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Protozoological Laboratory, Zoological Institute, University of Warszawa, Warszawa 64, Poland

Zdzisław RAABE

Ordo *Thigmotricha (Ciliata — Holotricha)*

II

Familia *Hemispeiridae*

I consider here, in the second part of my monographic study, the family *Hemispeiridae* as a group, of which the more primitive representatives I recognized as exit forms for *Thigmotricha* in general. These representatives, *Ancistrumina* especially, are on the one hand distinctly connected with *Pleuronematidae*, and on the other give series both in the family *Hemispeiridae* and in other groups of *Thigmotricha* leading to a further specialization.

Familia *Hemispeiridae* König, 1894
em. Chatton et Lwoff, 1949, em. Raabe, 1967

syn.: *Ancistridae* Issel, 1903; *Protophryidae* Cépède, 1910; *Ancistrumidae* Kahl, 1930; *Boveriidae* Pickard, 1927; *Thigmocomidae* Kazubski, 1958.

The studies on ciliates included presently to this family were initiated by O. F. Müller 1788, when he mentioned the finding of ciliate in *Mytilus edulis* named by this author *Trichoda ciliata*. It seems possible that it was here the question of *Ancistrum*, nevertheless this name must be treated as nomen nudum. Kent 1882 considers this ciliate as a representative of *Astomata*. Quennerstedt 1867 describes a ciliate *Opalina mytili* from *Mytilus edulis*, Maupas 1883 identifies it with ciliates found by him also in *Mytilus* and reports the description of the second species from *Venus gallina*; he creates for both species the genus *Ancistrum* and gives descriptions of *A. mytili* and of *A. venerisgallinae*, as first rather adequate definitions.

Stevens 1901 initiates the second as yet independent path by the description of a new genus and species *Boveria subcylindrica* from *Holothuria*. Issel 1903 soon observes its connection with *Ancistrum*: he introduces *Plagiospira* as an intermediate genus. These three genera: *Ancistrum, Plagiospira* and *Boveria* represent according to Issel 1903 an evolutionary sequence characterized by further spiralization and retrogradation of the two adoral kineties. Pickard 1927 who did not know the work of Issel, creates for *Boveria* a new family *Boveriidae* and includes it to *Heterotricha*!

The third even more separated way initiates Fabre-Domergue 1888 by his description of a peculiar ciliate *Hemispeira asteriasi* from *Asterias rubens* considered by the author as a representative of *Urceolariidae*. Similarly König 1894 includes to *Urceolariidae* his genus and species *Hemi-*

speiropsis comatulae creating for it and for *Hemispeira* a separated subfamily *Hemispeirinae*. However Wallengren 1885 does not consider them as *Peritricha* and Fauré-Fremiet 1934 connected *Hemispeirinae* with *Ancistridae*. Consequently Kahl 1934 includes them as a subfamily *Hemispeirinae* to the family *Ancistrumidae* and sets them up among *Thigmotricha*. This insertion is finally motivated by the considerations of Chatton et Lwoff 1949, which put *Hemispeira* at the end of the evolutionary sequence embracing *Ancistrospira* Ch. Lw., *Plagiospira* Issel and *Cheissinia* Ch. Lw. (= *Tiarella* Cheissin).

The fourth way leading to the knowledge of *Hemispeiridae* was that which conducted to the description by Kofoid 1903 of a form supposed to be a mouthless ciliate *Protophrya ovicola* ranged by this author to the family *Opalinidae* (!). For this reason *Protophrya* was located among *Astomata*, in a seperate family *Protophryidae* Cépède, 1910. Cépède indeed finds in the *Protophrya* a rudiment of mouth supposed to be seen by him and suggests the existence of a relationship between *Protophrya* and *Ancistrum*. At the same time he reports a description of a similar species *Isselina intermedia* which has also a mouth: however it seems that he confounded several species and failed to elucidate the problem. Only Chatton et Lwoff 1949 and lately Fenchel 1965 report redescriptions of *Protophrya ovicola*. It results that this species is distinctly approximate to *Ancistrum* and it may find its place in the same family.

In this way the four paths, initially independent, led to the integration of a family, which Kahl 1934 named *Ancistrumidae*. Kahl differentiates three subfamilies: *Ancistruminae* with the genera *Ancistruma* (pro *Ancistrum*) and *Euporterion*, *Boveriinae* with genera *Ancistrospira*, *Plagiospira* and *Boveria* and *Hemispeirinae*, to which he includes for unknown reasons besides *Hemispeira* and *Hemispeiropsis* also *Protophrya* and *Isselina*. A safe principle of division (however not always consequently applied) has been for Kahl 1934 the degree of spiralization and retrogradation of adoral kineties.

It seems that Chatton et Lwoff 1949 discovered only a real criterion of division of a phylogenetic value considering the family named by them *Hemispeiridae* König, 1894. This is the essential characteristic of the thigmotactic zone of ciliature: situated at the left body side and open, or dorsal and closed in système sécant. Chatton et Lwoff 1949 consider the following genera in their monograph: *Protophrya* Kofoid (= *Isselina* Cépède), *Ancistrum* Maupas (= *Ancistrina* Cheissin = *Eupoterion* McLennan et Connell), *Proboveria* Ch. Lw. and *Boveria* Stevens ranged to the subfamily *Protophryinae*, and *Ancistrospira* Ch. Lw., *Plagiospira* Issel, *Cheissina* Ch. Lw. (pro *Tiarella* Cheissin) and *Hemispeira* Fabre-Dom. (= *Hemispeiropsis* Koenig) — included to the subfamily *Hemispeirinae*.

Raabe 1959 revises the group of ciliates approximate to *Ancistrum*, therefore embraced according to Chatton et Lwoff 1949 by the subfamily *Protophryinae*. This revision is supported mainly on the promotion of two process: spiralization and retrogradation of adoral kineties. The author maintains or creates the following genera, which he defines with possibly available precision and objectivity: *Ancistrum* Maupas, *Ancistrumina* Raabe (pro *Ancistrina* Cheissin), *Protophrya* Kofoid, *Protophryopsis* Raabe, *Ancistrella* Cheissin, *Eupoterion* McLennan et Connell, *Proboveria* Ch. Lw. and *Boveria* Stevens. Raabe 1959 accepts and recognises the scope and character-

istic suggested by Chatton et Lwoff 1949 for the subfamily *Hemispeirinae*.

The basic change occurred in the systematic structure of *Hemispeiridae* when Kazubski 1958 described a very peculiar species *Thigmocoma acuminata*. He describes it from the renal organ of the terrestrian *Pulmonata* and he creates for it a separate family *Thigmocomidae*. Kazubski 1963 points out that the range of differentiation of *Thigmocomidae* and *Protophryinae* or *Hemispeirinae* is not higher than that one between the two subfamilies. He suggests the recognition of *Protophryinae*, *Hemispeirinae* and *Thigmocomidae* as three separate but strictly connected families. However this problem may be approached in another way (see part I p. 32) and the taxons can be recognized as subfamilies of one family *Hemispeiridae*.

Corliss 1961 includes all groups and genera discussed above to *Hemispeiridae*: he considers here also *Orchitophrya* Cépède, which has nothing in common with *Thigmotricha*, and *Protanoplophrya* Miyashita; I isolated for the last one a separate family *Protanoplophryidae* Raabe, 1967.

In spite of the diversity of shape which is revealed in the higher specialization and growing adaptation to the parasitic way of life, *Hemispeiridae* preserved the common character of the general architectonics and common characteristic feature of their structure:

The body is more or less distinctly flattened laterally, especially in the anterior part, where on the left or dorsal side lies the thigmotactic area. This area consists of the anterior sections of kineties of the left body side running to its posterior suture (subfamilia *Ancistrinae*) or rather of dorsal kineties sections, which do not continue backwards and constitute a system closed by the joint kineties of the general ciliature (subfamily *Hemispeirinae*). Sometimes the kineties in the left anterior part of the body are not continued but are also unclosed in système sécant (subfamily *Thigmocominae*).

The general ciliature has a different density. It seems that these forms may be considered as more primitive which reveal a rather rare ciliature (20—30 kineties). The densification of kineties (40—60) may be considered as a secondary symptom (vide part I. p. 11—14). The ciliature is very dense in the thigmotactic area, also generally in the anterior part of the body, but is rarefied in the posterior one. The kineties system consists evidently of two parts, a right and a left one, corresponding in its range only generally to the right and left body side. The boundary of the two sides, consist: the anterior suture running along the anterior body margin and less distinctly the posterior suture which runs virtually along the posterior margin. This suture becomes elongated in *Hemispeirinae* and is displaced to the anterior part along the dorsal margin — this in turn causes the abbreviation of the dorsal kineties and the rising of système sécant. The boundary of the two parts of the kinetic system on the ventral margin constitutes the naked peristomal area (at least in the more primitive forms); this limit undergoes divergencies in the forms, in which occurs the retrogradation and spiralization of the adoral kineties.

There are two adoral kineties: one is the stomatogenic kinety, running originally in a parallel position to the further kineties of the right body system; it describes an leiotropic arch in the posterior body end. The second kinety is the prostomal kinety — it runs first, paralelly to the stomatogenic

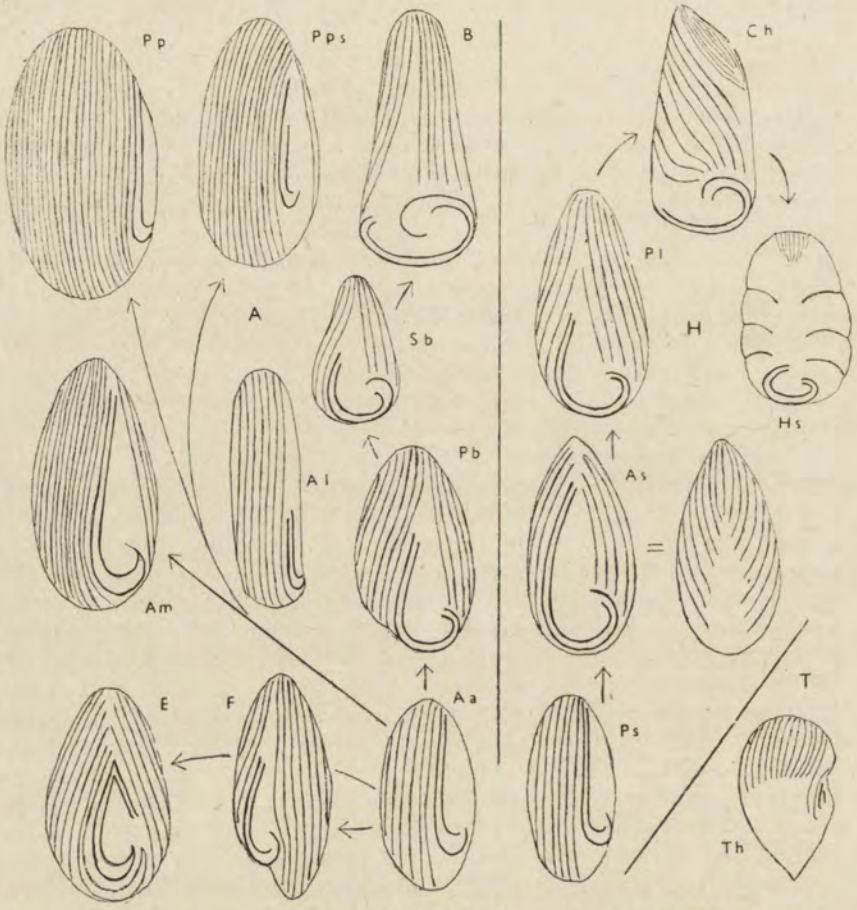

Fig. 1 Scheme of the system and evolution of *Hemispeiridae*: A — *Ancistrinae*, Aa — *Ancistrumina*, F — *Fenchelia*, E — *Eupoterion*, Al — *Ancistrella*, Am — *Ancistrum*, Pp — *Protophrya*, Pps — *Protophryopsis*, Pb — *Proboveria*, Sb — *Semiboveria*, B — *Boveria*; H — *Hemispeirinae*, Ps — *Protospira*, As — *Ancistrospira*, Pl — *Plagiospira*, Ch — *Cheissinia*, Hs — *Hemispeira*; T — *Thigmocominae*, Th — *Thigmocoma*

kinety, then becomes more distant from it the posterior body part and forms a somewhat smaller arch. The stomatogenic kinety is doubtless a haplokinety; the prostomal kinety seems to be in its trophic stages a haplokinety, however over certain periods (division) it reveals the features of diplokinety, or even of polykinety.

In more plesiomorphic forms both adoral kineties start in the ventral end of the anterior suture, near the anterior body pole and almost reach the body end describing a larger or narrower loop. However this initial location undergoes further modifications in the evolutive process of *Hemispeiridae* according to tendencies: retrogradation and spiralization, therefore the regression of elements of the adoral kineties from the anterior end of the body and their rolling round the posterior body pole.

These two tendencies may be realized not uniformly and not simultaneously: only retrogradation may occur — the adoral kineties become short and run in the posterior part of the peristomal area (*Protophrya, Protophryopsis, Ancistrella*). Spiralization and retrogradation occur especially distinctly in *Boveria* within *Ancistrinae*, in *Cheissinia* and *Hemispeira* within *Hemispeirinae*. This parallelism in both discussed families observed by Chatton et Lwoff 1949 (vide part I p. 23) is a very characteristic phenomenon and may be presented in the following completed Table 1 which considers 4 stages of this process.

Table 1

The evolutionary parallelism in the subfamilies *Ancistrinae* and *Hemispeirinae* in respect of the retrogradation and spiralization of the adoral kineties

Subfamily	*Ancistrinae*	*Hemispeirinae*
Initial position of adoral kineties	*Ancistrumina*	*Protospira*
I degree of the both processes	*Proboveria*	*Ancistrospira*
II „ „ „ „ „	*Semiboveria*	*Plagiospira*
III „ „ „ „ „	*Boveria*	*Cheissinia*
IV „ „ „ „ „	—	*Hemispeira*

Due to retrogradation and spiralization of the adoral kineties they take a vertical position toward the initial orientation considering the run of the kineties of the adoral ciliature. In such forms as *Hemispeira* the displacement involves also a shifting in the run of these kineties so, that they (distinctly rarified) accept rather a parallel arrangment than a meridional run. A particular apical location follow also the kineties of the thigmotactic ciliature on the anterior body pole.

The path of the adoral kineties in *Thigmocominae* is different, however nothing can be stated concerning the evolutionary tendencies within this group since it is monospecific. In this case the adoral kineties do not undergo spiralization and retrogradation processes, but rather are slightly shortened and more distinctly separated from kineties of the general ciliature. They change also their position toward one another: the stomatogenic kinety begins in the middle of body length, the prostomal kinety at its apex. In spite of this differentiation the morphogenetic processes in *Thigmocoma* (Kazubski 1963) strictly correspond to those which are observed in *Ancistrinae* or *Hemispeirinae* (and in *Conchophthirinae* too — Raabe 1963).

The phenomenon connected with the paths of the adoral kineties in *Hemispeiridae* is the involvement of the adjoining kineties of the general ciliature, mainly the kinety 1 (kinety 2 according to Chatton et Lwoff), and in several cases also the kinety 2 and the kineties n and n—1. This occurs distinctly in *Ancistrumina, Ancistrum, Boveria* (*Ancistrinae*) and *Cheissinia* (*Hemispeirinae*). It seems that this process is distinctly marked in *Eupoterion*, in which kineties 1 and 2 form something like a second adoral spiral, and kineties n and $n-1$ from the limitation of the peristomal area on the left side. Chatton and Lwoff 1949 (p. 231—232) observed a higher density of kinetosomes on kineties 1 and n; Raabe 1959 stressed strongly this phenomenon. The kinety 1 seems also to play an important role in *Thigmo-*

coma. It is distinctly longer than the further, shortened kineties of the general ciliature and surrounds the peristomal area; similarly the kineties n and $n-1$ are longer and more strong.

There are different evolutionary tendencies of the thigmotactic zone in *Hemispeiridae*. It is formed virtually of the anterior fragments of kineties of the general ciliature on the left body side. In *Ancistrinae* the undifferentiated thigmotactic ciliature ranges from the kinety lying on the dorsal margin to nearly the last kinety $n-2$, these kineties continue backwards to the posterior suture. In *Thigmocominae* in connection with a nearly complete reduction of ciliature on the posterior body side, the thigmotactic zone is cut, and its kineties do not continue backwards. In *Hemispeirinae* the differentiated thigmotactic ciliature occupies rather a dorsal position; the thigmotactic kineties break off nearly the middle of body length and are closed as in brackets by the nearest kineties of the general ciliature.

The difference revealed and stressed by Chatton et Lwoff 1949 in the position and shape of the thigmotactic zone in *Ancistrinae* (*Protophryinae according* Ch. et Lw.) seems very essential in this problem. It may be so really in extreme cases, as in *Cheissinia* or *Hemispeira*. However the origin of the closed and dorsally located système sécant in *Hemispeirinae* is very simple and its initial forms only slightly deviate from the outline of the structure of the dorsal zone in *Ancistrinae*. By now this problem is not elucidated for this reason, that Chatton et Lwoff and their successors applied at most the wet silver method, which fails to show the fibrills. Meanwhile this question may be elucidated by Klein's dry method (nota bene this method is also satisfactory on sea material).

The initial stage arising of the dorsal système sécant and of anterogradation of the posterior suture occur in *Protospira*, in its presently only single species *P. mazurica* Raabe, 1968. *Ancistrospira* and consequently *Plagiospira* represent a somewhat further stage. The dorsal part of the posterior suture (Fig. 2) shifts forewards in these forms, the kineties nearly the dorsal margin are shortened gradually and slightly buckle. It must be stressed that the kineties belonging to the left part of ciliary system are mainly involved in the arising of the thigmotactic système sécant. In the figures of Chatton et Lwoff 1949 the kineties of the thigmotactic area break off, therefore

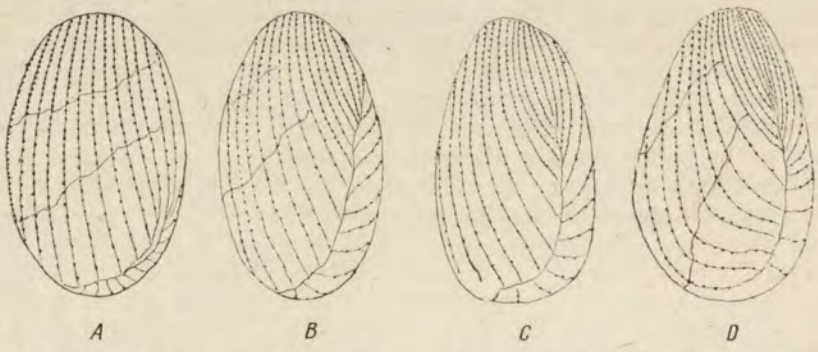

Fig. 2. Schemes of origin and formation of the parenthetical system (système sécant) of the thigmotactic ciliature in *Hemispeiridae*: A — stage *Ancistrumina*, B — stage *Protospira*, C — stage *Ancistrospira*, D — stage *Cheissinia*

they are monopolar; this picture results only from the fact that the posterior ends of the kineties are poorly occupied by kinetosomes which do not appear on the preparations effected by wet silver methods. In principle, as it is revealed on the preparations impregnated by Klein's method, all these kineties reach in one way or another the posterior suture, so that they are bipolarly connected with the whole argyrophilic system.

There are following problems which I am presently not able to decide generally and which probably cannot be generally taken for granted. These are: the structure of adoral kineties, especially of the prostomal kinety and the structure of the thigmotactic kineties.

Chatton et Lwoff 1949 consider that the prostomal kinety is ever a diplokinety and consists of two parts A and B (+C) and they present it always in that way especially on the schematized drawings. The followers of these authors generally maintain this view, but it seems that in many cases this support is rather conventional (i.e. Fenchel 1964, 1965). I have been never able to confirm it univocally. In view of my own preparations it results that, at least in the trophic stage (interphasal) the prostomal kinety is a haplokinety however sometimes with densified kinetosomes and occasionally aranged in zigzag. I have never met a distinct division in two segments A and B. However its fragmentation or rather the origin of the kinety from several elements of the polykinetic character occur in divisional stages. This problem will be discussed in the last parts of this study.

Fenchel 1965 touched the problem of the different structure, namely the question of diplokinety, of the thigmotactic system — he claimed that at least in *Hemispeirinae* the kineties of système sécant are diplokineties. According to my observations their kinetosomes virtually differ from the kinetosomes of the general ciliature. Their outlines are bleared or spread, but I could never state their binary system. However it seems possible that this diploidy may occur in several species with a more differentiated thigmotactic area.

Hemispeiridae are commensales or parasites mainly of the respiratory surface of *Mollusca*, both *Bivalvia* as *Gastropoda*, from sea and freshwater. Some species migrate to another host (*Boveria* on *Holothurioidea*, *Hemispeira* on *Asteroidea* and *Ophiuroidea*) or to another organs (*Ancistrumina limnica* in the intestine of *Gastropoda*, *Boveria subcylindrica* in the water lungs of *Holothurioidae*). Their specificity in relation to the hosts is not uniform — from a very large one, when the species appears in various types of hosts (*Boveria*) to the very narrow one, then its occurrence is limited to one species of host only.

Among the genera of *Hemispeiridae* it seems interesting to pay attention on two genera: *Ancistrumina* and *Boveria* quite different as in the remaining genera. Whereas the other genera are virtually monospecific (8 genera) or include 2 or 3 species (3 genera), the genus *Ancistrumina* has yet about 20 with many forms, the genus *Boveria* — 3 but also with numerous forms from various hosts. Both genera include not only many species, but they reveal also a high variability within the unities recognized as species. Moreover these genera are not specific according to groups (*Ancistrumina* occurs in *Lamellibranchiata*, *Prosobranchia* and *Pulmonata*, *Boveria* occurs in *Mollusca* and *Holothurioidea*) nor concerning the organs (*Ancistrumina* — the bran-

chial cavity and intestine, *Boveria* — the branchial cavity of *Mollusca* and water lungs of *Holothurioidea*).

This situation concerning *Ancistrumina* may be explained by the primitiveness and a limited morphophysiologic specialization and consequently by the evolutionary and ecological dynamism, which is reflected in the high radiation of the genus. Other genera, more specialized in different directions, mono- or oligospecific, reveal a distinctly lower evolutionary dynamics and are more specific in relation to their hosts.

The problem of the genus *Boveria* seems all the more strange; this genus reveals the last, final evolutionary possibilities at least in two features, retrogradation and spiralization of the adoral kineties. It may be admited in this case that the attainment of the level of "*Boveria*" in the evolution of *Ancistrinae* does not limit its adaptative possibilities but on the contrary, provided larger ones — a new radiation results on another level.

Hemispeiridae have a distinctly expressed thigmotactism in connection with their life mainly on the respiratory surfaces of *Mollusca*. This thigmotactism is especially realized thanks to distinctly developed ciliary thigmotactic zone and, in several forms, thanks to the appearance of a protrusion in the anterior body pole, called by Chatton et Lwoff "bouton adhesif". This "bouton" is clearly visible in many *Ancistrum* and *Ancistrumina* and may serve for the anchorage of the ciliate to the host's epithelium over a period of time. This bouton according to Chatton et Lwoff has besides its actual significance also a prospective one forming an anlage of suçoir of *Ancistrocomidae*.

Hemispeiridae move fast in general, often along a spiral or an arch. The majority of *Hemispeiridae* perform more frequent or scarce circular motions around their axis; this frequency depends on the shape and on the diversity of the ciliature. The displacement of the thigmotactic ciliature to the anterior, and of the adoral spiral to the posterior body pole in *Hemispeira* involved a different way of movement, namely a rotary gliding one, slightly similar to (certainly only convergently) the movement of *Urceolariidae* (*Peritricha*).

It seems that all *Hemispeiridae* feed up with bacteria or some other minute organic particles apart from the host's organism. Their relationship to the host, which is parasitism from the ecologic point of view, may be determined rather as a commensalism in the physiologic aspect.

Reproduction of *Hemispeiridae* occurs by an equal division. The divisional processes were examined by many authors in the aspect of nuclear transformations and cytokinesis; Chatton et Lwoff 1936, 1939 examined the cortical changes mainly on the example of *Proboveria loripedis* Ch. Lw., partly on *Plagiospira*, Kidder 1933 on *Ancistrum mytili* Quen., Raabe 1963 on the example of *Ancistrumina limnica*, Raabe and Kazubski 1963 on the example of *Thigmocoma acuminata* Kazubski. These problems, especially the interesting process of the stomatogenesis of the mentioned ciliates, will be discussed in the final chapters of this work. The conjugation was not very often observed, mostly by Nelson 1910 in *Boveria subcylindrica* Stevens, by Kidder 1933 and Fenchel 1965 in *Ancistrum mytili* Quen. — it occurs rarely but in mass. Raabe 1934 observed the winter incystation of *Ancistrum mytili* Quen., and Chatton et Lwoff 1937 described cysts of *Proboveria loripedis* Ch. Lw.

In view of the preservation of a common basic plan and type of the structure, the definition of the family *Hemispeiridae* may be rather clearly stated. However the former definitions are not adequate here considering separately *Ancistridae* and *Boveriidae*. The definition of C o r l i s s 1961 is also inadequate: "Cytostome at or near the posterior pole, buccal ciliature may extend anteriad on ventral surface. Thigmotactic ciliature near anterior pole. General somatic ciliature typically quite dense". A better support gives the definition of C h a t t o n et L w o f f 1949 (p. 196):

"Fam. *Hemispeiridae* König, 1894, emend. Ch. et Lw. Ciliés Holotriches commensaux, inquilins ou phorétiques, appartenant à l'ordre des *Thigmotricha* Ch. et Lw. La ciliature buccale est constituée par deux cinéties prostomiennes: l'une homologue de la cinétie stomatogène ou cinétie 1 porte une seule rangée de granules infraciliaires. Elle est douée de continuité directe par élongation. L'autre porte deux rangées de granules basaux. Elle est formée par les granules infraciliaires de la cinétie stomatogène. Les deux cinéties péristomienne 1 et B décrivent une courbe à concavité gauche; à leurs extrémités postérieurs se trouve l'infundibulum buccal tapissé de cinéties issues de 1".

Taking into consideration the former tests, all the present state of studies and the range of family (including *Thigmocomidae*) — definition of the family *Hemispeiridae* my be determined as follows:

Familia *Hemispeiridae* König, 1894
emend. Chatton et Lwoff, 1949, emend. Raabe, 1967

syn.: *Ancistridae* Issel, 1903; *Protophryidae* Cépède, 1910; *Ancistrumidae* Kahl, 1930; *Boveriidae* Pickard, 1927; *Thigmocomidae* Kazubski, 1958.

Thigmotricha of a mean body size (20—90 μ), with a rather scarce and usually differentiated ciliature; number of kineties 12—65. The thigmotactic ciliature separated from the general ciliature more feebly when consisting of anterior sectors of the kineties of the left body side (subfam. *Ancistrinae*) or more strongly when consisting of left complex of kineties court and paralell to each other (subfam. *Thigmocominae*) or a separate, rather dorsal complex of kineties, closed by the adherent kineties of the general ciliature in a paranthetical system (subfam. *Hemispeirinae*). The anterior suture runs on the front body margin; the posterior one runs in the more primitive forms along the hind margin. Two strong adoral kineties, stomatogenic and prostomal kinety, run in the more primitive forms from the ventral end of the anterior suture to the posterior body part, where they form a left-directed loop; in many cases, in the more evolutionary advanced forms, the adoral kineties undergo retrogradation and spiralization, in the extreme cases marking a loop on the distal body pole. The nuclear apparatus: 1 Ma, 1 Mi. 1 CV. Division equal, conjugation of the equal individuals. Parasites or commensals of the mantle cavity, of the intestine and of the renal organ of marine, fresh-water and terrestrial *Mollusca*, of the water-lungs of *Holothurioidea* and of the body surface of *Echinodermata*.

Typus familiae: genus *Hemispeira* Fabre-Domergue, 1888.

As it was formerly thoroughly discussed and visualized in the definition of the family, familia *Hemispeiridae* König 1894 may be divided into three subfamilies which realize different evolutionary paths within them. These are:

Subfamilia *Ancistrinae* Issel, 1903 = *Protophryinae* Cépède, 1910.
Subfamilia *Hemispeirinae* König, 1894
Subfamilia *Thigmocominae* Kazubski, 1958

Subfamilia *Ancistrinae* Issel, 1903

syn.: *Ancistridae* Issel, pro familia, *Protophryidae* Cépède, 1910 em. Chatton et Lwoff 1949.

On the basis of former discussion, the definition of the subfamily *Ancistrinae* may be presented in the following way:

Subfamilia *Ancistrinae* Issel, 1903

syn.: *Ancistridae* Issel, 1903 pro familia; *Protophryinae* Cépède, 1910, emend. Chatton et Lwoff, 1949.

Thigmotricha — *Hemispeiridae* of a thigmotactic area formed by anterior sectors of the kineties of the left part of the general ciliature; those kineties preserve their normal continuation at the hind part of the body; lack of the parenthetical system. The adoral kineties, taking in the more primitive forms an almost longitudinal position, tend to retrogradate and spiralize; in the extreme cases they make around the distal pole a spiral of an arc more than 360°. Parasites of the mantle cavity and of the intestine of marine and fresh-water *Mollusca* and of the water-lungs of *Holothurioidea*.

Typus generis: *Ancistrumina ovata* (Cheissin, 1930, 1931).

The systematics in the scope of the subfamily *Ancistrinae* is rather a difficult problem and occasionally the features differentiating the various species are slightly outlined. Raabe 1959 revised this subfamily and attempts to separate the genera on the basis of possibly objective and formalized criteria, taking into consideration three evolutionary tendencies: the retrogradation of adoral kineties, their spiralization on the posterior body pole and the tendency to rarefy the system of kineties of the general ciliature. However it would be more correct to treat this last differentiating feature inversely (according to that what was said in part I p. 30, 31), namely as a tendency to the polymerization of kineties of the general ciliature. As a result Raabe 1959 separated 8 genera and I add presently two new genera differentiated on the basis of the same criteria, namely the genus *Fenchelia* g. n. standing between *Ancistrumina* and *Proboveria*, and the genus *Semiboveria* g. n. standing between *Proboveria* and *Boveria*.

The Table 2 and diagram on Fig. 3 present the relations among the differentiated genera. In this diagram the genera *Protophrya* and *Protophryopsis*

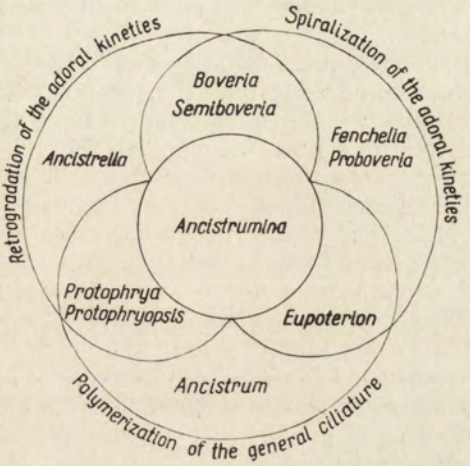

Fig. 3. Diagram of the evolutionary tendencies in *Ancistrinae*. The figure does not represent three circles which encroach one upon another (as in fig. 14, part I), but the epicardioide; the central field does not indicate therefore the coexistence of the three tendencies, but the lack of any of them

similarly as the genera *Fenchelia* and *Proboveria* and finally the genera *Semiboveria* and *Boveria* were in the common compartments. It results from it that they reveal coincidence in pairs according to the differentiating features and they diverge within the pair in other respects.

A discussion may arise on the differentiation of two genera, virtually vere near from one another: *Ancistrum* and *Ancistrumina*, introduced by

Table 2

The characteristic of the particular genera of the subfam. *Ancistrinae*

Genus	Number of kineties of the general ciliature	Density of the general ciliature	Retrogradation of adoral ciliature	Spiralization of adoral ciliature	Number of species
Ancistrumina	12—30	—	—	—	20
Ancistrum	40—60	+	—	—	3
Protophrya	ca. 65	+	—	—	1
Protophryopsis	50—60	+	—	—	1
Ancistrella	15—20	—	+	—	1
Eupoterion	40—50	+	—	+	1
Fenchelia g. n.	ca. 26	—	—	+	1
Proboveria	14—22	—	—	+	3
Semiboveria g. n.	ca. 20	—	+	+	1
Boveria	20—30	—	+	+	3—5

Raabe 1959. I continue to support this opinion in spite of the lack of endorsement by others authors.

The genus *Ancistrum* Maupas, 1883 was created for the species *A. mytili* (Quen.) with a simultaneous description of *A. venerisgallinae* Maupas. Issel 1903 described several species in this genus and among the others he reported the description of *A. mytili* Maupas which then Kahl 1931 and Kidder 1933 considered as a separate species *A. isseli* Kahl 1931. Then were described further species of this genus (Chatton et Lwoff 1926, 1949, Raabe 1936, Uyemura 1937). The genus *Ancistrina* was created by Cheissin 1931 for the species *A. ovata* Cheissin, 1930, 1931 from the snails of the Baikal Lake as a first freshwater representative of the family. Next were described several species involved to the genus *Ancistrina* on the basis of their similarity rather to *Ancistrina ovata* than to *Ancistrum mytili* (Bush 1937, Raabe 1947, 1950). Chatton et Lwoff 1949 recognized *Ancistrina* (similarly as *Eupoterion* MacLenn. et Conn.) as a synonym of *Ancistrum* but they give diagrams at the same time of the species properly corresponding to *Ancistrina*, but not adequate to *Ancistrum*.

Raabe 1959 revising the subfamily *Ancistrinae* (= *Protophryinae*) followed the former division, namely the genus *Ancistrum* and *Ancistrumina* (instead of the preoccupied *Ancistrina*) and he recognized that:

1. Typical species of both genera essentially differ from one another.
2. Only two species — *A. venerisgallinae* Maupas and *A. isseli* Kahl sensu Kidder — of the remaining species embraced by the genus *Ancistrum* sensu lato — correspond to the features of the well known *A. mytili* (Quen.), meanwhile the rest (except several species unsufficiently known) correspond to the features of *A. ovata* Cheissin or *A. limnica* Raabe.
3. Although the species of both suggested genera constitute a sequence starting from the minute ones having much less kineties to the bigger ones with numerous kineties; after all there is a disjunction in this sequence which separates three species ranged among the genus *Ancistrum*: besides the size and number of kineties the continuity is not revealed in such features as abundant or scarce evolution of the argyrophilic network on the adoral field (Fig. 4 and 5).

It should be added that I consider the abundance of ciliature revealed by the species included by Raabe 1959 to the genus *Ancistrum* as a secondary characteristic, as an expression of tendency to polymerization of kineties of the general ciliature (Raabe 1967). Therefore I consider the species of the genus *Ancistrum* as more advanced in the evolutionary tendencies, as more apomorphic ones.

The opinion of Raabe 1959 has not been recognized by Corliss 1961 nor by Fenchel. Fenchel 1965 writes: "The division cannot be maintained since some of the characters used by Raabe to separate the two proposed genera occur together in some species". However he gives descriptions in 1965 of 8 species (5 new within it) closely corresponding to the features of the genus *Ancistrumina* besides the species *A. mytili* and *A. crassum*, differentiated here by myself as a new genus *Fenchelia*.

I maintain therefore my opinion of 1959 and I differentiate two genera of *Ancistrum* Maupas and *Ancistrumina* Raabe (pro *Ancistrina* Cheissin) and I relate their definitions in appropriate places.

It is interesting to draw attention to the mentioned evolutive sequence

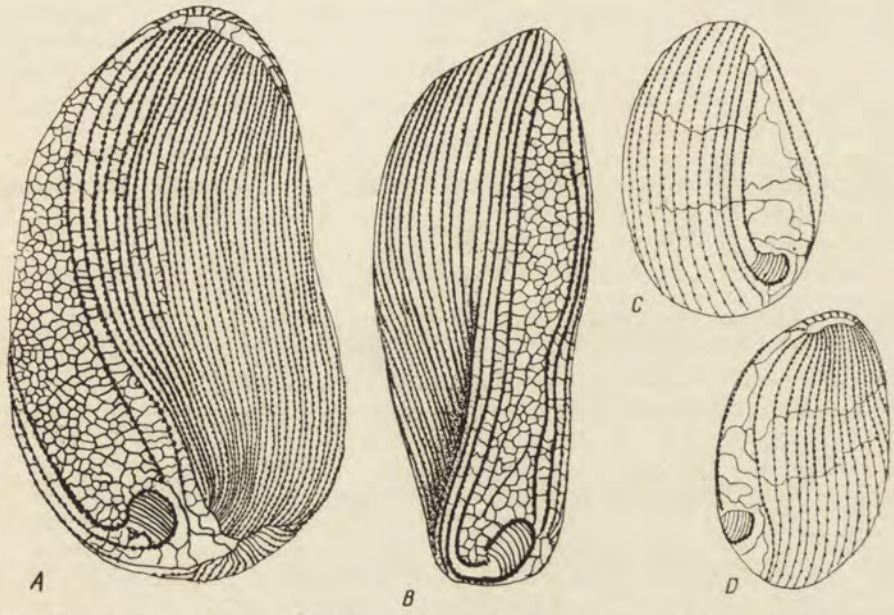

Fig. 4. Comparison of the cortical structure of the representatives of the genera: *Ancistrum* (*A. mytili* — A, B) and *Ancistrumina* (*A. tihanyensis* — C, D). From Raabe. ×1000

within the subfamily *Ancistrinae* which is revealed in the genera *Ancistrumina, Proboveria, Semiboveria* and *Boveria* (see Table 1 and Fig. 1). This sequence is consequently realized by parallel evolutive tendencies: the retrogradation and spiralization of the adoral kineties. Besides this row the development of other genera continued in rather different directions.

Genus *Ancistrumina* Raabe, 1959

syn.: *Ancistrina* Cheissin, 1930, 1931, *Isselina* Cépède, 1910; *Protophrya* part. in Cépède 1910.

The name of the genus *Ancistrumina* was introduced by Raabe 1959 in order to replace the name *Ancistrina* Cheissin, 1930, 1931, preoccupied by the name *Ancistrina* Goede, 1927 (*Lepidoptera*). This genus initially called *Ancistrina* has been created by Cheissin 1930, 1931 for the simultaneously described species *A. ovata* from the gills of *Benedictia* sp. sp. and *Choanomphalus* sp. from the Baikal Lake. *A. ovata* differs distinctly from the more typical representatives of the genus *Ancistrum* Maupas by its smaller size, rarity and scarce number of kineties; it has been the first fresh-water species in this group what was stressed by Cheissin. By the way this statement could confirm the supposition concerning the marine origin of the Baikal's fauna (Cheissin 1931, versus Raabe 1959). However virtually further studies led to the discovery of two freshwater species corresponding to the character of the genus *Ancistrina* (*A. limnica* Raabe, 1947 and *A. tihanyensis* Raabe, 1950) and also to the description of the marine species *A. kofoidi* Bush, 1937. Finally the figures and data published by Chatton et Lwoff 1949

concerning several representatives of the genus *Ansistrum*, and especially of *A. macomae* Ch. Lw. 1949 indicated that they correspond to the features of the genus *Ancistrina* Cheissin and not to the characteristic of *Ancistrum* Maupas. Similar characteristic reveal the majority of species described by Fenchel 1964, 1965.

The definition of the genus *Ancistrumina* Raabe, 1959 (pro *Ancistrina* Cheissin, 1931) may be according to the statement of Raabe 1959 formulated as follows:

Ancistrumina Raabe, 1959

pro: *Ancistrina* Cheissin, 1930, 1931; syn.: *Pleuronema* partim: *P. anodontae* Kahl, 1930—1933; *Ancistrum* pro parte Chatton et Lwoff, 1949 et auctores; *Issellina* Cépède, 1910; *Protophrya* in Cépède, 1910;

Hemispeiridae — *Ancistrinae* of a relatively scarce ciliature and rather small dimensions of the body (20—40 μ). Number of kineties 12—30. Two adoral kineties begin at the small distance from the apical suture and, running backwards, make a large loop at the distance of about 1/4 from the hind body pole. The argyronemes of the naked peritomal field are scarce; only two meridional argyronemes. Parasites of the intestine and the mantle cavity of fresh-water and marine *Gastropoda* and *Bivalvia*.

Typus generis: *Ancistrumina ovata* (Cheissin, 1930—1931).

The genus *Ancistrumina* includes presently (in the definition quoted here) circa 20 species however their range is not uniform. According to the authors' descriptions, several of them seem specific for the particular species of hosts and can be distinctly differentiated morphologically. However the other ones are rather slightly specific, they occur in many species of hosts, even in the species being distant one from the another (i.e. *Gastropoda* and *Bivalvia*). These more ubiquistic species (i.e. *A. cyclidioides* Issel or *A. limnica* Raabe) give in various hosts rather separate populations, differentiated by size, number of kineties and shape. These populations may be properly recognized as distinct species; the differences between them are often of the same rank as these which are decisive according to other authors for separation of their "good" species (Fig. 5). This is a problem for decision which will mainly depend on the personal opinion of the author.

Among the species which according to my criteria could be assigned to the genus *Ancistrumina* and have been described as representatives of the genus *Ancistrum*, I presently disengaged: *A. crassum* Fenchel, 1965 in a new species *Fenchelia* g. n.; I doubt about two species: *A. compressum* Issel, 1903 and *A. scrobiculariae* Ch. Lw., 1926.

Ancistrumina arcopagiae (Fenchel, 1965)

This is the smallest one and having the less numerous kineties from all the species known by now. The body dimensions: length 30—32 μ, width 16—20 μ. The body is pear-shaped, strongly sharpened in its front. Ma is ovoid (7—8 μ), Mi ?. There are 12 kineties of the general ciliature, 5 on the right and 7 on the left body side (Fig. 6 A).

The host: *Arcopagia crassa* (Penn.) Gullmarfjord, Sweden.

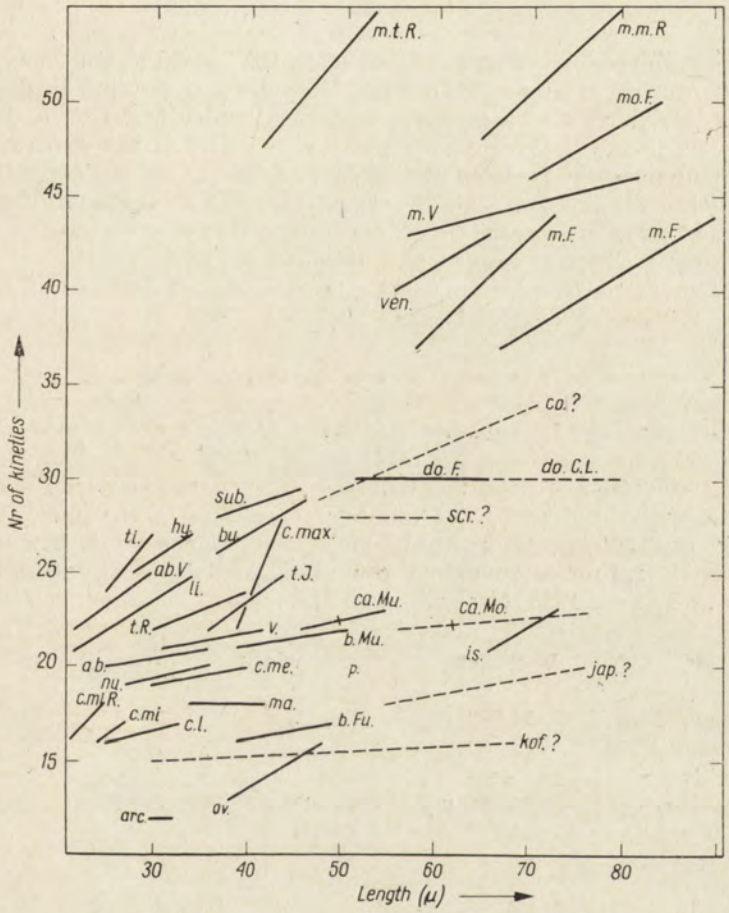

Fig. 5. Dispersion and ranges of the characters of the particular species and forms of the genera *Ancistrumina* and *Ancistrum*. The lines joint the point of the minimal number of kineties and the minimal length of the body with the point of the maximal number of kineties and the maximal length of the body of the given species or form. Indications: arc. — *A. arcopagiae*, ov. — *A. ovata*, c.mi.R. — *A. cyclidioides f.minor* — Raabe, c.mi. — *A. cyclidioides f. minor* — Issel, c.l. — *A. cyclidioides f. lata* — Issel, c.me. — *A. cyclidioides f. media* — Issel, c.max. — *A. cyclidioides f. maxima* — Issel, ma — *A. macomae*, b.Fus. — *A. barbata, Fusus*, b.Mu. — *A. barbata, Murex*, nu. — *A. nuculae*, ab. — *A. abrarum*, ab.V — *A. abrarum*, Varna, p. — *A. purpurae*, v. — *A. venerupis*, t.I. — *A. tellinae* — Issel, t.R. — *A. tellinae* — Raabe, li. — *A. limnica*, ti — *A. tihanyensis*, hy. — *A. hydrobiae*, sub. — *A. subtruncata*, bu. — *A. bulgarica*, ca. Mu — *A. caudata, Musculus*, ca.Mo. — *A. caudata, Modiolus*, is — *A. "isseli"* — Issel, jap. — *A. japonica*, kof. — *A. kofoidi*, scr. — *A. scrobiculariae*, do. F. — *A. dosiniae* — Fenchel, do C.L. — *A. dosiniae* — Chatton et Lwoff, co. — *A. compressa*, m.F. — *A. mytili* — Fenchel, m.t.R. — *A. mytili f. typica* — Raabe, m.m.R. — *A. mytili f. magna* — Raabe, m.V. — *A. mytili, M. galloprovincialis*, Varna, mo. — *A. modioli*, ven. *A. venerisgallinae*

Ancistrumina ovata Cheissin, 1931

The body dimensions: length 38—48 μ, width 15—20 μ; the body is ovoid, slightly sharpened in its posterior part. Ma spherical, located in the anterior part of the body. Mi 1—2 μ is usually located before Ma. CV in the posterior body part inside the vacuolized plasma. Kineties of the general ciliature are 13—16 in number, 5—6 on the right and 10—11 in the left side of the body. The cilia are 5 μ long, the adoral ones 10—12 μ. The adoral spiral is in the posterior body part; there are scarce connective argyronemata on the oral field, one equatorial argyronema (Fig. 6 B,C,D).

Hosts: *Benedictia baicalensis* (Gerst.), *B. limneoides* (Schr.) and *Choanomphalus* sp., the Baikal Lake, Siberia.

Ancistrumina cyclidioides (Issel, 1903)

The body size highly variable (see below). The body is oval, the shape rather variable, narrow or considerably broad. Ma is spherical in the anterior body part, Mi (2.5—3 μ) situated before it. CV in the posterior part of the body in the vacuolized zone. The number of kineties of the general ciliature is variable (see below), at the body end "un' unica setola". The species is conspicuously ubiquistic, revealing separate populations in different hosts, according to Issel 1903, Neapolitanian Bay:

Host	Body length	Body width	No. of kineties	"Forma"
Tellina exigua Poli	24—27 μ	12—14 μ	16—17	*minima*
Chiton olivaceus Sp.	25—33 μ	13—20 μ	16—17	*lata*
Capsa fragilis L.	—	—	—	*lata*
Donax trunculus L.	34—40 μ	14—19 μ	19—20	*media*
Tapes desussata L.	30—37 μ	12—19 μ	—	*media*
Natica hebraea Mart.	39—44 μ	19—24 μ	22—28	*maxima*

Issel 1918 reports *A. cyclidioides* from *Littorina neritoides* L. and *L. punctata* Gm. without further discussion. Raabe 1936 recognizes as *A. cyclidioides* f. *minima* the ciliates found in *Mya arenaria* L. Dimensions: Ma spherical, 6—7 μ, the number of kineties of the general ciliature is 17, no "setola" are observed at the end of the body. South Baltic Sea.

A. cyclidioides must be recognized as a highly ubiquistic and variable species, or as "collective" species in which may be separated a number of taxons with a rank of species or subspecies in the ecologic or parasitologic sense, not in a zoogeographic one. In the meantime, until the decision will be pronounced in this question, it seems possible in my opinion to maintain the identification of variety by the name "form" — this one seems the less constraining; it cannot mark any concrete taxonomic category (Fig. 6 G,H).

Ancistrumina barbata (Issel, 1903)

The body size: length 37—51 μ; width 14—22 μ, the body elongated, with parallel margins. Ma round, big, in the anterior part of the body, Mi 5.5 μ is located before Ma. CV in the posterior part of the body. There are 16—22 kineties of the general ciliature; strong cilia of the adoral kineties (Fig. 6 F).

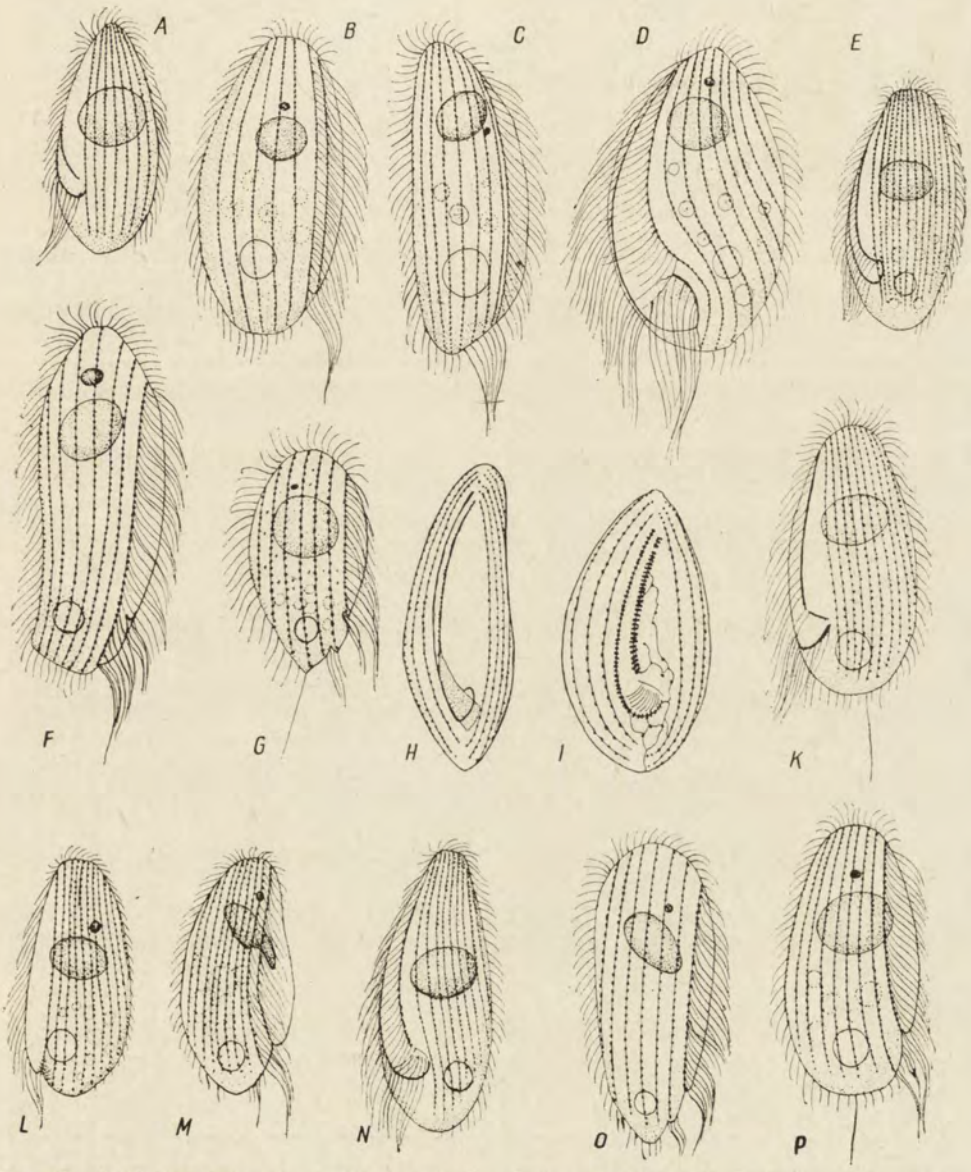

Fig. 6. *Ancistrumina: A* — *A. arcopagiae* (after Fenchel), B,C,D — *A. ovata* (a. Cheissin), E — *A. nuculae* (a. Fenchel), F. — *A. barbata* (a. Issel), G,H — *A. cyclidioides* (a. Issel), I — *A. macomae* (a. Chatton et Lwoff), K — *A. macomae* (a. Fenchel), L — *A. abrarum* (a. Fenchel), M — *A. hydrobiae* (a. Raabe), N — *A. venerupis* (a. Fenchel), O — *A. tellinae* (a. Issel), P — *A. tellinae* (a. Raabe). All drawings ×1000

The species reveals according to Issel a certain variability depending on the host. Neapolitanian Bay:

Host	Body length	Body width	No. of kineties
Fusus syracusanus L.	37—49 μ	14—17 μ	5+11—12
Murex trunculus L.	39—51 μ	15—22 μ	5+16—17

Ancistrumina nuculae (Fenchel, 1965)

The body oval, 27—36 μ long, 14—16 μ wide. There are 19 kineties of the general ciliature (Fig. 6 E).

Host: *Nucula turgida* Lack et Marsk, Gullmarfjord and Oeresund (Kattegat).

Ancistrumina venerupis (Fenchel, 1965)

The body is ovoid, slightly flattened in the posterior part, 31—42 μ long, 14—19 μ wide. The mouth lies 1/4 from the posterior end of the body. Ma is ovoid (11 μ), located in the median part, spherical Mi lies before Ma. The general ciliature — 21 and 22 kineties (Fig. 6 N).

Host: *Venerupis aurea* (Gmel.) Gullmarfjord, W. Sweden (with *Fenchelia crassa*).

Ancistrumina hydrobiae (Raabe, 1936)

The body is oval, its length 28—34 μ, width 14—17 μ. Ma elongated with irregular outline (10.5 μ) lies in the anterior body part, Mi (1 μ) lies in its proximity. The mouth in 3/5 length of the body. CV in the posterior body part; numerous alimentary vacuoles. 26 kineties of the general ciliature (Fig. 6 M).

Hosts: *Hydrobia ulvae* (Penn.), *H. ventrosa* (Mont.) but not in *H. jenkinsi* Smith, Bays of Gdańsk and of Puck, South Baltic Sea.

Fenchel 1965 reports *A. hydrobiae* from *Hydrobia ulvae* coming from Frederikshavn and N. Oeresung, Kattegat by 100% infestation, marking that the characteristic of his ciliate coincide with the original description of Raabe.

Ancistrumina macomae (Chatton et Lwoff, 1949)

The original description of Chatton et Lwoff 1949 is highly inadequate and may be related to many species of the genus *Ancistrumina*. The only data that is the number of kineties of the general ciliature — 18 in that case.

Host: *Macoma (Tellina) tenuis* Da Costa, Roscoff.

Fenchel 1965 as *A. macomae* Ch. Lw. considered the ciliates found by himself with the following features: the body ovoid, 34—42 μ long, 14—18 μ wide. Ma ovoid (11 μ) lies in the anterior part of the body, Mi next to Ma. CV in the posterior part of the body; there is also a long caudal thread. The oral loop lies 1/3—1/4 from the posterior body pole. 18 kineties of the general ciliature (Fig. 6 J).

Host: *Macoma baltica* L. from Askö (Baltic Sea), Oeresund (Kattegat).

Fenchel 1965 suggests that the ciliate found by him correspond to *A. tellinae* Issel in the description of Raabe 1936, however they do not correspond to the original description of Issel 1903.

Ancistrumina tellinae (Issel, 1903)

The original description of Issel refers: the outline of the body is ovoid, narrower in the posterior part, than in the anterior one, 36—44 μ long, 14—19 μ wide. Ma elongated lies in the posterior or medium part of the body. 22—25 kineties of the general ciliature (Fig. 6 O,P).

Host: *Tellina exigua* Poli (= *Macoma tenuis* Da Costa) — Napoli (with *A. cyclidioides*).

Raabe 1936 recognized for *A. tellinae* the ciliate found by himself in *Macoma baltica* in Baltic Sea. Its characteristic: the body ovoidal, the length 30—40 μ, width 18—22 μ; 22—24 kineties of the general ciliature. Ma oval (10—20 μ) in the anterior body part, Mi (1—2 μ) next to Ma.

Thus the description of Raabe 1936 corresponds to the data of Issel 1903, however it does not correspond with the data of Fenchel 1965 concerning also the ciliate from *Macoma baltica*.

It could be considered on the basis of the partial correspondence of the hosts, similar body outlines and similitude in other features, that *A. macomae* (Chatton et Lwoff, 1949) is a synonym of *A. tellinae* (Issel, 1903). The problem of separate number of kineties of the general ciliature may be less essential for the reason of considerable variability of these number in different populations. However it could seem strange that *Macoma baltic* from the South and Western Baltic Sea have separate parasites.

Raabe 1936 considered that also *A. dautzenbergi* Ch. Lw., 1926 could be a synonym of *A. tellinae* Issel; after the elucidation of the problem by Chatton et Lwoff 1949 this suspicion proved as inequitable (vide *Protophryopsis dautzenbergi*). A real mystery is the problem of *A. caulleryi* Ch. Lw., 1949 mentioned only from its name (p. 191), therefore being nomen nudum.

Ancistrumina abrarum (Fenchel, 1965)

The body is ovately outlined, with a more convex dorsal margin then the ventral one — this feature according to Fenchel 1965 differentiates distinctly this species from the others (??). The length of the body 25—36 μ, the width 5—14 μ, Ma ovoid (6—8 μ×5—6 μ) lies in the middle of the body, spherical Mi (1 μ) next to Ma. 20—21 kineties of the general ciliature. The ciliate swims quickly and by jerks. Fig. 6 L.

Host: *Abra nitida* (Müller) — Gullmarfjord, W. Sweden, infestation in 30—75%; the lack of this species in *Abra alba* (Wood).

I found quite near ciliate in *Abra ovata* (Phil.) in the brakish lake adjacent the Black Sea at Varna, Bulgaria. My data: length of the body 22—30 μ, width 11—15 μ. Ma ovoid, ca 6 μ in diameter, lies in the front part of the body; spherical Mi next to Ma. 22—25 kineties of the general ciliature.

It seems that *A. abrarum* is near to *A. tellinae* what is not strange considering the allied hosts and their ecologic vicariantism.

Ancistrumina limnica Raabe, 1967

syn.: *Pleuronema anodontae* Kahl, 1926 (?).

I described this species as *Ancistrina limnica* from several species of *Gastropoda Pulmonata* and *Unionidae* from different regions of Poland. It has been the second fresh-water species (after *A. ovata*) from the group *Ancistrum-Ancistrina*. I found it in my latter studies very often in different *Pulmonata* as well as in *Unio* and *Anodonta* from Poland and from the Balaton Lake (Hungary) R a a b e 1950. I found *A limnica* in Balaton lake in the *Anodonta* and *Unio*, describing at the same time a separate species *A. tihanyensis* Raabe, 1950 from *Lithoglyphus naticoides*, therefore from the representative of *Prosobranchia*. Finally I ranged to the species *A. limnica* the ciliates found in 6 species (among 23 studied) of *Gastropoda-Prosobranchia* from the Ohrid Lake (Yugoslavia); they were all molluscs strictly endemic for the Ohrid Lake.

The studies proved that the populations of *Ancistrumina* occur in different hosts and different regions varying within them by size and proportions, and especially (statistically) by the number of kineties of the general ciliature. The recognition of these populations as different species or even the differentiation of them as separate forms could not have any reason here, because the range of variability of their characters coincide with one another. Therefore *A. limnica* must be recognized as a highly cosmopolitic and ubiquistic species, very plastic and variable. The situation is similar as in the case of *Ancistrumina cyclidioides* (Issel, 1903) from the marine molluscs.

A. limnica occurs as a rule in the mantle cavity of their hosts. However Janina R a a b e (personal communication) found it quite regularly in the posterior segment of the intestine's loop in *Spiralina vortex* L. (*Pulmonata*) from the Polish river Pilica. In the intestine of these molluscs *A. limnica* occur sometimes in mass and they perform quick motions. Besides the cosmopolity and ubiquity, this character, the lack of topic specificity, may indicate the primitiveness of *A. limnica* (see p. 123).

A. limnica has been precisely studied by R a a b e 1947 on the preparations stained by dry silver method; it constitutes an object impregnating itself quite satisfactory and giving clean pictures (comp. part I Fig. 6 A, 7 A, 8 A).

Generally *Ancistrumina limnica* Raabe, 1947 may be characterized as follows:

The body is small, rounded on its anterior part, sometimes distinctly sharpened on its posterior end. A long polar cilium on the posterior body end. Typical dimensions: length 20—35 μ, width 11—18 μ. Ma elipsoidal, with diameters 8—10 μ and 6—8 μ lies in the anterior part of the body. Mi near Ma. CV at the body end. The length of the cilia of the general ciliature is ca. 5 μ, of the adoral ones — ca. 15 μ. 21—28 kineties of the general ciliature. The adoral kineties reach ca 3/4 of the body length and form a wide loop (Fig. 7 A).

Hosts: *Spiralina vortex* L., *Bathyomphalus contortus* L., other small *Pulmonata* and *Unio* sp. sp and *Anodonta* sp. sp. from the European Lowland; *Pseudamnicola consociella* Frau., *P. sturanyi* West., *Horatia ochridana* (Pol.), *H. brusinae* Radoman, *Lyhnidia gjorgjevici* Hadż., *Gocea ohridana* Hadż. — Lake Ohrid (Yugoslavia).

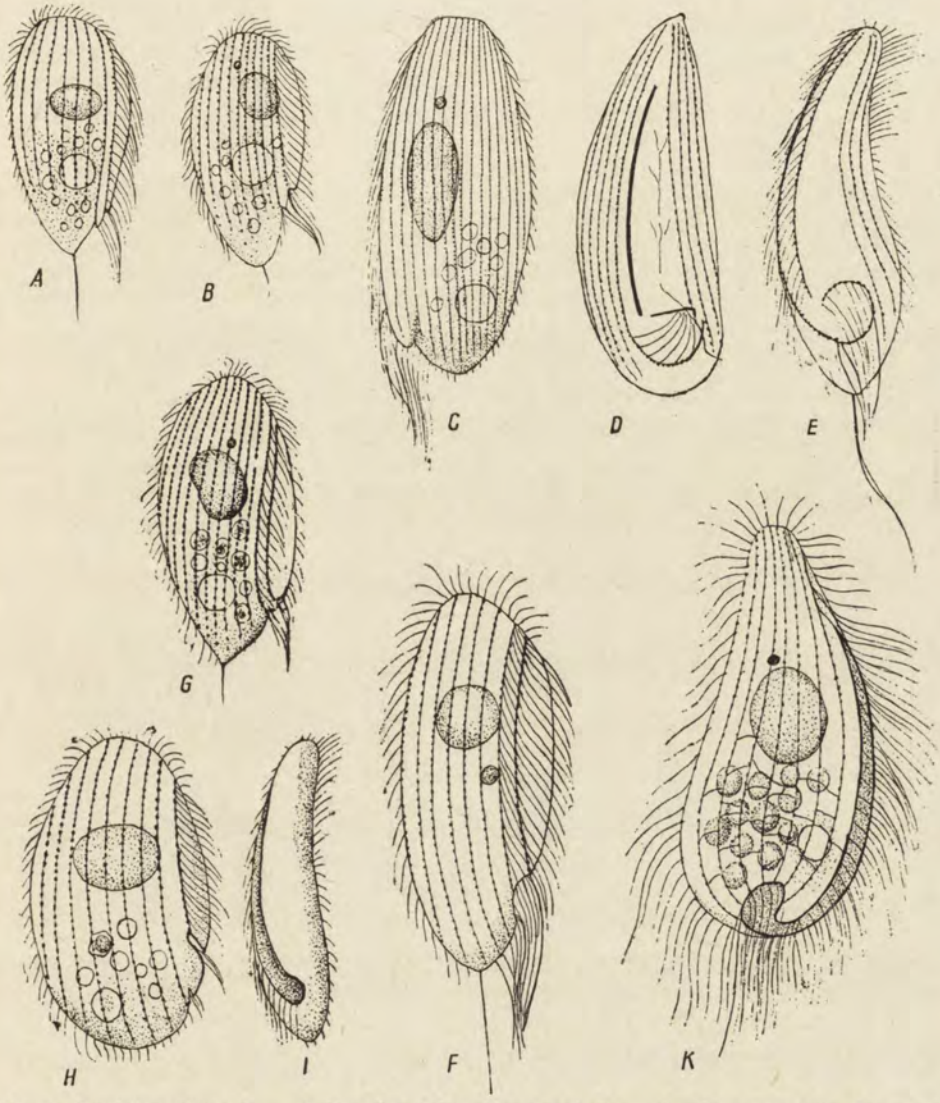

Fig. 7. *Ancistrumina*: A — *A. limnica* (after R a a b e), B — *A. tihanyensis* (a. R a a b e), C,D, — *A. caudata* (a. F e n c h e l), E,F, — *A. kofoidi* (a. B u s h), G — *A. bulgarica* (orig.), H,I — *A. subtruncata* (a. I s s e l), K — *A. japonica* (a. U y e m u r a). All drawings ×1000

K a h l 1935 repeats his description of *Pleuronoma anodontae* Kahl, 1926 occurring "selten in *Anodonta*, aber regelmaessig in *Sphaerium*-Arten". The description of K a h l 1935 (p. 389) runs as follows: "Gr. 55 µ. Schlank oval, hinten zugespitzt, vorn schwach zugespitzt, oder stumpf gerundet. Hinten mit halb Körperlanger Borste, die neben den Pol steht. Perst. typisch, auf dem mittleren Drittel. Wp. in dichten Reihen, kurz, vorn haking gekrümmt und derber als hinten. Hinter dem Perst. auf der l. Seite eine Raihe längerer Borsten. c.V.r., dors. von der Mdgr."

I suppose that K a h l had to do with *Ancistrumina*, but perhaps with its another species as *A. limnica*; I have never observed *Ancistrumina* in *Sphaerium* or *Pisidium*. The description of K a h l "prepared for *Pleuronema*" as well as the drawing give any idea concerning the probable differences.

Ancistrumina tihanyensis Raabe, 1950

I describe this species from *Lithoglyphus naticoides* (*Prosobranchia*) from the Balaton Lake, Hungary, because it differs from *Ancistrumina* get in Balaton from *Unionidae*, which I recognize as *A. limnica*. After recognizing the high variability of *A. limnica*, its wide geographic range and the scope of its hosts I do not exclude that *A. tihanyensis* may prove as a synonym of *A. limnica*.

The body ovoid with following dimensions: length 25—30 μ, width 13—17 μ, cilia ca 6 μ, adoral ca 16 μ. Ma elipsoid. diameter ca 8 μ, lies in the anterior body part. Small Mi next to Ma. CV in the posterior part of the body. 24—28 adoral kineties like in *A limnica*. *A tihanyensis* gives perfect pictures on the preparations stained by dry silver method (Fig. 4 C, D).

Host: *Lithoglyphus naticoides* (C. Pf.), Lake Balaton (Hungary).

Ancistrumina subtruncata (Issel, 1903)

The body is oval, the dorsal margin more convex than the ventral one; length 37—46 μ, width 20—25 μ. Ma ovoid, lies across, on the 1/3 height of the body length, Mi (4.5 μ) near it. CV in the posterior part of the body. 18—19 kineties of the general ciliature. A long "setola" at the posterior body end (Fig. 7 H, J).

Host: *Tapes decussata* L. Neapolitanian Bay.

C h a t t o n et L w o f f 1949 mention only the name (p. 191) *Ancistrum tapetis* n. sp. from *Tapes pullastra*; may be it has something in common with *A. subtruncata* (Iss.) — at any rate this is an evident nomen nudum.

Ancistrumina kofoidi Bush, 1937

The body ovoid, elongated, the length 30—70 μ, width 12—20 μ. Ma spherical, in the anterior or medium part of the body. The cilia 7 μ, oral ones till 15 μ. 15—16 kineties of the general ciliature. CV in the posterior body end. The observed division, and conjugation with 3 intensity periods over a year (Fig. 7 E,F).

Host: *Petricola pholadiformis* Lam. San Francisco Bay.

B u s h 1937 compares *A. cofoidi* only to *A. ovata* Cheissin, 1931 and knows, as it seems, merely the work of C h e i s s i n, for she does not mention any other species of *Ancistrum*. It is a striking fact that the spread of body length is very high (240%), anywhere observed. Perhaps it is the matter of dividing individuals?

Ancistrumina japonica Uyemura, 1937

This species was described by U y e m u r a 1937, evidently without the knowledge of the group and really in a fantastic way: the spiral is drawn as a clockwise, the photographs indicate that the author displaced the focus on preparations performed with iron haematoxylin!

The data of U y e m u r a: the body has two shapes: the oval type and the elongated type. The individuals from *Cyclina sinensis* are 55—75 μ long, 14—29 μ wide. Ma large, 11—16 μ, lies in the body medium. Mi — 1.5—4 μ. CV one or two in the posterior body end, 18—20 kineties of the general ciliature, the cilia are 6—10 μ long, the peristomal ones 20—28 μ (Fig. 7).

Hosts: *Meritrix meritrix* L., *Paphia philippinarum* Ad. et Reeve, *Cyclina sinensis* Gmelin, *Mactra veneriformis* Reeve, *Mactra sulcataria* Reeve and *Dosinia bilnulata* Gray. The coasts of Japan.

The diversity of hosts, the separation of two types by the author, a high spread of dimensions (even among the individuals from one host) seem to indicate that we have to do with a collective species, or with a confusion of several species. The description of U y e m u r a 1937 provide no background for their identification or for any analysis.

Ancistrumina purpurae (Chatton et Lwoff, 1926)

The body is ovoid, 50 μ long, 18—22 μ wide. The adoral spiral 360°. Kineties distant from one another by 3 μ on the right side, on the left by 1.5 μ (perhaps there are about 20 kineties Z.R.). The other data are not essential — no figures.

Host: *Purpura haemastoma* L. Banyuls-sur-Mer, Mediterrané.

Ancistrumina caudata (Fenchel, 1964)

The body elongated, the length 46—76 μ, width 17—30 μ. Ma elongated, 15 μ, lies in the body medium, Mi (2 μ) before it. The mouth in the posterior part of the body, the cytostom is covered "by a characteristic tongue-like prolongation". The cilia of the adoral ciliature are long, they reach 25 μ on the adoral spiral. The author distinguishes the forms from *Musculus*: length 46—55 μ, width ± 20 μ, and from *Modiolus*: length 56—76 μ, width 17—30 μ. The kineties of the general ciliature 22—23 (the same number in both forms). The mass conjugations were observed (Fig. 7 C,D).

Hosts: *Musculus niger* (Grey) and *Modiolus modiolus* (L.) — Oeresund and Gullmarfjord.

F e n c h e l 1965 indicates that *A. caudata* is very near to *A. isseli* Kahl, described by I s s e l 1903 as *A. mytili* from *Modiolus modiolus* from Napoli, however the last one has no long oral cilia. The species of I s s e l of course is not *A. mytili* Quen. F e n c h e l considers that the ciliate described by K i d d e r 1933 as *A. isseli*, from *Modiolus modiolus* from New York and Woods Hole also does not correspond to the description of I s s e l 1903 and therefore is not *A. isseli*. As a result F e n c h e l 1965 considers, that *A. isseli* (= *A. mytili* sensu Issel) and *A. caudata* are the forms of the same species.

The data of I s s e l 1903 for his "*A. mytili*" are: length 66—73 μ, width 25—40 μ (so considerably larger). Ma spherical, Mi 2.5 μ. The number of kineties of the general ciliature 21—23. The scarce number of kineties is striking in this description in comparison to a considerable width of the body, so I s s e l exaggerated this width or he did not appreciate the number of kineties (see *Ancistrum modioli* n. nov. p. 146).

I think that it possible to agree with F e n c h e l 1965 and to recognize that *A. mytili* sensu Issel, 1903 is a synonym of *A. caudata* (Fenchel, 1964).

Ancistrumina dosiniae (Chatton et Lwoff, 1926)

According to the description of Chatton et Lwoff 1926 — the body is subovoidal, the length 65—80 μ, the width 30—40 μ. "Pôle postérieur moins nettement tronqué sur le profil. Peristome courbé en arrière à 180°. Un long cil au pôle postérieur". The lack of drawings.

Host: *Dosinia exoleta* (L.) — Aux de Terennès, Baie de Morlaix.

Fenchel 1965 reports: length 52—65 μ, width 24—33 μ. Blunt posterior end. 30 kineties of the general ciliature. Ma in the anterior part, near body medium (Fig. 8 A,B). Host: *Dosinia exoleta* (L.) — Gullmarfjord, W. Sweden.

Ancistrumina bulgarica sp. n.

I describe this species in order to differentiate the ciliates found by myself in the mantle cavity of *Mytilaster* from the Black Sea at the Bulgarian coasts. The mussels were taken from stones in the spray-zone where they occur in dense colonies. The infestation amounted to ca 25 per cent of mussels. It seems characteristic that *A. bulgarica* does not occur on *Mytilus galloprovincialis*, living near or even within the colonies of *Mytilaster*; however *Ancistrum mytili* occurs in *M. galloprovincialis* (see p. 145).

The body of *A. bulgarica* sp. n. is ovoid, sharpened in vivo on its posterior pole; length 37—47 μ, width 15—20 μ. Ma ovoid, or irregular lies in the anterior part of the body and has 8—12 μ in diameter; Mi lies beside Ma. CV in the posterior body part. The number of kineties of the general ciliature 28—28. The adoral kineties form a large loop in the distance of hardly 1/5 from the posterior body pole (Fig. 7 G).

Host: *Mytilaster lineatus* (Smel.). W. Black Sea.

Ancistrumina (?) *scrobiculariae* (Chatton et Lwoff, 1926)

The body ovoid, distinctly flattened, length 50—60 μ, width 25—30 μ. The distance between the kineties of the general ciliature: 2.5 μ on the right side of the body and 1.3 μ on the left (it seems, that there are near 28 kineties — Z. R.). The adoral spiral describes 180°. "Sur la face dorsale la ligne de convergence des stries ciliaires se prolonge en arrière jusqu'auprés du pôle posterieur".

Host: *Scrobicularia plana* Da Costa (= *S. piperata*), Vimereux.

The characteristic mentioned by the authors concerning the shifting of the anterior suture to the back along the dorsal margin is likely to be a phenomenon opposed to that which occurs in the arising of système sécant in *Hemispeirinae* and is, as it seems, quite specific for *A. scrobiculariae*. However its description is very laconic and is not illustrated by any drawings; it does not provide idea concerning this problem. If this characteristic would be confirmed by further studies, then *A. scrobiculariae* Ch. Lw., 1926 could be qualified to create a separate genus.

Ancistrumina (?) *compressa* (Issel, 1903)

The body elongated and distinctly flattened, wided in its anterior part, narrower to the posterior; length 48—71 μ, width 18—25 μ. Ma elongated placed obliquely in the anterior part of the body, measures ± 1/3 of the body

length; Mi 3—3.5 μ lies near Ma. CV in the back of the body. 29—34 kineties of the general ciliature. The adoral cilia 10—15 μ long (Fig. 8 C,D).

Host: *Capsa fragilis* L., Napoli.

The drawings of Issel 1903 (especially Fig. 2, Taf. 4) suggest the existence of système sécant! This suggestion must be verified, because in the other respects the species *A. compressa* diverges both from the type of the genus *Ancistrumina* and *Ancistrum*, and probably refers to *Protospira* or *Ancistrospira* from the subfamily *Hemispeirinae*.

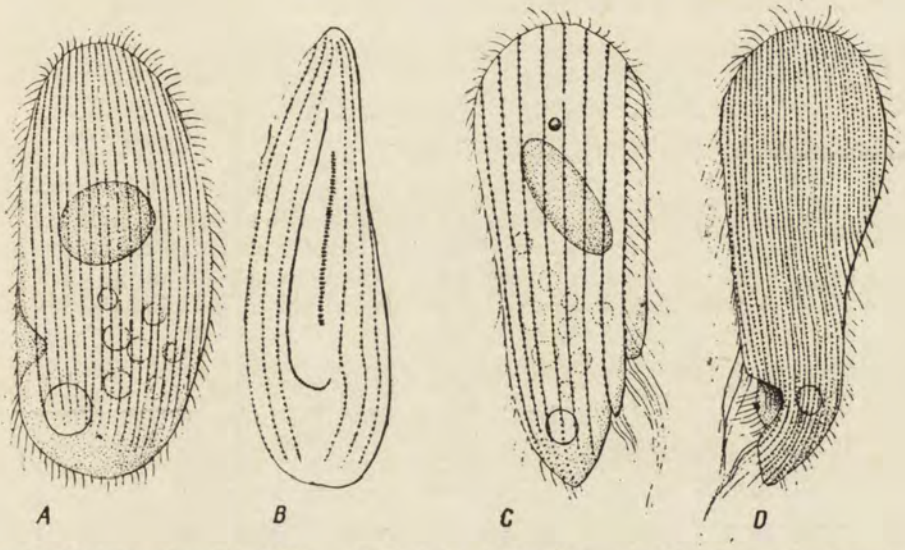

Fig. 8. *Ancistrumina* (?): A,B — *A. dosiniae* (after Fenchel), C,D — *A. compressa* (a. Issel). All drawings ×1000

Genus *Ancistrum* Maupas, 1883

syn.: *Ancistruma* Strand, 1926[1].

This genus was created by Maupas 1883 and involved then two species: *A. mytili* (Quen.) and *A. venerisgallinae* Maupas. Issel 1903 describes then some species of these genus, namely: *A. barbatum*, *A. cyclidioides*, *A. tellinae*, *A. subtruncatum* and *A. compressum* and gave his own description of the species named by him *A. mytili*. Kahl 1931 recognized the last one as a separate species and named it *A. isseli*.

Subsequently have been described other numerous species assigned to the genus *Ancistrum* (Chatton et Lwoff 1926, 1949, Uyemura 1937, Raabe 1936) or the species assigned to the created by Cheissin 1931 genus *Ancistrina* (presently *Ancistrumina* Raabe). Recently Fenchel 1964, 1965 describes some further species. After the revision effected by Raabe

[1] The name *Ancistruma* introduced Strand 1926 unnecessarily and unjustly in order to replace a supposedly preoccupied name *Ancistrum*. Chatton et Lwoff 1949, Raabe 1959 and Corliss 1961 recognized this mistake and they reestablished to the genus the old, proper name *Ancistrum* Maupas, 1883.

1959 and presently revealed (see p. 128) scarcely 3 species can remain in the genus *Ancistrum* corresponding by their body dimensions, number of kineties and the character of the cortical system to the type of the genus, namely *A. mytili*. These are *Ancistrum mytili* (Quen., 1867) Maupas, 1883 as a type species, *A. venerisgallinae* Maupas, 1883 and the species described by Kidder 1933 as *A. isseli* Kahl and which I take as *A. modioli* nom. novum after the elucidation of the problem (p. 146).

According to the principles of division proposed by Raabe 1959 and repeated in this work, the diagnosis of the genus *Ancistrum* Maupas, 1883 may be formulated as follows:

Ancistrum Maupas, 1883

syn.: *Ancistruma* Strand, 1926.

Hemispeiridae — *Ancistrinae* of a dense and abundant ciliature and rather large dimensions of the body (50—80 μ). Number of kineties 40—60. Two adoral kineties run from the apical suture nearly till the posterior pole and form a large loop at its end. A dense net of argyronemes occurs on the naked peristomal field and numerous transversal argyronemes join the kineties of the general ciliature. Parasites of the mantle cavity of marine *Lamellibranchiata*.

Typus generis: *Ancistrum mytili* (Quennerstedt, 1867) Maupas, 1883.

The species of the genus *Ancistrum* Maupas, 1833 s. str. seems to be highly specific in relation to their hosts.

Ancistrum mytili (Quennerstedt, 1867)

syn.: *Ancistrum musculi* Delphy 1938.

The biggest and the more densily ciliated ciliate of the genus *Ancistrum*; the body dimensions seems highly variable, mainly in relation to the salinity of the sea water, *A. mytili* accompanies its host *Mytilus edulis* in different zones of the occurrence of the last one. The juxtaposition of the Table 3 visualizes this variability. The body is generally elongated and distinctly flattened laterally, ovately shaped or considerably narrowed. This characteristic depends on population. The particular authors (Raabe 1934, 1936, Fenchel 1965) differentiate numerous forms separated conspicously from one another. Raabe 1965 calls these varieties by a not obligatory name "form" and applies their names. Fenchel 1965 considers that the differentiation of forms is dispensable, however he gives on his drawings the same differentiation of the shape of the species as Raabe (Fig. 9 C).

Delphy 1938 reports a ciliate from *Mytilus edulis* from the region Arcachon (Gulf of Biscay) and he named it *Ancistruma musculi*. A quite inadequate description does not report virtually any data and the drawings

Table 3

The characteristic of the particular forms of *Ancistrum mytili* (Maup.)

Name of form	Length	Width	No. of kineties	Host	Locality	Author
	65—70			*M. edulis*	Alger	Maupas
	70—100			*M. edulis*	Sylt	Kahl
f. *typica*	42—55	25—32	48—54	*M. edulis*	Gdańsk Bay	Raabe
f. *elongata*	ca. 30	ca. 13	„	„	„	„
f. *claviformis*	ca. 30	ca. 15	„	„	„	„
f. *magna*	65—80	25—32	„	„	„	„
f. *longissima*	ca. 75	ca. 18	„	„	„	„
	52—72	20—38	→53	*M. edulis*		Kidder
great form	69—88	22—41	38—44	*M. edulis*	Gullmar-sfjord	Fenchel
small form	55—73	23—42	„	„	Baltic See	„
	57—82	17—45	43—46	*M. gallo-provinc.*	Black See	Raabe

are on a scandalous level, the author reports only the dimension 110 μ (opposing it to 70 μ that were reported for *M. isseli*) and the nucleus shape "en forme de boudin, repié sur lui même". It may be supposed that Delphy had to do with a large form of *A. mytili*. Similarly, his fantastic *Anoplophrya mytili* is probably also *Ancistrum*.

The plasma of *A. mytili* is generally highly granulated, Ma is ordinarily reniform and relatively to the size of the body measures 10—25 μ in its oblong axis and 7—17 μ in the cross one. Ma lies in the anterior body part. Mi is spherical — near 2 μ, lies close to Ma. CV lies in the posterior part of the body. The medium part is filled with numerous nutritive vacuoles.

The ciliature of *A. mytili* is abundant, the number of kineties is distinctly high, however some fluctuations may exist in this scope correlated with the body size. However not all numerical data of the authors merit a confidence. Kidder 1933 i.e. does not report in his text the number of kineties of *A mytili*. This number may be differently read off: there are 15 on one figure in the right side and 20 on the left side of the body, on the other figure in the apical side — 34 in sum; on the figure of a microscopic section, this number amounts to 53 (a similarly striking inexactness is repeated on the figures concerning *A. "isseli"* — see *A. modioli* n. nov.). As it seems, Kidder 1933 does not lay a great stress upon this characteristic, and the figure of the strip seems to be the most precise and probable (Fig. 9 A,B,D). Raabe 1936 gives a similar number of kineties of the general ciliature, while Fenchel 1965 a somewhat reduced number; Lom et al. 1968 give a number of kineties as ca 50.

The kineties of the general ciliature run more or less meridionally from the anterior suture to the posterior one, however they do not reach the posterior suture indirectly. They link together in groups before reaching this suture. The kinetosomes are densely located, but are distinctly rarified in the posterior

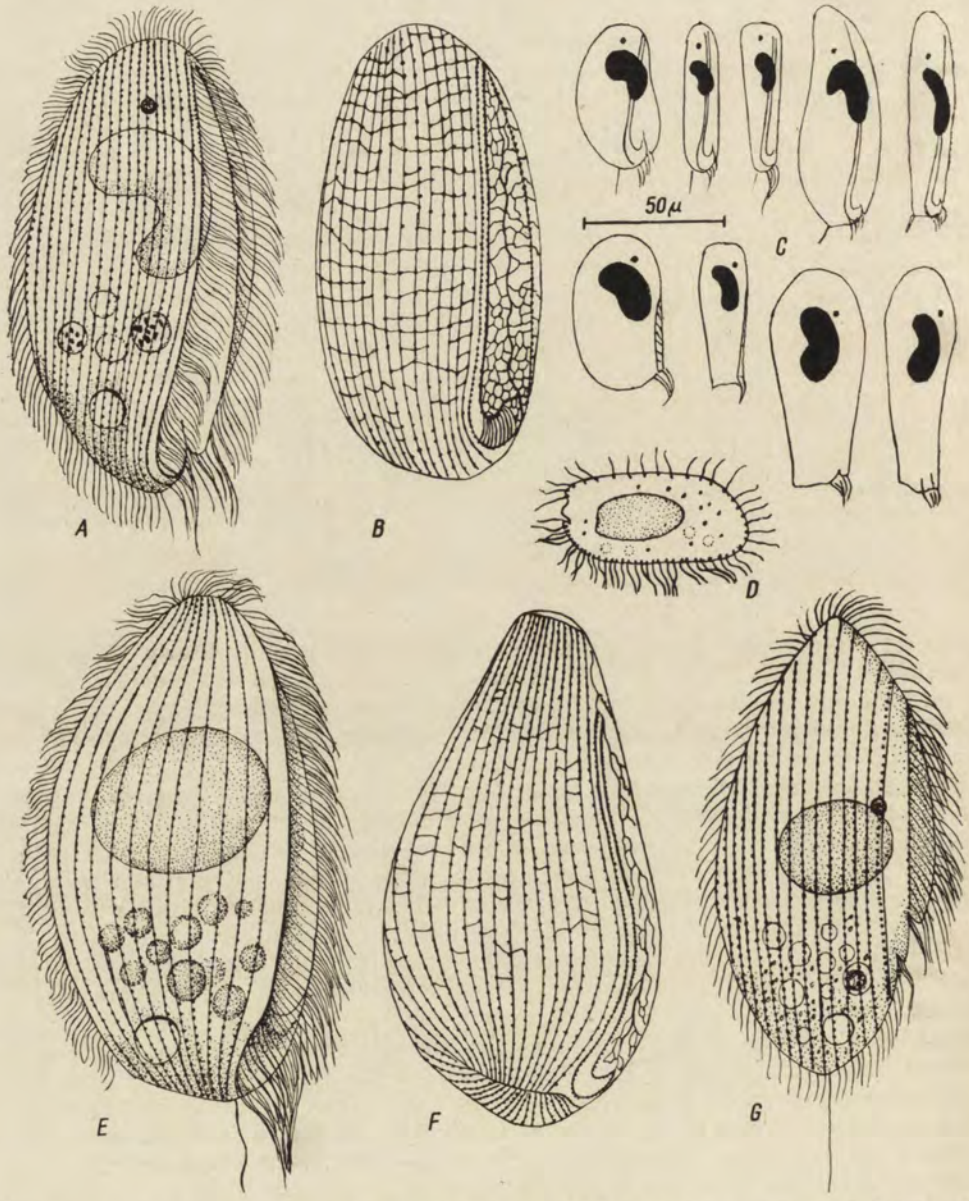

Fig. 9. *Ancistrum*: A,B — *A. mytili* (after Kidder); C — size and forms of *A. mytili*: above — forma *typica, elongata, claviformis, magna* and *longissima* after Raabe, below — forms from Ascö and Gullmarfjord after Fenchel: D — section of *A. mytili* (a. Kidder); E,F — *A. modioli* (a. Kidder), G — *A. venerisgallinae* (a. Issel). All drawings (with the exception of C) ×1000

part of the body. The adoral kineties run in *A. mytili* almost from the apex and go along the naked peristomal field near the body end, where they describe a large loop. It is chracteristic not only for *A. mytili* that the first kinety of the general ciliature is approximate to the stomatogenic kinety, the cilia are more dense at the end and it follows in its run the adoral spiral. Similarly the kineties n and $n-1$ distinguish themselves by densification of kinetosomes, slightly deviated from the former ones (Fig. 4 A,B).

It is revealed on preparations impregnated by dry silver method that the naked peristomal field is covered by a dense fibrillar network; comissure fibrills rather large in number but short, join irregularly some kineties in different regions of the body.

In spite of the amount of study, not elucidated are the occurrence and character of a single individual cilium or their bundle, or even of the bristle near the posterior body pole. Kahl 1931 mentions the "plasmatische Stachel" in his big forms of *A. mytili*, Raabe 1936 observed in his smaller forms (f. *typica*, f. *claviformis*, f. *elongata*), a separate cilium, in bigger forms (f. *magna*, f. *longissima*) a more stiff bristle. However it failed (similarly as in *Ancistrumina*), to discover and localize the insertion of this organelle applying silver impregnation method.

The variability of the size of *A. mytili* as it had been mentioned, seems to depend on the environmental conditions. Raabe found his smaller forms in *Mytilus edulis* from the port's piles and shallow places in the Gdańsk Bay and larger forms in *Mytilus* from deeper places (about 10 m): Fenchel 1965 observed smaller forms in Baltic waters (Askö), however bigger organisms in more salted waters (Gullmorfjord). The wide geographic distribution and a high ecologic tolerancy of the main host *Mytilus edulis* may probably exert influence on the higher diferentiation of *A. mytili* in various media.

A. mytili accompanies his main host *Mytilus edulis* nearly on the whole areal of its occurrence (Raabe 1949). Raabe 1934, 1936 found it in the Baltic Sea, similarly before him Quennerstedt 1867, and then Fenchel 1965 (Askö), Fenchel 1965 in the Atlantic coasts of Europe (Roscoff) similarly to De Morgan 1925 (Plymouth), Kahl 1931 (Sylt), Maupas 1883 in the coasts of Algeria, Kidder 1933 in the Atlantic coasts of North America. Similarly, Lom et al. 1968 (San Juan Island, Washington). It seemed that *A. mytili* is a specific parasite only in *Mytilus edulis* since it has not been found in *M. galloprovincialis* nor in *M. minimus* (data of Issel 1903 and Raabe 1949). During my investigations on the parasites of *Mollusca* in the Bulgarian coasts of Black Sea, I succeeded to observe the occurrence of *A. mytili* in *Mytilus galloprovincialis*. The absence of *A. mytili* in the Mediterranean Sea seems therefore more strange.

Ancistrum venerisgallinae Maupas, 1883

syn.: *Ancistrum veneris* — auctorum.

According to Maupas 1883: the body outline is eliptic, lateraly flattened, its length 55—65 μ, dense ciliature, the space between kineties 1.5 μ (7 kineties for 10 μ), strong thigmotactic zone. The mouth not in the body end, as in *A. mytili* but near to its front. Ma spherical in the body medium, Mi near Ma. The figures 12 and 13 on table XX give no information.

Host: *Venus gallina* L. — Alger.

According to Issel 1903: the body shape ovoid, length 50—60 μ, width 27—32 μ. The number of the kineties of the general ciliature 12—13 on the right side, 28—30 on the left side (Fig. 9 G).

Host: *Venus gallina* L. — Napoli.

Ancistrum modioli nomen novum

pro: *Ancistrum isseli* Kahl, 1931 sensu Kidder, 1933.

As it has been mentioned (vide *Ancistrumina* caudata p. 139) the description of Issel 1903 concerning his "*A. mytili*" cannot be referred to this species, therefore Kahl 1931 distinguished properly a new species *A. isseli*. But under this name Kidder 1933 describes a ciliate (in a rather right manner), which surely does not correspond to the form seen by Issel. This circumstance was pointed out by Fenchel 1965 and he identifies the Issel's species with his *A. caudata*. As an open question remained the problem of species, which considered Kidder and which corresponds to the character of genus *Ancistrum* sensu Raabe. I give a new name to this species: *Ancistrum modioli* nomen novum. The description of Kidder 1933 is quite unsufficient for taxonomic purposes: the figures do not elucidate the problem but rather permit to commet the situation (Fig.9 E,F).

The data of Kidder 1933 are as follows:

The body is ovoid, length 70—88 μ, width 31—45 μ, Ma "enormous", ovoid (according to the figure ± 30 μ), is located somewhat before body medium. Mi lies in the anterior, CV in the posterior part of the body. Kidder does not mention the number of kineties, he gives on the Figures: 11 or 16 on the right side, 17 or 22 on the left one. Since he presents on the drawings of *A. mytili* openly reduced number of kineties in comparison with real state, it seems likely that also the figures concerning "*A. isseli*" do not appreciate this number. Kidder describes on the basis of silver impregnated preparations a distinct posterior suture (which he does not see in *A. mytili*!), a rather abundant fibrillar network in the peristomal field and numerous (however not so numerous as in *A. mytili*), diagonal commissures between kineties. In my opinion these features correspond to the characteristic of genus *Ancistrum*. The ciliate occurs according to Kidder in large amounts and distinguish oneself by quick undirectional movements.

Host: *Modiolus modiolus* (L.), Woods Hole, N. America (Kidder), San Juan Island, Washington (Lom et al. 1968).

Fenchel 1965 reports (p. 101): "In *Modiolus modiolus* an *Ancistrum* species, which is very similar to *A. mytili*, was found occasionally. The length of this form is 75 μ (70—84 μ) and the width about 35 μ. There are 19—20 somatic rows on the left side and 27—30 on its left side. Other details of ciliature are identical with those in *Ancistrum mytili*". It seems doubtless that the form in question is *A. modioli* n. nov.

Genus *Protophrya* Kofoid, 1903

This genus has been created by Kofoid 1903 for the simultaneously described ciliate *P. ovicola*, found according to the author in brood sac of *Littorina rudis* Don. The descriptions and figures of the author give no idea of the ciliate's structure except for that it has a flattened body of an eliptic outline concave on one side, Ma is spherical with clinging Mi, CV is in the

posterior part of the body and the ciliature is dense and uniform. Kofoid included *Protophrya* in the family *Opalinidae* together with the *Anoplophrya*, *Hoplitophrya*, *Discophrya*, *Opalinopsis* and *Opalina* (!!!).

In view of that unclear description, several authors attempted to assign the name *Protophrya ovicola* Kofoid to some other ciliates. Ciliate considered by Cépede 1910 as *Protophrya ovicola* and *Isselina intermedia* do not correspond to *P. ovicola* sensu Kofoid, neither to *P. ovicola* sensu Chatton et Lwoff, but certainly to a representative of the genus *Ancistrumina*, probably *A. cyclidioides* (Issel) — Raabe 1949. Issel 1918 found this species in *Littorina neritoides* and *L. punctata* and Raabe 1949 in *L. neritoides* from Split (Yugoslavia). Rossolimo 1925 considers also that if the corrections of Cépède are valid, so both *Protophrya* and *Isselina* have to be included among *Ancistridae*.

Only Chatton et Lwoff 1949 report that they found in *Littorina rudis* and *L. obtusata* from Roscoff et Vimereux (nota bene they assign this discovery to Kofoid — p. 199) the ciliates corresponding in their general shape and way of life to *P. ovicola* Kofoid. The authors recognized them as representatives of this species. Finally the descriptions and figures of Fenchel 1965 convince only about the identity of these forms.

According to this state I think that further discussion on this subject is not necessary and I recognize (Raabe 1959) the determination of Chatton et Lwoff 1949 completed slightly by the data of Fenchel 1965.

From the description and figures of Chatton et Lwoff 1949 and Fenchel 1965 it results that *Protophrya* may be quite undoubtedly included among the *Hemispeiridae* — *Ancistrinae* (= *Protophryinae* Ch. Lw.). However there are some virtual reservations concerning the possibility of recognizing *Protophrya*, as would like Chatton et Lwoff, as a primitive form of this family. I suppose on the contrary tha it is an apomorphic form specialized and specific in relation to its host.

Chatton et Lwoff 1949 pay a considerable attention to the "zone de séquence" in the anterior body part and to "système sécant" between kineties 7—12 and kinety 1. These structures are not specific for *Protophrya*, the first one corresponds to the anterior suture shifter slightly backwards along the ventral margin, the latter corresponds to the confluence of kineties towards the posterior suture.

On the basis of data of Chatton et Lwoff 1949 and the supplement of Fenchel 1965 the diagnosis of the genus *Protophrya* may be set as follows:

Protophrya Kofoid, 1903 emend. Chatton et Lwoff, 1949

Hemispeiridae — *Ancistrinae* of a strongly flattened body and of oval outline; the left body side concave. The ciliature dense, the number of kineties of the range of 65. Both adoral kineties begin at a level of 1/3 of the body length from the apical end and form a small arc in the hind part of the body. The naked peristomal field is very narrow. Parasites of the mantle cavity (and the brood-sac) of marinae *Gastropoda* — *Prosobranchia*.

Typus generis: *Protophrya ovicola* Kofoid, 1903, emend. Chatton et Lwoff, 1949.

From the genus *Protophrya* has been described one species having features of the genus:

Protophrya ovicola Kofoid, 1903

The body of the elipsoidal outline, distinctly flattened laterally, and even concave at its left side. The length according to Kofoid 1903 is 88—102 μ, width 71—77 μ, thickness 30—35 μ. Ma oval, lies somewhat in the anterior part of the body and is 20—25 μ, Mi lies near Ma or before it and is 3—5 μ. CV in the posterior body part. Neither Chatton et Lwoff 1949, nor Fenchel 1965 give measurements. The body is densely covered with delicate cilia placed in kineties which run meridionally: their full number is not revealed by the authors. Fenchel 1965 mentioned that there are 35 kineties on the right side of the body; on the drawing of the left side are over 40 kineties. Everything goes to show that virtually there are more of them, probably about 80 kineties of the general ciliature. The naked peristomal area is very narrow, the adoral kineties run nearly parallel to the kineties of the general ciliature, in the posterior body part sharply curved, more or less of equal length (Fig. 10 A,B,C).

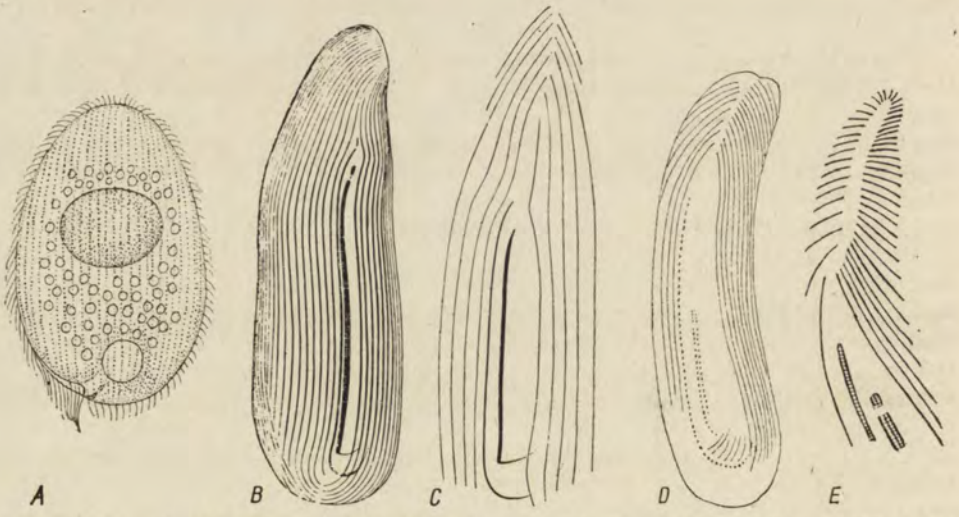

Fig. 10. A — *Protophrya ovicola* (after Fenchel ×500), B,C — *P. ovicola* (from Chatton et Lwoff, ×1000 and 2000), D,E — *Protophryopsis dautzenbergi* (from Chatton et Lwoff, ×1000 and 2000)

According to original data of Kofoid 1903, *Protophrya ovicola* would be a parasite of brood sac and eggs of the viviparous species *Littorina rudis* and of the mantle cavity of the species *L. palliata* and *L. littorea*. The data of Cépède 1910 cannot be taken into consideration, because we do not know if he really had to do with *Protophrya*. Chatton et Lwoff 1949 did not elucidate the problem of localization of *P. ovicola*, but it could be supposed that they found it rather in mantle cavity of the studied species of *Littorina*. Fenchel 1965 considers that *P. ovicola* virtually lives in the

mantle cavity of its hosts (however it occurs more often in females than in males) and only secondarily infests brood sac, what may be important in the spreading of parasite.

The hosts: numerous species of the genus *Littorina*, however not in all places of their occurrence: *L. saxatilis* (Olivi) = *L. rudis* Don. — New Port, North America (Kofoid 1903) and *L. littorea* (Chatton et Lwoff 1949 p. 198 attributes to Kofoid the discovery of *P. ovicola* in uterus and in the mantle cavity of *L. rudis* Maton and *L. obtusata* (L.) in Roscoff what is a real mistake!), *L. saxatilis* (Ol.), *L. obtusata* (L.) and *L. littorea* (L.) — Roscoff (Chatton et Lwoff 1949), *L. saxatilis* (Ol.) and *L. littorea* (L.), but not *L. obtusata* (L.) Frederikshavn and Gullmarfjord — Kattegat (Fenchel 1965).

Genus *Protophryopsis* Raabe, 1959

This genus was created by Raabe 1959 for the species *Ancistrum dautzenbergi* Ch. Lw., 1926 which varies distinctly from other numerous representatives of *Ancistrumina* or *Ancistrum* by features bringing it near to *Protophrya* such as: narrow adoral zone, poorly developed and abbreviated adoral kineties and an abundant ciliature. This species differentiates from the genus *Protophrya* Kofoid sensu Chatton et Lwoff in this way that the prostomal kinety is two times shorter than the stomatogenic one.

In the systematic arrangement accepted by Raabe 1959 within the subfamily *Ancistrinae*, these features constitute a difference of the genus range.

This permits to differentiate the genus of the following characteristic:

Protophryopsis Raabe, 1959

Hemispeiridae — Ancistrinae of a dense and equal ciliature; the number of kineties of a range of 50—60. The adoral kineties begin at uneven distance from the anterior body pole: the stomatogenic kinety at a distance of 1/3 of body length from apex, the prostomal kinety in the middle of the body length. The stomatogenic kinety makes a small loop in the vicinity of the hind pole. The naked peristomal field is very narrow. Parasites of the mantle cavity of marine *Mollusca*.

Typus generis: *Protophryopsis dautzenbergi* (Chatton et Lwoff, 1926) Raabe, 1959.

The genus *Protophryopsis* includes one species:

Protophryopsis dautzenbergi (Chatton et Lwoff, 1926)

The body elongated and flattened. The length 30—45 μ, width 14—18 μ, thickness 10—13 μ. The ciliature is abundant: the space between the kineties on the left side is 1 μ, on the right side 2 μ. The number of kineties of the

general ciliature — 55. The peristomal area is narrow, the adoral kineties of different length: the stomatogenic kinety begins in the 1/3 from the anterior body pole, the prostomal one in the middle of the body length (Fig. VIII Ch. Lw. 1949), (Fig. 10 D,E).

Host: *Macoma* (*Tellina*) *tenuis* Da Costa — Terrenès, Baie de Morlaix.

Genus *Ancistrella* Cheissin, 1930, 1931

This genus was created by Cheissin 1930, 1931 for the species *A. choanomphali* simultaneously described by him from *Prosobranchia* of the Bajkal Lake. Cheissin properly stress both the specific elongated shape of the body of this species, and above all a distinct abbreviation of the adoral kineties, than in other *Hemispeiridae*. As yet nobody met any other species which could correspond to the genus *Ancistrella* Cheissin, so it is not excluded, that it is an endemic genus for the Baikal Lake and for its specific *Prosobranchia*.

The definition of the genus on the basis of the data of Cheissin may be stated as follows:

Ancistrella Cheissin, 1930—1931

Hemispeiridae — Ancistrinae of an elongated body; the ciliature rather scarce, number of kineties 15 to 20. Both adoral kineties begin in the posterior half of the body, reach almost the posterior body pole, and from there a small loop. The argyronemes of the naked peristomal field and those joining the kineties are feebly developed. Parasites of the mantle cavity of Baikal *Mullusca*.

Typus generis: *Ancistrella choanomphali* Cheissin, 1930, 1931.

The genus *Ancistrella* embraces one species:

Ancistrella choanomphali Cheissin, 1930, 1931

The body strongly elongated, length 55—90 μ, width 18—20 μ. The ciliature is uniform, the spece between kineties on the left side 2.5—3.5 μ, on the right 1.5—2 μ. 16—17 kineties of the general ciliature. The adoral kineties are strongly retracted backwards, they begin in 2/3 of the body length and measure 18—20 μ. The peristomal area is narrow. Cilia of the general ciliature — 5 μ, the adoral ones about 10 μ. Ma spherical in the body medium, often in fragments. Mi before it (Fig. 11 A).

Host: *Choanomphalus* sp. — Baikal Lake.

Genus *Eupoterion* MacLennan et Connell, 1931

This genus was created by MacLennan and Connell 1931 for simultaneously described species *E. pernix*, approximate to *Ancistrum*, but differentiated by rather basic characteristics. It is striking that the American

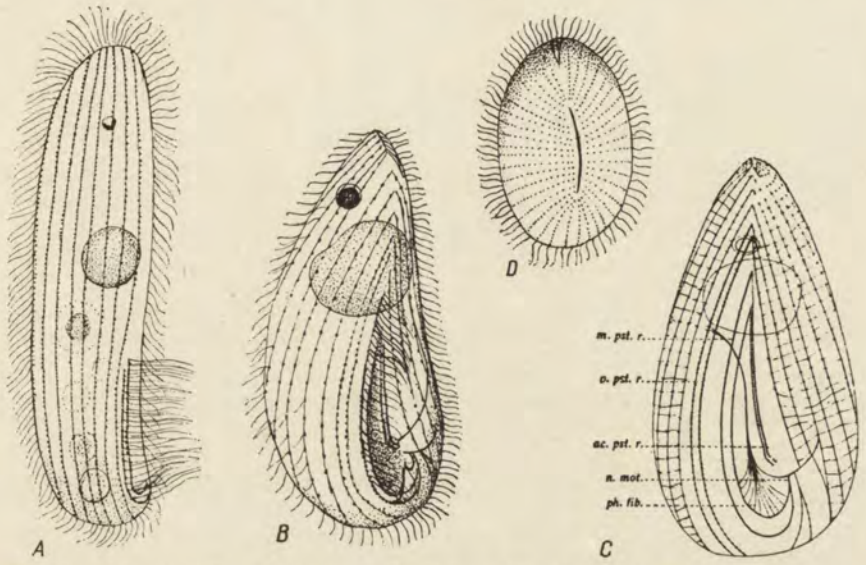

Fig. 11. A —*Ancistrella choanomphali* (after Cheissin), B,C,D — *Eupoterion pernix* (a. MacLennan and Connell). ×1000

authors do not give in the index of literature and do not refer to any position concerning *Ancistridae*, but include only those positions which refer to parasites of the alimentary tract of *Echinodermata* (*Cryptochilum*) — they also compare their species to the last one. Thus it is not excluded that for the reason of the lack of familiarity with the problems of this group they could do some inexactness in their description.

The structure of the ciliary adoral apparatus of *Eupoterion pernix* is rather striking in the descriptions and drawings of MacLennan and Connell 1931. Besides the two adoral kineties describing an arch in the posterior body end, the adoral system is strengthened by two kineties of the general ciliature of the right body side, which form a second spiral and also by two adjoining kineties of the left side (n and $n-1$). Chatton et Lwoff 1949 having doubt about the precision of these data, consider that MacLennan and Connell 1931 describe "d'une manière précise un *Ancistrum* typique". Lom et al. 1968 are in agreement with this opinion.

In my opinion the striking data of American authors cannot be taken for granted and considered as truthless statements. How much essential are the differences between *E. pernix*, described by them, and other *Hemispeiridae* their details are not in a virtual contradiction to the evolutionary tendencies existing among *Protophryinae* or *Ancistrinae* (Raabe 1959). In all *Ancistrinae* the kinety adjoining the stomatogenic kinety (kinety 1 according to Raabe 1959, kinety 2 according to Chatton et Lwoff) tends to accompany the adoral spiral. In many forms, in *Ancistrum mytili* among the others, kineties n and $n-1$ reveal a similar tendency, daviating from the adjoining ones and showing a difference in their run and density of ciliature. Therefore the data of MacLennan et Connell may be considered as probable in this field, and the described features as expressing evolutionary tendencies among *Ancistrinae* (see part I, p. 15, 23).

In this view the genus *Eupoterion* MacLennan et Connell, 1931 may be recognized provisionally as a real and separated unity which reveals features sufficiently deviating from the features of other genera of the subfamily *Ancistrinae* which is presumably connected with internal, intestinal, parasitism.

The definition of the genus *Europerion* may be stated as follows:

Eupoterion MacLennan et Connell, 1931

Hemispeiridae — *Ancistrinae* of a pear-shaped body and of an abundant ciliature; number of kineties 40—50. Two adoral kineties begin at a distance of 1/3 of the body length from the anterior body pole and make a large arc near the end of the body. The two first kineties of the general ciliature (kinety 1 and 2) includes themselves to the adoral complex and form a second arc behind the arc of the adoral kineties. Also the kineties n and $n-1$ are stronger and limit the naked peristomial field from the left side. Parasites of the intestine (!) of marine *Bivalvia*.

Typus generis: *Eupoterion pernix* MacLennan et Connel, 1931.

Genus *Eupoterion* includes one species:

Eupoterion pernix MacLennan et Connell, 1931

The shape of the body is oval, narrower in its anterior part. Length 38—58 μ, width 25—40 μ. Ma ovoid, 15—20 μ×10—15 μ situated transversaly in the anterior body part, Mi — 3—6 μ lies before Ma. The general ciliature is rather abundant. The authors do not report the number of kineties; there are 42 on the drawing of apex, it is unknown if it is not only a schematic approach. The cilia are 5—8 μ long, adoral ones 10—15 μ. The adoral ciliary apparatus consists of 4 pairs of kineties, namely: 1. "main peristomial rows", beginning in the 1/4 from the body anterior end and running parallely beckwards, they describe an arch round the cytostom — there are in our terminology adoral kineties; stomatogenic kinety and prostomal kinety; 2. "accessory peristomial rows", short kineties, beginning there where are the first ones, oriented to the back in the left side of the naked peristomial area — in our opinion these kineties are the differentiated kineties n and $n-1$; 3. "outer peristomial ciliary rows" running to the right from "main peristomial rows" and also describing an arch beyond the arch of the adoral kineties; in our opinion there are the two first kineties of the general ciliature (kinety 2 and 3 according to Chatton et Lwoff); 4. "two rows of cilia of the ordinary short peripheral type arising from the anterior ends of the outer peristomial rows", therefore the kineties $n-2$ and $n-3$, virtually not isolated from the general ciliature and only for the symmetry of system differentiated by the authors. "Main accessory" and "outer peristomial" rows have distinctly more densely arranged kinetosomes and larger cilia. The authors refer that there are pharyngeal fibrills in the cytostome confluent to the anterior part and a neuromotorium (?!) existing at the upper lip of the mouth (Fig. 11 B,C,D).

Host: *Acmaea persona*, intestine (!) — Stinson's Beach, California (MacLennan et Connell); *Acmaea digitalis*, the gut, San Juan Island, Washington (Lom et al. 1968).

Genus *Fenchelia* genus novum

pro: *Ancistrum crassum* Fenchel, 1965.

I create this genus according to the accepted scheme in order to separate the species *Ancistrum crassum* Fenchel, 1965. Fenchel 1965 himself marked that "*A. crassum* is in several ways not typical for the genus" and these untypical features depend on following: "the adoral rows start relatively far back, about 1/3 of the body length from the anterior end", "the UM form a big loop with radially arranged strips", "on the dorsal side the body continues posteriad in a cone-shape prolongation".

The retrogradation of the adoral rows corresponds rather to the state in *Proboveria*, however there is another arrangement of the peristomial loop before the body end and not on its posterior pole. I would like to preserve objectiveness in systematical position of the species within the subfamily *Ancistrinae*, I consider the species *A. crassum* Fenchel, 1965 as divergent from other species of the genus *Ancistrumina* in a sufficient degree to recognize it as typical for the new genus — I devote it to my Scandinavian colleague — Tom Fenchel.

The definition of the genus *Fenchelia* g. nov. may be stated as follows:

Fenchelia genus novum

Hemispeiridae — *Ancistrinae* of an elongated, slightly flattened body. Number of kineties, ca 26. Two adoral kineties start about 1/3 of the body length from the anterior pole and make a big loop near the hind body pole. On the dorsal side the body continues posteriad in a cone-shape prolongation. Parasites of the mantle cavity of marine *Mollusca*.

Typus generis: *Fenchelia crassa* (Fenchel, 1965) comb. nova.

The genus *Fenchelia* g. n. embraces one species only:

Fenchelia crassa (Fenchel, 1965)

The body elongated with a distinct protrusion on the posterior body pole. Length 63—82 μ, width 21—41 μ, thickness about 20 μ. The peristome lies far backwards, however behind it occurs a dorsal, cone-shape prolongation of the body. Fenchel points out a clear visibility of oral stripes even in living individuals. Ma is ovoid, 24—30 μ, Mi near Ma, measures 4 μ. There are 26 kineties of the general ciliature, 12 on the right and 14 on the left body side. Cilia of the general ciliature — 10 μ, adoral — 15 μ. *Fenchelia*

crassa does not move on the spiral but along a straight line by rotational movements round the body axis (Fig. 12 A,B).

Hosts: *Venerupis aurea* (Gm.) and *V.pullastra* (Mont.), 1—2 m of deepness, near Kristinaberg Zool. Station, Kattegat.

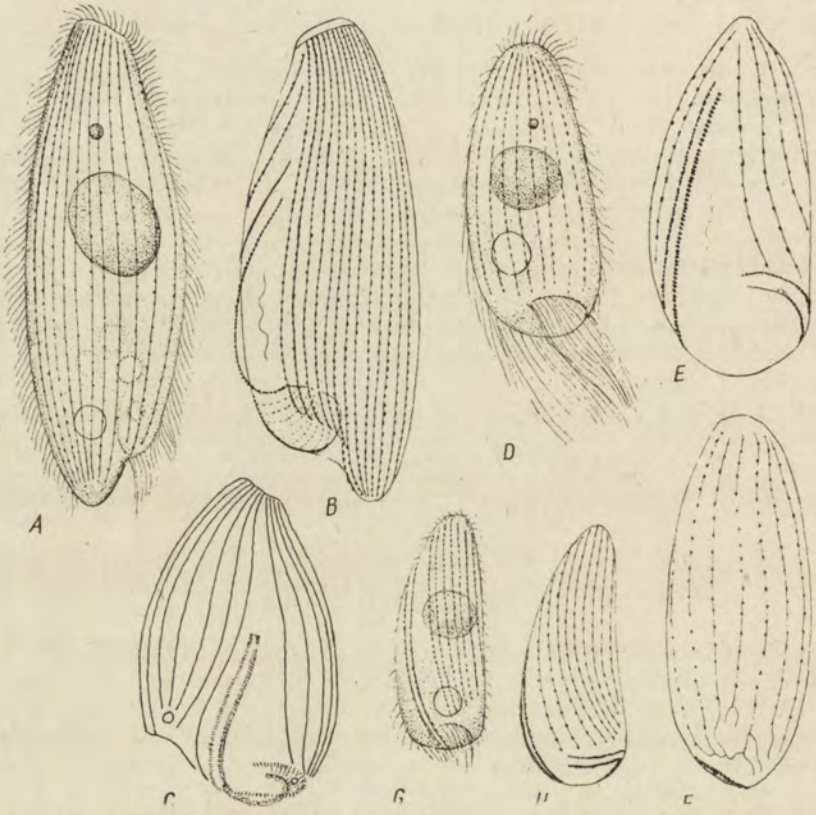

Fig. 12. A,B — *Fenchelia crassa* (after Fenchel), C — *Proboveria loripedis* (a. Chatton et Lwoff), D,E,F — *Proboveria thyasirae* (a. Fenchel), G,H — *Proboveria triangularis* (a. Fenchel). ×1000

Genus *Proboveria* Chatton et Lwoff, 1936

This genus has been created by Chatton et Lwoff 1936 for the species *P. loripedis* Ch. Lw., 1936 being an intermediate form between the representatives of the genus *Ancistrum* Maupas and *Boveria* Stevens considering the structure and arrangement of the adoral kineties. This species does not correspond to *Plagiospira crinita* Issel, 1903 for which Issel drew and described the convergence of thigmotactic kineties. Chatton et Lwoff 1949 forjudged arbitrally, but undoubtedly rightly, that the name of Issel must be ranged to the species which has this système sécant. For the species with the similarly folded adoral kineties but having continuous thigmotactic kineties reaching the body end, Chatton et Lwoff stated a new name for the genus and species: *Proboveria loripedis*. This problem has been more

intricate since both species occur in the mantle cavity of the same host: *Loripes lacteus* (see p. 168 *Plagiospira*).

In this way *Proboveria* (not *Plagiospira*, as supposed Issel 1903) for the reason of lack of the "système sécant" may be considered both as representing a grade between *Ancistrum* (rather *Ancistrumina*) and *Boveria*, and then also as representing a phylogenetic link between these two genera.

The definition of the genus *Proboveria* Chatton et Lwoff, 1936 may be stated as follows:

Proboveria Chatton et Lwoff, 1936

Hemispeiridae — *Ancistrinae* of an oval, feebly flattened and feebly ciliated body. Number of kineties 14—29. Two adoral kineties begin at a level of 1/3 of the body length from the anterior pole and make a large spiral on the hind body pole. Parasites of the mantle cavity of marine *Mollusca*.

Typus generis: *Proboveria loripedis* Chatton et Lwoff, 1936.

The genus *Proboveria* includes presently 3 species:

Proboveria loripedis Chatton et Lwoff, 1936

Chatton et Lwoff 1936 and 1949 did a large analysis of the genus, however they give only scarce information concerning the species *P. loripedis*. The leck full description is solely to some extent compensated by drawings which are rather schematic and have to illustrate the features and the morphogenesis of the genus. The dimensions are not reported. There are 20—22 kineties of the general ciliature, the body is cone-shaped (Fig. 12 C).

Host: *Loripes lacteus* Poli. Roscoff, Sète.

Proboveria thyasirae Fenchel, 1965

The body is ovoid, 33—46 μ long, 16—19 μ wide. Ma is ovoid, its size is 9 μ — located in the medial part of the body; Mi 1.5 μ lies before Ma. CV in the posterior body end. There are 7—8 kineties of the general ciliature in the left body side, 6 at the right side of the body, therefore 13—14 in general. The adoral cilia, 30 μ long, form a membrane, 25—30 μ in extend, somewhat obliquely oriented (Fig. 12 D,E,F).

Hosts: *Thyasira sarsi* (Phil.) — in large amounts together with *Plagiospira crinita*; also *Thyasira* sp. together with *Proboveria triangularis*. Gullmarfjord, W. Sweden.

Proboveria triangularis Fenchel, 1965

The body is cone-shaped, the left side somewhat concave. The length 28—35 μ, width 14—15 μ. There are 5 kineties of the general ciliature in the right side, and 9 in the left side of the body, so 14 in sum. Ma ovoid — 5 μ. The adoral cilia relatively short. CV in the posterior body part (Fig. 12 G,H).

Host: *Thyasira* sp. (with *P. thyasirae*) — Gullmarfjord, W. Sweden.

Genus *Semiboveria* genus novum

pro: *Boveria stavensi* Issel 1903.

I create this genus for the species *B. stevensi* Issel, 1903 separated from the genus *Boveria* for the reason of a different run of adoral kineties in this species (known only from the description of Issel 1903). The run of these kineties distinctly reveals intermediary state between *Proboveria* and *Boveria*: in *Proboveria* the adoral kineties start in 1/3 of the body length, in *Boveria* in the posterior body pole and in *B. stevensi* 2/3 from the body anterior pole. All other species of the genus *Boveria* reveal the equal final degree of the spiralization and retrogradation of the adoral kineties, and they differ from each others by smaller details.

The genus *Semiboveria* g. n. occupies in the evolutionary row of *Ancistrinae* the same place as in the row of *Hemispeirinae* occupies *Plagiospira* between *Ancistrospira* and *Cheissinia*; its differentiation consitutes all the more a claim of objectiveness and provides a more clear phylogenetic system.

The definition of the genus *Semiboveria* g. n. may be referred as follows:

Semiboveria genus novum

Hemispeiridae — *Ancistrinae* of an conical, posteriad enlarged body. The ciliature not abundant, number of kineties ca 18. The adoral kineties begin at a distance of about 2/3 of the body length and make a big loop around the posterior body pole. Parasites of the mantle cavity of marine *Mollusca*.

Typus generis: *Semiboveria stevensae* (Issel, 1903) comb. nova.

The genus *Semiboveria* g. n. includes presently one species:

Semiboveria stevensae (Issel, 1903) comb. n.[2]

The shape of the body slightly elongated, conical, obliquely cut in the posterior end. The length 24—29 μ, width 13—17 μ. The kineties are relatively reduced in number: 8 in the right, 9—10 in the left body side. The adoral kineties start in about 2/3 of the body length, they run obliquely and form a wide loop on the obliquely cut posterior pole. Ma ovoid, big, lies rather in the anterior part of the body. Mi besides it. CV in the middle of the body (Fig. 13).

Host: *Galeomma turtoni* Soverby, Napoli (Issel), Plymouth (MacKinnon and Ray).

[2] By the way I would like to correct Issel's mistake of nomenclature: he evidently dedicates his species to Mrs. Nettie Maria Stevens — therefore it ought to have a female ending as follows: *stevensae* and not *stevensi*.

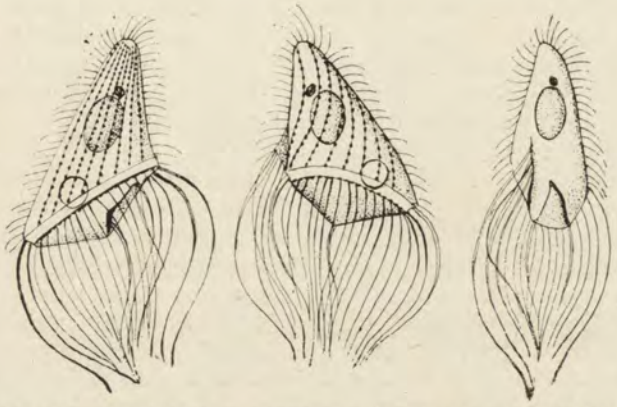

Fig. 13. *Semiboveria stevensae* (after Issel). ×1000

Genus *Boveria* Stevens, 1901

This genus is created by Stevens 1901 for the simultaneously described species *B. subcylindrica* Stevens, 1901 from the respiratory organ of *Holothuria californica*. Issel 1903 has stated the same (in his opinion) species in mantle cavity of 10 species of marine *Lamellibranchiata* from the Neapolitanian Bay and describes a new form *B. sybcylindrica* var. *concharum*. This form was then recognized by Stevens 1903, 1910. Issel 1903 describes at the same time a new species from *Galeomma turtoni* Sov. under the name of *B. stevensi*; he points to its distinct separateness in comparison to all populations of the former species.

Subsequently appear further descriptions: *Boveria labialis* Ikeda et Ozaki, 1918, *B. teredinidi* Nelson, 1923. Pickard 1927 compares these four described forms adding her own studies on *B. teredinidi*, concerning mostly the neuromotor apparatus. It is worth to mention that Pickard did not know the work of Issel 1903 (even she assigns the authorship of *B. subcylindrica* var. *concharum* to Stevens) neither she knows other works devoted to *Ancistridae*, nor the publications of Chatton et Lwoff. Consequently she finds the affinity between *Boveria* and *Stentor* and she places *Boveria* in the family *Boveriidae* among *Heterotricha*!!! Finally in subsequent years appear the descriptions of: *B. tapetis* Delphy, 1938 nomen nudum and *B. zenkevitchi* Levinson, 1941. This last species corresponds completely to *B. teredinidi* Nelson, 1923, but the author in spite of quoting the publications of Nelson and Pickard, describes it precisely as a new species. Finally Chatton et Lwoff 1949 analyse the structural pattern of the adoral apparatus of *Boveria* and the homology of its adoral kineties and those in *Ancistrum* and *Proboveria*.

In spite of these studies, or perhaps as result of different methods applied in them, the situation within the genus *Boveria* is not adequately elucidated.

The particular authors attempt to find specific differences in the compass of the adoral spiral. Stevens 1904 e.g. refers that her *B. subcylindrica* from *Holothuria californica* has a loop describing "one turn and 290° of a second turn"; the form from *Mollusca* therefore *B. subcylindrica* var. *neapolitana* (= *B.subcylindrica* var. *concharum* Issel, 1903) is supposed to have a loop

describing "one turn and 210° of the second turn". But Issel 1903 draw such relations for *B. subcylindrica* var. *concharum* as gives Pickard for the forms from *Holothuria* and similarly present this problem Chatton et Lwoff 1949 (Fig. 14 K—N). Moreover it is virtually difficult to determine how many degrees describes the adoral spiral of Boveria, where its medial point must be marked; the same arch may then be differently approached by various authors. Similarly fail other diagnostic features given by the authors, e.g. the shape and position of Ma which may be very different even within one population, depending on the elongation of the body. Also failed the attempts of differentiation of species on the basis of chromosomes' number in the dividing Mi which, as it seems, are 4 as a rule.

Among the described species of the genus *Boveria*, *B. stevensae* Issel, 1903 consitutes doubtless a separate unity. I segregate it in a new genus *Semiboveria* g. n. on the basis of a scarce retrogradation of adoral kineties. Other species reveal a full retrogradation and spiralization of the adoral kineties. The individuality of *B. teredini* Nelson, 1923 is supported by the turn of the beginning of adoral kineties in an inverse direction to the further course of the spiral; this is also confirmed by Levinson 1941 for his *B. zenkevitchi*. The separateness of *B. labialis* Ikeda et Ozaki, 1918 is also supported by "labial" protrusion described by the authors. The rest of species and varietates do not reveal distinct diagnostic characteristics (Fig. 14).

It seems that *B. subcylindrica* is a high ubiquistic and cosmopolitic species, occurring both in the waterlungs of *Holothurioidea* and in the mantle cavity of many *Lamellibranchiata*. It seems that in different hosts this species may give populations differing (at least statistically) by the body dimensions and other details. Ikeda and Ozaki 1918 describe also *B. labialis* from *Holothuria* and *Lamellibranchiata*. It is difficult to value the differences occurring among various populations of *B. subcylindrica* — this problem is difficult to solve because of the great variability of these forms within one population from one species, more over from one mollusc specimen.

Evidently the systematics within the genus *Boveria* is not clearly determined and deserves a more detailed revision similarly as it has been signalized for the genus *Ancistrumina*.

Independently of these difficulties the genus *Boveria* is distinctly outlined and may be characterized as follows:

Boveria Stevens, 1901

Hemispeiridae — *Ancistrinae* of a pear-shaped often strongly elongated body. The ciliature not abundant, number of kineties 20 to 30. Long adoral cilia. Two adoral kineties begin in the vicinity of the enlarged, posterior body pole and make around it a large, involutive spiral, lying then perpendicularly to the kineties of the general ciliature. Parasites of the mantle cavity of marine *Bivalvia* and of the respiratory organ of *Holothurioidea*.

Typus generis: *Boveria subcylindrica* Stevens, 1901.

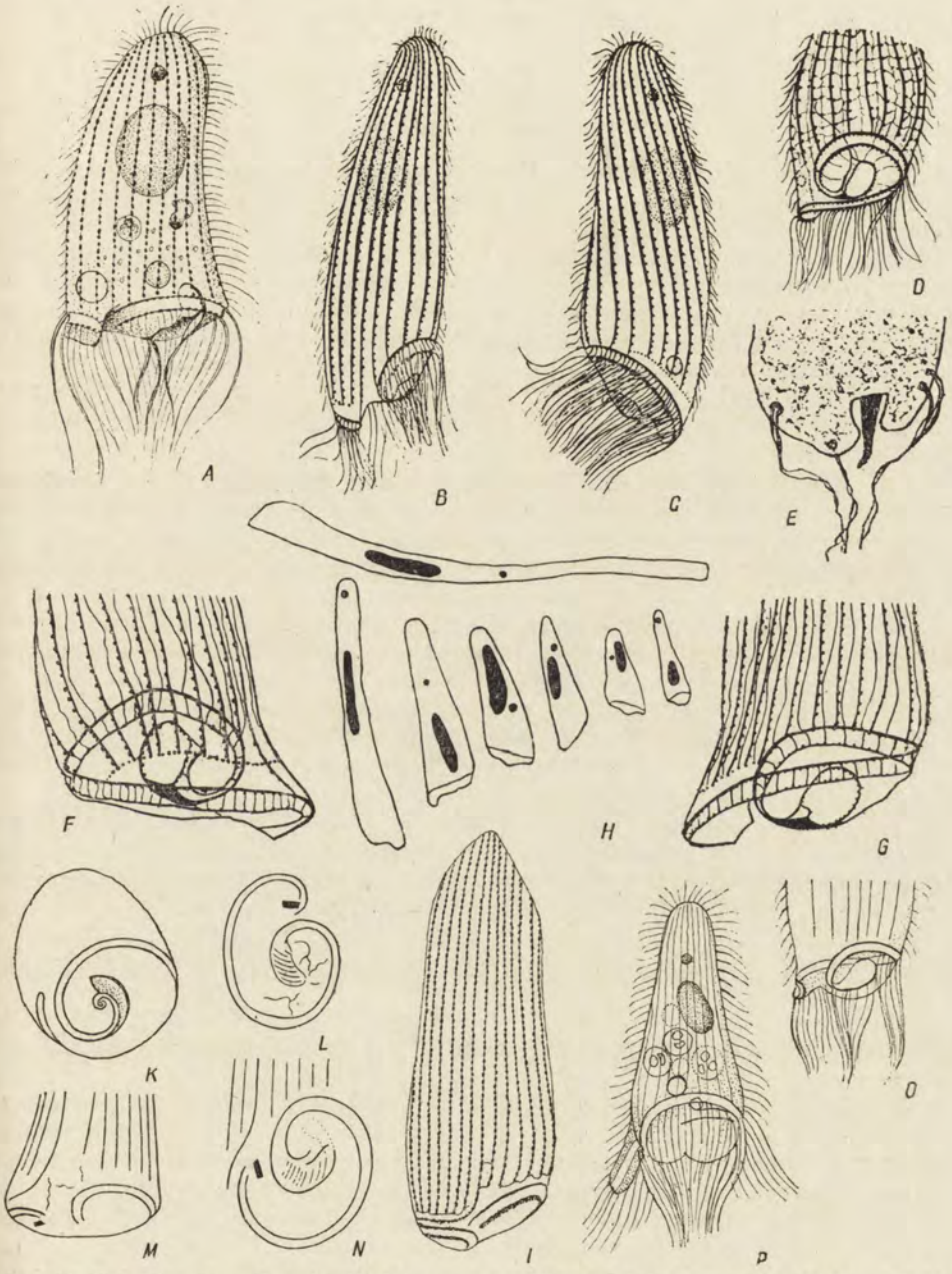

Fig. 14. *Boveria*: A — *B. subcylindrica* var. *concharum* (after Issel), B,C,D — *B. teredinidi* (from Pickard), E,F,G — *B. zenkewitchi* (from Levinson ×2000), H — length variations in *B. zenkewitchi* (f. Levinson, ×500), I — *B.* sp. (a. Fenchel), K — *B. subcylindrica* var. *concharum* — adoral spiral (a. Issel), L,M,N, — *B. subcylindrica*, adoral spiral (f. Chatton et Lwoff), O — *B. subcylindrica*, adoral spiral (a. Poljansky), P — *B. labialis* (a. Ikeda and Ozaki). All drawings, with the exception of E — H, ×1000

The genus *Boveria* includes presently 3 (?) species:

Boveria subcylindrica Stevens, 1901

syn.: *Boveria tapetis* Delphy, 1938 — nomen nudum.

The first species of the genus *Boveria* was described by Stevens 1909 from *Holothuria californica*. In her subsequent works the author returns to *Boveria* and differentiates the forms from molluscs as *B. subcylindrica* var. *neapolitana*; she gives some remarks on the division and conjugation in 1904 and is concerned more precisely on the conjugation and chromosomes in 1910. In view of the issued work of Issel 1903 with the description of *B. subcylindrica* var. *concharum* from the molluscs from Napoli, Stevens 1910 recognizes the priority of the Issel's name. In subsequent periods Poljansky 1951 gives the data concerning *B. subcylindrica* from *Holothurioidea* and Chatton et Lwoff 1949 gives data on *B. subcylindrica* var. *concharum*.

Stevens 1904 refers as differences between the typical *B. subcylindrica* and *B. subcylindrica* var. *concharum* the existence in the form from *Holothuria* of "a disc of denser cytoplasm between the micronucleus and the aboral end" which has not the form from *Mollusca*; this form reveals also another compass of the adoral spiral; namely 360°+290° in the typical forms and 360°+210° in the form from *Mollusca*. The first feature seems slightly outlined and depends on the applied methods of conservation and staining, the second one is difficult to measure and is questioned by other authors. Moreover Poljansky 1951 suggests that his *B. subcylindrica* from *Holothurioidea* has its initial segments of kineties buckle in an inverse direction to the spiral (as it is given for *B. teredinidi*) and that these kineties are situated on the protruding body lobe (as it is given for *B. labialis*).

This situation does not provide a detailed knowledge concerning the differentiation and the taxonomic rank of the forms from *Mollusca* in relation to the form from *Holothurioidea*, it also does not allow to distinguish separate units among various populations from different molluscs (Fig. 14).

Boveria subcylindrica Stevens, 1901 — typical form from *Holothurioidea*

The body is cylindric, elongated in different degree. The length 54—81 μ (Stevens), 25—90 μ (Polj.), the width in the aboral region 9—18 μ. 20—27 kineties of the general ciliature, arranged somewhat spirally. Ma oval, lies about the body medium, Mi near the body apex. CV besides the peristome. The double adoral spiral describes an arch "one turn and 290° of a second turn" (Stevens). According to Poljansky 1951 the starting segments of the adoral spiral buckle in an inverse direction to its course, therefore leiotropic. The adoral cilia are 15—30 μ long (Polj.). "A disc of denser cytoplasm between the micronucleus and the adoral end" (Stevens).

Hosts: *Holothuria californica* Stimp. — Pacific Grove, California (Stevens); *Parastichopus californicus*, Friday Harbour on San Juan Island, Washington (Lom et al. 1968); *Stichopus japonicus*, *Cucumaria* sp. sp. Vladivostok (Poljansky).

I have *Boveria* in my material from the water lungs of *Cucumaria plancki* Brdt. from Split (Yugoslavia) 1947. My individuals correspond in general

to the characteristic related here however I could find neither "a disc of denser cytoplasm" nor as inverse buckling of the beginning of adoral kineties, nor a protrusion corresponding to them. The dimensions of my specimens are: the length 37—43 μ, width in the oral region 20—23 μ, in the aboral region circa 10 μ.

Boveria subcylindrica var. *concharum* Issel, 1903

syn.: *B. subcylindrica* var. *neapolitana* Stevens, 1904, *B. tapetis* Delphy 1938 n. nudum.

The body strongly elongated, with highly variable proportions which grow out of a high variability of the length (27—169 μ) — see Table 4. It is difficult to size the scope of the arch of adoral spiral both in this species as in

Table 4

Characteristic of *Boveria subcylindrica* var. *concharum* Issel

Host	Locality	Length	Width	Author
Tellina exigua Poli	Napoli	37—102	19—21	Stevens
Capsa fragilis L.	„	65—121		„
Pinna nobilis L.	Napoli	37— 51	20—26	Issel
Capsa fragilis L.	„	32—155	20—31	„
Tellina exigua Poli	„	27—113	20—32	
Tellina nitida Poli	„	58—102	20—34	„
Loripes lacteus L.	„	66—169	27—33	„
Monia petalliformis (L.)	Kristinaberg	50— 65	20	Fenchel
Arca noae.	Adria	55— 75	20—23	Raabe
Anomia ephippium.	Monaco	37— 55	17—20	„
Tapes decussatus.	Arcachon	30—250		Delphy
Tapes fuscus	„			„

other ones. Stevens 1904 refers it as 360°+210°, Issel 1903 (p. 96) refers "il peristoma inoltre è molto più lungo nei miei individui, poichè ancho tralesciondo la porzione di spira entro la citostoma (che non è reppresentata nei disegni di Stevens) percorre due giri di spira anzichè 1 1/2, come si osserva nella *Boveria* della *Holothuria californica*". However virtually Issel refers on the drawing (fig. 28) the external part of the spiral similarly to Stevens 1901, and even rather longer than Stevens 1904 refers for her var. *neapolitana*, Chatton et Lwoff 1949 (p. 209) design it also similarly (Fig. 14).

As mentioned before, the evaluation of the arch of the adoral spiral is very difficult and uncertain. It seems that there occurs a high individual population variability in this field. The age of the concerned ciliate ought to be taken into consideration since the spiral rolls greatly during its individual life starting from the divisional period and then unrolls in the predivisional period. In that way in spite of many descriptions *B. subcylindrica* var. *concharum* deserves more detailed studies concerning above all the determination of its variability and the occurrence of possible separated forms in some *Mol-*

lusca and in different environments. This variety seems to be highly ubiquistic and cosmopolitic, what is confirmed by the list of hosts:

Hosts: *Tellina nitida* Poli, *T. planata* L., *T. exigua* Poli, *Capsa fragilis* L., *Donax politus* Poli, *Venus gallina* L., *Tapes decussata* L., *Loripes lacteus* Blain., *Cardita sulcata* Brug, *Pinna nobilis* L. — Napoli (Issel part. Stevens): *Anomia ephippium* L., *Loripes lacteus* Bl. — Sète (Ch. Lw.); *Monia patelliformis* (L.) — Skagerrak (Fenchel). I have some material from *Arca noae* L. from Adriatic and from *Anomia ephippium* L. from Monaco.

Boveria teredinidi Nelson, 1923

syn.: *Boveria zenkewitchi* Levinson, 1941.

This species has been described by Nelson 1923 in a rather superficial way. Pickard 1927 gives her redescription, she did a detailed analysis of the complex of cortical compositions: therefore of neuromotorium, of cilia and fibrils, named by her the neuromotoric apparatus[3]. Levinson 1941 describes from the same host — *Teredo navalis* — a new species *Boveria zenkewitchi*, discussing largely the analysis of its cortical system, considered as a statomotoric apparatus. This different denomination by both authors of the same structures results from a different approach: on the one hand it is considered as conducting apparatus and on the other one as an essentially skeletal apparatus. In spite of the attempts of Levinson concerning the separateness of his species it must be recognized as a synonym in relation to *B. teredinidi* Nelson. The differences stressed by Levinson 1941 are not essential, or depend on a different interpretation of the observed structures or are quite unlike as e.g. the occurrence in the mouth of *B. zenkewitchi* of membranella undulans! — Fig. 14 G.

In the related description I take into consideration the data of Nelson 1923, Pickard 1927 and Levinson 1941:

The body of an conical outline, less or more elongated, so that the relation of the width to the length amounted from 1:1 to 1:12, the more often 1:3—3.5. The anterior part of the body is flattened, the posterior is round in its section (the oral disc). The dimensions of the body are: length according to Pickard 27—173 μ, according to Levinson 12.3—242 μ, the width at the posterior body end according to Pickard 12—31 μ, according to Levinson 8.2—32 μ. Ma ovoid — its length is depending on the length of the body, sometimes strongly pulled out. Mi lies before Ma; according to Levinson it reveals during the division the presence of two unequal chromosomes (perhaps these are bivalent Z.R.). The kineties of the general ciliature run along the body somewhat spirally; their number according to Pickard 19—22, according to Levinson 18—25. The cilia of the general ciliature measure according to Levinson 4—7 μ, in the anterior part of the body up to 12 μ what Levinson takes for a species difference. However it may be considered as a not essential characteristic, since the cilia of the thigmotactic area, especially after fixation, may seem longer in many *Ancistrinae*.

The adoral kineties and the spiral formed by them were described in de-

[3] Pickard 1927 considered *Boveria* as a representative of *Heterotricha*, then with a marked tendency, she compares *Boveria* to *Stentor*, and even tries to find in it some features of *Stentor* and apply the interpretation appropriate for the genus.

tails by both of the authors. Pickard 1927 writes: "The two adoral fibres give rise to the adoral ciliary lines, making a double dexiotropic spiral about the peristomal field which ends distally a short leiotropic loop of half a spiral turn". (p. 420). Levinson disputes in his forms the feature differentiating the forms studied by Pickard from other species of the genus *Boveria*; he recognizes the lack of this characteristic as an essential difference between *B. zenkewitchi* and *B. teredinidi*. But in his drawings (Fig. 12 and 14 by Levinson) he shows distinctly a leiotropic loop corresponding closely to the data of Pickard. Levinson reports that his *B. zenkewitchi* has a wide triangular membranella undulans in its mouth's loop, on the margin of cytopharynx! This feature would be essential according to this author for the differentiation of his *B. zenkewitchi* not only from the other *Boveria* but also from all "*Ancistrumidae*". It seems obvious to me that there is a virtual misunderstanding here and this membranella does not exist. The cilia of the adoral spiral are long, according to Pickard 1/3 to 1/5 of the body length, therefore about 25 μ, Levinson reports there length 20.5—32 μ (Fig. 14).

Levinson 1941 reports some biometrical- statistical data. He finds in the population of *B. zenkewitchi* the greatest number of smaller forms measuring about 32 μ; the next top falls to the size 70 μ, the third one forms about 130 μ, and then the number of specimens representing the particular size classes falls down rapidly. Levinson suggests that this polyculmination is not a distinct mark of the differentiation of the species but an aspect of its life cycle: the individuals 85—95 μ long undergo reproduction, the larger ones do not divide. According to Levinson *B. zenkewitchi* which virtually lives on the surface of the host's gills, can also sink profundly in its tissues.

Host: *Teredo navalis* L., San Francisco Bay (Pickard), Sevastopol Bay (Levinson), Atlantic Coast of North America and in *Bankia* (Nelson).

Boveria labialis Ikeda et Ozaki, 1918

The body trumpet-shaped, aboral end rounded, oral strongly widened, equipped with a distincly protruding lobe, on which the adoral kineties start their course. This lobe is 10—15 μ long. The length of the body 31—100 μ, the width at the oral end 16—26 μ. Ovoid Ma lies in the middle of the body length, Mi in front of Ma, contains 4 chromosomes. CV in the posterior part of the body. The general ciliature arranged in 20—26 kineties. The adoral kineties describe a spiral 360+290° ($1^5/_6$ turns). The encystations in the lungs-tissue of host are observed (Fig. 14).

In view of the juxtaposition of hosts and their similarity concerning the structure it seems that *B. labialis* may prove as a synonym of *B. subcylindrica*. The specific feature which constitutes the lobial protrusion in the region of the adoral disc was also observed by Poljansky 1951 in *B. subcylindrica*. Attention must be paid to the fact that Poljansky has been concerned with the host from Vladivostok therefore from the places near to the Japan Sea.

Hosts: *Cucumaria* sp., *Stichopus japonicus*, *Tellina* sp.

Subfamilia *Hemispeirinae* König 1894, em. Chatton et Lwoff, 1949

On the basis of the accomplished determinations and according to Chatton et Lwoff 1949 the definition of the subfamily may be settled as follows:

Subfamilia *Hemispeirinae* König, 1894,
emend. Chatton et Lwoff, 1949

Thigmotricha — *Hemispeiridae* of a thigmotactic area formed by rather dorsal kineties reduced to their anterior sectors and embraced by the adherent left and right kineties of the general ciliature, making a parenthetical system (système sécant). The adoral kineties, taking in more primitive forms almost longitudinal position, tend to retrogradate and spiralize; however, even in extreme cases of the shifting on the distal body pole, they do not form a fully spiral, but an arc ca 180°. The general ciliature tends to take the oblique or even meridional position and to reduce the number of kineties. Parasites of the mantle cavity of marine and fresh-water *Mollusca* and of the integument of Echinodermata.

Typus subfamiliae: genus *Hemispeira* Fabre-Domergue, 1888.

The systematics within the subfamily *Hemispeirinae* is relatively very simple, it may rest on the degree of a parallel advancement of two features: spiralization and retrogradation of the adoral kineties followed by a simultaneous maintenance and anterogradation of the thigmotactic zone, differentiated here in système sécant. On account of these features the particular genera assigned here reflect properly and undirectional evolutionary row; from the form approximate to *Ancistrumina* which is *Protospira*, up to the highly modificated form as *Cheissinia* and finally *Hemispeira*. The whole subfamily is involved in the evolutionary row parallel to the row *Ancistrumina-Proboveria-Semiboveria-Boveria* which form several genera of *Ancistrinae* (see Table 1, Fig. 1).

The initial form of this evolutionary row, in which I am able to recognize *Protospira* Raabe, has the general features of *Ancistrumina*, however the système sécant of the thigmotactic zone is distinctly marked. This système sécant is caused by displacement forewards of the anterior end of the posterior suture along the dorsal margin of the body (Fig. 2). This subsequently caused the shortening of the kineties mainly of the left part of the ciliary system. In that way the système sécant is not so absolutely dorsal as would like Chatton et Lwoff 1949 but is rather originating from the left part of the ciliature. *Ancistrospira*, *Plagiospira* and *Cheissinia* continue properly these tendencies of the retrogradation and spiralization of the adoral kineties on the posterior body pole and simultaneously the tendency to anterogradation of the thigmotactic zone. The genus *Hemispeira* consists here not only a serious step but a real leap in this direction. The adoral spiral is shifted to the body pole, the thigmotactic zone is placed apically; this involves a nearly parallel arrangement of kineties of the general ciliature and a simultaneous distinct reduction of their number. This spring-up of *Hemispeira* from the gradually changing schematic structure of other genera, corresponds also to a distinct spring-up in the field of their parasitologic peculiarity. So far as all preceding species are connected with *Mollusca*, so *Hemispeira* which embraces two species, is related to *Echinodermata*, with the external surface of their body.

Genus *Protospira* Raabe, 1968

This genus has been isolated (Raabe 1968) for the species from *Theodoxus fluviatilis* (L.) described simultaneously as *Protospira mazurica* Raabe, 1968. This species very similar to *Ancistrumina*, has a système sécant of the thigmotactic ciliature which qualifies it to the subfamily *Hemispeirinae*. Owing to the preparations silvered by the dry method, it is seen distinctly that the système sécant arises as a result of elongation and shifting forewards of the posterior suture, to which converge kineties more and more shorter towards the middle of the thigmotactic field, mainly the kineties belonging to the left side system. There is a lack of more distinct symptoms of retrogradation of adoral kineties; they are not revealed although their loop falls near on the posterior part of the body. Opposed to *Ancistrospira* or *Plagiospira*, kineties n and $n-1$ do not break off on the arch of the adoral spiral but are slightly buckled, and reach the posterior suture. This genus may be considered therefore as a model of an exit form in the evolutionary line which constitutes the subfamily *Hemispeirinae*.

The definition of the genus *Protospira* may be stated as follows:

Protospira Raabe, 1968

Hemispeiridae — Hemispeirinae of an ovoid, elongated body and medium dense ciliature; number of kineties ca 25. The adoral kineties start just at the anterior body pole and make an arc just at the hind pole of the body. The thigmotactic ciliature consists of several kineties of the left part of the ciliary system, shortened and forming a parenthetical system. The kineties n and $n-1$ reach the end of the body and the posterior suture. Parasites of the mantle cavity of fresh-water *Gastropoda*.

Typus generis: *Protospira mazurica* Raabe, 1968.

The genus *Protospira* involves one species only:

Protospira mazurica Raabe, 1968

The body ovoid, narrow, slightly flattened laterally somewhat buckled to the left side, sometimes slightly twisted in a spiral. Length 40—45 μ, width 15—20 μ. Ma — 8 μ, lies in the anterior part of the body. Mi next to it. The number of kineties of the general ciliature 26—29 and approximately 8—10 kineties make up the thigmotactic ciliature closed in the système sécant. The adoral kineties begin close to the anterior body pole, they run to the posterior along the naked, narrow peristomal field and twist a large loop close to the posterior body pole. Kineties n and $n-1$ reach the posterior suture (Fig. 15).

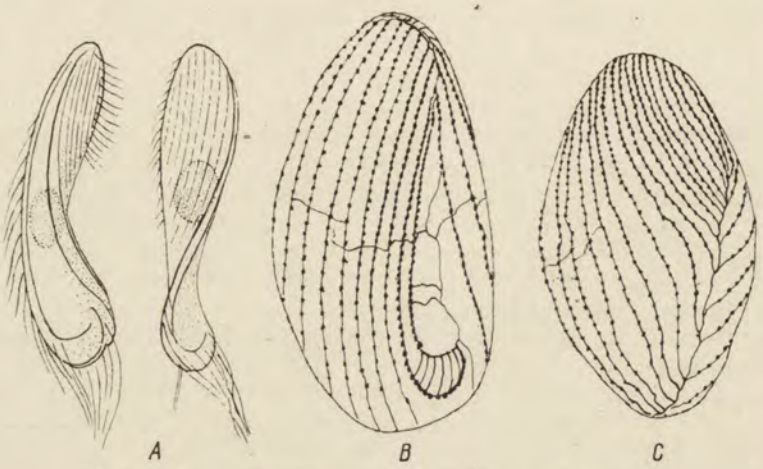

Fig. 15. *Protospira mazurica* (from Raabe). ×1000

Host: *Theodoxus fluviatilis* (L.) — Mazury Lakes (N. Poland). Raabe 1968 found *P. mazurica* only in several populations of *Theodoxus* from several Mazury Lakes lying near from one another (similarly Kazubski — personal communication); he did not met this species before in spite of his studies on *Theodoxus* from South Baltic Sea, from salt and brackish waters of Poland, from Balaton Lake, from rivers and lakes of Yugoslavia (including the Ohrid Lake) and from the waters of Bulgaria.

Genus *Ancistrospira* Chatton et Lwoff, 1926

This genus has been created by Chatton et Lwoff, 1926 for the simultaneously described species *A. veneris* Ch. Lw., 1926, characterized by a specific system of thigmotactic and adoral ciliature. This is a ciliate resembling generally the representatives of the genus *Ancistrumina*, but connected by numerous features to *Plagiospira* Issel, 1903. Chatton et Lwoff 1926 distinguish *Ancistrospira* from *Ancistrum* in the following way: "la ciliature n'est plus méridienne; le champs péristomien est légèrement hélicoidal dextre. Il marque la face ventrale. La partie ventrale de la face gauche est nue. Sur la partie dorsale les stries sont à peu près méridiennes. Sur la partie dorsale de la face droite elles deviennent hélicoïdales sénestres, tandis que sur la partie centrale de catte même face elles sont hélicoïdales dextres. Il en résulte que sur la ligne médiane de la face droite les stries dextres et les stries sénestres entrent en sécance en formant des arceaux brisés à sommet postérieur, inscrits les uns dans les autres. L'aire thigmotactique est dans système de sécance et en occupe la moitié antérieure. Ses stries étant elles-mêmes sécantes entre elles ne se continuent point en arrière. Cette aire est donc fermée". Chatton et Lwoff 1949 agree that this description must be completed by drawings which they presently enclose (Fig. XII, p. 213).

In view of the definition of the genus *Protospira* Raabe, 1968 and owing to the fact that the genus *Ancistrospira* has its place between *Protospira* Raabe and *Plagiospira* Issel, the diagnosis of the genus must be somewhat modified and may be defined as follows:

Ancistrospira Chatton et Lwoff, 1926

Hemispeiridae —*Hemispirinae* of an ovoid body and medium dense ciliature; number of kineties ca 25. The adoral kineties start at a distance of about 1/3 of the body length from the anterior body pole and, limiting the waste peristomal field, make a large arc just at the hind pole of the body. The thigmotactic ciliature consists of several short kineties, forming a parenthetical system. The last kineties of the general ciliature (kineties n, $n-1$, $n-2$) fail to reach the posterior suture and terminate on the rand of the adoral spiral. Parasites of the mantle cavity of marine *Bivalvia*.

Typus generis: *Ancistrospira veneris* Chatton et Lwoff, 1926.

The genus *Ancistrospira* embraces presently only one species:

Ancistrospira veneris Chatton et Lwoff, 1926

The body subovoid, the anterior body pole narrowed, the posterior one widened, slightly flattened. The body length 50—60 μ, width (along the body equator) 22—28 μ, thickness 18—22 μ. The distances between kineties are: 2 μ on the right ventral side, 3 μ on the dorsal side, 1 μ on the thigmotactic field. The adoral kineties form an arch of 360° in the posterior body pole — they do not pass to the dorsal side. CV on the height of the peristome is opened on the dorsal side. There are no data on the nuclear apparatus (Fig. 16).

Host: *Venus fasciata* Da Costa, Banyuls-sur-Mer.

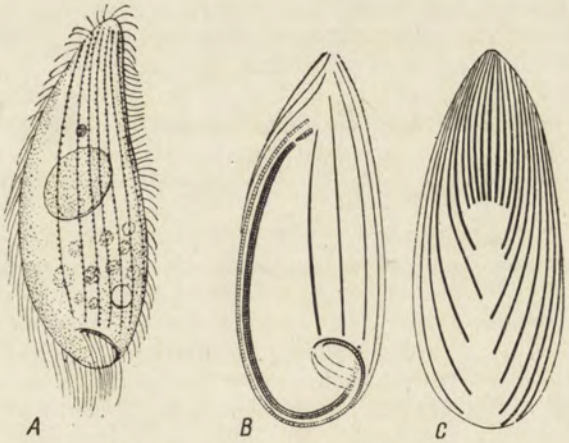

Fig. 16. *Ancistrospira veneris*: A — after Fenchel, B, C — after Chatton et Lwoff ×1000

Genus *Plagiospira* Issel, 1903

syn.: *Boveria* in Mac Kinnon et Ray, 1931.

This genus was created by Issel 1903 for the differentiation of the species *P. crinita* Issel, 1903 constituting in his opinion a transitory form between the representatives of the genus *Ancistrum* Maupas and the genus *Boveria* Stevens. The material gathered and studied by Issel originated from *Cardita calyculata* and *Loripes lacteus*; the drawings show the ciliates from the latter host. Comparing to the data of Chatton et Lwoff 1949 it seems that Issel had to deal with two species living one next to another and characterized by a similar shifting toward the posterior and a similar spiralization of the adoral kineties, namely both with *Plagiospira crinita* Issel and *Proboveria loripedis* Ch. Lw., 1936. Thus the intravital drawings of Issel (Fig. 21, 22, 23, 27) correspond more closely to the forms described by Chatton et Lwoff 1949 as *Proboveria*, whereas the drawings performed rather from preparations and illustrating the arrangement of the kineties of the thigmotactic ciliature (Fig. 95, 96) are consistent with the form considered by Chatton et Lwoff 1949 as *Plagiospira*. The peculiar system of thigmotactic ciliature (système sécant) Issel considers as a sign of predivisional transformations.

Since:

1. I consider as too much troublesome and as setting up a chaos the present recognition of the genus' name *Plagiospira* and the species' name *P. crinita* as nomina dubia,

2. Issel drew in the shape corresponding to *Plagiospira* sensu Chatton et Lwoff, some virtual and important details such as the fail or existence of système sécant decisive for the generic (and subfamilial) belongings,

3. the descriptions and drawings of Chatton et Lwoff 1949 are strict, popularized, are generally accepted and they prejudge the matter univocally (comp. Fenchel 1965).

I consider for right and I propose:

1. to recognize as *Plagiospira* Issel, 1903, or in a specific case as *P. crinita* Issel, 1903 the form in which the adoral spiral is shifted to the back and embraces by its end the posterior body pole and whose thigmotactic zone has kineties isolated in système sécant,

2. to recognize as *Proboveria* Ch. Lw., 1936 and in the specific case as *P. loripedis* Ch. Lw., 1936 the form in which the adoral spiral is shifted to the back and embraces by its end the posterior body pole; it has a thigmotactic zone with long kineties reaching the body end, which do not form the système sécant.

Plagiospira therefore, according to the pioneer thesis of Issel 1903 virtually represent a step in the organization of adoral apparatus lying between the level represented by *Ancistrum* and this which is represented by *Boveria*. However in spite of the thesis of Issel 1903 and according to the thesis of Chatton et Lwoff 1949 *Plagiospira* cannot be considered as a phylogenetic link connecting *Ancistrum* and *Boveria* because of the existence of the système sécant. For this reason *Plagiospira* may be considered as a phylogenetic link between *Ancistrospira* Ch. Lw., 1926 and *Cheissinia* Ch. Lw., 1949 (= *Tiarella* Cheissin).

The definition of the genus *Plagiospira* may be presented as follows:

Plagiospira Issel, 1903 emend. Chatton et Lwoff, 1949

syn.: *Boveria* pro parte Mac Kinnon et Ray, 1931.

Hemispeiridae — Hemispeirinae of an ovoid body and medium dense ciliature; number of kineties of the range of 25—30. The adoral kineties begin behind the middle of the body length, run backwards making a wide arc and roll a half of turn around the hind body pole. The thigmotactic ciliature consists of several short kineties reaching as far as 1/2 of the body length and embraced from both sides by more and more longer, arc-like bent kineties, forming a parenthetical system. The last kineties of the general ciliature (kineties *n*, *n*—1, *n*—2) fail to reach the end of the body and terminate on the rand of the adoral spiral. Parasites of the mantle cavity of marine *Bivalvia*.

Typus generis: *Plagiospira crinita* Issel, 1903, Chatton et Lwoff, 1949.

The genus *Plagiospira* includes by now one species:

Plagiospira crinita Issel, 1903

The body cylindrical, somewhat sharpened in its anterior part, widened to the posterior. The length according to Issel 32—58 μ, according to Fenchel 61—72 μ, its width relatively 18—34 μ and 14—25 μ. Ma spherical or somewhat elongated — 11—17 μ, lies in the anterior body part, Mi 1—3 lies in front of Ma. CV in the posterior part of the body. There are 16—18 kineties of the general ciliature according to Issel, who did not count the thigmotactic kineties. Chatton et Lwoff 1949 report 29—32 kineties. The thigmotactic kineties form a système sécant in the left dorsal part of the body; the evaluation of their number provides some difficulties since they gradually transform in a general ciliature. Chatton et Lwoff value their number as 7—9. Along the ventral margin the first and last kineties deviate from each other and form in this way a naked peristomal field in the posterior part of the body. The adoral kineties begin about 2/3 of the body apex, bent to the right and describe a loop in the posterior pole. The cilia of the adoral spiral are specially strong and long, according to Issel 1903 three times longer than the body width; they are distinctly shorter on the drawings of Fenchel 1965 (Fig. 17).

There is a contradiction between the descriptions and drawings of Chatton et Lwoff 1949 and Fenchel 1965. Chatton et Lwoff consider that the kinety 2 on the ventral margin and further kineties began at the apex, but kineties *n*—5, *n*—4, *n*—3, *n*—2, *n*—1 and *n* origin more and more far to the posterior and are gradually shorter (kinety *n* runs only along 1/3 of the body length). Fenchel considers that this concerns the two last kineties only and mentions distinctly a difference. On the drawings of Issel the situation is intermediate. I agree with Fenchel 1965 that

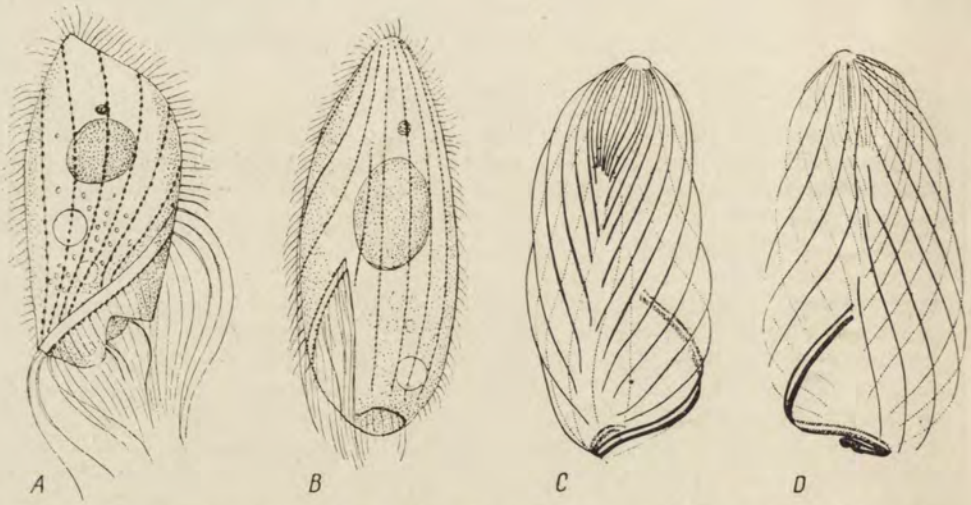

Fig. 17. *Plagiospira crinita*: A — after Issel, B — after Fenchel, C,D — from Chatton et Lwoff. ×1000

"those differences do not justify (at least presently) the erection of a new species". Both descriptions agree that kineties *n* and *n* —1 do not reach the posterior suture but break off before the arch of adoral kineties.

Host: *Loripes lacteus* (Poli), *Cardita calyculata* Brug, Napoli (Issel) and Roscoff (Ch. Lw.), *Nucula nucleus* (L.), *Divericella divericata* (L.), Roscoff (Ch. Lw.), *Thyasira sarsi* (Phil.), *T. flexuosa* (Mont.), *T.* sp., *Nucula turgida* Leck. Marsh. Gullmarfjord, Oeresund — Kattegat (Fenchel).

Genus *Cheissinia* Chatton et Lwoff, 1949

syn.: *Tiarella* Cheissin, 1930, 1931.

The genus name introduced Chatton et Lwoff 1949 in order to replace the name *Tiarella* Cheissin, 1930, 1931, preoccupied by *Tiarella* Swanson, 1840, *Mollusca*. The genus *Tiarella* Cheissin has been created by Cheissin 1930 for the species described at the same time as *T. baicalensis* Cheissin, 1928, 1931 from *Benedictia* sp. sp. of the Baikal Lake.

Cheissin 1930, 1931 did not pay a larger attention to the système sécant of the thigmotactic area of *T. baicalensis*, although he visualized it in details on his drawings. He did not describe exactly and did not show on his drawings how and when begin the adoral kineties — these details may be rather considered when compared with other genera as among the others *Plagiospira* and *Hemispeira*. In spite of some defficiencies in this description, the genus *Cheissinia* Ch. Lw. is distinctly outlined and its features correspond closely to the evolutional row, represented by *Protospira, Ancistrospira, Plagiospira*. These features constitute a farther stage of the development of evolutional tendencies in this scope. The genus *Cheissinia* constitutes a distinct link leading to *Hemispeira* F.-Dom.

The diagnosis of the genus *Cheissinia* may be determined as follows:

Cheissinia Chatton et Lwoff, 1949

syn.: *Tiarella* Cheissin, 1930, 1931.

Hemispeiridae — *Hemispeirinae* of a conical body and medium dense ciliature; number of kineties, 25—30. The adoral kineties begin in the posterior part of the body and make a spiral of about 300° around the posterior body pole. The first and last kineties of the general ciliature come together in front of the naked peristomal field. There exist about 18 thigmotactic kineties forming a closed system. The stomatogenic kinety is accompanied on a great distance by the first kinety of the general ciliature. Parasites of the mantle cavity of Baikal *Gastropoda*.

Typus generis *Cheissinia baicalensis* (Cheissin, 1930, 1931) Chatton et Lwoff, 1949.

The genus *Cheissinia* includes by now one species:

Cheissinia baicalensis (Cheissin, 1930, 1931)

The body is conical, more or less elongated, the length 50—72 μ (the length of the thigmotactic zone 28—35 μ, the rest of the body along the dorsal margin 30—32 μ), the width 13—20 μ in the anterior, 25—35 μ in the posterior body pole. Ma spherical, in the body anterior part, amounts to 12—14 μ and often in fragments. Mi measures 1—2 μ and lies before Ma. CV in the posterior part of the body. The kineties of the general ciliature run obliquely and buckle in the posterior part of the body so that these which are near the adoral kineties run parallel to them in their posterior segments. The general number of kineties of the general ciliature amounts to 27—29. The distances between kineties in the thigmotactic area amount to about 1 μ (there are 15—19 thigmotactic kineties); the space between kineties in the pasterior part of the body — 8 μ. The kineties are connected by the anterior suture shifted far backwards along the ventral margin, posterior suture and two transversal fibrills, arranged parallely. The adoral kineties begin their run (similarly as *Boveria* from among *Ancistrinae*) virtually in the posterior, widened body pole and describe an arch of about 180°. They are closed by a loop in which are located the cross fibrills (Fig. 18).

Cheissin distinguishes two forms of *Ch. baicalensis*: the form *typica* with the dimensions typical for the genus and the form *elongata*, occurring only in *B. limneoides*. The dimensions of this form are: length 105—130 μ, width 30—38 μ in the posterior and about 20 μ in the anterior part of the body.

Host: *Benedictia baicalensis* (Gerst.) and *B. limneoides* (Schr.) — Baikal Lake.

Genus *Hemispeira* Fabre-Domergue, 1888

syn.: *Hemispeiropsis* König, 1894.

The genus *Hemispeira* was created by Fabre-Domergue 1888 for simultaneously described species *H. asteriasi* and assigned to *Urceolariida* (*Peritricha*). Cuénot 1891 described from *Antedon* a different species as

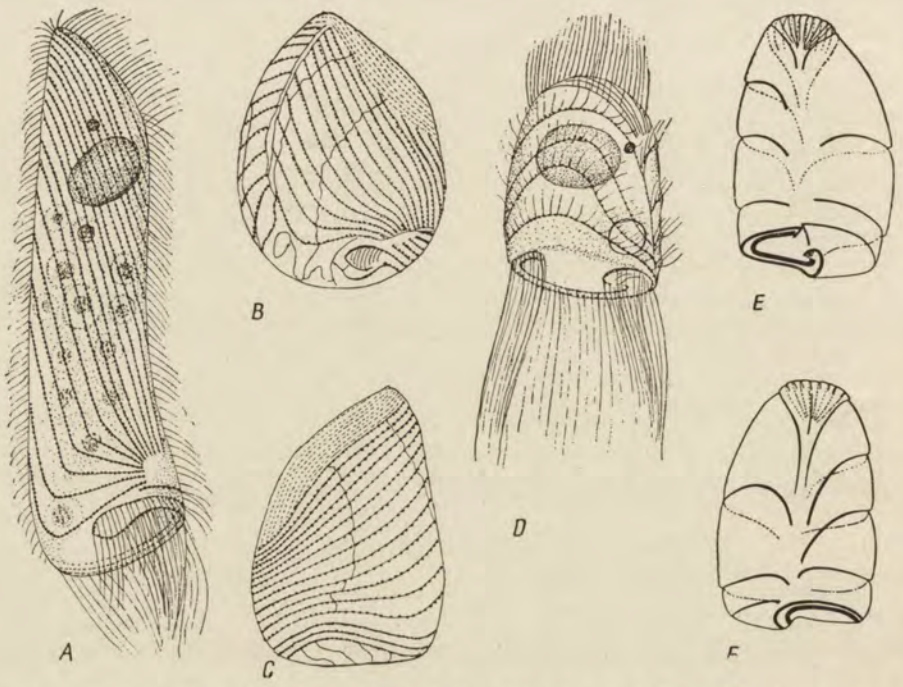

Fig. 18. A, B, C — *Cheissinia baicalensis* (after Cheissin), D — *Hemispeira comatulae* (after Fenchel), E, F — *Hemispeira asteriasi* (from Chatton et Lwoff). ×1000

Trichodina antedonis which several authors, finally Chatton et Lwoff recognized as belonging to the genus *Hemispeira*. Since the description of Cuénot is inadequate and since it is likely that *Urceolariidae* occur also on the *Antedon*, the name Cuénot's must be recognized as nomen nudum, or the species as species inquirenda. However the species described by König 1894 as *Hemispeiropsis comatulae* also from *Antedon* must be recognized, according to Chatton et Lwoff 1949 as belonging to the genus *Hemispeira*. According to the opinion of these authors I think that it is not necessary to maintain the differentiation of the genus *Hemispeiropsis*; the differences which occur between the typical species of both genera do not express the differences in the general organization of their body as well as in the grade of their evolutionary advancement; they express only the minute divergencies in the realization of evolutionary tendencies on the same development level (Fig. 19).

The known representatives of the genus *Hemispeira*, therefore *H. asteriasi* F.-Dom. and *H. comatulae* (König) are distinctly different from the other *Hemispeirinae* concerning numerous features of the structure; however these all represent the final results of development of features typical for the evolution of *Hemispeirinae*: shifting of kineties on the distal body pole, the displacement of the thigmotactic area nearly to the apical pole and their distinct separation from the general ciliature, a strong reduction of the number of kineties of the general ciliature and their arrangement in a nearly parallel

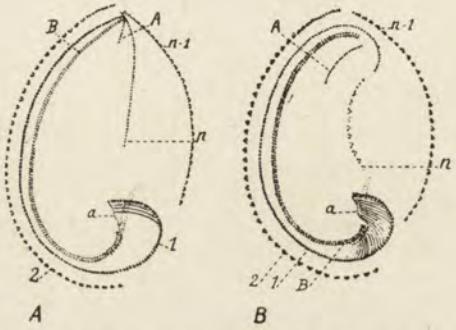

Fig. 19. *Hemispeira*: A — *H. asteriasi*, B — *H. comatulae* (from Chatton et Lwoff). ×2000

position. It must be stressed that in comparison to the evolutionary row which among the *Ancistrinae* represent *Ancistrumina*, *Proboveria*, *Semiboveria* and *Boveria*, there is no such an intensive spiralization of the adoral kineties as in *Boveria*; on the contrary — in *Hemispeira* they take nearly the same shape and the same scope as in *Ancistrospira* — therefore about 180°.

By now two species of the genus *Hemispeira* reveal exceptional, scanty body size 20—30 μ, so such one which is scarcely reached by several species of the genus *Hypocomella* or *Hypocomidium* (*Ancistrocomidae*) with a highly reduced ciliature. Doubtless the scanty dimensions collaborate in the reduction of the general ciliature in *Hemispeira*.

The evolutionary tendency represented by *Hemispeira* leads to a distinct transformation of the body so that consequently appears its separate biologic orientation. The animal according to the descriptions of the authors, uses its thigmotactic area completely shifted to the anterior body pole, as the adhesive-sucking apparatus by which it is oriented to the substrate. Hence arise presumably all attempts to bind *Hemispeira* with *Urceolariidae*. The adoral spiral shifted to the inverse dorsal body pole is provided with exceptionally long cilia forming a high pseudomembranella. Kineties of the general ciliature run nearly parallely in both sides of the body, four on each side, but the fifth kinety on the left side (kinety *n*) lies on the adoral field on the posterior, flattened body pole.

The proper orientation of the body of *Hemispeira* seems to be easier since Chatton et Lwoff 1949 proposed an outlook from this adoral field in which relations may be compared to those prevailing on the adoral field of *Ancistrospira*. In that case it is distinctly visible that on the right of the adoral kineties runs the kinety 1 of the general ciliature (2 according to Chatton et Lwoff), moreover the 2, 3, 4 run unvisible from the oral side, then on the body apex run the thigmotactic kineties, then kineties $n-4$, $n-3$ and $n-2$ on the left body side, finally the kinety $n-1$ visible from the oral side. Besides the kinety *n* lying on the oral area, Chatton et Lwoff design in it the segment A of the prostomal kinety. Both species of the genus *Hemispeira* differ according to these authors by a different rolling of the stomatogenic kinety and a different situation of the kinety A and kinety *n* on the oral area (Fig. 19).

In view of the characteristic presented here the diagnosis of the genus *Hemispeira* may be given as follows:

Hemispeira Fabre-Domergue, 1888

syn.: *Hemispeiropsis* König, 1894.

Hemispeiridae — *Hemispeirinae* of a conical body and strongly reduced general ciliature. The adoral kineties are totally located on the posterior, wide body pole, making an arc of about 180°, and are then directed perpendicularly to the long axis of the body. The thigmotactic kineties, short and dense, ca 10 in number, occupy a lens-shaped area shifted nearly on the apical body pole. The kineties of the general ciliature, ca. 8 in number, are situated obliquely and even almost meridionally. The kinety 1 of the general ciliature accompany the stomatogenic kinety on a great distance. Live on the integument of *Echinodermata* (*Asteroidea* and *Crinoidea*).

Typus generis: *Hemispeira asteriasi* Fabre-Domergue, 1888.

After including here the genus *Hemispeiropsis*, the genus *Hemispeira* includes two species:

Hemispeira asteriasi Fabre-Domergue, 1888

The body has a shape of a spherical and somewhat laterally flattened cone. The length (height) of the body 20—30 μ, width ca 20 μ. Ma generally ovoid, often fragmented, Mi ?. The adoral kineties start next to one another and describe an arch around the oral field, forming a large loop. According to Chatton et Lwoff 1949 the kinety A is short, kinety *n* runs straight on from the initial point of the adoral kineties towards their loop. Besides it: 4 kineties on the right, 4 on the left side of the body. The thigmotactic area includes 8—10 kineties arranged densely (Fig. 18 E,F, 19 A).

Host: *Asterias rubens* L., *Martasterias glacialis* (L.) — Concarneau (F.-Dom.), Banyuls sur Mer (Ch. Lw.); *M. glacialis* — Gullmarfjord (Wallengren), Frederikshavn (Fenchel). Fenchel 1965 writes: "the surface of the seastar, especially the sides of the arms, was quite covered with the ciliates".

Hemispeira comatulae (König, 1894)

syn.: *Hemispeiropsis comatulae* König, 1894, syn. (?) *Trichodina antedonis* Cuénot, 1891.

According to the detailed description of Fenchel 1965: the body has a shape of a cone, which is rounded and distinctly flattened laterally. Length 23—27 μ according to König, about 30 μ according to Fenchel, width about 18 μ, thickness 8 μ. Ma measures 10 μ and lies in the middle of the body, Mi, 1—2 μ, lies before Ma. The adoral kineties form an arch round the adoral area and describe on it a large loop. The beginning of the stomatogenic kinety protrudes according to Chatton et Lwoff before the beginning

of the prostomal kinety with a short arch. The kinety A is longer than in *H. asteriasi*, the kinety *n* rolls an arch parallel to the arch of the adoral kineties. Besides it: 4 kineties of the general ciliature on the right and 4 on the left body side; they are strongly buckled in arches oriented to the body apex. The thigmotactic field includes ? kineties. According to König 1894 kineties of the general ciliature are virtually enforcing fins and they have not cilia; according Chatton et Lwoff 1949 (p. 230) "les cineties sont accompagnées sur l'un des leurs côtes d'une crête cytoplasmatique assez saillante. Mais elles n'en pourtant moins des cils longs et fins" (Fig. 18 D, 19 B).

Host: *Antedon mediterranea* Lmk. — Concarneau, Port Vendres (Ch. Lw.); *Antedon petasus* Döb. Koren, Gullmarfjord (Fenchel). Fenchel 1965 writes that the antedons were "quite covered by the ciliates, it was specially numerous in the corners between the arms and the pinnake".

Subfamilia *Thigmocominae* Kazubski, 1958

This monogeneric and monospecific subfamily was created as a separate family *Hemispeiridae*, however he admits in the case of appearing of definite this problem in his detailed study, Kazubski 1963. Henceforth nobody was concerned with the representatives of this subfamily and no form was described which could be ranged among this group.

Corliss 1961 ranks provisionally the genus *Thigmocoma* among the family *Hemispeiridae*, however he admits in the case of appearing of definite Kazubski's study, the creation of a separate family, which could be characterized as follows: (Corliss 1961, p. 151): "Cytostome nearly midventral. Thigmotactic ciliature quite distinct. Ciliation reduced on the posterior parts of the body. Single genus".

Kazubski 1963 assigns a place to the family *Thigmocomidae* among *Thigmotricha* not only according to principle of morphological similarities but also on the basis of coincidence of morphogenetic processes, concerning especially the adoral kineties. Kazubski considers *Hemispeiridae* as the nearest to *Thigmocomidae* and he states that the differences existing between *Hemispeirinae* and *Protophryinae* (= *Ancistrinae*) are no lesser than these which occur between each of these subfamilies and the family *Thigmocomidae*. As a result Kazubski 1963 suggests an equal range for following taxons: *Hemispeirinae*, *Protophryinae* and *Thigmocomidae*. I confirm for them the range of subfamilies within the family *Hemispeiridae* König, 1894 as I stated it before (part I, p. 32, 33, this part p. 119).

Virtually *Thigmocominae* correspond to the characteristic of the family *Hemispeiridae* by their general scheme of structure, the presence of thigmotactic zone in the left side of the flattened body, the reduction of thigmotactic ciliature in its posterior part, the structure of the adoral kineties; they differentiate within the *Hemispeiridae* only by a specific shape of these elements. The features of this subfamily are as follows: the reduction of the thigmotactic ciliature only to the anterior left body part, and of the general ciliature nearly to the anterior right body part except several dorsal kineties going further. The adoral kineties are strongly modified but they undergo in their development similar stages to those revealed in other *Hemispeiridae* or in *Conchophthirinae*.

Nothing can be said on the development trends within this monogeneric and by now monospecific subfamily, it is also difficult to determine the paths

of its divergency from the other more plesiomorphic groups of *Hemispeiridae*. The specific characteristic of the structure of *Thigmocoma* may be related to the specific way of its life in the excretory system of terrestrian *Pulmonata*. Kazubski 1963 reports some interesting data, concerning the morphology, morphogenesis, biology and ecology of *Thigmocoma acuminata* which I intend to use in the final chapters of the present study, devoted to these problems. On the basis of single examinations of *Kazubski* 1958, 1963 and his definition, the diagnosis of the subfamily *Thigmocominae* may be stated only provisionally and considering the possibility of finding other species corresponding to it.

Subfamilia *Thigmocominae* Kazubski, 1958

pro: familia *Thigmocomidae* Kazubski, 1958.

Thigmotricha — *Hemispeiridae* of a thigmotactic area formed by anteriorly located kineties not continued towards the posterior body pole. The general ciliature of the right body side show a strong reduction and are limited to the anterior body part. The adoral kineties are lying on the ventral body margin and are shortened and modified. Parasites of the renal organ of *Gastropoda*.

Typus subfamiliae: genus *Thigmocoma* Kazubski, 1958.

The subfamily *Thigmocominae* is represented meanwhile by one genus only:

Genus *Thigmocoma* Kazubski, 1958

The characteristic of this genus founded on the data of Kazubski but in a slightly modified form adjusted to our scheme, may be presented as follows with some reservations:

Thigmocoma Kazubski, 1958

Hemispeiridae — *Thigmocominae* of a strongly flattened body and differentiated ciliature. The meridional kineties, ca 60 in number, of the general and thigmotactic ciliature show a strong reduction and occupy only the anterior body part; the thigmotactic kineties are mostly shortened. The posterior ciliature of the right and the left side tends to a considerable reduction or even to complete absence. Unciliated zone is present between the general and thigmotactic ciliature. The adoral kineties are lying on the ventral margin, the cytostome — more or less in the middle of the body length. Parasites of the renal organ of terrestrial *Gastropoda* — *Pulmonata*.

Typus generis: *Thigmocoma acuminata* Kazubski, 1958.

The genus *Thigmocoma* includes by now one species:

Thigmocoma acuminata Kazubski, 1958

The body ovately outlined, wide and spherical in the anterior part, sharply ended in its back; the ventral margin reveals in its middle a distinct oral incision; the right side convex, the left, especially in its anterior part, strongly concave, (thigmotactic area). The length 45—100 μ, most often 60—75 μ; width 26—68 μ most often 30—50 μ. Ma spherical in young individuals, 8—33× ×7.5—30 μ, mostly 13—22×14—29 μ; it lies somewhat before the middle of the body length and undergoes deformations in older individuals. Mi small, 1—1.5×2—2.5 μ, adjacent to Ma. CV lies in the posterior body part, among the vacuolized plasma (Fig. 20).

Fig. 20. *Thigmocoma acuminata*: A,B — general view from the right side and from the ventral margin, C,D — argyrophile system from the right and the left sides, E — scheme of the adoral ciliature. After and from Kazubski. ×1000

These data refer to the specimens from *Oxychilus orientalis*; in *O. syriacus* and *O. duboisi* the protozoans have somewhat smaller dimensions.

The ciliature is generally strongly reduced: the kineties of the thigmotactic area are reduced only to the anterior left part of the body, they reach scarcely 1/3 or even 1/4 of its length. The general ciliature occupies also only the anterior part of the right body side reaching in young individuals 2/3, in older 1/2 and finally scarcely 1/3 of the body length. The end of the body gets only some dorsal kineties of the right side: somewhat further to the back run also the first and second kinety of the general ciliature (kinety 2 and 3 according to Chatton et Lwoff) and kinety *n*. Besides kineties, their fibrills

are drawn to the back without kinetosomes, forming in the back of the body a scarce irregular net. In this net, both on the right as on the left side of the body, there are concentrations of kinetosomes in the shape of short rows (of several kinetosomes). The adoral kineties lie in the naked field on the ventral body margin. The stomatogenic kinety starts far in the back in the half of body length, is very short and ended by a minute oral loop. The prostomal kinety starts in the anterior body part at anterior suture, but ends next to the half of body length.

T. acuminata are living from several to a dozen days or so (7.5 on the average), growing over this period. The conjugating individuals are small, the dividing ones are rather of the middle size; further growth indicates the senil period. They occur sometimes in large quantities, amounting even several hundreds of individuals, in the renal apparatus and ureter of the snails.

Hosts: *Oxychilus orientalis* (Cless.) from Polish Carpathians (till 100% infestation of the population of snails), *O. syriacus* (Kob.) from Libanon, *O. duboisi* (Mouss.) from Caucasus.

Summary

The second part of the monograph on *Thigmotricha* comprises the elaboration of the family *Hemispeiridae*, of which the more plezjomorphic genera are recognized as models of exit forms for other *Thigmotricha*. The characteristics of the family is given as well as the evolutionary paths and the division in three subfamilies: *Ancistrinae*, *Hemispeirinae* and *Thigmocominae*. The paper comprises the descriptions and definitions of the genera and the diagnoses of the species. In the subfamily *Ancistrinae* two new genera are erected: *Fenchelia* g.n. for *Ancistrum crassum* Fenchel and *Semiboveria* g.n. for *Boveria stevensae* Issel and the new species *Ancistrumina bulgarica* sp.n. from *Mytilaster* from the Black Sea are described.

STRESZCZENIE

Druga część monografii *Thigmotricha* zawiera opracowanie rodziny *Hemispeiridae*, której bardziej plezjomorficzne rodzaje traktowane są jako modele form wyjściowych dla innych *Thigmotricha*. Podano charakterystykę rodziny, omówiono kierunki ewolucyjne w jej obrębie i podział jej na podrodziny: *Ancistrinae*, *Hemispeirinae* i *Thigmocominae*. Podano opisy i diagnozy rodzajów i opisy gatunków. W obrębie podrodziny *Ancistrinae* wyodrębniono nowe rodzaje: *Fenchelia* g.n. dla *Ancistrum crassum* Fenchel i *Semiboveria* g.n. dla *Boveria stevensae* Issel oraz opisano *Ancistrumina bulgarica* sp.n. z *Mytilaster* z Morza Czarnego.

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Department of Zoology, University of Warsaw, Warsaw 64, Poland

D. KOWALSKA and J. KACZANOWSKA

Studies on topography of cortical organelles of *Chilodonella cucullulus* (O.F.M.) II. Topographical relations of the total number of kineties to the disposition of CVPs

Studia nad topografią organellów kortykalnych *Chilodonella cucullulus* (O.F.M.) II. Zależność między liczbą kinet a rozmieszczeniem otworków wodniczek tętniących

In the previous study (Kaczanowska and Kowalska 1969) two patterns of CVPs (contractile vacuole pores) disposition have been stated.

The aim of the present study may be brought to the following points:

1. Examination whether the capability to differentiation of CVPs is restricted to definite interkinetal spaces.
2. Following on a possible various material whether the same number of kineties occurs between the upper CVPs.
3. Following the quantitative relations of kineties number of the right sector of ciliature (i.e. the meridians from right margin of the ventral surface up to CVP-1), this of the median sector (i.e. meridians between CVP-1 and CVP-2) and of the left one (i.e. from CVP-2 up to left margin of the ventral surface).
4. Description of the cases of changes in total number of kineties and changes of CVPs induction in dividers.

Material and methods

The material, methods and abbreviations used in the present study are the same as described in part I (Kaczanowska and Kowalska 1969). The stock B and K are used from samples which were the progeny of one single individual. Stock K was supplied to us by Mr. S. Radzikowski M. Sc. to whom we express our vivid thanks. This stock was isolated from the sewers of Kraków. Stock C and clone B_1 were used in the second, third and fourth sections of this article. From the initial stock B a random sample was taken (at least 100 individuals) and cultivated for three weeks in substarvation conditions. Those ciliates were fed with a reduced portion of yeast suspension, twice less frequently then the other stocks. This culture was inoculated once

a week. The starving individuals were transparent, flat, thin and motile. However no death of the cells was observed.

After three weeks of substarvation this sample was divided into two parts: one of them was fixed, while the other one was subjected to intense feeding again. The well fed ciliates were fixed after two following weeks.

The analysis of the whole material embraces the study of the total number kineties and of CVs visualized by their pores. Transmission of the total number of kineties and sites of new CVPs in relation to the old ones was analyzed in 150 dividers.

Cautionary remarks and abbreviations

The meridians supply opportunity for an adequate description of the cortical topography in the symmetric forms. Nanney 1966 a introduced and developed the study on corticotypes of such forms. The study of corticotype allowed the evaluation of both induction and field angles (Nanney 1966 b. Klug 1968).

In the assymmetric *Chilodonella* with its ciliated ventral surface only, the application of the corticotype notion and calculation of the angles is impossible. More so, the dilation of the distance between kineties fails to indicate a direct increase of the ventral surface, because it may also be connected with a casual smoothening of weell-fed specimens, or with stretching of the interkinetal ridges.

Folding of cortex makes also impossible (at the time being) the presise determination of the position of CVP in the interkinetal space. Considering the above restriction, the determination of the mid-point and mean position of CVPs was neglected in the case of double pores.

All the distances were expressed by the total number of interkinetal spaces. The median sector (i.e. distance from CVP-1 and CVP-2) expressed the number of interkinetal distance between the peripheral pores of CVPs in the cases of doublings.

The width of the median sector is stabilized during induction of CVPs i.e. before the morphogenetic movement of the kinety A_4 of opisthe which supplements the total number of kineties (phenomenon of "evolution par decalage" after Chatton et al. 1931, Radzikowski 1966, Kaczanowska and Kowalska 1969). This ingrowth of kinety A_4 shifts leftwards the CVP-2″, CVP-2a″, and CVP-3″ and eventually CVP-2b″ or CVP-2c″ not impairing the disposition of CVP-1″, for example: after this dislocation the five-kinetal distance during induction of CVP-1 and CVP-2 is kept unchanged in proter but it is modified to the six-kinetal distance in opisthe.

The distance from CVP-1 to CVP-2 does not represent the maximum of width of the sector undergoing differentiation. There are some cases when CVP-2b broadens this sector. However this pore appears only in some opisthes, therefore we decided to neglect its existence for a while. Howewer, this problem will be considered in discussion.

The disposition of CVP-3 differs in proter and opisthe. Some abnormality of its disposition can be observed in some individuals, for example: two kinetal distance between CVP-2 and CVP-3 in proter or tri-kinetal distance CVP-2 and CVP-3 in opisthe. These abnormalities are marked in the abbreviated form as CVP-3 oIIo in the first case, or CVP-3 oIIIo in the second

case. The lack of this mark in CVP-3 record indicates a regular and proper pattern for proter or opisthe in disposition of this pore.

According to the previous study (Kaczanowska and Kowalska 1969) the terms "proter" or "opisthe" were also applied to nondividers if they represent a regular pattern of CVPs topography.

A summary record for an individual expressed in three numerals e.g. 7—5—4 with no additional explanations, or marks indicates that the individual has 16 kineties being a proter (five-kinetal median sector) with seven kineties in the right and four in the left sector. This kind of abbreviations indicates simultaneously a regular disposition of CVP-3 for proter. The total number of kineties never includes the kinety X.

Results

Variability of the total number of kineties and of the CVPs number

It follows from the Table 1 that in stock B the group of individuals with 19 and 20 kineties was prevailing (94.2%). 94% of sample represents the regular pattern of width of the median sector (five-kinetal distance for proter, or six-kinetal distance for opisthe).

For the samples of stock K Table 2 the dominating group are the individuals with 17 kineties (63.1%). The regular pattern of CVPs disposition represents 96.2% of the sample. There is a complete absence of opisthes with CVP-2b. About 4% of individuals show a tri-vacuoles pattern of underdevoloped opisthe (see Kaczanowska and Kowalska 1969).

Table 1

Number of CVs and total number of kineties (stock B)

Number of kineties / Number of CVs	18	19	20	21	Total
3	5	48	40	4	97
3 (CVP-3 oIIo)	—	1	3	—	4
4	—	37	45	1	83
5	1	4	3	—	8
Total	6	90	91	5	192

Table 2

Number of CVs and total number of kineties (stock K)

Number of kineties / Number of CVs	16	17	18	19	Total
3	4	41	14	—	59
3 (CVP-3 oIIo)	2	2	—	—	4
4	2	22	15	1	40
Total	8	65	29	1	103

In the substarved sample of stock B (Table 3) a considerable extension of the variability of kineties number was observed. There appears e.g. the kinetome 16 which is not represented in the initial stock. Dominating group is represented now by the 18 kineties individuals amounting up to 53.5% of the sample. The amount of 19- and 20-kineties individuals fell down to 15.4%. About 7.7% of individuals represent underdeveloped tri-vacuoles opisthes and in four cases of two-vacuoles pattern of proter without CVP-3 was found.

Table 3

Number of CVs and total number of kineties (stock B substarved)

Number of kineties / Number of CVs	16	17	18	19	20	21	Total
2	2	1	1	—	—	—	4
3	2	21	32	13	—	—	68
3 (CVP-3 oIIo)	4	7	—	—	—	—	11
4	—	10	33	5	3	3	54
5	—	1	3	1	—	—	5
Total	8	40	69	19	3	3	142

In the group of individuals which have been well-fed for the second time (Table 4) the range of variability of the total number of kineties returns to the exit value of stock B. However the group of 18-kineties individuals still dominates amounting up to 44% of the sample. The percentage of 19- and 20-kineties individuals amounts up to 48%. Two individuals with four vacuoles pattern but with abnormal disposition of CVP-3 oIo were found. They are of unknown origin.

Table 4

Number of CVs and total number of kineties (stock B-refed)

Number of kineties / Number of CVs	18	19	20	21	Total
3	10	8	8	4	30
3 (CVP-3 oIIo)	3	—	1	—	4
4	8	2	4	—	14
4 (CVP-3 oIo)	—	1	1	—	2
Total	21	11	14	4	50

The width of the median sector

In Table 5 the width of the median sector is given for all the individuals together. Comparison of the data indicates that the distance of 5 kineties for proter and 6 for opisthe respectively is realized in 95.2% of 17- and 18-kineties individuals regardless of their origin. In those with a higher

Table 5

The middle sector (in number of kineties) and total number of kineties (all individuals)

Number of kineties / Width of middle sector		16	17	18	19	20	21	Total
Proters	5	7	59	65	74	51	8	264
	6	—	—	2	10	15	3	30
Opisthes	5	—	—	—	1	2	—	3
	6	4	34	58	59	63	2	220
	7	—	1	8	6	7	4	26
	8	—	—	—	1	4	—	5

total number of kineties an increase of the number of individuals with a broader distance between the pores of upper vacuoles is observed. Out of 151 individuals with 19 kineties — 11.2% are those with a broadened median sector, whereas in the group of 142 individuals with 20 kineties this distance is broadened in 18.3% of cases.

The same tendency was observed in each individual sample but the number of individuals is too small for any description of this relationship in a more homogeneous sample.

The heterogeneity of material different degree of the cortex extension are possible factors of diversity of the topographic relation in different protozoa lines. However the above results clearly indicate that there are no definite kineties specialized for CVPs differentiation.

Another conclusion from these data is the inclination to extension of the median sector being associated with an increase of the total number of kineties.

If the same data as in Table 6 were expressed as the mean width of the median sector in each group of individuals with the some total number of kineties, the Table 6 would be gained. The comparable calculation was made also for homogenous sample B (Table 7).

The results obtained, excluding only a few individuals with 21 kineties — aligne in a regular diminishing sequence in both cases (Table 6 and 7).

It follows from the above Tables that there is a certain stability of the five-kinetal distance between CVP-1 and CVP-2 despite of indefined set of conditions, heterogeneity of our stocks which could influenced upon the cortical pattern. (Nanney 1966 b, 1968, Heckmann and Frankel 1968).

Table 6

The mean ratio of the width of the middle sector to total number of kineties (al individuals)

Number of kineties	16	17	18	19	20	21
Proters	0.312	0.294	0.280	0.269	0.261	0.251
Opisthes	0.375	0.355	0.340	0.321	0.308	0.317

Table 7

The mean ratio of the width of the middle sector to total number of kineties (stock B)

Number of kineties	18	19	20	21*
Proters	0.278	0.269	0.260	0.338
Opisthes	0.389	0.327	0.313	0.333

* This observation was made only on five specimens.

Comparison of the number of kineties in right, median and left sectors

The mechanism of morphogenesis of *Chilodonella cucullulus* (evolution par decalage — after Chatton et al. 1931) is based on transmission of kineties of right kineties and "decalage" of the remaining kineties. A question arises whether the median sector occupies a definite position related to the kinetome of the cell. In other words what variants occur in the width of right, median and left sector.

The collective Tables 8 and 9 demonstrate the variability of the right (R) and left (L) number of kineties in the individuals of the same total number of kineties. The ciliature patterns of the individuals with an increased median sector are recorded in parentheses. It follows from these Tables that some patterns are much more numerous. Considering the patterns which occur most frequently, the data of the Table 10 were selected.

It could be observed that if we postulate the number of kineties in the left sector being n, then the following pattern occurs more frequently:

For proters if: L = n so R = n+1 for the even total number of kineties

L = n so R = n for the odd „ „ „ „

and for opisthes if: L = n so R = n+1 for the odd „ „ „ „

L = n so R = n+2 for the even „ „ „ „

If these regularities are not caused by heterogeneity of our material it would prove that the median sector has a rather definite disposition in relation to the ventral surface.

Therefore, a question arises what is the mechanism regulating the disposition of the median sector on the ventral surface. For this reason dividers were observed in which the old pattern of CVPs disposition and the daughter patterns of CVPs disposition may be observed simultaneously.

The mechanism of changes of the total number of the kineties and of CVPs disposition

In some slides individuals occur with a shortened marginal kinety of the right sector. Underdeveloped kineties were also observed in some of the other kineties as in the case in Pl. I 3. Similar underdeveloped kinety was also observed by Leofer et al. 1966 in *Tetrahymena*. The underdevelopment of some kineties involves presumably a diminished total number of kineties in progeny.

In three cases of dividers in early cytokinesis stage even two left marginal kineties failed to divide, so the opisthe had in sum less kineties than proter. These cases require however further investigation because it has not been ascertained whether this kinety would be not regained in the interdivisional period.

Table 8

The total number of kineties and the number of kineties in the right (R) and left (L) sectors of proters

(Abnormal patterns of ciliature marked in parentheses)

Number of kineties in sectors L-R	Total number of kineties											
	16		17		18		19		20		21	
	n	f	n	f	n	f	n	f	n	f	n	f
4 7	1	14.3										
5 6	5	71.4										
6 5	1	14.3										
5 7			9	15.2	2 (5-6-7)	3.0						
6 6			50	84.8								
6 7					62	92.6	4 (6-6-7)	4.7				
7 6					3	4.4	6 (7-6-6)	7.0				
6 8							9	10.7				
7 7							65	77.6	15 (7-6-7)	22.7		
7 8									30	45.5	3 (7-6-8)	27.2
8 7									21	31.8		
7 9											1	9.0
8 8											7	63.8

n — number of specimens. f — frequency in %.

Inversely, if all the kineties (including the left extreme one) participate in division then the opisthe gains one kinety more than proter.

The ingrowth of kineties after injury (Dobrzańska-Kaczanowska 1965) may also be the cause of alternation in the total number of kineties.

The changes in the total number of kineties in the right or left sector led also to the changes in ratio of width of sectors. Observations of dividers indicate that disturbances in proportion of the width of sectors evoke changes in the induction pattern during divisional morphogenesis. These cases of changes in the pattern of CVPs induction are visualized in Table 11 and additionaly in Pl. I 4.

On the Table 11 the two first cases concern the ciliates with a normal pattern of kinetome, with a broadened median sector. The total number of kineties is rather high and in the progeny of those forms these patterns will persist without any changes.

Table 9

The total number of kineties and the number of kineties in the right (R) and left (L) sectors of opisthes

(Abnormal patterns of ciliature marked in parentheses)

Number of kineties in sectors L-R	Total number of kineties											
	16		17		18		19		20		21	
	n	f	n	f	n	f	n	f	n	f	n	f
4 6	2		1	2.9								
4 7			1	2.9	6 (4-7-7)	9.0						
5 6			33	94.2	2 (5-7-6)	3.0	1 (5-8-6)	1.5				
5 7					53	80.1	6 (5-7-7)	9.0	4 (5-8-7)	5.2		
6 6					5	7.9						
5 8							5	7.4	4 (5-7-8)	5.2		
6 7							50	74.6				
7 6							4	6.0	2 (7-7-6)	3.9		
6 8									38	50.2	3 (6-7-8)	50.0
7 7							1 (7-5-7)	1.5	25	32.9	(7-7-7)	16.6
6 9									1 (6-5-9)	1.3	2	33.4
7 8									1 (7-5-8)	1.3		

n — number of specimens, f — frequency in %.

Two subsequent cases concern the patterns with the enlarged median sector and with CVP-3 oIIIo in the opisthe. In the progeny, one of the offsprings preserves the parenthal pattern whereas in the second component reduction in the median sector takes place.

The fifth case concerns the individual with a double pore for CVP-2 (at the distance of 5 and 6 kineties from CVP-1) and abnormal CVP-3 oIIIo a high disproportioned the left and right sectors. In the future proter of the next generation a regulation and symmetrization of pattern leads to formation of unusually broad middle sector and still irregular position of CVP-3 oIIo. The future opisthe keeps the anomaly of the parental pattern.

Table 10

The most frequent patterns of ciliature

Number of kineties of parent	Proters		Opisthes	
	pattern L—R	frequency %	pattern L—R	frequency %
16	5 6	71.4	4 6	100.0
17	6 6	84.8	5 6	94.2
18	6 7	92.6	5 7	80.1
19	7 7	77.6	6 7	74.6
20	7 8	45.5	6 8	50.2
21	8 8	63.8		

Table 11

Anomalies in CVPs induction pattern in dividers

No.	Parental specimen		Proter		Opisthe	
	pattern	total number of kineties	pattern	total number of kineties	pattern	total number of kineties
1	7—6—7	20	7—6—7	20	7—7—6	20
2	7—7—6	20	7—6—7	20	7—7—6	20
3	8—7—6 CVP-3 oIIIo	21	8—5—8 CVP-3 oIIo	21	8—7—6 CVP-3 oIIIo	21
4	7—7—6 CVP-3 oIIIo	20	7—6—7 CVP-3 oIIo	20	8—6—6 CVP-3 oIIIo	20
5	8—5—6 CVP-3 oIIo double CVP-2	19	6—7—5 CVP-3 oIIo	19	8—6—5 CVP-3 oIIIo	19
6	8—6—4	18	6—7—5	18	8—6—5	19
7	7—7—4	18	8—5—5	18	7—7—4	18
8	8—7—5	20	8—5—7 CVP-3 oIIo	20	8—6—6	20

The sixth case concerns the opisthe with a highly disturbed equilibrium of width in the left and right sectors. Similarly as in the former case, asymmetrization occurs and leads to enlargement of median sector at the expense of the width of the "conservative" right sector. Such a broad median sector is still abnormal in the case of such a low total number of kineties. The pattern in the future opisthe will be preserved, but the degree of anomaly will fall by the participation of the left extreme kinety in division. In this way, the future opisthe will possess more kineties than the parental individual.

The seventh case presents diminution of the median sector and increase of the right one in the future proter. This is an inverse situation of the two

former. Proter keeps the asymmetry of pattern and opisthe continues to develop the high anomaly of the parental pattern.

The eighth case shows a complete renormalization of the future opisthe and a partial renormalization of the proter with an abnormal disposition of CVP-3 oIIIo.

All the above cases prove that during the induction stages, a renovation of the proportion of the sectors width for the offsprings takes place. The induction is regulated by many factors but the above observations indicate that among the others the total number of kineties and tendency to central disposition of the median sector act upon it, without the necessity of preserving the parental pattern.

The remaining 170 dividers analyzed failed to show changes in induction of CVPs pattern. This seems to prove that the regular patterns of width of the sectors are followed in the next generations without any changes.

Discussion

The results reported above seem to indicate that there exists an essential relation between the total number of kineties and the disposition of CVPs.

In the course of induction of new CVPs in dividing individuals, a tendency is manifested to keep the definite proportion between the width of the left and right sectors. Broadening of the median sector accompanies the cases of a high total number of kineties. However a considerable stability of the five-kinetal distance between CVP-1 and CVP-2 is observed.

The origin of this phenomenon may be related to genetic character of some individuals to the cycle of culture, or may reflect more or less the sharp angle of induction. The solution of these problems is beyond the scope of this study.

However some other factor of tendency to keep the stable five-kinetal distance of induction of CVP-1 and CVP-2 may be postulated. Perhaps the increase of the total number of kineties is really accompanied by a proportional increase of the distance between CVP-1 and CVP-2 measured in absolute units in the moment of induction of CVPs. However the rarefication of the cortical ridges and furrows causes in these cases the reduction of the number of kineties involved in the activated space. It would lead to some "conservative" stability of five-kinetal distance of induction between CVP-1 and CVP-2.

It should be added that in our comparison the existence of CVP-2b in some opisthe was neglected. CVP-2b enlarges the sector involved in the formation of CVPs at the induction stage.

We decided to omit its existence and to postpone of the description at really existing sector of induction of CVPs, because CVP-2b is represented only in some opisthe. It is not quite sure, if its appearance would not be associated with the character of some stocks, or with more favourable set of conditions. There is no case of doubling of its pores, similarly as in the case of CVP-2a which exists only in opisthe and is often underdeveloped. It is worth to remark that a stable CVP-2 is represented in the same interkinetal space as less stable CVP-2a.

All the above observations would clearly indicate that besides all the factors concerned i.e. genic properties, the shift of cultures, cytotactic action,

set of conditions, less or more sharp induction angle, some stability of five-kinetal distance of CVP-1 and CVP-2 induction and total number of kineties which could influence on the topography of CVPs induction still another topographical factors may be related with it.

Some experiments on injured cells indicate such a possibility. (Kaczanowska 1969).

Summary

The topographic relations between the total number of kineties and the disposition of the contractile vacuole pores (CVPs) were investigated in *Chilodonella cucullulus* (O.F.M.).

Some tendency to broadening of the distance between CVP-1 and CVP-2 (two upper vacuoles) has been found in the individuals with a higher total number of kineties. However a considerable stability of the five-kinetal distance of induction between these CVPs is manifested in majority of individuals.

The variability and the most frequent pattern of the kineties number of the right, median and left sector of ciliature were determined.

An attempt was made to follow the mechanism of regulation of CVPs topography in dividers.

STRESZCZENIE

W orzęsku *Chilodonella cucullulus* (O.F.M.) badano zależności między liczbą ogólną kinet a położeniem otworków wodniczek tętniących. (CVPs)

Stwierdzono pewną tendencję do zwiększenia się liczby kinet między CVP-1 a CVP-2 (między otworkami przednich wodniczek tętniących) w osobnikach o zwiększonej liczbie ogólnej kinet. Jednakże dominował stały wzór pięciokinetalnego rozstępu indukcji między tymi wodniczkami.

Zbadano zmienność liczby kinet w prawym, środkowym i lewym sektorze ciliatury i określono najczęstsze wzory orzęsienia. Podjęto również próbę prześledzenia mechanizmu regulacji wzoru rozmieszczenia CVPs na osobnikach dzielących się.

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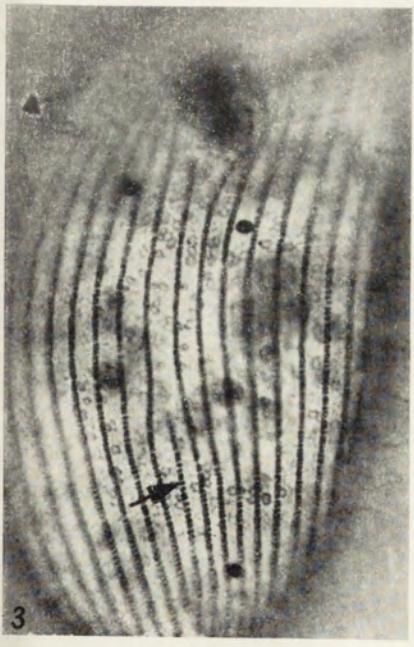
EXPLANATIONS OF PLATE I

1: Ciliature of the ventral surface of *Chilodonella cucullulus*. Four-vacuolar individual (opisthe) with enlarged distance between CVP-1 and CVP-2. Chatton-Lwoff silver impregnation.
2: Ciliature of the ventral surface of *Chilodonella cucullulus*. The case of reduced distance between CVP-1 and CVP-2. Photomicrographs made from the dorsal side so the system is inversed. Chatton-Lwoff silver impregnation.
3: Individual with one of the postoral kineties underdeveloped. Ventral surface of *Chilodonella cucullulus*. Chatton-Lwoff silver impregnation.
4: Ventral surface of divider of *Chilodonella cucullulus*. The case of changes in the pattern of CVPs topography during induction period. The changes in the right, 'conservative' system. Chatton-Lwoff silver impregnation.



D. Kowalska et J. Kaczanowska auctores phot.

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Department of Zoology, University of Delhi, Delhi-7, India

G. R. SAPRA and C. M. S. DASS

The cortical anatomy of *Stylonychia notophora* Stokes and morphogenetic changes during binary fission

Die corticale Anatomie von *Stylonychia notophora* Stokes und die morphogenetische Änderung während der Binarteilung

The development of the cortical structures preparatory to binary fission and during regeneration, in ciliate protozoa has been studied in a number of forms. Considerable diversity in this regard has been recorded (see reviews Tartar 1967, Hanson 1967). It is generally agreed that presence of the macronucleus is obligatory for the formation and early development of cortical structures; yet, sufficient attempts have not been made to establish any chronological correlation between the activities of the two. Katashima 1959, Wise 1965 a, and Diller 1966 made a correlative study of the two events during cojugation and asexual reproduction in *Euplotes*. Similar studies, demonstrating the relationship between macronuclear changes and cortical morphogenesis have been carried out in *Stentor coeruleus* (Tartar 1961, De Terra 1960) and *Urostyla* (Jerka-Dziadosz 1967). A more fruitful approach on these lines is possible in the hypotrichous ciliates (Wise, 1965 a), where the condition of the macronucleus can be designated precisely with reference to the course of Cell Cycle. It is possible to do so, due to the fact that, detailed information is now available about the synthesis of nucleic acids in the macronucleus of ciliates like *Euplotes* (Prescott 1967; Review) and *Stylonychia* (Dass and Sapra 1965; unpublished data). In the present paper, morphogenetic activity of the various primoridia of the cortex and their relationship with the macronuclear activity during the course of binary fission, in *Stylonychia notophora* Stokes, has been reported.

Material and methods

Stylonychia notophora, used in the present study, belonged to the clone E, isolated from mass culture obtained from Okhla, near Delhi. The cultures of the ciliate were maintained in hay infusion periodically fortified with Horlick's malted milk. The taxonomic position of the animal was ascertained by its body measurements, distribution of the cirri on the ventral surface and by its nuclear cytology. In all these respects, the animals corresponded closely to Kahl's description of this species (see Kahl 1935). Animals during logarithmic phase of growth were fixed and used for silver impregnation studies.

Silver impregnation

Various techniques were employed to reveal the cortical pattern of the ciliate, and of these, Corliss's 1953 wet silver impregnation technique proved to be the most satisfactory. Yet, in comparison to *Euplotes*, *Stylonychia* did not yield good preparation by this technique. However, by changing the concentration of silver nitrate from 3% as suggested by Corliss 1953 to 10% and also lengthening the time of exposure to sunlight for 4—5 hrs., satisfactory results were obtained. After dehydration, the specimens were cleared in xylene for 2 hrs. and mounted in DPX neutral mountant. Preparations thus obtained clearly showed the presence of replication bands in the macronuclei as well as densely argentophilic bases of the cirri and the cilia. Although such preparations did not show the micronuclear division stages clearly, it was, however, possible to assess them accurately by locating the position of the replication bands in the macronuclei (Dass and Sapra 1965). This system, therefore, afforded a precise correlation between the development of various primordia of the cortex and the corresponding macro- and micronuclear division stages. In addition, living animals were immobilized in methyl cellulose solution and observed under phase-contrast microscope to determine the extent of formation of new adoral zone of membranelles of the opisthe in relation to the position of the replication band in the macronuclei.

Observations

Salient features of the cortical anatomy of non-dividing *Stylonychia*

An interesting feature of the pellicle of *Stylonychia notophora* is the complete absence of silverline-network or pellicular network in the dorsal, ventral as well as in the buccal cavity surface of the animal. Such an absence of silverline network has also been noted in *Oxytricha* by Hashimoto 1961 and in *Urostyla* by Jerka-Dziadosz 1963, 1965. Yet, silver line network is very prominent in all species of *Euplotes*.

The dorsal surface of the animal shows six rows of short cilia ("sensory bristles") which can be seen in the living animal by phase contrast microscopy. These rows are uniformly spaced and have varying number of cilia (Fig. 2). The bases of the cilia are argentophilic and those of the same row are interconnected by a faint argentophilic line. No connection between two different rows could be seen.

The ventral surface of the animal shows three categories of silver impregnated structures (Fig. 1, Pl. I 1). These are (a) the adoral zone of membranelles, (b) undulating membrane or paroral membrane and (c) characteristic but asymmetrically placed bases of the cirri.

The adoral zone of membranelles (AZM) consists of membranelles arranged in parallel rows and extends up to nearly one half of the total body length (Fig. 1, Pl. I 1, 2). The number of membranelles consisting AZM varies from 28—37 in different individuals, the most frequent number being 32—34. According to the terminology of Yocum 1918 used for the description of AZM in *Euplotes* (see also Wise 1965 a), this structure in *Stylonychia* can be divided into "collar" and "lapel" regions. The collar region of the AZM is

located anterodorsally in the animal and comprises of 7 membranelles. The lapel region of AZM starts at a place where the collar bends sharply towards the ventral side of the animal, extends longitudinally downwards and tapers to a narrow point at the cytostome. The largest membranelles are situated in the lapel region just before it turns dorsally to become the collar region. The group of membranelles shortest in size are found where the AZM ends near the cytostome. The number of ciliary rows and the kinetosomal arrangement in a single membranelle is not clearly seen in the light microscope using silver impregnation method. However, Roth 1957 in his electron microscopic study of *Euplotes* found that each membranelle had two rows of cilia with a third and a shorter row occasionally present.

The undulating membrane (or the paroral membrane) which appears as a drawn out membranous structure in living animals supports a field of cilia arranged in rows (Fig. 1 um). This membrane extends to more than half the length of the right side of AZM while one of the ends terminates near the cytostome. In silver stained preparations it appears as a uniformly stained membranous structure.

The bases of the marginal cirri (mc) appear as rectangular silver depositions and are distributed along the right and left margins of the ventral surface of the animal (Fig. 1, Pl. I 1). The continuity of the marginal cirri along the margin of the ventral surface is broken by 3 caudal cirri (cc). The silver stained bases of the caudal cirri do not differ in any respect from those of the marginal cirri. The total number of marginal cirri in different specimens varies from 44 to 48, the most frequent number being 46.

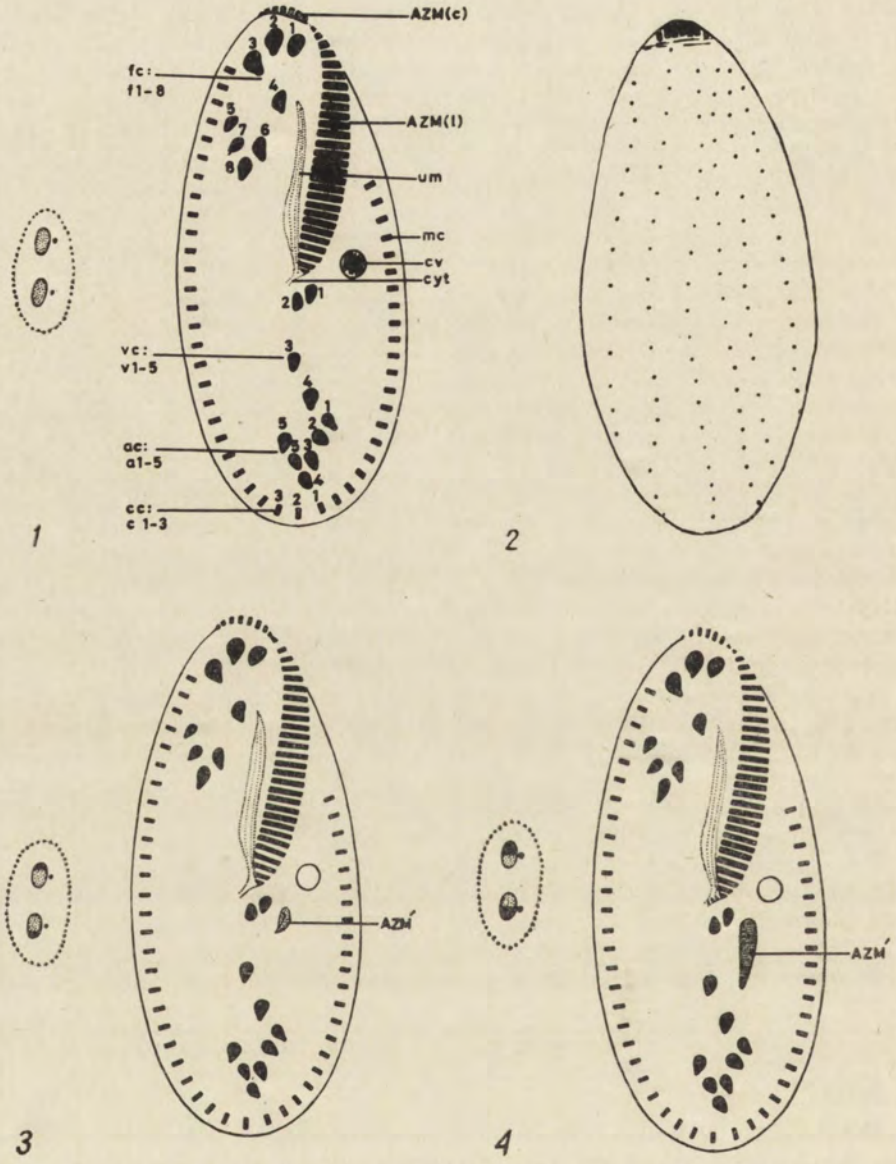
Another set of 18 cirri, is asymmetrically placed, yet exhibits a characteristic and constant pattern. Of the several hundred specimens examined, none showed any deviation in the relative positions of these cirri. The cirri have been classified into three categories on the basis of their position (Fig. 1). These are: 8 frontals (fc 1—8), 5 ventrals (vc 1—5) and 5 anals (ac 1—5). The silver impregnation methods do not reveal individual kinetosomes which collectively form the cirral base but electron microscopic studies of Roth 1957 on *Euplotes* and Chakraborty 1967 on *Oxytricha* clearly showed their composite nature — several cilia bound by a common outer sheath. The contractile vacuole pore is revealed by the deposition of silver and lies betwen the tapering end of AZM (1) and left margin of the animal.

Morphogenetic events preceding division

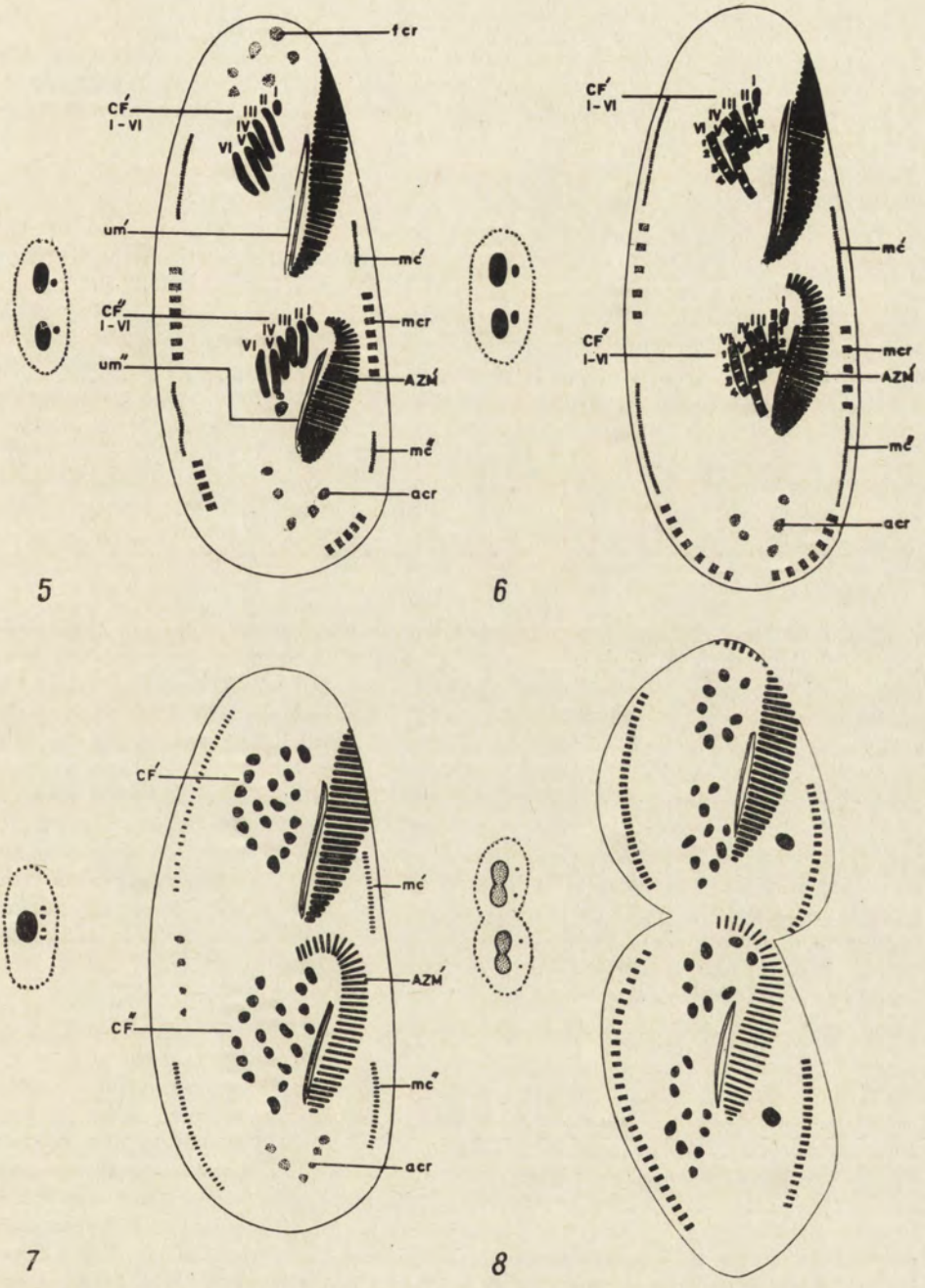
Stages of division

The division cycle has been divided into seven stages designated 0 through 6 on the basis of the appearance and progression of replication bands in the macronuclei and changes in the cortex.

In an animal preparing for division, four general categories of primordia may be distinguished (Fig. 5, Pl. I 3, 4, II 5) (1) The Oral Primordium destined to be the adoral zone of membranelles of the opisthe (AZM′), (2) Cirral Field I (CF′; CF″) consisting of two identical groups of differentiating regions, one lying anterior and the other posterior to the mid-line of the parent animal. (3) Cirral Field II (mc′; mc″) consisting of four sets of argentophilic stripes destined to differentiate into the marginal and the caudal cirri for the two sister-cells. They appear in groups of two, on each side of the animal.



Figs 1—8. Camera lucida drawings of the silver impregnated whole mounts of *Stylonychia notophora*. The nuclear stages corresponding to the cortical events are shown on the left side of the animal. In the macronuclei, the duplicated regions distal to the replication band are shown as dark areas while the unduplicated regions are marked with light stipple. 1 Ventral view of the animal in stage 0. 2 Dorsal aspect of the cortex of above animal. 3 Animal in stage II showing the presence of early appearance of oral primordium. 4 Late stage II. Kinetosomes aligned in definite membranellar pattern. 5 An animal in stage III showing silver stained primordial structures. 6 Stage IV animal. The cirral field I shows break up of the primordia into neo-cirri. Parental cirri (fcr; mcr; acr) start undergoing active resorption. AZM′ continues to expand. 7 Stage V animal showing expansion of neo-cirri in the longitudinal direction. 8 Animal in stage VI. Neo-cirri have come to occupy their relative position



AZM (c) — Collar region of adoral zone of mebranelles; AZM (1) — lapel region of adoral zone of membranelles; ac — bases of anal cirri; cc — bases of caudal cirri (numbered 1—3); cv — contractile vacuole pore; cyt — cytostome; fc — bases of frontal cirri (numbered f 1—8); mc — base of a marginal cirrus; um — undulating membrane of a vegetative individual

Stages 0 and I

Stage 0 represents the non-dividing or vegetative animal whose cortical anatomy has been described above. During stage I, replication bands appear at outer ends of the macronuclei but the cortex does not show any signs of morphogenetic activity.

Stage II

This stage is marked by the appearance of an oral primordium on the ventral surface (Fig. 3, Pl. I 3). The position of this primordium is fixed; it always arises at a specific position to the left of the lower end of the parent AZM. This primordium does not appear to be related to the existing AZM and is spatially separated from it. Its formation is preceded by the de novo appearance of a group of scattered kinetosomes which later organize into a cone-shaped area. It does not show any differentiation into membranelles etc. The pattern of origin of oral primordium corresponds to the characteristics of "de novo kinetosome stomatogenesis" Corliss 1967. The replication bands traverse 20—25% of the length of the macronucleus. No activity is observable in the micronuclei which lie in close proximity of the macronucleus.

Stage III

The AZM′ (new adoral zone of membranelles) increases in length and shows signs of differentiation in the form of parallel alignment of membranelles (Fig. 4). Primordia for the new undulating membranes develop from the posterior extremity of the AZM and AZM′ and increase linearly towards the right side of the animal (Fig. 20). The undifferentiated primordia for the cirral field I (CF′ and CF″) make their appearance, one anterior, and another posterior to the midline of parent cell. Each of these consists of 6 rows of undifferentiated densely stained argentophilic patches of varying size. Later the primordia of the cirral field II (mc′, mc″) also appear. They are in the form of fine argentophilic stripes, two on each side of the body along the margin. Afterwards, these stripes divide transversely into 30—40 closely packed segments of equal size. The replication bands at this stage have traversed nearly 60% of the length of the macronucleus. The micronuclei move away from the macronuclei and show an increase in volume.

Stage IV (Fig. 6, Pl. II 7)

With its further expansion the AZM′ is more or less completely developed with recognizable alignment of the membranelles. Three rows of cilia constituting new undulating membranes (um′, um″) for the proter as well as for the opisthe appear at this stage. These ciliary rows arise at the base of the cytostome and extend anteriorly on the right side of the AZM and AZM′. The cirral field I (CF′ and CF″) composed of undifferentiated argentophilic patches in stage III, now differentiates into distinct individual cirri. There is a slight asynchrony in the rate of differentiation between the anterior and posterior cirral field I. The posterior field generally differentiates earlier (Pl. II 7). The number of cirri (or "neo-cirri" as termed by Wise 1965 a for *Euplotes*) contained within rows I to VI are 1, 3, 3, 3, 4, 4 respectively. Similar differentiation occurs in the posterior Cirral Field I (CF″). Thus,

each group will contribute 18 new cirri, which will eventually be positioned as the frontals, ventrals and anals.

The replication band traverses 80—90% of the length of the macronucleus. The micronuclei are in distinct prophase. The marginal cirral field stretches further, so that the space between the individual cirri increases. In addition, there is increase in the dimensions of the each of marginal cirrus base.

Stage V (Fig. 7, Pl. II 8, III 9)

This stage is easily recognizable in living as well as silver impregnated individuals. It is marked by the fusion of two macronuclei after the replication bands have completely traversed the entire length of the macronucleus. The membranelles of AZM′ stain heavily with silver and attain the thickness comparable with those of vegetative individuals. AZM′ starts acquiring the definitive shape, particularly in the anterior region. There is further stretching of cirral fields, so that the cirri can now be classified into frontals, ventrals and caudals. The marginal cirri come to acquire the final shape and size of that of a vegetative individual. The mitotic division of the micronuclei is completed.

Stage VI (Fig. 8, Pl. IV 12—15)

As the morphogenetic processes leading to the formation of organelle complex for two individuals is already completed, the time spent in this stage is for the ultimate shaping of the AZM′ in relation to the advancing cytokinetic furrow. As division furrow deepens, the anterior part of AZM′ undergos a rotation of 180°, so that it comes to lie on the anterior dorsal edge of the opisthe, now becoming the collar region of the new AZM. Wise 1965 a has discussed the significance of a similar phenomenon in *Euplotes*. Division of the macronuclei is also completed by this time. The old cirri are also resorbed at the same time. This stage lasts for about 20 minutes.

Discussion

The study of morphogenetic processes in *Stylonychia notophora* preparatory to division, shows that like other hypotrichs (see review, Tartar 1967) the primordia for ciliature of the ventral side of the body arise in definite places strictly in relation to the existing cortical organelles. For instance, the anlage of the buccal apparatus arises from a group of kinetosomes which appear at a point strictly in relation to the existing AZM. Similarly, primordia for other cirri appear at specific sites. This leads to the question of the factors responsible for (a) the determination of primordial site, (b) developmental fate of a given primordium and (c) initiation and maintenance of development in question. To answer the questions raised above and to explain the control of morphogenesis in ciliates in general, two major hypotheses have been put forward. The "kinetosome hypothesis" (Lwoff 1950, Weisz 1954), held that kinetosomes were the basic units of the cortex which were capable of self-replication and responsible for the organization of the cortex through differentiation under the influence of their surroundings. On the other hand, according to Tatar's 1961 "pattern hypothesis" the existing cortical structures are responsible for the determination of site, initiation and

course of development. How far are these hypotheses applicable to *Stylonychia* in explaining the cortical morphogenesis of this organism?

In *Stylonychia* the ciliary rows of the dorsal surface resemble the somatic kineties of holotrichous ciliates. Therefore, the mechanisms involved in the ciliary increase may be similar to those controlling the production of cilia in simpler forms like *Paramecium* (Sonneborn 1963). The ventral surface of *Stylonychia,* however, presents a completely different picture. There is a precise arrangement of the cirri and membranelles which are asymmetrically placed; but the pattern is highly specific and reproducible during division and vegetative reorganization. The primordia for these structures appear at sites in specific relation to the existing structures. Even during starvation when *S. mytilus* undergoes repeated reorganizations (Dembowska 1938) the geometric relationship of the primordia to the existing topography of the cell is maintained. Regeneration experiments on *Stylonychia* (Dembowska 1925, Frick 1968) also show that after removal of the presumptive primordial site, the primordium later appears on the cortex of the operated animal. This shows that the site of the primordium formation is not irrevocable, but is determined in relation to the existing cortical pattern. Therefore, the concept of the pattern hypothesis is applicable to *Stylonychia* like in *Stentor* (Uhlig 1960, Tartar 1961) and *Blepharisma* (Suzuki 1957) to explain the determination of the primordium site.

The results of the present study indicate that kinetosomes of the anlagen of the various organelles arise without any relationship, direct or indirect, with the kinetosomes of the parental structures. In that event, the question of the origin of new kinetosomes arises. Lwoff 1950 and his school have stressed upon the genetic continuity of the kinetosomes. But recent studies have not provided any direct evidence for the self-replication of the kinetosomes (Tartar 1967). Moreover, this hypothesis does not explain the initial appearance of kinetosomes at the site of primordium formation in *Stylonychia* as noted in the present study, and in *Euplotes* (Wise 1965 a). To account for these exceptions, the proponents of the "Kinetosome hypothesis" predicted the presence of "cinetosome eratiques", scattered over the non-ciliated surface of the cortex of hypotrichs (Chatton et Sequela 1940). However, such scattered kinetosomes could not be demonstrated in ciliates like *Euplotes patella* and *E. eurystomus* (Chatton 1942). In such cases, the above authors noted that the kinetosomes were derived from the "argentophilic substance" of the silver line network, a view originally proposed by Klein 1936. This view though useful in accounting for the origin of kinetosomes in *Euplotes* and many other hypotrichs, is untenable in *Stylonychia* where the silver line network is completely absent.

The demonstration by Seaman 1960 and Argetsinger 1965 of the presence of DNA, RNA, proteins and a large variety of enzyme systems in *Tetrahymena* kinetosomes is of considerable interest. Similarly Smith-Sonneborn and Plaut 1967 have provided evidence for the presence of DNA in the pellicle of *Paramecium.* Sukhanova and Nilova 1965 have reported that kinetosomes of *Opalina* are capable of incorporating labelled thymidine into DNA and labelled uridine into RNA. From these observations, kinetosomes may appear as autonomous and self-generating entities; they may yet be subject to the control of macronucleus for regulative or modulating influence regarding the appearance, development and growth of cortical primor-

dia. The interrelationship between the cortical activity and macronuclear activity is obvious, from the present study in *Stylonychia* where the formation of cortical primordia is always preceded by the appearance of replication bands in the macronucleus. Such an interrelationship of the two events has already been reported in *Euplotes*. (Wise 1965 a, Diller 1966). Supporting evidence comes from numerous regeneration studies on various hypotrichs (Dembowska 1925, Yow 1958, Hashimoto 1961). Any injury to the cirral bases of these cells induces them to discard the existing ciliary organelles and form a new set of organelles. The process of vegetative reorganization is strikingly similar to the process of division reorganization with the difference that only one set of primordia is formed in the former instance (except in *Euplotes*; Yow 1958). Nevertheless, the behaviour of the macronuclei in respect of the formation and migration of replication bands during regeneration is similar to that seen during binary fission (However, see Yow 1961). Additional evidence that these two processes are related comes from the experiments of Wise 1965 b on *Euplotes*. It was observed by this author that irradiation of the primordial cortical organelles results in the resorption of all primordial structures and this is associated with the arrest or disappearance of macronuclear replication bands. A similar effect was obtained in *Stylonychia*, where mitomycin C causes the arrest of progression of the replication bands and with this the cortical morphogenesis is also inhibited, resulting in the blockage of division (unpublished). In fact, completion of cortical primordium development nearly coincides with the end of progression of replication bands in the macronuclei. This stage, actually has been indentified as the "stabilization point" in the division cycle of the animal (Dass and Sapra 1968). Moreover, there is further evidence to suggest that the substances (RNA) responsible for the primordium development, are released from the macronucleus, prior to the condensation of the macronucleus (Dass and Sapra 1966, Jerka-Dziadosz 1967), a stage which follows the replication phenomenon. What could be the significance of appearance and progression of replication bands in cortical morphogenesis?

Cytochemical studies on *Stylonychia* and autoradiographic studies on *Euplotes* (Prescott and Kimball 1961) have indicated that as the replication band traverses through the macronucleus, previously synthesized RNA is not detectable in the regions distal to the replication bands. The fate of this RNA has not been followed. As suggested by Wise 1965 a this RNA could well be a functional trascription for the specific proteins required for the initiation and development of the cortical primordia. The proposition finds support by observation in *Tetrahymena* that synthesis of DNA-dependent RNA is a pre-requisite for the onset and maintenance of cortical development (Frankel 1965, Whitson and Padilla 1965, Nachtway and Dickinson 1967). In that case the release of RNA in stages as marked by the structural modifications in the macronuclei of *Stylonychia* may be indicative of necessary templates for the development in question, before and during the process.

Summary

Cortical morphogenesis of *Stylonychia notophora* was studied in relation with nuclear activity, during the fission cycle. The pattern of morphogenesis is in conformation with that recorded for the hypotrichida. The course of

cortical development is closely related with the macronuclear activity and the end of development nearly coincides with the termination of DNA synthesis in the macronuclei. From these results it is concluded that stomatogenesis in *Stylonychia* is of the de novo kinetosomal type (Corliss 1967). Further, the initiation and maintenance of morphogenetic activity of the cortex in relation to the appearance and progression of the replication bands in the macronuclei is discussed.

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ZUSAMMENFASSUNG

Es wurde die corticale Morphogenese von *Stylonychia notophora* Stokes in dem Verhältnis zur Kerntätigkeit während des Teilungszyklus untersucht. Das Muster der Morphogenese entspricht dem Verlauf dieses Prozesses bei den Hypotrichen. Der Gang der Corticalentwicklung ist streng mit der Tätigkeit des Makronucleus verbunden, und das End der Entwicklung übereinstimmt fast mit dem Schluss der Synthese von DNA in dem Makronucleus. Auf Grund dessen wurde festgestellt, dass die Stomatogenese bei *Stylonychia* einen "de novo" Kinetosomaltyp (Corliss 1967) darstellt. Auch der Beginn und das Benehman der morphogenetischen Aktivität des Cortecs im Zusammenhang mit der Erscheinung und dem Fortschreiten der Replikationsbänder in dem Makronucleus wurde besprochen.

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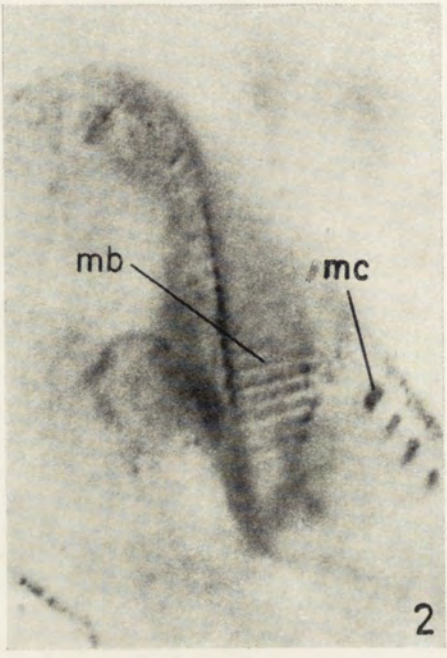
EXPLANATION OF PLATES I—IV

Stylonychia notophora, photomicrographs of silver stained specimens

1: Animal in stage 0. Ventral surface of the cortex showing bases of various cirri. ×750
2: Part of AZM at a higher magnification of an animal in stage 0. mb — membranellar band; mc — base of a marginal cirrus. ×1100
3: Early appearance of the oral primordium (AZM′) marking the beginning of stage 2. ×750
4: Animal in stage 3. Note the differentiation of membranelles in AZM′ and primordia of cirral field I (CF′; CF″) in early stages of differentiation ×750
5: Animal in stage 3. The undifferentiated primordia of the cirral field I (CF′ and CF″) and cirral field II (mc′ and mc″) can be seen. ×750
6: Cirral field I of the above stage at a higher magnification. Note the early indications of the division of primordia into individual cirri (I—VI). ×1500
7: An animal in stage 4 showing the division of cirral field I primorida into individual cirri (arrow). The synchrony between the anterior and posterior field is not exact. ×1 500
8: Animal in early stage 5 showing the complete break up of cirral field I primordia into individual cirri. ×900
9: Animal in late stage 5 showing the complete development of the bases of new organelles. New undulating membrane of the proter (um′) as well as that of opisthe (um″) is clearly visible. ×750
10, 11: Focus at higher magnifications on the new undulating membrane (um′) and part of the differentiated anterior cirral field I. Fig. 10 ×900; Fig. 11. ×1500
12: Animal in stage VI. Neo-cirri have almost assumed their final position. ×750
13: AZM′ in the process of acquiring final shape during cytokinesis. ×750
14: Above animal at a different focus showing final stages of macronuclear division. ×750
15: Opisthe at the end of division. ×750

1



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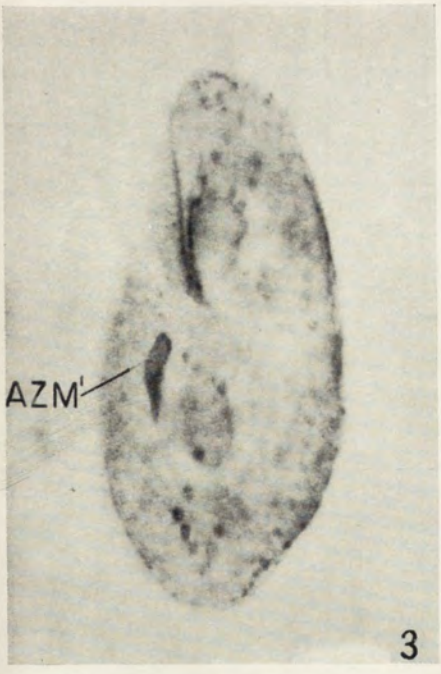

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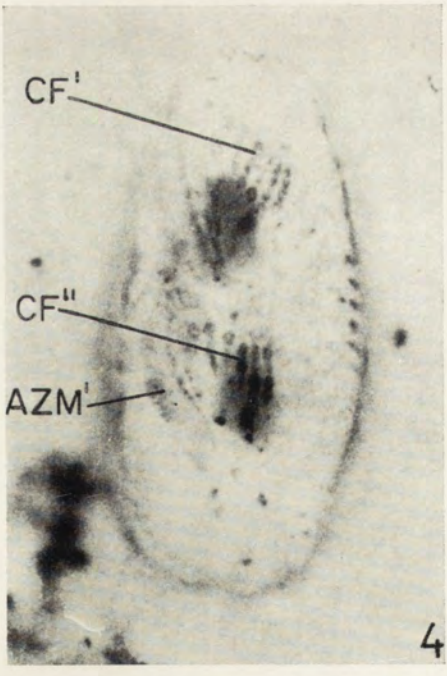

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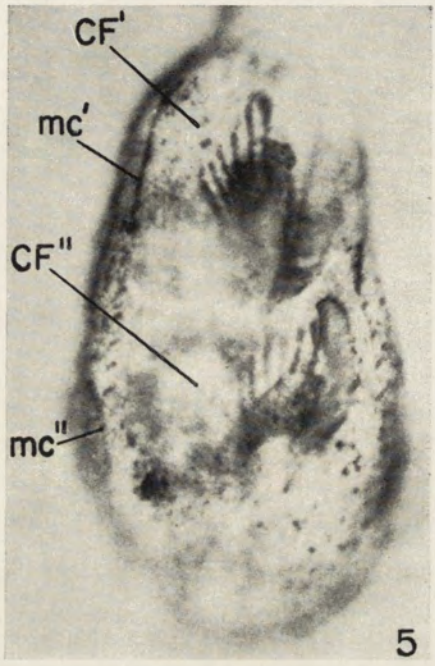

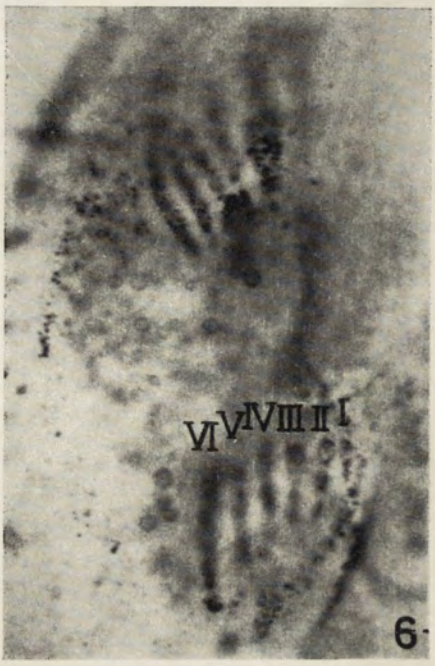

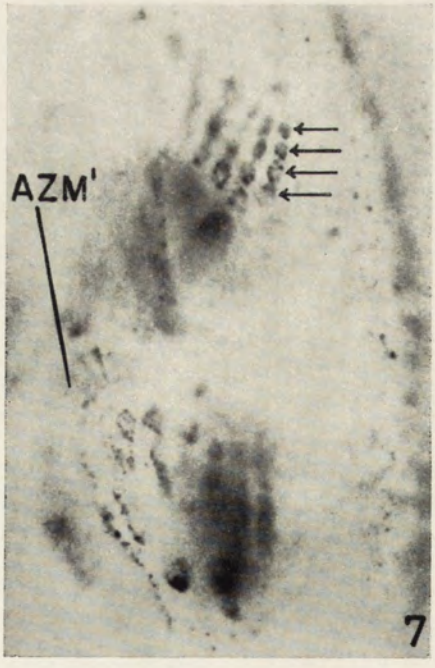

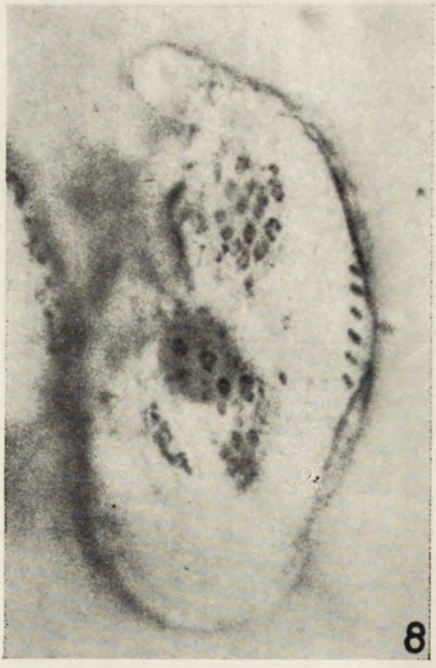

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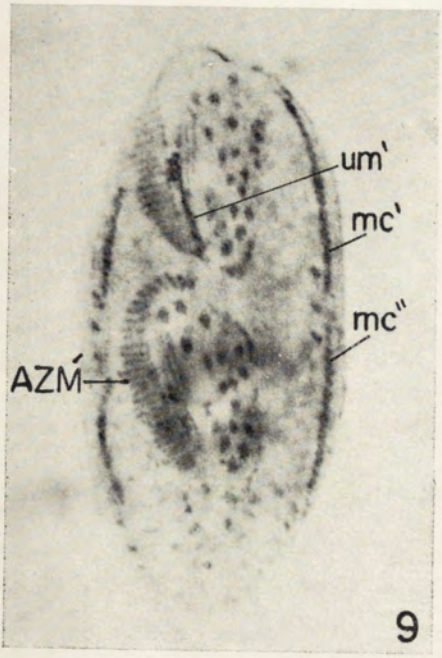

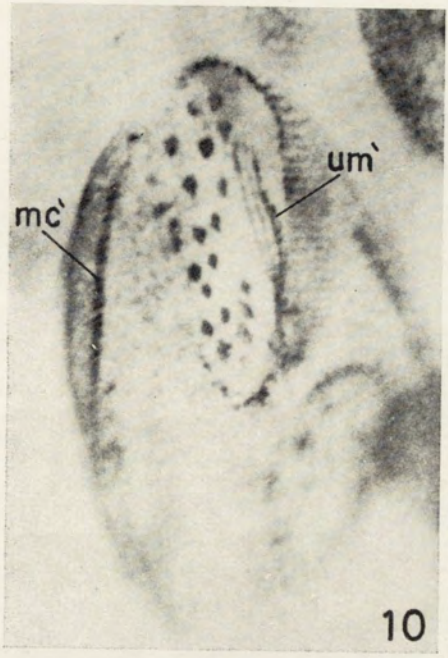

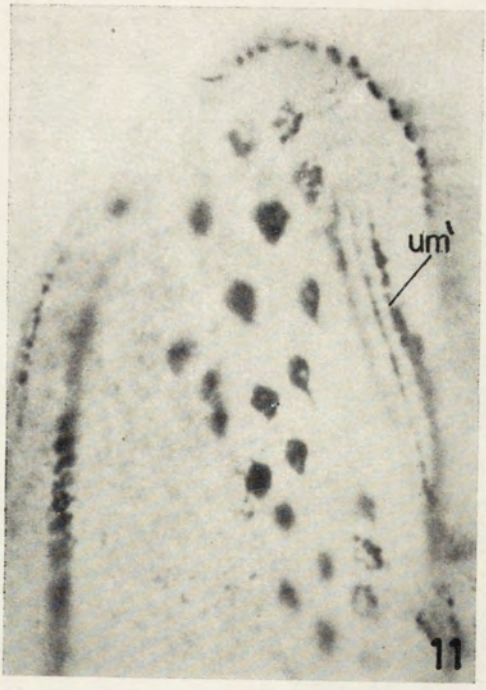

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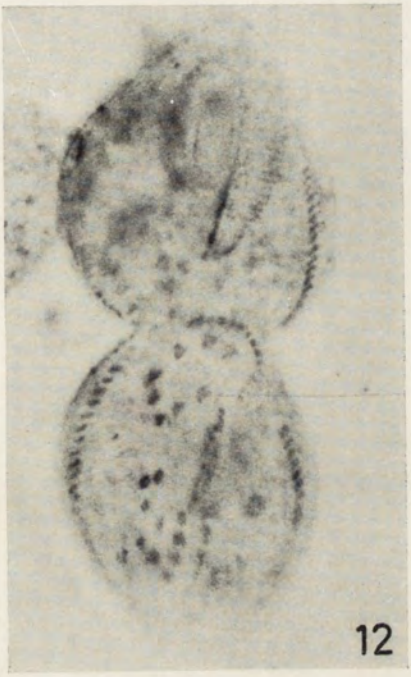

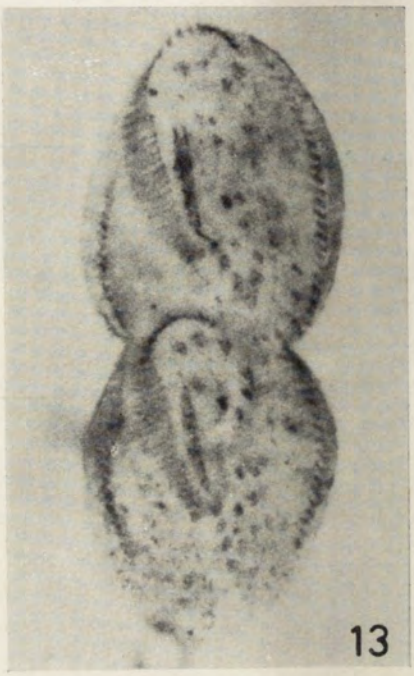

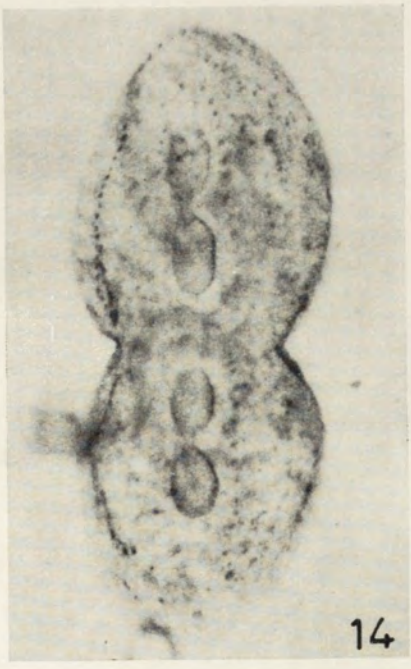

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Department of Zoology, University of Warsaw, Warsaw 64, Poland

Andrzej KACZANOWSKI

Morphological studies on Opalinids I. Staining and fragmentation of the pellicle of *Opalina ranarum*

Badania morfologiczne nad *Opalinata* I.
Barwienie i fragmentacja pellikuli *Opalina ranarum*

The cortex of opalinids consists of longitudinal pellicular ribs and kineties. These structures were detailed studied in electron microscope (Pitelka 1956, Blanckart 1957, Noirot-Timothée 1958, 1959, 1966, 1967, Wessenberg 1966). Inside the pellicular ribs there are parallel microtubules. The falx is an area of close lying kinetosomes stretching along the front margin of the protozoan's body. Its fine structure was revealed by Wessenberg 1966.

The present study was aimed at:

1. Finding suitable silver-staining methods for further studies on the morphology and growth of opalinids.
2. Study of some mechanical properties of the pellicle (fragmentation).
3. Comparison of the structure of the falx area in the species studied, with the results of Wessenberg 1961, 1966 obtained for *O. virguloidea* and *O. obtrigonoidea*.

Material

The following opalinids were studied: *Opalina ranarum* from *Rana temporaria*, *Cepedea dimidiata* from *R. esculenta*, *Protoopalina caudata* and *P. intestinalis* from *Bombina bombina*. Besides the trophonts of all the mentioned species all the life cycle stages of *O. ranarum* were also investigated. Pellicle fragmentation was studied on the trophonts of *O. ranarum*, *C. dimidiata* and *P. intestinalis* the greater part of study being based on *O. ranarum*.

Methods

Whole specimens and pellicle fragments were examined. For the study of pellicle fragmentation and of the structure of the falx area washing out the inner cytoplasm with 1% digitonin after Child and Mazia 1956 was employed. Then whole specimens as well their fragments were silver stained. Two methods of staining were used:

1. The Wessenberg 1961 method (modification of that of Rio Hortega).

2. Protargol staining. For this purpose the protozoans were Bouin-fixed, and after having been washed embedded in gelatin after Chatton. Decolorizing in $KMnO_4$ and $H_2C_2O_4$ was ommitted. Well hardened but moist gelatin smears proved better than dried ones. Further procedure was made according Tuffrau 1967 including fixation of embedded smears in mixture of alkohol+formalin and alkohol. Some fragments were shadowed by chromonickel for electron microscope.

Results

Silver staining after Wessenberg of whole specimens reveals the kinetosomes and sometimes also the longitudinal pellicular ribs, in keeping with the results of Fernandez-Galiano 1947, or lower situated transverse striae. Protargol usually stains the longitudinal ribs and subpellicular striae (Pl. I 1—3). These striae were previously seen by some authors (Maier 1903, Metcalf 1909, Konsuloff 1922, Ten Kate 1927, Overbeek de Meyer 1929, Fernandez-Galiano 1947). They are similar to those observed by Sergeieva 1963 as a result of staining of *Opalina* for indication of the presence of acid phosphatase in the cell. Also the argentophilic subpellicular striae in *Opalina* correspond to the subpellicular bands composed of little membranous bodies visible in electron microscope (Pitelka 1956, Noirot-Timothée 1958, 1959, Wessenberg 1966) which recognized as micropinocytic vesicles by Noirot-Timothée 1966.

After protargol staining the kineties and the falx are visible against the background of the darker pellicle as light lines and zones. Usually the kinetosomes remain undarkened while the interkinetal zones are already heavily stained. Thus the protargol-stained opalinids are negative very clear pictures of kinetome, but particular kinetosomes often can not be discerned (Pl. I 1, 2). These pictures are very useful for considerations of the growth of the body surface and are similar in all examined opalinids. The study of *O. ranarum* life cycle show the longitudinal pellicular ribs in all stages. These ribs were visible also inside the cysts in *O. ranarum* (protargol-stained) as well as in *C. dimidiata* (stained after Wessenberg). The transverse striae were observed in protargol-stained tomonts, tomits (progamonts), gamonts and protrophonts of *O. ranarum*. It is not clear whether they are also present in cysts and microgametes.

Phase contrast observations of digitonin ghosts similarly as silver staining reveal the longitudinal pellicle ribs, kinetosomes and cilia irrespective of the staining method used. The transverse striae are absent in digitonin treated material showing that not being fibrillar in structure they are washed out. The following observations confirm this view. Live protozoans, placed in the extracting fluid consisting of 0.01% digitonin, 0.12 M KCl, 0.003 M EDTA die almost instantly. Some cytoplasm content is than washed out and they undergo spontaneous fragmentation. These fragments were immediately examined with a phase-contrast microscope. The transverse striae were then visible similarly as in living specimens in contrast with the fragments obtained after Child and Mazia 1956. Ghosts protozoans fragment readily under the influence of simplest factors like pressing cover glass, drawing into fine pipette etc.

The pellicle most easily fragments along the kineties into longitudinal bands. Each band consists of one kinety running along its edge and of longi-

tudinal pellicular ribs situated between this kinety and the next one (Pl. I 4, 5). In further fragmentation isolated kineties can be obtained, next the pellicular ribs separate from each other. Such a single kinety retains its integrity (does not fall into separate cilia) and rigidity (Pl. I 6). A band of pellicle deprived of its kinety is not rigid and easily becomes transversaly folded (Pl. I 7, 8). Separated pellicular ribs becomes easy twisted (Pl. I 7, 9). These observations were confirmed by electron-microscopic investigation of shadowed pellicle fragments. The pellicular ribs appear as flat bands (Pitelka 1956). Pl. I 10 shows the twisted pellicular ribs separated on some sections of their length. The foregoing implies that those are the kineties which are responsible for rigidity of the pellicle.

A different fragmentation pattern was obtained sometimes in half-dried smears of *Cepedea* after protargol staining. At the surface adhering to the glass, kineties and separated thick bands between them formed by joined pellicular ribs are visible (Pl. I 11).

Further observations of pellicle fragments with preserved falx area shown:

1. Closely set of short rows consisting 5—6 kinetosomes on the average (Pl. I 12), the falx is narrowing towards both the dorsal and ventral body margins to 1—2 kinetosomes.

2. Above kinetosomes a fine reticulum of small meshes is visible resembling the argyrom of ciliates.

This reticulum is identical with the pellicular collars described by Wessenberg 1966 (Pl. I 13; the black dot on the photograph is an artifact). This confirms the role that while staining in the protozoan cells, the silver is deposited along the prominent ectoplasmic ridges. Examination of whole silver-stained specimens of *C. dimidiata* and *P. intestinalis* proved that in these species the falx is also the zone of densely lying kinetosomes, in according with the results of Fernandez-Galiano 1947. It was found, too, that the distribution of falx kinetosomes in *C. dimidiata* is identical with that of *Opalina*, i.e. that in the middle part of the falx short rows of 5—6 kinetosomes occur and the falx narrows greatly towards the ventral and dorsal body's margins. It may be presumed, therefore that the structure of the falx is all the species studied the same.

Discussion

The data presented confirm the view that various opalinid species exhibit the same structure of the somatic and of the falx pellicle. The absence of local differences in the cortex characteristic of particular species (as in the case with the buccal ciliature for example) offers difficulties for the systematics of opalinids within this group. The specific and generic differences consist in the general body proportions. These in turn are result from particular principles governing the growth of the cortical zone which is uniform in structure. Hence this point requires further research, as precise as possible.

Summary

Author presents his results on silver staining of pellicle and pellicular fragments of opalinids cells. For staining the protargol metod and Wessenberg method (the modification of Rio Hortega method) were used. The pellicle

most easily fragments along kineties into longitudinal bands. During further fragmentation kineties and pellicular ribs are separated. In the falx area kinetosomes and theirs pellicular collars were visible.

I wish to express my thanks to Prof. dr. Z. Raabe for his help during the course of work.

STRESZCZENIE

Autor przedstawia wyniki barwienia pellikuli opalin, oraz jej fragmentów. Preparaty barwiono metodą protargolową, oraz metodą Wessenberga, która jest modyfikacją metody Rio Hortega.

Stwierdzono, że pellikula najłatwiej fragmentuje się wzdłuż kinet na podłużne pasy pellikularne. Przy dalszej fragmentacji pasy te rozpadają się na poszczególne pasma pellikularne i kinety.

W strefie falksowej obserwowano kinetosomy i kołnierzyki pellikularne, które je otaczają.

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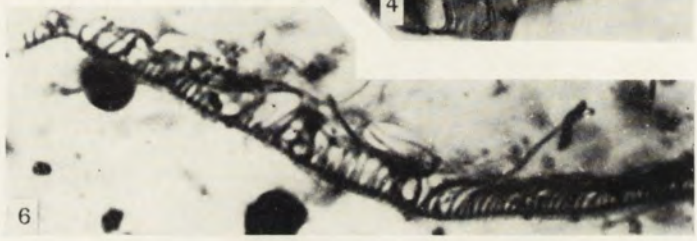
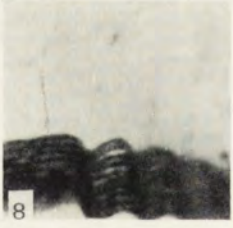
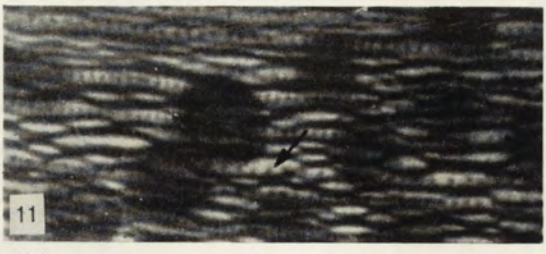
EXPLANATION OF PLATE I

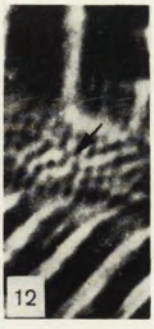
1: Protargol staining of *Opalina ranarum*. Kineties and falx area are visible as the light lines. Magnification ×2000
2: Protargol staining of *Opalina ranarum*. Pellicular ribs. Magnification ×2000
3: Protargol staining of *Protoopalina intestinalis*. Subpellicular striae. Magnification ×2000
4 and 5: Fragmentation of the pellicle of *Opalina ranarum* after protargol staining. Magnification ×1500
6: Isolated kinety. Digitonin treatment followed by staining after Wessenberg. *Opalina ranarum*. Magnification ×2000
7, 8 and 9: Folding and separation of the pellicular ribs after digitonin treatment and staining after Wessenberg. *Opalina ranarum*. Magnification ×2000
10: Splitting of the pellicular ribs in electron microscope after shadowing by chromonickel. *Opalina ranarum*. Magnification ×15000
11: Fragmentation of the pellicle of *Cepedea dimidiata* on the surface adhering to the glass after protargol staining. Magnification ×2000
12 and 13: Fragments of the falx area after digitonin treatment and Wessenberg's staining. Kinetosomes (12) and pellicular collars (13) are visible on this same fragment. *Opalina ranarum*. Magnification ×3300

The arrows in Figs 11 and 12 show the kinetosomes









A. Kaczanowski auctor phot.

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Institute of Cytology of the Academy of Sciences of USSR, Leningrad, USSR

L. P. OVCHINNIKOVA

Variability of DNA content in micronuclei of *Paramecium bursaria*

Изменчивость количества ДНК в микронуклеусе *Paramecium bursaria*

In *Paramecium bursaria*, Chen 1940 a,b,c,d,e revealed several races differing in the size of their micronuclei and intensity of staining according to Feulgen. Small micronuclei contained lesser chromosomes than large ones. The smallest chromosome number Chen observed was 80 which he thought to be a diploid number. In other races the chromosome number ran up to several hundred which allowed the suggestion that these micronuclei were polyploid. It was difficult to prove their polyploidy since it was uncertain whether 80 was a diploid number.

Chen menaged to observe the way how *P. bursaria* races with polyploid micronuclei arise. Most often a difference in polyploidy of micronuclei was a results of anomalous behavior of pronuclei during conjugation.

By means of cytophotomeric studies it was shown (Cheissin et al. 1964) that in *P. caudatum* micronuclei of different lines vary in their DNA content. This difference proved to be rather considerable. The largest macronuclei with the highest DNA content were 8-fold greater in size than small nuclei containing insignificant amounts of DNA.

It is possible that in other ciliates micronuclei vary by the amount of DNA within a species. Ray 1958 has shown that in one of the clones of *Tatrahymena pyriformis* the micronucleus is tetraploid.

It was interesting to know whether the strains of *P. bursaria* vary not only in their chromosome number, but also in the content of DNA within natural populations, and, in case differences exist, to determine the role of conjugation in their origin.

Material and methods

Seven strains of *Paramecium bursaria* were used for the present study. Strains 3R, 7R and 8R (4 19/8, 11 16/9 and PG VIII, according to Bomford) belong to the mating types III, VII and VIII. These strains were taken from water basins of Edinburgh (Scotland). Strains 4R and 5R belong to the mating types IV and V. They were obtained by crossing PG (VIII) and PK (V) (Bomford 1966, 1967 a). The four strains were kindly placed at our disposal by Dr. Bomford. We took two subcultures from 5R and designated them respectively as 5R1 and 5R2.

Strains 5D and 14D belong to the mating types I and II. They were collected in the basins in Peterhof in the vicinity of Leningrad. All the seven strains of *P. busaria* belong to syngene 4. Bomford attributed strains 5D and 14D also to syngene 4 and determined their mating types during his work at our laboratory.

All the paramecia were cultivated on the lettuce media at room temperature. *Aerobacter* was used as food.

To obtain conjugation equal quantities of ciliates were poured together on the 4th day after feeding. This procedure caused an immediate agglutination and formation of pairs. Each crossing yield 50 pairs. Each pair was placed in a microaquarium marked by an ordinal number. To avoid a possible abortive conjugation we used only those pairs which had been swimming for no less than 24 hours (Chen 1946, Bomford 1967 b).

After the separation of pairs the exconjugants were placed in microaquaria designated by the number of a corresponding pair of conjugants to which the number 1 and 2 were added.

For photometry we used paramecia of all the survived pairs of exconjugants. Paramecia from newly obtained clones were fixed after Nissenbaum's and stained according to Feulgen's procedure (hydrolized 6 min.). 50 representatives of each clone were measured.

The DNA content of macronuclei (Ma) and micronuclei (Mi) was measured on a microspectrophotometer MUF-4 by the scanning method in the monochromatic green light (line 546 mμ). The photometric and subsequent treatment of the experimental material were described in our previous publications (Ovchinnikova and oth. 1963, Cheissin and oth. 1963, Raikov and oth. 1963).

The DNA amount (Q) and the areas (S) of Ma and Mi were determined in paramecia in both the presynthetic and postsynthetic periods. However, for the analysis of changes in the DNA content in Ma and Mi we used only those data which were in agreement with the presynthetic Ma and Mi. This method is based on the works of Raikov and oth. 1963 and Cheissin and oth. 1964. The DNA amount (Q) is expressed in arbitrary units and the area (S) in $cm^2 \times 10^{-6}$. The ratio of DNA quantity in Ma and Mi is defined as $\frac{Q_{Ma}}{Q_{Mi}}$.

The writer is indebted to the deceased prof. Cheissin for his guidance and assistance, to Dr. Bomford who supplied us with strains and assisted us in our work, and also to T. D. Vlasova for her technical help.

Results

Micronuclei of initial strains

The DNA content in the micronucleus was determined in strains used for conjugation. Some of them had micronuclei with small amount of DNA, while the others, on the contrary, contained large micronuclei with a high DNA content.

By the increasing DNA content in Mi all the strains can be arranged as follows:

1	strain	8R	— 0.109 arb. units	5	strain	14D	— 0.183 arb. units
2	„	3R	— 0.118 „ „	6	„	4R	— 0.183 „ „
3	„	5D	— 0.156 „ „	7	„	5R1	— 0.199 „ „
4	„	5R2	— 0.176 „ „	8	„	7R	— 0.309 „ „

Hance, the quantity of DNA in the original strains vary from 0.109 to 0.309, i.e. about three times. Out of 8 strains one may single out three groups with (1) small (0.109—0.118 arb. units), (2) average (0.156—0.199 arb. units), and (3) great (0.309 arb. units) amount of DNA in Mi.

Individuals with both identical and different DNA content in Mi participate in the process of conjugation. Of all conjugations strains, strains 5D and 14D were found to be similar by their DNA content in Mi (Table 1). These strains belong to the second group with an average amount of DNA in Mi. Other pairs of conjugating strains belong to the first and second groups differing more than the pairs 5D×14D. Thus 3R×5R1 (0.118 and 0.199 arb. units, Table 2), 8R×5R2 (0.109 and 0.176 arb. units, Table 3) and 8R×14D (0.109 and 0.183 arb. units, Table 4) were found among conjugating pairs. The strongest differences in the DNA content in Mi were revealed in a pair with one conjugant (strain 7R) belonging to the third group (0.309 arb. units), and the other (strain 4R), to the second group (0.183 arb. units) (Table 5).

Macronuclei of initial lines

By their DNA content in Ma all the strains are similar with only one exception of the strain 7R containing a larger amount of DNA than other strains.

Dna content in all the strains vary from 2.04 arb. units to 2.90 arb. units and only in the strain 7R Ma contains 3.29 arb. units of DNA. In all cases this quantity of DNA is determined approximately by 3—4 mitoses.

Changes of DNA content in Mi in different clones of exconjugants

a. First we will consider changes in the DNA content of Mi in clones grown from exconjugants of the strains 5D and 14D similar in the DNA content of micronuclei. Five pairs of exconjugant clones were studied in detail. The remaining 45 pairs of these clones died yielding no mass cultures. Of interest is the fact that almost in all the clones the DNA content in Mi was lower than in parental forms, and in one clone only it more or less coincided with that in the parents.

Among parental pairs we may single out three groups of ciliates differing in the DNA content in Mi. The strains 5D and 14D belong to the second group with an average DNA amount in Mi. However, the exconjugant clones obtained by the DNA content in Mi should be attested to the first group with the lowest DNA content. In these clones the amount of DNA in Mi varied from 0.084 to 0.142 arb. units. One exconjugant clone had no micronucleus. As can be seen from Table 1, in each pair of exconjugant clones the DNA content in Mi is similar.

b. Progeny obtained after the conjugation of infusoria from strains of the first and second groups (3R×5R1, 8R×5R2 and 8R×14D) showed a diversity in the DNA content in Mi (see Tables 2, 3 and 4). Out of 26 exconjugant clones 11 had low DNA content i.e. they belonged to the first group. The DNA amount of these clones varied from 0.096 to 0.125 arb. units. The second group with the DNA content of Mi varying from 0.140 to 0.190 arb. units included the same number of clones.

Table 1

Changes of DNA content in Ma and Mi obtained after conjugation of strains 5D×14D in *Paramecium bursaria*

Designations	Q_{Ma}	Q_{Mi}	S_{Ma}	S_{Mi}	$\frac{Q_{Ma}}{Q_{Mi}}$
5D	2.59±0.198	0.157±0.014	0.040±0.007	0.0031±0.0003	16.8
14D	2.90±0.250	0.183±0.015	0.041±0.003	0.0036±0.0002	15.8
9_1	2.48±0.181	0.084±0.009	0.042±0.002	0.0023±0.0001	29.5
9_2	3.54±0.192	—	0.045±0.003	—	—
6_1	2.35±0.175	0.112±0.011	0.037±0.002	0.0026±0.0006	20.98
6_2	2.41±0.230	0.088±0.021	0.037±0.002	0.0028±0.0002	27.4
3_1	2.03±0.046	0.106±0.010	0.032±0.002	0.0032±0.0003	19.2
3_2	1.87±0.330	0.095±0.001	0.030±0.003	0.0030±0.0003	19.7
7_1	3.24±0.320	0.111±0.010	0.044±0.004	0.0026±0.0008	29.0
7_2	3.26±0.370	0.127±0.013	0.044±0.001	0.0027±0.0002	25.7
1_1	2.30±0.155	0.142±0.007	0.030±0.003	0.0030±0.0002	16.2
1_2	1.96±0.129	0.128±0.009	0.027±0.001	0.0026±0.0002	15.3

Table 2

Changes of DNA content in Ma and Mi after conjugation of strains 3R×5R1 in *Paramecium bursaria*

Designations	Q_{Ma}	Q_{Mi}	S_{Ma}	S_{Mi}	$\frac{Q_{Ma}}{Q_{Mi}}$
3R	2.40±0.31	0.118±0.007	0.031±0.0038	0.0026±0.0002	20.3
5R1	2.81±0.42	0.199±0.012	0.038±0.0031	0.0044±0.0003	12.7
17_1	3.38±0.35	0.212±0.015	0.049±0.0029	0.0043±0.0003	15.9
17_2	3.60±0.46	0.362±0.009	0.044±0.0066	0.0055±0.0004	9.9
29_1	2.38±0.05	0.096±0.007	0.039±0.0020	0.0027±0.0002	24.8
29_2	4.85±0.32	0.226±0.019	0.060±0.0045	0.0054±0.0003	21.5

Only in 4 clones the DNA content in Mi was more than 0.2 arb. units (0.212—0.362 arb. units). In the clones of exconjugants from one pair both similar and strongly differing indices of the DNA content were found. Thus there occurred pairs of clones one of which contained 0.074 arb. units of DNA in Mi, while the other, 0.220 arb. units. In other case one clone contained 0.096 arb. units, and the other, 0.226 arb. units. By their DNA content these clones were found to belong to the first and third groups, whereas parental strains which differed less, belonged to the first and second groups. If in these clones the difference was considerable, in other clones it did not exceed the limits of the first two groups (Table 2, 3, 4).

c. Progeny obtained after the conjugation of ciliates from strains of the second and third groups (7R×4R) were found to belong to the same second and third groups. Out of 10 clones 4 contained more than 0.200 arb. units of DNA in Mi and in 6 clones this amount varied from 0.154 to 0.198 arb. units. In 9 out of 10 cases (except $26_{1,2}$) exconjugant clones contained almost the same quantity of DNA in Mi.

Table 3

Changes of DNA content in Ma and Mi after conjugation of strains 8R×5R2 *Paramecium bursaria*

Designations	Q_{Ma}	Q_{Mi}	S_{Ma}	S_{Mi}	$\frac{Q_{Ma}}{Q_{Mi}}$
8R	2.42±0.87	0.109±0.0054	0.035±0.0037	0.0031±0.0003	22.2
5R2	2.65±0.19	0.176±0.012	0.029±0.0051	0.0033±0.0003	16.99
12_1	1.80±0.21	0.101±0.0089	0.022±0.0025	0.0025±0.0002	17.8
12_2	2.37±0.18	0.131±0.012	0.033±0.0021	0.0028±0.0002	18.0
5_1	1.52±0,21	0.125±0.011	0.027±0.0023	0.0031±0.0002	12.2
5_2	1.35±010	0.122±0.012	0.024±0.0021	0.0029±0.0002	11.06
8_1	2.13±0.13	0.144±0.011	0.034±0.0019	0.0028±0.0002	14.79
8_2	2.70±0.59	0.154±0.0035	0.037±0.0025	0.0032±0.0002	17.53
16_1	2.54±0.212	0.167±0.0097	0.037±0.0019	0.0032±0.0002	15.24
16_2	1.48±0.141	0.117±0.009	0.029±0.0029	0.0027±0.0003	12.65
6_1	2.09±0.19	0.074±0.0096	0.036±0.0029	0.0023±0.0002	28.24
6_2	2.26±0.24	0.220±0.017	0.033±0.0031	0.0039±0.0004	10.27

Table 4

Changes of DNA content in Ma and Mi after conjugation of strains 8R×14D *Paramecium bursaria*

Designations	Q_{Ma}	Q_{Mi}	S_{Ma}	S_{Mi}	$\frac{Q_{Ma}}{Q_{Mi}}$
8R	2.42±0.87	0.109±0.0054	0.035±0.0037	0.0031±0.0003	22.2
14D	2.90±0.25	0.183±0.015	0.041±0.003	0.0036±0.0002	15.84
2_1	1.77±0.10	0.118±0.0024	0.031±0.002	0.0026±0.0002	15.00
2_2	2.23±0.18	0.152±0.0098	0.031±0.003	0.0031±0.0002	14.67
8_1	1.93±0.19	0.121±0.0095	0.032±0.002	0.0031±0.0003	15.95
8_2	2.31±0.15	0.190±0.023	0.036±0.002	0.0040±0.0003	12.16
9_1	2.85±0.20	0.125±0.011	0.040±0.002	0.0028±0.0005	22.80
9_2	2.44±0.25	0.148±0.010	0.040±0.004	0.0034±0.0002	16.49
11_1	1.54±0.24	0.104±0.014	0.029±0.006	0.0023±0.0002	14.81
11_2	1.75±0.20	0.140±0.015	0.030±0.003	0.0029±0.0002	12.50
12_1	1.03±0.13	0.134±0.032	0.016±0.002	0.0031±0.0004	7.69
12_2	1.56±0.14	0.156±0.009	0.026±0.003	0.0033±0.0002	10.0
15_1	1.79±0.16	0.110±0.010	0.031±0.006	0.0025±0.0003	16.27
15_2	1.85±0.31	0.152±0.014	0.030±0.002	0.0030±0.0002	12.17

DNA content in macronucleus of exconjugant clones

In all exconjugant clones the DNA content in Ma varied less than in Mi. In the clones of exconjugants obtained from the conjugation of ciliates similar in the DNA content in Mi (5D×14D) we encountered macronuclei with varying DNA content. However, in all the cases it was found to be determined by 3—4 endomitoses. The variability of the DNA content in Ma ranged between

1.8 and 3.26 arb. units, and there was no relationship between the DNA content in Mi and Ma. In the lines in which Mi contained little DNA (0.084 arb. units), the DNA content in Ma was the same (2.48 arb. units), as in clones with greater DNA amount in Mi (0.142 and 2.30 arb. units).

The variability in the DNA content of Ma in clones obtained by conjugation of strains with different amount of DNA in Mi was approximately the same as in the first category of clones. The DNA content in Ma of these clones varied from 1.03 to 3.60 arb. units. In one of the clones Ma was particularly large and contained 4.85 arb. units of DNA. No relationship between the DNA content in Ma and Mi was revealed. In those exconjugant clones in which Mi contained little DNA (e.g. 0.074, 0.096, 0.1 etc.) the macronucleus had the same amount of DNA as the clones with a higher DNA content in Mi.

A certain shift toward an increase of the DNA content in Ma was observed in clones obtained from conjugants of the lines 8R×4R belonging to the second and the third groups according to the DNA content in Mi. In 10 clones Mi showed a high DNA content, while Ma of all these clones contained more DNA than the other clones (Table 5). The DNA content varied from 2.41 to 4.05 arb. units. In all cases, however, the DNA content in Ma was determined by 4 endomitoses.

Table 5

Changes of DNA content in Ma and Mi after conjugation of strains 8R×4R *Paramecium bursaria*

Designations	Q_{Ma}	Q_{Mi}	S_{Ma}	S_{Mi}	$\frac{Q_{Ma}}{Q_{Mi}}$
7R1	3.29±0.216	0.309±0.027	0.039±0.004	0.0044±0.0002	10.65
4R	2.04±0.205	0.183±0.016	0.034±0.007	0.0037±0.0004	11.15
5_1	2.41±0.034	0.174±0.013	0.034±0.003	0.0036±0.0002	13.85
5_2	2.25±0.296	0.173±0.013	0.034±0.002	0.0036±0.0005	13.0
14_1	3.42±0.259	0.189±0.005	0.051±0.006	0.0045±0.0002	17.30
14_2	2.67±0.259	0.154±0.009	0.041±0.004	0.0037±0.0006	17.34
26_1	3.27±0.30	0.241±0.018	0.041±0.002	0.0041±0.0003	13.57
26_2	2.78±0.21	0.172±0.010	0.037±0.003	0.0036±0.0003	13.60
28_1	3.05±0.207	0.198±0.016	0.039±0.003	0.0034±0.0006	15.40
28_2	3.13±0.068	0.226±0.015	0.039±0.003	0.0035±0.0003	13.85
29_1	3.38±0.033	0.290±0.018	0.052±0.002	0.0056±0.0002	11.65
29_2	4.05±0.249	0.243±0.019	0.051±0.004	0.0054±0.0010	17.61

Dimensions of micronuclei

The dimensions of micronuclei (Pl. I) containing different amounts of DNA sometimes appear to be identical. However, micronuclei comprising little DNA as a rule are lesser in size than the macronuclei rich in DNA. Whatever insignificant these differences might be they are nevertheless obvious. Thus the macronuclei containing:

0.074 arb. units had the area of 0.0023 $\times 10^{-6}$ cm^2
0.101 —„— 0.0025 „
0.112 —„— 0.0026 „
0.125 —„— 0.0028 „

But at the same time the macronuclei containing:

0.150 arb. units had the area 0.0031 $\times 10^{-6}$ cm^2
0.167 —„— 0.0032 „
0.174 —„— 0.0036 „
0.189 —„— 0.0045 „

The macronuclei of the third group with the DNA content exceeding:

0.200 arb. units had the area 0.0041 $\times 10^{-6}$ cm^2
0.212 —„— 0.0043 „
0.362 —„— 0.0055 „

Discussion of results

The data on the DNA content in Mi show that in result of the conjugation in different clones obtained from exconjugants one may observe a great diversity of micronuclei which seems to be caused by a difference in the behavior of pronuclei during conjugation. Sometimes one may observe unilateral migration of a pronucleus. In this case a synkaryon is formed in one of the conjugants due to the fusion of three pronuclei (two miggrating pronuclei and one stationary). The nuclear apparatus of the other conjugant is restored at the expense of one stationary pronucleus ("hemikaryon"). Four pronuclei may fuse if a part of "haploid" meiosis products do not undergo an ordinary degeneration and in one of the conjugants more than two pronuclei occur. Such anomalies in the koryogamy during conjugation lead to the formation of polyploid micronuclei (C h e n 1940 a, b, c). More often migrating pronuclei were found to exchange. In such case the DNA content in micronuclei of exconjugant clones is levelled.

The DNA content in Mi of different clones varies from 0.074—0.08 to 0.362 arb. units, and their areas range from 0.0023 to 0.0056$\times 10^{-6}$ cm^2. By the DNA content in Mi crossing strains are less diverse than their progeny. For example, the DNA content in Mi of conjugants was 0.109—118 arb. units, while in exconjugant clones it was much lesser. It can be suggested that the quantity of DNA in Mi equal to 0.074—0.08 and even to 0.10 arb. units should correspond to the "diploid" chromosome number. Such kind of clones were very few. Among the conjugants only the strain 8R proved to be "diploid" and yielded 4 such clones in progeny. Similar "diploid" clones (5) appeared among exconjugants as a results of crossing two strains, 5D and 14D (The second group of conjugants) and also by crossing strains 3R$\times$5R1 belonging to the first and second groups. One "diploid" clone resulted from them. However, out of 10 clones obtained from the conjugation of 7R$\times$4R containing Mi with a high DNA content not a single clone was "diploid". Hence, only 11 clones of 46, obtained from the conjugation of strains belonging to the first and second groups with small and average amount of DNA in Mi, may be regarded as "diploid".

Issuing from C h e n's observations and his suggestions concerning the anomalous karyogamy during cojugation one can easily explain the result we obtained in our experiments. When crossing the strains 7R2$\times$5R2, the

first of which is probably "tetraploid" and the second is "diploid", we can expect to obtain many "triploids" since in one conjugant pronuclei must be "haploid", whereas in the second — "diploid". If the nuclear apparatus of one of the conjugants is restored at the expense of one of the "diploid" pronuclei the progeny should be "diploid". In fact, in 26 clones the bulk of strains were "triploid" and only few "diploid" (4). Moreover, there were several "tetraploids" (5) which could arise from fusion of the stationary "haploid" pronucleus and two migrating ("haploid and diploid") pronuclei.

The crossing of two "tetraploid" strains 5D and 14D yields mainly "diploids" and very few "triploids". In this case, one might suppose that Mi could develop at the expense of hemikaryons only, though this supposition needs thorough proof.

The crossing of "tetraploid" and "octoploid" strains 7R1×4R results in the formation of "tetraploid" and "hexaploid" clones, as one can expect when analyzing the behavior of pronuclei. The combination of "diploid" and "tetraploid" pronuclei gives a "hexaploid" synkaryon, while restoration of the nuclei at the expanse of one "tetraploid" pronucleus gives accordingly a "tetraploid" line. Thus in the population of *P. bursaria* there are micronuclei differing in their ploidy. For these ciliates the species polymorphism is no less considerable than, in *P. caudatum*.

These data on the DNA content of Mi are in good agreement with Chen's observations about the polyploidy of Mi in *P. bursaria*. Now it is difficult to decide whether Chen's "diploid" races with 80 chromosomes are corresponding to our strains which are "diploid" in teir DNA content. It is important that micronuclei differ not only in the chromosome number, but also in their DNA content. Therefore, it may be suggested that Chen's races with small chromosome numbers correspond to our strains containing the smallest amount of DNA. If we arbitrarily designate the race Fd (according to Chen) as "diploid", the maximum degree of ploidy in other races would not exceed 8 n. The same with the DNA content. Assuming that the minimum quantity of DNA is 0.08—0.10 arb. units, the maximal degree of DNA ploidy should be also 8 n.

What is the ploidy of macronucleus assuming that a "diploid" Mi may contain up to 0.1 arb. units of DNA?

The relation of DNA content in Ma to the "diploid" DNA content in Mi varies from 15 to 30, and the degree of ploidy is found to be 30—60 n. No less than 3 or 4 endomitoses are required for the formation of necessary DNA amount in Ma. In our earlier work (Cheissin et al. 1964) the data on the ploidy of Ma in *P. bursaria* it was equal to eight, while in the work of Bomford 1967 b, to sixteen. It is evident that such low ploidy of Ma was obtained because we had not taken into account a possible polyploidization of Mi. Perhaps, we must check anew the degree of ploidy in Ma in *P. caudatum*, since the macronucleus in different lines was found to contain various amounts of DNA, and there was an 8-fold difference in the DNA content between the lines with small and large Mi.

Summary

The cytophotometric study of Mi in *P. bursaria* revealed the presence of different strains containing unequal amounts of DNA.

By the DNA content we may single out five groups of "diploid", "triploid", "tetraploid", "hexaploid" and "octoploid" clones.

Clones containing Mi with the lowest DNA content are designated as "diploid". Assuming that "diploids" comprise from 0.074 to 0.10 arb. units one must admit that "tetraploids" contain 0.16—0.20 arb. units. It is known that "triploids" usually contain about 0.12—0.15 arb. units. Micronuclei containing from 0.32 to 0.40 arb. units are regarded as "octoploids", and those with DNA ranging from 0.24 to 0.30 arb. units — "hexaploid".

As a result of conjugation between "diploid" and "tetraploid" strains, a great diversity of clones was obtained beginning from "diploid" up to "octoploid" ones.

The crossing of strains 3 n×2 n yielded clones with 2, 3 n (8 clones) and two with 4 n.

The crossing of strains with 2 n×4 n yielded clones with 3 n (9 clones), 2 n (2 clones) and with 4 n — one clone.

The crossing of strains 3 n×4 n resulted in the formation of one clone without a micronucleus, clones with 3 n (3 clones), with 2 n (7 clones), 4 n (2 clones), and with 8 n (1 clone).

The crossing of strains 4 n×8 n yielded 6 clones with 4 n and 4 clones with 6 n.

These data are in accord with Chen's observations on the polyploidy of Mi, and a most plausible explanation lies in the anomaly of karyogamy during conjugation.

Processes of polyploidization in Ma and Mi proceed independently.

In clones with "diploid", "triploid" and "tetraploid" Mi the DNA contents in Ma are similar, and the ploidy amounts 30—60 n. The latter is determined by 3 or 4 endomitoses.

РЕЗЮМЕ

Результаты цитофотометрии показали, наличие разных штаммов *Paramecium bursaria*, содержащих неодинаковое количество ДНК в Ми. Для сравнения использовались данные по содержанию ДНК только в пресинтетических Ма и Ми. В конъюгацию могут вступать особи как сходные по количеству ДНК, так и различающиеся. В результате конъюгации получилось разнобразие клонов по содержанию ДНК в Ми. При скрещевании штаммов 2 n × 3 n получились клоны с 2, 3 и 4 n. При скрещевании 2 n × 4 n получились клоны с 2, 3 и 4 n. При скрещивании штаммов 3 n × 4 n получились клоны без микронуклеуса, с 2, 3, 4 и 8 n. При скрещивании штаммов 4 n × 8 n получились клоны с 4 и 6 n.

Эти данные подтверждают наблюдения Чена о полиплоидности Ми и могут быть объяснены аномалиями кариогамии во время конъюгации.

Процессы полиплоидизации Ми и Ма протекают независимо друг от друга.

В клонах с ди, три и тетра „плоидными" Ми содержание ДНК в Ма примерно одинаково и плоидность равна 30—60 n. Это определяется наличием 3—4 эндомитозов.

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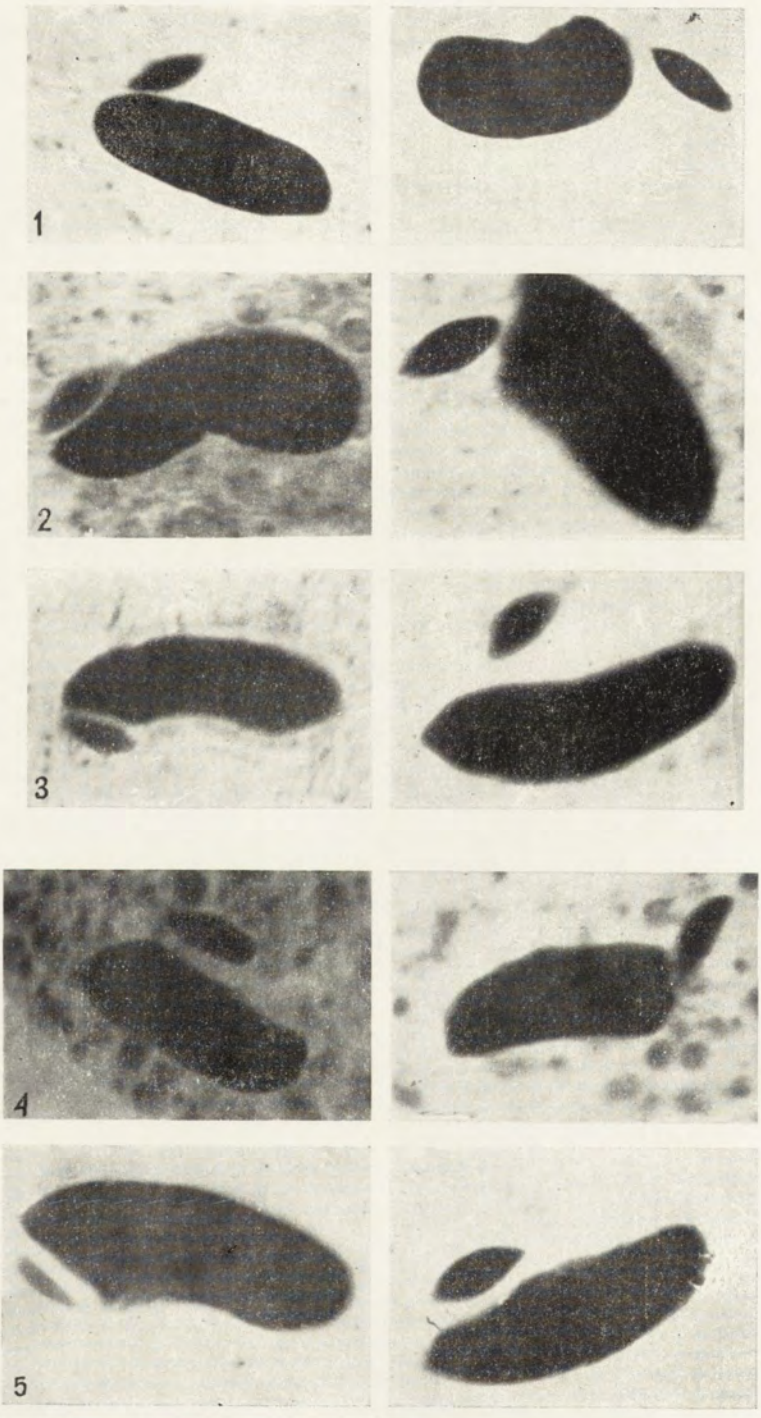
EXPLANATION OF PLATE I

Conjugating strains of *Paramecium bursaria* and most diverse clones obtained as a result of conjugation (two nuclear apparatuses from each clone)

1: Original strains 3R×5R 1
2: Exconjugant clones 17_1 and 17_2
3: Exconjugant clones 29_1 and 29_2
4: Original strains 5R2×8R
5: Exconjugant clones 6_1 and 6_2



L. P. Ovchinnikova auctor phot.

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Department of Systematic Zoology, University of Łódź, Łódź, Nowopołudniowa 14/16, Poland

Maria WOLSKA and Henryk PIECHACZEK

Some intestinal ciliates from American tapir (*Tapirus terrestris* L.)

Niektóre orzęski jelitowe tapira amerykańskiego (*Tapirus terrestris* L.)

A long list of ciliates found in the intestine of *Equidae* (Strelkov 1939) suggests a similar aboundance in the related family — *Tapiridae*. But till now, only three species have been described from the intestine of American tapir. This inclined the authors to search the ciliates in the only available material — feces of tapirs in Zoological gardens.

Material and methods

Samples of feces were fixed in 10% formalin and some in Schaudinn's fluid. During the period of one year (from May 1966 to May 1967) the following materials were gathered in Zoological gardens: 6 samples in Łódź, 2 samples in Płock, and 4 samples in Warszawa. All tapirs were kept in captivity from several years.

After rinsing and condensation the ciliates were stained with Meyer's acid hemalum or they were impregnated with silver following the method applied by Wolska 1966 a.

Historical review

In the year 1918 Da Cunha described a new species and genus *Prototapirella intestinalis* from the coecum of American tapir and classified it in the family *Cycloposthidae*. Da Cunha and Muniz 1925 described a new species and genus *Buissonella tapiri*, from the coecum and colon of the American tapir, and ranged it within the family *Buetchliidae*. Next, these authors (Da Cunha and Muniz 1928) described a new ciliate from the coecum of American tapir — *Bundlea batypharyngea*, also a new genus and species in the family *Buetschliidae*. Da Cunha and Muniz 1927 described the process of division in *Prototapirella intestinalis* Da Cunha, and expressed their opinion on the origin of this species. The comparison between the structure of *P. intestinalis* and the representatives of the related genera of the family *Cycloposthidae* was made by Hoare 1937. Ever since there have been no other reports on the intestinal ciliates of tapirs.

Results

In the samples taken from tapirs in the Zoological garden of Łódź three species of ciliates were found. One of them, *Buissonella tapiri* Da Cunha et Muniz, is known from American tapir, two others, *Balantidium coli* Malmsten and *Blepharocorys cardionucleata* Hsiung, have been described from the horse. In tapirs from ZOO in Płock only *B. coli* was found. The samples from Zoological garden of Warszawa were deprived of ciliates.

Buissonella tapiri Da Cunha et Muniz (Fig. 1)

The body pear-shaped, with anterior part narrowed, somewhat curved and contractile. It may be estimated by comparison of the body shape in a number of fixed specimens. The anterior contractile part is delimited from the rest of the body by distinct line, a groove according Da Cunha and Muniz 1925. The buccal overture lies terminally at the anterior pole, cytopyge at the posterior pole. The vacuole with concretions occurs in the forebody. The macronucleus is horseshoe-shaped, elongated; small, round micronucleus pressed close to the concave side of Ma. The position of the nuclear apparatus in cytoplasm is not very stable. One contractile vacuole (?) is seen usually in the posterior part of the body, the other one, sometime visible, in the median part. The line separating the anterior contractile part of the body runs before the vacuole with concretions, on the opposite body side it descends somewhat backwards. The body is covered with cilia arranged in meridional rows from the border of the buccal overture to cytopyge. The number of kineties is about 60. Individual kinetosomes are fairly big and distinctly delimited from one another. They are more dense in the anterior body part, rare near to cytopyge.

Dimensions of the body (in extension): length 43—74 μ, width 32—56 μ.

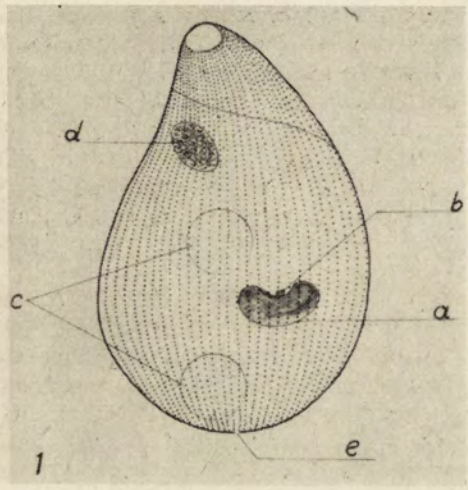
Balantidium coli Malmsten (Fig. 3, Pl. I 5, 6)

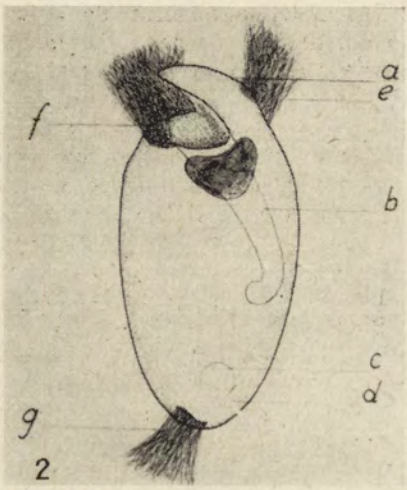
The body eggs-shaped with narrowed anterior end. The buccal overture somewhat shifted ventrally lies on the anterior end; cytopyge lies ventrally on the posterior end. Elongated, horseshoe-shaped macronucleus occurs usually in the median part of the body; small, round micronucleus pressed close to the concave side of Ma. Two contractile vacuoles (?) are present: one lies near to cytopyge, the other in the middle or in the anterior body part. The body covered with meridional rows of cilia. At the posterior pole these rows coincide around cytopyge and the kinetosomes are arranged rarely and irregularly. At the anterior pole the kineties of the dorsal side reach up to the buccal overture, at its margins they begin to penetrate gradually into a deepening vestibulum. The ventral side of the vestibulum is covered with anterior segments of ventral kineties. The kinetosomes of these segments are somewhat bigger than the somatic ones and very dense (Pl. I 5, 6).

Dimensions of the body: length 42—77 μ, width 39—64 μ.

Blepharocorys cardionucleata Hsiung (Figs. 2, 4—8, P. I 1—4)

The ciliate is elongated, with irregular body outline, flattened laterally, asymmetrical. On the anterior part of the body, more closely to the dorsal side protrudes the frontal process. Ventrally to it large buccal overture occurs.





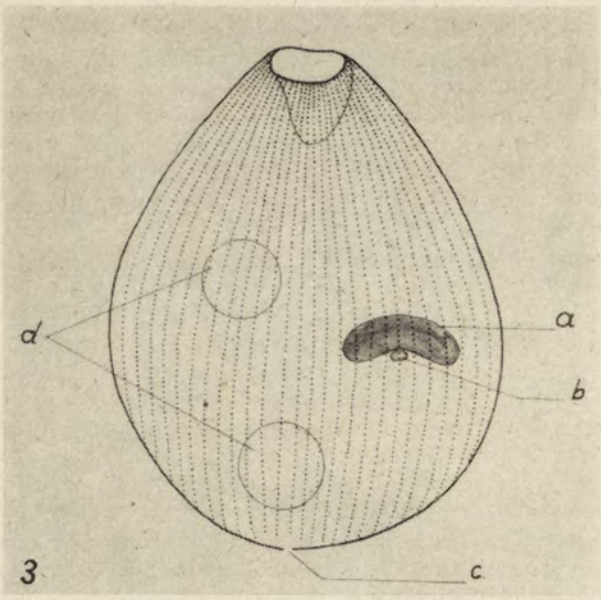


Fig. 1. *Buissonella tapiri* Da Cunha et Muniz. a — macronucleus, b — micronucleus, c — contractile vacuoles, d — vacuole with concretions, e — cytopyge

Fig. 2. *Blepharocorys cardionucleata* Hsiung, view from the left side. a — frontal process, b — infundibulum, c — contractic vacuole, d — cytopyge, e — group of cilia of the frontal process, f — cilia at the border of the buccal overture, g — caudal cilia

Fig. 3. *Balantidium coli* Malmsten. a — macronucleus, b — micronucleus, c — cytopyge, d — contractile vacuoles

Cytopyge lies dorsal on the posterior body part. Heart-shaped macronucleus occurs in the anterior body part. Small, round micronucleus is pressed into the dorsal lobe or near the median part of Ma. One contractile vacuole (?) lies near cytopyge. The buccal overture leads to an ample hollow narrowing into long funnel. The funnel is directed dorsally but its distal part bends to the ventral body side. It reaches somewhat behind midlength of the body. Almost the whole body is nude, the ciliature is limited to few groups of cilia only. One group is located in a depression at the base of frontal process, other one encircles the greater part of the buccal overture. A small group of caudal cilia occurs in a depression at the posterior pole, ventrally to cytopyge. The dorsal wall of the buccal cavity is also lined with cilia.

Dimensions of the body: length 33—53 μ, width 17—28 μ.

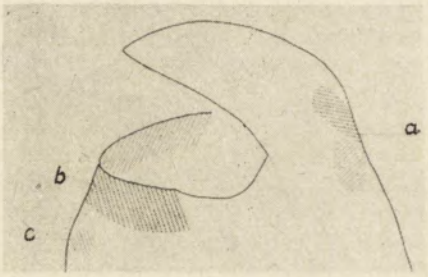
Since the description of *Blepharocorys cardionucleata* by Hsiung 1930 and Strelkov 1939 is too short and unsufficient, we are giving here the results of our observations on this species. Our investigations are not exhaustive, but they provide more information concerning infraciliature. The frontal process of *B. cardionucleata* has been drawn as a hollow structure rising dorsally above the buccal overture and spreading on its left and right side as well. In the descriptions of Hsiung 1930 and Strelkov 1939 this structure is represented as a curved lobe, with its convex side directed to the left, and a concavity to the right. We are not quite sure whether our representation of this process is right because the ciliate is rather small, flattened, and some details of structure are difficult to observe.

We observed in *B. cardionucleata* a small protuberance on the ventral side, backwards from a zone of cilia encircling the buccal overture (Pl. I 1). Such protuberance was observed by Wolska 1966 b, 1967, 1968 in other representatives of the family *Blepharocorythidae*. She recognized it as a rudimental concretion vacuole.

On silver impregnated preparations the following groups of somatic kinetosomes can be distinguished: a zone of oblique kineties at the base of the frontal process, lying in a depression partially on the left, partially on the right side; a zone of oblique kineties encircling the margin of the buccal overture; a small group of kineties on a protuberance, backwards from the former (Fig. 4, Pl. I 1), and a small zone of oblique kineties in a depression at the posterior pole, ventrally to cytopyge (Fig. 5).

The buccal kinetosomes form two groups: a zone of short, oblique kineties in the anterior part of the buccal concavity, lying partially in a hollow of the frontal process — the anterior buccal zone, and a group of long kineties running along the buccal funnel (Fig. 6, Pl. I 2). These long kineties originate near the anterior zone on the left wall and immediately run bacwards to the right wall (Fig. 6). Their kinetosomes are big and dense.

The fibers, running transversally over the nonciliated wall of infundibulum, are impregnated also with silver. These fibres begin at one of the longitudinal kineties as in other representatives of the genus *Blepharocorys* (Grain 1966, Wolska 1966 b). This kinety runs the most ventrally in the anterior part of the funnel on its left wall. In a deeper part of the funnel it becomes the most dorsal, running on the right side. So the fibers in the anterior part of the funnel begin on the left wall, then they pass on to the ventral and partially to the right wall. Opposite situation exists in a deeper part of the funnel; the fibers begin on the right side, pass on to the dorsal



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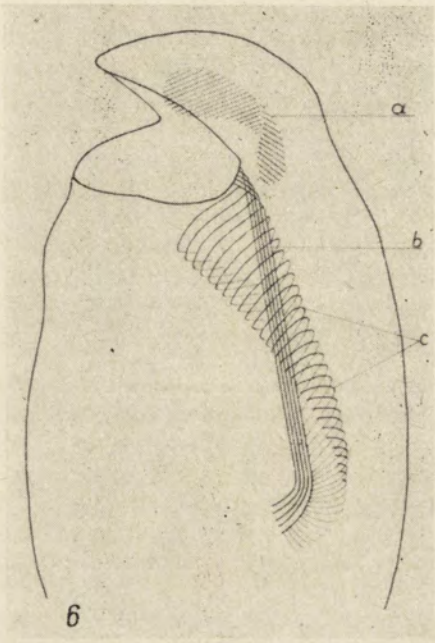

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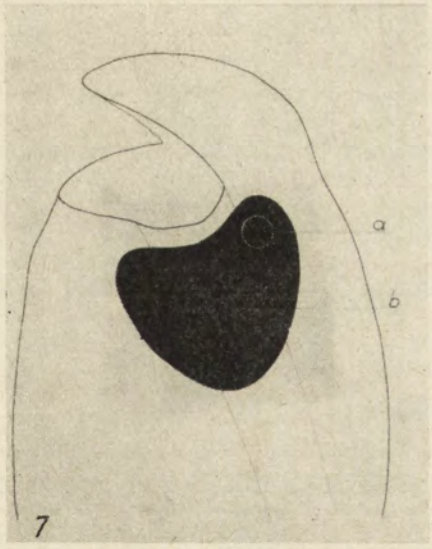

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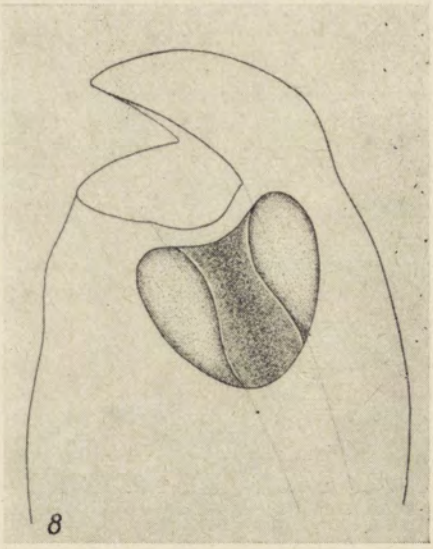

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Figs 4—8. *Blepharocorys cardionucleata* Hsiung. Fig. 4 — anterior part of the body. a — ciliary zone of the frontal process, b — kineties at the border of the buccal overture, c — a group of kineties on the ventral protuberance, Fig. 5 — posterior part of the body, a — contractile vacuole, b — cytopyge, c — zone of caudal kineties, Fig. 6 — anterior part of the body, a — anterior buccal zone of kineties, b — a group of long buccal kineties, c — semicircular fibers, 7 — strongly stained nucleus, a — micronucleus, b — macronucleus, Fig. 8 — weakly stained nucleus

and partially to the left wall. Another group of fibers (we could not detect their beginning) spread like a fan towards the anterior buccal zone (Pl. I 2). Nothing can be said about the ladder-like fibers described by Wolska 1966 b in *B. jubata*.

Short kineties occurring on the surface of the ventral protuberance may be packed sometimes without any order, or they are arranged in regular parallel rows (Fig. 4). Sometimes they are divided into two unequal groups. The irregular arrangement of these kineties results probably from the folding of the convexe surface over the so called rudimental concretion vacuole. Similar slats and grooves occur on the surface of the vacuole with concretions in the representatives of *Buetschliidae* examined by Grain 1966. It is difficult to say whether in *B. cardionucleata* cilia are present on these kinetosomes. It seems that in some specimens very short cilia are present. Probably the situation is the same as in other *Buetschliidae* studied by Grain 1966 under the electron microscope. This author found very short cilia hidden in the grooves between ectoplasmic slats ("crêtes ectoplasmiques)".

The nucleus of *B. cardionucleata*, described usually as heart-shaped in reality is elongated and bent. Usually its median part lies in the right side of the ciliate, on the plane parallel to the body sides, and its bent ends are directed towards the left and forwards. Looking at the ciliate from the side (normal position in the preparation caused by lateral flattening) we distinguish the heart-like outline of the nucleus (Fig. 7) Most frequently the nucleus embraces the anterior segment of the infundibulum with its bent ends (Fig. 8).

We observed the dividing specimens, some of them furnished good pictures of the division vacuoles with the primordia of new ciliature. In one specimen represented on Pl. I 3 three vacuoles are visible: one on the dorsal side of the ciliate (right side on photograph), containing the primordium of the frontal process ciliature; second one on the ventral side (left side on photograph), containing the primordium of the ciliature encircling the buccal overture, and the third median vacuole with the primordium of the anterior buccal zone. On the opisthe the outline of long buccal kineties is visible. Another dividing specimen, in more advanced stage of division, is represented on Pl. I 4. Here the primordium of the ciliature of the frontal process base is well visible (left side on photograph), medially to it lies the primordium of the anterior buccal zone, bent arch-like.

Discussion

Balantidium coli Malmsten was found in all the samples taken from tapirs in two Zoological gardens. So the species might be regarded as a permanent inhabitant of the American tapir intestine. But on the other hand this species is not specific and may be acquired by tapirs from any other animal in Zoological gardens. In the tapirs from ZOO in Warszawa the ciliates were not found. Probably it was due to drugs given to these animals.

Blepharocorys cardionucleata Hsiung, unless it is the repsesentative of the fauna characteristic of tapirs, could be acquired only from a horse. But it seems to be hardly likely because we do not know whether it forms the cysts, and the conditions in Zoological gardens are not favourable to

infect other host species with unencysted stage. In general, *B. cardianucleata* would be a nonspecific parasite not only for its host species but also for its host family.

Summary

Three species of intestinal protozoa found in American tapir (*Tapirus terrestris* L.) are described. These are: *Buissonella tapiri* Da Cunha et Muniz, *Balantidium coli* Malmsten, and *Blepharocorys cardionucleata* Hsiung. Special anttention was paid to the infraciliature of *B. cardionucleata.*

STRESZCZENIE

Autorzy opisują trzy gatunki pierwotniaków jelitowych występujących w przewodzie pokarmowym tapira amerykańskiego (*Tapirus terrestris* L.). Są to: *Buissonella tapiri* Da Cunha et Muniz, *Balantidium coli* Malmsten oraz *Blepharocorys cordinucleata* Hsiung. Specjalną uwagę poświęcono orzęsieniu *B. cardinucleata.*

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EXPLANATION OF PLATE I

Blepharocorys cardionucleata Hsiung

1: The ciliate in optical section
2: Anterior part of the body from the left side. Two ciliary zones are seen on the left side of photograph, a zone of frontal process and, medially to it, the anterior buccal zone. On the right a zone of cilia encircling the buccal overture is seen
3: Early stage of division. Three division vacuoles are seen
4: Advanced stage of division

Balantidium coli Malmsten

5: Anterior part of the body from dorsal side
6: The same specimen in optical section

Magnification: 1, 3—6 — 1000×, 2 — 2000×


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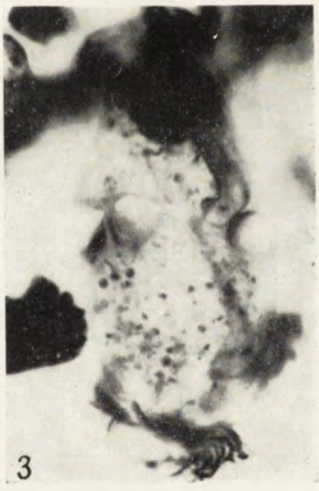
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M. Wolska et H. Piechaczek M. Wolska phot.

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Department of Zoology, University College, Swansea, U. K.

W. Allan EVANS and R. G. ELIAS

The life cycle of *Malamoeba locustae* (King et Taylor) in *Locusta migratoria migratoides* (R. et F.)

Le cycle évolutif de *Malamoeba locustae* (King et Taylor) dans *Locusta migratoria migratoides* (R. et F.)

Malamoeba locustae, the only known species of the genus *Malamoeba*, was first described by King and Taylor 1936 who reported the occurrence of this parasitic amoeba in the Malpighian tubules of the common grasshoppers *Melanoplus differentialis*, *M. mexicanus* and *M. femur-rubrum*. The morphology and distribution of the parasite, the condition of a typical section of infected tubule and certain aspects of the pathology of the infection were described. Later, a more detailed account of the pathology was given including a description of large scale infections in midgut tissue (Taylor and King 1937).

No further work on the parasite was published until Prinsloo 1960 described the occurrence of *Malamoeba locustae* in various tissues of *Locustana pardalina*, the brown locust. Infected regions were said to be the lateral oviducts, tunica propria, epidermal cells, wing muscles and femora, and between developing spermatozoa. Prinsloo 1962 later described nuclear and chromosomal variation in the amoeba after examining smears obtained from infected tubules of the brown locust. Venter 1966 found that individuals of the brown locust infected with *Malamoeba locustae* failed to mature sexually. Species previously known to act as hosts for *Malamoeba* include a few *Gryllidae* and several *Acrididae* including the economically important *Schistocerea gregaria*, the desert locust, and *Locusta migratoria migratoides*, the African migratory locust (Corbel 1967).

Although *Malamoeba* is such an important parasite of laboratory locust cultures, its complete life cycle has not been described. The present report is a detailed description of the life cycle of *Malamoeba locustae* in *Locusta migratoria*, including details of infection at various time intervals after the experimental introduction of cysts.

Materials and methods

From a laboratory stock of parasite-free *Locusta migratoria* 52 newly fledged male adults were removed and infected orally using a suspension of malamoeban cysts in insect saline. The cyst suspension was prepared by removing heavily infected Malpighian tubules from dead, dying or heavily

infected locusts and macerating the tubules in saline using a M.S.E. blender. The resultant suspension was then centrifuged in a Gallenkamp Junior centrifuge for 20 min at 1500 g after which the supernatant suspension was decanted from the tissue debris. By means of a Neuberg haemocytometer it was found that the supernatant fraction contained an average of 10 600 cysts per ml. A sample of the suspension was examined microscopically to ensure that cyst contents were apparently normal and viable, and that no other parasites were present which could interfere with the life cycle in any way.

For 24 hours prior to infection all experimental locusts were deprived of food and water and maintained at a temperature of 32—34°C as opposed to the normal 28—30°C. These altered conditions were conducive to an increase in water loss and it was found that locusts so maintained would readily take in and retain any available fluid, in this case the infective suspension. Each locust was anaesthetized with carbon dioxide and while in a comatose condition, individually infected with a known quantity of suspension. Infection was effected by inserting a fine plastic tube (diameter 0.5 mm) into the buccal cavity of each experimental locust, the tube being connected to a screw syringe containing the suspension. A volume of 0.2 ml of the suspension, containing approximately 2100 cysts, was thus introduced into the alimentary canal of each animal.

After infection all locusts were kept separately and examined in order to ensure that no regurgitation of the suspension occurred. They were separated into two batches. The first batch of 24 was kept in isolation and animals were killed off at hourly intervals in groups of four from the first to the sixth hour after infection. The gut contents of each group at each hourly period were examined in saline under phase contrast in order to observe the natural process of cyst breakdown with the resultant release of living trophozoites.

The second batch of 28 were transferred to a large laboratory locust cage and maintained at optimum conditions of temperature (28—32°C), humidity (40—60% (r.h.)) and under a light:dark regime of 8:16 hours. Fresh washed grass and bran were supplied as food and a fresh source of drinking water was made constantly available. Individual animals were killed off at 12 hourly intervals for the first 7 days and at 24 hourly intervals for the following 14 days. In each case the whole alimentary canal and the associated Malpighian tubules were dissected into aqueous Bouin's fixative, dehydrated in alcohol, cleared in xylene, embedded in paraffin wax (M.P. 56°C) and sectioned at 6—10 μ. Slides were stained alternatively with Ehrlich's haematoxylin and alcoholic eosin, and with a chromosomal stain, usually crystal violet (La Cour 1937) or Feulgen's stain (Feulgen und Rossenbeck 1924). Other staining procedures, such as Giesma's stain (Gelei modification), were also tried but the results of these methods were less satisfactory since both host nuclei and parasites gave similar staining rections. Ehrlich's haematoxylin and eosin however, gave a differential result. Host nuclei were stained dark blue, a typically basophilic result, while parasitic nuclei and cytoplasm gave a pinkish red, acidophilic reaction. Crystal violet and Feulgen stains were employed to detect and show parasitic nuclei in a state of division.

The numbers, positions and morphological characteristics of the parasitic stages were then recorded at various times after infection, and from these data the sequence of events in the parasitic life cycle was elucidated.

Results: observations and description of the life cycle

Penetration of gut wall

The parasitic stages occurring in the midgut are shown diagrammatically in Fig. 1 and the entire life cycle in Fig. 2. Phase contrast observations on untreated contents of fore- and midgut regions removed from living locusts of the first batch indicated that breakdown of the cyst wall began almost immediately the cysts were ingested and was well advanced by the time they had reached the midgut. It would appear that cyst wall breakdown depends on the activity of digestive enzymes in the crop. This was indicated by the failure of cysts to release trophozoites when placed in a gut which had been denatured by immersion in absolute ethanol, while the suspensions placed in excised but still active guts showed cyst wall breakdown. Over a period of 6 hours 42% of cysts in the living alimentary canals released trophozoites, compared with 0% in the denatured controls.

While digestive enzyme production in *Locusta*, as in all insects, is confined mainly to the midgut region, it has been shown that enzymes from this region pass into both fore- and hinduguts (Williams 1954, Eisner 1955, Evans and Payne 1964). The presence of these enzymes, together with salivary secretions, would explain the onset of cyst wall breakdown as far forward as the anterior crop region. The ovoid cysts of *Malamoeba* measured 9—11 μ by 6—7 μ and were characterized by the possession of from 2 to 30 spherical refractile globules (Fig. 3). These were 0.2—2.1 μ in diameter, depending on their abundance within the cytoplasm, and during the disappearance of the cyst wall then became very prominent, remaining so after the cyst wall had completely disappeared.

Under the influence of midgut digestive enzymes each cyst liberated a single trophozoite. These were originally elliptical, but commonly became secondarily spherical or irregular in shape (Fig. 3). On emergence the elliptical trophozoites measured 7—8 μ by 5—6 μ while the spherical ones had an average diameter of 6.8 μ. The dimensions of elliptical trophozoites were thus those of the cyst without the cyst wall.

In the 24 animals examined in the first batch there was a variation in the times of cyst wall breakdown and the release of trophozoites. Several factors would seem to influence the rates of cyst wall breakdown, including the concentrations of digestive enzymes in both fore- and midguts and the diluting and obstructing effects of any food matter still present in the guts. In most animals cyst wall breakdown was well advanced by the end of the first hour and by the third and fourth hour nearly all the cysts present had released their trophozoites. Between the fourth and sixth hours the greater part of the gut contents, including the cyst suspension, had passed into the hindgut. However, cyst wall breakdown still continued due to the effects of digestive enzymes passing with the suspension into the hindgut.

Once released from the cysts (Fig. 1 1) trophozoites were either passively carried, or may have undergone an active migration, to positions adjacent to midgut epithelial cells. Stages in the penetration of the brush border and entry into the cytoplasm of epithelial cells were seen both in smears examined by phase contrast and in paraffin wax sections (Fig. 1 2). Trophozoites libera-

ted near or in contact with the midgut epithelium were completely established within the cell cytoplasm in 6 hours. Those arising from cysts in the centre of the gut lumen, however, had first to reach the epithelium and they penetrated gradually during the next 3 days.

Food entering the midgut passes along the ventriculus and also into the six bilobed digestive caeca (see Fig. 2). In all experimental locusts 90%

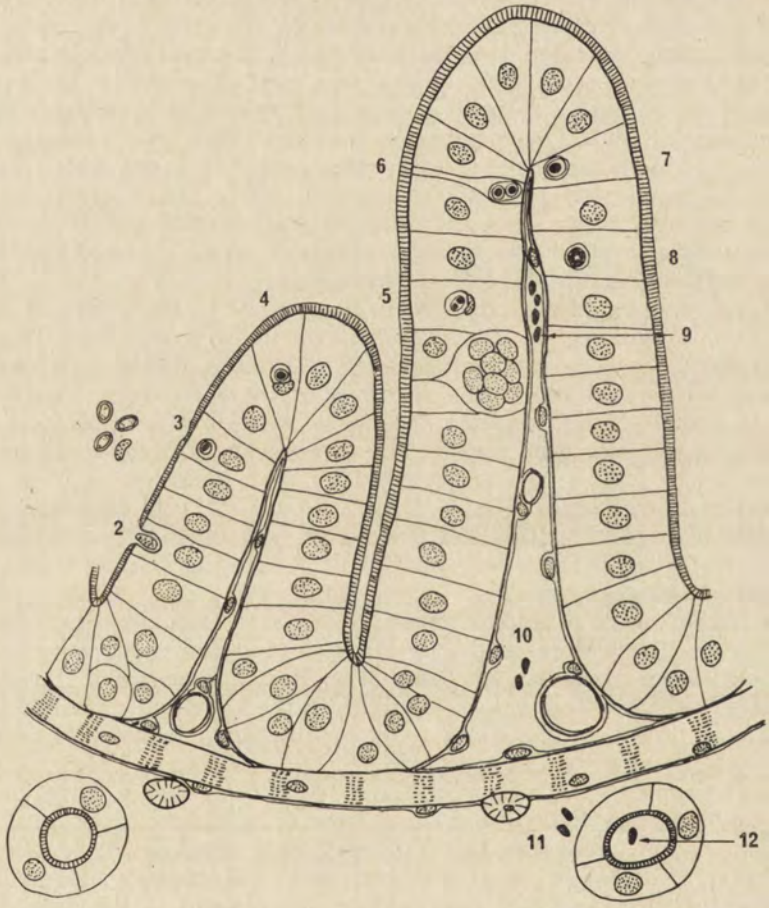

Fig. 1. Diagrammatic transverse section through 2 digestive caecal folds showing the stages of *Malamoeba* which occur in the gut cells: 1. Cysts break down in gut lumen releasing single trophozoites. 2. Trophozoite penetrates host epithelial cell brush border. 3. Primary trophozoite becomes established in a vacuole in the peripheral cytoplasm of the epithelial cell. 4. Cell nucleus destroyed as a result of the migration of the trophozoite and vacuole towards the basement membrane. 5. Primary trophozoite divides by binary fission. 6. Daughter cells in parent vacuole: these eventually separate and establish themselves in individual vacuoles. 7. Primary trophozoite enlarges at the expense of the host cell. 8. Primary trophozoite divides by multiple fission to form 4 or more secondary trophozoites. 9. Secondary trophozoites emerge into connective tissue. 10. Trophozoites migrate peripherally towards muscle coat. 11. Trophozoites penetrate muscle, emerge into haemocoel and attack Malpighian tubules. 12. Trophozoites having penetrated tubule cell become established in the tubule lumen

of penerating trophozoites were seen to be localized to the mid-anterior caecal lobe regions, few being observed in association with the epithelium of the ventriculus or the posterior lobes of the digestive caeca. The epithelium of the midgut regions is continuous and essentially similar throughout, but in the caeca it is folded to form prominent longitudinal ridges, which run the length of the lobes. The folded epithelium in this region increases the surface

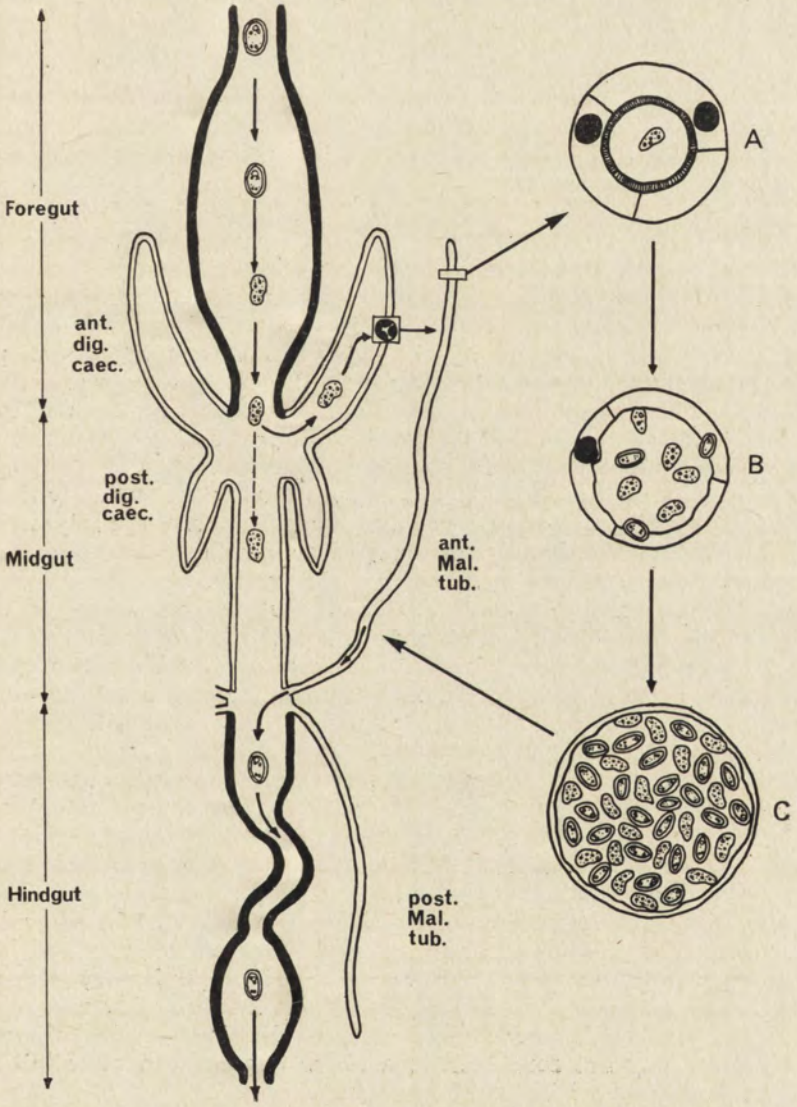

Fig. 2. Diagram to show the complete life cycle of *Malamoeba*, including the stages occurring in the Malpighian tubules, which are shown in diagrammatic transverse sections through the distal region of a tubule. A. Secondary trophozoites enter normal tubule from haemocoel (8 days after infection). B. Trophozoites destroy cytoplasm of tubule cells, some encyst (15 days after infection). C. Large numbers of trophozoites and cysts distend tubule (22 days after infection)

area available for attack and correspondingly reduces the size of the lumen. Also, since the caeca are closed diverticula, all parasites passing through the caecal lumen will make contact with the same region twice as the gut contents pass along and then back to the ventriculus. Even so, many trophozoites failed to penetrate the midgut epithelium and so passed out through the hindgut. Only a fraction of the original number of ingested cysts were seen to establish themselves successfully within midgut cells.

Early midgut infection

Once established within the host cytoplasm each trophozoite invariably became spherical and enclosed within a discrete vacuole (Fig. 1 3, Fig. 4). It was then observed to undergo a phase of growth and division, the diameter of the parasite increasing from the orignal 6—8 μ up to 10—14 μ, during which time the trophozoite would divide once or several times to produce two or more daughter cells. This was a nutritive stage and over a period of 2—8 days the vacuole and the trophozoite were seen to enlarge at the expense of the host cell. Meanwhile migration of the trophozoite and vacuole occurred, for the varying positions of trophozoites in midgut epithelial cells indicated that the parasite digested its way through the host cell cytoplasm, passing from the brush border of the cell towards the basement membrane.

Initially the host cell appeared to be unaffected, with the vacuole confined to the peripheral cytoplasm and the nucleus and remaining cytoplasm retaining their usual appearance and staining properties. Due to its large size (10—12 μ by 8—9 μ) the elliptical gut cell nucleus was eventually destroyed however since its central position was directly in line with the migration path of the parasite (Fig. 1 4). Rupture of the nuclear membrane and penetration of its peripheral areas resulted in its rapid disintegration and the dispersion of originally nuclear basophilic material throughout the remainder of the cell cytoplasm. Following the disruption of the nucleus the remaining cell contents rapidly deteriorated and the cell eventually collapsed, presumably under the pressure of adjacent healthy cells. The trophozoite continued its migration however, until it came to lie adjacent to the inner cell membrane and near the connective tissue core of the caecal fold.

During this migration the trophozoite was seen to divide once or several times, apparently by binary fission, to produce two or more daughter cells (Fig. 1 5, Fig. 5). The rate of division was variable and the governing factor in this process was not discovered. The two daughter cells produced as a result of each fission were small (average diameter 5.6 μ), but close replicas of the parent organism, initially occupying the same vacuole (Fig. 1 6). After division daughter cells showed a tendency to separate and eventually establish themselves independently in their own vacuoles (Fig. 1 7). Binary fission was observed in the gut epithelium from the 3rd post-infective day and continued even into the 21st day. The incidence of these divisions reached their maximum at the 8th day but continued to a lesser degree even after initial cyst reformation had occurred in the Malpighian tubules.

One prominent feature of the trophozoite's migration through the caecal wall its tendency to be confined to a single epithelial cell, the adjacent cells seeming to escape attack even though the host cell was completely destroyed. Only when the parent trophozoite underwent repeated binary fission was there any apparent invasion of new cells and even then this tended to be

restricted to those directly adjacent to the original host cell. If only a single binary fission occurred, however, daughter trophozoites tended to be confined to the original infected cell. During these early stages in the gut tissue the trophozoite was typically spherical, having an average diameter of 10.4 μ (range 6—14 μ). The trophozoite cytoplasm was typically clear, the large prominent karyosome normally occupying a central position.

Later midgut infection

From day $4^1/_2$ several of the larger trophozoites were seen to have divided to produce not two, but four or more daughter cells (Fig. 1 8). Detailed observations of these divisions indicated that this was probably a stage of multiple fission rather than binary fission, because the nucleus appeared to divide several times without division of the cytoplasm (Fig. 6), which eventually seemed to fall into as many parts as there were nuclei. Whether these divisions were mitotic or amitotic was not discovered. In some cases, especially when four cells were formed, the nuclei and the resultant daughter cells seemed identical, but when more than four nuclei were produced nuclear sizes varied. In all cases the nuclear material was distributed peripherally and a modicum of residual cytoplasm, often containing some nuclear material, was commonly seen to remain after separation of the daughter cells. As described in the preceding section the products of binary fission were exactly like the parent trophozoite, growing to form separate vacuoles but still remaining within the host epithelial cell or one adjacent to it. Products of multiple fission, however, showed several distinct differences from the parent trophozoite. They were, on average, slightly smaller (7—8 μ by 5—6 μ) and had a diffusely granular cytoplasm and an indistinct dispersed koryosome. As a result of the breakdown of the host inner cell membrane these numerous trophozoites came to lie in the connective tissue core which separated the two epithelial layers of the caecal fold (Fig. 1 9).

Because of the obvious differences between the trophozoites which migrated through the gut epithelial cells and their progeny, which occupied, and later migrated through the connective tissue and other regions, it seems necessary to distinguish between the two stages. It is suggested that the stages occurring in the gut cells be referred to as primary trophozoites and the consequent stages in connective tissue, haemolymph and Malpighian tubules, and in the gut lumen prior to the invasion of gut tissue, be known as secondary trophozoites.

It may be that the two stages of *Malamoeba* are essentially similar with a common fine structure, but there are demonstrable differences. For example, comparisons of nuclear material of the two stages using the Feulgen technique showed distinct differences in their staining properties. Primary trophozoites, especially those in a state of fission, gave strongly positive rections to the stain after one hour, but the nuclear material of the secondary trophozoites was unstained at this time and continued to be so even when the staining period was extended to 6 hours. The result of this test, at least, indicated differences in the properties of nuclear material in the two stages even though the corresponding cytoplasms gave similar results.

Migration of secondary trophozoites

Comparisons of gut sections at different post-infective times indicated that, after successfully penetrating into the connestive tissue core, the secondary trophozoites underwent a migration through this tissue (Fig. 1 10), and came to lie in a position adjacant to the thin circular muscle coat which surrounded and completely enclosed the caecal lobe (Fig. 7). In many cases initial penetration by primary trophozoites had occurred at the base of the caecal fold and in these circumstances no migration was necessary since secondary trophozoites emerged from the epithelium and were already adjacent to the muscle coat. In some sections up to 20 secondary trophozoites could be seen in an infected epithelial fold. Migration through the connective tissue was fairly rapid, the majority of the trophozoites reaching the muscle coat by the 9th day, having been observed initially from the 6th day. From this time onward secondary trophozoites were a constant feature at the bases of infected caecal folds but they became numerically less after the peak 9th day.

Individually, or in groups, the secondary trophozoites were seen to penetrate the peripheral muscle coat progressively until a section of the muscle layer was finally perforated and the trophozoites emerged into the haemocoel (Fig. 1 11). There they attacked the next host tissue, the Malpighian tubules (Fig. 1 12, Fig. 2 A). Of the 270—300 Malpighian tubules which arise from the ampullae of the hindgut about one third are anteriorly directed, reaching as far as, and often extending past, the anterior tips of the gastric caeca. The majority of these tubules are adjacent to, or even in direct contact with the midgut caeca, and because of this they are most vulnerable to attack from secondary trophozoites emerging through the caecal muscle coat.

When tubules were in contact with infected caecal tissue the parasite merely continued its migration from connective tissue, through the muscle and into the tubule cells. In tubules which were not in contact with the caeca, however, it was not established whether infection occurred as a result of active migration of the parasite or of passive transport by the haemolymph bringing it into accidental contact with a tubule. The latter seems probable but may be assisted to a limited extent by movement of the trophozoite, possibly in a response to a limited chemotactic field associated with the tubule tissue.

It was thus seen that trophozoites had appeared in association with Malpighian tubules 36 hours after the peak of secondary trophozoite incidence in the connective tissue at the caecal fold base. They were either attached externally to tubule basement membranes or actively penetrating the cells, or already established in the tubule lumen. These observations are indicative of a further migration, this time from the haemolymph through the tubule cells and into the tuble lumen.

Infection of Malpighian tubules

Once inside the lumen each trophozoite seemed to attack and destroy a section of the brush border of a cell (Fig. 8). It then became attached to, and commenced to feed on the exposed cytoplasm (Fig. 2 B). From this point

in the life cycle rapid and repeated binary fissions were observed and the trophozoites increased greatly in numbers, attacking and destroying an ever increasing number of tubule cells.

Initial invasions were confined to the anterior directed tubules. There was no observed attack on the posterior tubules, until the anterior infection was well advanced. Another salient feature of the initial invasion of tubule tissue was, that in all observed cases, the distal segments, that is those areas of tubules furthest from the ampullae, were invaded first. These segments are the ones normally in contact with or adjacent to the anterior caecal lobes. The proximal regions of the tubules lie alongside the ventriculus where the incidence of primary trophozoites was very low.

The large numbers of secondary trophozoites formed as a result of binary fission destroyed the cytoplasm and nuclei of tubule cells until only the peripheral basement membrane remained around the mass of parasites (Fig. 2 C). As the tubule cytoplasm, the parasite's food supply, became progressively used up, the infection spread down the tubules towards the ampulla region.

Observations on partly infected living tubules removed from parasitized hosts, which had been injected with 1% aqueous methylene blue, showed that, even though distal regions were infected and disrupted and were therefore unable to function properly the more proximal uninfected cells still excreted normally. So rapid was the binary fission of secondary trophozoites that tubule lumens, already increased due to the destruction of the cells, were seen to be packed and often distended with trophozoites. Because of this the diameter of infected tubules was often up to ten times its normal dimension. In this condition, although the tubule tissue had invariably been completely destroyed, the mass of trophozoites was usually enclosed by the still complete basement membrane (Fig. 9). This bounding membrane however, was often seen to have ruptured releasing large numbers of active trophozoites into the haemocoel through with they would be dispersed to various parts of the body. This is presumably the way in which other, as yet uninfected, tubules become infected and could explain the lapse in time between the appearance of large scale infections in the anterior and in the posterior tubules. The latter may become infected as a result of the extensive release of trophozoites from the previously infected anterior tubules.

Between the 15th and 18th day cysts appeared in the lumens of infected tubules (Fig. 2 B, C). The factors stimulating cyst reformation were not determined but it may have been induced by the decrease in the amounts or available food as the host tissue was destroyed. This effect would have been emphasized by the ever increasing numbers of secondary trophozoites competing for this food. Encystment was seen to be a simple process; the secondary trophozoites merely withdrew their blunt pseudopodia, became detached from the host cytoplasm, became elliptical and developed a cyst wall. In all but severely infected tubules an excretory current was still present in uninfected proximal regions. This tended to draw any fluids or loose particles, including the cysts and detached trophozoites, which had yet to develop cyst walls, from the lumen of the infected distal regions down into the proximal regions and thence into the ampullae and hindgut. Once in the hindgut cysts were compacted into faecal pellets, along with waste mater passed from the ventriculus, and eventually excreted via the rectal sac and rectum. Secondary trophozoites, as

well as cysts, were commonly seen in the ampulla and the hindgut. This would be due to the non-selective nature of the excretory current.

From the 15th to the 18th day an even increasing number of cysts became apparent in the hindgut and increasingly larger numbers of tubules were destroyed. The anteriorly directed tubules were totally destroyed and were completely non-functional for several days before most of the posterior tubules were even initially infected. For several days before death the locust is presumably dependent on an ever decreasing number of healthy or partially infected posterior tubules, until eventually these are so ineffective as excretory organs that they are inadequate to maintain the needs of the host. A gradual accomulation of excretory material must thus occur throughout the body, resulting in the slow but inevitable poisoning of the locust. By this time however, considerable quantities of cysts have passed out to infect further individuals (Fig. 2).

Table 1

Distribution of *Malamoeba* cysts and trophozoites in the anterior digestive caecal region over a 24 day period after infection. To obtain these figures counts were made of the stages visible in 10 transverse sections through the gut at the level of the mid-anterior gastric caeca. At this level the sections were of the proventricular valve, the 6 adjacent caeca and numerous Malpighian tubules. The numbers shown under Malpighian tubules were of parasites in 30 of the tubules, randomly selected

Time (days)	Number of parasites in gut lumen	Number of primary trophozoites in caeca:			Number of secondary trophozoites:		Number of cysts
		undivided	dividing by binary fission	dividing by multiple fission	in connective tissue	in Malpighian tubules	
1/2	291	89	—	—	—	—	—
1 1/2	83	236	—	—	—	—	—
3	14	315	56	13	—	—	—
4 1/2	—	387	56	51	—	—	—
6	—	522	96	80	17	—	—
8	—	658	197	156	81	26	—
11	—	332	116	77	296	167	—
14	—	491	78	89	841	349	—
17	—	375	52	88	647	1264	176
21	—	438	64	80	384	718	285
24	—	197	108	64	446	914	298

General discussion

The course of the malamoeban life cycle is reflected in Table 1 which indicates the time at which each parasitic stage was at its maximum. It can be seen that there is a gradual but maintained increase in the number of primary trophozoites up to the 8th day when their maximum incidence was recorded. This was coupled with a corresponding increase in the numbers of dividing trophozoites. The incidence of primary trophozoites dividing by both binary and multiple fission also reached its maximum at the 8th day. The percentage of trophozoites dividing increased twofold from 17.9% in

the 3rd day, when division was initially observed, to 34.91% at the peak 8th day. The maximum incidence of division, both binary and multiple, directly preceded the rapid rise in the numbers of secondary trophozoites present in the connective tissue which reached a maximum by the 14th day. This in turn preceded a substantial rise (approximately ×4) in the numbers of secondary trophozoites observed in Malpighian tubules from days 14—17. The final stage in the host, the infective cysts, first appeared on day 15 and were still increasing in number on day 24. All the stages occurring in gut tissue, that is, up to and including secondary trophozoites in connective tissue, showed a peak period of numerical incidence. In the tubules, however, there is a continuous increase in the numbers of both trophozoites and cysts until death.

The parasite life cycle, as described in the preceding section, involves a movement from one host tissue to another. The infective parasitic stage first enters the foregut but this region is unaffected, the cysts passing through its lumen. The midgut epithelium is the first tissue of the host to be attacked, in particular the cells of the anterior gastris caeca. Following a migration through the haemocoel the Malpighian tubules become infected. It has been shown that similar regions are affected in *Melanoplus* (Taylor and King 1937) but in this report the migration from midgut to Malpighian tubules via the haemocoel was not described. It seems likely, however, that the parasite moves from one tissue to the other in that host by a route similar to that in *Locusta*. This is suggested by similarites in gut structure (Hodge 1936, 1939) and also by the similar time period from initial infection to cyst production. In the present investigation cysts were excreted 15—18 days after experimental infection. King and Taylor 1936 reported the period to be 14—18 days in *Melanoplus*.

One main difference between the infection of *Melanoplus* and *Locusta* concerns the part of midgut witch is principally attacked. Large numbers of trophozoites, presumably secondary trophozoites, occur in the ventricular epithelium in *Melanoplus* (Taylor and King 1937) but these were not found in *Locusta*, nor were such large numbers of trophozoites recorded in the midgut epithelium of *Locustana by* Prinsloo 1962. There is thus a difference in this phase of the life cycle, possibly correlated with different structural or physiological conditions in the various hosts.

As described previously, two distinct types of trophozoite were observed to be a constant feature of the malamoeban life cycle in *L. migratoria*. Primary trophozoites occurred within the gut epithelium, had prominent central nuclei and were enclosed in a distinct vacuole. These gave rise to other generations of primaries by binary fission and to secondary trophozoites by multiple division. Secondary trophozoites occurred in gut connective tissue, the haemocoel and Malpighian tubules are preceded, and immediately followed, the cyst stage. It may well be that the two trophozoite stages exist in all host species although, until now, only a single trophozoite stage has been described. This could be explained by the fact that other workers have generally confined their descriptions of the parasite to one tissue only, the Malpighian tubules, and have consequently observed only the secondary trophozoite.

It is interesting to compare the life cycle of *Malamoeba* with that of *Malpighamoeba mellificae* Prell as described by Giordani 1959 and reviewed by Lipa 1963. This is the only other amoeban parasite of insect Malpighian tubules, it occurs as a parasite of *Apis mellifera* in which it has a life cycle

of 24—28 days. Malamoeban cyst formation in *L. migratoria* is some 10 days more rapid. The parasite life cycles would appear to be similar in that in both species trophozoites attack and destroy cells of the Malpighian tubules within which they replicate only by binary fission. The effect of *Malpighamoeba* on the tubules of *A. mellifera* (Giordani 1959) is essentially similar to that described above for *Malamoeba*, but little detail of the full life cycle is known (Lipa 1963). A full comparison of the life cycles is, therefore impossible because of the lack of detailed knowledge concerning stages in the ventricular epithelium of honey bees. It is merely reported that the gut wall is affected (Giordani 1959) and it is not known if *Malpighamoeba* has one trophozoite stage only or is similar to *Malamoeba* in the possession of 2 distinct trophozoite generations.

Summary

Cysts of *Malamoeba locustae* were introduced into the alimentary canals of healthy male *Locusta migratoria*. Digestive enzymes dissolved the cyst walls liberating trophozoites which penetrated midgut cells. Primary trophozoites passed through the epithelium dividing first by binary fission, and later by multiple fission to form secondary trophozoites. These emerged into the haemocoel and attacked the distal ends of anteriorly directed Malpighian tubules. Trophozoite numbers continued to increase by repeated binary fission as the infection spread along the tubule. Between 15 and 18 days after infection secondary trophozoites formed cysts which passed out via the hindgut. These infected other locusts.

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RÉSUMÉ

On introduit des cystes de *Malamoeba locustae* dans les tubes digestifs de males specimens salubres de *Locusta migratoria*. La dissolution des parois des cystes par les enzymes digestifis resulta dans la libération des trophozoïtes qui pénétrèrent les cellules de l'intestin moyen. Des trophozoïtes primaires entrèrent dans l'épithélium ou ils se divisèrent, en premier lieu par scission binaire, et en seconde par scission multiple, cette division resulta dans la formation des trophozoïtes secondaires. Ceux-ci passérent dans l'haemocoele ou ils attaquèrent les bouts distales des tubes de Malphigi orientés à l'antérieur. Le nombre de trophozoïtes augmentait sans cesse par continuelle scission binaire tandis que l'infection se propagait le long du tube. De 15 à 18 jours après l'infection, les trophozoïtes secondaires produirent des cystes qui sortirent via l'intestin posterieur. Ils infectèrent des autres locustes.

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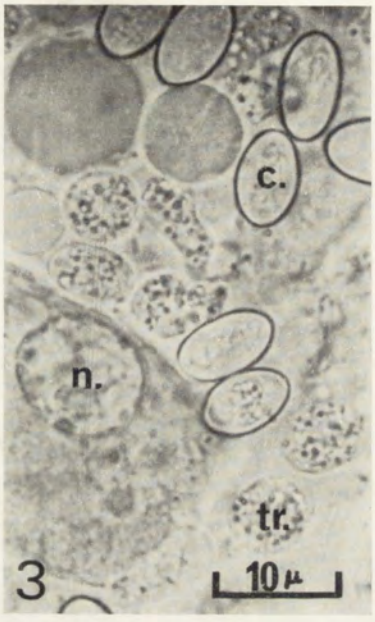
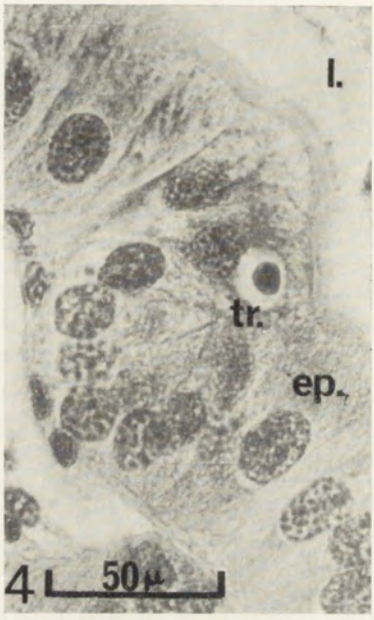
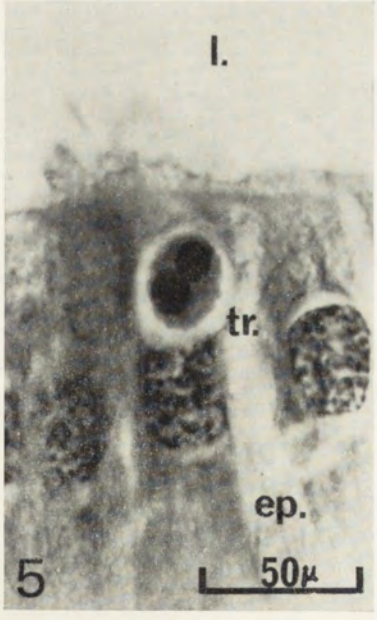
EXPLANATION OF PLATES I—II

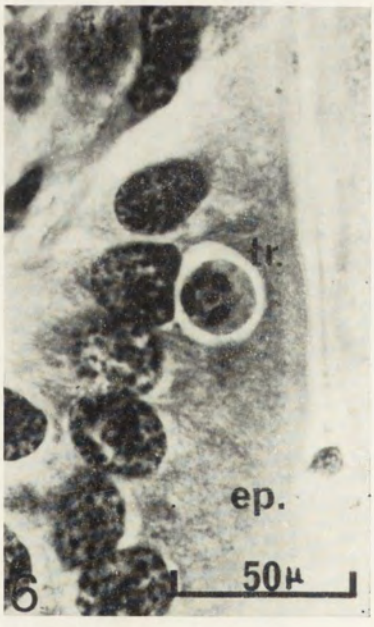
Malamoeba locustae (King et Taylor) in *Locusta migratoria migratoides* (R. et F.)

Fig. 3: Cysts (c.) and liberated trophozoites (tr.) in midgut contents extracted from a locust 1 hr after infection; n, nucleus of a detached epithelial cell (phase contrast).
Fig. 4: Spherical primary trophozoite (tr.) enclosed by a vacuole in the peripheral region of the epithelial cell (ep.) having penetrated the cell from gut lumen (l.). (T. S. anterior digestive caecum, 3 days after infection)
Fig. 5: Enlarged primary trophozoite (tr.) undergoing binary fission within its vacuole in an epithelial cell (ep.). (T. S. anterior digestive caecum, 6 days after infection)
Fig. 6: Primary trophozoite (tr.) undergoing multiple fission within an epithelial cell (ep.). (T. S. anterior digestive caecum, 9 days after infection)
Fig. 7: Transverse section through a caecal epithelial fold showing the gut lumen (l.) the columnar epithelium (ep.) and secondary trophozoites (tr.) migrating through the connective tissue (c.t.) core. (T. S. anterior digestive caecum, 12 days after infection)
Fig. 8: Early infection of a Malpighian tubule; trophozoites (tr.) have penetrated it from the haemocoel (h.) and destroyed the brush border and some cytoplasm while a tubule cell nucleus (n.) is not yet affected. (T. S. anteriorly directed tubule 14 days after infection)
Fig. 9: Malpighian tubule at a later stage of infection; all nuclei and cytoplasm of tubule cells have been destroyed and replaced by large numbers of encysted parasites (c.) which fill the region bounded by the still complete basement membrane. (T. S. anteriorly directed Malpighian tubule 18 days after infection)









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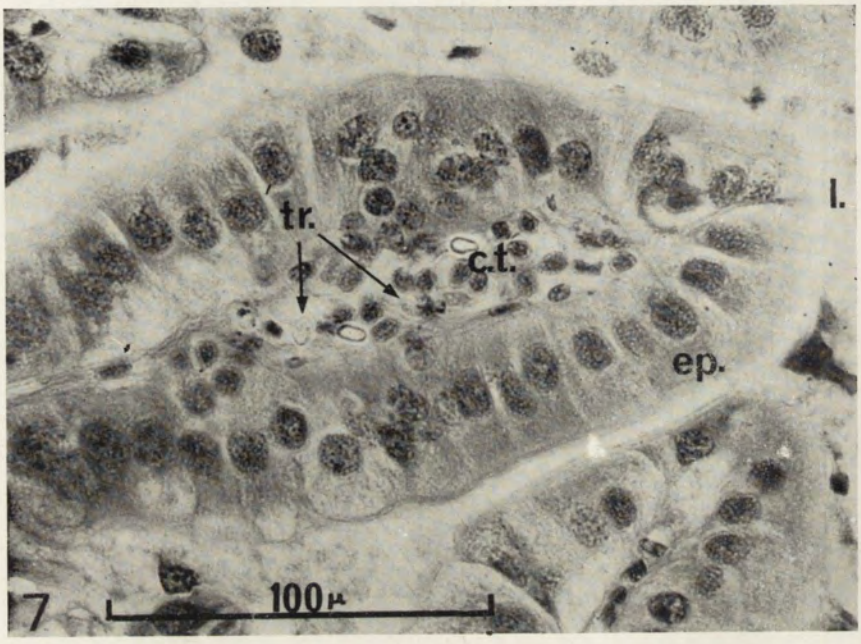

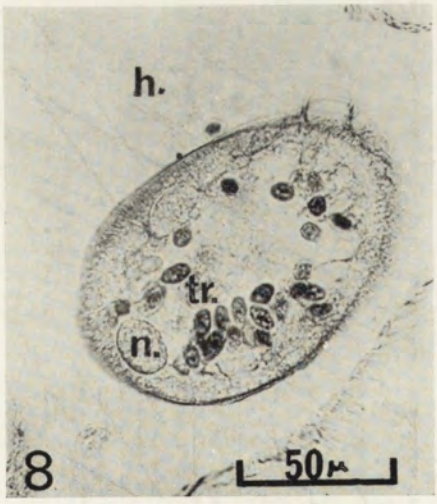

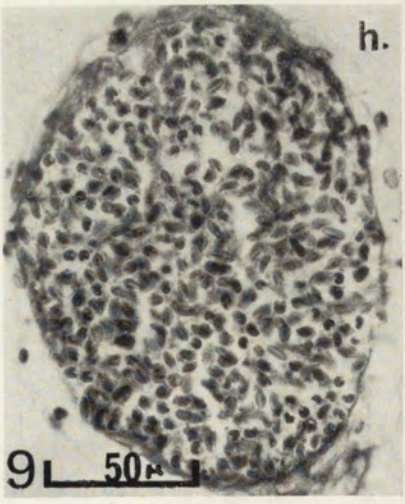

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Department of Zoology, University of London King's College, Strand, London WC 2, England

Tara N. GHOSH*

Studies on the growth and encystation of *Entamoeba invadens* Rodhain, 1934, in vitro

Studien über des Wachstum und die Encystierung von *Entamoeba invadens* Rodhain, 1934, in vitro

In the appropriate environmental conditions *Entamoeba*, completes its life cycle by periodic production of trophozoites, precysts, cysts and the metacysts. The process of encystation involves multiplication of the trophozoites and a series of metabolic events, not yet clearly understood. A few studies have been made of these cyclic changes of *Entamoeba* in monoxenic (Dougherty 1959) cultures. Therefore, in the following, attempts have been made to follow the course of population growth and encystation of *E. invadens* in monoxenic cultures.

Material and methods

Both non-clone and clone monoxenic cultures of *Entamoeba invadens* strain BC was used. The strain was derived from Rodhain's 1934 original strain via axenic culture of McConnachie 1956. *Escherichia coli* strain A′ (Dobell, Neal, Hoare 1952) was then added. The cultures were grown in biphasic "HSre+S" medium of Dobell, as quoted by Hoare 1948 and in Jones' 1946 medium, modified by incorporating horse serum slant, omitting buffers, and replacing "marmite" with yeast extract (Difco). The latter was designated as "HShsm+S". McCartney and universal screw-cap bottles, each with 25 ml capacity, were used and each vial contained 10 ml of inspissated horse serum (HS) slope overlayed with 10 ml ringer-egg-albumin (re) or the horse-serum-yeast-extrast (hsm) as the case may be, and 40 mg sterilized BDH rice starch (S).

Before these experiments were performed, both the non-clone and clone amoebae were allowed to adapt for several months in both the media used. Each vial, was "preconditioned" by incubating overnight at 37°C with 2 drops of 24-hour old culture of *Esch. coli* A′ in nutrient broth. The required number of amoebae were then inoculated from a four day old culture in the respective media, free from starch. All the cultures of *E. invadens* were incubated at 24°C.

The number of amoebae and cysts produced in the cultures was determined at intervals of 24 hours in a Bürker double ruled bright line chamber. Each

*Present address: Department of Protozoology, Calcutta School of Tropical Medicine, Chittaranjan Avenue, Calcutta-12, India.

calculation as recommended by Paulson 1932, was the mean value of five separate counts. Granular and degenerating amoebae were not counted.

To test the uniformity of distribution of amoebae and cysts, in the Bürker counting chamber, the ruled area of the chamber was replicated as 2 sets of 144 squares, cyclostyled on sheets of paper and the microscopic observations of each square of the counting chamber were plotted on the corresponding square of the paper. It was found that in most of the cases, the parasites were uniform in distribution throughout the ruled area of the counting chamber.

Results

Experiment 1. *E. invadens*, non-clone in HSre+S, Fig. 1

50×10^3 amoebae from a 4 day old culture were inoculated into the preconditioned medium and incubated. The average number of amoebae and cysts produced per ml of the liquid overlay was calculated and plotted against time in days. No count of the viable bacteria in the medium was made in any of the following experiments. Fig. 1 shows a steady increase of population from the 1st to the 8th day of incubation. On the 9th day a slight decrease in the number of amoebae was recorded, but subsequently the number increased and reached a maximum on the 14th day. After this period, the number of amoebae declined slowly. No further counting was possible after the 16th day, because the horse serum slant (HS) was found broken.

Cysts first appeared on the 5th day (1.53 per cent) and varied from 0.17 per cent (11th day) to 4.61 per cent (16th day) of the total population of trophozoites and cysts.

Experiment 2. *E. invadens* non-clone in HShsm+S, Fig. 2

A similar procedure to that described above was followed with an initial inoculum of 50×10^3 trophozoites in the preconditioned medium. Essentially, the course of population growth in this medium (Fig. 2) was similar to that described from HSre+S (Fig. 1). On the 9th day, a slight decrease in the number of amoebae, as seen in HSre+S medium (Fig. 1) was also found in this culture. The maximum number of trophozoites was attained on the 13 th day. On the 14th day a marked fall in the number of trophozoites was recorded, and this was followed by a further phase of decline on the 15th day.

Cysts first appeared on the 5th day and attained a maximum of $27.08 \times \times 10^3$ per ml on the 12th day, i.e. 10.19 per cent of the total population of trophozoites and cysts. No further observation was made beyond the 17th day of incubation.

The 2 culture vials, used in the above experiments (Expts. 1 and 2) were incubated together and treated similarly to minimize possible exogenous variations.

Experiment 3. *E. invadens* clone in HShsm+S, Fig. 3

29.66×10^3 amoebae were inoculated into the preconditioned medium. A steady rise in the population of amoebae was noticed (Fig. 3) from the 2nd to the 9th day. On the 10th day of incubation an appreciable fall of 39.1×10^3 amoebae per ml was noticed. On the 11th day a very slight rise of the tro-

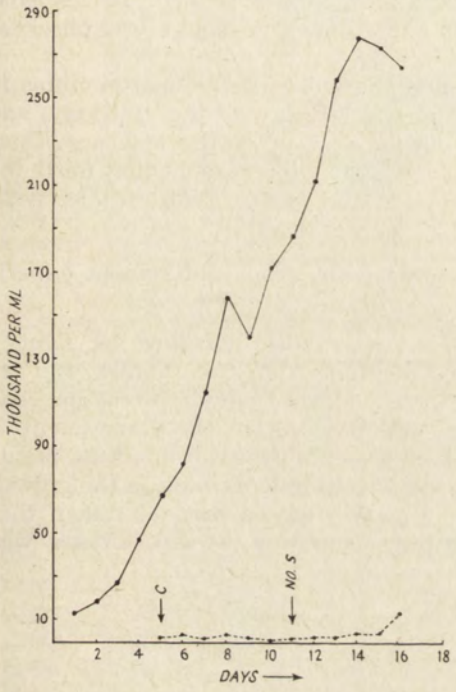

Fig. 1. *Entamoeba invadens* strain BC non-clone in HSre+S

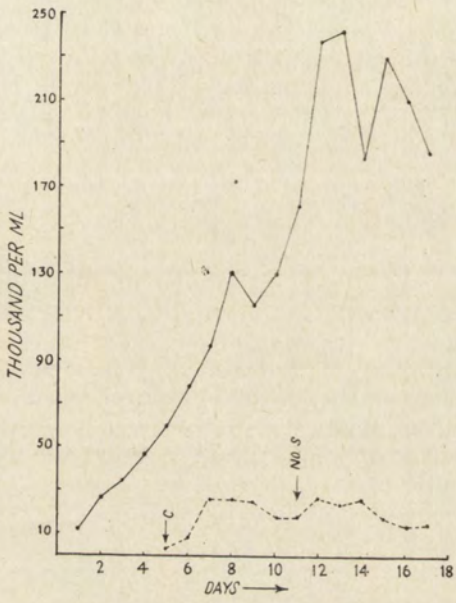

Fig. 2. *Entamoeba invadens* strain BC non-clone in HShsm+S

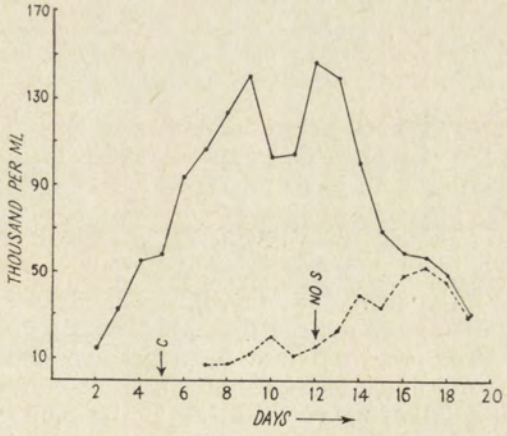

Fig. 3. *Entamoeba invadens* strain BC clone in HShsm+S

Marks and abbreviations used in Fig. 1—4: Trophozoites ·———·; Cyst ·····; C — cyst; No S — No starch outside amoebae; R — Replacement

phozoite count was observed which gave a maximum of 147.56×10^3 amoebae per ml on the 12th day. A rapid fall in the trophozoite count was observed on subsequent days.

Cysts first appeared on the 5th day and showed three distinct rhythms in population. It attained a steady rise from the 5th day to the 10th day, and a fall on the 11th day was followed by an increase up to the 14th day. Once again, a fall on the 15th day was followed by an increase lasting until the 17th day. On further incubation the cysts (and the trophozoites as well) rapidly disappeared from the culture.

Experiment 4. *E. invadens* clone in HShsm+S (The replacement experiment) Fig. 4

The experimental procedures were similar to those described for Expt. 3 except that 2 ml of undisturbed culture medium from the surface was replaced with fresh medium on the 9th day. The course of population of amoebae steadily increased from the 2nd to the 10th day. As in the Experiment 3, an appreciable decrease of amoebae was noticed before it attained the maximum on the 12th day. However, in this case (Fig. 4) a depression in the growth curve during the log phase appeared on the 11th day, a day later than that recorded in the Expt. 3. From the 13th day decline of the trophozoites was noticed.

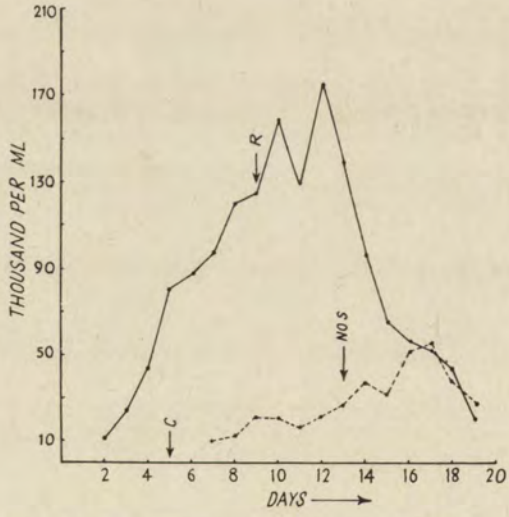

Fig. 4. *Entamoeba invadens* strain BC clone in Hshsm+S (Replacement expt.)

Cysts first appeared on the 5th day. Here again three waves in the course of the cyst population were noticed (Fig. 4). A steady, but slow rise was seen till the 9th day. This was followed by a gradual depression in the count until the 11th day. A gradual increase in the number of cysts was then observed up to the 14th day, following which a fall in the count was recorded on the 15th day. This was again followed by a growth phase lasting until the 17th day. Finally the number of cysts declined in the culture on the 18th and 19th days of incubation.

Discussion

Growth

The lag phase

In the foregoing experiments no attempt was made to work out the details of the lag phase of growth of *E. invadens*. However, it is reasonable to suppose that in the present experimental conditions, the lag phase of *E. invadens* extends from the time of inoculation to less than 24 hours of incubation.

The log phase

In the present series of experiments where the quantity of rice starch (40 mg), the liquid overlay (10 ml), and the temperature of incubation (24°C) remained constant, the maximum number of trophozoites were recorded between the 12th and 14th days of incubation.

On the growth curve of *E. invadens* in HSre+S (Fig. 1) and HShsm+S (Fig. 2), prior to the attainment of the maximum growth on the 14th or 13th days respectively, definite falls have been observed on the 9th day of incubation. In both the cases the initial inoculum was 50×10^3 amoebae. Using the clone cultures (Figs 3 and 4) of *E. invadens* and initial inoculum of 29.66×10^3 amoebae the similar falls in the growth curves occurred on the 10th (Fig. 3) and 11th (Fig 4) days of incubation. This indicates that the size of the initial inoculum has an effect on the final shape of the growth curve. Comparison of Fig. 2 and Fig. 3 discloses that the maximum population of 243×10^3 trophozoites per ml (Fig. 2) can be obtained (13th day) from a culture having an inoculum of 50×10^3 amoebae, while not more than 147×10^3 amoebae per ml can be obtainable from a nearly identical culture with an initial inoculum of 29.66×10^3 amoebae (Fig 3).

Apart from the inocula used to construct Fig. 2 and 3, there is also a little difference in the history of the strain (BC) used. The culture for Fig. 3 was a clone (trophozoite) culture derived from the mother strain BC of *E. invadens* which provided the culture for Fig. 2. Therefore, the possibility of variation in the clone of *E. invadens* strain BC in its response to growth in vitro cannot be ruled out. Among the other possible sources of variation in the growth response of the 2 cultures (Figs. 2 and 3) the pH and bacterial content deserve consideration. In both the experiments (Expts. 2 and 3) horse serum slants (HS) and 10 per cent horse serum (hs) enriching liquid everlay have been used. The buffering capacity of the horse serum, in both the coagulated and normal state, is very high (Dobell and Laidlaw 1926, Dobell, Neal, Hoare 1952), therefore, it is considered that the two cultures did not seriously differ in their pH during incubation. The technique of "preconditioning" the media with *Esch. coli* A′ was identical and the possibility of unequal bacterial population in the culture, which might cause effective variation of anerobiosis was remote. Balamuth 1963 has shown that *E. invadens* cease to multiply at a level of 2 per cent oxygen but remain viable up to 15 per cent oxygen.

From the above discussion it can be suggested that the size of the initial inoculum, at least under the present experimental conditions (Expts. 2 and 3) directly influences the final population of *E. invadens*. This however, does not agree with Meerovitch 1958 and Balamuth 1962. Balamuth

1962 using a monoxenic culture of *E. invadens* with *Clostridium perfringens* (30°C) concluded that "the size of the inoculum did not materially affect the final population reached". Without any control of the initial inoculum and counting of the population of *E. invadens* Meerovitch 1958 has claimed arriving at the similar conclusion.

The fall in the growth curve, referred to above, has been observed in both the non-clone and clone cultures of *E. invadens* in two different media. This clearly indicates that in these monoxenic cultures with *Esch. coli* A′ this fall in the log phase is reproducable. The significance of this is not yet very clear. The growth curves of *E. invadens* (Balamuth 1962, McConnachie 1955) and *E. histolytica* (Shaffer 1953) in vitro, so far published, do not show any such fall in the population of amoebae before the maximum number has been attained.

McConnachie's 1955 studies of two dixenic strains of *E. invadens* strain A and M in vitro are useful but she did not consider the following points: 1. controlled inoculum of amoebae and rice starch and 2. counting of the amoebae at intervals of less than 2—4 days.

However, the cause of the fall in the growth curve of *E. invadens* was believed to be related to the growth of the accompanying bacteria. Attempts to construct a parallel growth curve for the accompanying viable *Esch. coli* A′ with tetrazolium salts (Guha 1957) failed. As claimed by Guha, Dobell's strain of *Esch. coli* A′, which has been used in these experiments, failed to produce reddish formazan which could be seen under a light microscope. In one of the clone cultures (Expt. 4) on the 9th day of incubation, the number of bacteria was reduced by replacing 2 ml of the undisturbed surface culture medium with the same quantity of fresh medium (Fig. 4). This exchange caused the fall to shift to the 11th day instead of the 10th day as observed in the growth curve constructed from an otherwise identical culture (Expt. 3, Fig. 3). Therefore, the accompanying bacteria seem to be associated in shaping the growth curves of *Entamoeba*, at least in *E. invadens* under the present experimental conditions.

Further, to analyse the log phase of growth in *E. invadens* alone, without any viable associate in the culture, strain BC was re-isolated in the axenic medium of Diamond 1960 with the help of antibotics. This axenic strain of *E. invadens* has been maintained, with difficulty, for more that 3 months (24°C) without any viable associate. The yield of amoebae was so poor that the growth curve, following the techniques described above, could not be plotted.

The fall in the growth curves observed on the 9th to 11th day of incubation appears difficult to explain. Similar effects have been noted in bacteria due to adaptive enzyme formation. A simple case is that of a bacterial culture containing glucose and lactose. The organism first utilises glucose at the main energy source. When the glucose is exhausted, a new lag phase occurs which is followed by a log phase during which the lactose is utilised. This produces the so called diauxic growth curve (reviewed by Mandelstam 1956 p. 70 in which Monod's original works are cited). Such a curve would be possible to construct for *E. invadens*, if it could be counted more frequently than once (5 repeated countings) a day. In the present case of *Entamoeba* similar effect may be operating. Some nutrient may be exhausted selectively, and a lag appears during which adaptation to a fresh substrate takes place.

It is very difficult to understand the nature of these nutrients because of the complexity of the culture medium used. The use of chemically defined medium supporting the growth of *Entamoeba* in vitro would be the first step forward in solving this problem.

The stationary and declining phases

The stationary phase occupied not more than 2 days. After this, all the cultures showed a rapid fall in the population. The onset of decline occurred between the 13th and 15th days of incubation.

Encystation

Quantitative studies of encystation in monoxenic cultures of *E. invadens* BC with *Esch. coli* A′ have disclosed the pattern of changes from trophozoite to cyst. *E. invadens* BC produced cysts in both the HSre+S and HShsm+S media. There is however, no mass encystation, producing clumps of cysts, found in the polybacterial BAH and monoxenic BN strains of *E. invadens* (Neal 1961). On the 5th day of incubation (Figs 1—4) cysts of *E. invadens* BC appeared in the cultures irrespective of the composition of the media.

Both the HSre+S and HShm+S media support the growth of *E. invadens* but HShsm+S medium supports better cyst formation whereas the former supports growth of the trophozoites. HShasm+S medium contains vitamins arising from the added 'Difco' yeast extract. These additives may favour increased growth of *Esch. coli* A′ which in turn possibly influences the process of encystation.

The rate of production of cysts appears to be related to the population of the trophozoites. This is very much evident in the case of a clone culture (Fig 3). Close examination of this growth curve (Fig 3) reveals that after the 7th day, the points of the growth curve show a fall in the trophozoite count with a corresponding rise in the number of cysts,thereby indicating an inverse relationship between the two. The starch in the medium, outside the amoebae, was found completely utilized between the 11th and 13th days of incubation (Figs 1—4).

The maximum cyst formation invariably followed the depletion of starch in the medium. This observation agrees well with those of Balamuth 1962 and McConnachie 1955 but differs from those of Meerovitch 1958, who has noticed mass encystation in the cultures of *E. invadens* containing large amount of starch. Strain variation of the amoebae and differences in the method of cultivation may explain the difference of behaviour of *E. invadens* in vitro.

Although good growth of trophozoites and depletion of starch coincide with the cyst formation, neither can be implicated directly in the encystation process (Balamuth 1962).

Summary

The course of population growth and encystation of *E. invadens* strain BC in vitro, both clone and non-clone, have been studied in monoxenic cultures in association with *Esch. coli* A′. Under the present experimental conditions, where strict control was maintained on the quantity of rice starch, amount of

liquid overlay, and the volume of the solid slant (horse serum), the size of the inoculum of amoebae is directly related to the maximum population obtainable in the culture. A fall in the growth curves (trophozoites) of *E. invadens* has always been observed between the 9th and 11th days of incubation (24°C), that is prior to the attainment to maximum trophozoite count on the 12th to 15th days of incubation. A good growth of trophozoites and depletion of starch always occurred before the cysts in the culture formed appreciably.

Acknowledgement

I am grateful to Professor J. F. Danielli, F.R.S. for providing the facilities and for his interest and encouragement. It is my pleasure to thank Dr. R. A. Neal for the gift of initial cultures of amoebae and for his advice and help during the progress of the work. Financial assistance of the Calcutta University and the Wellcome Foundation, London is gratefully acknowledged.

ZUSAMMENFASSUNG

Der Verlauf des Populationswachstums und die Encystierung von *Entamoeba invadens* Stamm BC in vitro so in der klonalen wie auch in der nichtklonalen monoxener Kultur in Assoziation mit *Esch. coli* A' wurde untersucht. Während der Versuche wurde eine strenge Kontrolle der Menge der Reisstärke und der Reisstärke und der Grösse der übergelegten Flüssingeit, wie auch des Umfangs der dichten, schrägen Unterlage (des Pferdeserum) durchgeführt. Es wurde festgestellt, dass die Grösse des Inoculum der Amöben direkt von dem Populationsmaximum abhängt, welches in der Kultur zu erhalten möglich ist. Zwischen den 9-ten und 11-ten Tag der Inkubation (24°C) konnte man immer eine Absenkung der Wachstumskurve (Trophozoiten) der *Entamoeba invadens* feststellen; es geschah also frühed als die Erreichung der maximaler Zahl der Trophozoiten, die erst zwischen den 12-ten und 15-ten Tag der Inkubation vorkommt. Ein reiches Wachstum der Trophozoiten und die volle Verwendung der Stärke fand ständig statt, eher sich die Cysten in der Kultur massenhaft ausgeformt haben.

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Institute of Parasitology of the Czechoslovak Academy of Sciences
Department of Protozoology, Prague 2, Vinična 7, Czechoslovakia

Petr BEDRNÍK

Cultivation of *Eimeria tenella* in tissue cultures II. Factors influencing a further development of second generation merozoites in tissue cultures

Kultivace kokcidie *Eimeria tenella* v tkáňových kulturách
II. Faktory ovlivňující další vývoj merozoitů druhé generace v tkáňových kulturách

Our knowledge of the requirements of coccidia on the living conditions has been derived only from their behaviour in animals. Conditions under which they are able to grow in tissue cultures have been only little known till present. We tried to follow several factors which could influence the development of coccidia in tissue culture. For our study we chose 2nd generation merozoites of *Eimeria tenella* which enabled us to achieve the most promising results by cultivation in tissue culture. Second merozoites inoculated in tissue culture developed to both the 3rd and the 4th asexual generation and to sexual stages which, after fertilization, gave rise to zygotes and oocysts (Bedrník 1967 a, b).

Material and methods

Obtaining of merozoites, kinds of tissue culture used, methods of inoculation of tissue culture etc. were described in detail by Bedrník 1969.

The used kinds of inoculum

In addition to N and N TRYPS inoculum described in the previous paper the following types of inoculum were used:

Nonpure inoculum N_1.

The N inoculum was centrifuged at 600 G for 2 minutes. Sediment containing merozoites was resuspended in a supernatant. This served as a control to P_1 inoculum.

Nonpure inoculum N_2.

The N inoculum was repeatedly centrifuged, at first 2 minutes at 600 G and afterwards for 4 minutes at 800 G. This served as a control to P_2 inoculum.

Pure inoculum P_1.

After centrifugation of the N inoculum at 600 G for 2 minutes the superna-

tant contained still many merozoites whereas the other elements present in the N inoculum sank to the sediment. This supernatant was used as P_1 inoculum containing only merozoites and a small amount of erythrocytes.

Pure inoculum P_2.

P_1 inoculum was centrifuged for 4 minutes at 800 G. The sediment consisting only of merozoites and erythrocytes was resuspended in a suitable amount of fresh medium.

Pure inoculum P_2+lysis.

When preparing the P_1 inoculum the sediment contained still several free merozoites, released caecal cells, erythrocytes, macrophages etc. These elements were lysed by means of a small amount of deionised water. After 15 minutes the normal osmotic pressure was restored with the help of PBS. In preparing the P_2 inoculum merozoites sedimented in this fluid after second centrifugation were resuspended.

Pure inoculum P_2+blood.

Merozoites sedimented after second centrifugation were resuspended in a medium containing a small amount of blood from chickens in which merozoites were developed. The blood was obtained from cut carotides. After resuspendation of merozoites this suspension was kept for 3 hours at 39°C and then inoculated into the tissue culture.

Pure inoculum P_2 — TRYPS.

This inoculum was prepared from N TRYPS inoculum by a method used in producing the P_2 inoculum.

Pure inoculum prepared by filtration through membrane filters Pf.

The cut caeca containing merozoites were agitated in a large amount of PBS (minimum 500 ml). The obtained suspension was strained through membrane filters PUFS (middle size of pores 4 μ). The filtered fluid contained only merozoites and a small amount of erythrocytes. It was re-filtered through RUFS filter (middle size of pores 1.5 μ) (both filters were products of the Czechoslovak firm Synthesia. This filter retained most of the merozoites. Immediately after filtration the filter used was agitated in a necessary amount of medium. This type of filter did not retain all merozoites; besides a large number of them adhering to it could not be released by agitation in the medium. This was why before filtration the RUFS filters were in some instances overcovered with a fine film of $MgCO_3$ which prevented retainment of merozoites within the filter enabling thus their 100% yield. Particles of $MgCO_3$ had no influence upon merozoites or cells. Nevertheless the pH in the inoculated culture increased and it had to be balanced by a larger amount of fresh medium when changing the inoculum.

Pure inoculum filtered and trypsinized P_f TRYPS.

The fluid obtained after trypsinization of infected caeca and containing merozoites (see preparation of N TRYPS inoculum) (Bedrník 1969) was diluted by PBS approximately ×30 and filtered by a method mentioned above.

Inoculum Pf TRYPS+caecal cells.

The cut caeca of healthy chickens were repeatedly washed in PBS, then put in 1.5% trypsine with 500 u. of penicillin and streptomycin per ml., agitated at intervals and kept at 39°C for one hour. The obtained cells were resuspended in Pf TRYPS inoculum.

Inoculum Pf TRYPS+CHE cells.

A small amount of cells derived from 10 up to 13-days-old chicken embrya was added to Pf TRYPS inoculum.

Results

Factors studied by using N and N TRYPS inoculum

The influence of the type of cells used

The used type of cells was of paramount importance for further development of 2nd merozoites inoculated into tissue cultures. In He-La cells we observed the development of 3rd generation merozoites only very sporadically, the sexual stages developing not at all. In all three types of cultures derived from chicken embryos CHE-A, CHE-B and CHE-C, a further development was incomparably more frequent. Both the asexual and sexual stages were noted, the latter giving rise to oocysts. The development of asexual stages took place in fibroblasts, epithelial cells and macrophages. The largest quantity of sexual stages was recorded in CHE-C cultures which contained the largest number of epithelial cells. The small physiological differences among embryonal epithelial cells from various organs or the great dedifferentiation of these cells in tissue culture enabled the development of sexual stages also in CHE-B cultures derived from chicken embryos the digestive tract of which was removed before trypsinization. Sexual stages developed also in macrophages from N or N TRYPS inoculum attached to pure slides. These observations have completed the results obtained by L o n g 1965, 1966 who had ascertained that sporozoites of *E. tenella* inoculated in the allantois of chicken embryo, can develop to all stages of the life cycle including oocysts. The development of sexual stages need not necessarily be limited to glandular epithelium of caecum.

The influence of merozoites used

The developmental properties of merozoites originated from various chickens differed considerably. Using the same number of merozoites of various origin for inoculation of tissue culture we observed either the development: 1. only of asexual stages, 2. only of sexual stages, 3. both stages in most varied quantities 4. no development at all.

Merozoites obtained from chickens on the 6th day after inoculation differed from those obtained on the 5th day following the inoculation by their ability to develop into sexual stages. When undergoing further development in tissue culture, the first ones gave rise to sexual stages in all instances, whereas the second ones only in some instances (Table 1).

A large difference was also noted among merozoites from the N inoculum, i.e. merozoites obtained from caecal contents and merozoites from the N TRYPS inoculum which were released from caecal cells. In tissue cultures inoculated with N TRYPS inoculum a substantially more intense development was observed than in cultures inoculated with N inoculum from the same chicken. Merozoites from caecal contents were probably partly damaged whereas in merozoites released from caecal cells all necessary properties were preserved.

Table 1

Survey of developmental properties of merozoites

Experiment No.	Merozoites obtained on the		Further development of merozoites in tissue culture 24 hours after inoculation	
	5th day	6th day	asexual stages	sexual stages
30	+		0	0
31	++		+	+
33	++		+	+
37A	+		+	+
38A	+		+	+
45/1	+		+	+
45/2	+		+	+
46		+	+	+
47	+		+	+
48		+	+	+
49	+		+	0
50/1	+		+	+
50/2	+		+	+
51/1	+		+	+
51/2	+		+	0
51/3	+		+	+
51/4	+		+	+
56	+		+	+
64	+		+	0
66/1	+		+	0
66/2	+		0	0
78/1	+		+	0
78/2	+		+	0
80/1	+		+	0
80/2	+		0	0
80/3	+		0	0
81/1		+	+	+
82/1	+		+	0
82/2		+	+	+
82/3		+	+	+
83/1	+		+	0
83/2		+	+	+
83/3		+	+	+
83/4		+	0	0
84/2		+	+	+
85/1	+		0	0
85/2	+		0	0
85/3		+	+	+
87/1		+	+	+
87/2		+	+	+
88/1		+	+	+
88/2		+	+	+
88/3		+	+	+
89 tryps		+	+	+
96 tryps		+	+	+
97 tryps		+	+	+

0 = no development; + = dovelopment

The influence of cultivation temperature

The inoculated tissue cultures were kept either at 37°C or at 39 up to 40°C. In both instances we observed multinucleated schizonts and rosettes of new 3rd merozoites after 24 hours. The temperature within these limits did not exercise a different influence on further development of 2nd merozoites. For a more correct approach to conditions in vivo the temperature was kept at 39 up to 40°C in all subsequent experiments.

The influence of inoculation and cultivation medium

We used PBS and PBS + 10% calf serum as inoculation media for comparison with the tissue culture medium + 10% calf serum without noting any difference. We followed also the influence of single compounds of the tissue culture medium and PBS on merozoites. In all examined compounds of the medium as well as in PBS, clusters of merozoites were observed after several hours. These merozoites showed morphological changes which however, the same as agglutination, were not a sufficient criterion of their vitality. Single merozoites from samples in which all individuals displayed morphological changes not only entered the cells but also were developing further within them. In a slightly hypotonic medium merozoites became shorter, thicker and lost their mobility. When normal osmotic pressure was renewed their normal morphological appearance and mobility were restored, their developmental properties being also preserved as proved by further course of the experiment. For the only criterion of the vitality of merozoites we must take their further development in tissue culture. As regards the single intracellular merozoites we cannot say with certainty whether they were not phagocytized by cells. A 10% native serum lysed merozoites within 1 hour at 4° the same as at 40°C, a 2% native human serum lysed them partly within 3 hours.

The substitution of calf serum by horse serum in tissue culture did not result in a different influence upon the development of inoculated merozoites.

Factors studied in using other types of inoculum

In a subsequent study we attempted to prepare pure inoculum containing only 2nd merozoites. P_1 and P_2 inocula prepared by means of centrifugation contained merozoites which were mobile and invaded cells but their further development was either limited or did not take place at all. Merozoites from control inocula N_1 and N_2 which were also centrifuged developed as usual. The loss of developmental properties of merozoites in P_1 and P_2 inocula could not be caused by their damage during centrifugation. It was necessary to ascertain the factors preventing further development.

The following was taken into consideration:

1. Presence of a stimulating chemical substance the concentration of which was lowered to an ineffective degree by dilution and centrifugation. To keep this substance in the inoculation medium we prepared the P_2 inoculum + lysis (pure merozoites resuspended in fluid obtained by lysis of all other elements present in the N inoculum). The negative results of experiments with this inoculum disproved the original supposition on the presence of a chemical stimulating substance in the N inoculum.

2. According to a second assumption the presence of blood cells in the

inoculum was necessary. Inoculum P_2 + blood was prepared and an interaction between merozoites and cells was made possible for 3 hours at 39°C, before inoculation into tissue culture. Also this assumption was incorrect, further development being minimal again.

3. A third line of experiments started from the assumption that only those merozoites developed in tissue culture which, enclosed within the caecal cells released from infected chicken, were introduced to cultures. This was supported by results of other authors reporting transmission of sporozoites within macrophages and a probable necessity of this transport for a further development of sporozoites in animals. (Challey and Burns 1959, Van Doornick, Becker 1957, Patillo 1959). However our observation of the N inoculum prior to the infection of the tissue culture contradicted this assumption. Only once during our study we noted an intracellular merozoite in a macrophage. Other coccidial developmental stages "misintroduced" into the tissue culture within the released caecal cells (small schizonts) could be differentiated from coccidia developed in proper tissue culture cells. We tried to release intracellular merozoites from caeca by means of trypsinization. Though the N TRYPS inoculum exceeded our expectation (merozoites from it developed much better than from the N inoculum) in the P_2 TRYPS inoculum merozoites developed only to a limited extent.

4. A careful quantitative evaluation of the number of merozoites 4 hours after inoculation disclosed the decreasing of the number of intracellular merozoites in centrifugated inocula in some instances (Table 2). This observation resulted in the preparation of Pf and Pf TRYPS inocula using membrane filters for purification of merozoites. These merozoites penetrated in cells in

Table 2

Number of intracellular merozoites in tissue culture following infection with different types of inocula

Experiment No.	Type of inoculation	Hours after inoculation	Number of merozoites in inoculum in millions per 1 ml	Number of intracellular merozoites per field of view (ocul. ×10, obj. ×100, magnif. ×1350).		Assumed number of intracel. merozoites at a uniform concentration 1 million merozoites per 1 ml of inoculum
				Number of fields of view	Number of merozoites	
49	N	4	1	70	10	10
49	P_2	4	1	70	10	10
51/1	N		1	552 (100)*	126 (22.6)	22.6
	P_1		1.4	612 (100)	55 (8.9)	6.3
	P_2		1.7	586 (100)	171 (29.1)	17.1
51/2	N		0.5	100	16	32
	P_2		1.0	100	16	16
51/4	N	8	1.26	30	50	38
	N_2	8	1.5	90	63	42
	P_2	8	1.2	60	31	25.8

* Numbers in parentheses indicate the assumed number of intracellular merozoites per 100 fields of view.

a large quantity, they increased in size and became coiled within the cells, but they developed further only very occasionally.

With the exception of oocysts, in tissue cultures inoculated with pure inocula we observed all developmental stages of coccidia the same as in cultures infected with nonpure N or N TRYPS inocula.

5. The conducted experiments revealed that a further development of merozoites within the tissue culture was strongly influenced by the presence of free cells originated from the caeca of infected chicken. We prepared two more inocula; Pf TRYPS + caecal cells, containing cells derived from caeca of healthy noninfected chickens and Pf TRYPS + CHE cells, containing cells derived from 10-days-old chicken embryos. In both instances a further development of coccidia was minimal.

Discussion

The obtained results revealed that further development of 2nd merozoites took place mainly in tissue cultures infected with nonpure inocula containing, besides merozoites, also different other cells which were released from the inflamed caecum of the infected chicken and "misintroduced" into cultures. The main importance of these cells in which inoculated merozoites may also develop, for the cultivation of merozoites in tissue culture, consists in the possibility of existence of some unknown growth factor within these cells. Together with them, this factor, supporting in a decisive measure a further development of the inoculated merozoites in proper tissue culture cells, was "misintroduced" into the tissue culture. If such cells were not present (P inocula) the inoculated merozoites developed as well, but only in minimal quantities.

From several experiments we can deduce some of the properties of this assumed growth factor. In the first place we must exclude a lack of vitamins, essentially for the development of *E. tenella*. All necessary vitamins are present in the used cultivation medium TC 199 (Warren 1968).

The preparation of N TRYPS inoculum has proved that this factor is not damaged by long-term trypsinization. Destruction of cells present in the inoculum by lysis resulted also in the destruction of this factor.

Attempts at transferring coccidia developed in tissue culture into fresh cultures indicate that the aforementioned factor can evidently be transferred into fresh cultures.

Even a very small number of "misintroduced" cells is sufficient to support sensibly the growth of merozoites in inoculated cultures.

This factor is not present either in cells of healthy caeca or in cells of chicken embryos (see the results of experiments with inocula Pf TRYPS + caecal cells and Pf TRYPS + CHE cells).

In this connection it is possible to present some more facts. Burns 1958, 1959, Sharma and Foster 1964 and Strout and Holman 1965 ascertained that homogenate of caecal scrapings of chickens infected with *E. tenella* is lethal for rabbits, whereas the homogenate of caecal crapings of healthy chicken has proved as harmless. Extract from oocysts of *E. tenella* had the same effect. For chickens and other laboratory animals this toxic substance was harmless. Sublethal dosis of this substance provoked an immune response.

In cultivating *E. tenella* sporozoites in tissue culture derived from chicken embryos the infected tissue fell off the slides after 24 or 48 hours after inoculation. This phenomenon was also observed by Patton 1965. He explained it by the influence of high temperature at which the cultures were kept (41°C). Our own experience from experiments with cultivation of sporozoites revealed that as long as the tissue is not infected with coccidia the falling off of the cell layer takes place only in some instances and it may moreover be due to a large amount of cells mounted on the slides (Bedrník 1969). When using the N inoculum with 2nd merozoites the reaction of chicken cell cultures was rather similar. It is interesting that in infected He-La cell cultures the falling off of the cell layer did not take place, the action of this factor being probably specific.

In this respect we consider as very important the results of Gresham and Cruickshank 1959 who were the only authors observing the development of asexual stages in macrophages in vivo. They reported the following histochemical results: "... the histological appearance suggests an enhanced ribonucleoprotein production within chick macrophages parasitized by *E. tenella* and, furthermore, that this new protein is incorporated into newly formed merozoites." Furthermore, Doran and Vetterling 1967 mention that "factor responsible for activation is not present in all types of cells" so that sporozoites of *E. meleagrimitis*, *E. necatrix*, *E. gallopavonis* and *E. acervulina* develop only in some kinds of tissue culture or not at all. Also Fayer and Hammond 1967 were searching for the cause of the fact that sporozoites of *E. bovis* were transformed into trophozoites only in a secondary culture of bovine kidney cells, not in their primary one, in the absence of the inhibiting factor or in the presence of some conditions or substances which were acting as a stimulus.

To conclude we wish to attempt a synthesis of all quoted facts and considerations. Our "growth factor" is not present in the caeca of healthy chicken, it must therefore be introduced to them during infection, most probably from oocysts during their excystation. The influence of an inoculum containing sporozoites and of the N inoculum containing second merozoites is very similar so that the cause may be identical. The fact that cells fall off only in tissue culture derived from a natural host can be explained by conditions unfavourable for the spread of this factor in nonspecific tissue culture. If it is impossible for the factor to spread, no development of sporozoites or merozoites takes place. The changes in secondary cultures of bovine kidney cells may be more favourable for this factor enabling thus transformation of sporozoites into trophozoites. Antigenic properties of the toxin substance obtained by Burns and other authors, production of ribonucleic substance in the infected macrophages and evidence of an "adhesive capacity" of our "growth factor" to intact cellular structures may represent three different qualities of one substance.

Summary

The type of cells used was of paramount importance for further development of inoculated 2nd merozoites. The largest number of developing coccidia was observed in the tissue derived from digestive tracts and livers of 17 up to 19-days-old chicken embryos.

Merozoites originated from different chickens showed different developmental properties. Merozoites obtained from chickens on the 6th day after infection developed to sexual stages in all instances, those obtained on the 5th day after infection gave rise to sexual stages only in some instances. Merozoites obtained from caecal cells showed a more intense growth than those obtained from caecal contents.

No differences were noted in the development of coccidia grown at 37 or at 40°C.

The effect of inoculation media PBS and PBS + 10% calf serum and TC medium 199 + 10% calf serum upon the number of merozoites invading the cells was uniform. Agglutinations and morphological changes of merozoites were observed after several hours in single compounds of the medium and even in PBS. However these changes cannot be considered as a sufficient criterion of the vitality of merozoites.

The substitution of calf serum by horse serum in the medium showed no difference in the effect upon the growth of coccidia.

Further development of 2nd merozoites took place mainly in tissue cultures inoculated with nonpure inocula containing — besides merozoites — also different cells from the inflamed caecum of the infected chicken. An unknown factor which in a decisive measure supports a further development of merozoites is harboured by these cells and introduced into the cultures with them. Character of this factor is discussed in comparison with the results presented by other authors.

SOUHRN

Byly sledovány faktory, o nichž se předpokládalo, že mohou ovlivňovat vývoj kokcidií v tkáňových kulturách:

1. Používaný druh buněk má zásadní význam pro vývoj kokcidií. V He-La buňkách se vyvíjela jen vzácně asexuální stadia, v buňkách odvozených od kuřecích embryí se vyvíjela jak asexuální tak sexuální stadia, ale sexuální stadia se vyvíjela pouze v epitheliálních buňkách a někdy v makrofágách.

2. Vlastnosti merozoitů pocházejících z různých kuřat jsou velmi různé. Některé se v tkáňových kulturách nevyvíjejí vůbec, některé pouze v asexuální stadia, některé pouze v stadia sexuální a některé v obojí stadia v nejrůznějších kvantitativních poměrech.

Merozoity odebírané z kuřat 6. den po infekci se vyvíjely vždy v sexuální stadia, merozoity odebírané 5. den po infekci kuřat se vyvíjely v sexuální stadia jen občas. Merozoity získávané z coek nakažených kuřat trypsinisací měly lépe zachovány vývojové schopnosti než merozoity z coekálního obsahu.

3. Inokulační teplota v rozmezí 37—40°C neovlivňovala rozdílně vývoj kokcidií v tkáňových kulturách.

4. Inokulační media PBS, PBS + 10% telecího séra a TC medium 199 + 10% telecího séra neměla rozdílný vliv na počet merozoitů proniklých do buněk. Jednotlivé složky kultivačního media i PBS působily po několika hodinách aglutinace a morfologické změny merozoitů, které však nejsou dostatečným kriteriem jejich životaschopnosti.

5. Nahražení telecího séra v mediu koňským sérem neovlivnilo rozdílně další vývoj merozoitů.

6 Další vývoj merozoitů 2. generace probíhá hlavně v tkáňových kulturách

infikovaných N a N TRYPS inokuly, kde mimo merozoitů jsou zanášeny do tkáňových kultur i různé buňky ze střeva infikovaného kuřete. Spolu s těmito buňkami je do kultur zanášen blíže neznámý faktor, který rozhodující mírou napomáhá dalšímu vývoji inokulovaných merozoitů. Je diskutována povaha tohoto faktoru a konfrontován s výsledky jiných autorů.

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Laboratory of Microbiology, Biological Research Institute of Leningrad Strate University, Leningrad V-164, USSR

B. V. GROMOV and K. A. MAMKAEVA

The culture of *Aphelidium chlorococcarum* Fott f. *majus* f. nova

Культура *Aphelidium chlorococcarum* Fott f. *majus* f. nova

The genus *Aphelidium* was described by Zopf in 1885 as a genus of algal parasites which is characterized by the intracellular plasmodial body dividing into uniflagellated zoospores after maturation without the formation of intermediate cyst (Zopf 1885). These organisms were placed by Zopf into the order *Gymnococcaceae* of Monadinea group. Zopf described one species — *Aphelidium coleochaetae*; later Scherffel 1925 described 3 new species: *A. melosirae*, *A. chaetophorae*, and *A. tribonemae*. Recently Fott 1957 described the fifth species *A. chlorococcarum*. All species differed mainly by the host on which they were observed. All data concerning these organisms were obtained from natural material.

During the summer of 1967 we observed the development of *Aphelidium*-like organism in the large scale cultures of *Kirchneriella obesa* (West) Schmidle, carried out in the Biological Institute by V. V. Pinevich with coworkers (1968).

It was possible to observe the development of the parasite in laboratory cultures of *K. obesa*, inoculated with infected material. The double culture of the parasite with algae free from contamination was obtained. Studies of the parasite in artificial culture have shown that it is related to *A. chlorococcarum* Fott, but some new additional features were observed. We succeeded to determine a possible host range for the parasite. The examination of the culture gave us the possibility to consider it as a specialized form of *A. chlorococcarum* Fott.

Material and methods

Algae and parasites were cultivated in the standard medium 6 (Gromov 1965) which proved to be favourable for inoculation. The medium was as follows (gramm per liter of distilled water): KNO_3 — 1; K_2HPO_4 — 0.2; $MgSO_4\ 7H_2O$ — 0.2; $CaCl_2$ — 0.15; $NaHCO_3$ — 0.2; micronutrient solution — 1 ml.

Microorganisms were cultivated at 25°C and constand illumination from fluorescent lamps (light intensity on the surface of the medium about 2500 lux).

Aphelidium did not multiply in the algae which grew on the surface of solid media, while only rarely it developed in the semisolid media (with 0.6—0.7% of agar). If it developed in semisolid media, it formed local zones in which algae were destroyed. Several *Aphelidium* clones were obtained from such zones but they contained numerous bacteria. The double culture free from contamination was obtained by means of repeated inoculations from dilutions of rough culture of pure algal cultures to which 4 000 unit/ml penicilline was added. Purified culture was maintained by the inoculations of pure cultures of *K. obesa* in liquid medium. The culture was stable and needed reinoculations no more than every 1—2 months. Material from the cultures of different age was examined under phase-contrast microscope.

Available cultures of different algae grown in liquid medium were inoculated with the parasite for the examination of its possible host range. The inoculated cultures were examined microscopically in 10 and 20 days. 248 cultures of different algae from our collection (Gromov 1965) and from the collection of Indiana University (Starr 1964) were tested.

Results and discussion

The zoospores of *Aphelidium* are round 2—3 μ in diameter with one small refractive globule (Pl. I 1). They have no tendency to the amoeboid movement and acquire flagellum when formed in the destroyed algal cell. The flagellum is massive with basal part 7—9 μ and thin whiplash (akronema) about 5 μ long. The spores move rapidly by means of the sharp beat of flagellum. On the opposite side of the spore little conic stiletto or sting is faintly visible. It serves as a point of contact with the host cell.

When attacking alga the parasite forms a round body connected with the host cell by short stalk which is evidently formed from stiletto (Pl. I 2). Parasite's protoplasm flows into the host cell through the stalk and hole in the host cell wall underneath of it. The empty bag remains outside the host cell attached to the algal cell wall (Pl. I 3). Vegetative body grows by digesting the algae till the whole space under the wall is occupied by the parasite (Pl. I 4). A little vacuole with yellow excrete globule is situated in the center of the vegetative body (Pl. I 5). One alga is often infected by several zoospores. They sometimes penetrate into alga in several places and form several vegetative bodies which grow separately, but then fuse forming single mature sporangium. More usual is penetration of parasite into the infected cell through the empty bag formed by its precursor. Evidently it is more simple for the parasite to inoculate alga through the recently formed hole than to penetrate into thick algal cell wall. Hyperinfection is especially characteristic of the cultures in which numerous zoospores are formed but only few algae remain. Ten or more zoospores can be joined with first one and a peculiar "tree" is formed on the surface of algae in this case (Pl. I 6). The biological nature of this phenomenon is obscure. More zoospores are sometimes trying to attack algal cell than would be formed after its decay.

Mature vegetative body of the parasite subdivides into zoospores which leave the destroyed cell. The cell wall of destroyed algae with parasite's excrete globule has a very characteristic appearance which allow us to indicate the presence of parasites in ill algal cultures (Pl. I 7—8). Sometimes

Table 1

Examined algae resistant to *Aphelidium*

	Number of species	Number of strains
Type *Chlorophyta*		
Order *Volvocales*		
Chlamydomonas	5	7
Haematococcus	1	1
Order *Chlorococcales*		
*Ankistrodesmus**	16	16
Characium	1	1
Chlorella	11	81
Chlorococcum	16	17
Coelastrum	3	3
Coccomyxa	2	2
Dictyosphaerium	2	3
Muriella	1	1
Neutococcus	1	1
Neochloris	4	4
Oocystiy	2	3
Pediastrum	2	2
Protococcus	2	2
Scenedesmus	14	76
Scotiella	2	5
Tetraedron	2	2
Trebouxia	4	4
Order *Chlorosphaerales*		
Chlorosarcinopsis	1	1
Order *Ulotrichales*		
Hormidium	4	5
Stichococcus	5	7
Order *Zygnematales*		
Closterium	1	1
Mesotaenium	1	2
Type *Chrysophyta*		
Order *Heterococcales*		
Chloridella	1	2
Chlorocloster	1	1
Pleurochloris	1	1
Order *Heterotrichales*		
Heterococcus	1	1
Tribonema	1	1

* The sensitive strains of this genus are listed in the text.

mature vegetative body does not divide but contracts and excretes thick wall to form dormant spore (Pl. I 9). Yellow fat globule is pushed out of the body into the space between algal and parasite walls.

Aphelidium developed only in the cells of 6 strains of algae from 248 examined. They were *Kirchneriella obesa* (West) Schmidle, *K. subsolutaria* West, *Ankistrodesmus amalloides* Chod. et Oettli, *A. arcuatus* Korsch., *A. Braunii* Brun., *A. spiralis* (Turn.) Lemm. Taxones proved to be resistant are listed in Table 1. It is evident that this parasite is very specific, it can attack only chlorococcous algae and only of the genera *Kirchneriella* and *Ankistrodesmus*. Only few *Ankistrodesmus* strains are sensitive, but they represent 4 different species. On the other hand, one strain of *A. braunii* is sensitive but 3 others are resistant. It seems that parasite is not specific to definite species of algae but can grow in the cells of some strains of certain genera.

The consideration of the systematical position of the organism in question is very complicated. Never before such organisms were cultivated under experimental conditions. The descriptions of *Aphelidium* species were very superficial, especially from the point of view of their possible hosts. The criteria for species differentiation do not exist. This organism is morphologically similar to *Aphelidium chlorococcarum* Fott and attacks the cells of algae which belong to the same group — *Chlorococcales*. Fott did not note whiplash and stilleto in zoospores but he used a usual light microscope where these structures were not visible. Zoospores of our form are round and bigger than are those of *A. chlorococcarum* — 3—4 μ in diameter instead of 1.5 μ and flagellum up to 14 μ instead of 8 μ. Zoospores in Fott's drawings are oval.

Fott described *A. chlorococcarum* as a parasite specific to the chlorococcous algae but *Kirchneriella* and *Ankistrodesums* were not mentioned as its hosts. At the same time, some genera, for instance, *Scenedesmus*, listed by Fott as sensitive and examined by us did not contain sensitive strains (76 *Scenedesmus* strains were examined).

It is possible to consider the organism in question as a specialized form of *Aphelidium chlorococcarum* Fott. In accordance with its bigger size we designated it as *Aphelidium chlorococcarum* Fott forma *majus* f. nova.

A. chlorococcarum f. *majus* is characterized by round zoospores 3—4 μ in diameter with stilleto and one flagellum. Flagellum consists of thick basal part 7—9 μ long and whiplash about 5 μ long. This form attacks algae of the genera *Kirchneriella* and *Ankistrodesmus*. One strain was isolated from the mass culture of *Kirchneriella obesa* near Leningrad.

Summary

The double culture of *Aphelidium chlorococcarum* Fott with sensitive alga free from contaminants was obtained from the mass culture of *Kirchneriella obesa*. *The parasite* has round zoospores with stilleto and single flagellum. The flagellum is composed of thick basal part and thin whiplash. The parasite infects algae of the genera *Kirchneriella* and *Ankistrodesmus*. On the basis of morphology and specificity of the strain obtained it is considered as a new form — *Aphelidium chlorococcarum* Fott forma *majus*. f. nova.

РЕЗЮМЕ

Из массовой культуры *Kirchneriella obesa* получена свободная от загрязнений культура паразитического организма *Aphelidium chlorococcarum* Fott. Свободная стадия паразита представлена круглыми зооспорами с „носиком" и одним жгутиком. Жгутик состоит из толстой базальной части и тонкой акромены. Паразит развивается в клетках водорослей родов *Kirchneriella* и *Ankistrodesmus*. На основе особенностей морфологии и специфичности выделенный штамм описан как новая форма — *Aphelidium chlorococcarum* Fott forma *majus* f. nova.

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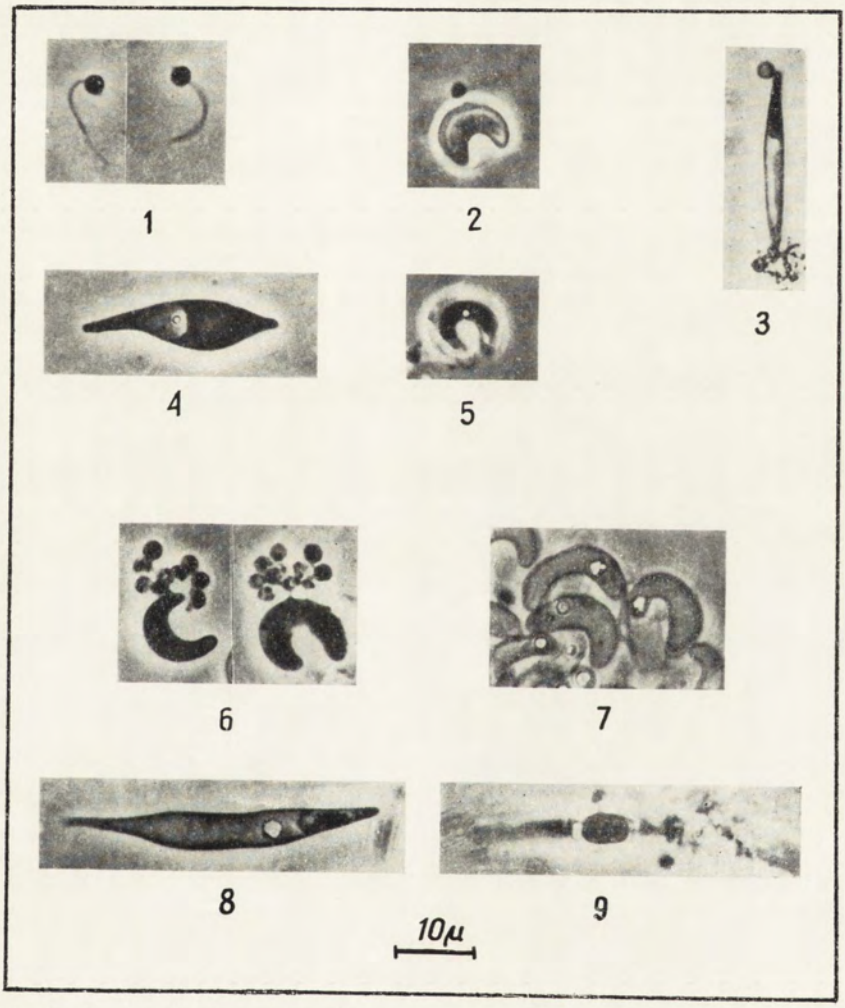
EXPLANATION OF PLATE I

1: *Aphelidium* zoospores
2: *Kirchneriella* cell attacked by *Aphelidium* zoospore
3: Infected *Ankistrodesmus* cell. The body of parasite developed inside alga and empty wall of zoospore are visible
4: Vegetative body of *Aphelidium* in the cell of *Ankistrodesmus*. Excrete gobule in the center of body is visible
5: The cell of *Kirchneriella* occupied by mature body of parasite
6: *Kirchneriella* hyperinfected by *Aphelidium* zoospores
7: *Kirchneriella* cells digested by parasite
8: *Ankistrodesmus* cells digested by parasite
9: Dormant spore of *Aphilidium* in the cell of *Ankistrodesmus*

All microphotographs in the magnification 1000×, phase-contrast



B. V. Gromov et K. A. Mamkaeva auctores phot.

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Department of Biology, Military Medical Academy, Łódź, Pl. 9 Maja 1, Poland

Julia ROSTKOWSKA*

Studies on the infective stage of *Balantidium coli* (Malmsten) for hamsters

Badania nad stadium inwazyjnym *Balantidium coli* (Malmsten) dla chomików

In my present studies I attempt to elucidate the experimental conditions of cyst formation in *Balantidium coli* (Malmsten), their excystment and infectivity, and to follow the viability of the trophozoites in extraintestinal medium, the possibility of infection with vegetative stages, the resistance of *B. coli* to environmental conditions and the influence of various factors on the variability of this protozoan. The studies have been made also on the infection of golden hamsters (*Mesocricetus auratus* Waterh.), the nonspecific host for *B. coli*.

Material and methods

In order to investigate the viability of trophozoites and cysts of *B. coli*, their encystment and excystment, the following method was applied: the feces and intestinal content of 50 pigs from the slaughterhouse in Łódź were examined. Feces were taken from pigs before killing. Afterwards a segment of the large intestine, about 30 cm long, was examined. The feces and intestinal content were examined for the presence of trophozoites and cysts with the aid of decantation and flotation methods. The feces were examined after 4, 12, 18, 24, 48, 72, 96, and 120 hours. The samples were maintained at a temperature of 20°C. The intestinal content was examined only once. The trophozoites were inoculated in the medium of Pavlova and the cultures were kept at a temperature of 37°C. To examine the cyst formation, the cultures of balantidia were placed at 37°, 20°, 14°, and 4°C temperatures.

The development of *B. coli* in animals infected with cysts and vegetative stages was studied in hamsters. The animals 24—30 days old were used for experiments. Infection was made per os with the aid of pipette. Each hamster received about 500 cysts. Each experiment comprised a series of 25 to 30 hamsters. Sections were made in 10, 30, 90, and 180 days after infection. The alimentary tract was divided in 3 parts: stomach, small intestine and large intestine. Each part was placed separately in a glass with 0.85% natrium chloride solution. The intestinal content of each part was carefully examined under the microscope.

* Present address: Biological Institute, Silesian University, Katowice ul. Krasińskiego 2.

Experimental part

The results of examination of the feces and intestinal contents of pigs are given in Table 1. The specimens of *B. coli* from particular pigs differ in shape and size. The mean size of round protozoans, counted on 200 specimens, is 84×79 μ, and of oval ones 140×117 μ. Within one population, comprising the ciliates of various shape, the mean size (counted on 200 specimens) of the large form is 145×113 μ, and of the small, round form 65×58 μ. In high intensity infection balantidia occurred on the intestinal walls, in the lumen

Table 1

Incidence of *Balantidium coli* in feces and intestinal content of pigs

Number of pigs examined	Number of pigs infected	Shape of trophozoites			Incidence of trophozoites		Incidence of cysts	
		round	oval	various	in feces	in intestinal content	in feces	in intestinal content
50	46	11	29	6	45	46	4	—

of the intestine and in intestinal content. In such a population balantidia differed in shape and size. Big trophozoites dominated on the intestinal wall; out of 100 specimens gathered there only 7% were small, while in the intestinal content 43% were small. The preparations were made from the intestinal wall of pigs with high infection of large and small forms of *B. coli*. On sections balantidia were not found in submucosa or in deeper layers of the intestinal wall. Experiments were made in summer when the per cent of pigs infected is high. In the winter season the per cent of infection is distinctly lower.

Single cysts appeared in samples of feces not earlier than 4—5 hours after defecation. The greatest number of cysts was formed in feces in which the protozoans of different size occurred. It seems, on the base of my observations, that the majority of *B. coli* leave the pig intestine as trophozoites, and, under the influence of low temperature, cysts able to infect another animals are formed.

To investigate whether the size and shape of these protozoans are stable I maintained the cultures of balantidia taken from the large intestine of some pigs. After 12 and 24 months (Table 2) the shape and size of the protozoans in culture changed as well as their numerosity and sensibility to atebrine.

Moreover, I investigated the influence of temperature on the longevity of passages of *B. coli*. Nelson 1935, Lom 1956 and others reported various periods of one passage longevity at a temperature of 37°C. I maintained *B. coli* cultures at 37°, 20°, and 4°C. To some cultures maintained at 20°C *Escherichia coli* was added. The mean of 10 passages from all cultures is represented on Fig. 1. Similar results were obtained by Lom who maintained the passages during 17 days at 37°C. The longevity of *B. coli* in one passage was examined taking into account the period of laboratory culture of the strain. Fig. 2 represents the longevity of balantidia 6 days after they have been taken from pig intestine, and after 12 months and 24 months of breeding.

Table 2
Variability of *Balantidium coli* in culture

Time in months	Round forms			Oval forms		
	size in μ	number of specimens in 1 ml	sensibility to 0.003 M atebrine	size in μ	number of specimens in 1 ml	sensibility to 0.003 M atebrine
0	98×80	10 300	9.84±2.91	143×117	6 200	12.73±3.61
12	90×77	7 460	23.16±3.88	128×104	3 300	29.6±4.58
24	79×68	4 780	46.2±4.46	112×90	2 150	53.4±5.75

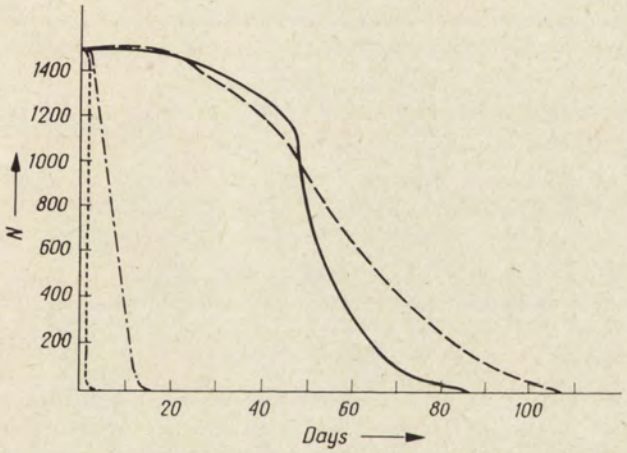

Fig. 1. Influence of temperature on the longevity of *Balantidium coli* population, —·—·— temperature 37°C, — — — temperature 20°C, ——— temperature 20°C in culture with *Escherichia coli*, ····· temperature 4°C

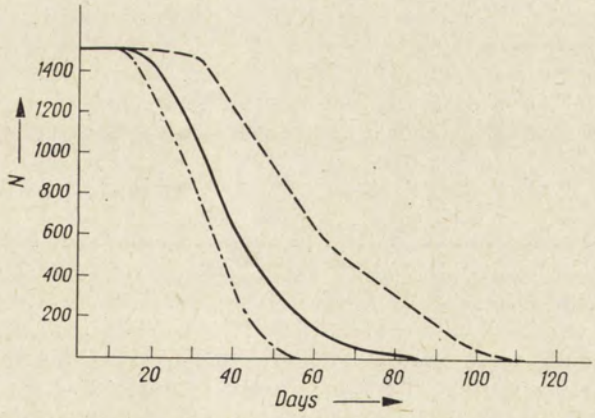

Fig. 2. One passage longevity depending on the period of previous laboratory culture of *Balantidium coli*, — — — population after 6 days of breeding at 37°C, ——— population after 12 months of breeding at 37°C, —·—·— population after 24 months of breeding at 37°C

There are also some differences between the longevity of balantidia of different shape and size. The results are represented on Fig. 3.

My further observations concern the influence of various temperatures on cysts formation in laboratory conditions. Populations of *B. coli* trophozoites, differing in shape and size, were maintained at various temperatures (Table 3). Most of the cysts were formed in the populations of small, round or big, oval trophozoites. The cysts are round, measuring 54×58 μ.

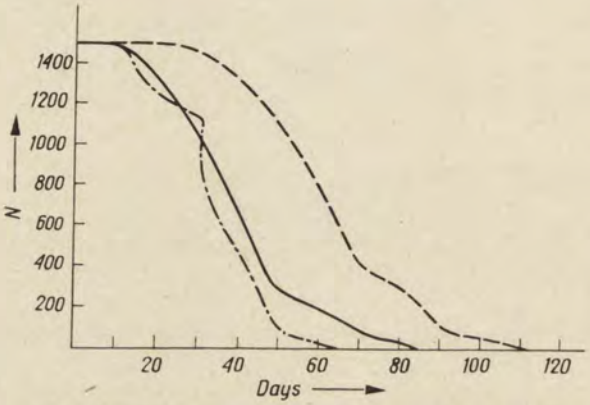

Fig. 3. The longevity of *Balantidium coli* population on shape and size of trophozoites, —·—·— round form, ——— oval form, — — — various forms

The cysts were placed in a medium in which balantidia were bred at a temperature of 37° or 20°C, but excystment did not occurred in any experiment. Beacuse of the failure of these experiments attempts were made to infect with cysts the laboratory animals — white mice, rats and hamsters, free from balantidia. I did not find any excysted balantidia in the intestine of white mice and rats. The hamsters, 25 in number, were infected with cysts formed at 4° and 20°C. In this experiment I obtained positive results in 12% of hamsters infected with cysts formed at 4°C, and in 84% of hamsters infected with cysts formed at 20°C. These results show the advantage of the cyst formation at 20°C. Possibly not all the cysts formed at 4°C were able to excyst. Next, the hamsters were infected with cysts at various ages, formed at 20°C (Table 4). The most infective appeared 11-day-old cysts. The first dissection, 10 days after infection, showed the lowest per cent of infected animals.

There are frequent reports in the literature on the possibility of infection with vegetative stages of *B. coli*. I attempted also to infect hamsters with trophozoites. Two series of experiments were made. In the first one the hamsters were infected with balantidia after various periods of laboratory culture. The results were unsatisfactory (Table 5). It seems that in the conditions of laboratory culture the resistance of protozoans to digestive enzymes of the host is diminished, or there are some essential changes in the protozoan cell preventing their adaptation to the hamster intestine. The hamsters were infected also with protozoans living at various temperatures. The most positive results were obtained with the ciliates maintained at 20°C. This strain,

after isolation from pig intestine, was maintained in culture during 64 days. Balantidia which appeared infective for 32% of animals had been maintained during 18 days at 20°C. The protozoans maintained at 37°C became infective only for 20% of animals. It suggests that the life cycle stages of balantidia become more resistant and infective when they are maintained at the temperature lower than in their natural habitat.

Table 3
Influence of temperature on cyst formation in *Balantidium coli*

Time in hours	Number of cysts at temperature of				
	37°C	20°C	14°C	4°C	1°C
24	0	0	0	+	0
48	0	0	0	++	0
72	0	0	+	++	–
96	0	0	+	++	–
120	0	+	++	++	–
144	0	+	+++	++	–
168	0	++	+++	++	–
192	0	++	+++	++	–
216	0	++	++++	++	–
240	0	++	++++	++	–
264	0	++	++++	++	–
288	0	++	++++	++	–
312	0	+++	++++	++	–
336	0	++++	++++	++	–
360	–	++++	++++	++	–
388	–	++++	++++	++	–

\+ single cysts in 0.1 ml.
++ 3—5 cysts in 0.1 ml.
+++ 5—10 cysts in 0.1 ml.
++++ more than 10 cysts in 0.1 ml.

Table 4
Excystment of *Balantidium coli* depending on cyst age

Time after infection in days	Number of cysts	4-day-old cysts	11-day-old cysts	14-day-old cysts	20-day-old cysts
10	500	0*	4	2	2
30	500	0	5	4	3
60	500	1	6	6	3
90	500	1	6	5	4
180	500	2	6	4	4
Number of hamsters infected		4	27	21	16
%		13.3	90.0	70.0	53.3

* in each experiment 6 hamsters were examined.

Table 5

Infectivity of vegetative stages of *Balantidium coli*

Time after infection in days	Period of *Balantidium coli breeding in medium*			
	3 days	3 months	6 months	12 months
10	0*	0	0	0
30	2	1	0	0
60	2	1	0	0
90	2	2	1	0
180	3	1	2	1
Number of hamsters infected	9	5	3	1
%	36.0	20.0	12.0	4.0

* in each experiment 5 hamsters were examined, except 3 days series in which 6 hamsters were used.

Discussion

Already Stein 1867 reported the occurrence of large, oval and small, round forms within the populations of *Balantidium elongatum*. He found also that in some populations small forms prevailed, while in other ones the large formes dominated. Similar observations were reported by Sukhanova 1959 for *B. elongatum*, *B. duodenale*, and *B. entozoon*.

Hegner 1926, 1934, Rees 1930, Wenrich 1960 described the populations of balantidia of various body size. According to Stein the opisthe, formed after division of the small form of *B. elongatum*, is twice as small as the proter. It is round in shape and forms the so-called precystic stage. Only such stages can encyst and excyst. The occurrence of the precystic stages in *B. entozoon*, *B. elongatum*, and *B. duodenale* was confirmed by Lom 1956 and Sukhanova 1959. The forms differing in size were found by Jirovec 1930 in *B. elongatum*.

My experiments confirm the suggestions of these authors. Within the populations of *B. coli* the differentiation of shape and size occurs, but unequal division in this species has not been observed. I accept the opinion of Stein, Sukhanova and others that in the development of *B. coli*, similarly as in other representatives of the genus, the annual cycle occurs. These authors stage that in *B. elongatum*, *B. entozoon* and *B. duodenale* the division rate and precystic stage formation occur in spring and summer. It is connected with the life cycle of their hosts. In *B. coli* in the same period of the year the per cent of pigs infected is higher and the number of small forms greater. Probably in this case it is the direct influence of the temperature. In lower temperatures the trophozoites die quicker, and the cysts are less infective.

In my further studies I observed the cyst formation in pig feces at 20°C. A similar phenomenon of cyst formation in water was observed by Stein 1867, Sukhanova 1955 and Jirovec 1930 in *B. entozoon*, *B. duodenale*, and *B. elongatum*. It seems that the influence of the temperature on the life of *B. coli* is significant because its vegetative stages live longer at room temperature, up to 111 days, than at a temperature of 37°C — 16 days. In laboratory

conditions the cyst formation in *B. coli* is evoked by reducing the temperature. The highest number of cysts, living and able to excystment, is formed at 20°C. Sukhanova 1959 and Mihalčenko 1958 observed the influence of temperature on the longevity of vegetative forms, cyst formation and their viability in *B. entozoon* and *B. elongatum*. But the results of their experiments are not comparable with mine because of different habitat (poikilo- and homothermic vertebrates) of these closely related protozoans.

Sukhanova 1960 and Mihalčenko 1958 infected frogs with vegetative stages and cysts of *B. duodenale* with positive results. Westphal 1939 infected rabbits and rats with *B. coli*. Bailey and Wiliams 1949 described the case of *B. coli* occurring in the dog. In my studies the attempts to infect rats and rabbits were negative, instead I obtained infection in hamsters with the cysts and vegetative stages of *B. coli*.

The greatest per cent of hamsters examined was infected with 11-day-old cysts, formed at 20°C, and with trophozoites maintained in culture 72 hours after isolation from the pig intestine. One can suppose that in the cysts of *B. coli* some changes occur making them the most viable after 11 days. Possibly similar changes occur in vegetative forms which in laboratory conditions lose their virulence and become less resistant.

Summary

Balantidium coli in pigs occurs in two forms differing in shape and size. Cyst formation occurs in feces at 20°C. The longevity of *B. coli* vegetative stages in culture lasts 111 days at 20°C and only 16 days at 37°C. The shape, viability, infectivity and sensibility of *B. coli* to chemical agents change in laboratory conditions.

The cysts able to excystment were obtained in the laboratory conditions at 20°C. The cysts were most infective after 11 days. Attempts to infect white mice and rats were negative. Positive results were obtained in hamsters infected with cysts and vegetative stages as well.

STRESZCZENIE

Balantidium coli występuje u świń w postaciach różniących się między sobą kształtem i wielkością. Stwierdzono tworzenie się cyst w wydalonym kale w temp. 20°C. Postacie wegetatywne *B. coli* w kulturze żyją dłużej w temp. 20°C (111 dni), gdy w temp. 37° tylko 16 dni. W warunkach laboratoryjnych zmienia się kształt, żywotność, inwazyjność i wrażliwość *B. coli* na związki chemiczne.

Otrzymano w warunkach laboratoryjnych w temp. 20°C cysty zdolne do ekscystacji. Cysty te okazały się najbardziej inwazyjne po 11 dniach od powstania. Nie udało się nimi zarazić białych myszek i szczurów; otrzymano natomiast zarażenie chomików i to zarówno cystami jak i formami wegetatywnymi.

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Институт цитологии Академии Наук СССР, Ленинград Ф-121, проспект Маклина 32, СССР
Institute of Cytology of the Academy of Sciences of the USSR, Leningrad F-121, Prospect Maklina 32, USSR

И. Г. БЕРЕЗИНА
I. G. BEREZINA

Цитохимическое исследование некоторых дегидрогеназ у *Amoeba proteus*, адаптированных к высокой и низкой температуре

Cytochemical study of some dehydrogenases in *Amoeba proteus* adapted to high and low temperatures

Известно сравнительно немного работ, посвящённых действию высокой и низкой температуры на амёб (Скадовский 1955, Danielli 1959, Hirshfield 1959, James 1959, Hawkins and Danielli 1963. Лунц 1963, Полянский и др. 1967, Суханова 1968). Проведённое недавно изучение температурных адаптаций у *Amoeba proteus* выявило ряд особенностей, отличающих их от ранее исследованных простейших. У амёб при адаптации к низкой положительной температуре не обнаружено стойкого, постоянного снижения темлоустойчивости, которое характерно для ряда инфузорий, опалинид и жгутиконосцев (Полянский и др. 1967).

В предыдущей нашей работе (Семёнова 1967) было показано, что перенос амёб из относительно высокой температуры в более низкую не влечёт за собой в отличие от инфузорий изменений в цитоплазме количества полисахаридов и белков. Активность сукцинатдегидрогеназы не меняется при смене температурного режима. Полученные данные ещё больше подчеркнули своеобразную, отличную от инфузорий реакцию амёб на действие температурного фактора. У инфузорий, как известно, культивирование их при низкой положительной температуре связано с накоплением в цитоплазме запасных питательных веществ и с увеличением активности некоторых дегидрогеназ: сукцинатдегидрогеназы, глюкозо-6-фосфатдегидрогеназы, лактатдегидрогеназы и α-глицерофосфатдегидрогеназы (Полянский 1963, Ковалёва 1968, Суханова 1968).

В задачу настоящей работы входило: 1. выявление у *Amoeba proteus* ферментов цикла Кребса: малатдегидрогеназы и α-кетоглюторатдегидрогеназы, и глютаматдегидрогеназы — ключевого энзима аминокислотного обмена, 2. изучение влияния относительно высоких (25°C) и низких температур культивирования (4°C) на активность этих дегидрогеназ. Также прводилось исследование по действию указанных температур на активность сукцинатдегидрогеназы в связи с тем, что в раннее проведённой работе (Семёнова 1967) выяснялось влияние только низких температур на активность этого фермента.

Материал и методика

Объектом исследования были выбраны клоны *Amoeba proetus* — B, C, A. Амёбы всех клонов культивировались по методике, принятой в Лаборатории генетики опухолевых клеток Института цитологии АН СССР (Оленов и др. 1961). Средой для культивирования служил раствор Прескотта (Prescott and James 1955). Смена культуральной среды производилась ежедневно. Корм (*Tetrahymena pyriformis*, штамм GL) давался через день. Культуры велись при 4 ± 0.5°C („холодные"), при 17 ± 0.5° („контрольные" и при 25 ± 0.5°C („теплые"). Для опытов брались амёбы, адаптированные к указанным температурам не менее одного месяца.

Изучение распределения дегидрогеназ проводилось на материале, фиксированном жидким азотом. Перед замораживанием в каплю с амёбами добавлялась защитная среда — поливенилпирролидон (конечная концентрация 3.5%). Сукцинатдегидрогеназа выявлялась по методу Нахласа (Пирс 1962). Оптимальные условия инкубирования были описаны нами в предыдущей работе (Семенова 1967). Контрольные опыты проводились в среде, лишенной субстрата — сукцината натрия. Для выявления НАД и НАДФ-специфических дегидрогеназ (малатдегидрогеназа, глютаматдегидрогеназа и α-кетоглюторатдегидрогеназа) были выбраны три метода: 1. Пирса (Пирс 1962), 2. Хитземана (Hitzeman 1963) и 3. Лилли (Lillie 1965). Лучше результаты для всех изученных дегидрогиназ были получены при применении метода Лилли. Введение в инкубационную среду NaCl или $MgCl_2$ по прописям Пирса и Хитземана не дает никаких преимуществ. Параллельно с опытом ставились два контроля. В одном из них цитохимическая реакция проводилась в инкубационной среде, лишенной субстрата; в другом — в среде, не содержащей кофактора — НАД или НАДФ.

При определении оптимумом pH для каждой исследуемой дегидрогеназы приготовлялся инкубационный раствор с разной кислотностью (pH 6.0, 6.9, 7.4, 7.6, 7.8, 8.0, 9.1, 10.0, 11.0). С целью выяснения оптимальных условий инкубирования как для теплых, так и для холодных амеб были поставлены опыты на различные сроки и при разной температуре инкубирования (15, 30, 45 и 60 минут при 37°C; 0.5, 1.0, 1.5, 2.0 и 2.5 часа при 18—20°C; 2.0 и 4.0 часа при 4°C). Каждый опыт повторялся несколько раз. Инкубационная среда приготовлялась непосредственно перед употреблением. В Таблице 1 представлены полученные для каждой дегидрогеназы наилучшие условия инкубирования и оптимум pH. Сравнение активности и локализации ферментов у *Amoeba proteus*, адаптированных к различным температурам, проводилось на препаратах амеб, окрашенных по методу Лилли с учетом поправок, указанных в Таблице 1.

Митохондрии исследовались на тотальных препаратах, окрашенных по методу Кулля (Ромейс 1953).

Рисунки сделаны с помощью рисовального аппарата и микроскопа МБР-3. Фотографирование препаратов осуществлялось при использовании микроскопа МБР-3 и микрофотонасадки МФН-8.

Результаты исследования

При помощи цитохимических методов у *Amoeba proteus* была выявлена активность следующих дегидрогеназ: малатдегирогеназы, α-кетоглюторатдегидрогеназы, сукцинатдегидрогеназы — энзимов цикла Кребса и глютаматдегидро-

Таблица 1

Оптимальные условия для цитохимического выявления дегидрогеназ по методу Лилли у *Amoeba proteus*

The optimal conditions for cytochemical demonstration of dehydrogenase activities after Lillie in *Amoeba proteus*

	Опт. pH Opt. pH	Время инкубирования Incubation time	
		в 18—20°C (час) at 18—20°	в 37°C (мин.) at 37°C
НАД-специфическая глютаматдегидрогеназа NAD linked glutamate dehydrogenase	6.9—9.1	1	45
НАДФ-специф. глютаматдегидрогеназа NADP linked glutamate dehydrogenase	6.9—9.1	2	60
НАД-специф. малатдегидрогеназа NAD linked malate dehydrogenase	7.9	2	60
НАДФ-специф. малатдегидрогеназа NADP linked malate dehydrogenase	9.1	2	60
НАД-специф. α-кетоглюторатдегидрогеназа NAD linked α-ketoglutorate dehydrogenase	9.1	1.5	45
НАДФ-специф. α-кетоглютаратдегидрогеназа NADP linked α-ketoglutorate dehydrogenase	9.1	1.5	45

геназы — фермента, принимающего участие в обмене аминокислот. Малатдегидрогеназа у амёб связана как с НАД (оптимум pH 7.8), так и с НАДФ (опт. pH 9.1), но наибольшую активность показала НАД-специфическая малатдегидрогеназа. Глютаматдегидрогеназа также работает как при участии кофактора НАД, так и НАДФ (опт. pH 6.9—9.1). Однако, реакция на НАД-специфическую глютаматдегидрогеназу значительно сильнее, чем на НАДФ-специфическую глютаматдегидрогеназу. α-Кетоглюторатдегидрогеназа у *Amoeba proteus*, по-видимому, связана не с НАД или НАДФ, а с каким-то другим кофактором. Этот вывод вытекает из следующих данных: у амёб интенсивность реакции на α-кетоглюторатдегидрогеназу не меняется в зависимости от введения в инкубационную среду НАД или НАДФ или заменяющего их в контрольном опыте раствора трис-буфера.

Из всех исследованных дегидрогеназ, выявленных при помощи метода Лилли (Lillie 1965), наибольшую активность обнаружила НАД-специфическая глютаматдегидрогеназа. Реакция на α-кетоглюторатдегидрогеназу более слабая. Наименьшую активность показали НАД и НАДФ-специфические малатдегидрогеназы. Сукцинатдегидрогеназная активность (метод Нахласа) столь же высокая, что и НАД-специфическая глютаматдегидрогиназная активность. Оптимум pH всех исследованных энзимов лежит в щелочной зоне pH (6.9—9.10).

При изучении распределения дегидрогеназ у *Amoeba proteus* (контрольные амёбы) было обнаружено, что сукцинатдегидрогенза у амёб локализуется только в митохондриях диаметром около 1.2μ (Рис. 1, Табл. I 1). Малатдегидрогеназа и α-кетоглюторатдегидрогеназа содержится как в митоходриях, так и в более мелких цитоплазматических частицах — α-гранулах диаметром около 0.3—0.4μ Рис. 2, Табл. I 3). Глютаматдегидрогеназа также связана с гранулами двух типов: α-гранулами и более крупными частицами, размер которых приблизительно колеблется от 0.7μ до 1.6μ (Рис. 3, Табл. I 4, 5). Реакция цитоплазмы всегда отрицательная.

Сравнение препаратов *Amoeba proteus*, адаптированных к 25°С, 17°С и 4°С, показало, что интенсивность цитохимической реакции на малатдегидрогеназу, α-кетоглюторатдегидрогеназу, сукцинатдегидрогеназу и глютаматдегидрогеназу не зависит от температуры культивирования амёб. Смена температурного режима оказывает влияние на характер распределения дегидрогеназ. У амёб, живущих при 25°С, все вышеуказанные ферменты локализуются в митохондриях. Соответствующие гранулы относительно мало варьируют по своим размерам (Рис. 1 А, 2 А, 3 А, Табл. I 4) или по своей активности. Изредка на препаратах, главным образом окрашенных на глютаматдегидрогензу, встречаются более мелкие частицы (диаметр 0.6—0.8 μ). Реакция α-гранул у теплых амёб очень слабая.

По характеру распределения ферментов цикла Кребса контрольные амёбы мало отличаются от теплых. Малатдегидрогеназа, α-кетоглюторатдегидрогеназа и сукцинатдегидрогеназа локализуются в митохондриях диаметром около 1.2μ (Рис. 1 В, 2 В, Табл. I 1). Наблюдается лишь немного более выраженная гетерогенность митохондрий по размерам и по активности: изредка на препаратах встречаются небольшие фермент-содержащие частицы (диаметр 0.6—0.8 μ). Реакция α-гранул у контрольных амёб немного более сильная по сравнению с реакцией этих же гранул у теплых амёб. В отличие от ферментов цикла Кребса, глютаматдегидрогеназа содержится у амёб, адаптированных к 17°С, в небольших частицах диаметром 0.6—1.0 μ и в α-гранулах (Рис. 3 В, Табл. I 5). Редко встречаются слабоокрашенные набухшие гранулы диаметром до 1.6 μ.

Перенос амёб из 17°С в 4°С приводит к резко выраженным изменениям в характере распределения ферментов. Сукцинатдегидрогеназа у холодных амёб содержится в митохондриях диаметром около 1.2 μ; (Рис. 1 С), размеры которых практически не отличаются от размеров митохондрий у контрольных простейших. Малатдегидрогеназа и α-кетоглюторадегидрогеназа у амёб, культивируемых при низкой положительной температуре, локализуются в крупных слабокрасящихся гранулах (диаметром около 1.4—3.2 μ) и в более мелких темноокрашенных частицах диаметром 0.4—0.8 μ; Рис. 2 С). Крупные гранулы, по-видимому, являются набухшими митохондриями, какое отношение мелкие частицы имеют к α-гранулам сказать пока трудно. Глютаматдегидрогеназа у холодных амёб связана с α-гранулами и с более крупными глобулками (диаметром 0.6—1.2 μ (Рис. 3 С). Таким образом, мы видим, что у холодных амёб малатдегидрогеназа и α-кетоглюторатдегидрогеназа по характеру распределения отличаются от сукцинатдегидрогеназы. В свою очередь все три указанных фермента цикла Кребса резко отличаются по местам своей локализации от глютаматдегидрогеназы. Адаптация амёб к относительно высокой температуре (25°С) не вызывает столь глубоких изменений распределения ферментов в фермент-содержащих гранулах как перенос простейших из 17°С в 4°С. Наоборот, все исследованные дегидрогеназы у теплых амёб локализуются, в основном, в митохондриях и в α-гранулах.

Распределение и размеры гранул, окрашенных по Куллю (Табл. I 2), совпа-

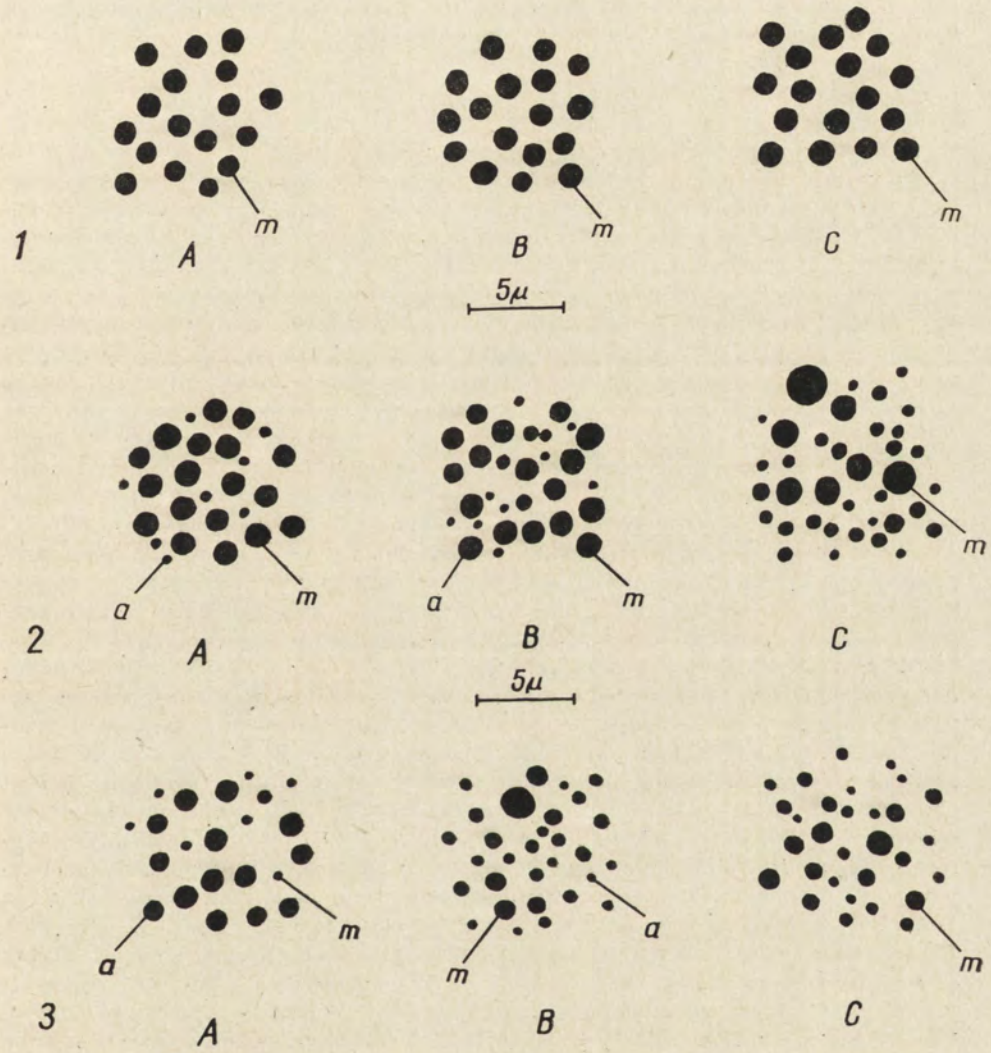

Рис. 1—3. 1 — Митохондрии у амёб, адаптированных к 25°С (А), к 17°С (В) и 4°С (С) (реакция на сукцинатдегидрогеназу); 2 — Митохондрии и α-гранулы, содержащие малатдегидрогидрогеназу и α-кетоглюторатдегидрогеназу, у амёб, адаптированных к 25°С (А), к 17°С (В) и к 25°С (С); 3 — Митохондрии и α-гранулы, связанные с глютоматдегидрогеназой у амёб, адаптированных к 25°С (А), к 17°С (В) и к 4°С (С). М — митохондрии, а — α-гранулы

Figs 1—3. 1 — Mitochondria in amebas adapted to 25°C (A), to 17°C (B) and to 4°C (C), reaction for succinate dehydrogenase; 2 — Mitochondria and alpha granules containing malate dehydrogenase and α-ketoglutorate dehydrogenase in amebas adapted to 25°C (A), to 17°C (B) and 4°C (C); 3 — Mitochondria and alpha granules containing glutamate dehydrogenase in amebas adopted to 25°C (A), to 17°C and 4°C. M — mitochondria, a — alpha granules

дают с размерами и распределением митохондрий, содержащих сукцинатдегидрогеназу. Никаких иных частиц, кроме митохондрий, на препаратах амёб, обработанных вышеуказанным способом мы не наблюдали.

Обсуждение

При цитохимическом изучении *Amoeba proteus* обнаружены существенные различия в активности исследуемых дегидрогеназ: наибольшую активность показала НАД-специфическая глютаматдегидрогеназа — ключевой энзим аминокислотного обмена, активность малатдегидрогеназы и α-кетоглюторатдегидрогеназы (цикл Кребса) значительно слабее. Выявленная очень сильная реакция на глютаматдегидрогеназу по сравнению с другими исследуемыми ферментами указывает на важную роль аминокислотного обмена в дыхании амёб. В литературе нет точных данных относительно субстратов дыхания у *Amoeba proteus*. Однако полученные цитохимические результаты дают основание предположить, что одним из основных субстратов дыхания служат протеины. В пользу этого предположения говорит присутствие в цитоплазме у амёб большого количества экскреторных кристаллов, состоящих из триурета, который является, по-видимому, конечным продуктом белкового обмена (Griffin 1960, Anderson 1967).

Наблюдение над распределением дегидрогеназ у *Amoeba proteus*, адаптированных к 25°С, показало, что у амёб сукцинатдегидрогеназа содержится только в митохондриях. Малатдегидрогеназа, α-кетоглюторатдегидрогеназа и глютоматдегидрогеназа локализуются наряду с митохондриями и в α-гранулах. В α-гранулах содержится также и цитохром-оксидаза (Семёнова 1967). Обнаруженная связь α-Гранул с типичными митохондриальными ферментами представляет большой интерес. α-гранулы, природа которых пока неизвестна, являются цитоплазматическими частицами амёб диаметром до 0.5 μ. В отличие от митохондрий они окружены одной мембраной и не красятся Янусом-зеленым (Mast and Doyle 1935a, 1935b, Byrne 1963, Daniels 1964). В цитоплазме многих простейших (*Eupletus pattela, Stentor coerules, Blepharisma undulans, Tetrahymena pyriformis, Spirostomum ambiguum*) были выявлены микротельца (диаметром до 0.5 μ), обладающие некоторыми митохондриальными свойствами (Weisz 1960, Roth 1957, Hogg and Kornberg 1963, Müller and Hogg 1967, Березина 1969). Единого мнения о природе этих гранул и об их связях с митохондриями пока не существует. Предполагается, что эти гранулы могут быть предшественниками митохондрий Wohlfarth-Botterman 1957, Rouiller 1960, Rooydyn 1967). Обсуждается также вопрос о родстве этих микротелец у простейших с пироксисомами — новым типом цитоплазматических частиц (Roth 1957, Raudhuin et al. 1965, Müller and Hogg 1967). У нас нет оснований идентифицировать α-гранулы с пироксисомами или с предшественниками митохондрий; их природа остается пока неизвестной. Однако, локализация в них окислительно-восстановительных ферментов, а также наблюдаемое увеличение в них активности малатдегидрогеназы и α-кетоглюторатдегидрогеназы при понижении температуры культивирования амёб, дает возможность предполагать активное участие α-гранул в дыхании *Amoeba proteus*.

При изучении распределения исследуемых дегидрогеназ у амёб, адаптированных к 25°С, 17°С и 4°С, было показано, что смена температурного режима по-разному влияет на митохондрии содержащие: 1. сукцинатдегидрогеназу,

2. малатдегидрогеназу и α-кетоглюторатдегидрогеназу и 3. глютаматдегидрогеназу. При понижении температры культивирования амёб размеры митохондрий, связанных с сукцинатдегидрогеназой почти не меняются; в то же время диаметр гранул, содержащих глютаматдегидрогеназу значительно уменьшается уже при переносе простейших из 25°С в 17°С. Существенные изменения в размерах малатдегидрогеназных или α-кетоглюторатдегидрогеназных гранул наблюдаются только при понижении температуры окружающей среды до 4°С. Поскольку все выше указанные дегидрогеназы локализуются у теплых амёб в типичных митохондриях, описанных ранее многими авторами (Mast and Doyle 1935a, 1935b, Pappas 1959, Daniels 1964), у нас нет оснований предполагать присутствие в цитоплазме *Amoeba proteus* каких-либо иных гранул (диаметром больше 0.5 μ), связанных с дегидрогеназами и отличных от митохондрий. Полученные результаты, вероятно, более правильно объяснять гетерогенностью митохондрий *Amoeba proteus* по ферментативному составу. Аналогичные наблюдения о неравномерном распределении дегидрогеназ цикла Кребса в различных митохондриях у простейших, в частности, у *Paramecium caudatum*, описаны Наточиным и Серавиным (1962). Данные о гетерогенности митохондрий по отношению к дегидрогеназам (в том числе и к сукцинатдегидрогеназе) были получены также при помощи биохимических методов и электронно-микроскопической цитохимии на клетках различных многоклеточных организмов (Felix et al. 1954, Seligman 1966, Seligman et al. 1967, Португалов 1963).

Явление набухания митохондрий, наблюдаемое при адаптации амёб к 4°С, и появление небольших фермент-содержащих гранул — дегенерирующих митохондрий — свидетельствуют о повреждающем действии низкой положительной температуры культивирования (4°С) на амёб. Процесс увеличения размеров гранул (набухание) является обратимым процессом, в то время как дегенерация митохондрий указывает на их необратимые изменения (Rouiller 1960). У простейших набухание митохондрий часто наблюдалось при их культивировании в условиях относительно высокой температуры (Wohlfarth-Botterman 1958, Машанский 1961, 1962, Суханова 1963, 1968). Явление дегенерации митохондрий подробно исследовано на инфузориях, в частности на *Tetrahymena pyriformis*, при их голодании, а также у стареющих культур (Elliot and Bak 1964, Leve and Elliot 1968). На этих инфузориях было показано, что дегенеративные митохондрии одновременно являются важным источником метаболитов для эндогенного дыхания (Leve and Elliot 1958). Несомненно, что оба описанных процесса играют значительную роль в жизнедеятельности одноклеточных организмов.

У *Amoeba proteus* наиболее чувствительны к действию низких температур культивирования оказались митохондрии, содержащие глютаматдегидрогеназу, т.е. связанные с белковым обменом. Наименее чувствительными оказались митохондрии, содержащие сукцинатдегидрогеназу. Возможно, именно гетерогенностью митохондрий по составу дегидрогеназ и различной чувствительностью отдельных митохондрий, содержащих неодинаковые наборы ферментов, к действию температурного фактора объясняется своеобразное поведение *Amoeba proteus* при адаптации к низкой положительной температуре. Известно, что у амёб, в отличие от инфузорий и опалинид, при переносе их из относительно высокой в более низкую температуру культивирования отсутствует постоянное, стойкое снижение теплоустойчивости, причем одновременно не наблюдается и резко выраженных изменений в содержании в цитоплазме белков, полисахаридов и волютина (Полянский и др. 1967, Семёнова 1967).

Резюме

Цитохимическими методами у *Amoeba proteus*, адаптированных как к 25°С, так и к 17°С и 4°С, выявлена относительно высокая активность глютаматдегидрогеназы и более низкая активность дегидрогеназ цикла Кребса: сукцинатдегидрогеназы, α-кетоглюторатдегидрогеназы и малатдегидрогеназы.

Малатдегидрогеназа, α-кетоглюторатдегидрогеназа и глютоматдегидрогеназа локализуется в митохондриях и в α-гранулах, сукцинатдегидрогеназа только в митохондриях.

Перенос амеб из относительно высокой температуры культивирования (25°С) в более низкую (17°С или 4°С) приводит к частичному набуханию или дегенерации отдельных митохондрий.

Обнаружена резко выраженная гетерогенность митохондрий по составу дегидрогеназ и по их реакции к действию температурного фактора. Наиболее чувствительны к действию низких положительных температур оказались митохондрии, связанные с глютаматдегидрогеназой, наименее чувствительны митохондрии, содержащие сукцинатдегидрогеназу.

SUMMARY

A cytochemical study of the respiratory enzymes in *Amoeba proteus* adapted to 25°C, 17°C and 4°C was performed. Amoebae showed high glutamate dehydrogenase activity and a comparatively low activity of the tricarboxylic cycle enzymes: α-ketogluterate dehydrogenase, malate dehydrogenase and succinate dehydrogenase. The activity of malate dehydrogenase, α-ketogluterate dehydrogenase and glutamate dehydrogenase was found to be associated with the mitochondria and with the alpha granules. Succinate dehydrogenase was found to be localized exclusively in the mitochondria.

The transfer of amoebae from the relatively high temperature at which they had been cultivated to the lower temperature of 17°C or 4°C caused partial swelling or degeneration of individual mitochondria. It was found that amoebae mitochondria were heterogeneous by dehydrogenase composition as well as their reaction to low temperature. The mitochondria which contained glitamate dehydrogenase appeared to be the most sensitive to the action of 4°C. The mitochondria containing succinate dehydrogenase were found to be the least sensitive to the same low temperature. No significant changes of dehydrogenase activity were observed in amoebae adapted to 4°C, 17°C and 25°C.

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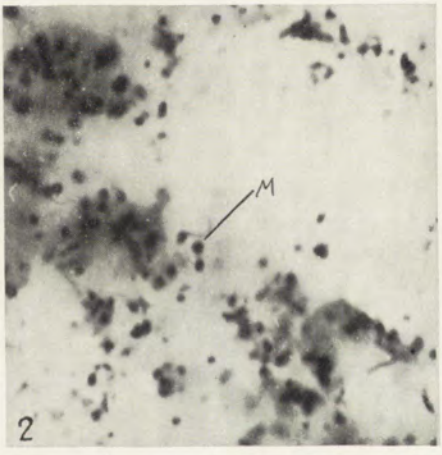
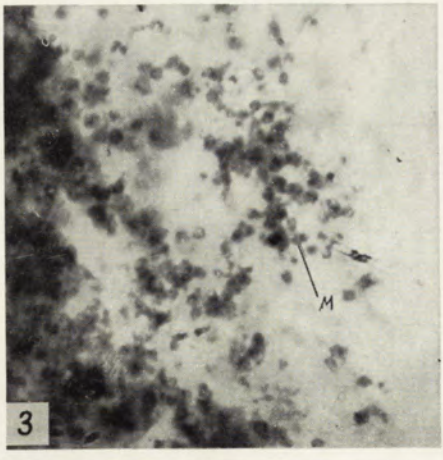
ОБЪЯСНЕНИЕ ТАБЛИЦЫ I

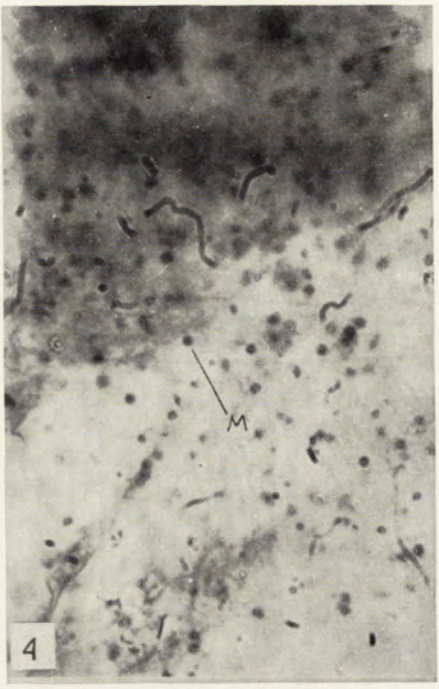
1: Митохондрии (М), окрашенные по Куллю, у амёб, культивируемых при 25°С. (срез)
2: Реакция на сукцинатдегидрогеназу у амёб, адаптированных к 17°С
3: Реакция на α-кетоглюторатдегидрогеназу у амёб, культивируемых при 25°С
4: Митохондрии (М), содержащие глютаматдегидрогеназу у амёб, адаптированных к 25°С
5: α-гранулы (реакция на глютаматдегидрогеназу)

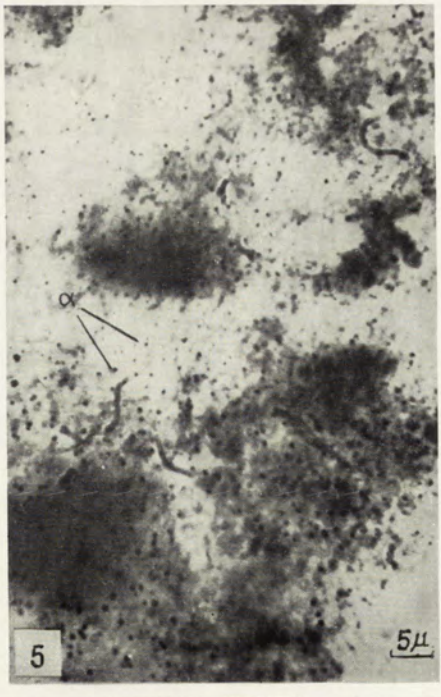
EXPLANATION OF PLATE I

1: Mitochondria (M) stained by Kull's method in amoebas cultivated at 25°C (section)
2: Succinate dehydrogenase test in amoebas adapted to 17°C
3: α-ketoglutorate dehydrogenase test in amoebas cultivated at 25°C
4: Mitochondria (M) containing glutamate dehydrogenase in amoebas adapted to 25°C
5: Alpha granules (glutamate dehydrogenase test)









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