other one, except perhaps colorimetric method. The results presented in this report are the first direct and continuous measurement of intracellular K activity in the protozoan cell with using of K^+-selective microelectrode.

The electrochemical K equilibrium potential (E_m') of −65.0 mV has been calculated from direct measurements of ionic activities of K ions on both sides of the cell membrane in *Stentor* (Table 2). As found earlier (Fabczak 1980, Margenhagen 1971, Wood 1973) and in the present paper (Table 2) the transmembrane potential difference in cell of *Stentor* is considerably lower (from −30 to −50 mV) than the calculated equilibrium potential for potassium (−65 mV). If the K electrochemical equilibrium potential, calculated from measured extra- and intracellular K activities, is not equal to the membrane potential within experimental error, than the cell membrane is not in equilibrium with respect to K ions, and the K

electrochemical gradient may be maintained by the permeabilities of ions other then K, or it may be maintained by specific active transport of potassium ions. Both mechanisms are known in the cells of higher organisms while in the protozoan cells at present state of a knowledge it is impossible to conclude which one is present in cell of *Stentor*. The possibility of application of microelectrodes with use of other than K^+-selective ion-exchangers would allow to gain insight into the mechanism of ionic regulation in protozoan cell of *Stentor*.

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ACTA PROTOZOOLOGICA
Vol. 22, No. 2, pp. 183–189 (1983)

Effect of External Agents on Cytoplasmic Streaming in *Paramecium*. I. Influence of Carmine Suspension

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Received on 22 November 1982

Synopsis. The influence of carmine suspension concentration on cytoplasmic streaming velocity and food vacuole formation rate within *Paramecium bursaria* cells have been studied. In range from $2.7–5.3 \times 10^6$ particles per ml considerable acceleration of cytoplasm flow is observed. Over and above increase of endocytosis rate, however not as sharp, is noticed. The results suggested that there is correlation between both examined cells activities. It is probable that this relation is the expression of the cell control over the food vacuole intake process. It is suggested that food vacuole formation rate depends on cytoplasmic streaming velocity and is facilitated by it.

Among *Protozoa* the rotational cytoplasmic streaming has been detected only within *Paramecium* cells. Different intracellular organelles like mitochondria, uninserted trichocysts, food vacuoles and other particles, flow within moving cytoplasm in constant direction. All these structures move along rotational path with velocities related to the cytoplasm in case of small particles, while larger ones move slower due to the obstacles of the "channel" (Sikora 1981, Sikora et al. 1979).

There is no doubt that food vacuole immediately after formation moves rapidly to the posterior end of the cell along postesopharyngeal fibrils (Lund 1941). Later, cytoplasmic streaming seems to be responsible for food vacuole movement (Wasik and Sikora 1981), however, it is not clear yet, is it the only mechanism of their propulsion. It is likely, that transportation of food vacuoles and other organelles is the chief function of cytoplasmic streaming in *Paramecium* cells. Among them disc-shaped vesicles, the probable main source of membrane for food vacuole formation (Allen 1974, Allen 1978) might be transported to the cytopharyngeal region not only by the microtubular ribbons as Allen and Staehelin (1981) suggested but also by cytoplasmic streaming.

Sikora and Jurand (in press) put forward the hypothesis, that particles present

in the surrounding medium stimulate surface receptors of the *Paramecium tetraurelia* cells while feeding and induce cytoplasmic streaming. In experiments reported, only presence of cilia and their activity have been taken into account.

The question arises, whether particulate matter and its variable concentration in surrounding medium would induce changes in cytoplasmic streaming velocity. It is known that increase of food vacuole formation rate is stimulated by the increase of suspension concentration (Bozler 1924, Frisch 1937). Therefore it is probable that there is correlation between the rate of food vacuole formation and velocity of cytoplasmic streaming.

Material and Methods

Paramecium bursaria grown on the Scottish grass medium inoculated with *Enterobacter aerogenes* 535i at room temperature 19±2°C was used. Paramecia were cultured in darkness to reduce number of intracellular zoochlorellas, which hindered observations of cytoplasmic streaming within the cells.

Carmine suspension (BDH) was prepared by rubbing of 0.5 g of carmine powder and suspending it in 200 ml of maintenance solution (MS), consisting of 5 mM TRIS-HCl buffer (pH 7.25) with 1 mM KCl and 1 mM $CaCl_2$.

Suspension remained in supernatant was decanted after 24 h and left for consecutive 24 h. The suspension of this last supernatant was the initial source of carmine particles for experiments. Aliquots of carmine suspension were kept frozen before used.

The concentration of carmine particles of initial suspension was 1.07×10^9 particles per ml, measured by means of Bürker's hematocytometer. Successive dilution of initial suspension was prepared by means of MS from 1.07×10^9 to 1.07×10^5 particles per ml and are expressed as 100% to 0.01% concentrations.

The sizes of carmine particles ranged from 0.5 to 1.5 μm in diameter well corresponding to those of bacteria.

Before experiments *Paramecium bursaria* was kept overnight in MS. Afterwards in experiments the cells were incubated for 3 min at given concentration of carmine suspension. Control paramecia were incubated in MS only. Then the cells were immobilized by means of 0.5 mM of $NiCl_2$ for 10 min according to method of Sikora and Wasik (1978). During the first 30 min after immobilization, rinsed in MS paramecia, were examined in vaseline mounted microscopic preparations under bright light microscope.

The velocity of cytoplasmic streaming was measured by the estimation of the time for movement of small particles (1–3 μm in diameter) to flow between two points on ocular eye-piece graticule.

The number of carmine vacuoles was counted in 3% glutaraldehyde-fixed cells.

All experiments were carried out at room temperature 19±2°C.

Duncan's test of analysis of variances for uncorrelated data was used to estimate significance, on at least 1% level, of differences between particular measurements.

Results

The purpose of our study was to estimate the influence of the different concentrations of carmine suspension in range from 1.07×10^5 to 1.07×10^9 particles

per ml (p.p.m.) on food vacuole formation rate and effect on cytoplasmic streaming velocity within *Paramecium bursaria* cells.

Time of incubation of *Paramecium bursaria* cells in carmine suspension has been chosen upon the preliminary experiments. As a result of exposing the ciliates to the medium with particles for longer than 3 min, too large number of food vacuoles were formed, limiting reliability of counting.

In Fig. 1 the effect of carmine concentrations on cytoplasmic streaming velocity is shown. Ciliates incubated in low concentrations of carmine suspension (0. 01%–

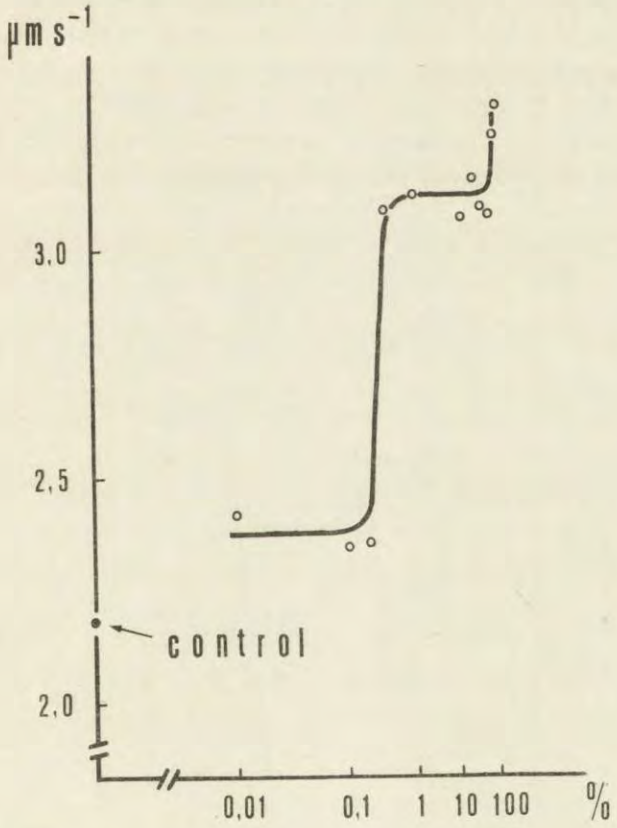

Fig. 1. Influence of different carmine suspension concentration (abscissa) in range from 0.01% to 100% i.e., 1.07×10^5 to 1.07×10^9 particles per ml on cytoplasmic streaming velocity (*ordinate*) within *Paramecium bursaria* cells. Velocity of cytoplasm is expressed in $\mu m\ s^{-1}$. Time of incubation in carmine suspension was 3 min followed by $NiCl_2$ immobilization

0.25% i.e., 1.07×10^5–2.67×10^6 p.p.m.) show a slight but significant increase of cytoplasmic streaming velocity in comparison with the control paramecia. The increase of carmine concentration in the medium from 0.25% (2.67×10^6 p.p.m.) to 0.5% (5.35×10^6 p.p.m.) caused distinct acceleration of cytoplasmic flow. Fur-

ther increase of concentration from 0.5% (5.35×10^6 p.p.m.) to 70% (7.49×10^8 p.p.m.) did not show significant changes, while in the range from 70% (7.49×10^8 p.p.m.) to 90% (9.63×10^8 p.p.m.) following increase of streaming velocity is noticed. The results shown in the Fig. 1 suggested that the carmine particles in surrounding medium in concentrations above 5.35×10^6 p.p.m. lead to dramatic increase of cytoplasmic streaming velocity.

The recorded values of streaming velocity did not significantly change along the 30 min testing period.

Investigation of the effect of varying the carmine particles concentrations from 0.01% (1.07×10^5 p.p.m.) to 0.25% (2.67×10^6 p.p.m.) demonstrates that the rate of food vacuole formation is not significantly different (Fig. 2). Incubation in media containing particles in concentrations above 0.25% (2.67×10^6 p.p.m.) leads to gradual increase of number of food vacuoles formed. Maximal number of food vacuoles is formed in 10% (1.07×10^8 p.p.m.) carmine. Its decrease is in higher carmine concentrations slight, however, statistically significant.

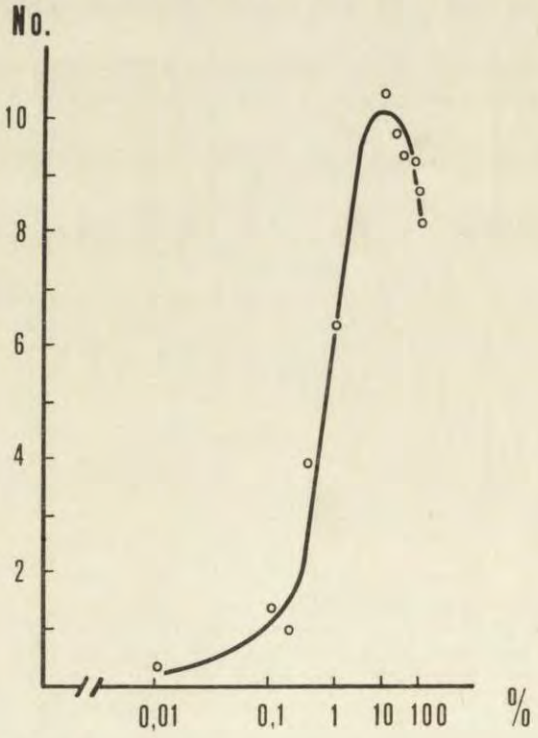

Fig. 2. Influence of different carmine suspension concentration (*abscissa*) in range from 0.01% to 100% i.e., 1.07×10^9 particles per ml on food vacuole formation rate (*ordinate*) within *Paramecium bursaria* cells. Rate of food vacuole formation is expressed in number of food vacuoles formed within 3 min incubation in carmine suspension

Paramecia exposed to the MS without particulate material, have not produced visible food vacuoles, and no acceleration of cytoplasmic streaming has been observed.

In all experiments mentioned above paramecia were incubated in media containing particulate material and followed by immobilization by means of nickel chloride solution. To test whether Ni^{2+}-immobilization influence the food vacuole formation, immobilized and rinsed in MS paramecia were incubated in media with carmine particles. Both low and high concentrations of carmine particles induce neither food vacuole formation nor acceleration of cytoplasmic streaming.

Discussion

In *Paramecium* some physical and chemical alterations of environment lead to increase of number of food vacuoles formed. Mast (1941) in his comprehensive study on *Paramecium*, has suggested that kind of particles play an important role in food vacuole formation and agreed with Bozler (1924) and Frisch (1937) about the significance of suspension concentrations in this process, too.

Commonly used model of the suspension are the carmine particles. These particles "... are readily taken up by the cells and they are incorporated into all vacuoles formed in their presence..." (Nilsson 1976). The carmine food vacuoles are relatively simple to count in living and fixed cells (Chapman-Andresen and Nilsson 1968).

As it is expressed in Results carmine suspension concentrations have a distinct role not only in increasing the rate of food vacuole formation but also accelerate *Paramecium bursaria* cytoplasmic streaming (Fig. 1 and 2).

In low concentrations of carmine particles both food vacuole formation rate and cytoplasmic streaming velocity show little but significantly distinct increase in comparison with the control. In 0.5% of suspension concentration the rapid acceleration of cytoplasmic flow is noticed (Fig. 1). Further increasing of carmine particles concentrations in range from 0.5% to 70% do not cause visible differences (Fig. 2). While cytoplasmic streaming velocity increases considerably, food vacuole formation rate is slower, attaining its highest level in 10% of carmine concentration (Fig. 2).

As it is shown on Fig. 1 and 2, carmine concentration over 10% reduces food vacuole formation rate, while over 70% consecutive increase of cytoplasmic streaming velocity takes place. This part of relation will be discussed elsewhere.

The cytoplasmic streaming and endocytosis in *Paramecium* are both accelerated by means of particulate material in medium. This is distinctly seen on Figs. 1 and 2, where curves are sloping sharply. There are no doubts that there is correlation between these two processes in a certain range. It seems that acceleration of streaming caused by carmine particles is precedent to the endocytosis. Therefore the

external agent, carmine suspension, might probably stimulate the outer surface of *Paramecium* cells, close to cytostome, where the hypothetical receptores exist (Sikora and Jurand, in press). The response of the cell to the increasing particle's number in medium is the acceleration of the cytoplasmic streaming flow. It is highly probable that the relation between the acceleration of cytoplasmic streaming with the following increase of food vacuole formation rate is the expression of the *Paramecium's* control of the food vacuole ingestion.

As it is expressed in Results paramecia immobilized prior to the incubation in medium containing carmine particles do not show any visible differences in their streaming velocity in comparison with the control. Food vacuoles are not formed, either. The results support suggestion about the role of cilia in cytoplasmic streaming flow control.

The acceleration effect is maintained by the cell over at least 40 min after stimulations. Therefore the effect of presence of food vacuoles within the cell on cytoplasmic streaming activity could not be excluded for certain.

According to Allen (1974, 1978) and Allen and Staehelin (1981) food vacuoles in *Paramecium* are formed by fusion of disc-shaped vesicles, transmitted along microtubular ribbons to the cytostome–cytopharynx region. Results described here suggest that cytoplasmic flow may perform an important role in providing required amount of the membrane pool for new vacuole formation, too.

ACKNOWLEDGEMENTS

I gratefully acknowledge Dr J. Sikora for his comments and suggestions on this work and for critically reading the manuscript. I would like to thank Mr W. Sikora for his assistance with the statistic analysis of results.

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ACTA PROTOZOOLOGICA
Vol. 22, No. 3, pp. 191–195 (1983)

The First Stages of the Frontal Part of *Physarum polycephalum* Plasmodium Derived from Endoplasmic Drops

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Received on 18 November 1982

Synopsis. Observations of endoplasmic drops from *Physarum polycephalum* made with both light and electron microscope have revealed that activity is connected with the arrangement of their internal structure. During the period in which the drop transforms into a microplasmodium the central endoplasmic part is reduced. Electron micrographs of the frontal part fixed during the surge of endoplasm show a large amount of filamentous material which may arise as a consequence of unfolding of the membrane.

Motility in the acellular slime-mold *Physarum polycephalum* has been investigated on both the molecular and structural levels. Contractile proteins responsible for the movement of the endoplasm and participating in its regulation have been isolated and characterized (Hatano 1973, Hinssen 1981, Isenberg and Wohlfarth-Bottermann 1976, Kuźnicki et al. 1979, Nachmias 1981, Wohlfarth-Bottermann et al. 1976). In studies on the endoplasmic stream a search for steering systems and those responsible for shuttle-streaming has been undertaken (Baranowski 1976, Grębecki and Cieślawska 1978, Grębecki and Kołodziejczyk 1983). Morphological studies have revealed the structure of endoplasmic channels which form a system of dichotomus veins (Achenbach et al. 1979, Wohlfarth-Bottermann 1974).

Drops of endoplasm obtained by puncturing a vein are a particulary good model for morphogenetic studies. A "naked" bleb of protoplasm regenerates a cytoplasmic membrane within a few seconds (Wohlfarth-Bottermann and Stockem 1970). During the next few minutes differentiation of the interior of the drop is initiated. Vesicles appear and fuse with each other and at the same time join the membrane giving a system of invaginations characteristic for the structure of the vegetative form of *Physarum* (Achenbach et al. 1979).

The development of the membrane system is accompanied by increased viscosity of the endoplasm which under light microscope is manifested by the appearance of a large number of fibrils and in the electron microscope — by filaments. These filaments are, at first, unordered and are observed in the whole volume of the endoplasm. After about 10 min of the life of the drop the filaments arrange themselves under the membrane, along the invaginations (Achenbach et al. 1979, Isenberg and Wohlfarth-Bottermann 1976). This internal organization of the drop is a manifestation of its ability to initiate contractile activity (Götz von Olenhusen and Wohlfarth-Bottermann 1979). Thus, during its first 20 min the drop undergoes a complete cycle of transformations resulting in a specialized organism.

The beginning of locomotory activity is connected with the formation of pseudopodia and the structural transformation of small endoplasmic channels into veins. Rhythmic contractions force the endoplasm towards the margin. It is, at present, dificult to say which part(s) are destined to take over the frontal part of the slime mold. It is possible that imperceptible changes of the environment may modify the membrane to an extent facilitating the transport of endoplasm in this direction.

The purpose of this paper was to follow the differentiation of a drop into a microplasmodium with particular focus on the architecture of the frontal part of *Physarum*.

Material and Methods

Physarum polycephalum was cultivated on moist filter paper and was fed with rolled oats. The culture was maintained at room temperature in the dark.

Endoplasmic drops were obtained by means of puncture with a glass needle. The drops were then put on unsupplemented agar surface. Observations under stereoscopic microscope allowed the selection of appropriate developmental stages which were then fixed in:

(1) 2.5% glutaraldehyde in 0.04 M cacodylate buffer (pH 7.4) for 60 min at room temperature, then postfixed in 1% OsO_4 in the same buffer (40 min at 4°C)

(2) with a mixture of 2.5% glutaraldehyde with 1% OsO_4 in 0.04 M cacodylate buffer on ice.

After fixation the specimens were dehydrated in graded series of ethanol, followed by two series of propylene oxide, then embedded in Epon 812 resin. Semi- and ultrathin sections were cut on LKB microtome. Semithin sections were stained in methylene blue and the ultrathin ones in saturated solution of uranyl acetate and lead citrate. Semithin sections were examined with Ergaval microscope and ultrathin ones with JEM 100 B electron microscope.

Results and Discussion

Plate I 1 shows the characteristic transverse section of a drop able to contract. The inner endoplasmic part is enclosed by a ring of invaginations. The outer part contains numerous small channels which conduct the endoplasm during the contractions (Pl. I 2). This structure resembles a vein in which the inner part of endoplasm is surrounded by a system of invaginations (Pl. II 5).

The structure seems to be a primary one which the regenerating organism strives to recreate first at all. Those which do not show any contractile activity even 2 h after their removal from the vein are distinguished by strongly vacuolized interior and the absence of internal order (Pl. II 6). The formation of a vein is preceeded by a small marginal swelling which transforms first into a pseudopodium and then into vein (Pl. I 3). Elongation of the vein and transport of the endoplasm is accompanied by reduction of the inner part of the drop. The uniform mass of protoplasm is replaced by oval, membrane-enveloped structures (Pl. I 4) which are probably remnants from the withdrawing endoplasm.

Movement of the plasmodium on the substrate is accompanied by reduction of its posterior part with simultaneous expansion of the front. During the shuttle-streaming of the endoplasm towards the front an excess of cytoplasm remains in the frontal part of the plasmodium. This part of *Plasmodium* is forced to increase its volume. The incorporation of vesicles into the frontal part of the membrane leading to its development and expansion has been postulated (Achenbach and Wohlfarth-Bottermann 1981, Hrebenda 1981). Fixation of this part of the plasmodium during the flow of endoplasm towards its front has revealed the occurrence of considerable amount of filamentous, material which is arranged either parallely or perpendiculary to the membrane (Pl. III 7, 8). It has been observed that the filamentous material is mainly localized along the invaginations (Pl. IV 9, 10).

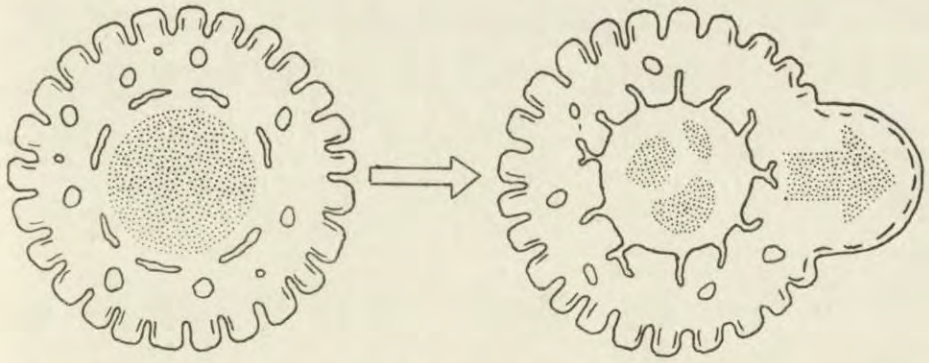

Fig. 1. The diagram showing changes which occurs in the *Physarum polycephalum* endoplasmic drop during the front formation. Bundles of microfilaments accompanying surface of invaginations are marked by short lines. Dotted arrow represents endoplasm came from central part of young endoplasmic drop

The presence and arrangment of those material beneath the membrane of the very tip of pseudopodium may suggest that in this place during the inflow of endoplasm investigations are unfolded (Fig. 1).

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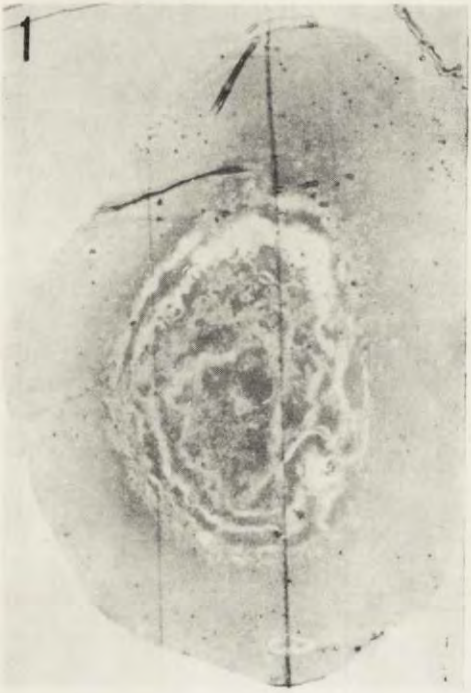
EXPLANATION OF PLATES I–IV

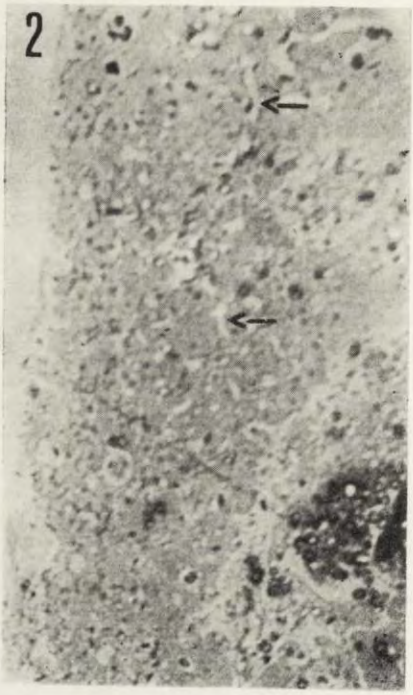
Pl. I. Light microscopy of *Physarum polycephalum* endoplasmic drops fixed by mixture of 2.5% glutaraldehyde and 1% OsO_4

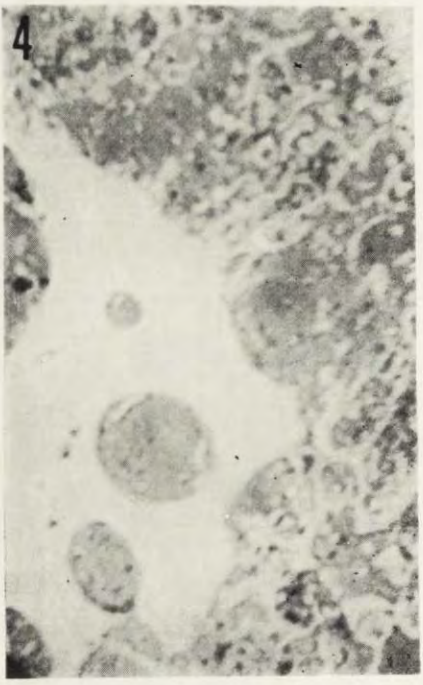
1: Transversal section of the drop of *Physarum polycephalum* aging 20 min. The circular invaginations around the endoplasm are visible. Magnification 128×
2: Higher magnification of the margin of the drop. Arrows indicate the small channels. Magnification 254×
3: Part of advancing pseudopodium. Magnification 128×
4: The inner part of the drop developing into microplasmodium. Note the membrane bound endoplasm remainings. Magnification 128×

Pl. II–IV. Electron microscopy of *Physarum polycephalum* endoplasmic drops fixed with 2.5% glutaraldehyde and postfixed with OsO_4

5: The longitudinal section of the small vein. Magnification 3600× en — endoplasm, i — invagination
6: Non-developing drop of 2 h age. Note the aboundant of invaginations and vesicles without visible order. Magnification 6840×. v — vesicles
7, 8: The invaginations accompanied by the layer of filaments. Magnification 36 000 and 45 600× respectively
9, 10: The filamentous layer beneath the membrane at the front of plasmodium. Magnification 30 000 and 45 000× respectively. f — filaments







B. Hrebenda auctor phot.

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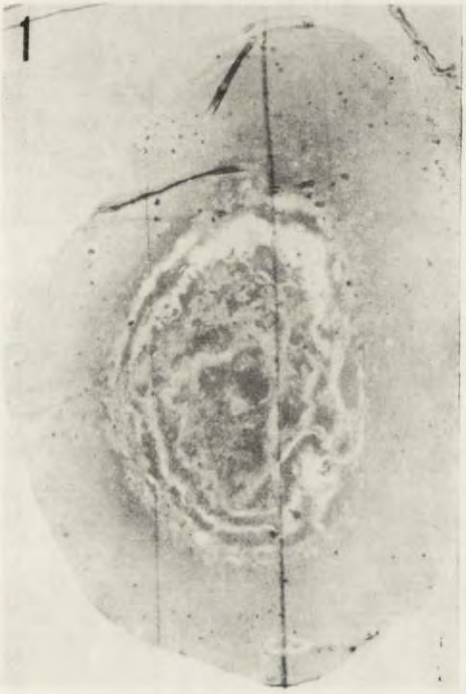

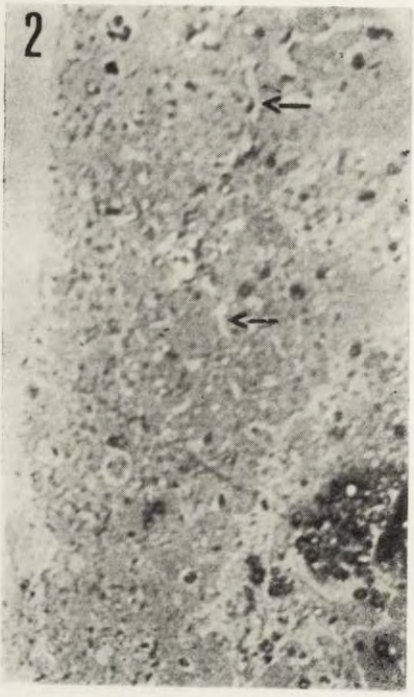

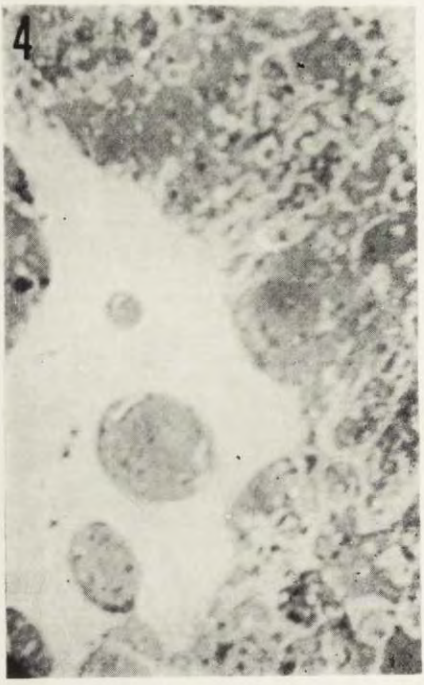

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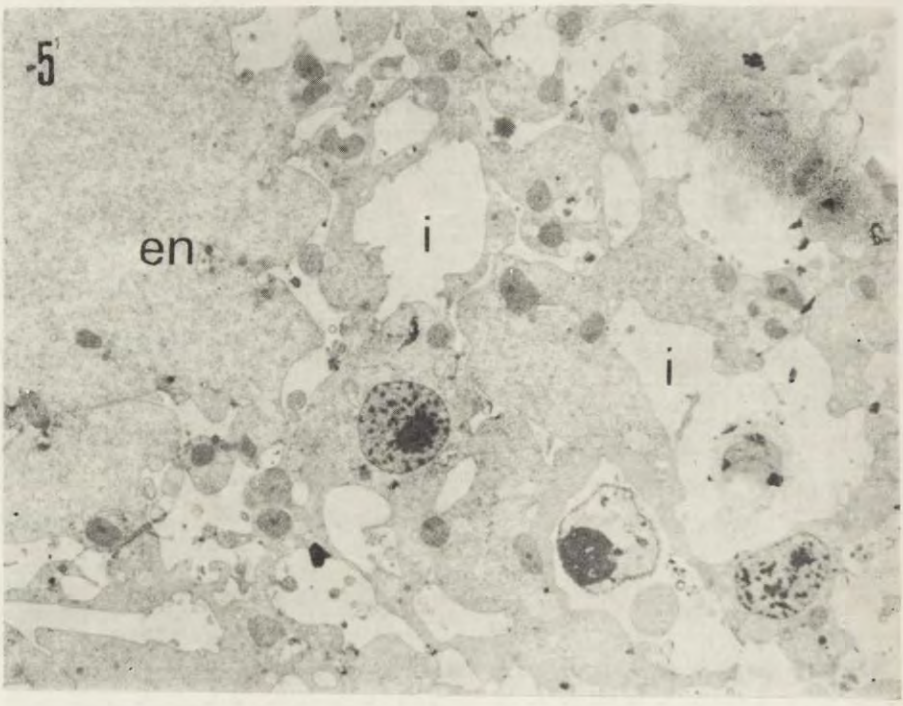

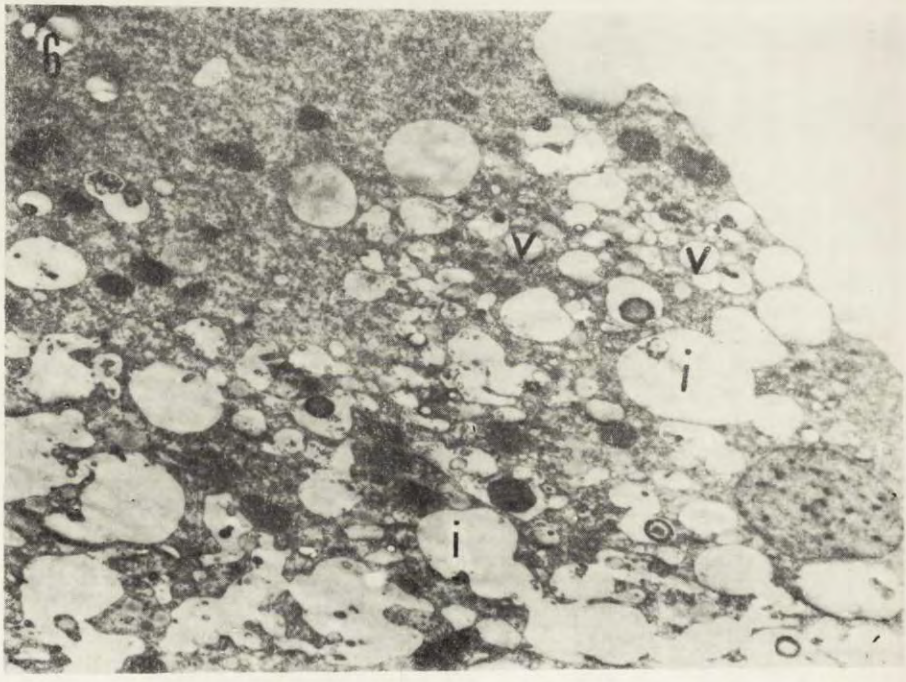

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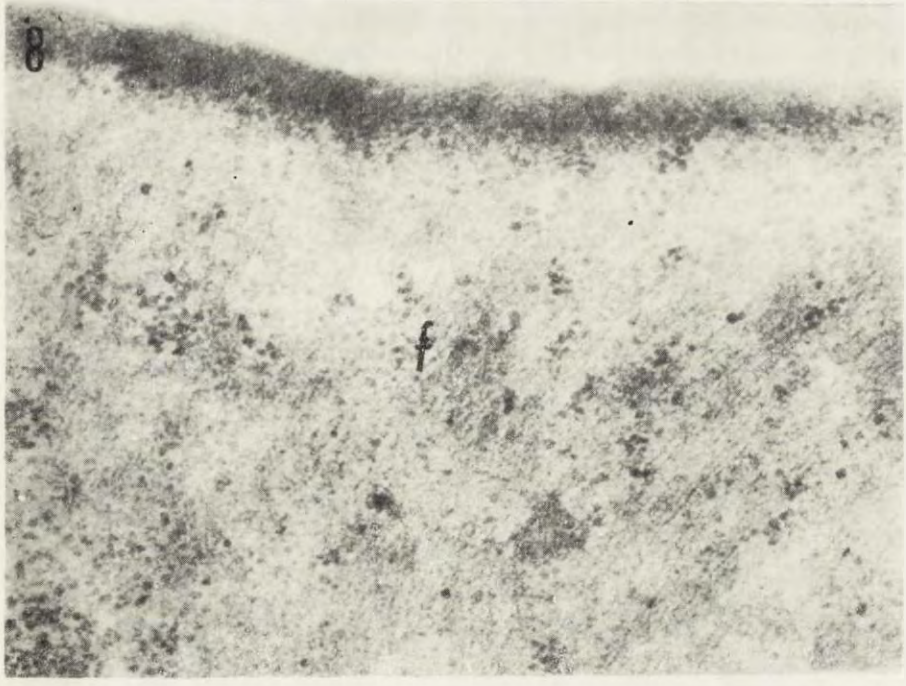

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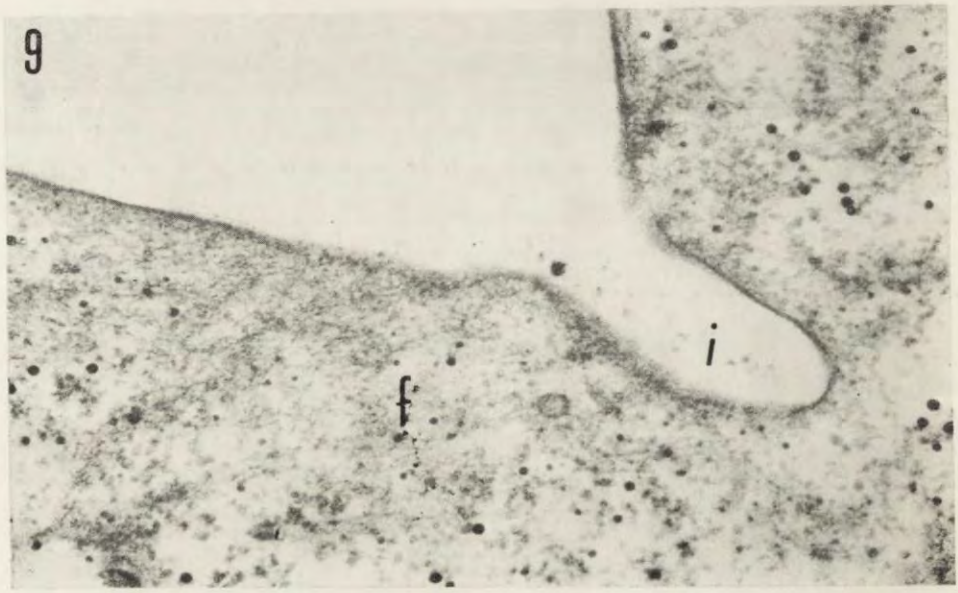

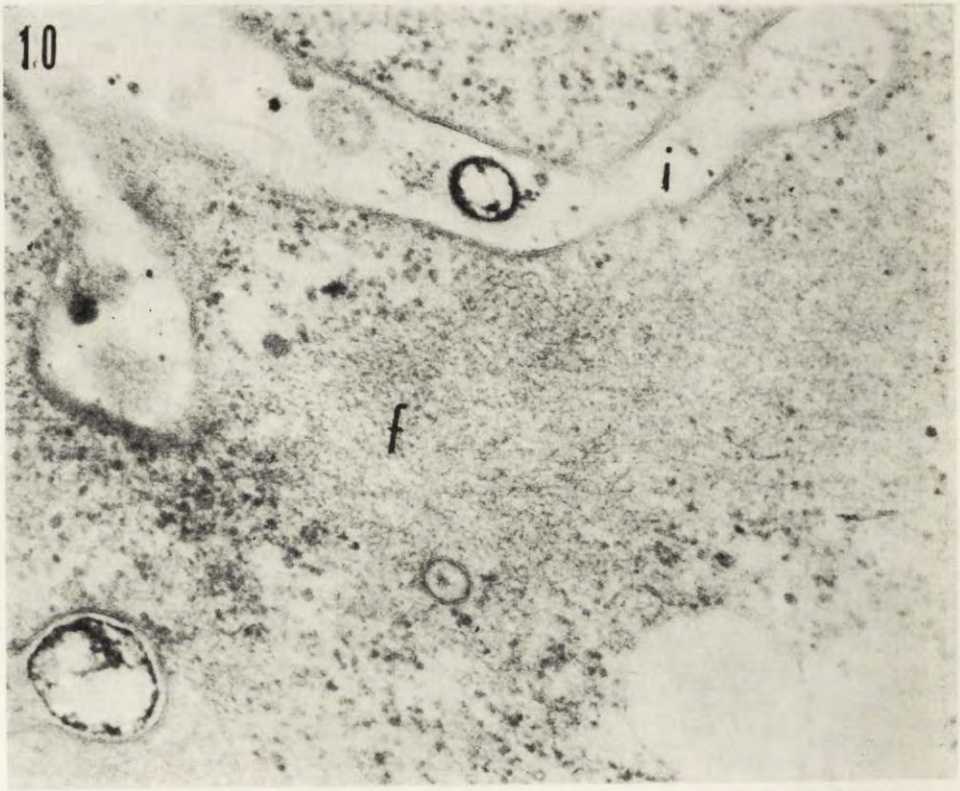

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BOOKS RECEIVED

THE PROKARYOTES
A Handbook on Habitats, Isolation, and
Identificaton of Bacteria
Editors: Starr M. P., Stolp H., Trüper H. G., Balows A., Schlegel H. G. 1981. XLVIII, 2596 pages (In two parts, not available separately). 6510 g. Cloth DM 880,—: approx. US $ 410.00 Berlin–Heidelberg–New York: Springer-Verlag
ISBN 3-540-08871-7

"The Prokaryotes" is an extensive monograph of bacteria. The idea of its writing crystallized in the course of a cycle of symposia under the title Enrichment, Culture and Selection of Mutants, which started in Göttingen in 1964. The amount of work involved may be realized from the list of about 200 authors and almost as many scientific editors who participated in the edition. The whole comprising two volumes was published by the publishing house Springer-Verlag. Although the monograph is limited to the discussion of the present state of knowledge about bacteria, particularly their occurrence, methods of isolation and identification, a careful reader will find there some general information from the field of physiology, molecular biology or genetics. The extensive factographic material is divided into 169 chapters grouped in 23 sections (A–W). The first including six chapters is an introduction, the remaining ones discuss the main tautological groups of bacteria. The particular chapters, with a few exceptions, correspond to the description of the particular species. As basis for division into groups was adopted the classification in the 8th edition of Bergey's "Manual of Determinative Bacteriology", with the introduction, however, of a number of changes resulting from new aspects of bacterial systematics. For instance in this new publication a description of new species is introduced, not included in Bergey's book, e.g., *Cyanobacteria*, *Prochloron*, *Stella*, *Legionella*. *Cyanobacteria*, described so far for physiological and ecological reasons as algae are actually prokaryotic organisms and have been defined as such by the Judicial Commission of the International Code on Systematic Bacteriology in 1979.

The arrangement of the monograph and the information contained in it are to some extent a reflection of the dilemmas set before bacteriologists when trying to establish a consistent hierarchic system of classification of bacteria. An explanation is due here. Early attempts at classification were limited to the description of phenotypic similarities between bacteria, without taking into account the natural relations between living organisms. It was not till the forties of this century that taxonomy

of bacteria began to consider the latter aspect. Investigations on the natural relations are conducted at various levels, which may, hovewer, be reduced to two: the strictly genetic one where the general organization and the sequences of homologous DNA bases are compared, and the epigenetic one, where similarities between the organisms are searched for at the translation or else in a smaller degree at the transcription level. Hence, beside "classical" information characterizing the species, that is data from the fields of morphology and physiology, results of most recent research may be found, giving for instance the G+C base ration (mol % guanine + cytosine) in the chromosomal DNA of the particular species, data concerning DNA hybridization *in vitro* (DNA from two compared species). Genetic investigations are also reported on interspecific hybridization *in vivo* and epigenetic studies documented by results obtained with the use of immunological and chemical techniques. Moreover, each chapter describes the techniques of isolation of bacteria from the natural environment, their culture and enrichment of pure cultures, the composition of bacteriological culture media, methods of storage of strains and sets of biochemical tests. Determination of species is facilitated by dichotomically arranged keys and comprehensive tables.

The characteristic of the particular bacterial biotypes gives numerous ecological data. They allow, on the one hand, to localize the given species in a definite microecosystem, and on the other to understand the relation established in the course of evolution between the environmental condition and the general organization of the cell and its metabolism. Descriptions of the relation host-prokaryotic organism (in the case of symbiotic and pathogenic bacteria) have a place by themselves in the monograph. Two main types of pathogenic bacteria are distinguished here, that is phytopathogenes and pathogenes of humans and animals.

The readers of Acta Protozoologica may be interested in information concerning the objects of their research contained in the monograph. Protozoans appear together with bacteria in many ecosystems, and in some, as for instance the rumen of herbivorous animals they exclusively constitute the microflora. Relations between these two groups may be studied on the examples discussed in this book of symbiosis, for instance the symbionts *Clostridium symbiosum* and *Entamoeba histolitica*, bacteria and protozoans of the rumen microflora, finally symbionts isolated in *Paramecium* strains. The nature of the killer factor (Alpha, Gamma, Delta, Kappa, Lambda, Mu, Nu, Pi and Sigma) transmitted during conjugation of *Paramecium* is discussed in a separate chapter. The same chapter gives, beside a characteristic of the isolated bacterial strains contaminated with the killer factor, a number of methodical indications.

While recommending this book to protozoologists, I would like also to call their attention to certain implications resulting from the similarity and universality of a number of biological systems. I have in mind here similarities of structural cell components, the universality of ATP as elementary biological energy load, the universality of the genetic code, and of the distribution of degradation pathways, of

the respiratory chain and of the foundations of metabolism. Hence all basic research conducted on the model of bacterial cells (evolutionally the oldest ones) may be to some extent transposed to the eukaryotic cell and *vice versa*. In investigations conducted at the molecular level the same techniques may be applied perhaps somewhat modified and advantage can be taken of reciprocally acquired experience and generalization. The monograph in point gives the reader an orientation in fundamental research on all groups of bacteria and the opportunity to find the eventual similarities with the study model interesting to the reader. For illustration I would like to quote an example from the monograph. Investigations on the contractile elements of the cell are intensively conducted on the model of an eukaryotic cell. Similar structures may be found, though very seldom, also in bacteria. Some *Spirochaeta* (*Pillotina*) possess microtubules (although somewhat smaller than the typical ones of eukaryotic cells). Protein isolated from *Pillotina* cells combines with specific immunofluorescent antibodies of eukaryotic microtubules and show the same migration velocity in electrophoresis on acrylamide gel as thet from microtubulins isolated from brain cells. It would seem on this basis that the protein of bacterial tubulins is the archaetype of the contractile proteins of flagella of eukarytotic cells.

The publication of "Prokaryotes" is no doubt a major scientific event, encompassing in its scope all circles of biologists. I believe that scientists will treat this monograph as a specific kind of guide facilitating discernement in the complicated problems of microbiology, while university lectures and students have received in it encyclopaedic compendium of information on bacteria.

Both volumes are very carefully prepared editorially. The particular chapters are provided with references, numerous photographs, tables and diagrams which illustrate the text. Access to the included information is facilitated by a subject and an authors index.

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Springer Verlag
Wien, New York

CELL BIOLOGY MONOGRAPHS, VOL. 9: RAIKOV, THE PROTOZOAN NUCLEUS

VOL. 9: THE PROTOZOAN NUCLEUS. MORPHOLOGY AND EVOLUTION.

By Dr. Igor Borissovich RAIKOV, Institute of Cytology of the Academy of Sciences of the USSR, Leningrad, USSR.
Translated from the 1978 Russian edition by Nicholas Bobrov and Marina Verkhovtseva, Moscow, USSR.
1982. 1 portrait. 116 figures. XV, 474 pages. 1320 Gramm.
Cloth S 1.720. DM 245, US $ 98,00

Contents: 1. Morphology of Eukaryotic Protozoan Nuclei, 2. Mitosis in Eukaryotic *Protozoa*, 3. Chromosomes of Eukaryotic *Protozoa*, 4. Nuclear Apparatus of Mesokaryotic *Protozoa*, 5. Meiosis in *Protozoa*, 6. Polygenomic Nucleic of Radiolaria, 7. Nuclear Dualism in *Protozoa*, 8. Polyploid Macronuclei of Ciliates, Conclusion, References, Subject Index, Index of Protozoan Genera and Suprageneric Latin Names

Academy of Sciences of the USSR
Proceedings of the Zoological Institute
Vol. 107

THE EVOLUTION AND PHYLOGENY OF THE UNICELLULAR ANIMALS

Contents: S. S. Schulman, V. V. Reshetnyak. The phenomenon of convergent similarity in floating *Sarcodina*, V. I. Mikhalevich. The parallelism and convergence in the evolution of the foraminiferal skeleton, M. V. Krylov, L. A. Kostenko. Morphofunctional organization of the *Sporozoa*, I. V. Issi. Recent research into life cycles of the microsporidians, Z. S. Donets. Formation of myxosporidian fauna in the southern seas of the USSR, A. W. Jankowski. New species, genera and families of tentacled *Infusoria* (class *Suctoria*), D. V. Ossipov, S. A. Podlipaev. The cytological aspects of the intranuclear symbiosis in *Protozoa*

In preparation:

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