ACTA PROTOZOOLOGICA

VOLUMEN IX
Fasciculi: 9–14

W A R S Z A W A 1 9 7 1

INSTYTUT BIOLOGII DOŚWIADCZALNEJ IM. M. NENCKIEGO
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ACTA PROTOZOOLOGICA

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ACTA PROTOZOOLOGICA

VOL. IX WARSZAWA, 15.XII.1971 FASC. 9

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Ordo *Thigmotricha* (*Ciliata — Holotricha*)

IV

Familia *Thigmophryidae*

I would like to discuss the family *Thigmophryidae* in the following fourth part of my monographic analysis in the sense and range indicated in Part I, so covering the former families: *Thigmophryidae* Ch. Lw., 1926, *Conchophthiridae* Kahl, 1931 and *Peniculistomatidae* Fenchel, 1965. By now I consider these families as subfamilies.

The family *Thigmophryidae* refers in many ways to *Hemispeiridae*, particularly to their representatives with an abundant general ciliature. The development of this family tends to a further polymerization of the general ciliature and a slight retrogradation of the adoral kineties. It is also observed that the more or less numerous kineties are engaged in the service of foodtaking apparatus under the form of a ciliated infundibulum.

Familia *Thigmophryidae* Chatton et Lwoff, 1926, em. Raabe, 1967

syn.: *Conchophthiridae* Kahl, 1931; *Peniculistomatidae* Fenchel, 1965.

Ehrenberg 1838 initiated the examination of ciliates ranged by now to this family. He described a big ciliate named *Leucophrys anodontae* from *Anodonta* of the river Ob — Siberia. Stein 1861 created for it the genus *Conchophthirus* and described the second species of this genus, namely *C. steenstrupi* from the slime of terrestrian *Pulmonata*. A series of further species ranged to the genus *Conchophthirus* have been described after years however they did not always correspond to the character of this genus (vide genus *Conchophthirus*, p. 144).

Independently of these researches concerning to a great extent the parasites of fresh-water and terrestrian molluscs, Chatton et Lwoff 1923 describe a new species and create a new genus: *Thigmophrya bivalviorum*; they describe in 1926 the two further species of this genus, and recognize the *Thigmophrya* as "l'un des tout premiers stages de l'evolution des Holotriches Hyménostomes à ciliature indifférenciée vers

Ancistridés". Chatton et Lwoff 1926 differentiate for this genus a new family *Thigmophryidae* and they set it at the origin of the evolutional tree of *Thigmotricha*.

Kahl 1931 creates the family *Conchophthiridae* among *Thigmotricha* for the numerous species of the genus *Conchophthirus* described in the meantime. This family virtually embraces one genus *Conchopthirus*, with unquestionable addition the genus *Thigmophrya* (Kahl 1931, p. 285–288). However the genus *Conchophthirus* appeared as an heterogenous and collecting unit; consequently Raabe 1934 a, b differentiates from it the genus *Kidderia* (for *C. mytili* de Morgan) and genus *Myxophyllum* (for *C. steenstrupi* Stein). Nearly at the same time Kahl 1934 differentiates the species *C. mytili* in a new genus *Morgania* which then constitutes a synonym of *Kidderia*.

Raabe 1936 accomplishes an intimate revision both of the genus *Conchophthirus* and of the families *Conchophthiridae* and *Thigmophryidae* proposing the following solution:

Fam. *Thigmophryidae*: the lack of a naked peristomial field and of the differentiated "adoralen Reihen" with genera: *Thigmophrya* Ch. Lw., 1923, *Conchophyllum* Raabe, 1936 (for *Conchophthirus caryoclada* Kidder) and *Myxophyllum* Raabe, 1934;

Fam. *Conchophthiridae*: a naked peristomal field and "adoralen Raihen" occur with genera: *Conchophthirus* Stein, 1861 and *Kidderia* Raabe, 1934.

Raabe 1936 discovers in his examination the existence of a ciliated infundibulum both in *Conchophthirus* and in *Thigmophrya* or *Myxophyllum*; and homologizes the adoral apparatus of *Conchophthirus* and *Kidderia* with the adoral kineties of *Ancistrum*; however he does not perceive and does not describe the adoral kineties in *Thigmophrya* and *Myxophyllum*.

Chatton et Lwoff 1949–1950 accept the data of Raabe 1936 and they recognize the including of *Conchophthiridae* to *Thigmotricha* and even consider it as an development degree elucidating the way from *Thigmophryidae* to *Hemispeiridae* (=*Ancistridae*). Therefore *Thigmophryidae* are always considered in their deliberations as initial forms for *Thigmotricha* for the reason of a slight differentiation of the thigmotactic ciliature and lack of adoral kineties. This view of the French authors taken by them at the outset corresponds to the opinion of Raabe 1936. He was writing: "Die Familie *Thigmophryidae* kann auf Grund der Merkmale der als Typus der Familie anzusehenden Gattung *Thigmophrya* Ch. et Lw. nicht zwischen die Familie *Conchophthiridae* und *Ancistrumidae* eingerückt werden wie es Kahl 1934 will. Sie muss vielmehr an Anfang des Subordo *Thigmotricha* stehen, als diejenige Familie, welche sich am schwächsten in den Richtungen spezialisiert hat, in welchen jene zwei, in der Differenzierung des Wimpersystems wichtige, gemeinsame Merkmale aufweisende Familien sich entwickelt haben".

This position attributed to *Thigmophryidae* within *Thigmotricha*, and especially in the relation to *Conchophthiridae* was resolutely shaken when Fenchel 1964 finds the existence of adoral kineties in *Thigmophrya*, hollowed in the infundibulum

in the trophic stage, undergoing during the division morphogenetic processes completely convergent to *Conchophthirus* (according to Raabe 1963). Additionally Penn 1958 shows on some faint photographs of the divisional stages the existence of similar and similarly shaped structures in *Myxophyllum*. Both this feature and the more detailed analysis of the possible development ways of *Thigmotricha* induce me to revise my former opinions in the I part of this work and recognize *Thigmophryidae* not as a plesiomorphic primitive family but on the contrary as a highly specialized one and distinctly approximate to *Conchophthiridae*.

The path of one of the species was rather peculiar, namely of *C. mytili* de Morgan, 1925 originally ranged to the genus *Conchophthirus*, examined more precisely by Kidder 1933. On the basis of a distinct individuality in relation to the species typical of the genus *Conchophthirus* and other approximate species *C. mytili* was differentiated by Raabe 1934 in a new genus *Kidderia* and a bit later by Kahl 1934 in a new genus *Morgania*. Jankowski 1964 replaces these both names as younger homonyms with the name *Peniculistoma*, referring at the same time new details concerning the buccal apparatus. Fenchel 1965 forcibly emphasizes the individuality of *P. mytili* and creates for it a new family *Peniculistomatidae*.

Corliss 1961 recognizes two families of the discussed group, namely:

Fam. *Thigmophryidae* Chatton et Lwoff, 1923: "Cytostome posteriorly located. Anterior thigmotactic field composed of closely set cilia derived from several somatic rows. General body ciliature uniform. Two genera: *Conchophyllum* Raabe, *Thigmophrya* Ch. Lw.";

Fam. *Conchophthiridae* Kahl in Doflein and Reichenow, 1929 (syn. *Conchophthiriidae*): "Cytostome in posterior half of the body. Body laterally compressed. Ciliation uniform. Five genera: *Andreula* Kahl, *Cochliophilus* Kozloff, *Conchophthirus* St. (syns. *Conchophthirius*; possibly *Kidderia* [hom.] and *Morgania* [hom.]), *Conchoscutum* Raabe, *Myxophyllum* Raabe".

Obviously the division of individual genera between these two families applied by Corliss 1961 is quite arbitrary, their definitions convey almost nothing and virtually they do not differ from one another. The genus *Andreula* Kahl, examined by Raabe 1938 cannot be find here; its place is among *Spirotricha*, certainly in the family *Plagiotomatidae*. It has to be admitted that the rest of genera mentioned by Corliss are closely related to one another, however they differ distinctly in substantial though various characters.

This situation prevails on me (Raabe 1967) to determine one family for all of them which would be named according to the principle of priority as the family of *Thigmophryidae*. Three subfamilies at most may be differentiated in it namely: *Thigmophryinae*, *Conchophthirinae* and *Peniculistomatinae*.

What may be said about the general characteristic of the family *Thigmophryidae*?

Thigmophryidae are in general big organisms, their body is 100–200 μ long, in general strongly flattened laterally, a rather strong thigmotactic field exists in the anterior part of its left side. Morphologically this surface does not distinguish

itself, it is simply formed of the anterior part of kineties of the left body side, only somewhat densely arranged and densely filled with kinetosomes. The ciliature of the whole body is remarkably dense: the number of kineties amounts from 80 to 270 nearly uniformly arranged; in general kinetosomes more rarely arranged are in the posterior body part.

The system of kineties of the general ciliature consists of two parts according to the initial scheme typical for *Thigmotricha*: the right and left one corresponding by their range to the right and left body side. The kineties of both parts reach the anterior suture in the anterior part of the body, somewhat at its left side lengthened fibers constitute this suture. Kineties of both parts reach the posterior suture at the posterior body end lying along the posterior body margin somewhat or even strongly at its right side. This suture consists of lengthened fibers, the net of these fibers or — in the extreme case — the point to which converge kineties. Both parts of the system of kineties, the right and the left one are separated by: the arcuated anterior suture, the posterior suture and the gap on the ventral margin that is the naked peristomal field, bordered on one side by the first kinety of the right part, and on the other one — by the last kinety of the left part of the general ciliature. The adoral kineties are arranged on this field. On the dorsal margin of the body both parts of the general ciliature are in general closely joint to each other.

In numerous representatives of the family *Thigmophryidae* some kineties of the general ciliature close to the naked peristomal field, they enter the peristome and build a ciliary infundibulum gutter- or funnel-shaped. Various kineties may contribute to the creation of funnel, they run in many ways. These are the first kineties of the right part of the general ciliature in the representatives of the genus *Conchophthirus*, these kineties enter the peristome from the back, rove its walls and go out forming a top over naked peristomal field. In *Myxophyllum* the kineties are rather the marginal ones of the left body side which enter and go outside as first kineties of the right part of the general ciliature. The reverse of this happens with *Thigmophrya*: kineties of the right part enter from the back to the peristomal gutter and they go outside joining the left part of ciliature. Fenchel interprets their run in a different way, he assumes that all of them in *Thigmophrya* belong to the left ciliary system (Fig. 1).

The adoral kineties occur in a constant number of two: stomatogenic and prostomal kinety, usually parallely arranged and rather short as a rule. They were found first of all in the representatives of the genus *Conchophthirus*, in *Peniculistoma mytili* (de Morgan), in *Myxophyllum steenstrupi* (Stein) and in the representatives of the genus *Thigmophrya*. The situation of the adoral kineties in *Cochliophilus* Kozloff is not very clear, they were not perceived and described in other genera. However their existence cannot be definitely denied for the reason that in *Thigmophrya* or *Myxophyllum* these kineties are inserted into the peristomal funnel, they are slightly visible and they were not perceived for a long time.

The adoral kineties lie always on the area of the naked peristomal field formed

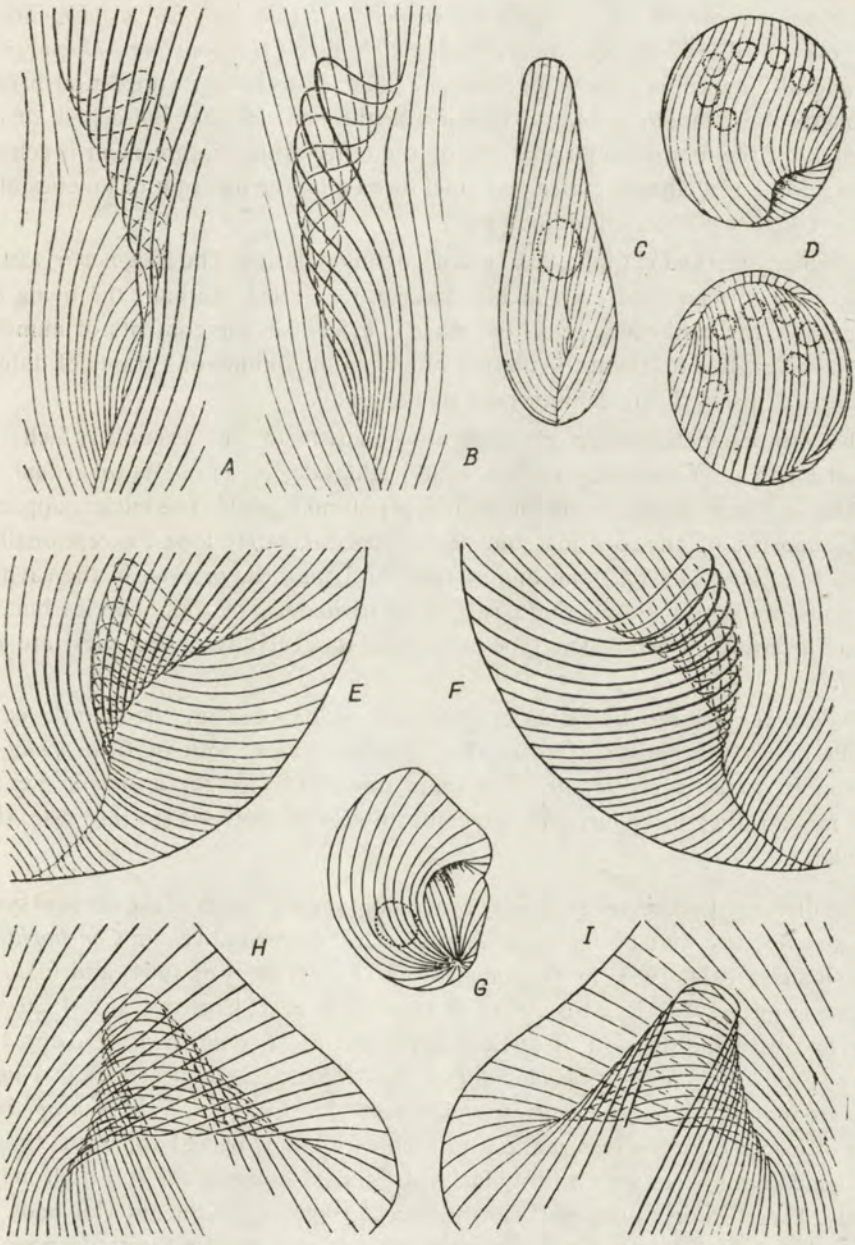


Fig. 1. Infundibulum of some *Thigmophryidae* from the surface and from the inner side: A, B, C — *Thigmophrya macomae*; D, E, F — *Myxophyllum steenstrupi*; G, H, I — *Conchophthirus anodontae*. From Raabe

by the gap of kineties of the left and right part of the general ciliature. This field may remain on the surface of the body (*Peniculistoma*, *Cochliophilus*, *Conchophthirus*) but it may partly (*Conchophthirus*) or completely dive (*Myxophyllum*, *Thigmophrya*) with adoral kineties into the infundibulum. There is an inverse correlation between the length and strength of adoral kineties on the one side and the degree of reinforcement of the food-taking apparatus on the other. This reinforcement is achieved by the arising of a ciliated gutter or funnel formed by the invasion of kineties of the general ciliature to the infundibulum.

A strongly marked cytopharynx runs deep to the plasma. The *Thigmophryidae* are able to take formed food even of big size, i.e. the scaled (perhaps the living ones too) cells of the epithelium of a host what is evident in the contents of numerous food vacuoles. Besides them the plasma often contains numerous grains of different size, located distinctly in definite part of the body.

The osmoregulative-excretory apparatus consists of one vacuole which finds its outlet either on the body surface at its right side, or as in *Myxophyllum*, and probably in *Thigmophrya*, inside the ciliated peristomal funnel. The nuclear apparatus usually consists of one, big macronucleus, spherical, rarely lobar, exceptionally of several Ma (*Myxophyllum*) and one or two Mi, lying near or even in the cavity of Ma. Some authors mention the existence of trichocysts in some species but they do not determine with which type of forms so generally called they are concerned.

Thigmophryidae are living as commensals or parasites in the mantle cavity, on gills or on the body surface of their hosts-molluscs, both of *Gastropoda* and *Bivalvia*. It occurs exceptionally that some species (*Thigmophrya annella* Fenchel, 1965) move rather secondarily to the commensales of molluscs (in this case *Malacobdella*).

The divisional processes especially concerning the evolution of the cortical system in *Thigmophryidae* were examined in many of their representatives: in *Conchophthirus* Stein (Raabe 1934, 1963) in *Thigmophrya* Ch. Lw. (Fenchel 1964) and in *Myxophyllum* (Penn 1958, Raabe 1970). Rossolimo et Jakimowitsch 1929 were concerned with the division of nuclear apparatus in *Myxophyllum*; Kidder 1933, 1934 in *Peniculistoma* and *Conchophthirus*. The stomatogenetic processes in *Thigmotricha* are individual, but they correspond in general to the relations in *Hemispeiridae*. In all of examined cases occurs the transition of the new buccal apparatus through ambihymenium system with a formula 1AM+4AZM however the run itself may be various. Therefore in *Conchophthirus* the buccal apparatus in the anterior body part falls to the proter, while the buccal apparatus of the opisthe forms de novo; its anlage arises on the nonciliary fibril which constitutes the elongation of the stomatogenic kinety of the paternal individual. It is evident from the description of Fenchel 1964 as well as from his drawings that the buccal apparatus moved far to the back and remains at the opisthe in *Thigmophrya*, but in proter it arises de novo. It seems

evident from some data of Jankowski 1964 and Fenchel 1965 that the adoral kineties lying in *Peniculistoma* on the ventral surface of the body margin, divide in the middle, and their parts falling to the proter and to the opisthe undergo some transformations similarly to *Proboveria* (vide: part II, p. 154). I intend to discuss these processes in a more detailed way in one the last parts of my monography devoted to the morphogenesis, and especially to the stomatogenesis of *Thigmotricha*.

In my proposed approach the character of the family *Thigmophryidae* may be reported as follows:

Familia *Thigmophryidae* Chatton et Lwoff, 1923

Thigmotricha of a significant body size (70–200 μ) and various shapes of the body, with a dense, equal general ciliature; the number of kineties of a range 80–300. The thigmotactic ciliature morphologically not separated. The anterior suture lies along the anterior body margin, the posterior one is customarily shifted on the right body side. The buccal apparatus, shifted forewards or backwards; there are two reduced adoral kineties on the naked peristomal field. In the number of species a number of the kineties of the general ciliature enter into the peristome, forming a ciliated infundibulum. The nuclear apparatus common: 1 Ma, 1–2 Mi. C. V. opening on the surface of the right body side or in the infundibulum. Division equal; the old oral apparatus remains by the opisthe (*Thigmophryinae*) or by the proter (*Conchophthirinae*) or is divided and reorganized both in proter and opisthe (*Peniculistomatinae*). Parasites of the mantle cavity and the gills of *Mollusca*.

Typus familiae: genus *Thigmophrya* Chatton et Lwoff, 1923.

As it was mentioned before the family *Thigmophryidae* in my approach originated by the combination of two, or even of three families: *Thigmophryidae* Ch. Lw., *Conchophthiridae* Kahl and *Peniculistomatidae* Fenchel. As it has been said the criteria dividing both according to the approach of Chatton et Lwoff 1949 and of Raabe 1936 the two first families become unactual. The most important criterion would constitute the lack of adoral kineties in *Thigmophryidae*, and their presence in *Conchophthiridae*. But when it appears that both *Thigmophrya* and *Myxophyllum* have these kineties this feature lost its meaning. It is true that so far there is a lack of data concerning the occurrence of the adoral kineties in some species (*Conchophyllum*) or details concerning their character (*Cochliophilus oncomelaniae* Tchang) but the same lack concerned until quite lately the *Thigmophrya* or *Myxophyllum*. The only representative of the third family, *Peniculistoma mytili* (de Morgan) differs from *Conchophthiridae* or *Thigmophryidae* virtually only by the individual shape

of the adoral kineties to the same extent as differs *Thigmocoma acuminata* Kazubski from *Ancistrinae* or *Hemispeirinae* in the range of the family *Hemispeiridae* (vide Part I, p. 25 and Part II, p. 175).

Since I suggested the individuality of three discussed groups as subfamilies would be left in the range of the family *Thigmophryidae* then the only virtual criterion of division could be the position, the character and the system of division and of the morphogenesis of the adoral kineties:

In *Conchophthirus* — a short adoral apparatus is transmitted to the proter in the division process — we are able to range this character as one of the subfamily *Conchophthirinae*, in *Thigmophrya* the adoral apparatus is propagated to the opisthe similarly as in *Myxophyllum* and it may be approached as a character of the subfamily *Thigmophryinae*, in *Peniculistoma* a long adoral apparatus undergoes a division in its medial part; both proter and opisthe get their fragments which later on undergo a reorganization — this may be recognized as a character of the subfamily *Peniculistomatinae*.

Subfamilia *Thigmophryinae* (Chatton et Lwoff, 1926)

This subfamily has been created by Chatton et Lwoff 1926 in the rank of the family *Thigmophryidae* however without an adequate description or diagnosis. One can be familiar with the character of this taxon merely from the description of *Thigmophrya*, the only genus of the family in the approach of Chatton et Lwoff. Raabe 1936 supported by these data reported a more detailed description of *T. macomae* Ch. Lw., paying attention on the occurrence of the peristomal gutter. Chatton et Lwoff 1949 adopted this detail in their drawings.

Raabe 1936 ranged to the family *Thigmophryidae* the genus *Myxophyllum* on the base of similarity of structure of the peristomal funnel and on the other hand as it been accepted at this time basing on the lack of adoral kineties, and also the less known genus *Conchophyllum*, which has created for the species *Conchophthirus caryoclada* Kidder, 1933.

Later examinations, namely of Fenchel 1964 revealed the presence of adoral kineties in *Thigmophrya*, however slightly developed and concealed in the infundibulum. On the photographs of Penn 1958 the same structures are revealed also in *Myxophyllum* what is confirmed by the later investigations of Raabe (1970). Unfortunately by now there are no available data whether the adoral kineties occur also in *Conchophyllum*; this problem does not result from the descriptions and drawings of Kidder 1933.

On the other hand ciliates described by Kozloff 1945 as *Cochliophilus* sp. sp. would correspond to the character of the subfamily *Thigmophryinae*, although they have no infundibulum and the adoral kineties lie on the body surface. The third species included to the genus *Cochliophilus* namely *C. oncomelaniae* Tchang, 1957

is not so clearly described; there are some doubts concerning the structure of its peristome and the entering into it "4–5 bands de cils ondolés et membraniformes". This character as well as the others does not allow to maintain this species in the range of the genus *Cochliophilus* Kozloff; I create for it a new genus *Cochliodomus* g. n. which I leave provisionally in the subfamily *Thigmophryinae*.

In the present situation, the diagnosis of the subfamily *Thigmophryinae* may be approached as follows:

Subfamilia *Thigmophryinae* Chatton et Lwoff, 1923, emend.

Thigmotricha — Thigmophryidae of a significant size (70–150 μ) and various shapes of the body, customarily laterally flattened, with a dense, equal general ciliature; the number of kineties of a range of 80–300. The anterior suture lies along the anterior body margin, the posterior one is customarily shifted on the right body side. The buccal apparatus is shifted backwards and does not have any nacked, external field. In several genera a number of kineties enter into the infundibulum, and in the further course they appear again on the surface; inside the infundibulum there exist two reduced and short adoral kineties. The nuclear apparatus ordinary (1 Ma and 1 Mi) or composed (Ma ramified or in several particles); one C. V. Division equal; the old peristome remains by the opisthe. Parasites in the mantle cavity and slime of the terrestrial and water (marine) *Mollusca*.

Typus subfamiliae: genus *Thigmophrya* Chatton et Lwoff, 1923.

Genus *Thigmophrya* Chatton et Lwoff, 1923

Research findings on the representatives of this genus go back as it seems to the first half of the XIX century when Ehrenberg 1838 described a ciliate from *Mya* under the name of *Paramecium compressum*; its shape strictly corresponds to the known by now representatives of the genus *Thigmophrya*. Schuberg 1889 (p. 67 and 84) mentions about it and he suggests that (according to Bütschli 1887 p. 1720) it may be the question of some species of *Conchophthirus*. This line of exploration has not been continued and the species pass into silence.

Only Chatton et Lwoff 1923 describe an elongated paramecium-shaped ciliate from *Mactra solida* L. (and from *Tapes pullastra* Ment.); they create for it a new genus *Thigmophrya* and they describe it under the name of *T. bivalviorum*. The description of Chatton et Lwoff 1923 has been rather laconic and confusing because the authors oriented defectively *Thigmophrya* (taking the back for the front)

and they rectified it only in the description of two further species (Chatton et Lwoff 1926), namely *T. macomae* and *T. tapetis*. They also did not report a definition of the genus in any of their monograph, like the definition of the family *Thigmophryidae* [1].

In spite of the fact that the descriptions of French authors had not any drawings Raabe 1936 was able to identify as *T. macomae* Chatton et Lwoff, 1926 the ciliates found by himself in Baltic Sea and report their detailed description. The report concerning the structure of the funnel or rather of the peristomal gutter and the way in which a number of kineties deep into this gutter constitute the basic value of this description, also very important was the homologization of these structures with similar ones in *Myxophyllum* and in *Conchophthirus*. Finally Chatton et Lwoff 1949 report the drawing of *Thigmophrya bivalviorum* and a run of kineties to the infundibulum very similar to the examinations of Raabe. Neither Raabe nor Chatton et Lwoff did notice the adoral rows of *Thigmophrya*.

Fenchel 1964 did a real turn in the study on *Thigmophrya* by finding the presence of adoral rows in *T. saxicavae* deeped into the peristome so that they appear only in the divisional processes. The adoral kineties of *Thigmophrya* proved very similar to these of *Conchophthirus*. Also their morphogenesis is very approximate to the former one but in *Thigmophrya* the mouth pocket of the paternal individual remains at the opisthe but arises de novo in the proter.

Genus *Thigmophrya* distinguishes oneself distinctly from the other by the paramecium-like shape of the body of its representatives. These ciliates are big, of a length over 100 μ. The anterior, narrower body end is somewhat flattened laterally, on the left side the thigmotactism is marked. The posterior widened end of the body is rather round in the section. In 3/4 of the body length occur a strong peristomal concavity stretched meridionaly or somewhat obliquely.

The general ciliature of *Thigmophrya* consists of two parts as in all other *Thigmophryidae*: the right and the left one, however not corresponding to the right and left body side. The anterior suture runs indeed exactly along the anterior margin of the body flattened laterally in this part, but the kineties further run somewhat dextrorotatory so that peristome which lies at the border of the right and left part of the ciliature lies distinctly on the right lateral body side and not at its ventral margin. This torsion of the general ciliature may be weaker or stronger in different species.

The kineties of the general ciliature counting from the back enter the fissure-like oral concavity surrounding its walls, going out forwards they link again to the general ciliature. Raabe 1936 suggests that these kineties in the posterior part belong to the right side of the general ciliature, and after leaving the infundibulum they link to the left part of this ciliature. Fenchel 1964 finds that they belong on

[1] Nota bene. Chatton et Lwoff 1926 compared *T. tapetis* to "*Th. pelseneeri*", probably it was a mistake, certainly it was the question of *Th. bivalviorum*, because the species name "*pelseneeri*" belongs to *Ancistrocoma* — vide Part III p. 400.

their whole run to the left part of the general ciliature. In view of some difficulties in dry silver method for *Thigmophrya* as well as difficulties in the investigation of the decisive posterior suture the question is not easy to try. I do not abide by my opinion however this interpretation seems correct to me and more remainding the relations in *Myxophyllum*.

In any case genus *Thigmophrya* is characterized by the ciliate infundibulum in construction of which contribute several (about 7) kineties of the general ciliature; the possession of adoral kineties introverted deeply into the infundibulum and unseen from outside is also typical of this genus.

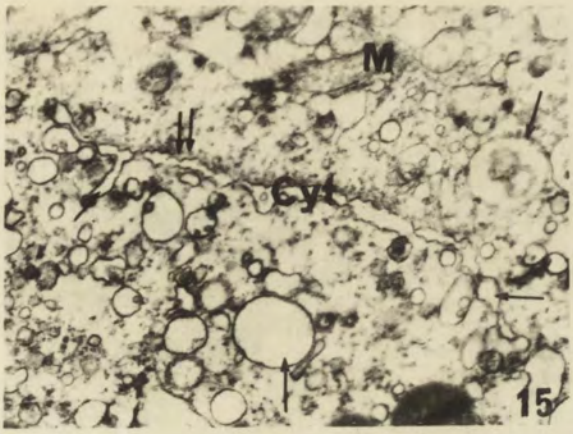
The problem of the trichocysts occurrence in *Thigmophrya* is not quite elucidated. Chatton et Lwoff 1926 report their presence in *T. tapetis* and their absence in *T. macomae*. One can suspect that there was the question of some other formations in *T. tapetis*.

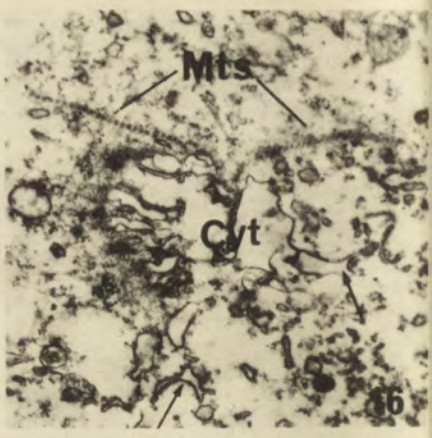
The nuclear apparatus in *Thigmophrya* consists of a usually spherical Ma lying in the middle of the body and of a minute Mi arranged closely to Ma. Single C. V. finds it outlet in the infundibulum near to its right margin. The plasma contains numerous nutritive vacuoles and often strong granulations.

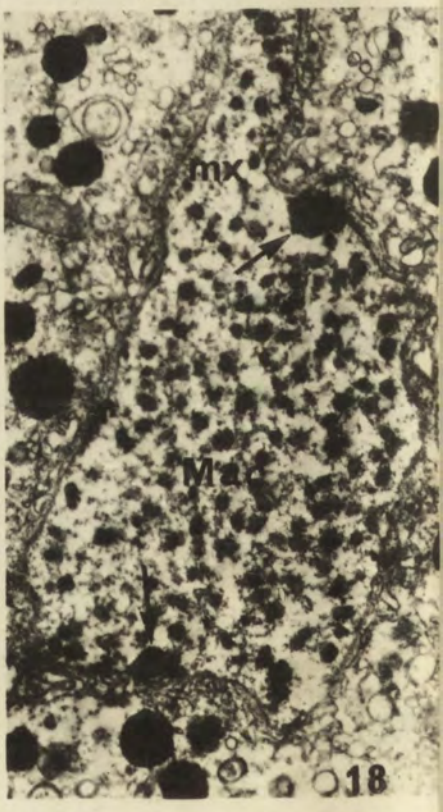
The divisional processes in the species *T saxicavae* Fenchel, 1964 were examined by Fenchel. He failed in the investigation of the first early stages of stomatogenesis and of the arising of morphogenetic fields of the adoral kineties. Although he does not state it expressis verbis, it appears from the drawings, that the peristome of the patternal individual falls to the opisthe and then undergoes an reorganization. It seems that the peristome of the proter arises de novo on the border of the left and right part of the general ciliature.

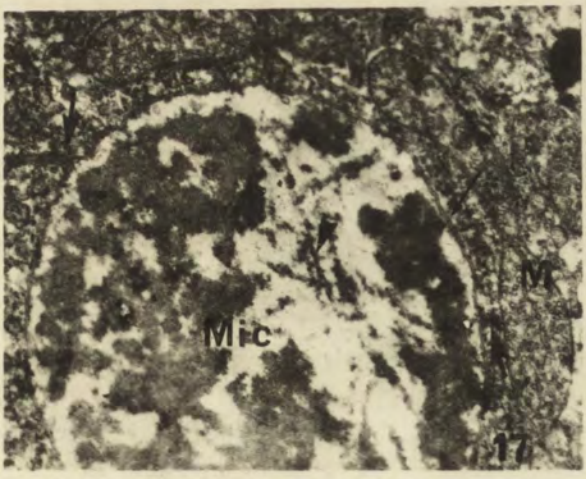
The conjugation was observed by Fenchel 1965. The partners couple together directed to one side, however one of them is somewhat retreated in relation to the other-one.

Among the described species of the genus *Thigmophrya*, four of them and several undescribed forms live in the mantle cavity of marine *Bivalvia* like most of *Thigmotricha*. However one species *T. annella* Fenchel, 1965 lives in the intestine of *Melacobdella grossa* Blainv. (*Nemertini*), a commensal of the mantle cavity of *Bivalvia*, concretely *Cyprina islandica* (L.). This ecologic desertion is not strange for other *Thigmotricha*. Jarocki 1935 describes the transition of several species of the genus *Hypocomella* (*Ancistrocomidae*, *Hypocomellinae* — vide Part III. p. 394). which are parasites of the mantle cavity of *Gastropoda* for symbionts of these snails, namely *Chaetogaster limnaei* (*Oligochaeta*). On the other hand there are some examples of transition of some *Thigmotricha* to the life in the intestine of their hosts. In that way could live *Ancistrumina limnica* Raabe according to the observations of Janina Raabe (vide Part II, p. 136). Levinson 1941 mentions about the internal parasitizing of *Boveria* (vide Part II, p. 163). Finally the whole family *Hysterocinetidae* and *Protoanoplophrya* occur in the intestine of *Gastropoda* and *Oligochaeta* (vide Part V).









M. A. Khan auctor phot.

Raabe 1936 reports a detailed description, among the others of the system of kineties and of the peristomal funnel. Size: length 100–170 μ, the greatest width 30–50 μ. The body somewhat twisted (±45°), the peristome at a distance of 3/4 from the beginning of the body. There are about 70 kineties, cilia 10 μ long. Ma:

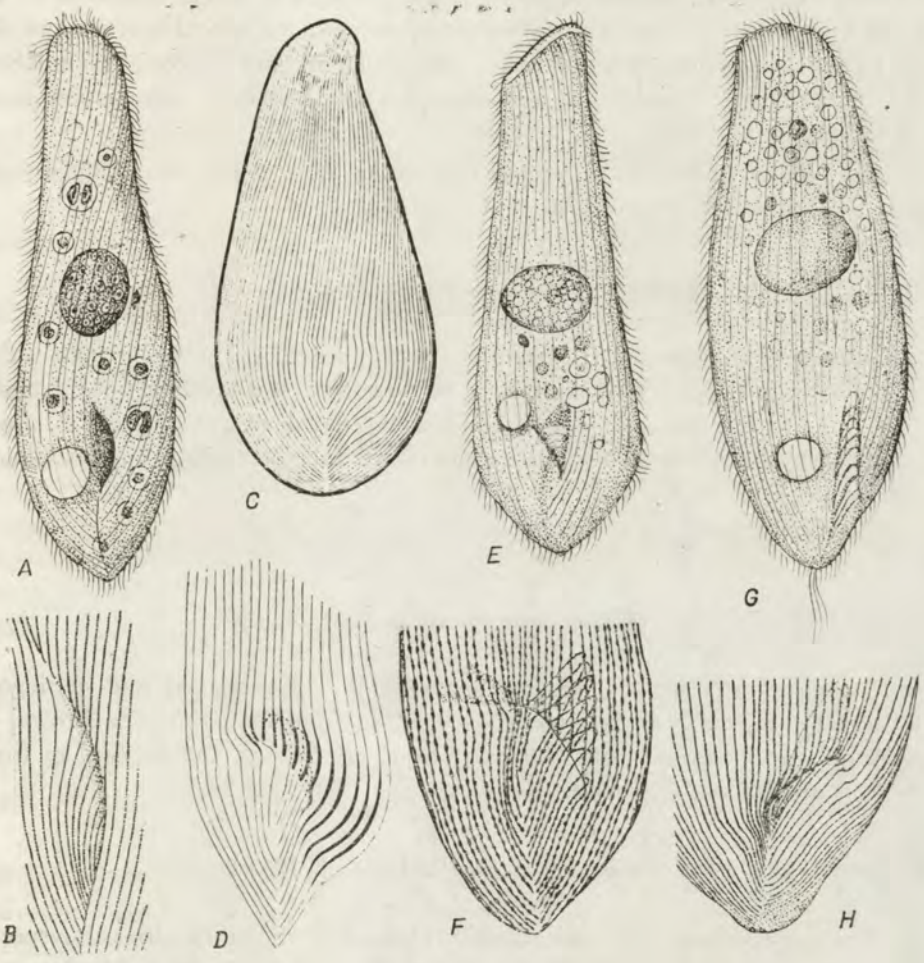


Fig. 2. *Thigmophrya*: A, B — *T. macomae* (after Raabe); C, D — *T. bivalviorum* (a. Ch. Lw.); E, F — *T. saxicavae* (a. Fenchel); G, H — *T. annella* (a. Fenchel). ×500 resp. 1000

20–40 μ × 17–30 μ, lies in the middle of the body length. Mi measures 3 μ and lies close to Ma. C. V. lies at the body back and presumably does empty to the peristome.

Fenchel 1965 reports following dimensions: length 90–142 μ, width 30–40 μ, ±70 kineties, the peristome stretched paralelly to the body axis (Fig. 2 A, B).

Host: *Macoma baltica* L. Vimereux, Pas de Calais, France (Ch. Lw.), S. Baltic Sea (Raabe), Oeresund (Fenchel).

Thigmophrya saxicavae Fenchel, 1964

Body length 126–160 μ, width 28–47 μ, 50–60 kineties of the general ciliature; about 7 kineties enter the infundibulum. The peristome is obliquely arranged to the body axis, the kineties are arranged nearly parallely to it. The outlets of C. V., in the number of 2–5 are in the vestibulum, between the adoral kineties and the first kinety of the general ciliature. Ma ovoidal with numerous nucleoles rich in RNA. The morphogenesis, especially stomatogenesis are described, conjugation mentioned (Fig. 2 E, F).

Host: *Hiatella* (=*Saxicava*) *arctica* (L.) and/or *H. striata* Fleur. Gullmarfjord. West Sweden — numerous.

Thigmophrya tapetis Chatton et Lwoff, 1926

Dimensions according to Chatton et Lwoff: length 130 μ, the greatest width 45 μ. The buccal aperture on the border of the second and third part of the body. C. V. runs to the infundibulum. Kineties somewhat dextrorotatory distant from each other by 1 μ. Between the kineties there are trichocysts (? ZR). The description unadequate; lack of drawings.

Host: *Tapes pullastra* Mont., Vimereux, Pas de Calais.

Thigmophrya sp. sp. — Fenchel, 1965

Thigmophrya sp. from *Cultellus pellucidus* (Penn.) — Kristinaberg and Helsingoer (Kattegat) — similar to *T. sexicavae*. *Thigmoprya* sp. from *Spisula elliptica* (Brown) — Oeresund (nec in Kristinaberg) — similar to *T. bivalviorum*. *Thigmophrya* sp. from *Cardium ovale* Sow., Oeresund.

Thigmophrya annella Fenchel, 1965

"*T. annella* differs from the typical *Thigmophrya* in morphological respects. The width greatest on the middle, and the posterior end is pointed. The length is 143 μ (100–171 μ) and the width is 33–60 μ. There are about 90 somatic kineties; they carry 6–7 μ long cilia. In the posterior end there are about three 25–30 μ long caudal cilia. The ovoid Ma measures about 30 μ 22 μ a spherical Mi is situated near it. The mouth pocket is smaller than in other *Thigmophrya* species, and the anterior end of the opening is directed obliquely to the left. About 10 somatic kineties run into the pocket. Adoral rows are observed in a silver impregnated individual which was in an early stage of division. The anterior half of the body is filled with feeding vacuoles" (Fig. 2 G, H).

Host: *Malacobdella grossa* Blainv. (*Nemertini*), a commensal in the mantle cavity of *Cyprina islandica* (L.) — Gullmarfjord. West Sweden, common and abundant.

Genus *Myxophyllum* Raabe, 1934

syn: *Conchophthirus* pro parte — Stein, 1861 et auctores.

This genus was created by Raabe 1934 for differentiation of the species *C. steenstrupi* Stein, 1861 from the genus *Conchophthirus*. This species diverges in many ways from the very cohesive genus *Conchophthirus*: it has a different arrangement of kineties of the general ciliature which enter the peristome an absence of naked exterior peristomal field, the run of C. V. into the peristomal funnel and complexity of the nuclear apparatus (7 Ma+1 Mi). Somewhat later Raabe 1936 included genus *Myxophyllum* to the family *Thigmophryidae* on the basis of a certain similarity of the peristomal funnel to the peristomal gutter of *Thigmophrya* and for the reason of a lack of adoral rows.

Penn 1958 indicated on his photographs the presence of adoral rows, however he did not notice them nor describe; additionally these kineties were in the stage of post-divisional morphogenesis. Farther examinations of my own (Raabe 1970) confirmed distinctly this suggestion. The existence of these rows in *Myxophyllum* allows to maintain its position among *Thigmophryidae* — *Thigmophryinae* since in the meantime the presence of adoral rows was proved in *Thigmophrya* (Fenchel 1965) and in *Cochliophilus* (Kozloff 1945).

Rossolimo et Jakimowitsch 1929 paid a deep attention to the genus *Myxophyllum* for the reason of its very specific nuclear apparatus. It appears a very specific phenomenon that the numerous Ma do not fuse together in the pre-divisional period but divide synchronically independently of each other. These processes were confirmed in my own unpublished observations as well as in the data of Penn 1958 [2].

Genus *Myxophyllum* is for the time being a monospecific genus. Although Stein 1861, and lately Kazubski (in preparation) indicate the occurrence of its representatives in the mantle cavity of numerous species of the terrestrial snails however any previous data enable for differentiation of any other species except for *M. steenstrupi* (Stein, 1861). Everything goes to show that this only species is a highly ubiquistic and cosmopolitic one.

It ought to be stressed that *Myxophyllum* is sticked to its habitat, namely not so much to the mantle cavity but to the slime covering the body of terrestrial *Gastropoda*.

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[2] Here a particular note may be taken: Penn 1958 reports the major part of his photographs and graphs in the specular position and it contributes to the wrong interpretation of pictures. Certainly it is the result of removing the best silvered side of the animal which is headed towards a coverglass.

The diagnosis of the genus *Myxophyllum* may be stated as follows:

Myxophyllum Raabe, 1934

syn.: *Conchophthirus* pro parte auctorum.

Thigmophryidae — Thigmophryinae of a strongly laterally compressed body, ellipsoidal in outline. The ciliature is dense and abundant, lack of the naked, external peristomal field. The kineties of the general ciliature run almost meridionally; the anterior suture lies along the anterior, the posterior one — along the posterior margin of the body. The peristome is located posteriorly and shifted somewhat on the right body side. Many kineties enter into the vestibulum, encircle its walls and go out as the kineties of the left part of the ciliature. The adoral kineties lie into the infundibulum. The nuclear apparatus composed of several (7) Ma and 1 Mi; C. V. entering into the infundibulum. Lives in the slime of terrestrial *Gastropoda — Pulmonata*.

Typus generis: *Myxophyllum steenstrupi* (Stein, 1961) Raabe, 1934.

Myxophyllum steenstrupi (Stein, 1861) Raabe, 1934

syn: *Conchophthirus steenstrupi* Stein, 1861 — auctorum.

The body strongly flattened laterally with an ovoidal outline, remarkably elastic and flexible. Dimensions: length 120 μ (100–150 μ) width 100 μ (80–110 μ), thickness ca. 30 μ. A delicate ciliature but very abundant: ca. 125 kineties; 70 of them belong to the right and ca. 55 to the left side of the ciliary system. The mouth pocket is arranged obliquely in the posterior part of the right side of the body at its ventral margin; 10–15 kineties of the general ciliature run into the pocket. C. V. centrally situated eliminates its contents to the reservoir and then to the infundibulum. Nuclear apparatus: 7 Ma in general, arranged arcuately parallely to the anterior body margin; 1 Mi, arranged inside the arch of Ma Ma. The number of Ma fluctuate to some extent: Stein 1861 reports their number 9–20 (undoubtedly in a division ZR). Penn 1958 reports 5–9, however most often 7 (76%).

M. steenstrupi moves slowly in the slime of its hosts bending its body, and even folding it when passing through narrow passages. It moves faster in the water and rather unidirectionaly.

Kazubski (in preparation) differentiates two forms among the populations of *M. steenstrupi*; he associates their vicariantic occurrence in different *Gastropoda* to the geographic disposition of hosts and the thermic conditions. Namely, in the lowland territories in different host species there occurs the form with more rounded

outline, 65–160 μ long, with ca. 50 kineties on the right body side, while in the mountain territories (Carpathians) there occur forms little pointed in the front part, greater, 90–230 μ long, with ca. 75 kineties on the right body side (Fig. 3).

The list of the hosts of *M. steenstrupi* is very large and permanently increasing. Stein 1861 mentions: *Succinea* sp., *Clausilia* sp., *Limax* sp., *Arion* sp. and *Cepaea*

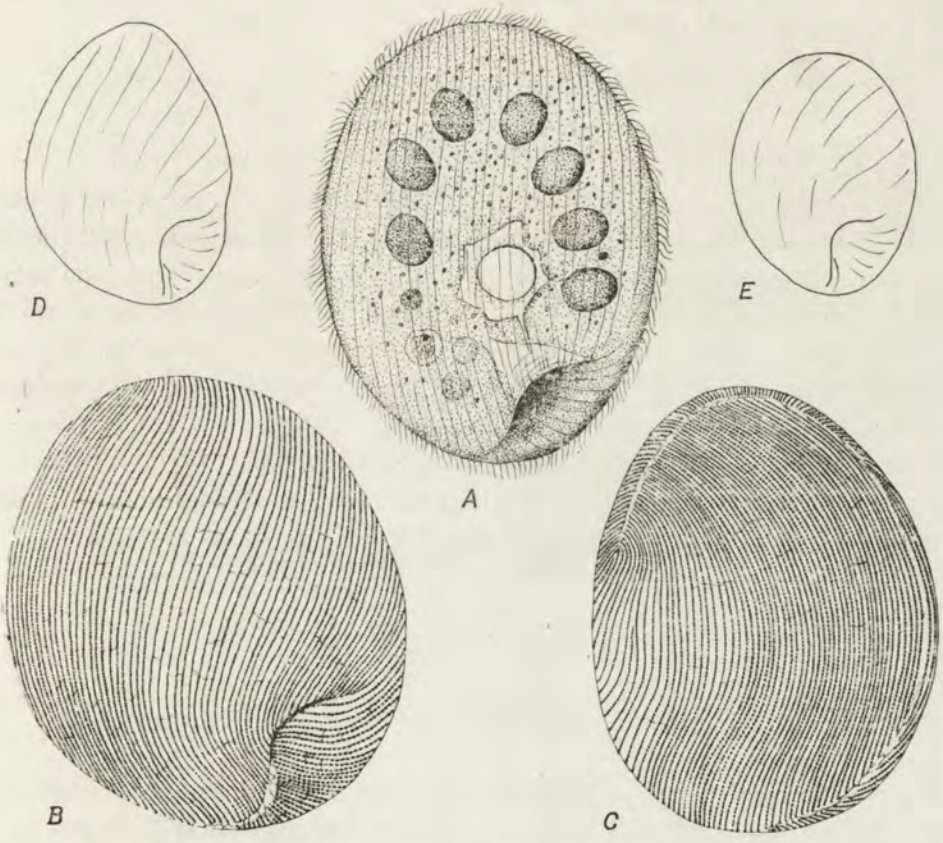

Fig. 3. *Myxophyllum steenstrupi*: A — total (after Raabe); B, C — ciliary system of the right and the left sides, $AgNO_3$ prepar. (from Raabe); D, E — schemata of two forms distinguished by Kazubski (a. Kazubski). × 500, D and E × 250

sp. Raabe 1934 examines it in *Succinea putris* (L.) as other European authors. Kazubski 1964 a, b found it in *Poiretia algira* (Brug.) and in two species of *Helicella* in Dagestan (USSR) and Greece. Penn 1958 reports from North America (Iowa, USA) as hosts: *Oxyloma decampi gouldii* (Say), *Anguispira alternata* (Say), *Triodopsis multilineata* (Say).

The large list of European hosts of *M. steenstrupi* from Poland reports Kazubski

(in preparation). These are: *Succinea putris* (L.), *S. pfeifferi* Rossm., *Cochlicopa lubrica* (Müll.). *Iphigena ventricosa* (Drap.), *I. tumida* (Rossm.), *I. plicatula* (Drap.), *Laciniaria biplicata* (Mont.), *L. plicata* (Drap.), *L. cana* (Held). *L. gulo* Rossm., *Discus rotundatus* (Müll.), *D. ruderatus* (Stud.), *Eulota fruticum* (Müll.), *Zenobiella vicina* (Rossm.), *Z. incarnata* (Müll.), *Perphoratella bidens* (Chemn.), *P. dibothrion* (Kimak.), *Trichia bielzi* (A. Schm.), *T. hispida* (L.), *T. lubomirskii* (Ślós.), *Helicigona faustina* (Rossm.), *Arianta arbustorum* (L.), *Isognomostoma personatum* (Lam.) and *Cepaea hortensis* (Müll.).

Genus *Conchophyllum* Raabe, 1936

This genus was created by Raabe 1936 for the species *C. caryoclada* Kidder, 1933 separated from the genus *Conchophthirus*. Kahl 1934 includes this species to the genus *Morgania* (=*Peniculistoma*) without any motivation. The typical genus was not described accurately so that even it is difficult to imagine the system of kineties in the oral region, the construction of the peristome, and so on. However the description of Kidder 1933 indicates on some features which at the time being allowed to left *Conchophyllum caryoclada* among the subfamily *Thigmophryinae* — in this subfamily it seems to be aproximate to *Myxophyllum* or *Cochliophilus*.

On the basis of the description of Kidder 1933 and of differentiation of Raabe 1936 the diagnosis of the genus *Conchophyllum* may be stated as follows:

Conchophyllum Raabe, 1936

syn.: *Conchophthirus* pro parte Kidder, 1933; *Morgania* pro parte Kahl, 1934.

Thigmophryidae — Thigmophryinae with a lateral flattened body of an oval outline. The ciliature is dense and uniform; lack of the naked peristomal field. The peristome lies on the right side in the hind body part, parallely to the hind body margin. The kineties run almost meridionally; in the hind part of the body the kineties run together from both sides at the margin of the peristome. Lack of data on the ciliation of the infundibulum and on the adoral kineties. Nuclear apparatus: 1 Ma, 1 Mi; C. V. postero-terminally. Parasites of the mantle cavity of marine *Bivalvia*.

Typus generis: *Conchophyllum caryoclada* (Kidder, 1933) Raabe, 1936.

Conchophyllum caryoclada (Kidder, 1933) Raabe, 1936

syn.: *Conchophthirus caryoclada* Kidder, 1933; *Morgania caryoclada* — Kahl, 1934.

Body nearly ovoid, measures: length 140–250 μ, width 90–160 μ. The peristome lies in the last quarter of the body length on its right side. Ma branched forth, lies in the medium part of the body, 1 or 2 Mi before Ma. C. V. — ? Numerous food vacuoles. Trichocysts occur.

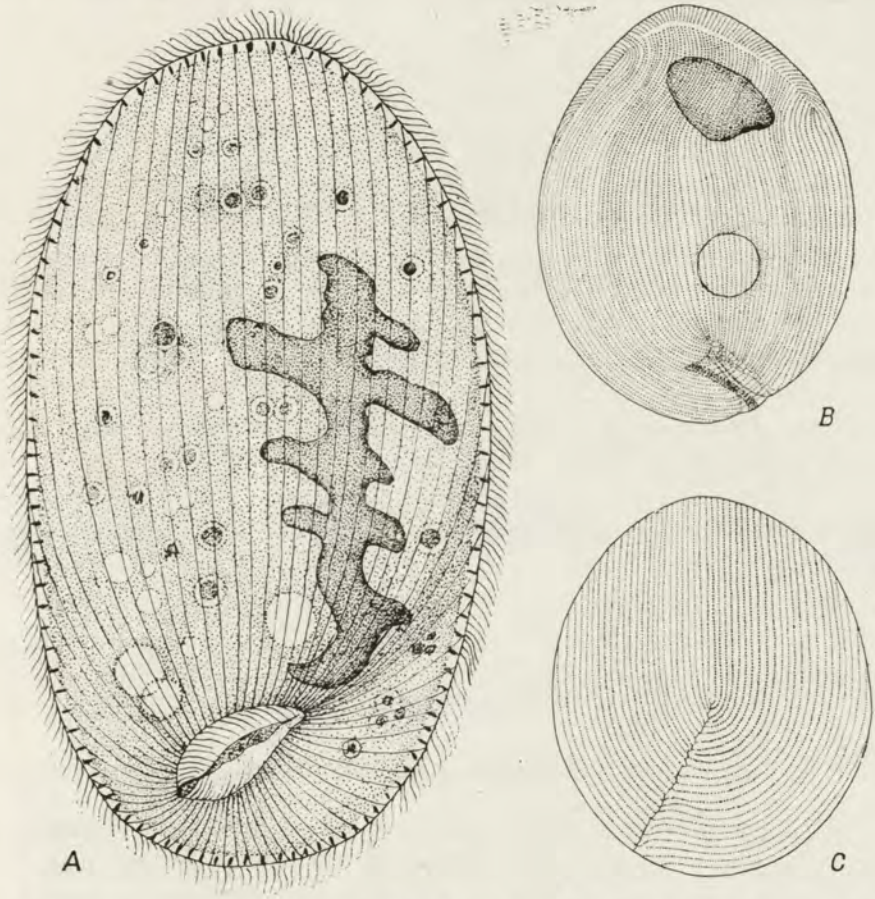

Fig. 4. A — *Conchophyllum caryoclada* (after Kidder); B, C — *Cochliodomus oncomelaniae* (a. Tchang). × 500

The ciliature is dense, slightly scarce in the posterior part of the body. The kineties of the left side run nearly meridionally and parallel to each other, they are connected in the anterior end of the body by the anterior suture lying along the anterior margin of the body. Kineties of the right side of the body converge in the back on the margin of the peristome reaching it from the front. Kineties entering

here from the left side of the body reach the peristomal margin from the back as well as from the ventral body side. "There is no deep peristomal groove opening into the cytostome, but the right end of the cytostome is pointed and continues into a narrow furrow". This furrow marks the ventral border of the right and left system of kineties. "The two edges of the cytostome are supplied with longer cilia that beat into the opening". Kidder does not report any data concerning the inside of the peristomal groove (Fig. 4 A).

Host: *Siliqua patula* Dixon, sandy beach at Seaside, Oregon, USA.

Genus *Cochliophilus* Kozloff, 1945

This genus was created by Kozloff 1945 for two very approximate species found in the mantle cavity of *Phytia setifer* (Cooper), *Pulmonata*, from the region of San Francisco. The author doubts about the insertion of *Cochliophilus* in the order of *Thigmotricha* for the reason of "the presence of membrane-like structure in the peristome", however he pointed on similar structures in "*Ancistrumidae*" and in *Conchophthirus* according to the examinations of Raabe 1932, 1934. These structures virtually not only object, but on the contrary, provide facilities for inclusion of *Cochliophilus* to the order of *Thigmotricha*.

Corliss 1961 includes *Cochliophilus* to the family *Conchophthiridae*. However the localization of the mouth in the posterior part of the body and a similar arrangement of the general ciliature approach *Cochliophilus* rather to *Thigmophryinae*. In spite of the lack of the ciliated infundibulum, *Cochliophilus* proves a certain similarity to *Myxophyllum*. There is also a certain convergence of hosts: the terrestrial *Pulmonata* in both cases.

The diagnosis of the genus may be stated as follows on the basis of the diagnosis of Kozloff 1945:

Cochliophilus Kozloff, 1945

Thigmophryidae — Thigmophryinae with a flattened, ovoid in outline body. The peristomal area is elongated and situated on the ventral margin in the posterior fourth of the body. The adoral kineties overlies a series of thick cilia; that part of the peristomal area posterior to the cytostome is naked. The peripheral cilia are disposed in longitudinal rows. Ma is centrally located, Mi is usually situated near the Ma. The C. V. opens to the exterior on the right side. Commensals of the mantle cavity of land *Pulmonata*.

Typus generis: *Cochliophilus depressus* Kozloff, 1945.

Cochliophilus depressus Kozloff, 1945

"Average size about 93 μ by 63 μ (70–107 μ×47–77 μ) the thickness being about one-sixth the length. The ciliary rows are 52 to 56 in number. The peristomal, membrane-like structure is motile. The Ma is round or oblong"—Kozloff (Fig. 5 A,B,C).

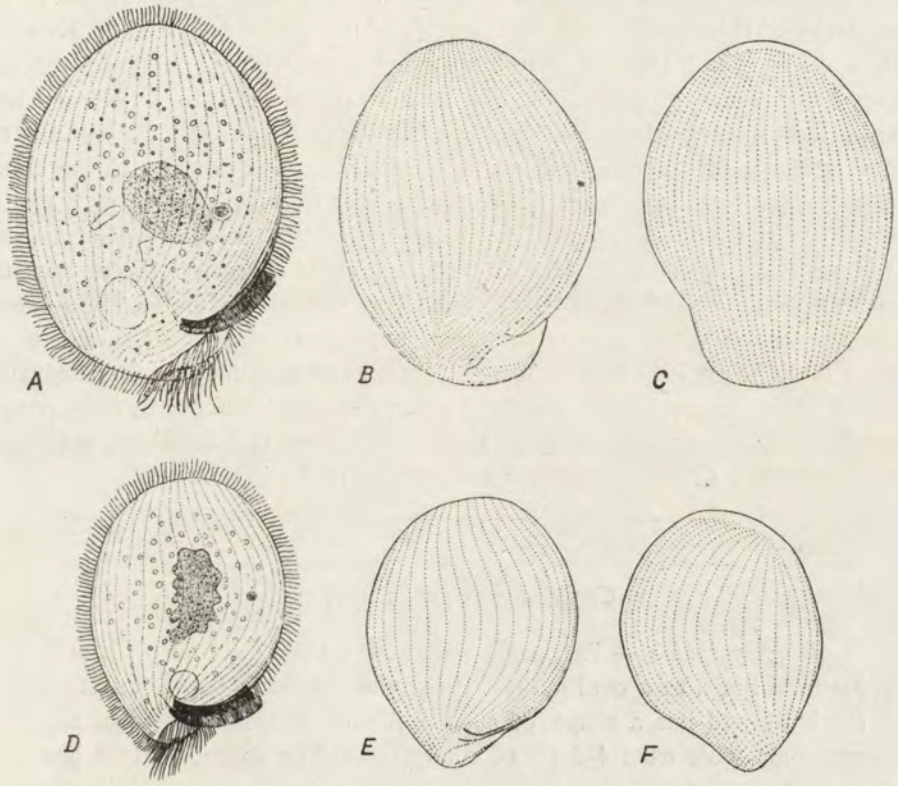

Fig. 5. *Cochliophilus*: A, B, C — *C. depressus* (from Kozloff); D, E, F — *C. minor* (from Kozloff). × 500

Host: *Phytia setifer* (Cooper), under matted vegetation and debris in salt marshes, San Francisco, USA.

Cochliophilus minor Kozloff, 1945

"Average size about 63 μ by 45μ (51–80 μ×33–56 μ), the thickness being about one-fourth the length. The ciliary rows are 36 — 38 in number. The peristomal membrane-like structure is apparently immotile, serving as a funnel directing food particles into the cytostome. The Ma is characteristically ramified" — Kozloff (Fig. 5 D,E,F).

Host: *Phytia setifer* (Cooper), under matted vegetation and debris in salt marshes, San Francisco, USA.

Genus *Cochliodomus* genus novum

pro: *Cochliophilus oncomelaniae* Tchang, 1957.

I create this species for the differentiation of the species *C. oncomelaniae* from the genus *Cochliophilus* on the basis of a series of characters that contribute to the dissimilarity of this species from both species of this genus described by Kozloff 1945. Tchang et coll. 1957 were not familiar as it seems with the references concerning *Thigmotricha*: they quoted only one position namely Kozloff 1945 and they compare their species only to *Cochliophilus*. It results from the Chinese text that the authors consider their species and genus *Cochliophilus* as belonging to the family *Thigmophryidae*. *Cochliodomus* g. n. is connected with *Cochliophilus* only by a general body outline and a dense ciliature, more dense after all than in *Cochliophilus*, and by the posterior arrangement of the peristome. These characters are at least the characters of the whole family of *Thigmophryidae*, and in any case of the subfamily *Thigmophryinae*. Strongly integrated buccal apparatus forming according to the diagram of Tchang 1957 a deep furrow or canal and its supply in a unadequately described ciliary arrangement — these characters distinguish sufficiently these genera. Although the description of Tchang 1957 does not allow to a familiarity with some details, the genus *Cochliodomus* may be characterized as follows:

Cochliodomus genus novum

Thigmophryidae — Thigmophryinae with a large (ca. 100 μ), lateral flattened body, of a oval outline. Peristome located in the rear part of the body and has a shape of a deep funnel or canal. Inside of the peristome there exist 4–5 (?) of ciliary rows. The anterior suture lies along the anterior body margin, the posterior one is located obliquely on the right body side. Ciliature abundant; there are ca. 100 kineties. Ma ovoid. V. P. in the rear part of the body. Parasites of the mantle cavity of the *Gastropoda*.

Typus generis: *Cochliodomus oncomelaniae* (Tchang, 1957).

Cochliodomus oncomelaniae (Tchang, 1957) comb. n.

Body outline ovoid. Average size: 92 μ×71 μ. Body densely covered by equal cilia, the stronger cilia occur only in the buccal region. About 100 kineties. The peristome elongated, it reaches the middle of the posterior body part. "Sur le plancher

du peristome, il y a 4–5 bands de cils ondolés et membraniformes". Ma large, somewhat triangular, lies in the anterior body part; Mi small spherical, lies near to it. C.V. in the middle part of the body, between Ma and the cytostome (Fig. 4 B,C).

Host: *Oncomelania* sp. — a freshwater snail, China.

Subfamilia *Conchophthirinae* (Kahl, 1931)

false: *Conchophtheridae* in Fenchel 1965.

This taxon created Kahl 1931 (p. 285) as familia *Conchophthiridae* first embracing in it one species only, namely *Conchophthirus* Stein, 1861 and adding at the end without discussion genus *Thigmophrya* Ch. Lw., 1923. This genus includes as he reports, one species only, however he names 3 of them. Kahl 1935 arranged the family *Conchophthiridae* between the families *Colpodidae* and *Parameciidae* in the suborder *Trichostomata*. In the "Nachtrag I" to his work Kahl recognizes the suborder *Thigmotricha* (this fact preceded the recognition of this suborder by Kahl 1934) and he arranged in it the fam. *Conchophthiridae*, in which he names following genera: *Conchophthirus* Stein, 1861, *Andreula* Kahl, 1934 (for *C. antedonis* André, 1910). *Myxophyllum* Raabe, 1934 and *Morgania* Kahl, 1934. Raabe 1936 mentions two species only in the family *Conchophthiridae*: *Conchophthirus* with 9 species and *Kidderia* Raabe, 1934 (corresponding to *Morgania* Kahl, 1934), and he creates in 1947 a new genus *Conchoscutum* arranged in this family.

Corliss 1961 ranges 5 genera in the family *Conchophthiridae*: *Andreula* Kahl, *Cochliophilus* Kozloff, *Conchophthirus* Stein, *Conchoscutum* Raabe, *Myxophyllum* Raabe and genera *Kidderia* [hom.] = *Morgania* [hom]. At the same time Corliss ranks to the family *Thigmophryidae* only these genera: *Conchophyllum* Raabe and *Thigmophrya* Ch. Lw.

From the genera mentioned by Corliss 1961, genus *Andreula* Kahl created by Raabe 1934 and enriched by Raabe 1938 by 4 new species is undoubtedly a representative of *Spirotricha* and refers to the family *Plagiotomidae*, so it cannot be examined among *Thigmotricha*. If we do accept the criteria proposed above by myself concerning the division of the family *Thigmophryidae* sensu lato, therefore we may left in the subfamily *Conchophthirinae* two genera only corresponding to its characters, namely the polyspecific genus *Conchophthirus* Stein, 1861 and monospecific for the time being genus *Conchoscutum* Raabe, 1947.

It seems very characteristic that as a result of a revision based on morphological grounds these genera and species remained in the range of the subfamily *Conchophthirinae* which are exclusively living in the mantle cavity of freshwater *Bivalvia*, *Unionidae* especially. The subfamily *Conchophthirinae* is also properly outlined from the ecologic view-point.

Considering the above discussed changes in the scope of the former family

Conchophthiridae and settlement of these taxon as a subfamily its definition may be reported as follows:

Subfamilia *Conchophthirinae* Kahl, 1931

Thigmotricha — Thigmophryidae of significant body size (100–200 μ), strongly flattened body with a dense and almost equal ciliature; the number of kineties is 80–270. The buccal apparatus lies in the vicinity of the ventral body margin; in the naked peristomal triangular field there are two adoral kineties, not entering to the infundibulum. However, several kineties of the general ciliature may enter into the infundibulum, forming a ciliated funnel. The nuclear apparatus: 1 Ma, 1–2 Mi. C. V. opening on the surface of the right body side. Division equal, the old peristome remains in the proter. Parasites of the mantle cavity of fresh water *Bivalvia*.

Typus subfamiliae: genus *Conchophthirus* Stein, 1861.

Genus *Conchophthirus* Stein, 1861

syn: *Plagiotoma* pro parte auctorum; false: *Conchophthirius* vel *Conchophtirus* — auctorum.

Schuberg 1889 presented perfectly the first period of researches on the representatives of this genus; for this reason I consider fit to quote a respective fragment of his work pp. 65–68.

"Der erste Beobachter der auf dem Körper der Najaden lebenden Infusorien dürfte wohl C. E. von Baer gewesen sein, welcher «zahlreich im äusseren Schleim, aber auch im Innern der Muscheln lebende Paramaecien» fand, die in der Mitte des Leibes einen Saugnapf besitzen sollten. Ehrenberg darauf entdeckte im Jahre 1829 im Wasser einer *Anodonta* des Ob bei Barnaul (am Altai Sibiriens) ein Infusorium, das er mit *Leucophra fluida* M. identisch glaubte und später (1838) als *Leucophrys anodontae* beschrieb. Seine Beobachtungen indessen erscheinen ihm selbst so unzureichend, dass er der Meinung war, «die Form könnte bei noch genauerer Untersuchung sich vielleicht zur Gattung *Bursaria* stellen lassen». Dujardin (1841) beschrankte sich darauf, einfach die Angaben Ehrenbergs zu wiederholen, ohne ihnen irgend etwas an eigenen Beobachtungen hinzuzufügen, und tritt nur im Speciellen für die Infusoriennatur der von Ehrenberg beobachteten Tiere ein, Steenstrup dagegen (1842) hielt sie für die Brut des *Aspidogaster conchicola*, eines in unseren Najaden parasitierenden Trematoden, obwohl sie ihn selbst "an *Paramaecium* oder *Colpidium* erinnerten", und obwohl er meinte, "das man sie wahrscheinlich zu einer Art dieser Geschlecher rechnen würde, falls man

die nötigen Hülfsmittel besässe". Perty, welcher unsere Infusorien an "*Anodonta rostrata* Kokeil und *Unio batavus* aus dem Bielersee und von Urtenen" auffand, reichte sie (1852) Dujardins Gattung *Plagiotoma* als *Pl. concharum* ein. Die von ihm gegebenen Abbildungen und Beschreibungen bieten gegenüber den früheren Angaben zwar wenig, immerhin aber etwas Neues. Ob seine *Plagiotoma difformis* gleichfalls hierher zu beziehen ist, kann bei der unzureichenden Darstellung und Abbildung nicht sicher entscheiden werden, erscheint mir indessen nicht unwahrscheinlich. Stein schliesst sich 1854 — im Zusammenhang mit seiner damaligen Ansicht, dass die Opalinen "die Larven von Tieren eines höheren Organisationsplanes darstellen" — der Ansicht Steenstrups durchaus an. Lieberkühn beobachtete unsere Tiere 1855, machte jedoch leider keine Mitteilungen über seine Forschungsergebnisse. Dagegen sprach sich noch im gleichen Jahre Wegener fur die Infusoriennatur der von Steenstrup beobachteten "paramaeciumartigen Wesen" aus, die er mit dem von Ehrenberg in *Mya* gefundenen "*Paramaecium compressum*" identifizieren möchte und von denen er einige Abbildungen giebt die zum Teil nach unregelmässig gestalteten Individuen hergestellt sein durften, immerhin aber die früheren bildlichen Darstellungen sowohl in technischer Ausführung, wie in Erkennung einzelner — allerdings nicht erläuterter und wohl auch nich verstandener — Details ubertreffen. 1856 stellte Stein die Ehrenberg'sche *Laucophrys anodontae* unter demselben Speciesnamen zur gattung *Bursaria*, unter gleichzeitiger Schilderung einzelner Organisationsverhältnisse. Claparède und Lachmann beschrieben 1858 eine von ihnen als *Plagiotoma acuminata* bezeichnete Art, welche sich auf *Tichogonia Chemnitzii* Fér. (=*Dreissena polymorpha* Van Ben.) findet, und von deren Verhältnis zu der uns hier beschäftigenden Species weiter unten noch die Rede sein wird. Stein hinwiederum stellt 1859 fest, dass die *Leucophrys anodontae* Ehrbg. zur Gattung *Plagiotoma* Duj. gehört, und giebt an verschiedenen Stellen seines Werkes Beobachtungen über diese Form wieder, welche er nun mit Perty als *Plagiotoma concharum* bezeichnet. War hierdurch, sowie durch die Angaben von 1856 schon angedeutet, dass Stein die noch 1854 von ihm vertretene Ansicht von der Unselbständigkeit der auf *Anodonta* lebenden Infusorien aufgegeben hatte, so wurde diese Korrektur seiner Anschauungen 1861 weiterhin dadurch bekräftigt, dass er jetzt für das "im Körper- und Nierenschleim der Unionen und Anodonten so häufig vorkommende Infusionstier", das er für ein "achtes holotriches infusionstier erklärte, die besondere Gattung *Conchophthirus* errichtete und die vorliegende Art als *C. anodontae* bezeichnete. Engelmann gab (1862) zum Teil im Anschlusse an die letzten Stein'schen Daten weitere Details, sowie die erste einigermassen brauchbare Abbildung von letzterer Species und stellte die neue Art. *C. curtus* auf. 1867 darauf suchte Stein vor allem die ihm wahrscheinlichen Verwandschaftsverhältnisse des *C. anodontae* festzustellen und vereinigte die *Plagiotoma acuminata* Clap. Lachm., sowie den *C. curtus* Engelm. mit der von ihm begründeten Species".

Conchophthirus metschnikoffi Certes, 1891 has been described in the later years, properly recognized by Kahl 1931 for a representative of the genus *Phacodinium*

(*Spirotricha–Heterotricha*), and considerably later *C. antedonis* Andre, 1910, individualized by Kahl 1934 to the genus *Andreula*, belonging undoubtedly to *Heterotricha* and most probably to the family *Plagiotomidae*.

After Stein 1861, 1867 it was Schuberg 1889 who has examined genus *Conchophthirus* leaving in the genus only *C. anodontae* and *C. steenstrupi* considering *C. acuminatus* and *C. curtus* as synonims of *C. anodontae*. The further described species were: *C. discophorus* Mermod, 1914, *C. elongatus* Ghosh, 1918, *C. lamellidens* Ghosh, 1918 properly corresponding to the character of the genus, and then *C. mytili* de Morgan, 1925, the first species from the marine mollusc.

Raabe 1933, 1934 reexamined the genus *Conchophthirus* reporting the exact descriptions of the ciliary system of *C. anodontae* Ehrbg., *C. curtus* Englm., *C. discophorus* Mermod, *C. acuminatus* (Clap. Lachm.) and the new species *C. unionis*; on the other hand he eliminated from the genus *Conchophthirus* the species *C. steenstrupi* (Stein) to the new genus *Myxophyllum*. The later described *C. magna* Kidder, 1934 and *C. cucumis* Uyemura, 1935 correspond to the definition of the genus *Conchophthirus* reported by Raabe 1934; but *C. striatus* Uyemura, 1934 and *C. caryoclada* Kidder, 1933 do not correspond to it. The first one originating from *Echinodermata* corresponds perfectly to the description of *Plagiopylella pacifica* Poljansky, 1951 and may be arranged in the family *Plagiopylidae* or *Entorhipidiidae*. The second one has been differentiated by Raabe 1936 in genus *Conchophyllum* and placed in the family *Thigmophryidae*.

Kahl 1935 did a considerable confusion in the nomenclature in the range of the genus *Conchophthirus* (p. 837). He writes on the *Conchophthirus anodontae*:

"Diese Bezeichnung muss nach den internationalen Regeln nach wie vor für die elliptische Form weiter geführt werden, obgleich sie anscheinend nicht in *Anodonta*, sondern nach Raabe und Engelmann nur in *Unio*-Arten vorkommt. Die Originalfigur Ehrenbergs entspricht ihr nämlich Völlig. Die Gattung *Unio* ist erst lange nach Ehrenbergs Arbeit von *Anodonta* abgetrennt worden; der von Ehrenberg gawählte Name besteht also derzait zu Recht. Ich schlage vor, als typische Zeichnung die jetzt reproduzierte Fig. (von Raabe als *C. unionis* bezeichnet) anzuerkennen. Meine eigene Fig. S. 278, 38, mag vielleicht von einer weiteren Art stammen".

The reasoning of Kahl is quite arbitral, moreover it includes a lot of cardinal errors. Firstly, the form described by Raabe 1936 as *C. unionis*, occurs also in *Anodonta* especially where *Anodonta* are in contact with *Unio*; the form recognized by Raabe as *C. anodontae* occurs only in *Anodonta*. Secondly the drawing of Ehrenberg (as well as of Engelmann), as it was at that time, is rather symbolic than naturalistic and does not present the shape of any concrete species (n.b. similarly to Kahl Fig. 38 page 278, which remembers rather a large form *C. unionis* — vide *C. unionis* p. 153). Finally, the more nonsensical question: genus *Anodonta* Lam. has been created in 1799, and genus *Unio* Philipsson in 1788; thus

not after Ehrenberg 1838, but 40 or 50 years before him![3] Additionally in the river Ob on Syberia from which originated the material of Ehrenberg, occur several species of the genus *Anodonta* [*A. cellensis* (Schr.), *A. piscinalis* Nilss., *A. anatina* (L.) and *A. cygnea* in IV-order], but does not occur *Unio*[4]. In this situation I leave here without any alterations the division as well as the nomenclature applied formerly by myself.

Genus *Conchophthirus* Stein, 1861 cleared in this way from foreign species and revised constitutes a very coherent and uniform taxon. Undoubtedly 10 described forms may be arranged to it, though some of them may be revealed as synonyms. Over the last 20 years were described additionally *C. ochridensis* Georgevitch, 1950 from *Anodonta* of the Ohrid Lake and *C. klimentinus* Raabe, 1965 from *Dreissensia* of the same lake.

Genus *Conchophthirus* includes large (100–200 μ) ciliates, with an ovoid outline of the body strongly flattened laterally, covered with a more or less uniform ciliature densely arranged in 80 to 275 kineties. This ciliature may be similarly as in other *Thigmotricha* divided in two parts: the right one corresponding more or less to the right body side and the left one first of all covering its left side. There is not a complete coincidence between the body sides and the lateral parts of the general ciliature. First of all the anterior suture having the shape of two parallel fibres, run along the anterior margin of the body but it is moved on its left side; the posterior suture is moved to the right side of the body. The posterior suture has a shape of a tract covered by a fibrillar net (sometimes among them there are stronger kinetosomes), or it constitutes one point to which converge kineties. In the anterior part of the left body side there is a stronger or weaker thigmotactic surface formed by the general ciliature, limited in front by the arcuately running anterior suture. The run of kineties of the left side of the body is more or less meridional and it is not disturbed by the fact that the last of them (kineties n, $n-1$ and s.o.) move on the right body side in their posterior run; also on the right body side pass the ends of kineties of the left part pointing to the posterior suture.

Kineties of the system on the right part undergo more considerable perturbations for the reason of the peristome which shifts there. The peristome constitutes a funnel to which enters a naked unciliated field, formed by a gap of the last kineties counting from the posterior suture of the left part of the general ciliature (kineties n, $n-1$, $n-2$ and s.o.) This field has therefore the form of a triangle, which by its sharp apex is directed backwards. On this field just at the first kineties of the right side of the general ciliature lie short adoral kineties running nearly and parallel to each other.

[3] Formerly the known species arranged by now to the genus *Unio*, have been assigned to genus *Mya*; these which found their place in the genus *Anodonta*, were in general placed in genus *Mytillus* — it was so in Linneaeus 1758.

[4] Žadin V. I. 1952: Molluski presnyh i solonovatyh vod SSSR. Akad. Nauk SSSR. Inst. Zool., pp. 376, Table on the p. 80–81.

The shift of peristome to the right side and its structure results in many changes. First of all, the kineties of the right side, pointing forewards from the posterior suture as they become more distant from the dorsal margin of the body they bend more and more to the ventral margin so, that they reach the anterior suture along the anterior-ventral body margin. The last of them turn off backwards forming over (or rather before) that peristome a typical fan.

The more distant from the dorsal margin i.e. the first kineties of the right part of the system, run into the infundibulum (at various number depending on the species), rove its walls and go outside, they link in the fan, or as it called Raabe 1932, to the eaves over the peristome. It is quite exceptional that these first kineties do not enter the peristome, but they break off on its margin (*C. klimentinus*). This break on the margin of the naked peristomal field or on the border of the eaves concern more often the last kineties of the left part which run forewards from the posterior suture in the close neighbourhood to the first kineties of the right part of ciliature.

Raabe 1934 first described the structure of the peristomal apparatus of *Conchophthirus* and the occurrence of adoral kineties (called then membranella undulans). Kidder 1934 believes "that Raabe must have mistaken the fibers of the peristomal basket for an undulating membrane". In view of posterior confirmation of data revealed by Raabe and homologization (Raabe 1936) of *Conchophthirus* adoral kineties as well as of *Ancistrinae* and *Peniculistoma* (=*Kidderia*) what accepted Chatton et Lwoff 1949 and other authors this remark of Kidder would be considered as evaded.

The character of the posterior suture and the eventual presence on its area of stronger kinetosomes, as well as the number of kineties of the general ciliature and the number of kineties entering the infundibulum, finally the spot of outlet of contractile vacuole — consist the more important features differentiating particular species in the range of genus *Conchophthirus*. The outlet of C. V. lies in a definite point for every species on the right side of the body between definite kineties counting from the peristomal field.

A well outlined cytopharynx runs arcuately inside the plasma oriented towards the back; it is able to carry off even large quantities of food; as it seems there may be even dead (or perhaps even alive) cells of epithelium of the host's gills. The large and numerous food vacuoles are gathered in the posterior part of the body but the plasma is in its anterior part before the cytopharynx and is strongly but minutely granulated.

The nuclear apparatus of *Conchophthirus* sp. sp. constitutes a large, massive, elipsoidal or spherical Ma and 1 or 2 Mi lying close to or in the concavity of Ma. The contractile vacuole lies in the middle or on the posterior part of the body and it leaks as it has been said at its right side.

A zone of a strongly granulated endoplasm occurs in the anterior part of the body of the representatives of the genus *Conchophthirus*, especially of *C. curtus*; this zone

lies under the thigmotactic surface. Beers 1962 describes it in that way: "The anterior third of the endoplasm, unlike the remainder, is relatively firm and without gastriole; it contains an extensive aggregation of specialized endoplasmic granules and is therefore called granulopalsm. On the surface ventral (left after my interpretation Z. R.) to the granuloplasm is a thigmotactic area that bears closely set, strongly adherent cilia. There is no evidence that the granules are intracellular microorganisms, they are Feulgen-negative and do not divide, nor do they stain like bacteria. Cytochemical tests show that the granules contain neutral fat, fatty acid, phospholipid, glycogen, and mucin. The evidence indicates that the principal function of the granules and granuloplasm is the production of mucin, which is supplied to the underlying thigmotactic cilia, thereby conferring on them their adhesive properties. Thus the granules and granuloplasm constitute a mucous organelle, and to the extent that they are osmiophilic and secretory they qualify as Golgi material respectively. Since endoplasmic granula is a general form for any of granules of protozoan endoplasm, it is recommended that the granules of the present study be called "muciferous granules".

The division of ciliates of the genus *Conchophthirus* has been observed and examined many times. Kidder 1934 was concerned on nuclear processes on the example of *C. anodontae*, *C. curtus* and *C. magna*. Kidder reports the descriptions and drawings of the division of Mi (i.e., *C. anodontae* has 12 chromosomes), and the division of Ma with the characteristic leaving of chromatine nonincluded to Ma, descending from residual Ma; however Beers 1963 reports it in another way. Raabe 1963 reveals essential evidence on the division of cortical system; he paid a special attention to the division of adoral apparatus and its reproduction in the descendants. During the division of representatives of the genus *Conchophthirus* the buccal apparatus of the parental individual falls on the whole to the proter. The adoral kineties of the opisthe arise in the way of neoformation from the nonciliary fiber constituting the backwards lengthening of the stomatogenic kinety of the parental individual. This fiber lies on the border line between the left and the right part of the general ciliature. The morphogenetic field produced there gives a new stomatogenic kinety and 4 membranellae of the AZM type arranged obliquely. The further transformation consists on the arrangement of AZM in one row, their partially reduction and the arising of prostomal kinety. The peristomal groove of the opisthe arises by bend of anterior sections of the kineties of the ventral ciliature on the right side of the body.

Penn 1958 reports the stomatogenesis of *Conchophthirus* in a different way. He writes: (p. 521): "During division, the peristomal area is reorganized: there is lengthening, constriction and fusion of the peristomal groove in the midregion of the old mouth. Two new mouths are formed at the point of constriction". This observation is obviously not supported by any evidence and seams to be a speculation based on data concerning other *Ciliata*!

The conjugation occurs among the representatives of the genus *Conchophthirus*

evidently seldom and is observed quite unusually. Raabe 1934 mentions about the conjugation of *C. discophorus* (Mermod), which in view of the existence of eaves protruding over the peristomes, is effected by mouths, but the partners are arranged according to scheme 69, that means, either of them is oriented in another side. A similar arrangement of individuals of *C. unionis* observed Kazubski (personal communication).

On the basis of a revision carried out by Raabe 1934, 1936 and in view of the present one, genus *Conchophthirus* may be characterized as follows:

Conchophthirus Stein, 1861

Thigmophryidae — Conchophthirinae of a great (100–200 μ). laterally compressed body, covered by a dense ciliature; number of kineties 80–270. The ventral body margin is somewhat concave in the middle, in the vicinity of peristome and the dorsal margin is convex; the left body side flat or even concave in the front part (thigmotactic area), the right one — somewhat convex. The buccal apparatus consists of the naked, triangle-shaped field, shifted somewhat to the right side and leading from it a vast funnel-shaped infundibulum. Several first kineties of the right body side enter to the infundibulum; they constitute the infundibular ciliature and go out on the body surface in the form of an "eaves" beyond the naked field. Two short adoral kineties, parallel to the first ones of the general ciliature, run on the naked field. The nuclear apparatus common (1 Ma, 1–2 Mi); the unique C. V. opens on the right body side. Division equal; conjugation of the equal individuals. Parasites of the mantle cavity and the gills of fresh-water *Bivalvia*.

Typus generis: *Conchophthirus anodontae* (Ehrenberg, 1838) Stein, 1861.

Genus *Conchophthirus* embraces at present 10 species corresponding properly to its characteristics. Only 7 are sufficiently described above all on the basis of the system of the kineties run what appears quite indispensable also for this genus. The following species examined by Raabe 1932, 1934, 1966 are well differentiated on this basis: *C. anodontae* (Ehrbg.) Stein, *C. unionis* Raabe, *C. curtus* (Englm.), *C. discophorus* (Mermod), *C. acuminatus* (Clap. Lachm.), *C. klimentinus* Raabe and *C. magna* Kidder well described by the author. *C. lamellidens* Ghosh, *C. elongata* Ghosh, *C. cucumis* Uyemura are inadequately described. Very awkwardly is described *C. ochridensis* Georgévitch which is doubtless a monster arised by the combination of *C. anodontae* and *C. unionis* in one description!

The examinations concerning the representatives of genus *Conchophthirus* indicate, that the individual species are more or less closely connected with their hosts — freshwater molluscs, but they are highly cosmopolitic and they are able to change their hosts according to different regions. Thus i.e. *C. curtus* is living in *Unio* sp. sp. in Europe and in *Anodonta* sp. sp. and respectively in *Anodonta lauta* in Japan, in *Anodonta* sp. in North America, as well as *Alasmidonta* and *Lampsilis* sp. sp. and does not reveal a more distinct variability. For this reason it seems possible that the inadequately described: *C. lamellidens* Ghosh, *C. elongata* Ghosh and *C. cucumis* Uyemura living in the mantle cavity of *Unionidae* from India and Japan correspond to *C. anodontae* and *C. unionis*. A definitive decision concerning this problem seems impossible without the Klein's method of dry silver impregnation. In european conditions at least in the areas explored by myself (Poland, Hungary, Yugoslavia, Bulgaria) — *C. anodontae* is specific for *Anodonta* sp. sp., however I did never met it in *Unio* sp. sp. even there where *Unio* occur together with *Anodonta*. *C. discophorus* is connected with *Pisidium* sp. sp. and *Sphaerium* sp. sp., *C. acuminatus* and *C. klimentinus* exclusively with *Dreissensia polymorpha*.

Conchophthirus anodontae (Ehrbg, 1838), Stein, 1861

syn.: *C. raabei* Kahl, 1935; *C. ochridensis* Georgévitch, 1950 pro parte.

As I did in my first work concerning *Conchophthirus* (Raabe 1932) and as I discussed it above (p. 146). I consider as *C. anodontae* (Ehrbg., 1838), Stein, 1861 this species which is specific for *Anodonta* sp. sp. in Europe and which Kahl called *C. raabei*. It is a species with a very typical body outline and an individual character of the posterior suture:

Body with an ovoidal outline, sharpened anteriorly, rounded on its posterior part, the length is 80–170 μ (average 120 μ), the width 40–120 μ (average 70 μ). The dorsal margin strongly convex, the ventral margin distinctly bulged before the peristome, and distinctly concave behind it. A large peristomal field in a shape of a triangle oriented forwards by its apex; a wide funnel runs from the peristome anterodorsally and is elongated in an arcuately running cytopharynx. Ma spherical measures 40–80 μ of diameter and lies in the posterior part of the body, moved towards the back; Mi lies in the concavity of Ma. C.V. is arranged more or less in the middle of the body.

About 80 kineties (the fluctuations do not exceed the number of 10) converge in the posterior suture, which in *C. anodontae* constitutes one point. Obviously they do not all reach this point directly but before they join by several together. Kineties of the right part of the system run forwards from this suture, parallel to the dorsal margin but they gradually are more bent towards the ventral margin. The last 10–15 kineties enter the peristomal funnel, rove their walls with gradually shallow arches and go outside forming a large eaves over the naked peristomal

field. Kineties of the left part of the system which are close to the right side run yet at the right side of the body, but they move in their anterior ends to the left side. The outlet of the C. V. lies on the interruption of 1–2 kineties, between kinety 8 and 12 counting from the peristome. The anterior suture runs parallely to the anterior margin somewhat on the left side of the body (Fig. 6 AB).

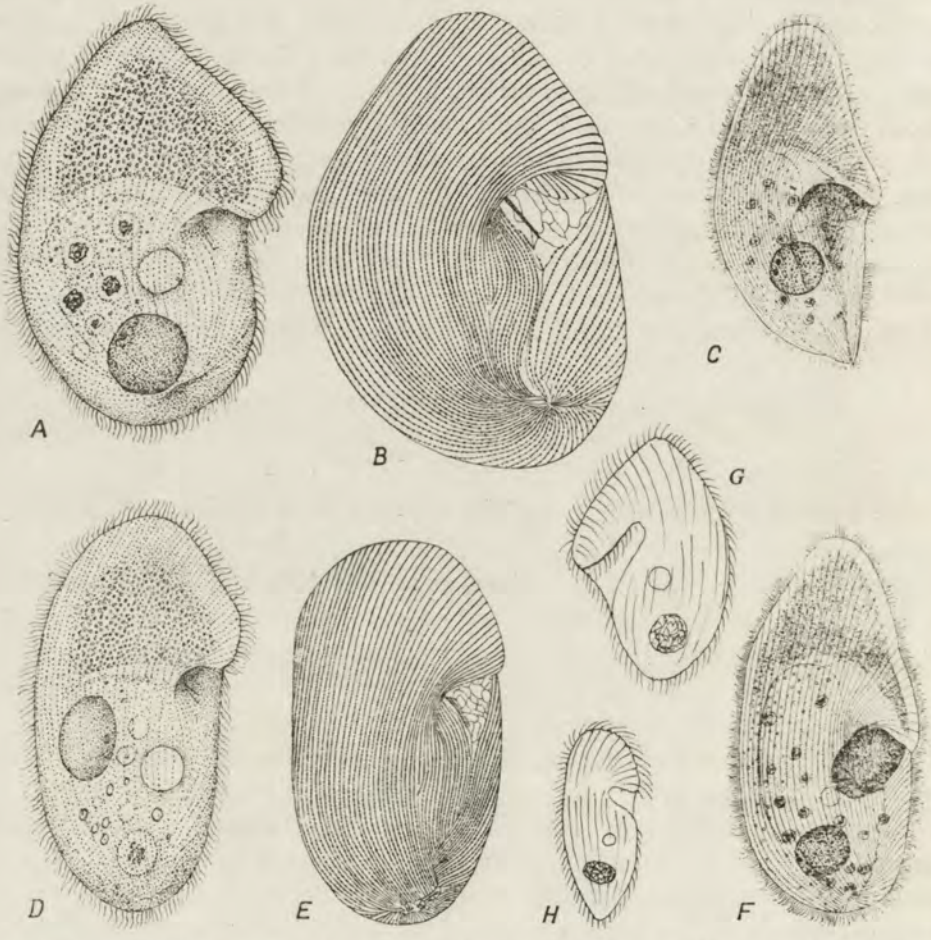

Fig. 6. *Conchophthirus*: A, B — *C. anodontae*, total and $AgNO_3$ prep. (after and from Raabe); C — *C. lamellidens* (from Uyemura); D, E — *C. unionis*, total and $AgNO_3$ prep. (after and from Raabe); F — *C. cucumis* (from *Uyemura*); G — *C. lamellidens* (after Kahl); H — *C. elongatus* (a. Kahl). × 500, G × 380

C. anodontae occurs in the mantle cavity and on the gills of its hosts sometimes in large quantities, especially when there are no *C. unionis* in them. Its characteristic shape with a pointed anterior body end is easy to differentiate from other commensals (*C. unionis* and *C. curtus*).

Hosts: *Anodonta cygnea* L. — Poland, Hungary, Bulgaria — Raabe; Kopenhagen region — Fenchel; *Elliptio complanatus* (Say) — 100% in great abundance, Woods Hole, Mass., U.S.A. — Kidder.

It may well be that the species described from India and Japan become a synonim of *C. anodontae*, namely:

Conchophthirus lamellidens Ghosh, 1918

In Kahl 1931: "Gr. 90 μ. Gestalt oval, am Ventralrand von der Mitte nach hinten ausgestutzt. Peristom scheint sich nach de Zeichnung nach links oder als Spalt ventralwäts zu öffnen. Peristomtrichter weit, schräg nach vorn gerichtet. Die Reihen scheiden die prästomale Ventralkante von beiden Seiten zu überschneiden. Plasma vorne mit dunkler Granulazona. Ma rund oder dreikantig, hinten liegend. C. V. etwas hinter der Mitte. In *Lamellidens*-Muscheln. Indien", (p. 287).

In Uyemura, 1935: "The body sharpened anteriorly and in its back, measures: length 90–108 μ (average 100 μ), width 35–54 μ (average 47 μ). The dorsal margin strongly convex, ventral straight. A wide peristome lies at the ventral margin and pass in a funnel oriented obliquely forwards. Ma spherical, measures about 17 μ with two Mi (2μ) adhesive to it, it lies in the posterior body part. C. V. lies in the middle of the body. An extensive aggregation of plasmic granules anterior to the peristomal funnel". Uyemura does not describe the run of kineties and their number; one may observe from the drawing that in the eaves over the peristome there are about 7 kineties. The convergence of kineties in the posterior end of the body is rather enigmatic.

Host: *Anodonta lauta* v. Martens, Japan, Saitama province.

Conchophthirus elongatus Ghosh, 1918

In Kahl 1931: "Gr. 50 μ (!! Z.R.). Gestalt hinten verjüngt, vorn dorsal etwas ausgeweitet; ventral beim Peristom etwas konkav. Dieses liegt vor der Mitte, hat eine kleine Depression und kein langgestrecktes Schlundrohr. C. V. nahe der Mediane deutlich hinter der Mitte. Ma ellipsoid, hinten. Die Reien sind besonders vorne deutlich und kerben den Rand. Häufiger als curtus in *Lamellidens* (Indien)," (p. 288) (Fig. 6 H).

Conchophthirus unionis Raabe, 1932

syn.: *C. anodontae* St. — Kahl 1931; *C. ochridensis* Georgévitch, 1950 pro parte.

As I did it in 1932 and repeated above (p. 146), I consider this species as *C. unionis* Raabe, 1932 which occurs regularly in *Unio* sp. sp., which often appears in *Anodonta* sp. sp. and differs distinctly from *C. anodontae* both by the shape of the body and a distinct structure of the funnel, as well of the posterior suture.

The body ovoidal, usually strongly elongated, rounded on both ends. The dorsal margin slightly convex, the ventral margin slightly concave in the region of peristome, lying somewhat before the half of the body length. Dimensions: length 80–170 μ (average 120 μ) width 30–100 μ (average 60 μ). The peristomal field relatively narrow and not very large; a ciliate not deep funnel runs to the back, elongated in a cytopharynx extended perpendicularly to the body axis towards its dorsal margin. Ma ovoid measures 30–40 μ×20 μ lies somewhat the half of the body length next to its dorsal margin. Mi lies in the concavity of Ma. C. V. lies in the middle of the body somewhat over the half of its length.

The kineties in number of 90–95 converge in the back of the body towards the posterior suture which consists of a thin net of fibres, obliquely arranged parallely to the posterior and ventro-posterior body margin; in this net are ranged about 8–10 larger kinetosomes consisting the basis of stronger cilia. Kineties of the right part of the system run forewards from the posterior suture somewhat declining slightly towards the dorsal margin; first of them very close to the peristome enter it in the number of 6, they take part in the forming of infundibulum and they go outside forming a slight eaves over the naked peristomal field. Kineties of the left part of the system pass to the left side of the body. The outlet of C. V. is the end of the interrupted kineties, of 12 and 13 numbering from the peristome (Fig. 6 D,E).

C. unionis occurs in the mantle cavity, on gills and it is numerous especially on labial palps of its hosts. It is evident at a number of occasions that two forms occur: a large one, about 150 μ, and a small one which measures ca. 100 μ. There are some transitions between these forms, there is a lack of considerable morphological differences. Kazubski (personal communication) thinks that there are some differences in the number of kineties of both forms. It seems, however, that in this respect, as in the size, there exist gradual transitions between the two forms. In any case, my drawings in this paper represent the small form. The problem needs more detailed elaboration.

Hosts: *Unio pictorum* L., *U. crassus* Retz., *U. tumidus* Retz., and *Anodonta cygnea* L. in the ponds, lakes and rivers of Poland, in the lake of Balaton (Hungary), in Bulgaria, Yugoslavia (including the lake Ohrid) — Raabe.

Conchophthirus cucumis Uyemura, 1935

The body sharpened in its anterior part, rounded at the back, strongly elongated, measures: 87–141 μ (average 112 μ), width 45–72 μ (average 60 μ). A funneled mouth lies in the middle of the body next to ventral margin. Ma rounded lies in the back of the body associated by a closely adhesive Mi or two Mi. C. V. lies anterior to Ma. The plasma is strongly and densely granulated in the anterior body part before the arcuately running cytopharynx, numerous food vacuoles in the posterior part of the body.

Uyemura does not report the description of the run of kineties. It may be concluded from the drawing (Abb. 7) that kineties of left and right side of the body converge in the back to a rather long posterior suture similarly as it is in *C. unionis*. It exists according to this drawing a slight eaves over the peristome, the peristome is however drawn as a cave interrupting the run of kineties, so evidently in a wrong way. Uyemura separates his species from *C. lamellidens* for the reason of a smaller peristome (?) rounded at the back of the body as well as for the size of the species. These differences are rather vague because of poor descriptions, unadequate drawings and photographs (Fig. 6 F).

It may seem doubtful if *C. cucumis* is virtually an individual species or perhaps it is a confusion of characters of *C. anodontae* and *C. unionis*.

Host: *Anodonta lauta* v. Martens — Japan, province Saitama — frequent and abundant infestation.

Conchophthirus curtus (Engelmann, 1862)

syn: *C. anodontae* Stein — Schuberg, 1889 pro parte.

Body elongated, strongly rounded on both ends, in petty individuals nearly clypeiform. Both margins, dorsal and ventral, nearly uniformly convex. Size: length 60–150 μ (average 120 μ), width 50–100 μ (average 70 μ). The peristomal field rather small, infundibulum shallow leads to an arcuately bent cytopharynx. Ma ovoid, measures ca. 30 μ of diameter and lies in the middle of the body close to cytophyrynx arch; Mi (1–2 μ) lies in the concavity of Ma. C. V. somewhat in the posterior part of the body.

Kineties in the number of 150–160 are very densely arranged and contain relatively minute kinetosomes. Nearly all kineties reach directly the posterior suture which is extended along the ventro-posterior body margin at its right side. A singular fibril constitutes this suture, in the posterior part that is a net of fibrills among which stick 20–30 big kinetosomes being as it seems a basis of stronger cilia. Kineties of the right part of the system run from the posterior suture towards the anterior and anterio-ventral margin; several of them (3–5) dive slightly into the shallow infundibulum and form a very scanty eaves over the peristomal field. The outlet of C. V. is at the end of breaked kinety, usually of the 19th, counting from the peristome (Fig. 7 A,B).

C. curtus occurs in small quantities in general associated by *C. anodontae* or *C. unionis*. It differs from those species in its shape, inconspicous plasma especially in the anterior part of the body and finally in slow movements.

Hosts: *Unio pictorum* L., other *Unio* sp. sp., *Anodonta cygnea* L. — Poland, Bulgaria, Hungary, Yugoslavia with the lake Ohrid — Raabe; *Anodonta lauta* v. Mart. — Japan — Uyemura 1935, in America: *Lampsilis siliquoidea* Barn, *Anodonta grandis* Say — Iowa, U.S.A. — Penn 1958, *Anodonta marginata* Say (10%),

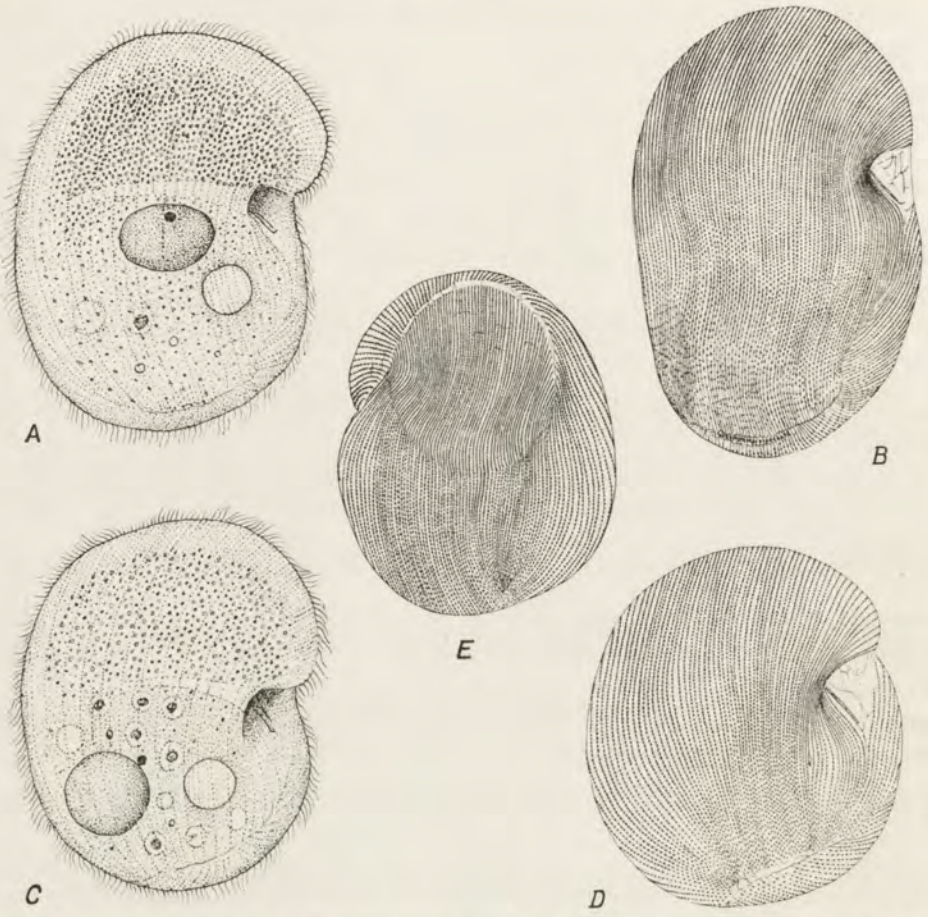

Fig. 7. *Conchophthirus*: A, B — *C. curtus*, total and $AgNO_3$ prep. (after and from Raabe); C, D, E — *C. discophorus*, total and $AgNO_3$ from the both sides (after and from Raabe). × 500

A. implicata Say, *A. cataracta* Say, *Lampsilis radiata* (Say), *L. cariosa* (Say), *Alasmidonta undulata* (Say) — Woods Hole, Mass., Lake Chautauqua, N. Y., U.S.A. — Kidder 1933.

Conchophthirus discophorus Mermod, 1914

Body with an ovoid outline, strongly rounded on both ends. Dimensions: length 60–110 μ (mostly ca. 90 μ), width 60–100 μ (mostly ca. 85 μ). Peristom lies somewhat before half of the body length; the peristomal field rather large and strongly concave. The right side of the body strongly convex, the left in the anterior part strongly concave forms a dive sucker. Ma elipsoidal or spherical, diameter ca. 20 μ, lies in the back of the body near its dorsal margin; Mi lies in its depression. C. V. lies in the posterior part of the body near the ventral margin. In the anterior part of

the body the plasm is strongly granulated, the posterior part contains numerous large food vacuoles.

The general number of kineties amounts to ca. 150. They are rather densely arranged, the most densely in the anterior part of the left side of the body on the area of the sucker, however any cortical structure is marked which would confine or seperate this adhesive apparatus. The sucker is anteriorly limited by the anterior suture. The posterior suture consists of several fibrils running along the posterior body margin and it does not contain larger kinetosomes. The majority of kineties of the general ciliature reach directly the posterior suture. Several kineties only form the peristomal funnel; the eaves over the naked field are slightly outlined (like in *C. curtus*). The outlet of C. V. is on the 14th kinety or so, counting from the peristome (Fig. 7 C, D, E).

C. discophorus occurs in *Sphaeriidae* of various species usually in poor quantities. Raabe 1934 observed a conjugation and a winter incystation of these ciliates in *Pisidium casertanum* Poli in small freezing reservoirs in the forests of Warszawa region.

Hosts: *Pisidium* sp. — Jura Vaudois — Mermod; *Pisidium casertanum* (Poli) — — Warszawa region — Raabe; *Musculium lacustre* O.F.M., *Sphaerium corneum* L., *Pisidium obtusale* Pfr. — small water reservoirs in the Mazury Lake-land (N. Poland) — Dobrzańska 1958.

Conchophthirus acuminatus (Clap. Lachm., 1858), Raabe, 1933

syn.: *Plagiotoma acuminata* Clap. Lachm., 1858; *C. anodontae* Stein-Schuberg, 1889 pro parte.

Body with an ovoid outline, sharpened anteriorly, rounded at its end. Size: length 50–120 μ (most often ca. 100 μ) width 30–60 μ (most often 50 μ). The peristome lies more or less in the middle of the body length and may be shifted strongly to its right side. The naked peristomal field is small and may be strongly inserted deep into the peristome together with adoral kineties lying on it. Ma ovoid measures ca. 40×30 μ lies in the posterior half of the body, rather somewhat dorsally; Mi close to Ma. C. V. in the posterior part of the body.

Ciliature of average density: the general number of kineties amounted to ca. 90. Kineties of the left side of the body run more or less meridionally; the last ventral pass to the right side of the body in their posterior parts. The posterior suture has a shape of a tract running obliquely and confined by two fibrills without kinetosomes. Among the kineties of the right side, counting from the dorsal side about 35 of them run from the posterior suture to the anterior and antero-ventral body margin. Several further kineties (ca. 10) enter the infundibulum and after going out of it they form eaves over the peristome; about 8 further kineties yet of the right part of the system, break before the eaves, and during their run join the other ones belonging to the left part of the system. The outlet of C. V. is located at the point,

where breake several kineties, in general the 10th to the 15th kinety, counting from the naked peristomal field (Fig. 8 A, B, C).

C. acuminatus is a specific parasite of *Dreissensia polymorpha* Pallas; it seems that this parasite is associated with its host all over the whole areal of its occurrence

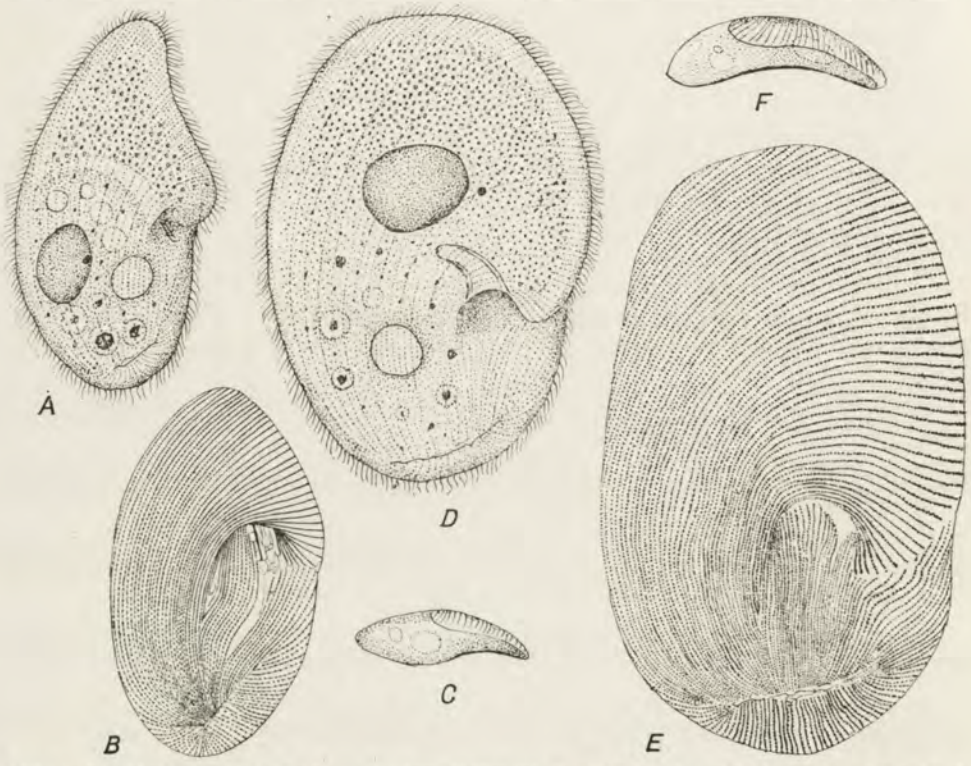

Fig. 8. *Conchophthirus*: A, B, C — *C. acuminatus*, total, $AgNO_3$ prep. and the scheme from the oral margin (after and from Raabe); D, E, F — *C. klimentinus*, total, $AgNO_3$ prep. and the scheme from the oral margin (after and from Raabe). ×500. C and F × 250

in Europe. Raabe 1934, 1950, 1965 observes it all over Poland, in Hungary (Balaton), in Yugoslavia and in Bulgaria. Fenchel 1965 in the region of Copenhagen.

A special attention must be paid to the presence of *C. acuminatus* in *Dreissensia* in the lake of Ohrid (S. Yugoslavia) — Raabe 1965; next to it occurs another species of the genus *Conchophthirus* that is *C. klimentinus* Raabe, 1965.

Conchophthirus klimentinus Raabe, 1965

The body with a rather regular ovoid outline, strongly flattended laterally and conspicuously concave at the left side. Dimensions: length 60–130 μ (most often ca. 100 μ), width 40–100 μ, (most often ca. 55 μ). The peristome is located in the posterior half of the body and strongly shifted to its right side. A triangle naked

peristomal field, adoral kineties squeezed deep into the peristome. Ma spherical or ovoidal lies in the anterior part of the body, 1 or 2 Mi close to Ma. C. V. in the posterior part of the body lies somewhat dorsally. The peristomal funnel oriented antero-dorsal and the cytopharynx going away from it are both strongly marked.

The general number of kineties amounted to ca. 160. The kineties of the left body side run more or less meridionally, parallel to each other; the most ventral of them overrun in the posterior end the ventral margin of the body and pass to the right side, like the ends of the remaining kineties of the left part. Among kineties of the right side, counting from the dorsal margin about 50 run from the posterior suture towards the anterior part and they reach the antero-ventral margin of the body. Several further kineties enter the infundibulum, rove its walls and go out backwards linking to the eaves over the peristome; the further 10–12 kineties which run from the posterior suture to the anterior end, break off before they run into the infundibulum. Finally the further 10 kineties belong already to the left system; they break like the formers on the boundary of the peristomal field or after the contact with the eaves. At last further 10 kineties continue their run forewards, pass to the left side of the body and reach the anterior suture. The posterior suture is long but it does not contain larger kinetosomes. The outlet of C. V. lies on the 15th kinety of the right system (Fig. 8 D, E, F).

C. klimentinus Raabe, 1965 approaches mostly *C. acuminatus*: the strong fan of *C. acuminatus*, the small naked peristomal area, a considerable number of kineties of the first right and last left system which break off before they reach the peristome, constitute features which in *C. klimentinus* have been strengthened.

C. klimentinus is a parasite of *Dreissensia polymorpha* Pallas but for the time being only from the lake Ohrid (Makedonia, Yugoslavia). It occurs in *Dreissensia* from the depth of 40 m in largest quantities and in populations consisting of the biggest individuals; less numerous and tiny individuals originate from the population of *Dreissensia* from the depth ca. 20 m and from littoral populations. In view of the fact that *C. klimentinus* was never and nowhere observed on the European lowland, Raabe 1965 set up a hypothesis, that this species could originate in the lake Ohrid under the influence of its specific conditions by means of intralacustrine speciation. The initial species could be a widely spread and associated with him — *C. acuminatus*.

I intend to discuss once more this problem in the last chapters of my study.

Conchophthirus magna Kidder, 1934

The body with an elongated ovoid outline, strongly flattened laterally; the anterior part of the body is wider towards the dorso-ventral margin than the posterior one. Size: length 123–203 μ (average 180 μ), width 63–116 μ (average 95 μ). The peristomal cavity and the naked field is narrow but long, it lies close to the ventral margin of the body. Ma of an irregular shape lies in the middle of the body close behind

the cytopharynx and measures 25–30 μ; 2 small Mi lie in the concavity of Ma C. V. lies near behind Ma somewhat dorsally (Fig. 9).

The ciliature is dense, the number of kineties (after the drawing of Kidder 1934, Fig. 7) amounts to 275; ca. 140 among them belong to the left and 135 to the

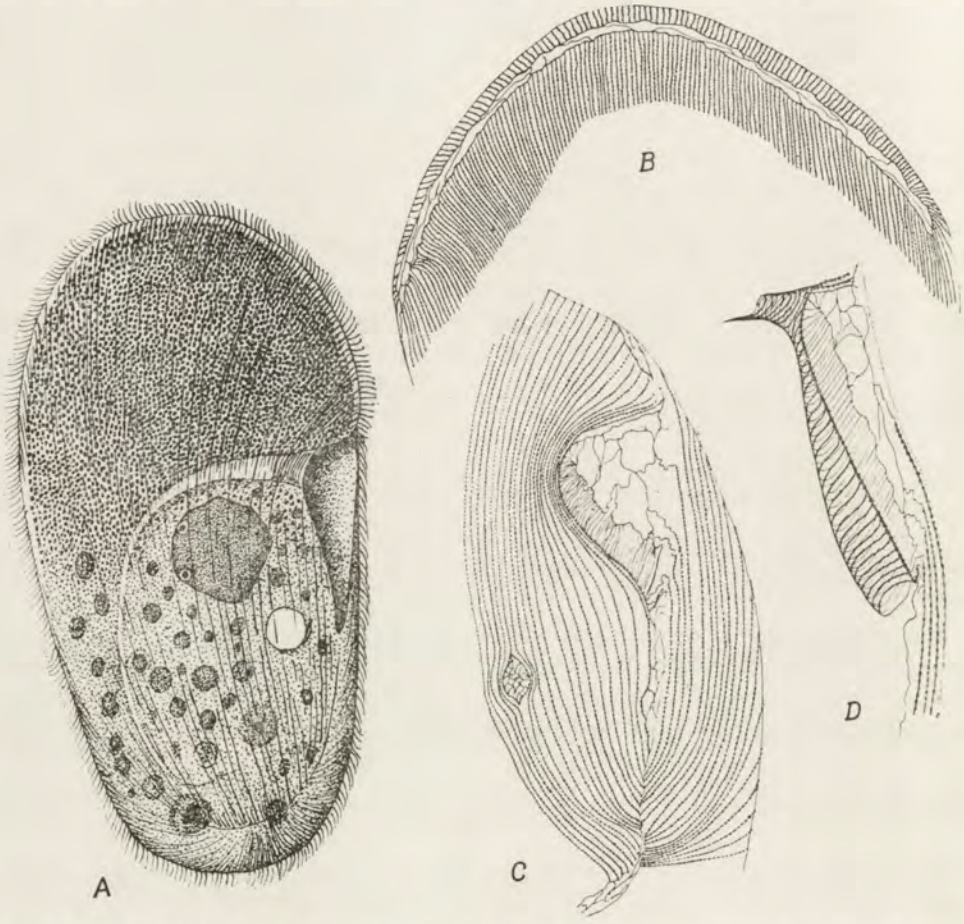

Fig. 9. *Conchophthirus magna*: A — total (after Kidder), B — the anterior suture, C — the peristomal region, D — the fibrillar system of the peristomal region in $AgNO_3$ preparations (from Kidder). × 500 resp. 1000

right system. The anterior suture is large and occupies the anterior body margin; the posterior suture is also long and rather wide with numerous large kinetosomes lying on it. Few kineties (1–3?) of the general ciliature enter very shallow to the peristome, on the margin of the peristomal field break off 4 kineties of the right system.

Host: *Elliptio complanatus* (Dill.), with *C. anodontae*, non-numerous (10–20 individuals in one host), in 1/4 of the examined Bivalvia — Woods Hole, Mass., U.S.A.

Genus *Conchoscutum* Raabe, 1947

This genus has been created by Raabe 1947 for the species *C. inversum* described at the same time which is very similar to the representatives of genus *Conchophthirus*; it differs from all of them in its individual nearly inverted arrangement of the peristome. The peristome with its naked field as well as its not very deep funnel and the adoral kineties, is placed near the ventral body margin and is shifted not to the right but to its left side. The other elements of the ciliary system, that is the thigmotactic surface, the anterior and posterior sutures, the outlet of C. V. are situated similarly in *Conchoscutum* as in *Conchophthirus*.

This specific arrangement of the peristomal apparatus appeared in the fact that kineties forming the eaves over the peristome originate and belong to the left (not to the right as in *Conchophthirus*) ciliary system, adoral kineties are parallely arranged close to the last kineties of the left part of the system (not of the first kineties of the right part) and they are bent in their posterior part also towards the ventral margin of the body, therefore to the right and not to the left.

Evidently this inversion may be treated very seriously, as a principal character giving evidence for a systematic individuality of the rank of ordo or even of a higher taxon. But in this case all other features and all convergencies existing among *Conchoscutum* and *Conchophthirus* and other representatives of the whole family *Thigmophryidae* would be neglected! Nota bene, the problem of the inverted forms is not very strange to zoological sciences: they may appear in many groups of animals in which is revealed a spiralization in some of the system as i.e., among *Gastropoda–Pulmonata*.

The individuality of *Conchoscutum* from *Conchophthirus* is not so high and is revealed as secondary to a high degree when the morphogenetic processes are studied and especially the stomatogenesis. However this problem is by now not exactly examined it seems that the adoral kineties are individualized in opisthe as in *Conchophthirus*, from the continuation of the stomatogenic kinety; this occurs somewhat on the right side of the body. (Raabe 1947).

The diagnosis of the genus *Conchoscutum* may be stated as follows:

Conchoscutum Raabe, 1947

Thigmophryidae — *Conchophthirinae* of a great (140 μ), strongly flattened body of an ellipsoidal outline, covered with a dense ciliature; number of kineties ca. 250. The left body side somewhat concave, the right — somewhat convex; the ventral body margin in the 1/3 of the body length somewhat sunk in the vicinity of the peristome. The buccal apparatus is from the ventral margin a little shifted to the left (!) body side; several kineties of the left system of the general ciliature

($n, n-1, n-2$ etc.) enter into the shallow infundibulum and go out making a feeble "eaves" beyond the small naked field. On the field — two short adoral kineties. Parasites of the mantle cavity of fresh-water *Bivalvia* (*Unionidae*).

Typus generis: *Conchoscutum inversum* Raabe, 1947.

Conchoscutum inversum Raabe, 1947

The body large with an elliptic outline, strongly flattened. Size: length ca. 140 μ, width ca. 100 μ. The peristome is marked as a slight incision of the ventral margin of the body in the distance of 1/3 from its anterior pole shifted somewhat to its left side. A wide cytopharynx extends from the peristome towards the back and somewhat to the end of the body. The plasm of the anterior part of the body is strongly and finely granulated, in the posterior part contains numerous food vacuoles. Ma ovoid, ca. 30×20 μ, lies in the middle of the body length, somewhat towards its dorsal margin; Mi close to Ma. C. V. lies also in the middle of the body length, but closer to its ventral margin.

The ciliature is very abundant, consists of minute, densely arranged cilia without a distinct rarefying of them in the posterior part of the body. There are ca. 120 kineties on the right and 110 on the left side of the body. The kineties of the right side run to the posterior suture which occupies a large space in the posterior part of the right side of the body towards the anterior suture, lying along the anterior margin at its left side; they run nearly directly and they do not link on their way. Only several kineties close to the 40th kinety counting from the peristome, break off in the middle of the body length or so marking the place of the outlet of C. V. The posterior suture has the shape of a tract filled with a net of fibrills among which stick ca. 50 larger kinetosomes. Kineties of the left side of the body run even more regularly meridionally from the posterior to the anterior suture; they twist at the ventral margin somewhat in its direction, several of them (6–7) enter the peristome and go out from it forming a slight eaves over the naked peristomal field. Two adoral kineties lie on this field, parallely to the last kineties of the left system (Fig. 10).

Conchoscutum inversum distinguish oneself distinctly from the species of the genus *Conchopthirus* with which it is associated not only in its size and inconspicuous granulated plasma but also in its behavior. Its movements are slow, stable, it often clings to the substrate by its thigmotactic surface. It occurs rarely and in poor quantities — several individuals in one mollusc.

The ecology of *C. inversum* seems very characteristic. Raabe found it firstly in *Unio pictorum* in a rather large lake of Żarnowiec (Pomerania, N. Poland) and in

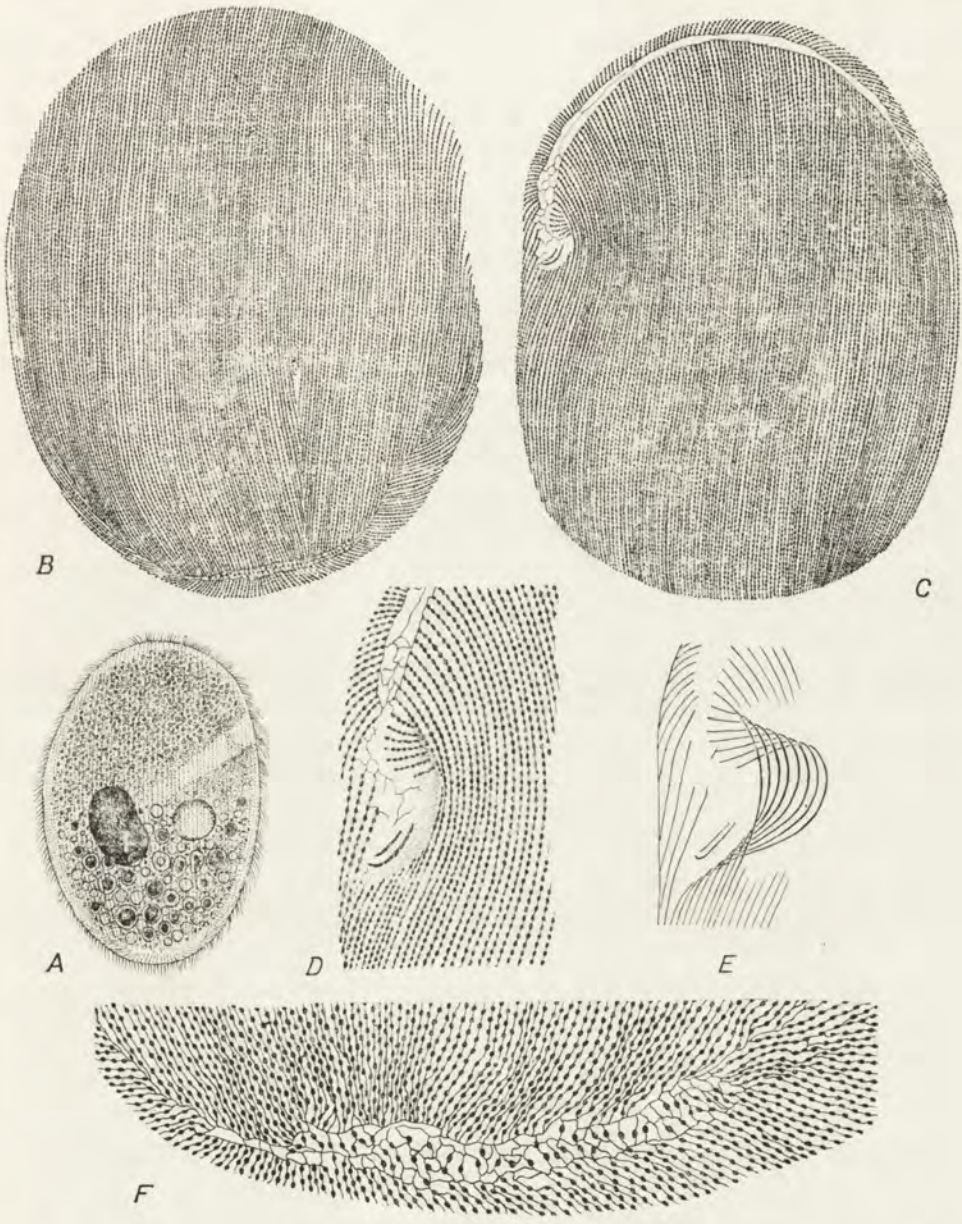

Fig. 10. *Conchoscutum inversum*: A — total, B, C — the ciliary system from the both sides, D — the oral region, E — the infundibulum, F — the posterior suture in $AgNO_3$ preparations (from Raabe). A×300, B, C×500, D, E, F — × 1000

Unio pictorum, *U. crassus*, *Anodonta cygnea* in vast Troki Lakes (Lituania), then he found it in *Anodonta cygnea* in the wide Balaton Lake (Hungary) and finally in *Unio crassus* in a very specific Lake Ohrid (S. Yugoslavia). In spite of large differences among lakes all of them are wide complexes with a different depth. However in spite of numerous exploration in small water bodies and rivers in Poland, Bulgaria and Yugoslavia I was able to find everywhere an abundant infestation by different species of *Conchophthirus*, never by *Conchoscutum*.

Hosts: *Unio pictorum* L., *U. crassus* Retz., *Anodonta cygnea* L. — larger European lakes.

Subfamilia *Peniculistomatinae* Fenchel, 1965

This taxon as familia *Peniculistomatidae* was created by Fenchel 1965 for the genus *Peniculistoma* Jankowski, 1964 differentiated from the family *Conchophthiridae*. Fenchel 1965 is writing: "The family *Peniculistomatidae* is erected in this paper to contain *Peniculistoma mytili* (de Morgan) (*Conchophthirus mytili* de Morgan), which in many important respects differs from the *Conchophthiridae*. The adoral rows are well developed and nearly identical with those found in *Pleuronematina*. The *Peniculistomatidae* must be considered as the most primitive family within the *Thigmotricha*". The diagnosis reported by Fenchel 1965 runs as follows: "Laterally flattened ciliates. Well developed mouth with a long undulating membrane (UM) and three distinct membranelles each consisting of several rows of kinetosomes. Dense ciliation. One genus: *Peniculistoma* Jank., 1964".

I cannot recognize the definition of *Peniculistomatidae* or *Peniculistomatinae* as "the most primitive family within the *Thigmotricha*" according to my previous statements and concerning the position of *Thigmophryidae* approached by Chatton et Lwoff (part I. p. 3). The primitiveness of an organism or of a group of organisms cannot be taken for granted on the basis of one plesiomorphic feature (in this case the character of adoral kineties). In the respect of the abundant general ciliature as well as other morphological aspects and on account for its high and close parasitological specificity, *Peniculistoma* is not primitive. Moreover it corresponds adequately to the characters of the family *Thigmophryidae* in the sense and range stated by myself. Subfamilia *Peniculistomatinae* occupies an analogous position within the family *Thigmophryidae* as subfamilia *Thigmocominae* (after all also monospecific) within the *Hemispeiridae* (vide Part I. p. 25). As I stated it before, the specificity of this subfamily consists on well developed adoral rows, however modified, they extend along the ventral body edge on a large surface and a lack even of a tendency to form an ciliated infundibulum. An additional character would be recognized that during the division the adoral rows divide and then undergo reorganization very likely to the *Pleuronematidae* and among the *Thigmotricha* — to the *Hemispeiridae*.

Therefore the definition of the subfamily may be as follows:

Subfamilia *Peniculistomatinae* Fenchel, 1965

Thigmotricha — *Thigmophryidae* of a great, strongly flattened body (ca. 120 μ) of an ellipsoidal outline with the depression on the ventral rand. The ciliature is dense and uniform; number of kineties of the range of 180. The peristomal field lies on the ventral margin of the body; two long adoral kineties, drawn aside and modified, lie along the naked field. The kineties of the general ciliature do not enter the infundibulum. The nuclear apparatus: 1 Ma, 1 or 2 Mi. C. V. in the hind body part. Division equal; the adoral kineties divide himself and fall both to the proter and to the opisthe. Parasites of the mantle cavity of marine *Bivalvia*.

Typus subfamiliae: genus *Peniculistoma* Jankowski, 1964.

Genus *Peniculistoma* Jankowski, 1964

syn.: *Conchophthirus* Stein, 1861 pro parte — De Morgan, 1925; *Kidderia* Raabe, 1934 [hom.]; *Morgania* Kahl, 1934 [hom.].

The name of the genus *Peniculistoma* sets up Jankowski 1964 as substitute of the genus name *Kidderia* Raabe, 1934 which like the introduced a month later name *Morgania* Kahl, 1934 were revealed as homonyms (Corliss 1960, 1961). The both changed names of the genus: *Kidderia* Raabe and *Morgania* Kahl were set for the differentiation of the species *C. mytili* de Morgan, 1925 from genus *Conchophthirus* Stein; this species does not correspond to the characteristic of the genus fixed by Raabe 1934 and Kahl 1934.

The species typical for the genus, *P. mytili* (the only so far) has been described by De Morgan 1925 quite unadequately and wrongly oriented: the author recognized its anterior part as the posterior one. Only Kidder 1933 reported an adequate and exact redescription of the species, however he left it within genus *Conchophthirus*. As it has been stated Raabe 1934 and Kahl 1934 differentiated this genus.

The only representative of the genus *Peniculistoma* is being a big ciliate with a reniform outline, strongly laterally flattened. The ciliature is abundant, uniform and arranged in ca. 170 kineties. Jankowski (personal communication after Fenchel 1965) "has also observed a dorsal thigmotactic field consisting of 7 kineties on the right side of the dorsal edge" what was observed neither by Fenchel 1965 nor by former authors. The nuclear apparatus consists of a large Ma in the middle of the body and of two Mi. C. V. lies in the posterior end of the body. The plasma contains numerous food vacuoles.

The most of different interpretations reveal the structure of the adoral rows of *P. mytili*, Kidder 1933 draws two kineties, differentiated and fragmented: one right long and describing a strong arch in the posterior part, and the left one shorter and more straight. Raabe 1934 homologizes these kineties with the adoral rows of *Ancistruma* and *Conchophthirus*. Chatton et Lwoff repeat it after him. Fenchel 1965 draws a long right kinety, he identifies it as UM, this kinety describes an arch in its posterior part; he draws also three other kineties, determined by him as homologues of AZM. These kineties are: initiating previous to UM (M_1) which runs to the midst of the body length and defines from the left the peristomal field, M_2 — rather long and wide kinety extending between UM and M_1, finally M_3, a zigzag kinety arranged behind M_2, in its continuation. Jankowski (after Fenchel 1965) considers the kinety identified by Fenchel as M_1 as the last thigmotactic kinety which occurs in his opinion among some *Pleuronematidae*, whereas he considers M_2 as combination of M_1+M_2. However, Jankowski 1966 does not analyze more exactly this matter and does not give any drawings. This is why the orientation remains very difficult (Fig. 11 B, C).

I have no opportunity to examine this problem, however in my view the drawing of Kidder presents rather a state of rest of the adoral apparatus of *Peniculistoma*, whereas the drawings of Fenchel mostly concern the reorganization stages. Fenchel 1965 observes rightly, that "a study of the stomatogenesis will probably clarify this problem". I intend to discuss this problem in that part of my monographic study which would be mostly concerned on the morphogenetic process in the *Thigmotricha*.

The monospecific genus *Peniculistoma* may be determined for the time being in the following way:

Peniculistoma Jankowski, 1964

syn.: *Conchophthirus* pro parte auctorum; *Kidderia* Raabe, 1934; *Morgania* Kahl, 1934 — homonyma.

Thigmophryidae — Peniculistomatinae of a diagnosis as the diagnosis of the subfamily. The body great (ca. 120 μ), strongly flattened, of the kidney-shape outline. Number of kineties ca. 108. The peristomal field lies on the ventral, concave margin of the body, the adoral kineties: stomatogenic kinety long, the prostomal kinety differentiates into several (3) parts. The nuclear apparatus: 1 Ma and 2 Mi, lies in the centre of the body. C. V. in the rear body part. Parasites of the mantle cavity of marine *Bivalvia*.

Typus generis: *Peniculistoma mytili* (De Morgan, 1925) Jankowski, 1964.

Peniculistoma mytili (De Morgan, 1925), Jankowski, 1964

syn.: *Conchophthirus mytili* De Morgan, 1925 — auctores; *Kidderia mytili* (De Morgan, 1929) — Raabe 1934; *Morgania mytili* (De Morgan 1925) — Kahl 1934.

The body large with an outline of an concave oval on the one (ventral) side, therefore of a somewhat reniform shape. The anterior part of the body more pointed, the end bluntly finished. Size: length 100–180 μ (according to Fenchel 110–176 μ) width ca. 120 μ (according to Fenchel 66–110 μ). The body is uniformly and densely covered with tiny cilia; the number of kineties amounted to 160–180 (Fenchel). A peristomal field lies on the concave body margin covering up to 1/2 of the body length. Adoral rows lie on this field, their structure reported for the genus. Independently of any interpretation these are as follows: a stomatogenic kinety, long and forming in the posterior part a rather wide loop and the prostomal kinety fragmented more or less distinctly in 3 parts. The nuclear apparatus consists of an oval Ma arranged by its longer axis across the body, in the middle of the body length or so, and of two Mi lying anterior to Ma. The size of Ma — ca. 35×20 μ, C. V. lies in the posterior end the body at its dorsal edge. There are numerous

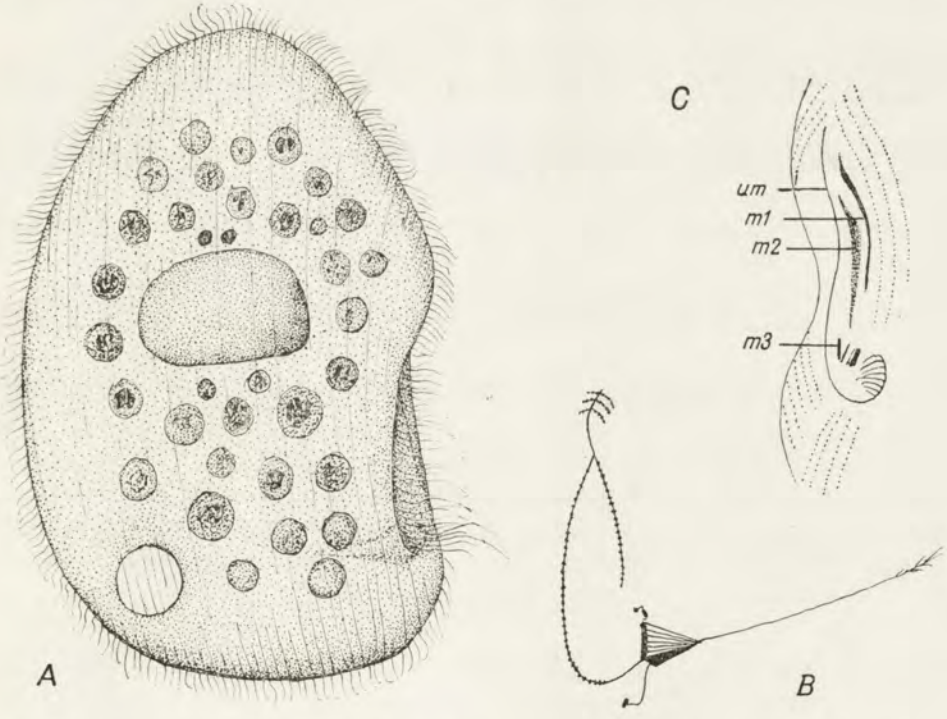

Fig. 11. *Peniculistoma mytili*: A — total (after various authors), B — the adoral kineties (from Kidder), C — the peristomal region (after Fenchell) × 500

granulations in the plasma and sometimes a mass occurrence of food vacuoles containing large food particles, originating as it seem from the epithelium of the host. An equal division, observed by Kidder 1933, Beers 1959 and Fenchel 1965, the conjugation observed by Kidder 1933 and Fenchel 1965 (Fig. 11).

Host: *Mytilus edulis* L. on the whole area of its occurrence as it seems: Raabe and Fenchel report it from Baltic and Kattegat, from Plymouth — De Morgan, from Barents Sea — Jankowski, from the Atlantic coasts of North America — Kidder and Beers.

The infestation of *Mytilus* by *Peniculistoma mytili* is in the opinion of different authors highly variable in its frequency and intensity, namely from 0–100 infestation of the individuals of the mussel population, from 0–300 ciliates in one mussel. The infestation grows distinctly with the size and age of mussel, with the deepness from which they originate and as it seems with the salting of the water; in the brakish waters the infestation is not so intense as in the full salted (data of Raabe, Beers, Fenchel).

Summary

The fourth part of the monograph on *Thigmotricha* comprises the elaboration of the family *Thigmophryidae* with the three subfamilies: *Thigmophryinae*, *Conchophthirinae* and *Peniculistomatinae*. The family *Thigmophryidae* characterize the abundant general ciliature, the tendency to the atrophy of the adoral kineties and, in many cases, the presence of the ciliated infundibulum. The characteristics of the family and the subfamilies are given. The paper comprises the descriptions and definitions of the genera and the diagnoses of the species. In the subfamily *Thigmophryinae* the new genus is erected: *Cochliodomus* g. n. for *Cochliophilus oncomelaniae* Tchang, 1957.

STRESZCZENIE

Czwarta część monografii *Thigmotricha* zawiera opracowanie rodziny *Thigmophryidae*, obejmującej trzy podrodziny: *Thigmophryinae*, *Conchophthirinae* i *Peniculistomatinae*. Rodzina *Thigmophryidae* charakteryzuje się obfitym urzęsieniem generalnym, pewnym uwstecznieniem kinet adoralnych i, w wielu przypadkach, występowaniem urzęsionego infundibulum. Podano charakterystykę rodziny i podrodzin. Podano opisy i diagnozy rodzajów i opisy gatunków. W obrębie podrodziny *Thigmophryinae* wyodrębniono nowy rodzaj *Cochliodomus* g. n. dla *Cochliophilus oncomelaniae* Tchang, 1957.

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ACTA PROTOZOOLOGICA

VOL. IX WARSZAWA, 15.XII.1971 FASC. 10

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Studies on the family *Blepharocorythidae* Hsiung. VI. Phylogenesis of the family and the description of the new genus *Circodinium* gen. n. with the species *C. minimum* (Gassovsky, 1918)

Badania nad rodziną *Blepharocorythidae* Hsiung.
VI. Filogeneza rodziny i utworzenie nowego rodzaju
Circodinium gen. n. z gatunkiem *C. minimum* (Gassovsky, 1918)

In my previous papers concerning the family *Blepharocorythidae* I have pointed out the possibility of its origin from *Buetschliidae* (*Gymnostomata*).

At first, my suggestions were based on the presence of similar groups of kinetosomes and their arrangement in both families. In *Buetschliidae* there are big kinetosomes (Wolska 1964) forming few short kineties on the surface of the vacuole with concretions ("Konkrementenvacuole" according to Dogiel 1929 a); they are called "cinéties sus-concretionnaires" by Grain 1966. In *Blepharocorythidae* there are big or fine kinetosomes (Wolska 1966 b, 1967 a, b, 1968, Wolska and Piechaczek 1970) arranged in short kineties on the surface of the vacuole without concretions, or, on the surface of the cytoplasmic protuberance, but always in the same position which in *Buetschliidae* occupies the vacuole with concretions. I consider this structure to be a remnant of the vacuole with concretions (Wolska 1966 b).

Then, after the genus *Raabena* Wolska, 1967, included in the family *Blepharocorythidae*, had been described, I have pointed out an analogy and probably a homology between the somatic ciliary zones of the genera *Raabena* and *Didesmis*. In the latter I have found (Wolska 1966 a) two posterior ciliary zones (similar to those in primitive *Blepharocorythidae*) and the origin of the anterior ciliary zone from two primordia (there are two anterior zones in *Blepharocorythidae*).

On the other hand, the idea of the relationship of *Blepharocorythidae* with highly specialized group *Entodiniomorphida* (Wolska 1967 a) and the possibility of the origin of *Entodioniomorphida* from *Buetschliidae* came to my mind (Wolska 1966 a, b).

The question of the possible relationships between these groups is the aim of the present work. It will be discussed on account of the character of the ciliature and the morphogenesis of these groups, the most essential features for recognition of phylogenesis in ciliates.

Evolution within the family *Blepharocorythidae*

The genus *Raabena* seems to be the most primitive among the components of the family *Blepharocorythidae*. Its primitiveness reveals in the character of the buccal ciliature connected with the somatic one, and in the character of the caudal zones constituting rich ciliature on the posterior end of the body.

The genus *Raabena* differs from all the genera of the family by its not independent oral ciliature but is closely related with all of them. Comparing the genera *Raabena* and *Pararaabena* we can notice that only slight reduction of cilia in the fronto-buccal zone and the presence of the processes of the posterior end of the body in *Pararaabena* distinguishes it from *Raabena*. Similar relations occur between *Raabena* and the genera *Charonina* and *Spirocorys* (strong development of the somatic ciliature in the latter is probably a secondary phenomenon). In *Blepharocorys* the oral ciliature is differentiated and divided into two groups, so the genus is more distant from *Raabena*.

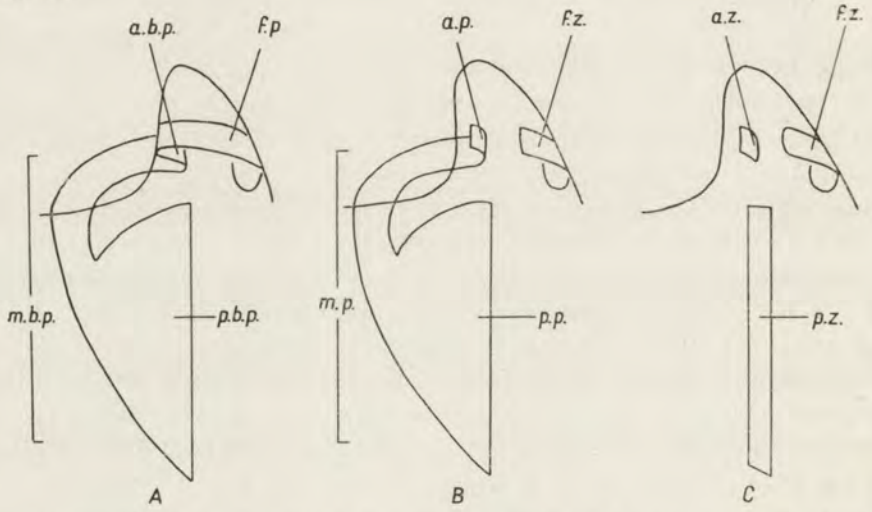

Fig. 1. Scheme of the development of the buccal ciliature in *Blepharocorythidae* on examples of the genera *Raabena*, *Pararaabena* and *Blepharocorys*. A — Fronto-buccal zone of *Raabena*. Frontal part (*f.p.*), anterior buccal part (*a.b.p.*), middle buccal part (*m.b.p.*), posterior buccal part (*p.b.p.*). B — Frontal zone and buccal zone of *Pararaabena*. Frontal zone (*f.z.*), anterior part of buccal zone (*a.p.*), middle part of buccal zone (*m.p.*), posterior part of buccal zone (*p.p.*). C — Frontal zone, and two buccal zones of *Blepharocorys*. Frontal zone (*f.z.*), anterior buccal zone (*a.z.*), posterior buccal zone (*p.z.*). In all drawings the fibers are omitted

The development of the oral ciliature in *Blepharocorythidae* may be characterized by gradual disappearance of some parts of the fronto-buccal zone in the most primitive form, leading to the development of independent buccal zone, and then, to the disintegration of this zone into the anterior and posterior oral zones (Wolska 1968). These changes are illustrated by the schemes of the buccal ciliature in three genera of the family *Blepharocorythidae* (Fig. 1). In the first stage of reduction a part

of the ciliature on the frontal segment disappears, dividing the homogenous fronto-oral zone (as in Fig. 1 A) into two parts — the frontal and the oral ones (Fig. 1 B). In the next phase the median part of the oral zone disappears dividing the oral zone into two parts — the anterior one composed of short kineties and the posterior one composed of long kineties (Fig. 1 C).

Taking into account the direction of the development of the buccal ciliature and caudal zones, it is possible to distinguish several evolutionary lines (Fig. 2) within the family *Blepharocorythidae*, originating from the most primitive form

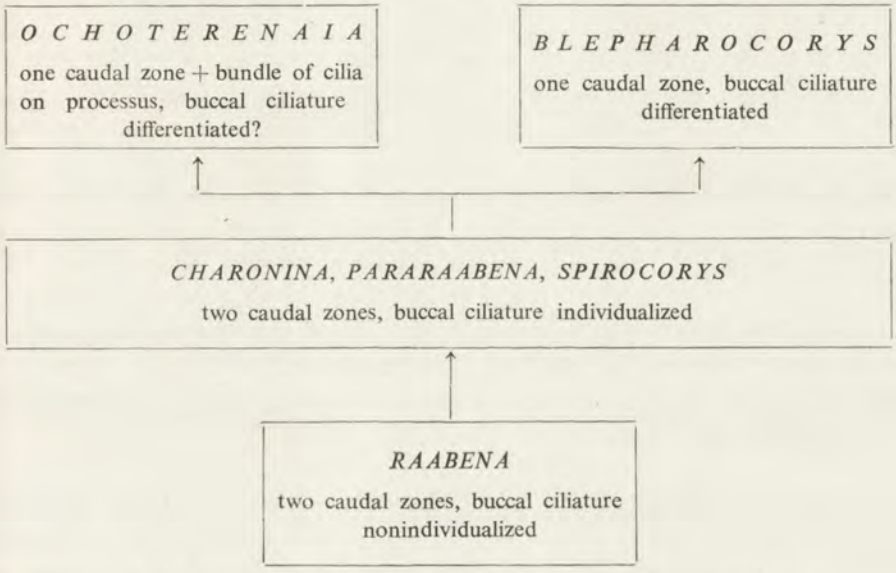

Fig. 2. The evolutionary lines within the family *Blepharocorythidae*

(*Raabena*). In these considerations the genus *Charonnautes* is omitted because its buccal ciliature has not yet been studied, but the genus *Ochoterenaia* is included due to its close relation with the genus *Blepharocorys*. The single representative of this genus was even included to *Blepharocorys* by Strelkov 1939, so we may expect great similarity in the structure of the buccal ciliature in both genera.

Within the genus *Blepharocorys* (the only the polispecific) the buccal ciliature is generally homogenous in character, but there is a marked tendency to the differentiation of longitudinal kineties by means of shortening and separation of one kinety from the others. It leads to total separation of this kinety. In *B. valvata* one kinety, somewhat shorter, is slightly separated from the group of kineties (Wolska 1971, Fig. 4). In *B. jubata* one shorter kinety deviates distinctly from the compact group of the remaining ones (Wolska 1971, Fig. 3). In *B. curvigula* one kinety is totally reversed and displaced to the zone of short kineties (Wolska 1971, Fig. 6). Here the separation of one kinety is so strongly marked and fixed that during

ontogenesis the primordium of this kinety appears independent of the primordia of the other longitudinal kineties. Such condition exists also in *B. cardionucleata* (Wolska 1971, Fig. 7).

Then we may say that the development of the buccal ciliature in the genus *Blepharocorys* proceeds from the species *B. uncinata* and *B. angusta*, with undifferentiated longitudinal kineties, through *B. valvata* and *B. jubata* to *B. cardionucleata* and *B. curvigula*, in which total separation of one longitudinal kinety occurs.

Possibility of origin of *Blepharocorythidae* from *Buetschliidae*

The family *Buetschliidae* (*Gymnostomata*) is rich in genera and species occurring in the intestine of herbivorous mammals, mainly *Perissodactyla*. Several species occur in the intestine of rodents and one in the rumen of ruminants (uncertain species are not counted).

In *Buetschliidae* the buccal overture occurs on the anterior pole of the body, cytopyge on the posterior one. The body is circular in cross section, except in the representatives of the genus *Didesmis* which have strongly flattened body. The ciliature covers either the whole body, or is reduced in some parts. The means of reduction of the ciliature and succeeding degrees of this process were given by Strelkov 1939, Fig. XXII. Usually one contractile vacuole occurs in the posterior part of the body, and the vacuole with concretions, characteristic of *Buetschliidae*, occurs in the anterior part.

On the surface of the vacuole with concretions there is always the characteristic group of kinetosome called the special kinetosomes (Wolska 1964). When the somatic ciliature covers the vacuole with concretions (in forms with abundant ciliature) the special kinetosomes are incorporated in it, but always they are well marked by their size and more compact arrangement. When the ciliature of the body does not cover the vacuole with concretion (in forms with reduced ciliature) the special kinetosomes form a distinct group. This is the situation in the genus *Didesmis*, where the special kinetosomes form clearly distinguished group, situated posteriorly to the anterior ciliary zone.

It is possible to show the similarity of *Blepharocorythidae* (at least the primitive species) to *Buetschliidae* with strongly reduced ciliature. This similarity reflects the relationship of both these families.

In *Blepharocorythidae* the somatic ciliature is confined to the groups of cilia in the anterior and posterior parts of the body, similarly as in the representatives of *Buetschliidae* with advanced reduction of ciliature.

The similarity of the somatic ciliature patterns in both families is best marked when we compare the primitive genera of *Blepharocorythidae* as *Raabena*, *Pararaabena* and *Charonina* with *Didesmis* from *Buetschliidae*. In the representatives of *Blepharocorythidae* two groups of cilia occur on the anterior and two on the posterior parts of the body. As a matter of fact such two groups of cilia in the

anterior and two in the posterior parts of the body occur also in *Didesmis*. The posterior groups are separated (they are well visible after silver impregnation) while the anterior ones form one zone, but their primordia are separated during morphogenesis (Wolska 1966 a). Another feature characteristic of *Blepharocorythidae* and rather extraordinary in *Buetschliidae* is the flattening of the body in *Didesmis*.

A vacuole with special kinetosomes on the surface, more or less separated from the anterior somatic zone, lies posteriorly to one of the anterior somatic zones in *Blepharocorythidae*. This structure corresponds to the vacuole with concretions in *Buetschliidae*. In *Blepharocorythidae* the vacuole does not contain any concretions. It seems most probable that the vacuole without concretions (vacuole "R"), or even a protuberance of cytoplasm with special kinetosomes, is a remnant of the

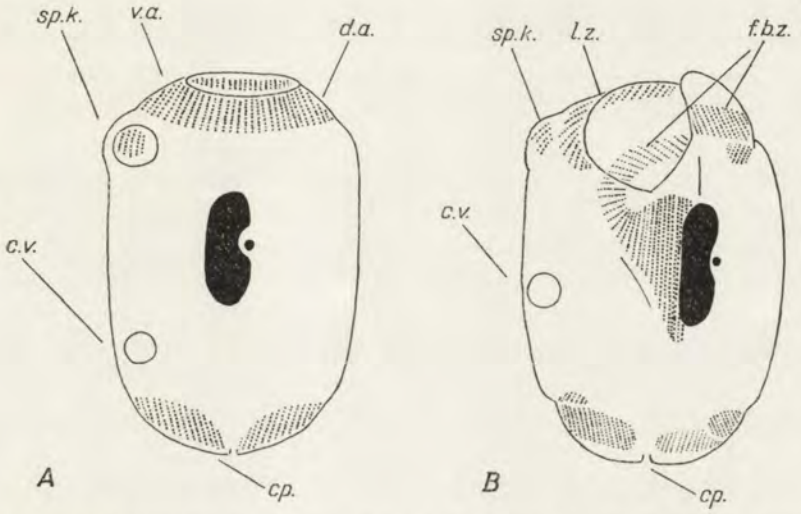

Fig. 3. Scheme of the structure and infraciliature in the species: A — *Didesmis ovalis*, B — *Raabena bella*. The anteroventral zone or ventral arc (*v.a*) and the antero-dorsal zone or dorsal arc (*d.a.*) in *Didesmis*. The labial zone (*l.z.*) and the fronto-buccal zone (*f.b.z.*) in *Raabena*. Special kinetosomes (*sp. k.*), contractile vacuole (*c.v.*), cytopyge (*cp.*)

vacuole with concretions. It is a vestigial organellum but it speaks forth for the origin of *Blepharocorythidae* from *Buetschliidae*.

Comparing the schemes (the fibers are not marked) of *Didesmis ovalis* (*Buetschliidae*) and *Raabena bella* (*Blepharocorythidae*) (Fig. 3) we may see the great similarity in the body shape, the character of the ciliary zones and the arrangement of other elements of the structure of these ciliates. Nothing is against the supposition that *Didesmis ovalis* is flattened laterally and not dorso-ventrally as it has been stated by Hsiung 1930 and Grain 1966. So in the drawing it is seen from the left side, similarly as *Raabena bella*. In consequence the contractile vacuole in *Raabena bella*

lies in the same position as in *Didesmis ovalis* and the vacuole "R" without concretions in *R. bella* equivalent to the vacuole with concretions in *D. ovalis*, at the ventral side of the body. The arrangement of the nuclear apparatus is also similar in both these species. The posterior end of the body does not show any significant difference in the compared species. In both species the cytopyge is situated terminally and the posterior ciliary zones ventrally and dorsally to the cytopyge. There is only a difference in the degree of development of these zones. The caudal zones in *R. bella* are weakly developed in comparison with *D. ovalis*, the dorsal zone is partly reduced on the left and the ventral zone on the right side of the body.

If *R. bella* originated from the representative of *Buetschliidae* of the type of structure of *Didesmis*, then the anterior end of the body would undergo greater changes.

Bearing in mind that the anterior ciliary zone in *Didesmis* is composed of two parts (arcs) converging at the sides of the body, we may regard one of these parts as the anterior ventral zone (or ventral arc, *v.a.*) and the other one as the anterior dorsal zone (or dorsal arc, *d.a.*). During transformation of the anterior part of the body, the anterior ventral zone would remain unchanged, on the surface of the body, partly surrounding the new, more extensive buccal overture. It would correspond to the zone of the ventral lip, or simply the labial zone (*l.z.*) in *Blepharocorythidae*. The anterior dorsal zone (dorsal arc) would change its course because of the formation of the frontal lobe. After encircling the frontal lobe this zone dives into the buccal concavity forming further the buccal ciliature. On the whole, it becomes the fronto-buccal zone in *R. bella* (*f.b.z.*). For detailed description of the fronto-oral zone and the accompanying fibers see Wolska 1967 b. The fibers of the cytopharynx in *Didesmis* become dislodged and the buccal concavity receives, in its nonciliated part, a lining of semicircular fibers connected with the kinetosomes of the buccal ciliature. Starting from this moment, i.e. from the type of structure of *R. bella*, the development of the buccal ciliature within *Blepharocorythidae* runs in the manner described in the part V of the present paper.

The development of the somatic ciliature on the posterior end of the body is characterized by a tendency to reduction. The course of the reduction is variable, beginning with diminishing of both caudal zones (e.g. *Charonina*), ending in total reduction of one of the caudal zones, usually the dorsal one. On the other hand, there is a marked tendency to form syncilia in the caudal zones (*B. curvigula*, *B. jubata*, probably *O. appendiculata*). In some *Blepharocorythidae* the ability appears to form various appendices on the posterior part of the body (*P. dentata*, *O. appendiculata*).

The buccal ciliature in the most primitive form *R. bella* is a continuation of the somatic ciliature. It corresponds to the vestibulum of *Trichostomata* but the continuation of the somatic ciliature into the buccal concavity, in the genus *Raabena*, is realized in another way. The vestibulum in *Trichostomata* is a concavity preceeding the cytostome into which sink the endings of somatic kineties (Fauré-Fremiet 1950 a, 1950 b, Corliss 1956, 1961). In *R. bella* newly formed numerous kineties

enter the concavity. These are not the endings of the somatic kineties, occurring on the body surface.

From the fact that in *R. bella* the buccal concavity similar to the vestibulum occurs, does not conclude in my opinion that this genus and the whole family ought to be retained in the order *Trichostomata*. *Blepharocorythidae* are not at the level of the development of *Trichostomata*. The type of morphogenesis in *Blepharocorythidae* (Wolska 1966 b, 1967 a, b), even in the most primitive genus, approaches them rather to the order *Entodiniomorphida*.

Not considering further the difficult problem of the systematic position of *Blepharocorythidae*, I want to emphasize that the origin of *Blepharocorythidae* from *Buetschliidae* of the *Didesmis* type of structure is very probable.

Comparison of *Blepharocorythidae* and *Entodiniomorphida* and possibility of their common origin from *Buetschliidae*

The relationships and systematics within the order *Entodiniomorphida* were discussed by numerous authors. The papers of Kofoid and MacLennan 1930, 1932, 1933, Kofoid and Christenson 1934, Dogiel 1927, 1947, Lubinsky 1957 a, b, c, Noirot-Timothée 1959, 1960, Latteur 1966 concerned the evolution within the family *Ophryoscolecidae*. Hoare 1937 discussed the relationships of some representatives of *Cycloposthiidae*, Strelkov 1939, gave a full system of *Cycloposthiidae* from equids.

Up to now, no investigations here been carried out on the origin of *Entodiniomorphida*. They are traditionally placed in *Spirotricha*.

It seems that *Entodiniomorphida* and *Blepharocorythidae* are closely related. It has been already shown in the previous papers of the author (Wolska 1966 b, 1967 a) that the great similarity in morphogenesis joins these groups. Other similarities may also be accounted between them.

It is worth to remind here the characteristic of *Entodiniomorphida*. The order belongs to *Spirotricha*. It was formerly divided into two families — *Ophryoscolecidae* and *Cycloposthiidae*, each with subfamilies. The contemporary authors divide it into some families (Strelkov 1939, Noirot-Timothée 1960).

This highly specialized group lives in the rumen of artiodactylan ruminants (*Ophryoscolecidae*) and in the large intestine of perissodactylans (*Cycloposthiidae*), also in rodents and elephants, one genus occurs in schimpanzee and gorilla.

The body of the ciliate is covered with rigid pellicle and usually it is laterally flattened. The ciliature is restricted to only one adoral zone (some *Ophryoscolecidae*), or there are also additional zones on various parts of the body. In *Ophryoscolecidae* from the rumen there is only one additional zone in the anterior half of the body, called the dorsal zone, while in other ciliates more zones may occur.

The adoral zone may be retracted into the so-called infundibulum, formed by invagination of the anterior part of the body. In some representatives of *Entodinio-*

morphida there is more or less developed skeleton under the pellicle. It is composed of small prisms of polysaccharide character (Noirot-Timothée 1960). The endoplast (according to Strelkov 1939) is delimited from the ectoplast by a fibrillar layer and forms a digestive space — the endoplasmic sac, communicating with the environment through the cytostom and cytopyge. One, usually elongated macronucleus lies in the ectoplast at the dorsal side of the body. The fibers are abundant in various parts of the body (Strelkov 1939, Holland and Batisse 1959, Noirot-Timothée 1960).

The adoral zone in *Entodiniomorphida* (and other zones also) is commonly named the zone of membranelles (or cirri by some authors) and is homologized with AZM in *Heterotricha* and *Hypotricha*. Such determination of the adoral zone in *Entodiniomorphida* is not right. In living protozoans, grouping of cilia into bundles may be observed but they form neither membranellae nor cirri. Most frequently, these groups disintegrate in single cilia in fixed specimen. Galei and Sebestyen 1932 proposed the name "syncilia" for these unstable bundles. Noirot-Timothée 1960, on the base of the electron microscope, came to conclusion that the adoral zone of *Entodiniomorphida* cannot be regarded as the zone of membranelles or cirri because such structures do not occur and only temporary brush-like groups of cilia — the syncilia are present in these ciliates. To each ciliary zone, according to Noirot-Timothée, corresponds the infraciliary zone composed of kineties oblique in relation to the long axis of the zone. Each kinety is supported by "baquette infraciliaire" in *Ophryoscolecidae*, but not in *Cycloposthiidae*.

Observations of the author (Wolska 1966 a) show also that the adoral zone in *Entodiniomorphida* is composed of oblique kineties, parallel to each other equally spaced. The opinion of Grain 1966 about the structure of this zone is similar to that of Noirot-Timothée. According to Grain the groupings exist only on the level of cilia, not of kinetosomes. Due to the investigations of Noirot-Timothée and Grain, the character of the ciliary zones in *Entodiniomorphida* is beyond any doubt.

Due to the works of Fernandez-Galiano 1958 a and Noirot-Timothée 1960 it is known that the adoral zone of *Ophryoscolecidae* is composed of two segments developing from distinct primordia during stomatogenesis (one part on the ventral and the other one on the dorsal side), uniting later into one.

Noirot-Timothée described small cilia occurring at the posterior margin of the adoral zone, on the ventral side. They were not found in silver impregnated preparations. Later, these cilia have been described by Bretschneider 1962 as "Paralabialorgan". The kinetosomes of these cilia have been found in silver impregnated preparations by Wolska 1965, who called them free cilia and homologized with "cinecias ventrales independientes" described by Fernandez-Galiano 1958 b in *Cycloposthium edentatum*. Thus, the adoral ciliature in *Ophryoscolecidae* is made up of three elements: ventral part of the zone of syncilia, dorsal part of syncilia, and free cilia (Fig. 4 A, Pl. I 1). Free segment of the dorsal

part of syncilia gets down (sometimes conspicuously) into a concavity lying posteriorly to the infundibulum and preceeding the digestive area. Grain 1966 compares this concavity with vestibulum of *Trichostomata*.

Three elements composing the adoral ciliature occur also in *Cycloposthiidae* (Fig. 4 B). Fernandez-Galiano 1958 b gave detailed description and drawings of the infraciliature in *Cycloposthium edentatum* using the method of silver impregna-

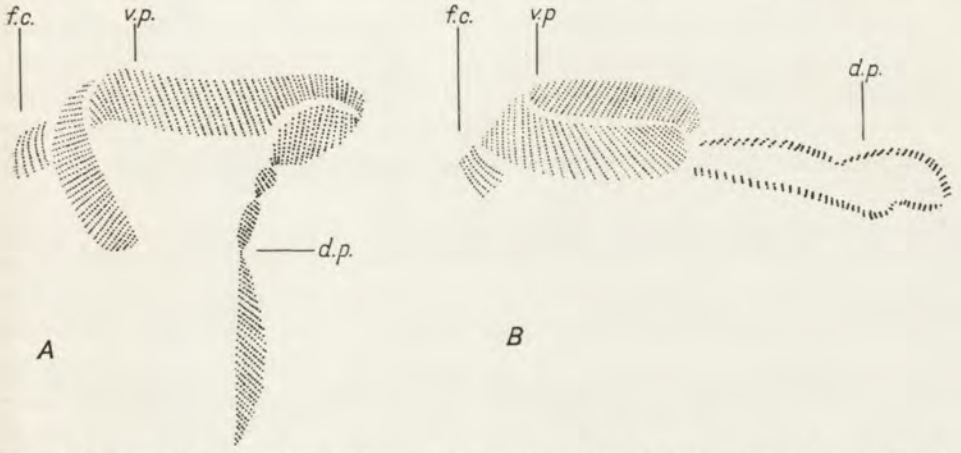

Fig. 4. Scheme of the adoral infraciliature. A — representative of *Ophryoscolecidae* (according to Noirot-Timotheé 1960, modificated), B — representative of *Cycloposthiidae* (according to Fernandez-Galiano 1958 b). Ventral part of the adoral zone (*v.p.*), dorsal part of the adoral zone (*d.p.*), free cilia (*f.c.*)

tion according to Rio Hortega. The author obtained similar pictures of infraciliature in *C. edentatum* and in other species of the genus *Cycloposthium* using the same, somewhat modified technique (Wolska 1965).

The most developed part of this ciliature, situated at the ventral wall, has the same structure as the bipartite zone of syncilia in *Ophryoscolecidae*. It is composed of parallel and oblique kineties. The zone was called "banda bucal" by Fernandez-Galiano. I regard this part to be homologous with the ventral part of the zone of syncilia in *Ophryoscolecidae*. The other part of the buccal ciliature, situated at the dorsal side, occurs in form of a narrow ribbon, composed of very short, thick and loosely distributed kineties. This part was called the zone of membranellae by Fernandez-Galiano. But similarly as in the ventral part, there are no groups of kineties so it cannot be regarded as the zone of membranellae. I regard this part to be homologous with the dorsal part of the adoral zone in *Ophryoscolecidae*. Third element in the adoral ciliature forms "cinecias ventrales independientes", called "free cilia" by the author (Wolska 1965). They are quite similar to free kineties in *Ophryoscolecidae* and are similarly situated (Pl. I 2).

Similar results have been obtained by silver impregnation of the ciliates of the genus *Triplumaria* from the faeces of an Indian elephant (unpublished data). Here

the adoral ciliature is more similar to that of *Ophryoscolecidae* because of the dorsal part (a narrow ribbon of kineties) getting down deeply in the "vestibulum" (Pl. I 3).

Then, to each one of the three elements of the adoral zone in *Ophryoscolecidae* corresponds an element of the adoral zone in *Cycloposthiidae*.

According to authors observations on silver impregnated material (Wolska, unpublished data) the adoral zone in remaining families of *Entodiniomorphida* is generally similar in structure. The genus *Triadinium* Fiorentini is an exception. In the species *T. caudatum*, the adoral zone is more simple, without any part corresponding to the dorsal part. I exclude the genus *Triadinium* from the present considerations, since it will be considered further. In this chapter only the families *Ophryoscolecidae* and *Cycloposthiidae*, the most extensive representatives of the order *Entodiniomorphida*, permitting to generalize the results of investigation, will be discussed.

Generally speaking the features characteristic of the buccal zone of *Spirotricha* are not present in the structure of the adoral zone of *Entodiniomorphida*. Thus *Entodiniomorphida* cannot be regarded as *Spirotricha* or as a group derived from any group of *Spirotricha*. Their ancestors ought to be looked for among *Holotricha* with simple structure of ciliature, without such structures of higher degree as membranellae. Such initial group for *Entodiniomorphida*, similarly as for *Blepharocorythidae*, might be *Buetschliidae* of the type *Didesmis*, the anterior ciliature of which with special kinetosomes might transform into the adoral zone of *Entodiniomorphida*. Deeper changes had to follow this process than in the case of *Blepharocorythidae*. There would be the particular differentiation of the cytoplasm and sinking of the ciliary zones into developing infundibulum.

In spite of these divergent trends in development *Blepharocorythidae* and *Entodiniomorphida* preserved a series of common features. They are as follows:

1. Rigid pellicle (a feature strongly expressed in *Entodiniomorphida*, weaker in *Blepharocorythidae*, although some species of the genus *Blepharocorys* have ridges on the body surface).
2. Flattening of the body.
3. Arrangement of the ciliary zones (on the anterior and posterior parts of the body in many cases, only on the anterior part in *Ophryoscolecidae*).
4. Structure of the ciliary zones.
5. Ability to form syncilia (common in *Entodiniomorphida*, only initial in *Blepharocorythidae*).
6. Formation of cytoplasmic lips around the ciliary zones (weakly marked in *Blepharocorythidae*, conspicuous in *Entodiniomorphida*).
7. Ability to form appendices at the posterior end of the body (frequent in *Ophryoscolecidae*, rare in *Blepharocorythidae*),
8. Presence of a distinct group of cilia on the ventral side (posteriorly to the zone of the ventral lip in *Blepharocorythidae* and posteriorly to the adoral zone in *Entodiniomorphida*).

What concerns the ciliature, the schemes of the anterior ciliature in *Didesmis* (Fig. 3 A) and in *Ophryoscolecidae* and *Cycloposthiidae* (Fig. 4 A, B) should be compared.

If we imagine the sinking of the ciliature of *Didesmis* into an infundibulum and pushing of the dorsal arc away on the left side from the median suture, we shall obtain a picture similar to that of the adoral zone in *Ophryoscolecidae* (Fig. 4 A). The vacuole with concretions had to disappear but the special kinetosomes remained. They have been drawn in together with the ventral arc into a concavity and remained there in the another configuration at the base of the zone.

As it was mentioned above the adoral zone in *Ophryoscolecidae* develops during ontogenesis from two primordia. Noirot-Timothée regards it as a special matter. I suppose this way of formation of the adoral zone in *Ophryoscolecidae* is inherited from *Didesmis* and may be regarded as a reflection of phylogenesis.

Then the following homologies may be accounted:

1. Ventral part of the somatic zone in *Didesmis* (*v.a.*) and the ventral part of the adoral zone in *Ophryoscolecidae* (*v.p.*).

2. Dorsal part of the somatic zone in *Didesmis* (*d.a.*) and the dorsal part of the adoral zone in *Ophryoscolecidae* (*d.p.*).

3. Special kinetosomes in *Didesmis* (*sp.k.*) and free cilia in *Ophryoscolecidae* (*f.c.*).

Similarly the anterior ciliature in *Didesmis* is homologous with the adoral ciliature in *Cycloposthiidae* (Fig. 4 B). The length and arrangement of the kineties on the dorsal part of the body in *Cycloposthiidae* change but the general pattern remains the same. Then, the homologies listed above may be spread over the whole order *Entodiniomorphida*.

They will be as follows:

1. *v.a.* in *Didesmis* and *v.p.* in *Entodiniomorphida*.

2. *d.a.* in *Didesmis* and *d.p.* in *Entodiniomorphida*.

3. *sp.k.* in *Didesmis* and *f.c.* in *Entodiniomorphida*.

This is the way, I suppose, of transformation of the anterior ciliature in *Didesmis* into adoral ciliature in *Entodiniomorphida*.

Such representatives of *Entodiniomorphida* were used as examples which, beside the adoral zone, have no other ciliary zones on the anterior part of the body (*Entodinium*, *Cycloposthium*). In the representatives of other genera of *Entodiniomorphida* an additional zone may occur on the anterior part of the body, on the dorsal side (more or less distant from the anterior end). It is called DZM (e.g. *Diplodinium* and *Triplumaria*). This additional zone has probably originated from the dorsal arc of *Didesmis*, by a process similar to differentiation of the frontal zone in *Blepharocorythidae*.

Comparing *Entodiniomorphida* and *Blepharocorythidae* we ought to admit that in *Entodiniomorphida* the additional zone is homologous with the frontal zone, the ventral part of the zone of syncilia with the labial zone, the dorsal part of the zone

of syncilia with the buccal zone or zones, and free cilia with special kinetosomes in *Blepharocorythidae*.

As a result from the above, *Blepharocorythidae* and *Entodiniomorphida* have probably originated from common ancestors. But direction of evolution of both these groups was different. In each group many particular features developed and many common features remained as well; however, they were not evently preserved in both these groups. Some features, common for *Blepharocorythidae* and *Entodiniomorphida*, had already occurred in their initial group (*Buetschliidae*), for example the structure and arrangement of the ciliary zones. Some common features had been more conspicuous in the initial group, then they disappeared in their derivatives. For example, the vacuole with concretions, formed always distinctly in *Buetschliidae*, became rudimental in *Blepharocorythidae* and only special kinetosomes remained in *Entodiniomorphida*. It is possible that *Entodiniomorphida* are not the direct derivatives of *Buetschliidae*. It is possible also that they originate from *Blepharocorythidae* preserving some features of this group in vestigial state and developing others to higher degree. This problem will be discussed in the next chapter.

It is difficult to follow all the details of morphogenesis in *Blepharocorythidae* and the data, I have to do with, characterizes it only in general.

Already Dogiel 1926 has noticed that the division in *B. ventriculi* begins not from circular constriction but from formation of the subcuticular, periferal channel, similar to that in *Ophryoscolecidae*.

During the studies on *Blepharocorythidae* (Wolska 1966 b, 1967 a, b, 1968) I had an opportunity to notice various stages of morphogenesis in the species examined. These facts are as follows: new ciliature develops in the intracytoplasmic vacuoles (Pl. I 4, 5); the buccal ciliature develops without any connection with the somatic ciliature (Pl. I 6); new ciliary zones, including the buccal zones, develop without contact with the paternal ciliature.

Such type of stomatogenesis ("de novo kinetosomal" according to Corliss's 1967 classification) occurs in *Entodiniomorphida*. It is of particular phylogenetic importance.

Blepharocorythidae and *Entodiniomorphida* had undergone similar evolution. Both originated from a primitive group (*Gymnostomata*) and, conserving some primitive features, achieved the type of stomatogenesis characteristic of the ciliates of the higher evolutionary level.

Posibility of the origin of *Entodiniomorphida* from *Blepharocorythidae* and the description of the new genus *Circodinium*

The recent studies of the author (Wolska, in press) on two species of the genus *Triadinium* Fiorentini from the family *Ditoxidae* gave an occasion to consider the possibility of the origin of *Entodiniomorphida* from *Blepharocorythidae*.

The family *Ditoxidae* was created by Strelkov 1939 for the genera *Ditoxum* Gass., *Tetratoxum* Gass., *Cochliatoxum* Gas., and *Triadinium* Fior., taken out from the family *Cycloposthiidae* Poche, 1913. This family is characterized by non-retractile adoral zone and lack of the skeleton.

The genus *Triadinium* differs distinctly from other genera of the family by the shape of the body. It is shortened and rounded in outline. Strelkov 1939 thought that *Triadinium* evolved from the elongated representative of *Ditoxidae* by bending of the body to the ventral side. My opinion is different. I have examined silver impregnated specimens of *Triadinium minimum* (Fig. 5, Pl. II 8) and *T. caudatum* (Fig. 6, Pl. II 9). It appeared that the typical buccal apparatus of *Blepharocorythidae* occurs in *T. minimum*. It has shape of a long funnel with characteristic long kineties and semicircular fibers (Pl. II 7). On account of these observations, a conclusion has been drawn that *T. minimum* evolved by coiling up of the body of a typical representative of *Blepharocorythidae* to the ventral side. Then the species ought to be included in the family *Blepharocorythidae*. This question will be discussed later. It is also my supposition that *T. caudatum* derives from the representative of *Blepharocorythidae* similar to *T. minimum*.

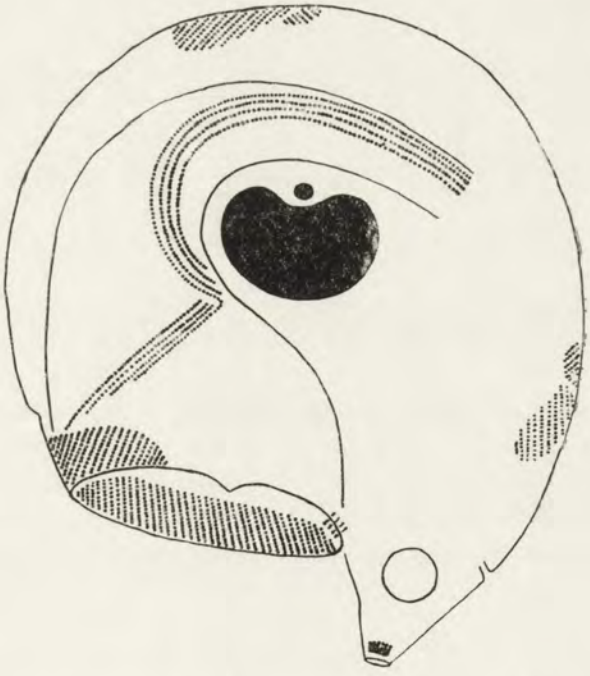

Fig. 5. *Circodinium minimum* comb. nov. from the left side, scheme of the infraciliature

Speaking about the transformations consisting in coiling up of the body of the ciliate I follow Strelkov's argumentation but related to another initial form and restricted to one species *T. minimum* only.

In the process of coiling up of the body of the representative of *Blepharocorythidae* the buccal overture is shifted backward to the ventral side together with the ciliary zone of the ventral lip. The special kinetosomes, situated posteriorly to the zone of the ventral lip in the initial form, change their position. They may occupy only

Fig. 6. *Triadinium caudatum* from the left side, scheme of the infraciliature

the place at the posterior end of the body, near to the dorsal side, a position in which some short kineties occur in *T. minimum*. Due to the shifting of the buccal overture to the posterior end of the body the buccal concavity becomes conspicuously bent. The frontal lobe disappears (probably losing its function of a protection of the mouth). The frontal ciliary zone assumes the terminal position.

The posterior end of the body of *T. minimum* bears a processus with a bundle of cilia.

There exists one species within *Blepharocorythidae* with the identical processus at the posterior end of the body. It is *Ochoterenaia appendiculata* Chavarria, 1933. It is easy to imagine that *T. minimum* evolved from the form similar to *O. appendiculata*. Then nothing would change at the posterior end of the body. The position of the processus, contractile vacuole and cytopyge is identical in both species. In both cases the cytopyge is situated dorsally in relation to the processus and the contractile vacuole lies at its base. The nuclear apparatus is also similar. In *T. minimum* the position of the nucleus in the internal curvature of the buccal concavity

results from the coiling up of the ciliate body. As a result of such bending, the direction of the long axis of the macronucleus is also changed. It turns about 90 degrees in the sagittal plane.

The buccal ciliature in *T. minimum* is formed by the longitudinal kineties only (Fig. 5), there is a lack of the zone of short kineties occurring in all the species of the genus *Blepharocorys*. Nothing is known about these structures in *O. appendiculata* because this species has not yet been silver impregnated. Certainly the longitudinal kineties occurs in this species. It results from the description of the species by Chavarria 1933. The zone of short kineties might have been reduced already in *O. appendiculata* or it disappeared later. In the first case, the buccal ciliature in *T. minimum* would be inherited without any change from the ancestor similar to *O. appendiculata*. In such situation the transformation of one form into the other would consist only on coilling up of the body.

As *T. minimum* does not exhibit the features of the genus *Triadinium* Fior. (created initially for *T. caudatum*) as well as the features of the order *Entodiniomorphida*, but shows the features characteristic of *Blepharocorythidae*, I suggest creation of the new genus *Circodinium* for this species and transfer of it to the family *Blepharocorythidae*.

Circodinium gen. nov., diagnosis

Blepharocorythidae. Body flattened laterally, rounded in the outline. The posterior end passes into a processus. Buccal overture in the posterior part of the body, on the ventral side. One contractile vacuole in the posterior part of the body. Cytopyge situated dorsally in relation to the contractile vacuole.

Four groups of somatic cilia are present. The first one partly surrounds the buccal overture, the second one, somewhat smaller, is situated terminally at the anterior end of the body. The third small group occurs on the dorsal side of the posterior part of the body, and the fourth one, in form of a small bundle, on the tip of the posterior processus.

The special kinetosomes are situated near the base of the processus. The buccal overture leads to the buccal concavity in form of strongly bent funnel provided with several long oral kineties. The nonciliated wall of the funnel is supported by semicircular fibers.

Type of the genus: *Circodinium minimum* (Gassovsky, 1918) comb. nov. a parasite of the intestine of horses.

I suppose that *T. caudatum* evolved from the form similar to *Circodinium minimum* by means of reduction of the buccal kineties and changes in the internal structure. Such presupposition would mark the evolutionary line *Blepharocorythidae* — *Entodiniomorphida* (leading to only one species — *T. caudatum*).

It is possible that *C. minimum* and *T. caudatum* evolved independently. *Circodinium* would evolve from any of the representatives of *Blepharocorythidae* and *Triadinium*

by coiling up of the representative of *Ditoxidae*, such as *Ditoxum* or *Tetratoxum* (according to Strelkov's opinion 1939). It would be followed by reduction of a part of the adoral zone and formation of the posterior precessus.

In the paper entitled "*Triadinium minimum* Gassovski — its phylogenetic importance" (in press) I tried to justify my opinion. I conceive the conviction of the origin of *T. caudatum* from *C. minimum* on account of striking ressemblance of the body shape, pattern of the somatic ciliature and arrangement of the special kinetosomes in both these species.

In this case, I suppose the similarity of the body shape cannot be regarded as a result of convergency. Among known ciliates from the intestine of herbivorous mammals the characteristic ciliated processus at the body end occurs only in *O. appendiculata* and in some species of *Triadinium* (within its former range). So it seems more justified to relate *T. caudatum* with *C. minimum* rather then with *Ditoxidae* having no processes.

Special kinetosomes occur in all *Entodiniomorphida*, so they are also in *Ditoxidae* but they differ in the character. They are arranged in irregular kineties. But in the primary arrangement of short, straight kineties, as in *T. caudatum* and *C. minimum*, they occur exactly in *Blepharocorythidae*.

In *C. minimum* the special kinetosomes are in the same position in which they ought to be after bending of the body of the ciliate. In *T. caudatum* they changed their position and lie at the anterior pole. This may be explained as follows.

It is known that the special kinetosomes are connected initially (in *Buetschliidae*) with the vacuole with concretions, and the fibers running out of these kinetosomes take a part in the structure of the wall of the vacuole (Grain 1966). In *Blepharocorythidae* the vacuole with concretions is vestigial (Wolska 1966 b), and in *Entodiniomorphida* it completely disappears, only the kinetosomes with short cilia are present. It is possible that in *Entodiniomorphida* these kinetosomes and cilia are not only a vestigial organ but they perform a new function. Bretschneider 1962 regards that they are a sense organ (indetermined) in *Ophryoscolecidae* and calls them "Paralabialorganellum". If his supposition is right, it becomes quite evident that in *T. caudatum* (the typical representative of *Entodiniomorphida*) the special kinetosomes displaced to the anterior end of the body taking the proper position for sensory organellum.

The contractile vacuole in *C. minimum* lies in the posterior part of the body, in the same position as in *O. appendiculata*, while in *T. caudatum* it is situated near to the macronucleus, in the anterior part of the body. In *Entodiniomorphida* the contractile vacuoles occur always (with a few exceptions) in the neighborhood of the nucleus (Strelkov 1939). It is not yet known what is the importance of the relations of the nucleus and contractile vacuole. It may be only ascertained that in *T. caudatum* the vacuole occupies the usual position in *Entodiniomorphida*. The shape of the macronucleus in *T. caudatum*, differs from that in *C. minimum*. The change of the shape of the macronucleus is evoked probably by its contiguity with the

contractile vacuole. It becomes not regular in shape due to the pressure of the vacuole on it.

In *Entodiniomorphida* the contractile vacuole as well as the nucleus occur in the ectoplast. Thickness of the layer of ectoplast differs in various parts of the cell. In *Ditoxidae* the ectoplast occurs mostly on the right side of the body, in the anterior part (Strelkov 1939), in other parts of the body it is nearly lacking. The relations of layers of cytoplasm in *Triadinium* corresponds with typical *Ditoxidae* according to Strelkov. (I suppose that the data obtained for *T. caudatum* have been generalized and spread over the whole genus because the drawing of the cross section referred only to *T. caudatum*. The sections of *T. minimum* have not yet been made). In *T. caudatum* the ectoplast is concentrated in the anterior part of the body, so that the nuclear apparatus and contractile vacuole could be situated only in this area. Then, the shifting of the nucleus and contractile vacuole from the position occupied by them in. *C. minimum* to that in *T. caudatum* is connected with the changes in the organization of the cytoplasm and attaining the characters of *Entodiniomorphida*. Such feature as the specialization of the layers of cytoplasm might have developed at the same hypothetical evolutionary line from *C. minimum* to *T. caudatum*. Such hypothesis cannot be proved directly but there is also no evidence of more probable origin of *Entodiniomorphida* from other groups.

If the origin of one representative of *Entodiniomorphida* from *Blepharocorythidae* is at least probable, it is worth to consider whether other *Entodiniomorphida* could be derived also from *Blepharocorythidae*. Here another evolutionary line from primitive *Blepharocorythidae* ought to be taken into account because the line *Circodinium — Triadinium* cannot be considered as initial for the whole order. The question is whether these hypothetical changes would result from the general tendency in the development of the ciliature in *Blepharocorythidae*.

The most primitive *Blepharocorythidae* have two ciliary zones at the posterior end of the body. In the majority of *Cycloposthiidae*, if they are derived from *Blepharocorythidae*, the ciliature of the posterioı pole would remain almost the same as in the initial group. Only a diminution of the zones and a formation of cytoplasmic lips around them would occur. In the development leading to formation of *Ophryoscolecidae* the reduction of both posterior zones would occur.

A tendency to the reduction of the posterior zones already occurs within the family *Blepharocorythidae*. In the genus *Blepharocorys*, comprising many species, only one zone occurs. It is possible that in the family *Blepharocorythidae* total reduction of the posterior ciliature occurs also, if we regard that the species *Charonina nuda* described by Hsiung 1932 would be a representative of this family. Unfortunately, the drawing and description of this species are not precise and, moreover, it has not been found any more.

The ability to form the appendices at the posterior end of the body is a common feature in *Blepharocorythidae* and *Ophryoscolecidae*.

It seem that the structure of the posterior end of the body in *Entodiniomorphida*

represents a further step in the development in relation to the structure of this part of the body in *Blepharocorythidae*. Generally speaking, the changes in the ciliature on the posterior end of the body consist in greater of lesser reduction.

The development of the anterior ciliature in *Blepharocorythidae* (beginning from the form nearest to *Buetschliidae*) is characterized by differentiation of strongly developed fronto-buccal zone into the frontal and oral ones, then the differentiation of the oral zone into two parts.

The differentiation of the oral zone follows the disappearance of the median part of the primary buccal zone. It leads to the formation of the oral ciliature of *Blepharocorys*, composed of two parts — the anterior oral zone (short kineties) and the posterior buccal zone (long kineties) (see Fig. 1). Basing on the same tendency we must accept only the disappearance of other parts of ciliature to imagine the transformations leading to the development of the ciliature of *Entodiniomorphida*. In such way only the supposition ought to be made about the sinking of the labial zone to the developing infundibulum and about the disappearance of the frontal lobe of *Blepharocorythidae*. The picture of such transformation is given in a scheme (Fig. 7). As a result of this process, the ciliature of the type *Entodinium* is obtained. After disappearance of a great part of the fronto-oral zone the remaining segment forms

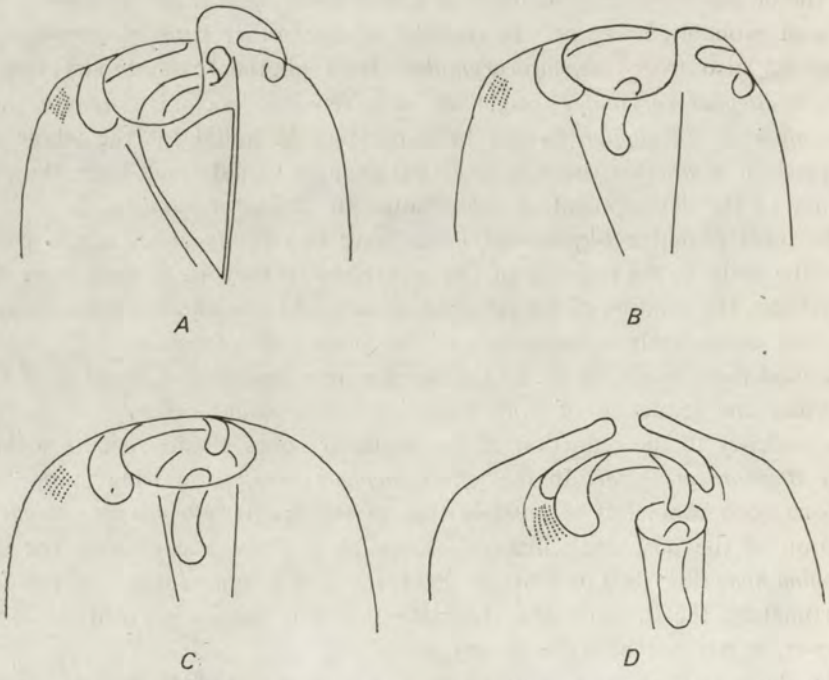

Fig. 7. Scheme of probable changes in the ciliature of a representative of *Blepharocorythidae* (A), leading to the development of the ciliature of the representative of *Ophryoscolecidae* (D). B and C — hypothetical intermediate forms

the dorsal part of the adoral zone in *Entodinium*. Special kinetosomes sink into infundibulum together with the labial zone which becomes the ventral part of the adoral zone.

Here we have to do with the three elements of the adoral zone in *Ophryoscolecidae*, each of them is homologous with a corresponding element of the ciliature of the representative of *Blepharocorythidae* (Part V, Fig. 1):

1. The ventral part of the adoral zone in *Ophryoscolecidae* (*v.p.*) is homologous with the labial zone in *Blepharocorythidae* (*l.z.*)

2. The dorsal part of the adoral zone in *Ophryoscolecidae* (*d.p.*) is homologous with the anterior oral zone in *Blepharocorythidae* (*a.b.z.*).

3. The free cilia in *Ophryoscolecidae* (*f.c.*) are homologous with the special kinetosome in *Blepharocorythidae* (*sp.k.*).

If the segment of the fronto-oral zone, remaining on the surface of the body (Fig. 7B), did not disappear, it would become the additional dorsal zone in *Ophryoscolecidae*, called DZM. According to the degree of development of this segment and the distance to which it would be displaced from the anterior pole of the body, the genera *Diplodinium*, *Epidinium* or *Ophryoscolex* might have evolved. This is the segment of the fronto-oral zone which constituted the frontal zone in *Blepharocorythidae* (Fig. 1C). Then, the so-called dorsal zone in *Ophryoscolecidae* (DZM) is homologous with the frontal zone in *Blepharocorythidae* (*f.z.*).

In similar manner we may imagine the development of the adoral ciliature in *Cycloposthidae*, however the dorsal part (the ribbon of short kineties) in this family has another shape and its course may be different from that in *Ophryoscolecidae*. The kinetosomes in particular kineties of this part are densely arranged. It is known also, on account of the observations on morphogenesis in *C. edentatum* (Wolska 1965), that the ribbon of short kineties develops from two primordia which approach later but do not unit completely. This discontinuity may be seen always in silver impregnated preparations of *Cycloposthium*. It may be seen also in a species of the genus *Triplumaria* (Pl. I 3). The discontinuity of the ribbon of short kineties may be explained by an increased tendency in course evolution to disintegration of the fronto-oral zone from which this ribbon develops.

In such way the evolutionary line of *Entodiniomorphida* might have branched from primitive *Blepharocorythidae* of the type of structure of *Raabena* and *Pararaabena*.

The evolution of *Blepharocorythidae* is characterized by the development of the buccal ciliature with dominating long kineties. When one of the parts of the oral ciliature disappears, it is the zone of short kineties (*Ochoterenaia*? *Circodinium*).

In contrast the evolution of *Entodiniomorphida* is characterized by the disappearance of the long kineties and the development of the zone of short kineties, they become the dorsal arm of the adoral zone of syncilia. In the species *Triadinium caudatum* both ciliary zones disappear. In consequence the dorsal part of the adoral zone is lacking. Total reduction of the dorsal arm may be realized at the same

evolutionary line, but it may be reached in another way as it was considered at the beginning of this chapter when the origin of *T. caudatum* was discussed. Another way of evolution of this species cannot be excluded.

The origin of the additional anterior somatic zone in *Cycloposthiidae* (anterior dorsal bundle or tuft in *Triplumaria*, *Tricaudalia* and other genera) may be explained by the same manner as the origin of the dorsal zones in *Ophryoscolecidae*. The additional anterior dorsal bundle in *Triplumaria* would be homologous with the frontal zone of *Blepharocorythidae*.

This hypothesis of the origin of *Entodiniomorphida* explains better the formation of the additional zones situated always at the dorsal side.

Certainly, it is possible that two segment are separated successively from the fronto-buccal zone, not only one. The first of them might have been displaced far backward. This would be the way of formation of the somatic zones pattern in *Prototapirella*.

The hypothesis about the origin of the dorsal ciliary zones in *Entodiniomorphida* by their separation from the adoral zone was at first expressed by Dogiel 1927, 1951, then it was stressed by Corliss 1956.

Consideration of the origin of additional zones in *Entodiniomorphida*, presented in this paper, does not contradict Dogiel's statement. It shows only that the splitting of the zones occurred in early stages of evolution of this group and it shows the course of splitting.

In such a way Dogiel's principle of polymerization of organellae, in this case the polymerization of the ciliary zones, could be realized.

Final remarks

Summarizing the above observations and discussion, the author wants to underline two points:

1. It is suggested that *Blepharocorythidae* originate from *Gymnostomata*.
2. Against the common opinion about the relationship between *Entodiniomorphida* and *Spirotricha*, a hypothesis is given about the origin of *Entodiniomorphida* directly from *Gymnostomata*, or indirectly, through *Blepharocorythidae*.

These suppositions result from the analysis and comparison of the ciliature and morphogenesis of the groups under discussion.

The initial form for such evolutionary line or lines would be the representative of *Buetschliidae* with the *Didesmis* type of structure.

Grain 1966 designated several evolutionary lines for *Gymnostomata*, leading to various genera and families and issuing from the primitive forms with toxycysts, mucoid trichocysts and nemadesms. In particular lines the development of one of these elements prevails; sometimes there are two or even three of these elements simultaneously. *Buetschliidae* are on the line of development characterized by the

presence of nemadesms only. Within the family *Buetschliidae* there are two directions of development according to Grain. It is the genus *Didesmis* which belongs to the different line of development than other genera of this family. This underlines the particular character and special position of this genus regarded in the present paper as the initial form for other groups.

The genus *Blepharocorys* differs from *Trichostomata* by some features of ultrastructure, so it is considered separately by Grain. The results obtained by this author seem to show some similarities in the ultrastructure of *Blepharocorys* and *Entodiniomorphida* (e.g. the structure of cytoplasm and kinetosomes). These data, at least, are not against author's hypothesis.

The evolutionary line of *Didesmis*, drawn out by Grain, would prolongate according to my hypothesis to *Blepharocorythidae*, branching at various levels and leading finally to *Entodiniomorphida*. At present it is impossible to choose any of the discussed possibilities of the origin of *Entodiniomorphida*. This question needs further studies.

The probable ways leading from *Gymnostomata* to *Entodiniomorphida* are given in Fig. 8. The lines run out from the form of the *Didesmis* type of structure to the

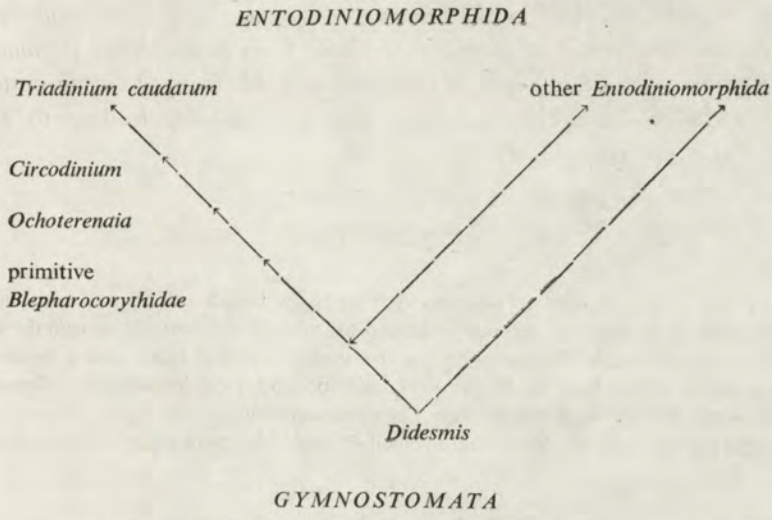

Fig. 8. Probable evolutionary lines leading from *Gymnostomata* to *Entodiniomorphida*

level of *Entodiniomorphida*. The first line runs in agreement with the evolutionary line established for *Blepharocorythidae*, through the genus *Ochoterenaia* to *Circodinium* and then to *Triadinium*. The other two lines have no corresponding contemporary genera on their course.

It would be worth to think about the systematic position of *Blepharocorythidae* and *Entodiniomorphida*. In the most primitive genus of the family *Blepharocorythidae*

the buccal concavity similar to vestibulum occurs, but in other genera the buccal ciliature is individualized and the morphonenesis in all the genera is different from that in *Trichostomata* and even in *Holotricha*. This would be a cause for separation of *Blepharocorythidae* from *Trichostomata* and *Holotricha*. Also *Entodiniomorphida* ought not to be any longer in *Spirotricha* because they do not show the features of this class in their ciliature. These two groups do not fall within the ranges of the present classes but their relationships are obvious.

To this opinion, which I have expressed for the first time in the year 1966, seems to adjoin Noirot-Timothée 1969. If it is right, a new class *Entodiniomorpha* ought to be establish, including *Blepharocorythidae*, raised up to the range of the order *Blepharocorythida*, and *Entodiniomorphida*. But further studies are needed to resolve finally this problem.

Summary

In the present paper the up to date results of the author's studies on the family *Blepharocorythidae* are summarized and the diagnosis of the new genus *Circodinium*, included in this family, is given.

It is the author's opinion that *Blepharocorythidae* and *Entodiniomorphida* are closely related. The origin of *Blepharocorythidae* from *Buetschliidae* (*Gymnostomata*) is discussed as well as the origin of *Entodiniomorphida* from the same initial group. Possible ways of the evolution within *Blepharocorythidae* leading to *Entodiniomorphida*, are also considered.

STRESZCZENIE

Praca jest podsumowaniem dotychczasowych wyników badań autorki nad rodziną *Blepharocorythidae*, zawiera też diagnozę nowoutworzonego rodzaju *Circodinium* włączonego do tej rodziny.

Autorka jest zdania, że *Blepharocorythidae* i *Entodiniomorphida* łączą bliskie związki rodowe. Autorka stara się udowodnić, że *Blepharocorythidae* pochodzą od *Buetschliidae* (*Gymnostomata*), z tego samego źródła wyprowadza też *Entodiniomorphida*.

W pracy są przedstawione różne drogi ewolucji *Buetschliidae*, prowadzące do *Entodiniomorphida*.

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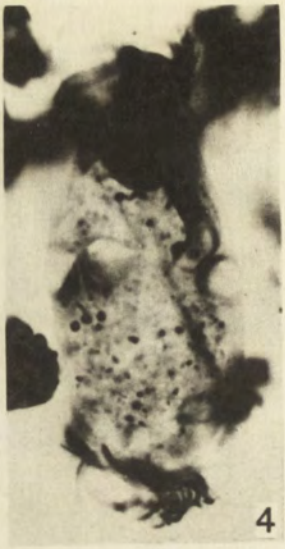
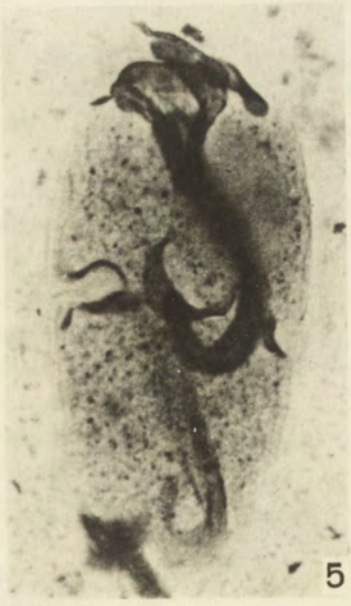
EXPLANTATION OF PLATES I–II

1: The adoral zone of a representative of *Ophryoscolecidae*, free cilia are visible
2: The adoral zone of a representative of *Cycloposthiidae* (*C. edentatum*), free cilia are visible
3: The adoral zone of a representative of *Cycloposthiidae* (*Triplumaria* sp.)
4: *Blepharocorys cardionucleata*, division. The division vacuoles are visible
5: *B. curvigula*, division
6: *B. jubata*, division
7: *Cicodinium minimum* comb. nov., long oral kineties are visible
8: *C. minimum*, general sight
9: *Triadinium caudatum*, general sight





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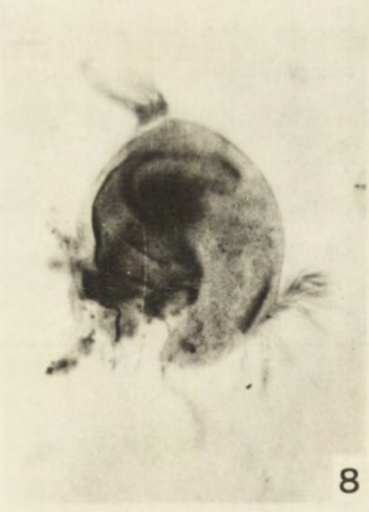

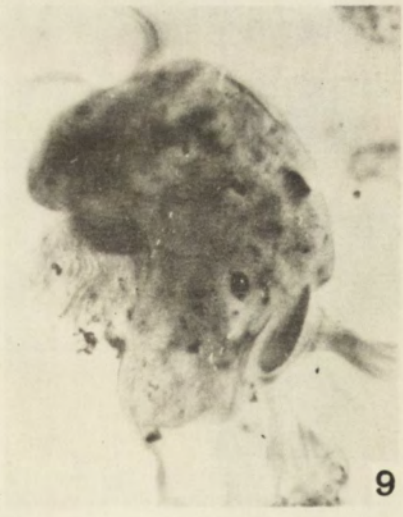

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ACTA PROTOZOOLOGICA

VOL. IX WARSZAWA, 15. XII. 1971 FASC. 11

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Ultrastructure of *Ancistrumina nucellae* Khan, an arhynchodine thigmotrichid ciliate

Ultrastructure de *Ancistrumina nucellae* Khan, un Ciliate arhynchodine thigmotrichide

This commensal ciliate from the mantle cavity of *Nucella lapillus*, the common dog whelk, belongs to the hemispeirid group of thigmotrichid ciliates. These are characterised by having a well developed posterior buccal apparatus which is joined by a shallow adoral groove coming from the anterior end and running along the ventral surface of the body. There are 18 somatic kineties in the general ciliature plus two adoral kineties one of which is a polykinety and is composed of two closely set ciliary rows (Khan 1970). The other is a stomatogenic kinety (=undulating membrane) (Lom et al. 1968) which is a haplokinety (p. 197) and runs posteriorly along the adoral groove, curves round the peristome and enters the buccal area. The thigmotactic field is formed in the anterior-ventral region of the body where 11–13 kineties are set closely in a narrowed anterior end to give rise to short, closely set cilia. The cytoplasm contains a large number of food vacuoles and there is also a contractile vacuole in the posterior region of the body. The macronucleus varies greatly in shape and is situated with a micronucleus in the anterior half of the body (Khan 1970) (Text-fig. 1).

The *Thigmotrichida* form a somewhat heterogenous group with commensal semiparasitic and parasitic representatives, but very little is known about their ultrastructure as Pitelka 1963 remarked. Recently Lom et al. 1968 made the first comparative studies on the buccal apparatus of *Ancistrum* and *Boveria* (Family: *Hemispeiridae*), in which they recognised certain homologies in the adoral ciliature with peritrichs and hymenostomes. The fine structure of *Ancistrocoma* has been studied by Khan 1969 as a representative of the sub-order *Rhynchodina*, and the present study, on one of the *Arhynchodina*, offers some interesting points for comparison. The genus *Ancistrumina* is related to *Ancistrum*, which was created by Raabe 1959.

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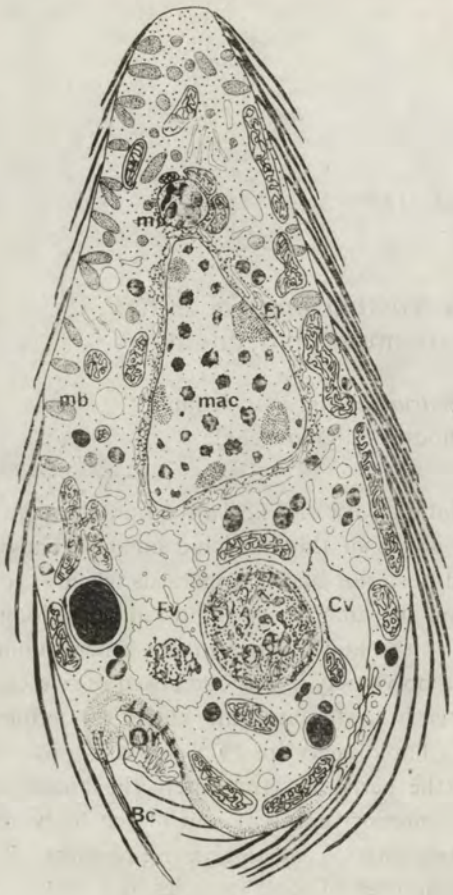


Text-fig. 1. Diagrammatical longitudinal section of *Ancistrumina nucellae* showing the anteriorly situated micronucleus (Mic) and the macronucleus (Mac) around which the endoplasmic reticulum (Er) forms cisternae. The mucigenic bodies (Mb) are abundant in the anterior thigmotactic region. The posterior region of the body contains a contractile vacuole (Cv) and a variety of food vacuoles (Fv). A number of smaller vesicles are also seen in the cytoplasm. The peristomial zone (Bc) forms a cleft posteriorly and there are well developed oral ribs (Or) in the bucal cavity

Materials and methods

The ciliates were collected from the host by washing the mantle cavity with sea water into a petri dish, and were picked up by a fine pipette under a binocular microscope. Concentrated drops of ciliates were dropped in 3% glutaraldehyde buffered to pH 7.2 with sodium cacodylate and 0.25 M sucrose (Sabatini et al. 1963). Fixation was carried out for 2 hours, followed by post-fixation in 2% osmium tetroxide for 2 hours, washing dehydrating and embedding in Epon 812 (Kay 1961, Luft 1961 and Richardson et al. 1960). Sections were cut on a Huxley Ultramicrotome using glass knives, and thin sections of grey and silver interference colour were picked up on 200 mesh grids and double-stained with 2% uranyl acetate for 15 minutes and with lead citrate for 2.5 minutes (Reynolds 1963). They were examined with either an Akashi Tronscope TRS-50 at 50 kv. or an AEI EM-6G operated at 75 kv. Thick plastic sections were cut in a similar fashion and stained with Azur II and methylene blue (Grimley et al. 1964) for light microscope examination. The electron micrographs were taken at original magnifications of from ×5000 to ×40 000.

Observations

Pellicle

The body is covered by a pellicle of double adielectronic layers which lies just outside a third membrane, the plasma membrane (Figs. 3 and 7). The latter is somewhat thicker than either of the pellicular layers, because there is a dense material attached to its inner surface. The second layer is folded inside periodically and joins with the third inner membrane thus forming bridges in the space between them (Fig. 7). Anteriorly, particularly in the antero-ventral thigmotactic area, the trilaminar body wall is thrown into sharp longitudinal folds, appearing in section as narrow elongated projections (Figs. 3 and 9). The cilia of the thigmotactic region and of the general ciliature emerge from deep furrows in between these folds. Posteriorly the height of the folds is gradually reduced, until they appear in section as small protuberances (Fig. 11) or disappear completely. In this region the pellicle seems to be less wavy and stretched more evenly over the surface of the body where the pellicle lines one of the lips of the peristome, the dielectronic gap between it and the plasma membrane widens and contains, in places, an electron-opaque substance and occasional lamellar structures. The pellicle continues inside the gullet and forms elongated projecting folds somewhat resembling those termed oral ribs by Lom et al. 1968 (Fig. 11).

Cilia

The cilia of the thigmotactic region are 6–8 μ long, whereas those of the adoral zone reach 15–20 μ but the fine structure of all the cilia is the same (Sleigh 1962). Each comprises nine doublet peripheral fibrils and two central fibrils, and is covered externally by the outer pellicular layer. The two central fibrils terminate proximally in a dense granule. A little deeper, two more partitions may be seen in a longitudinal section (Figs. 3 and 8). The distal partition is cup-shaped and the proximal one is a straight horizontal disc. These deeper partitions seem to demarcate the free cilium from its kinetosome. Like their cilia, the kinetosomes of different regions are uniform in structure. In a transverse section the peripheral triplets are arranged somewhat obliquely and fibres could be distinguished running from the triplets to the centre of the kinetosome to form the typical "cartwheel" structure. The central space of the kinetosome contains 6–8 large granules arranged in a zig-zag way longitudinally (Fig. 8).

Kineties

The stomatogenic kinety (=undulating membrane, UM) appears surprisingly similar in ultrastructure to kineties in the buccal apparatus of peritrichs (Eatmen et al. 1966, Faure-Fremiet 1962, Lom 1962, Lom and Corliss 1966, Lom 1968 and Rosenberg and Grim 1966), consisting of two rows of kinetosomes arranged in a zig-zag manner and forming a haplokinety, which is of typical

construction in that only one of each pair of constituent kinetosomes gives rise to a cilium whilst the other remains "barren". Each of the remaining kineties of the general ciliature consists of a single row of cilia-bearing kinetosomes. No peniculi have been observed in the buccal region.

Associated with each kinetosome of the somatic kineties is a group of 10–12 microtubules arranged in a row, arising from the left side, running towards the surface and coming to lie under the pellicle as the sub-pellicular microtubules (Figs. 4, 6 and 7). A similar set of microtubules in *Tetrahymena* have been referred as postciliary microtubules by Allen 1967, 1969. These form a layer embedded in the inside dense area of the pellicular membrane. No transverse microtubules or post-ciliary fibres have been noticed, however, although these are said to occur in *Ancistrum* (Lom et al. 1968). There are no kinetodesmal fibres either, but dense fibrous patches link pairs of neighbouring kinetosomes. Similar dense fibres can be seen joining the paired kinetosomes of the UM and the polykinety of the adoral zone (Figs. 4 and 5). The kinetosomes of each polykinety seem to give off a number of fine fibres basally, which run longitudinally in the walls of the adoral zone and continue into the walls of the buccal cavity. A band of fine fibres is also seen at the base of the oral ribs and a dense patch is seen on this between each pair of adjacent ribs.

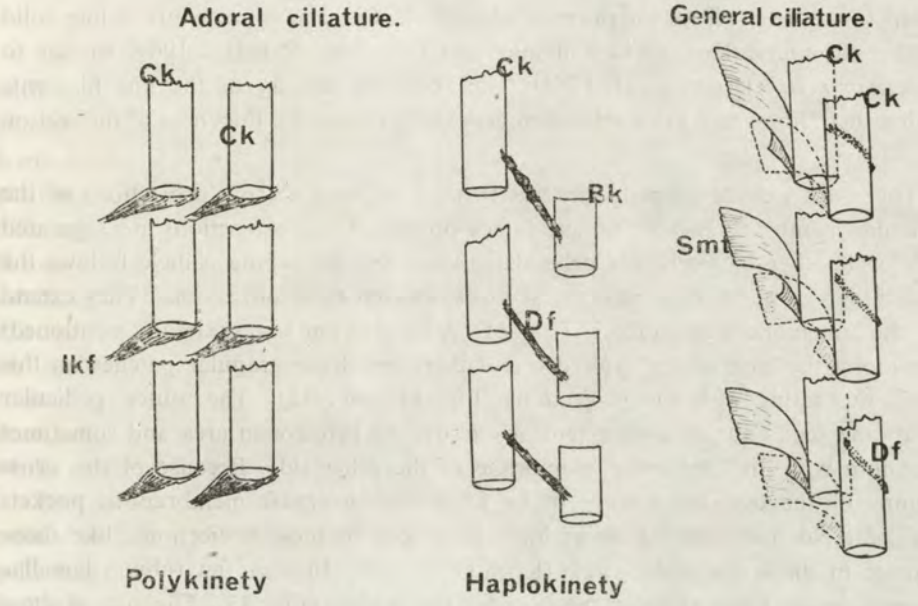
The kinetosomes of the UM do not give rise to any fibrillar or microtubular elements in their length, except in the buccal area, where fibrillar bundles are given off from the base of each kinetosome. These fibrils seem to join those of the adjacent polykinety (Text-fig. 2 and Figs. 5 and 11).

Parasomal sacs lie anterior to each kinetosome. Anteriorly, in the thigmotactic zone, tubular structures are seen close to the parasomal sacs. They seem to extend into the cytoplasm as small sinuous tubes. Sometimes they occur in large numbers in the cytoplasm underlying the thigmotactic field and form stacks of lamellar tubular structures with dielectronic spaces inside (Figs. 3 and 9). These elongated tubular structures are also seen posteriorly at the bases of the kinetosomes of the UM.

Cytoplasm

Perhaps the most prominent subpellicular structures, apart from the mitochondria, are capsule-shaped, electron-dense mucigenic bodies, lying in groups of 2 or 3 between adjacent kinetosomes (Figs. 3 and 8). They are found in large numbers, particularly in the anterior thigmotactic field, though very few of them are seen in the posterior part of the body. They do not possess a surrounding membrane, though they show an electron-dense thin cortex around them at high magnifications. They are thus similar to the mucigenic bodies of *Ancistrocoma* (Khan 1969), but differ from the similar sub-pellicular bodies (=mucocysts) of other holotrichs (de Puytorac 1964, Tokoyasu and Scherbaum 1965, Zebrun et al. 1967). They contain a uniformly fine granular material, but none has been seen ejecting its contents (Figs. 6 and 7).

Mitochondria also appear abundantly in the thigmotactic region and form a regular arrangement of longitudinal rows under the pellicle, parallel to the kineties (Fig. 4). They seem to be highly active and labile. Their cristae are of the familiar microtubular kind, which sometimes fully pack the space available or at other times are less numerous, leaving empty spaces. They are bounded by the usual



Text-fig. 2. A diagram showing the three types of kineties occurring in *Ancistrumina*, each having different fibrillar and microtubular systems associated with it. Bk, barren kinetosomes, Ck, ciliated kinetosomes, Df, dense fibres joining the adjacent kinetosomes of the adoral kineties, Ikf, infra-kinetosomal fibres arising from the bases of the kinetosomes of a polykinety, Smt, sub-pellicular microtubules arising from one side of the kinetosomes of the general ciliature

double membranes. Quite often they are seen surrounding the micronucleus on one side, or lying close to the contractile vacuoles (Figs. 8 and 17).

Buccal apparatus

A complex feeding apparatus is present in many bacteriophagous free and commensal ciliates. Its ultrastructure has been studied in detail in many holotrichs, including *Hymenostomatida*, *Gymnostomatida* and *Apostomatida*, and also in some peritrichs and hypotrichs (Bradbury 1965, Daniel and Mattern 1965, Eatman et al. 1966, Fauré-Frémiet et al. 1962, Jurand 1961, Kennedy 1965, King et al. 1961, Miller and Stone 1963, Paulin 1967, Rosenberg and Grim 1966). The buccal apparatus of *A. nucellae* shows some structures which seem to be homologous with those of peritrichs, at the same time also some features which may be characteristic of the arhynchodine thigmotrichs. The shallow adoral groove and its

ciliature enter the peristomial zone, curving round the posteroventral margin of the body, but the only kineties which enter the cytostomal area are the haplo-and the poly-kineties (Fig. 11). These kineties give rise to the bundles of fibrils already mentioned which run into the walls of the cytostome (Fig. 11). Electron micrographs of the cytostome, sectioned transversely and obliquely, show the kinetosomes of the UM and polykinety, to be surrounded by these fibrils (Fig. 14), the structure of which is different from the microtubular fibrils, for each is made up of fine solid fibrillar sub-units. They form a filamentous reticulum (Pitelka 1969) similar to *Ancistrum mytili* (Lom et al. 1968). The apparent scarcity of the fine filaments joining these fibres to form a reticulum is possibly due to the thickness of the section (Fig. 14).

There are well developed oral ribs formed by long narrow projections of the pellicular membrane and the underlying cytoplasm. These projections are separated from each other by fairly flat pellicular alveoli and the whole pellicle follows the underlying cytoplasm deep into the grooves between these projections. They extend into the lumen of the gullet freely. (Fig. 11). A band of fine fibres (already mentioned) runs along the base of the oral ribs, and there are dense granular patches on this band, alternating with the projections (Fig. 11 and 12). The outer pellicular membrane sends out sinuous extensions across the cytostomal area and sometimes these join with the pellicular membrane of the other side. Because of this cross sections of the cytostome seem to be filled with irregular membranous pockets (Fig. 12). No microtubular fibres have been seen in these projections, like those reported in *Ancistrum* and *Boveria* (Lom et al. 1968). Instead, few tubular lamellae are seen in the large alveolar space under the pellicle (Fig. 13). The size of these tubular structures does not suggest that they are microtubular. Each projection is filled by cytoplasm except apically, where there is a small pocket apparently formed as an invagination from the lumen of the buccal cavity.

The passage into the cytopharynx is narrowed at one point and the walls there seem to form a simple narrow canal in which there seems to be phagocytic activity (Figs. 15 and 16). There is a sheet of microtubular fibrils on one side of the wall of the cytostome, like those described in *Ancistrum* (Lom et al. 1968).

A contractile vacuole has also been seen and its features are similar to the one described in *Ancistrocoma* (Khan 1969) and other ciliates (Kitching 1967). The structure of the nuclei seems to be more conventional except the macronucleus varying greatly in its shape (Khan 1970). Rough endoplasmic reticulum is also seen active around it and the smooth ER frequently forms cisternae close to the nuclear membrane (Figs. 19 and 20). The micronucleus shows an irregular aggregation of dense granular material, with the space around it sparsely filled by a fibro-granular matrix (Fig. 17). The fibres of the matrix are sometimes seen attached to the inner nuclear membrane. In the dense granular material no distinction can be made between chromatin bodies and the nucleoli. The methyl green pyronin test shows

that the micronucleus is mostly filled with masses of DNA. There is always a zone of ribosomal aggregation around the micronucleus, but no cisternae of the ER are associated with it, (Fig. 17). A layer of mitochondria seems to surround the ribosomal zone around the micronucleus (Fig. 17).

Discussion

The ultrastructure of the oral apparatus in *Ancistrumina* seems to be homologous with that of peritrichs, which also have haplo-and poly kineties forming the adoral ciliature (Bradbury 1965, Eatmen et al. 1966, Faure-Fremiet et al. 1962, Lom 1962, Lom and Corliss 1966, Lom 1968). The idea of fairly close thigmotrich-peritrich affinities, as put forward by Lom et al. 1968, is thus supported. The differences in the fine structure of the buccal apparatus from that of *Ancistrum* (Lom et al. 1968) do not support the conclusions of Fenchel 1965 and fairly establish Raabe's views (Raabe 1959) on erecting this genus. In other respects this hemispeirid (sub-order *Arhynchodina*) recalls earlier studies (Khan 1969) on *Ancistrocoma*, although that is a member of the *Ancistrocomidae* (sub-order *Rhynchodina*) and very different in habitat, habits and organisation. *Ancistrumina* thus gives beautiful example of a morphological link with a different order of ciliates, whilst showing that certain characteristics are widespread within its own order.

The way the pellicular bridges are formed between the two outer and the inner pellicular membrane seems to be unique, for they are infoldings of the second pellicular membrane and thus completely different from the dense bridges described in *Tetrahymena* (Tokoyasu and Scherbaum 1965).

The thigmotactic field of *Ancistrumina* is surprisingly like that of *Ancistrum* (Lom et al. 1968) and *Ancistrocoma* (Khan 1969), in that the short thigmotactic cilia lie deep in the troughs of the characteristically folded pellicle. *Ancistrumina* however, lacks the elaborate anchoring system of microtubules seen in *Ancistrocoma* (Khan 1969), which may perhaps be associated with the fact that the pellicular folds of *Ancistrumina* are rather more numerous and relatively smaller, so may be less in need of support. The thigmotaxis of *Ancistrumina* is very efficient, perhaps because of the enormous increase in surface area caused by the folds of the pellicle and also because of the secretions of the large number of mucigenic bodies situated just under the pellicle in the thigmotactic field. There is no difference in structure between the cilia of the thigmotactic field and those of the rest of the body, suggesting again that the phenomenon of thigmotaxis is, possibly, nothing to do with the cilia themselves, contrary to the opinion of Chatton et Lwoff, and of other protozoologists who followed them (Chatton et Lwoff 1949, 1950, Raabe 1959). The prime specialisation of the thigmotactic field seems to be for production and secretion of mucoid substances in great quantities, which was also suggested by Beers 1962. These mucoid secretions presumably help the ciliate to adhere to the host surfaces. The underlying cytoplasm contains great number of ribosomes,

vesicles and mucigenic bodies. The stacks of tubular structures seen in the thigmotactic field may be golgi like sacs giving rise to the vesicles found in the cytoplasm. The exact function of these stacks of elongated tubes is yet to be investigated after which a more probable interpretation of these structures is possible.

The adoral kineties were studied in the thigmotrichs *Ancistrum* and *Boveria* by Lom et al. In *Ancistrumina* the UM is similar to those of *Ancistrum* and *Boveria* but the fibrous attachment which is seen in *Ancistrum* (Lom et al. 1968), joining the pairs of kinetosomes of UM, to the pairs of the polykinety, is lacking in *Ancistrumina*. Its polykinety is different from that of *Ancistrum* in having two kineties only and is thus surprisingly similar to the "atypical" polykinety of *Boveria* (Lom et al. 1968). The polykineties of peritrichs comprise more than three kineties, whilst *Ancistrum* has three (Lom et al. 1968). Those with three or more rows have been termed typical polykineties. In *Boveria* and in *Ancistrumina* the polykineties might be called diplokineties, but they are here called polykineties since they seem to be homologous with those of peritrichs. The similarity between *Ancistrumina* and *Boveria* links these two genera and at the same time separates them from the genus *Ancistrum* (see also discussion on the oral ribs below). This helps to establish *Ancistrumina* as a genus separate from *Ancistrum*, supporting Raabe 1959. In view of this, all species described by Fenchel 1965 need careful re-examination, to see whether their polykineties can be categorised as atypical or typical. If this character is linked with the possession of less than 33 kineties on the one hand or more than 33 on the other, then Raabe's conclusion that the genus *Ancistrumina* should be separated from *Ancistrum* is quite justified.

The fibrillar network in the walls of the buccal cavity and the cytostome may perhaps function in co-ordinating feeding activities, rather than being just supporting elements in the walls of the cytostome. Their association with the adoral ciliature and the oral ribs joining all these organelles to one another supports the idea of their being communication lines, which may co-ordinate the beat of the adoral ciliature, movement of the oral ribs and the opening and closing of the passage between the cytostome and the cytopharynx for ingestion of food.

The ultrastructure of the mucigenic bodies confirms that in the *Thigmotrichida* these bodies characteristically lack the surrounding membrane which the mucigenic bodies of other ciliates generally possess. The contents of these bodies seem to be slightly different from those of *Ancistrocoma* as they do not show any crystalline contents.

The buccal apparatus shows some features which are similar to those in *Ancistrum* (Lom et al. 1968), especially the structure of the cytostome, but the oral ribs with their associated fibrillar band differ markedly from the comparatively simple ribs of *Ancistrum* and even from the better developed ribs of *Boveria*. The important feature of the oral ribs of *Ancistrumina* is that the pellicle itself forms narrow, elongated projections into the gullet, whereas the oral ribs in both *Ancistrum* and *Boveria* do not seem to involve the pellicular layer, apart from pushing them

out slightly to form inconspicuous ridges. Instead, they are formed by projections of the inner plasma membrane which touches the pellicle only along the ridges and are separated by sub-pellicular alveoli. These sub-pellicular ribs may perhaps be only for strengthening the wall of the buccal cavity. The projecting ribs of *Ancistrumina*, however, may play a more complicated role as a feeding apparatus. They may perhaps gather the food particles and strain the contents of the buccal cavity allowing only small particles to enter the cytostome. These differences between *Ancistrumina* and the other two genera provide another character to support Raabe's view that the genus *Ancistrumina* should be distinguished from *Ancistrum*.

The ribosomal particles which aggregate and coat the nuclear membrane of the macronucleus may be parts of the nucleoli passed out through the pores of the membrane. The ER which forms cisternae close to the nuclear membrane, may provide a surface area to form the rough ER. Occasionally there is an extension from the anterior end of the macronucleus (Khan 1970). This variation in the shape suggests that at certain times the macronucleus may enlarge, with increased synthesis of its contents, and can be reduced in size when this activity is reduced.

Acknowledgements

Grateful thanks are due to Professor E. W. Knight-Jones under whose supervision and guidance this work was carried out. He has also helped in the preparation of the manuscript. This work is part of the thesis, submitted for the degree of Ph. D., to the University College of Swansea, University of Wales.

Summary

The ultrastructure of the thigmotactic region, the kineties, buccal apparatus and the nuclei of *Ancistrumina nucellae* Khan have been described in detail. Thigmotaxis appears to involve secretion from abundant mucigenic bodies, whilst the pellicular folds on the thigmotactic region are particularly long.

One microtubular sheet arises from the side of each kinetosome of the somatic kineties and runs with others longitudinally under the pellicle to form a layer of subpellicular microtubules. The haplokinety (=stomatogenic kinety) consists of a double zig-zag row of kinetosomes, of which each alternate kinetosome is "barren". The kinety which flanks one side of the haplokinety, comprises a double row of cilia-bearing kinetosomes. The kinetosomes of this polykinety give rise to bundles of infrakinetosomal fibrils, which run posteriorly in the walls of the adoral groove and enter the cytostome, spreading around it to form the filamentous reticulum and joining the kinetosomes of the haplokinety.

The buccal apparatus is peculiar in having a structure somewhat similar to but different from oral ribs. It is formed of elongated projections from the pellicle which lines the gullet. The bases of these are supplied with bundles of fine fibres.

The systematic relations of the genus *Ancistrumina* Raabe, 1969 are reassessed.

RÉSUMÉ

On a décrit en détail l'ultrastructure de la région thigmotactique, les kinètes, l'appareil buccal de *Ancistrumina nucellae* Khan et les noyaux. Thigmotaxie semble consister en la secretion des corps mucigéniques qu'ou trouve en abondance, pendant que les plis pelliculaires dans la région thigmotactique sont particulièrement longs.

Une couche microtubulaire se forme du côté de chaque kinetosome des kinètes somatiques et est située avec les autres d'une façon longitudinale sous la pellicule en formant ainsi une couche des microtubules subpelliculaires. La haplokinète (kinète stomatogène) se compose d'un double rang des kinetosomes en zig-zag chaque alternante kinetosome duquel est "stérile". La kinète située d'une côté de la haplokinète se compose d'une double rang des kinetosomes ciliées. Les kinetosomes de cette polykinète forment des faisceaux des fibriles infrakinetosomales situés des côtés postérieures des parois de la rainure adorale et entrent dans le cytostome en s'étendant autour de lui et formant le réticule composé des filaments et rejoignant les kinetosomes de la haplokinète.

L'appareil buccal est tout à fait particulier en ce qu'il possède une structure semblable à un certain degré, à celle des côtés orales mais différente. Elle est composée des projections alongeés de la pellicule qui double l'oesophage. Les bases de celles-ci possèdent des faisceaux des fibres fins. On a réevalué les relations systematiques du genus *Ancistrumina* Raabe, 1959.

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EXPLANATION OF PLATES I–V

Ultrastructure of *Ancistrumina nucellae* Khan.

Fig. 3. Slightly oblique section of the anterior tip showing the pellicular folds (Pf) in the thigmotactic region. The kineties (K) are situated in between these folds. The cilia (C) are cut transversely to show the peripheral and central fibrils in the ciliary and the kinetosomal regions. The small arrows show the two partitions at the distal end of the kinetosomes. Mucigenic bodies (mb) and parasomal sacs (Ps) lie adjacent to the kinetosomes. The pellicle comprises of two outer membranes, always seen wavy and somewhat loose (large arrow), and an inner, well stretched, dense membrane (arrow head). ×40 000

Fig. 4. A kinety and a parallel row of mitochondria (M) are seen in this longitudinal section. At the base, the kinetosome shows converging dense fibres from the triplets. The fibrous patches (large arrows) are seen joining the kinetosomes in pairs. The double arrows show the dense granules inside the kinetosomes. The subpellicular microtubules (arrow heads) arise from the side of the kinetosomes and run longitudinally to lie immediately under the inner pellicular membrane; mb, mucigenic bodies. ×48 000

Fig. 5. The kinetosomes of the undulating membrane (Um) show a zig-zag arrangement, in which the ciliated kinetosomes (CK) alternate with the barren kinetosomes (Bk). Infrakinetosomal fibres (Ikf) are seen, arising at the bases of a neighbouring polykinety (Pk) and joining together to form a bundle of fibres which runs posteriorly. There are dense fibres (e.g. large arrow) connecting adjacentki netosomes along the length of the UM and the polykinety; M, mitochondria. × 24 000

Figs. 6 and 7. High magnification electron micrographs of mucigenic bodies (Mb) show that they contain a dense, uniformly fine granular contents. Large arrow shows the dense peripheral core appearing as a membrane surrounding the body. Double-membraned bridges (small arrows) are seen crossing the space in between the two outer and the inner pellicular memebranes. The subpellicular microtubules (arrow heads) lie immediately under the denser inner pellicular memebrane. × 100 000

Fig. 8. A longitudinal section of a cilium showing the circular granule (large arrow) at which the central fibrils end. There are two more partitions (small arrows) underneath it. The mitochondria (M) and the mucigenic bodies (Mb) lie on the sides of the kinetosome. × 40 000

Fig. 9. A transverse section of the anterior region showing the pellicular folds (Pf) in the thigmotactic region (Thf). Bunches of thick-membraned canals (Cl), which lie under the bases of each kinety and seem to be extensions of the parasomal sacs, are a prominent feature of the thigmotactic field. × 28 000

Fig. 10. The outer pellicular memebranes (large arrow) surround a large space which is otherwise bounded by the inner membrane (arrow head) and filled with a finely granular liquid and occasional lamellar inclusions. Immediately inside the inner pellicular memebrane a dense band of infrakinetosomal fibres (Ifk) runs into one of the lips of the peristomial cleft. × 48 000

Fig. 11. When cut obliquely, the buccal apparatus shows well developed oral ribs (Or), formed by a row of finger-like projections of the pellicle lining the buccal cavity. There are large sub-pellicular alveoli (arrow heads) in the sides of each projection and deep furrows (small arrows) in between. The polykinety (Pk) enters the buccal cavity and turns an acute angel to come to lie on the side opposite to the oral ribs, where it gives off a thick network of infrakinetosomal fibres (Ifk). These fibres run through the edge of the wall of the cavity on one side and also join the fibres arising basally from the kinetosomes of the UM (double arrow). Stacks of thick-membraned channels (large arrow) are seen on the sides of the kinetosomes of both UM and polykinety × 24 000

Fig. 12. Deep inside the buccal cavity, the oral ribs give off sinuous pellicular extensions from their apices (arrow beads). The bases of the oral ribs (Or) are provided with a band of fine fibrils. The arrows show dense and clear patches on this band of fibrils. × 48 000

Fig. 13. Part of the oral ribs magnified to show the deep furrows in between two projections (small arrows). Membrane-bound spaces at the apices of the cytoplasmic projections (arrow heads) are perhaps formed by invagination of the outer pellicular memebrane. A few of the subpellicular alveoli contain tubular structures (large arrow). × 95 000

Fig. 14. The network of filamentous reticulum magnified to show that these fibres are solid. × 60 000

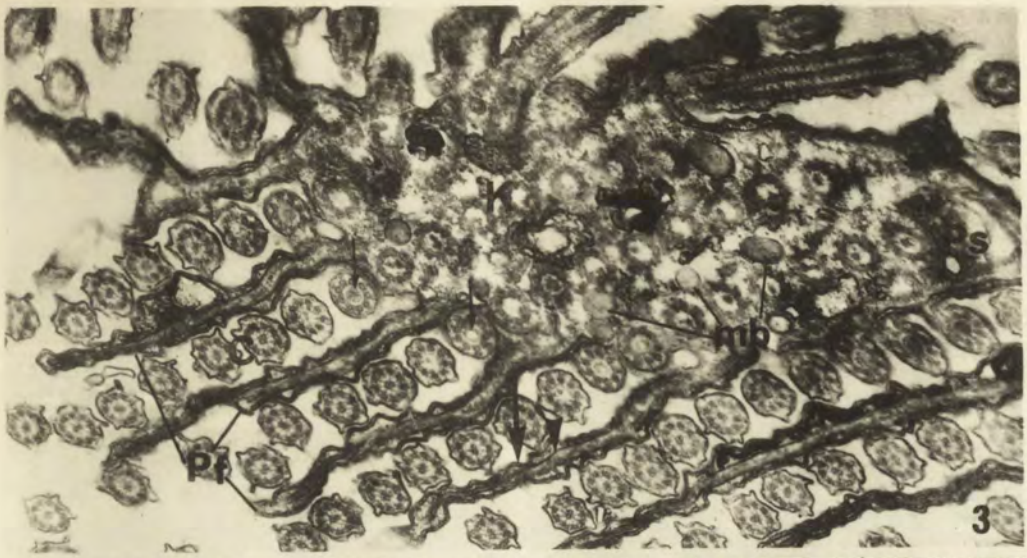
Fig. 15. A section passing through the cytostome (Cyt) shows it as a long canal or slit, with a sheet of microtubular fibrils supporting the wall on one side (double arrow). Phagocytic activity can be seen on the other side, which results in the formation of small food vacuoles (small arrows). Mitochondria (M) are always seen around the cytostomal walls. × 16 000

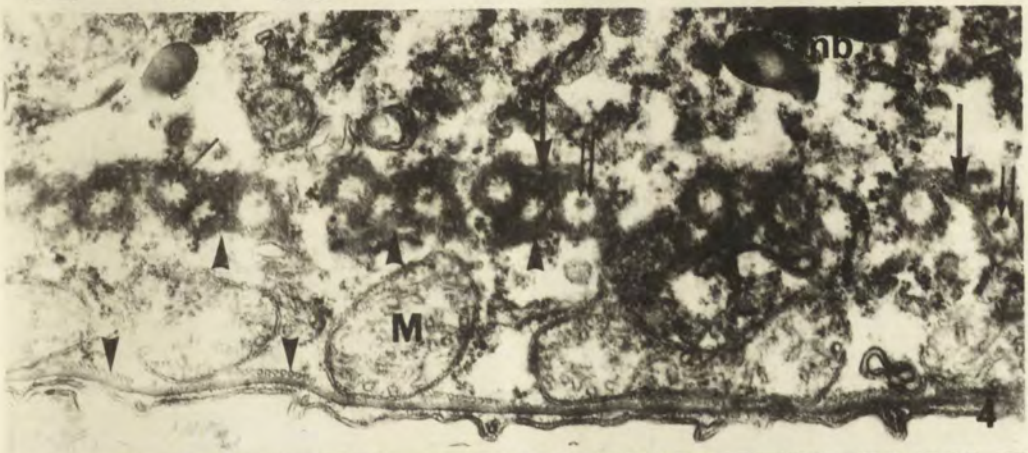
Fig. 16. Deep inside the narrow end of the buccal cavity the finger-like projections of the oral ribs have become reduced in size and the cytostome (Cyt) with its microtubular sheet (Mts) appears next to the buccal space. Arrows show sites of phagocytic activity in the walls of the cytostome. × 20 000

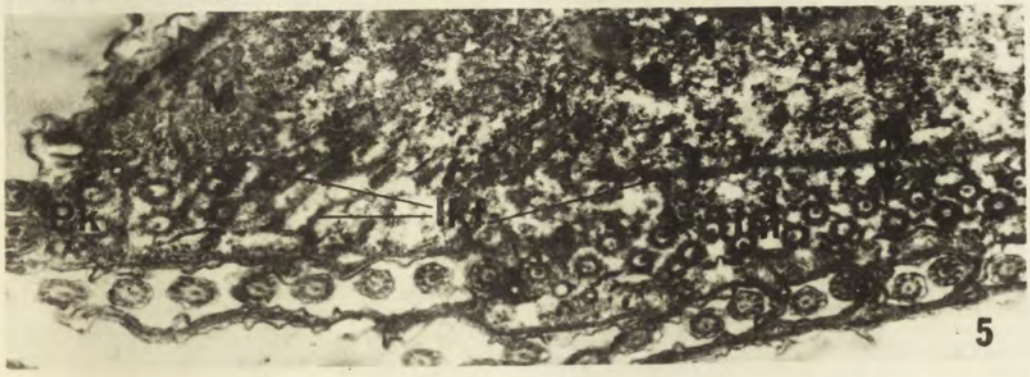
Fig. 17. The micronucleus (Mic) contains dense irregular masses of DNA, between which are spaces containing scanty matrix. What may be spindle microtubules (arrow head) may be seen in a space between masses of DNA. Pores in the nuclear membrane (small arrow) can be seen and at two places the nuclear membrane extends out with a dense material inside (large arrows). Around the micronucleus, a chain of mitochondria (M) is also seen. × 20 000

Fig. 18. The macronucleous (Mac) contains small spherical dense DNA bodies scattered in the nucleoplasm. Nucleoli are larger, coarsely granular bodies and are seen at the periphery, closely opposed to the nuclear membrane (large arrows). The matrix (mx) is granular and does not fill up the gaps between the DNA bodies. Around the nuclear memebrane, there is a net-work of cisternae of the endoplasmic reticulum. × 12 000

Fig. 19 and 20. Part of the nuclear membrane of the macronucleus is magnified to show the two types of pores (large arrows). Spherical DNA bodies separated by scanty, granular matrix (Mx). may also be seen with a large, coarsely granular nucleolus (Nu) lying close to the nuclear membrane. Both smooth and rough endoplasmic reticulum (Er, Rer) form cisternae around close to the nucleus. × 48 000 and 98 000







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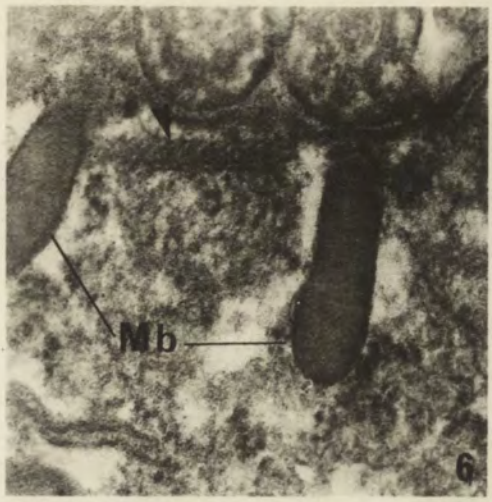

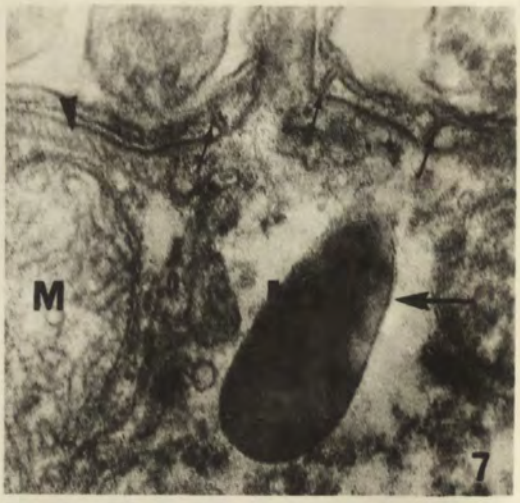

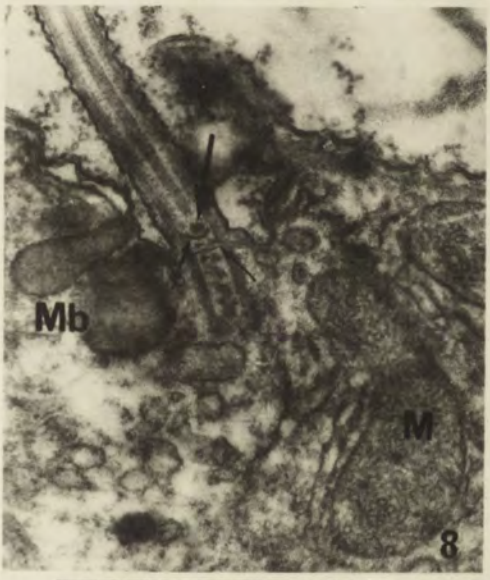

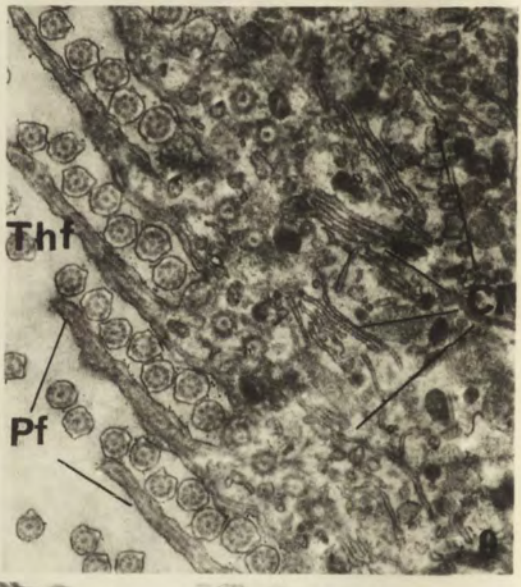

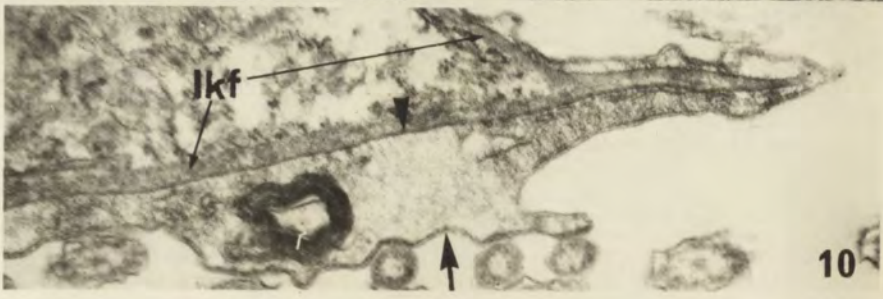

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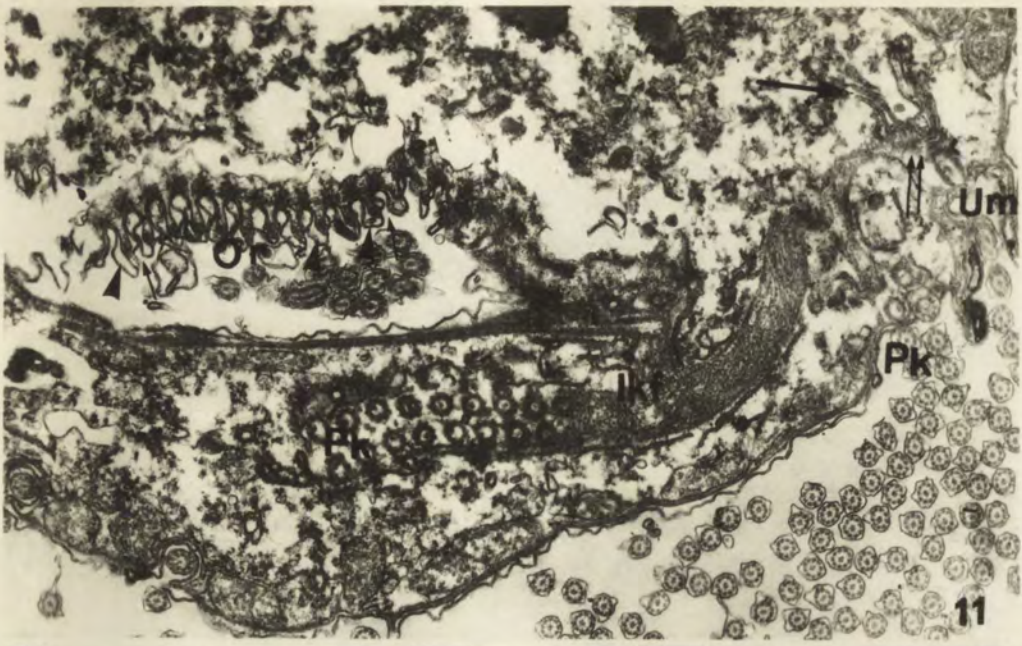

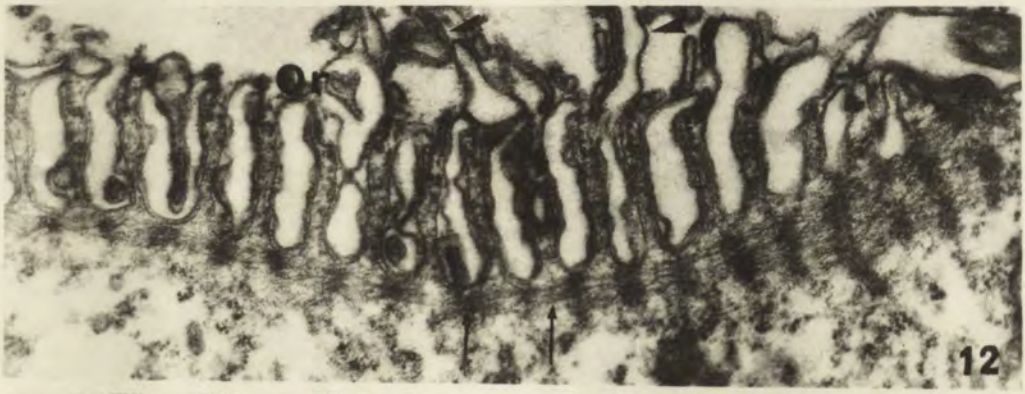

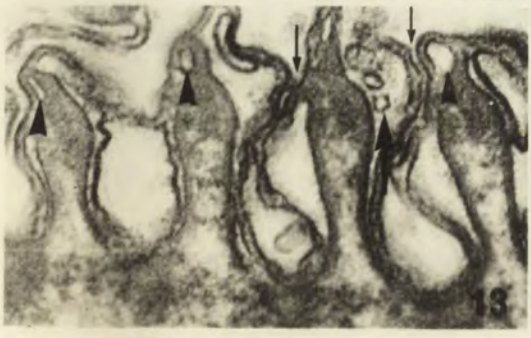

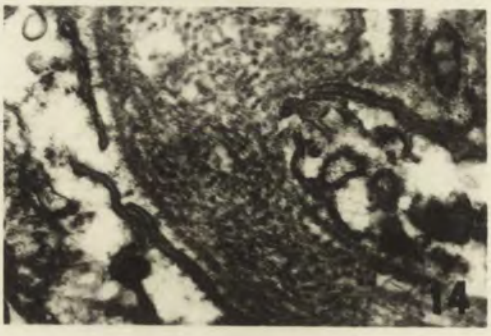

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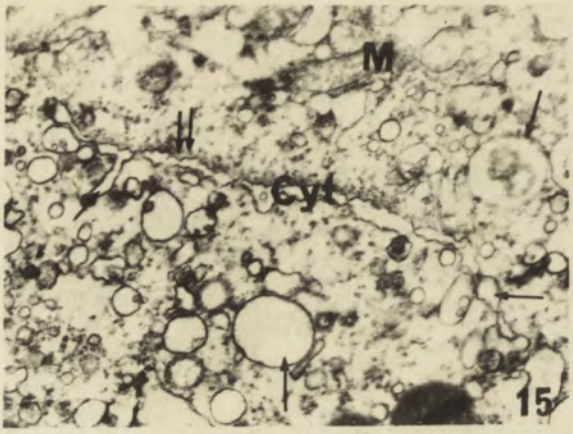

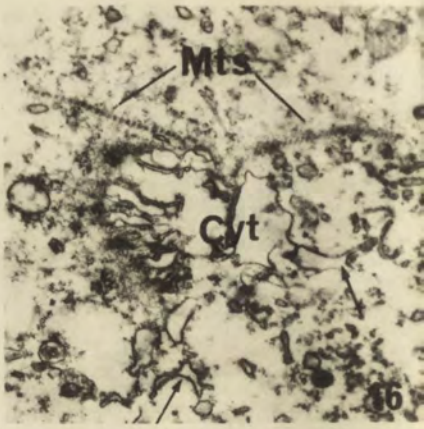

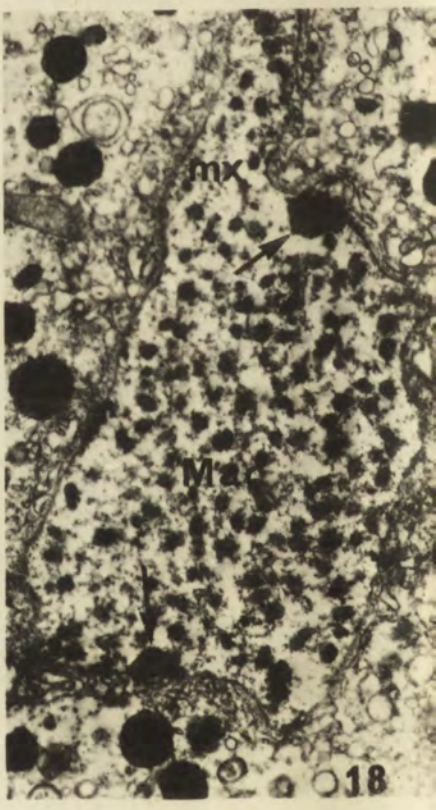

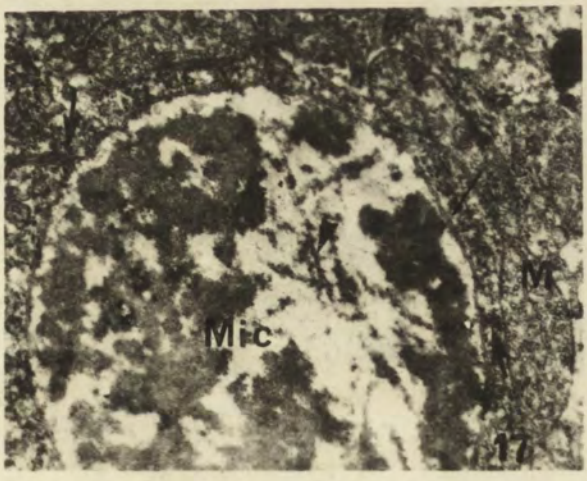

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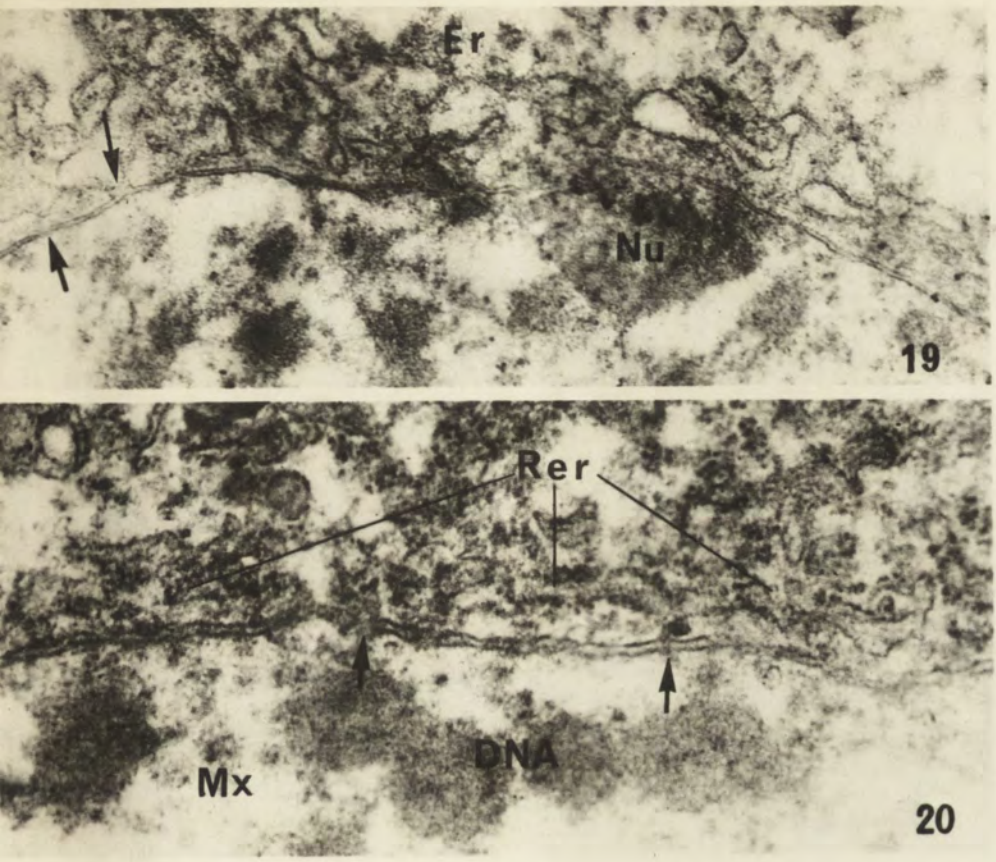

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ACTA PROTOZOOLOGICA

VOL. IX WARSZAWA, 15. XII. 1971 FASC. 12

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Mesodinium fimbriatum Stokes, 1887, a ciliate with bifurcated and barbed cirri

Mesodinium fimbriatum Stokes, 1887, ein Ciliat mit gezweigten Borsten

In October and November 1970 water from a slowly-moving water course in southwestern Indiana was searched for species of the genus *Halteria*. During the examination of this water a species of *Mesodinium* possessing bifurcated and barbed cirri was observed. Since the organism, tentatively identified as *Mesodinium fimbriatum*, remained available in good numbers in the culture dishes and had an unusual structure, the decision was made to subject it to study. It was hoped that such an investigation would also shed further light on the morphology of other *Mesodinium* species, and would produce more knowledge of the movements shown by members of this little-known genus.

A description of the structure, movements, and behavior of *Mesodinium fimbriatum* Stokes, 1887 follows.

Materials and methods

Water collected from the bayou to the north of the mill dam on Otter creek, in North Terre Haute, Indiana, was placed in large culture dishes. The water contained *Cabomba*, dead leaves and detritus. A good number of protozoan species, including *Halteria bifurcata*, and hydra, rotifers and crustaceans were present. At intervals of a few days a small quantity of distilled water and a little skimmed milk powder were added to the bayou water samples. Temperatures were kept near 20°C. In this way it was possible to maintain good populations of *M. fimbriatum* for 4 weeks.

In order to study the normal movements and the behavior of the *Mesodinium* species, a "lake" of water collected from the bottom of a culture dish and containing some detritus was placed on a slide and observed through the binocular microscope.

To make it possible to examine the structure and the means of movement of the *Mesodinium*, the afore-mentioned "lake" of water was shaken and then searched for jumping organisms. Specimens were captured with a micropipette and placed on another slide until a drop of water had been accumulated. The last was now covered with a coverglass and examined under the phase contrast microscope.

It was found advantageous to allow the "lake" of water to undergo considerable evaporation before gathering specimens from it. This tended to slow the deterioration of the specimens under the coverglass, and they did not always burst at their posterior ends before exhibiting periods of

immobility. The partial evaporation of the bayou water may also have facilitated microscopic observation by lowering the degree of activity of the otherwise rapidly-moving *Mesodinium* specimens.

Live specimens and parts of deteriorating specimens were studied and measured by phase contrast microscopy and through phase photomicrography.

Results

Body — general structure

The body is divided by a constriction into two portions, as is characteristic of the genus *Mesodinium*. The anterior section is cone-shaped and the much larger posterior section forms a globular flask (Pl. I 1). An anterior ring of cirri and a posterior ring of membranelles arise from the body constriction.

Anterior cone

The anterior cone exhibits dimorphism. Many specimens have a cone which tapers strongly toward its front end, where it bears the cytostome and the surrounding tentacular processes (Pl. I 2–4). The tips of such cones have a slight lateral bulge, and are indented centrally toward the cytostome, so that a shallow pre-oral depression is formed (Pl. I 4). The cytopharynx is indicated by a more translucent central region at the anterior end of the cone, behind the cytostome.

Other specimens possess a cone which is less tapered, and which terminates in a slightly convex anterior edge. At the front ends of these cones there are no signs of a mouth opening or of tentacular processes (Pl. I 5).

While the cone form of *M. fimbriatum* cannot yet be correlated with any aspects of the environment, microscopic observations suggest how the last, less-structured cone form may originate. In many coverglass preparations it was possible to observe a degeneration of the first-described, highly-structured cone type into the second form lacking a mouth structure and tentacular processes.

In one such specimen all of the cone except the anterior end was seen to bulge outward. This widening of the cone soon spread to the anterior end, and the two extruded tentacular processes became surrounded by bulging cytoplasm. The cone of this specimen then evolved into the structureless form.

Globules of cytoplasm frequently develop at the front end of a cone and break off, and the extruded tentacular processes disappear, as the cone degenerates into the structureless type.

In a number of coverglass preparations specimens with a variety of transitional cone forms were observed.

Nevertheless, it seems that specimens may have the structureless cone form without having undergone degeneration beneath the coverglass. This was evidenced in fresh coverglass preparations by specimens which possessed a typical structureless cone while concomitantly showing no signs of deterioration in the posterior section

of the body (Pl. I 5). Also, only during a particular time period were no tentacular processes seen on any of the organisms obtained from one culture dish.

Posterior flask

The larger, globular posterior body portion, which is truncated at its anterior end by the constriction, seems at first glance to have a surface marked by spiral grooves (Pl. I 1, 5). In reality this appearance is produced by the posterior ring of membranelles.

The cell cytopyge is located at the middle of the posterior end of the body, behind a small food vacuole.

Body — dimensions

It is difficult to obtain correct measurements of the body parts of a high number of specimens partly because deterioration sets in so early in most coverglass preparations. Not only do the anterior cones usually soon start to widen and lengthen, as has been described, but the posterior flasks quickly begin to undergo an even more pronounced enlargement. Nevertheless, reasonable estimates of typical body dimensions can be made by using both measurements taken of apparently undeteriorated specimens and upper-limit values obtained from presumably deteriorating organisms.

The following measurements were taken on an undeteriorated specimen with the structured cone form: total length (tip of cone to end of flask) 17–18 μ, length of cone 6–7 μ, width of cone base (close to the constriction) 9 μ, width of cone tip 4 μ, length of flask 10–11 μ, greatest width of flask 11 μ.

An undeteriorated specimen with a structureless cone yielded the following values: total length 18 μ, length of cone 6–7 μ, width of cone base 9 μ, width of cone tip 7 μ, length of flask 12 μ, width of flask 13 μ.

Specimens possessing structureless cones and probably undergoing various degrees of deterioration had these measurements: total length 21–22 μ, (5 specimens — the body length of 2 additional specimens was 18 μ and 25.2 μ respectively), length of cone 8–9 μ (4 specimens), width of cone base 7.5–11 μ (9 specimens — 5 of these had cone bases with a width of 9 μ), width of cone tip 5–7 μ (4 specimens), length of flask 12–16 μ (9 specimens), width of flask 13–18 μ (11 specimens).

The above measurements indicate that the typical body length approaches 20 μ. The typical length of cones can be considered to lie near 7 μ, and they are almost only half the length of the flasks. The greatest width of the flask appears to equal or slightly exceed the length of the flask.

Cirri

A single, anterior ring of almost always 21 cirri arises from the body constriction. However, when an organism is immobile, the cirri are divided into three groups

of 7 cirri each by the direction in which they are pointed (Pl. I 6, II 9). One group of cirri then points anteriorly, another extends sideways, and a third points posteriorly. Each one of every three consecutive cirri falls by an ordered repetition into a different one of the three groups. Therefore, as one focuses up and down with the phase microscope along the frontal aspect of a quiescent specimen, one clearly notices that each cirrus pointing posteriorly is further to one side of a cirrus pointing anteriorly than is an intervening cirrus extending sideways.

All the cirri of a specimen have the same structure. They are bifurcated and typically also bear two barbs, one below the other, on their stem (Pl. I 1, 8). However, the cirri of a few organisms were observed to only have the terminal bifurcation. Thus there appears to be some intraspecific variation in regard to the barbs. Since the barbs are small, and the proximal barb is close to the body, it is often difficult to see the proximal barb.

The length measurements obtained from the cirri of 17 specimens fell most frequently between 14–16 μ. Some cirri clearly approached a length of 16 μ. The cirri exhibit only a slight curvature.

The bifurcated portions of the cirri of 10 specimens, as measured from the apparently longer branch, were 7.5–9 μ long. Thus the cirri are bifurcated for about half their length.

The two barbs of a cirrus are of the same length, which was 2–2.5 μ in 6 specimens. The distance between the base of a cirrus and the proximal barb seems to be about equal to that between the two barbs.

Each cirrus is seen to be composed of 2 united portions near its base, but closer to its distal end no medial line dividing it into 2 parts is visible.

Membranelles

A single, posterior ring of membranelles also originates from the constriction. It was possible to determine the number of membranelles by counting the "notches" (bases of the membranelles) in frontal views of the ring of membranelles (Pl. II 9). There are typically 21 membranelles. Since in a few specimens 20 membranelles were counted, there appears to be some intraspecific variation in the number of membranelles.

The membranelles extend posteriorly around the flask in a counterclockwise direction, and terminate just beyond the posterior end of the body, as is the case in *Mesodinium pulex* (Bakker 1966). This causes the posterior end of the flask of undeteriorated specimens, in side-views, to seem to have a toothed border of membranelle tips.

The first indication of the deterioration of a specimen is the expansion of the food vacuole at the posterior end of the flask, and the resultant lengthening of the flask (Pl. II 13). This can produce the erroneous impression that in nature the membranelles do not reach to the posterior end of the body.

The curved membranelles of 6 specimens were found to measure between 9.5–12 μ in length. Those of the earlier-described undeteriorated specimen with the structured cone were 11–12 μ long. The membranelles arise from bases having a length of 3–4 μ.

Tentacular processes

M. fimbriatum can extrude at least 5 tentacular processes from the anterior end of its cone. Sometimes only 1 or 2 tentacular processes are extruded from the cone's perimeter, where it surrounds the cytostome. The tentacular processes are thrust forth at an outward angle, and in some cases extend to a noticeable degree in a sideways direction.

The tentacular processes divide into 5 branches at their distal ends. In one unusually favorable preparation a specimen presented a frontal view of the round-shaped anterior end of its cone, and attached with 3 tentacular processes to the coverglass. The 5 branches of each tentacular process together terminated in 5 points, which outlined a pentagon, on the coverglass. Thus 3 pentagons, outlined in dots, were visible.

As a result of their distal branching the tentacular processes appear to bear terminal enlargements. These enlargements sometimes seem to have an elongated capitate, or a drop-shaped form, and at other times assume to a lesser or greater degree the shape of a funnel (Pl. I 2–4). Presumably the shape of a terminal enlargement is determined by the extent to which the 5 branches are spread outward.

The tentacular processes, when fully extruded, are 3–4 μ in length.

Nuclei

In some deteriorated specimens it was possible to observe the 2 small, spherical nuclei reported by Penard 1922 to be present in a fresh-water *Mesodinium pulex*. The two similar nuclei (Pl. II 10) are located in the flask. They were found to each have a diameter of 2 μ in 2 specimens.

Contractile vacuoles

There are two contractile vacuoles, which are located laterally at about the middle of the anterior-posterior axis of the cone (Pl. I 2, 3).

The two contractile vacuoles alternate in expulsing fluid from the organism — as one vacuole expands the other undergoes systole (Pl. II 11, 12).

Food vacuoles

All observed specimens had a food vacuole (perhaps also with an excretory function) in the middle of the posterior end of the body, just anterior to the cytopyge.

This terminal vacuole, when empty, has a flattened, crescent-shaped appearance in a side-view (Pl. II 11). It soon enlarges in coverglass preparations (Pl. II 13).

In several specimens a large food vacuole was seen on one side of the anterior portion of the flask, while in one organism a large food vacuole containing an algal cell was noted in the central portion of the cone. Small food vacuoles were observed in deteriorating specimens.

Algae

None of the examined specimens contained symbiotic algae. However, algal cells were on occasion seen in food vacuoles.

One specimen had a spherical structure with a diameter of 6 μ in its posterior food vacuole (Pl. II 14). This structure was interpreted as an algal cell. Another organism contained 2 algal cells, one in a food vacuole in the central portion of the cone, the other in a food vacuole in the flask.

An ingested algal cell was noted to possess a central nucleus, from which strands of cytoplasm extended to the periphery.

Inclusions

Numerous small granules are typically present in both the cone and the flask (Pl. I 1, II 14). These granules are yellow and are seen with the phase microscope to have a clearcut, black border. They are generally essentially spherical or ovoid, have irregular bulges, and for the most part have a size of 2–3 μ. The described granules probably consist of reserve material.

The above granules appear to often undergo conglomeration into large masses. These are commonly elongated or irregularly oval in shape and have an internal structure. Such an apparent metaplasmic concretion is frequently observed in the posterior portion of the flask (Pl. II 15).

Two presumed concretions of granules respectively measured approximately 3×4.8 μ and 3.8×4.8 μ.

Movement

Forward movement

All forward motion, whether carried out on the substrait or through a volume of water, is produced by the cirri.

When an active, undeteriorated specimen is facing the coverglass, the cirri seen around the entire circumference of the organism move directly backward. At each portion of the circumference the backward stroke of one cirrus follows that of another. The consecutive backward movement of the cirri produces the effect of a mite-like crawling movement when an organism is seen from the side. Then on each side of a specimen one cirrus after another is seen to pass backward in a partial

circle from in front of the cone to a level considerably behind the body constriction (Pl. I 1). This mite-like crawling movement is the motion most commonly observed in side-views of *M. fimbriatum* in fresh coverglass preparations. It is often very rapid, and under the coverglass may be maintained for a long period.

Forward swimming through a volume of water by means of the described backward movement of the cirri was observed with the binocular microscope. Forward-swimming specimens spiral to the left, but in most cases the spiraling is not pronounced.

The membranelles remain inactive during forward movement.

Jumping

The forward movement of *M. fimbriatum* is from time to time interrupted by a backwards jump. These jumps are directed backwards in a straight line, and can vary greatly in length. They occur when the organism hits obstacles, the substrait or the water-air interface. Specimens also jump away (or do reversal away) from the limits of a high concentration of potassium chloride.

The saltatory movement is so rapid that it was not possible to determine the means by which it is accomplished.

Reversal

If their lake of water is shaken, they are exposed to a strong chemical solution, or when the terminal stage of deterioration sets in under the coverglass, specimens perform a rapid backward escape movement, reversal. This movement is produced by the membranelles, which then beat anteriorly, and thus also are directed anteriorly during much of their activity.

At the start of reversal the cirri are thrown forward so as to meet in front of the anterior end of the cone (Pl. II 15), and they do not move during the subsequent backward motion. The anterior "circum-cone basket" thus formed by the cirri should reduce to a minimum the resistance offered by the cirri to reversal. The "basket" may even act to stabilize reversal.

Reversal is a very fast movement, and is usually maintained long enough to cover a considerable distance. During it the organism spirals backward, but at 10×magnification with the binocular microscope backward motion is seemingly in a straight line.

Behavior

If *M. fimbriatum* is not disturbed it remains immobile for long periods on the substrait. In quiet "lakes" of water on slides the specimens were seen to rest on the glass with their cones directed downward. Presumably they used their tentacular processes to attach to the glass. When the organisms are in this position one observes their cirri, which are then a small distance above the substrait, to project outward beyond the circumference of the flask.

Some specimens were seen to attach sideways to leaf detritus on the slide.

If a "lake" of water is severely shaken the organisms rise to do long, straight lines of reversal. They then stop and perform forward movement through the water in the same general direction as the previous reversal. In many cases the specimens quickly go to the substrait (by forward movement) and, after doing a very small amount of the mite-like crawling movement on the substrait, come to rest with the cone downward. In other cases the spiraling forward movement through the water, produced by backward (crawling) strokes of the cirri, is more protracted and is occasionally interrupted by jumps.

If the disturbance of the water is less severe the organisms may rise from the substrait by means of the much slower, meandering forward movement. After traveling about erratically by this method, with some jumping, they then again settle to the substrait.

As a specimen contacts the substrait its forward motion becomes the mite-like crawling which always precedes inactivity. The duration of crawling is usually very short.

Less commonly a previously immobile organism will do a small bit of crawling and come to rest again; or a forward-moving specimen will reach the substrait, crawl over it, and then rise again by forward movement.

The three types of movement available to *M. fimbriatum*, if carried out in quick succession, can make specimens appear to be extremely mobile. They may then move in several planes within a short interval of time.

An interesting insight into the initiation of forward movement after immobility on the substrait can be gained from coverglass preparations. In many such preparations specimens seen to be totally immobile in frontal views first moved only one or several cirri before general movement of all the cirri began. This suggests that a spreading depolarization brings about general activity of the cirri.

In some specimens only one cirrus was temporarily moved, or only two or three showed some activity, while the others remained at rest.

Responses to chemical solutions

M. fimbriatum can show behavior which is reminiscent of PCR (periodic ciliary reversal) in other ciliates. This was determined by mixing a small quantity of water containing numerous specimens with a large volume of a solution of 0.01 molar potassium chloride in bayou water. At 10× with the binocular microscope most of these specimens were observed to do short lines of reversal, separated from one-another by momentary inactivity, until they died. When the specimens were watched at 70×, the cirri were seen to carry out the backward strokes of forward movement during the short intervals of apparent inactivity, but the specimens made little forward progress. Instead they remained more or less in place while performing a spiraling movement.

One specimen was seen to do continuous reversal in the 0.01 molar potassium chloride solution.

If a crystal of potassium chloride was placed in a "lake" of water on a slide and the slide was subsequently shaken, specimens doing reversal came to a stop at the limit of the dissolved potassium chloride. Some specimens then jumped away from the potassium chloride, some did reversal away, and others moved away by forward movement.

Deterioration in coverglass preparations

M. fimbriatum is very vulnerable to the conditions which produce deterioration under the coverglass.

Soon after the coverglass has been put in place, the posterior food vacuole, at the posterior end of the flask, begins to enlarge. This is accompanied by a gradual, continuing increase in the length and the width of the flask.

After the posterior food vacuole and the flask have started to increase in volume, the cone, if it is of the structured type, commonly also begins to deteriorate. This process has been described under Body-general structure.

During the initial deterioration outlined above a specimen beneath the coverglass chiefly exhibits the mite-like crawling movement, which is interrupted from time-to-time by a backwards jump. Sometimes, under favorable conditions, a specimen will also become immobile for a varying period. At a certain time, however, after considerable swelling of the flask has occurred, lines of reversal usually begin which often carry the organism into any fluid lying beyond the coverglass. In this drying fluid it quickly deteriorates and bursts.

If, after a series of reversals, the specimen remains under the coverglass, the cirri frequently show no further movement. The membranelles often continue to exhibit activity after that of the cirri is over. Then the posterior portion of the flask, which by this time is filled by the greatly-enlarged posterior food vacuole, suddenly bursts. This is followed by complete inactivity and final deterioration.

Discussion

The only previous description in the literature of a *Mesodinium* with branched cirri is that of Stokes 1887. This investigator reported *Mesodinium fimbriatum*, a fresh-water organism which he discovered in central New Jersey, in the United States, to possess setose cilia with three or more unequal, distal branches. Stokes attributed a body length of approximately 23 μ to his species, and indicated that in a frontal view its body presents a crenulated outline. The habitat was said to be standing pond water.

In many respects Stokes' observations differ from those reported in the present paper. Thus Stokes' drawing of *M. fimbriatum* shows the cirri to bear three quite

short branches close to their distal ends. The body is described as being divided into two unequal, subglobose regions. Also, Stokes reports a large, spherical contractile vacuole to be located on one side near the posterior extremity.

However, differences of similar scope exist between earlier descriptions of *Mesodinium pulex* and that given by Bakker 1966. For instance, Bakker, in an illustration, shows *M. pulex* to possess a single contractile vacuole, located in the anterior portion of the cone. All earlier descriptions of species of the genus *Mesodinium* place the contractile vacuole posterior to the body constriction. Penard 1922 reports his fresh-water *M. pulex* to have a large contractile vacuole slightly to one side of the posterior end, and Kahl 1930 echos this location for the contractile vacuole of *M. pulex*. In respect to the mite-like crawling movement, Bakker 1966, Penard 1922 and Borror 1963 report it to occur in *M. pulex*, but Noland 1937 never observed *M. pulex* to perform this movement. There are also disparities in the body lengths given for *M. pulex*.

The cone of *M. fimbriatum* might well have appeared subglobose to Stokes if he examined specimens whose cones tapered only beyond the basal portion (Pl. I 2–4) and had already undergone some deteriorative swelling. Furthermore, it may be justifiable to ascribe the other points of difference between Stokes' description and the present one to the unusual posterior food vacuole and its tendency to swell under the coverglass, and to the inferior microscopic equipment available to Stokes. Therefore it seems quite probable that Stokes' organism and the species dealt with in this paper are one and the same.

In view of the present finding that *M. fimbriatum* has two contractile vacuoles in its cone, and the observation by Bakker 1966 that *M. pulex* of marine origin possesses a contractile vacuole in its cone, it might be well to re-examine the location of the contractile vacuole(s) in all members of the genus *Mesodinium*. This idea is given impetus by Penard's 1922 description of the contractile vacuole at the posterior end of his fresh-water *M. pulex*. He reports a very large contractile vacuole which bursts in the manner of those of amoebas, like a large bubble with a delicate surface which breaks suddenly. The preceding description causes one to think of the posterior food vacuole and its behavior during deterioration.

The finding that a single population of *M. fimbriatum* shows dimorphism in its cone, and the observation of the evolution, beneath the coverglass, of the highly-structured cone form into the structureless type, are of interest. They shed further light on the probable origin of simple cones lacking cytostomes in other *Mesodinium* species.

Bakker 1966 reported that while *M. pulex* obtained from the Oosterschelde usually contained no symbiotic algae and had a conical anterior section with a cytostome and tentacular processes, *M. pulex* gathered in the Veerse Meer could be identified by the presence of symbiotic algae and possessed a semi-spherical anterior section generally lacking a cone-like elevation with tentacular processes. Bakker occasionally observed transitional specimens from the Veerse Meer which still

exhibited a small conical elevation and a few tentacular processes on the anterior body section.

Bakker suggested that the symbiotic relationship with algae results in the loss of the cytostome and the tentacular processes by an organism which has now become autotrophic. He speculated that the *M. pulex* population from the Veerse Meer may consist of obligatory symbiotes.

Kahl 1933 found that the *M. pulex* inhabiting the sand bottom of the bay of Kiel has a semi-spherical anterior section lacking a cytostome. In this anteriorly-convex front portion of the body Kahl 1933, 1935 observed black-appearing granules, while the posterior section held coarse colorless bodies. Kahl suspected that this modification of *M. pulex* contains symbiotic bacteria, and considered it a separate variety. He named it *M. pulex* f. *pupula*.

Fauré-Fremiet 1950 obtained the above variety at the beaches of Concarneau. He states that a revision of the genus *Mesodinium* based on thorough morphological and cytological studies by means of in vitro cultures will be needed to determine if *M. pulex* f. *pupula* should have a separate status.

In regard to *M. rubra* from the Baltic sea, Lohmann 1908 reported that small specimens having no symbiotic algae possess a cone with a cytostome, but that large organisms with symbiotic algae have a semi-spherical anterior section without a cytostome. When the several posteriorly-located algae of transitional specimens increased in number, the anterior cones of these specimens enlarged.

Kahl 1931 also noted that when its internal algal cells multiply, *M. rubra*'s anterior cone enlarges to become globose. A cytostome is then no longer visible in some specimens, while others still exhibit several tentacular processes.

In 1933 Kahl gave *M. rubra* the status of a variety of *M. pulex*, naming it *M. pulex* f. *rubrum*.

Ballard 1952 observed a change in the cone form of *M. acarus*. In addition, he found hyaline bubbles to be attached to the cones of a few of his specimens, but could not determine their significance.

Calkins 1901 stated that the oral extremity of his *M. cinctum* is "sometimes hollow, sometimes evaginated and convex". However, he considered this evidence of flexibility.

The present findings confirm that at least some *Mesodinium* populations are able to undergo a change in cone form. However, in the case of *M. fimbriatum* the observed dimorphism was not related to the appearance or multiplication of any internal symbionts. Further studies with in vitro cultures should be carried out to determine whether factors such as the state of nutrition, osmotic pressure, etc. might be responsible for a natural change in the cone form of *M. fimbriatum*.

It is interesting to note that Fauré-Fremiet 1945 has observed a polymorphism related to the state of nutrition in another genus of the *Didiniidae*, in *Monodinium* (*Didinium*) *vorax*.

The observation that the terminal enlargements of the tentacular processes of

M. fimbriatum may vary from close-to-capitate to funnel-like in shape, presumably depending on the position of the 5 distal branches of each tentacular process, may help to solve disagreements between investigators.

For example, Kahl 1930, 1931 states that the tentacular processes of *M. pulex* bear a distal suction disk. Borror 1963 corroborates this description, reporting the "stylets" of *M. pulex* to be "flat or slightly concave distally". On the other hand, Noland 1937 finds the tentacular processes of *M. pulex* to be trifurcate, and Bakker 1966 speaks of a tripartite sucker. The present results lend force to Noland's suggestion that Kahl's suction disk represents a misinterpretation of three terminal branches.

Also, Penard 1922 describes the tentacular processes of his fresh-water *M. pulex* as being distally enlarged into a pinhead or a ball. Noland 1937 finds only the tentacular processes of *M. acarus*, not those of *M. pulex*, to bear capitate tips. These, and other observations, cause Noland to suspect that Penard's organism is really *M. acarus*. The results on *M. fimbriatum* indicate that the shape of tentacular-process terminations may be generally labile, and that in *M. acarus* these terminations should be carefully re-examined.

The three types of movement exhibited by *M. fimbriatum* have their counterparts in other ciliates.

Forward swimming by means of the cirri is homologous to the common forward spiraling carried out through cilia or an adoral zone of membranelles by diverse ciliates. Forward swimming automatically becomes mite-like crawling when *M. fimbriatum* reaches the substrait. The mite-like crawling movement has previously been noted in other species of the genus *Mesodinium* (Bakker 1966, Borror 1963, v. Buddenbrock 1920, Calkins 1901, Noland 1937, Penard 1922, Stein 1862).

The backward jumping of *M. fimbriatum* serves the same function as the saltatory movement of *Halteria*, and is therefore equivalent to the avoidance reaction of other ciliates. Bakker 1966, Kahl 1930, 1931 and Penard 1922 all state that the jumps of *M. pulex* are produced by the membranelles. However, these investigators did not differentiate between jumping and reversal.

The reversal of *M. fimbriatum*, and presumably that of the other *Mesodinium* species, is unusually rapid. Thus reversal in the genus *Mesodinium* resembles the fast reversal shown by *Halteria*. However, while the reversal of *Halteria* is produced by an unusually well-developed adoral zone, that of *Mesodinium* results from the action of a highly-developed posterior zone of membranelles.

Bakker 1966 and Penard 1922 speak of an occasional flexible, cilia-like motion of the cirri in *M. pulex*. Ballard 1952 reports a similar wave-like motion by the cirri of *M. acarus*. During the course of the present research the stiff cirri of *M. fimbriatum* were never observed to perform an undulating movement.

Summary

Mesodinium fimbriatum Stokes cultured with skimmed milk powder had a body length of about 20 μ and a cone length close to 7 μ. The anterior, conical portion shows dimorphism, and was frequently observed to lose the cytostome and tentacular processes under the coverglass. An anterior ring of 21 cirri points into 3 different directions, and each cirrus is bifurcated and has 2 barbs. There is a posterior ring of 21 membranelles. The tentacular processes have 5 distal branches. The 2 contractile vacuoles lie in the cone, and the 2 similar, spherical nuclei in the posterior section.

Forward movement, jumping, reversal, normal behavior and the rapid deterioration under the coverglass are described.

ZUSAMMENFASSUNG

Mesodinium fimbriatum Stokes wurde mit getrockneter Magermilch kultiviert. Es hat eine Körperlänge von ungefähr 20 μ, und einen vorderen, konischen Teil (Mundkegel) von circa 7 μ. Der vordere Teil erscheint in zwei Formen, und es konnte beobachtet werden wie manche solche Teile den Mund und die Haftstäbchen unter dem Deckglas verloren. Ein vorderer Kranz von 21 Cirren ist in 3 verschiedene Richtungen gespreizt. Jede Cirre ist in zwei gegabelt und hat ausserdem 2 Ästchen. Es gibt einen hinteren Kranz von 21 Membranellen. Die Haftstäbchen zerteilen sich distal in 5 Zweige. Die 2 kontraktilen Vakuolen liegen in dem vorderen Teil, und die 2 ähnlichen, sphärischen Kerne in dem hinteren Teil.

Forwärtzbewegung, Springen, Rückwärtsschwimmen, normales Benehmen und die rasche Entartung unter dem Deckglas sind beschrieben.

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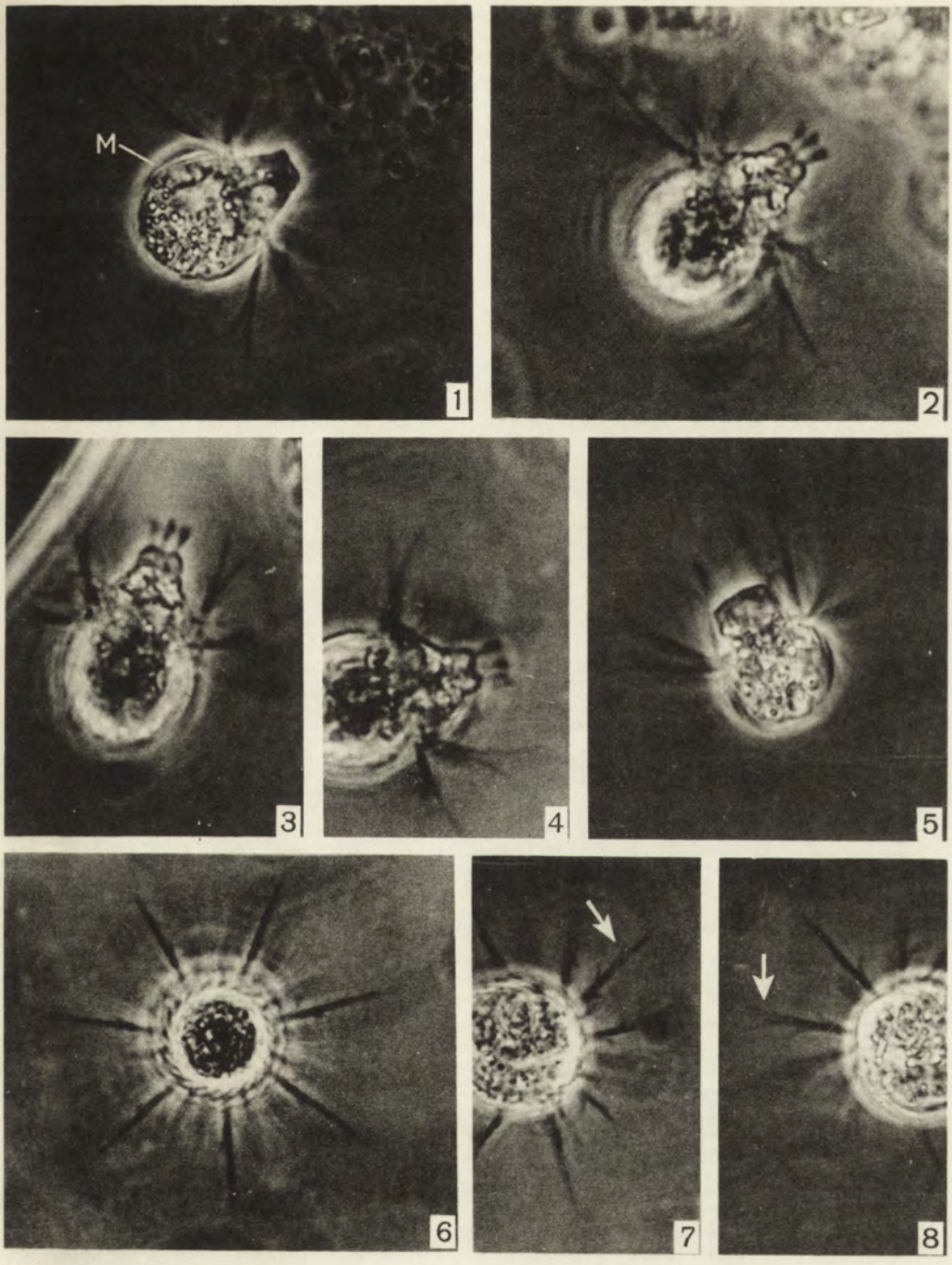
EXPLANATION OF PLATES I–II

Mesodiunium fimbriatum Stokes

1: Side-view of specimen doing mite-like crawling. The anterior cone is transitional. The membranelles (M) do not reach the posterior end, indicating some specimen deterioration
2: Side-view of specimen with highly-structured cone bearing a cytostome and tentacular processes. The cone tapers only beyond its basal portion and bulges laterally near its tip. Note subsidiary vesicles contributing to contractile vacuoles in cone
3: Another view of the same specimen
4: Side-view of a highly-structured cone, showing pre-oral depression and anterior lateral bulge. Four tentacular processes can be seen
5: Side-view of specimen with structureless cone. The membranelles reach the posterior end, indicating there has been no deterioration
6: Frontal view from anterior end of immobile specimen. Group of 7 cirri extends sideways
7: Bifurcated cirrus with 2 barbs
8: Bifurcated cirrus with 2 barbs
9: Frontal view from posterior end of immobile specimen. The bases of the 21 membranelles are in focus and the 7 cirri pointing posteriorly are visible
10: Two nuclei (N) in deteriorated specimen
11: Two contractile vacuoles in cone of deteriorating specimen. Membranelles (M), food vacuole (F)
12: Same organism as Fig. 11, a short time later. The lower vacuole has undergone systole, and the other has enlarged.
13: Enlarged food vacuole containing alga in deteriorating specimen
14: Algal cell (6 μ diameter) in enlarged food vacuole
15: The cirri are extended anteriorly in a deteriorating specimen doing reversal. An apparent metaplasmic concretion has come to be included in the enlarging food vacuole
(All photomicrographs by phase contrast. At 500×, exposure 1/125 sec with microflash, except Fig. 10. Tri-X film, D-76 developer)



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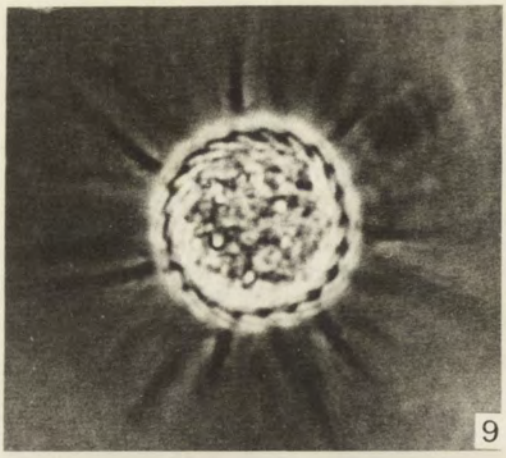

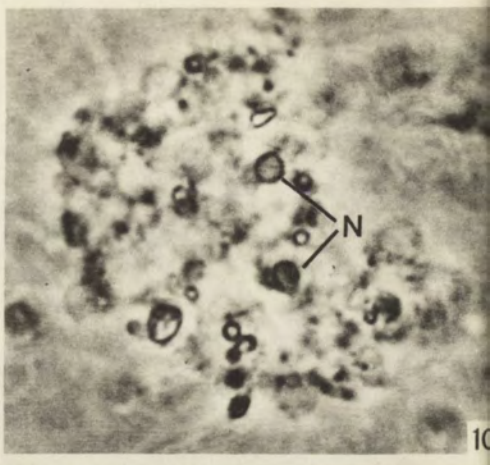

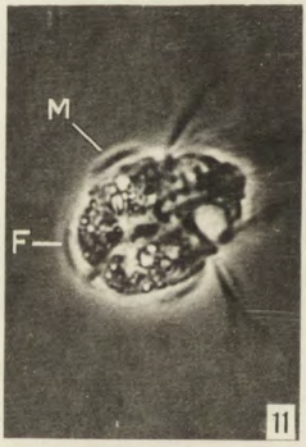

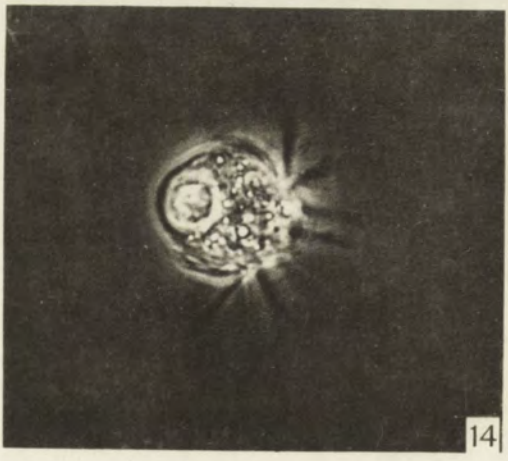

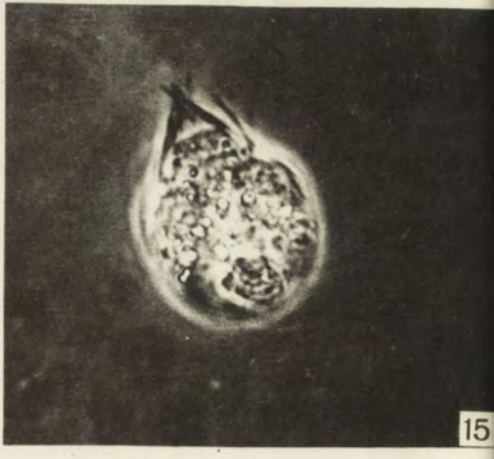

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ACTA PROTOZOOLOGICA

VOL. IX WARSZAWA, 15. XII. 1971 FASC. 13

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Endogenous stages of the life cycle of *Eimeria schamchorica* Musajev et Alijeva, 1961 (*Sporozoa, Coccidia*) the parasite of *Meriones erythrourus* Gray

Эндогенные стадии жизненного цикла *Eimeria schamchorica* Musajev et Alijeva, 1961 (*Sporozoa, Coccidia*), паразита краснохвостой песчанки (*Meriones erythrourus* Gray)

In the literature no reports were found on the full life cycle of particular *Coccidia* species of rodents. Some communications available are of a fragmentary character (Pinto 1928, Martin 1930, Becker and Hall 1931, Carini 1932, 1937, Gonzales-Mugaburu 1942, 1946, Doran and Jahn 1952, Pellérdy and Babos 1953, Pellérdy 1954 a, b, Rodhain 1954, Cordero del Campillo 1959, Levine and Ivens 1960, 1965, Parasad 1960, Ellis and Wright 1961, Zellen 1961, Černa 1962). More detailed data concerning this problem may be found in the works of Henry 1932, Roudabush 1937, Lapage 1940, Pellérdy 1960 as well as those of Musajev and Wejsov 1965.

Nevertheless the study of life cycles of the rodent coccidia presents an interesting subject. The rather recent monography of Musajev and Wejsov 1965 embraces a description of over 130 species of coccidia from 45 species of rodents, 20 out of them being the representatives of the Azerbaijan fauna. Unfortunately the bulk of the presented material is based on the study of one development stage of coccidia only. However even such uncomplete research permits to reveal the interesting fact that the oocysts of rodent coccidia are represented by a great variety of forms. The presence of a great number of species in one host indicates, that in rodents, the process of species formation of coccidia proceeds very intensely and is correlated with their distribution within the alimentary tract. For that reason the detailed study of the full life cycles of rodent coccidia is of a great scientific interest in connection with the problem of species formation of parasitic protozoa, as well as with the more extensive problem of mutual interrelations of parasite and host.

The present study is the first detailed investigation of life cycles of the rodent coccidia.

Material and methods

The individuals of *Meriones erythrourus* Gray, free of coccidia were cultivated in the vivaria of Institute of Zoology of Azerbaijan SSR. The infection of parasite-sterile animals was performed with a single oocyst of *Eimeria schamchorica*, which produced a genetically uniform material with all endogenic stages belonging without doubt to the species under study.

For the study of endogenous cycle of *E. schamchorica*, 3–5 host animals were dissected every day in the course of the whole prepatent period (5 days). The host animals were infected with a high numbers (3000–5000) of mature sporulated oocysts. The total number of dissected host individuals amounted 18. After dissection, the whole intestinal tract was examined from duodenum to rectum and the liver and bile-duct as well. Every 4–5 cm of the intestine tract length, small samples of its wall (1 cm) were cut out and fixed with the Zenker or Carnoy fixatives. Simultaneously smears were prepared from the corresponding regions of intestine and fixed with the Schaudinn's fluid.

The samples of intestine were embedded in paraffine and cut at 5–7 μ. Sections and smears were stained with iron haematoxylin of Heidenhain and with azur-eosine after Nocht-Maximov. Drawings were executed by means of the Abbe apparatus of the type PA-4 at the level of microscope stage (eyepiece 20×, and objective 90 ×). Microphotograms were executed using the microphoto camera MNF-8 and microscope MBI-6.

Results

The youngest stages of the agamic reproduction — schizonts, of the first and second generation are localized in duodenum at 2–8 cm distance from stomach. Later on, the schizonts of the second and third generations as well as macrogametes and microgametocytes are localized in jejunum and in the upper segment of ileum,

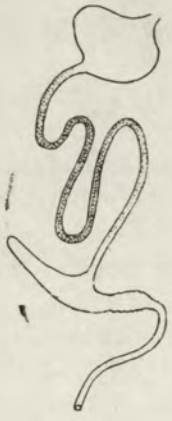

Fig. 1. A scetch of the intestinal tract of the red-tailed jird. Dotted are places of localization of *Eimeria schamchorica*

with the highest concentration at the distance of 5–15 cm from stomach (Fig. 1). In the region of duodenum, about 72 hr after infection, the number of parasites falls, whereas in jejunum — a considerable rise of the individual number at endogenous stages is observed. Further on, in ileum nearer the colon, the number of

endogenous stages gradually diminishes and — at the distance of 25–28 cm from stomach — no endogenous stages of *Eimeria schamchorica* are found.

Consequently in the 1-month-old host individuals with a 52–55 cm long intestine, the endogenous stages of *Eimeria schamchorica* infect the half of the intestine length. Schizonts of all the generations as well as gamonts are localized in the epithelium of villi over the nuclei of epithelial cells, i.e. in their proximal part (Pl. I 1–2). The first and second generations of schizonts are located along the villi, whereas the

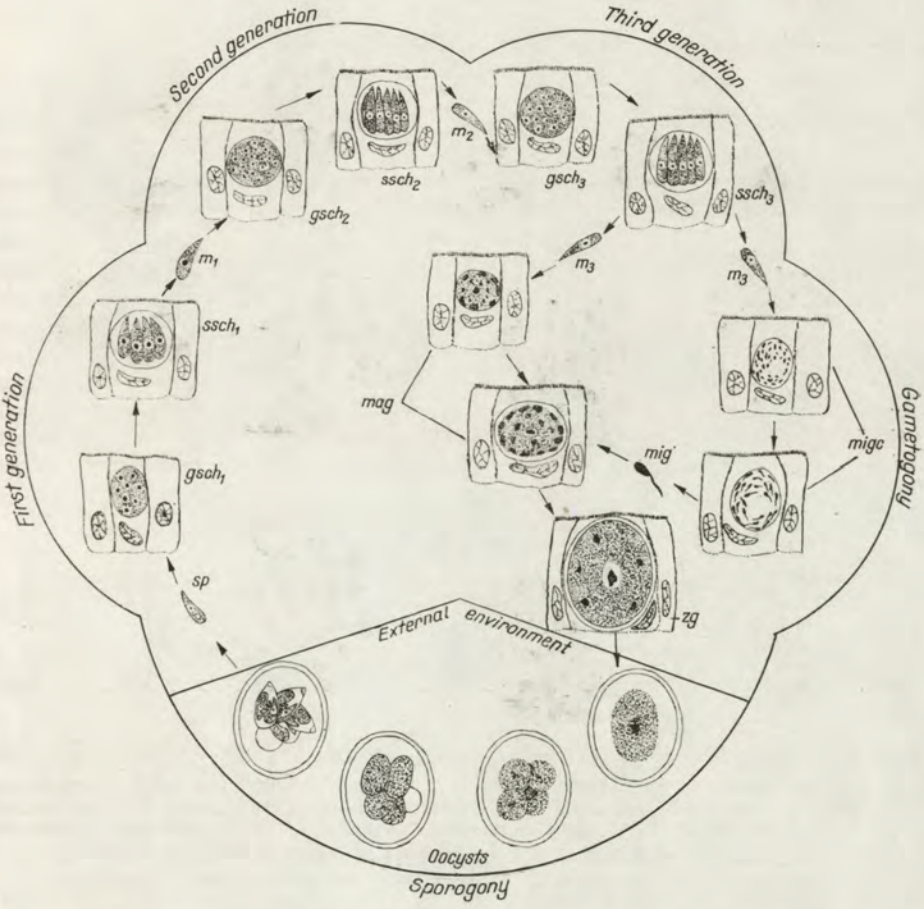

Fig. 2. A scetch of the life cycle of *E. schamchorica*: sp — sporozoite, gsch — growing schizont, ssch — segmented schizont, m — merozoite, migc — microgametocyte, mig — microgamete, mag — macrogamete, zg — zygote. Numerals near letters signify corresponding asexual generations

third generation — in their distal part. Gamonts are localized along the villi as well as and in their distal part (Pl. I 3–4). In the apical part of villi and in the crypts, single individuals of the endogenous stages are present. No one of the endogenous cycle stage of this species penetrates into the connective tissue of villi.

Schizonts of the first generation are not numerous, therefore toward the end of the first and beginning of the second day after infection, the majority of cells are free of parasites. Mature schizonts are of very small dimensions, and the epithelial cells are rather little changed under the influence of parasites at the period of their schizogony. Toward the end of the fourth and at the beginning of the fifth day, i.e. at the period of gametogony, the infection with the endogenous stages of *E. schamchorica* evokes a sharp deformation of the epithelial cells. In many cases, 2–3 macrogametes are present in one epithelial cell.

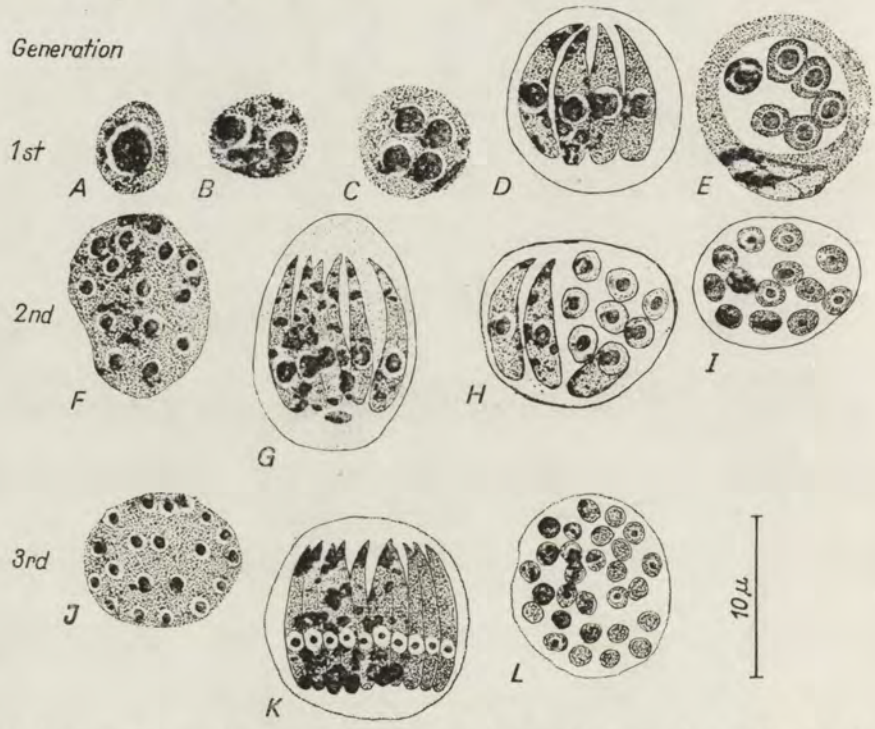

Fig. 3. Successive stages of asexual development (1st, 2nd and 3th generations) of *E. schamchorica*: A–C — growing first generation schizonts, D — mature first generation schizont, E — cross-sected first generation schizont, F — growing second generation schizont, G — mature second generation schizont, H–I — cross-sected second generation schizont, J — growing third generation schizont. K — mature third generation schizont, L — cross-sected third generation schizont

In the endogenous development period of *E. schamchorica*, three generations of schizonts may be distinguished (Fig. 2). The schizonts of the first generation begin their development 5–10 hr after infection. Towards the 40th hour, in the epithelium of duodenum, the schizonts of the first generation may be detected, with big merozoits (Fig. 3 A–E). Segmented schizonts are of ovoid shape. The number of merozoits in schizonts fluctuates from 4–22, on average 13. The dimensions of schizonts and merozoits are presented in Table 1. Single schizonts of the first

generation occur in epithelium up to the 52nd hour. In the host dissected 52 hr after infection, an insignificant number of young schizonts of the second generation may be detected (Fig. 3 F–I). In the schizonts of the second generation, 10–33 (on average 19) merozoits are formed and a residual body is present. The dimensions of schizonts and merozoits of the second generation are presented in the Table.

Table 1

Dimensions of the endogenous stages of *E. schamchorica*

Development stage	Dimensions in μ (average value in brackets)		Number of individuals measured
	length	width	
Schizonts of the first generation	11.2–16.8 (13.87)	8.4–14.0 (10.91)	47
Merozoits of the first generation	8.4–14.0 (11.59)	1.7–3.0 (2.19)	41
Schizonts of the second generation	12.6–18.2 (15.15)	9.8–14.0 (11.81)	66
Merozoits of the second generation	7.7–12.6 (10.06)	1.2–2.5 (1.74)	67
Schizonts of the third generation	14.0–19.6 (16.67)	9.8–15.4 (12.87)	40
Merozoits of the third generation	9.8–13.3 (11.51)	0.96–1.8 (1.30)	47
Microgametocytes	9.6–18.0 (13.44)	7.2–14.4 (10.69)	66
Microgametes	1.2–3.0 (1.82)	—	40
Macrogametes in the moment of envelop formation	13.2–18.0 (16.30)	10.0–15.6 (13.4)	50

Beginning with the 66–68 hr, the merozoits of the second generation begin to penetrate into the epithelium of villi. About the 88th hr, schizonts of the second as well as of the third generation may be observed in the epithelium of jejunum. Schizonts of the third generation are characterized by considerable dimensions, by narrow and elongated merozoits (Fig. 3 J–L). The number of merozoits in the schizonts of the third generation rises when compared with the preceding stage, up to 12–38 (22). The dimensions of schizonts and of merozoits of the third generation are also presented in the Table 1.

Schizonts of the third generation conclude their development at about 120th hr. Since the sporozoits penetrate into the epithelium not simultaneously and begin their development at different time, we found simultaneously merozoits and schizonts of different generations in the intestine of the host. Merozoits of the third generation give origin to the gamonts.

Gametogony initiates 86 hr after infection. Dissecting the host animal after 93–94 hr, we may detect young macrogametes (Pl. I 3, 4). The number of macrogametes exceeds considerably the number of microgametocytes. So, in the course of 116 hr, for 100 macrogametes occur only 8–9 microgametocytes.

The young macrogametes have a spheroid form. Since the very beginning of macrogametes development, in their cytoplasm accumulate inclusions in the form of granules. In the centre of nucleus of young macrogametes appear big karyosomes which stain intensely with iron haematoxylin (Fig. 4 A–C, Pl. II 3, 4).

The growth of macrogametes is accompanied by the rise of number and of dimensions of the cytoplasmic granules. Later on, they are spread over the periphery

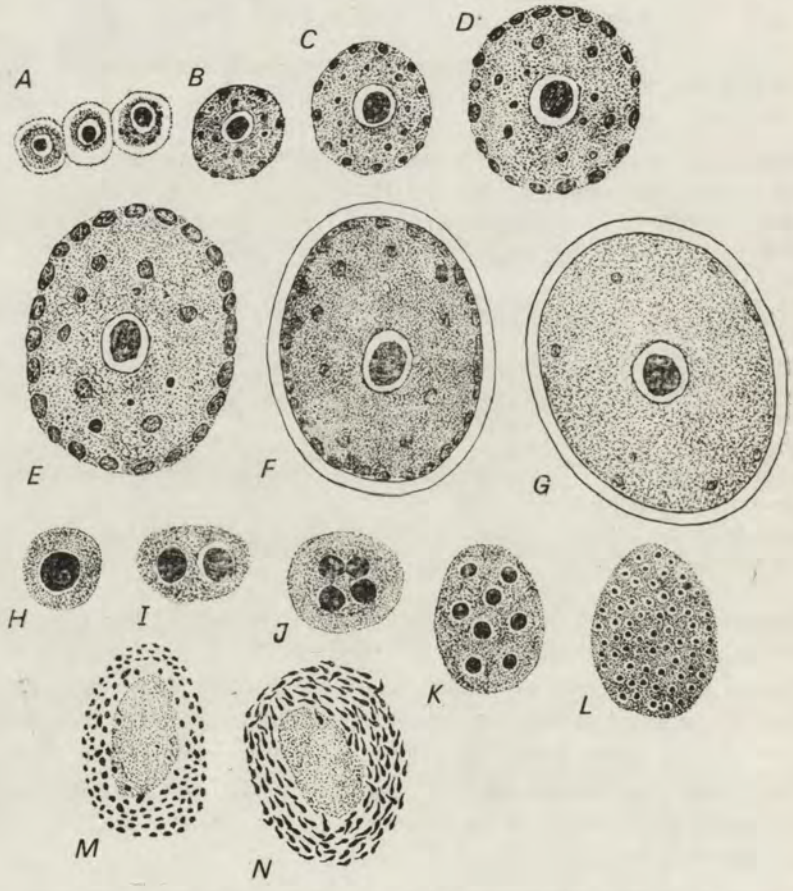

Fig. 4. Successive stages of sexual development of *E. schamchorica*: A–D — accumulation of cytoplasmic granules and their eventual arrangement on the periphery of a macrogamete, 5–6 — fusion of peripheral granules and formation of the envelope, G — oocyst, H–M — different steps of microgamete formation, N — a mature one-centered microgametocyte

of macrogamete (Fig. 4 D–E), while its nucleus enlarges simultaneously. At a later stage, when the nucleus begins to elongate assuming an ovoid shape, the peripheric granules gradually coalesce with one another producing the external envelope of the oocyst (Fig. 7 F–G). The granules left in cytoplasm seem to participate in the

formation of the internal envelope of the oocyst and that of spores in the sporulation process of oocysts. After the fertilization of mature macrogametes, oocysts are formed which break across the epithelium and penetrate into the gut lumen.

Microgametocytes begin their development similarly as macrogametes, in the epithelium of villi, over the nucleus of the epithelial cells. Early stages of microgametocytes development can be distinguished with difficulty from young schizonts. However, after staining with iron haematoxylin the binuclear microgametocyte has a more dark cytoplasm than schizont. The nuclei of young microgametocytes are big. Their dimensions considerably diminish in proportion as the number of nuclei increases after several divisions (Fig. 7 H–L). In the multinuclear gametocyte the nuclei are initially scattered at random in cytoplasm and then shift nearer to the periphery (Fig. 7 M), around the central cytoplasmic mass which becomes the residual body of microgametocyte in the subsequent formation of microgametes. Those are so-called unicentral (Fig. 7 N) microgametocytes. The nuclei of microgametocytes become located at the cell periphery. In the subsequent development they slightly elongate and assume the shape of a short comma. Those nuclei become the nuclei of microgametes.

Mature microgametocytes are of ovoid or symmetric oval form. Their dimensions fluctuate: length — 9.6–18.0 μ (average 13.44 μ), width — 7.2–14.4 μ (average 10.69 μ).

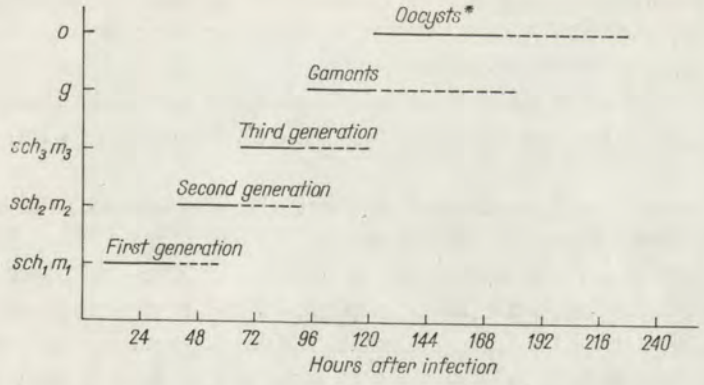

Fig. 5. Scheme and duration of particular stages of the endogenous development of *E. schamchorica*: sch — schizonts, m — merozoites, g — gamonts, o — oocysts. Numerals near letters signify corresponding generations. — — — — a few, ———— numerous oocysts, * — in feces

After 110–116 hr, in the gut epithelium occur in mass macrogametes and microgametocytes at various stages of growth and development as well as single schizonts of the third generation.

The endogenous development of *E. schamchorica* lasts up to 9 days. The first oocysts formed appear in the gut lumen 110–115 hr after infection (Fig. 5). After

the infection of host with an infignificant number of *E. schamchorica*, the first oocystes appear in the fecal mass at 118–122nd hr (the maximal time term of the preparent period). When significant doses have been applied, the prepatent period is shortened down to 112 hr.

Discussion

The investigation of life cycles of coccidia i.e. cifering the sequence of development of single parasite stages, is interesting from the point of a full characteristic of the species as well as in terms of the biological categories. Out of 232 species of coccidia of rodents of the *Eimeria* genus, the life cycles or single phases of development have been described fully only for comparatively few species. Endogenous stages of the life cycle of *E. caviae* — the parasite of guinea pig (*Cavia porcellus*) have been described by Henry 1932 and by Lapage 1940. Deciphering of the life cycle of *E. nieschulzi*, *E. separata*, *E. miyiardii*, the parasites of the roof rat (*Rattus rattus*) is given in the work of Roudabush 1937. The author described the form and dimensions of schizonts and of merozoits of all the generations of those species, followed the development of their macrogametes and microgametes as well as the formation of the envelopes of the oocysts. Pellérdy 1960 studied some endogenous development stages of *E. sideli* from *Myocaster coypus*. Webster 1960 studied the endogenous stages of the life cycles of *E. neosciuri*, a coccidium of *Sciurus carolinensis*. Musajev and Wejsov 1965 provided data about the terms of the prepatent and patent periods and, partly, about the endogenous development stages of *E. vinogradovi*, a parasite of *Meriones vinogradovi*.

Coccidia (*Eimeria*) of rabbit have been investigated most fully among the parasites of this genus. We owe this in the first place to the studies of Cheissin (1946, 1947, 1948, 1967).

In the present report the method of Cheissin was followed: the infection of the coccidia-sterile host with one oocyst.

It is known from the literature that in the majority of coccidia-species studied, the endogenous development lasts longer than the prepatent period i.e. after appearing of oocysts in the feces of animals, the development of endogenous stages (schizogony and gametogony) continues for some time in the host tissues. However Roudabush 1937 indicated that the endogenous development of *E. separata* and *E. miyiardii* may coincide in some cases with the preparent period. According to the results of this author, the endogenous period in *E. separata* concludes after 4 days and 12 hr and on the 5th day; the oocysts appear in the feces of the rat.

In our material, the endogenous development stages of *E. schamchorica* occur in the epithelium of villi even 3–4 days after appearing of the first oocytes in the feces of rat i.e. the endogenous period of *E. schamchorica* lasts 8–9 days after infection.

The endogenous development stages of the majority of the investigated coccidia species of rodents are localized in the thin part of the intestine. So e.g. out of 31

coccidia species of rodents, for which the localization place of endogenous stages is known —19 contain those stages in the small, 7—in the large intestine and 5—in both segments. The stages of development of the parasite appear, as a rule, in the epithelium of villi and of crypts, being located over or below their nucleus. In some species, penetration of gamonts and schizonts into the connective tissue of villi was observed.

According to our observations, the endogenous stages of *E. schamchorica* appear in the epithelium of villi of the small intestine. The development stages are located over the nucleus and no one stage penetrates into the conjunctive tissue.

It is known that among the coccidia of rodents in which the life cycle has been studied, only in 3 species (*E. nieschulzi*, *E. separata*, and *E. miyiardii*) the number of agamic generations has been determined. So in the first species, 4 generations were stated, in the 2nd and 3rd — 3 generations in each of them. The number of merozoits present in schizonts of a single generation and in different species is not the same. The highest number of merozoits (36–60) was noticed in the schizonts of the fourth generation of *E. nieschulzi*, the lowest one (2–6) in the schizonts of the third generation of *E. separata*.

In the endogenous phase of *E. schamchorica*, 3rd generations are developed. Schizonts of the single generation of this species — like in all the coccidia species studied by us — differ from one another in form, dimensions and number of merozoits contained in them. The smallest number of merozoits (4–22) was found in the schizonts of the first generation. This number was rising in the subsequent generations. So e.g. in the schizonts of the second generation the number of merozoits varies from 10–33 (19 on average), in the schizonts of the 3rd generation — from 12–33 (22 on average).

The development and structure of macrogametes and microgametocytes of *E. schamchorica* are in conformity with those signalized for other coccidia species of the genus *Eimeria*. It has been ascertained by us, that with the rise of the infection dose, the term of the prepatent period in *E. schamchorica* slightly shortens (for 6–10 hr). Shortening of the term of prepatent period in coccidia of rabbit, depending on the dose of infection was also observed by Cheissin (1946). According to his results, after increasing the infection dose the prepatent period is reduced in *E. performans* and in *E. media* to 5–7 hr, in *E. irresidus* to 8–18 hr, in *E. magna* to 8–12 hr, in *E. coecicola* to 2–3 hr, in *E. piriformis* to 5–7 hr. This may be accounted for by the fact that in the case of a high infection the peristaltics of intestine is intensified and the mature oocysts quickly leave the tissues and penetrate earlier into the external medium.

Summary

Endogenous development of *Eimeria schamchorica* occurs in the duodenum, jejunum and upper part of ileum of the red-tailed Lybian jird *Meriones erythrourus* Gray. All the intracellular stages lie in the proximal part of the host cell,

over the nucleus; no parasites were found in the connective tissue of the intestine.

Schizogonic development starts 5–10 hr following the infection with oocysts and proceeds as long as 9 days. Three asexual generations are distinguished in the life cycle.

Morphological patterns and sizes of the developmental stages are presented along with the schedule of successive phases of the parasite.

РЕЗЮМЕ

Эндогенные стадии развития *Eimeria schamchorica* локализуются в двенадцатиперстной кишке, тощей и в верхнем отделе подвздошной кишки. Все стадии эндогенного развития располагаются над ядром эпителиальных клеток ворсинок и ни одна стадия в соединительную ткань кишечника не проникает.

Шизогония у *E. schamchorica* начинается через 5–10 часов после заражения и продолжается до 120 часов, а гаметогония начинается на 4-е сутки и длится до 9 суток после заражения. В эндогенном периоде жизненного цикла *E. schamochorica* развиваются три генерации шизонтов.

В работе приводится детальное описание последовательных стадий роста и развития шизонтов разных генераций, макрогамет и микрогаметоцитов. Указаны сроки появления и развития всех стадий эндогенного развития, а также даны формы и размеры всех стадий.

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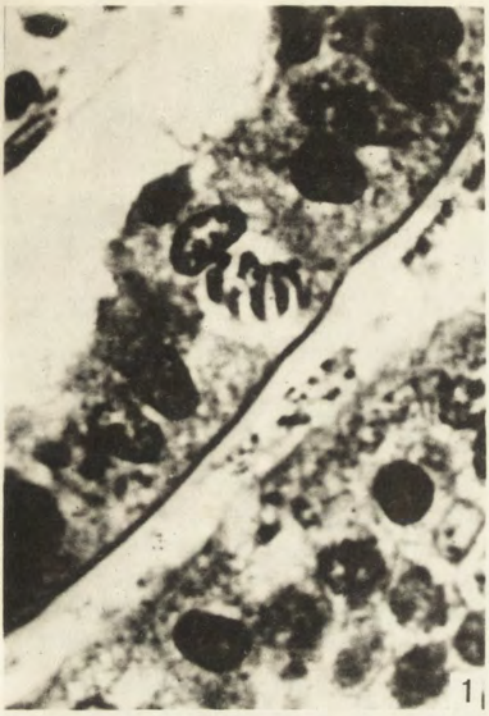
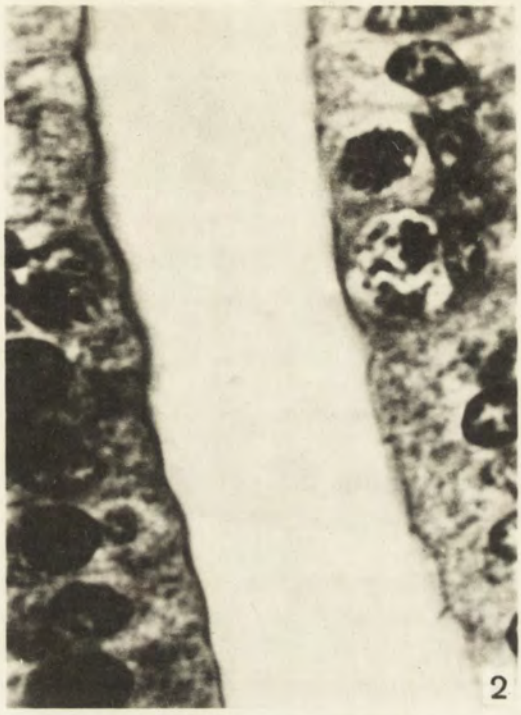
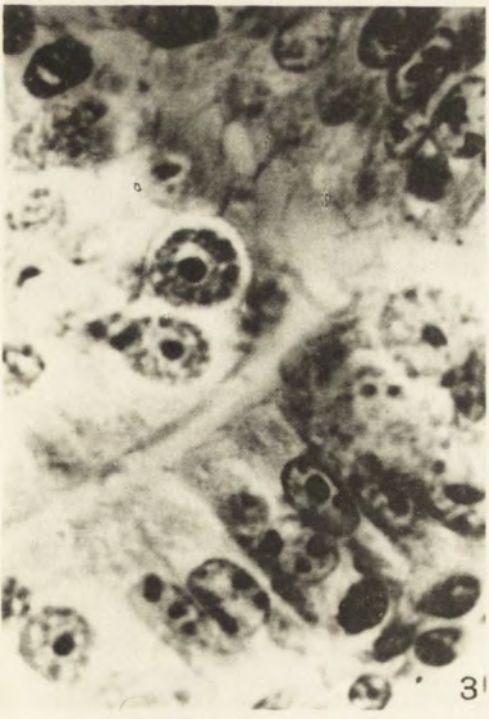
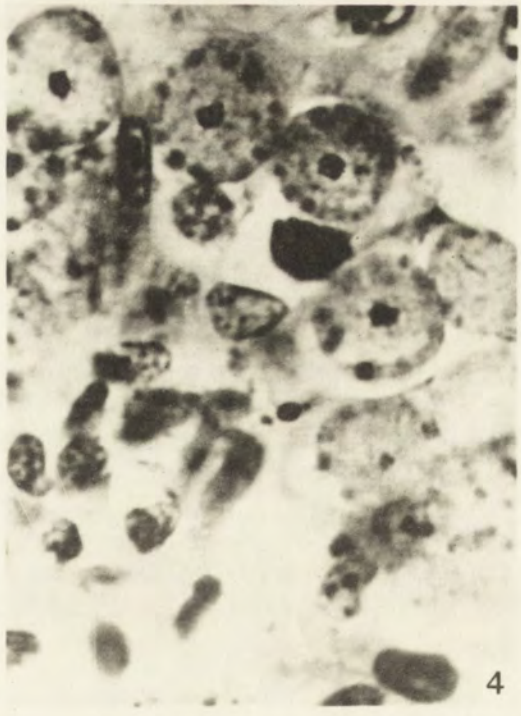
EXPLANATION OF PLATE I

1: A first generation schizont of *Eimeria schamchorica* in the intestinal epithelium of the red-tailed jird, 52 hr following the infection
2: Second generation schizonts of *E. schamchorica* in the intestinal epithelium, 71 hr following the infection
3: Macrogametes of *E. schamchorica* in the intestinal epithelium, 94 hr following the infection
4: The same as on photo 3, 116 hr following the infection









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ACTA PROTOZOOLOGICA

VOL. IX WARSZAWA, 15. XII. 1971 FASC. 14

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Chemotactic effects of cations and of pH on *Stentor coeruleus*

Wrażliwość chemotaktyczna orzęska *Stentor coeruleus*

The studies carried out till now on the phenomena of chemotaxis in *Protozoa* have concerned mostly *Paramecium*. The early observations were related to the tendencies of *Protozoa* to form gatherings in search for food.

The studies of Jennings 1904 were carried from this point of view, as he came to the conclusion that the "food-conditioned chemotropic" accumulation of protozoans takes place within the range of diffusion of slightly acid substances. This could be influenced by the CO_2 produced in medium as well by protozoa as by bacteria.

The experiments of Lozina-Lozinsky 1929 showed also the connection of "chemotropism" in *Paramecium* with the intake of food. The base of this conclusion was always the rise of formation rate of food vacuoles in the chemotropic positive substances and its fall in the chemotropic negative ones.

The phenomenon of positive chemotaxis towards food was also observed in *Dileptus* and *Peranema* (Chen 1950), in *Tetrahymena* (Corliss 1960) and in *Amoeba* (Shaffer 1957, Bovee 1960) although no chemical attractants of food for protozoa could be found.

The extensive studies on chemotaxis in *Paramecium* were carried out by Dryl (1952, 1959 a, b, 1960, 1961, 1963). He applied the method of quantitative study of this phenomenon which enabled him to analyse more exactly the course of this reaction. Dryl succeeded in determining the optimal pH for the chemotaxis reaction in *Paramecium* amounting 5.4–4.6. The results of his experiments concerning the chemotactic influence of chlorides of some alkalic metals upon *Paramecium*, permitted him to arrange cations according to the power of their negative chemotactic action: $Ba^{++} > K^{+} > Li^{+} > Mg^{++} > Na^{++} > Ca^{++}$.

In the present study an attempt was made to investigate the chemotactic sensitivity of *Stentor coeruleus* which, when compared with *Paramecium caudatum*, has a much more differentiated motor apparatus, and consequently a different manner of behaviour.

* Submitted in partial fulfillment of the requirements for the degree of Doctor of Biological Science in Nencki Institute of Experimental Biology under the guidance of Prof. dr. Stanisław Dryl.

Material and methods

The ciliates *Stentor coeruleus* were cultivated in the Pringsheim's medium of pH 7.2 at room temperature of about 22°C. Cultures were kept in high culture containers of a diameter approx. 20 cm, in conditions of a good gas exchange with the surrounding medium. Containers were closed with a sheer cork and kept in a moderate illumination, covered with parchment hoods. As food served *Tetrahymena pyriformis*.

For experiments the cultures was densified by using nylon sieves with approx. 35 μ diameter meshes because neither utilization of negative geotaxis nor densifying by centrifuge gave satisfactory results.

Experiments were executed by means of the photomacrographic technique described by Dryl 1958. A modification was applied consisting in the use of a deeper, several milimeter layer of liquid. In this way, adhering of *Stentor* to the bottom was avoided which occurred in the thin layers of liquid. A plate of dimensions 10 × 15 cm was applied.

On the test field (T) of a 1 cm^2 square the solution studied was dropped, while on the control field (C), the same quantity of the control solution was placed. After elapse of the fixed period of time (about 2–3 min), necessary for equal distribution of the animals in C and T, the protozoa on the plate were photographed 10 times at intervals of 15 sec. The time of exposure was 1/25 sec (Pl. I 1). Subsequently the movement of protozoans was registered by means of macrophotography in dark field (Dryl 1958, 1959). The time of exposure was 5 sec (Pl. I 2).

The quantitative data concerning the chemotactic sensitivity towards examined solution could be calculated by the analysis of the photographic negatives. By this method the percentage of individuals which failed to respond by the reaction of escape to the dropped solution was established. This percentage was related to the number of individuals in control which was treated as 100%. In several cases an unclear image was obtained because the stimulating substances with strong activity caused sometimes slackening of the forward movement of the ciliates under study. Only the careful analysis of character and rate of movement allowed to evaluate adequately the quantitative data.

In order to avoid any mistake, the behaviour of ciliates on the boundary of experimented substance in various ionic environments was studied by means of direct low-power microscope examination.

Basing on the data of previous investigations in this field especially those of Jennings 1905 and of Dryl 1961, the avoiding reaction of protozoans occurring at the contact with the stimulating solution, was assumed as the sign of negative chemotaxis.

In the case of positive chemotaxis the protozoan swimms without reaction into the tested medium but shows the avoiding reaction on the boundary between examined solution and experimental medium.

In the mass experiments, the number of individuals remaining within the reach of the action of the tested solution is the measure of the sensitivity degree to the definite chemical stimulus in the population of the protozoan species under study. It has been assumed therefore that the percentage of individuals remaining in the tested solution calculated in relation to the number of individuals in control one (assumed as 100%), may prove the degree of chemostatic sensitivity.

In many cases, the numbers of individuals in the tested and control fields were very significant and the action of the tested solutions was distinct. If the percentage of individuals in the square T fluctuated within the limits 85–115% related to the control square, the statistical significance of the difference between the number of individuals in C and T was calculated applying the Wilcoxon's signed-ranks test (after Siegel 1956). The "T" statistic was calculated at the postulated level of significance 0.05 and risk $p=0.5$. The non-significant results were interpreted as a lack of any chemotactic reaction, i.e. indifferent chemotactic response. The statistically significant result was

assumed as positive or negative chemotaxis according to its direction. The high number of individuals on the experimental plate (900–2000 on average) permits to consider the results as reliable.

The quantitative data were presented for C and T as the arithmetical means of the recorded numbers of individuals (m) and their standard deviations (σ) based on 10 times repeated experiments, When σ was higher than 5%, the series of experiments was repeated. Basing on the above means the percentage of non-responding individuals (%nR) was calculated.

Experiments

Chemotactic sensitivity of *S. coeruleus* to the changes of pH of the medium

In the initial studies, the tris-malate buffer solution was applied, acting at the pH range 5.2–8.6, embracing both the acid and alkaline ranges. It is only known buffer of such a broad pH range. However the analysis of movement of protozoa subjected to the action of this buffer for a more prolonged time (approx. 1 hour) revealed some far advanced disturbances evoked by the toxic action of maleic acid. It was therefore necessary to apply another buffer. The citric-phosphate buffer was selected for the study of pH range 3.8–7.0 and Tris+HCl for pH 7.2–9.0.

Experiments were executed in two steps. The first one concerned the study of protozoa sensitivity to the pH changes in the acid range. For this 1 milimolar solution of citric-phosphate buffer (Dryl 1959) was used with addition of 1 mM $CaCl_2$ per 1 l of solution. The solution of pH 6.96 was simultaneously the initial medium (in which *Stentor* was kept before starting the experiment) and the control solution in this cycle of experiments.

The second step of experiments concerned the study of sensitivity to the changes of pH in the alkaline range. The experiments were based on 1 milimolar solution

Table 1

Chemotactic sensitivity of *S. coeruleus* to changes of pH

Chemical composition of solution	pH	C m σ	T m σ	%nR	Significance of difference between T and C
citric-phosphate buffer +1mM/l $CaCl_2$	3.8	73.8+2.6	1.0+0	1.3	sign.
	5.2	77.1+2.7	3.5+0.5	4.5	sign.
	6.0	46.0+0.8	30.3+1.0	65.7	sign.
	6.9	56.8+1.5	55.0+2.1	96.4	non-sign.
Tris/HCl buffer+ +1 mM/l $CaCl_2$	7.2	52.8+1.4	53.1+1.0	100.5	non-sign.
	7.7	51.6+1.6	55.0+1.7	106.6	sign.
	8.4	77.6+1.1	60.5+1.7	78.0	sign.
	9.0	46.3+1.1	13.9+0.4	29.9	sign.

C – control field, T – test field, m – arithmetic mean from 10 experiments, σ – standard deviations, %nR – percentage of non-responding individuals to chemotactic sensitivity (number of individuals in control field = 100%).

of Tris+HCl buffer with addition of 1 mM$CaCl_2$ per 1 l of solution. In this case, the solution of pH 7.2 was the initial medium for experiments and served as control solution for all the experiments in this series.

Considering the fact that the pH range of buffers was difficult to be kept constant, some deviations from the assumed value of pH 7.2 were unavoidable. Therefore before every experiment in which the Tris+HCl solution of pH 7.2 was applied, the pH of medium and of the solution studied was controlled with pH-meter. If the deviations were significant, new solutions were prepared.

It became evident that ciliates manifest a typical negative chemotactic reaction to the highly acid medium whereas in the alkaline range parallel to the rise of pH, the chemotactic sensitivity to this stimulus increases (Table 1).

Chemotactic sensitivity to the ions of some alkaline metals

In all the experiments of this cycle, the solutions studied contained chlorides of the cations under study. The initial concentration with which the experimental cycle has begun, was 1 milimolar solution. Considering the results of this preliminary experiment, the subsequent ones were carried out with more diluted or more concentrated solutions. If at the concentration c = 1 mM/l a typical negative chemotaxis occurred, the solution was diluted until the chemotactic reaction became indifferent. In this way the thresholds of chemotactic sensitivity of protozoa to different ions of alkaline metals was determined.

As a threshold of chemotactic sensitivity, the concentration or range of concentrations has been assumed in which the negative chemotaxis appeared.

In the case of K^+, the threshold of sensitivity is in the concentrations 0.125–

Table 2

Chemotactic sensitivity of *S. coeruleus* to ions K^+

Concentration KCl in mM/l	C m σ	T m σ	% nR	Significance of difference between T and C
0.125	28.5+2.4	28.2+1.2	99.0	non-sign.
0.25	23.3+3.4	17.8+2.0	76.5	sign.
0.5	26.0+4.3	15.5+2.0	59.6	sign.
1.0	28.7+3.7	3.0+0.6	10.4	sign.
2.0	32.3+3.3	2.4+0.4	7.4	sign.
4.0	30.3+3.1	2.2+0.8	7.2	sign.
8.0	26.1+2.7	1.3+0.1	4.9	sign.
16.0	12.9+2.0	4.2+0.9	32.5	
32.0	15.9+2.9	6.3+1.8	39.6	
64.0	20.9+3.6	8.9+0.8	42.6	

Explanations – see Table 1.

0.250 mM/l. With the rise of K^+ concentrations in the tested solution — the negative chemotactic reaction increases gradually (Table 2).

The registration of movements of protozoa swimming in the zone of diffusion of KCl solution presents a typical phenomenon of negative chemotaxis. On the boundary of contact with the solution studied, protozoa retreat and swim in the new direction.

Experiments were also performed with higher concentrations of KCl (16 mM/l, 32 mM/l, 64 mM/l). However the analysis of movement distinctly showed disturbances which made impossible escape from the region of tested solution. High concentrations of potassium paralyse very quickly the motor system of protozoan and make it incapable to a normal response to chemotactic stimuli. The partial immobilization of protozoa within the area of action of high KCl concentration could give illusion of occurrence of positive chemotaxis. However the phenomenon of positive chemotaxis could be excluded taking in account the established facts of kinetic disturbances in the form of immobilization or very conspicuous slackening of the progressive movement:

Na^+ ions

The negative chemotaxis in the case of Na^+ appears at concentration range 0.25–0.5 mM/l. At this range the transition from the positive chemotaxis to the negative one is observed which is indicated by the statistically significant difference between T and C (Table 3). At NaCl concentration of 64 mM/l the negative chemo-

Table 3

Chemostatic sensitivity of *S. coeruleus* to ions Na^+

Concentration NaCl in mM/l	C m σ	T m σ	% nR	Significance of difference between T and C
0.25	62.9+3.3	74.7+3.8	118.9	non-sign.
0.5	76.1+3.6	63.7+4.4	83.7	sign.
1.0	53.5+5.0	42.4+3.1	79.2	sign.
2.0	47.3+5.0	26.1+1.3	55.2	sign.
4.0	36.9+2.6	16.9+1.7	45.8	sign.
8.0	40.2+5.0	17.5+3.1	43.5	sign.
16.0	31.3+4.5	2.6+0.9	8.3	sign.
32.0	35.5+2.2	2.2+0.7	6.2	sign.
64.0	54.7+2.6	5.0+1.0	0.9	sign.

Explanations – see Table 1.

taxis was nearly 100%, i.e. no one individual is present in the range of the stimulus activity. The analysis of movement registration failed to reveal any incidental locomotor disturbances.

Ca^{++} ions

As concerns Ca^{++} ions, the results indicate chemotactic threshold zone in the concentrations 1.5–2 mM/l. The negative chemotactic reaction rises parallel to increase of concentration of $CaCl_2$ (Table 4). No other movement disturbances connected with exposure of protozoa to calcium ions were observed.

Table 4

Chemostatic sensitivity of *S. coeruleus* to ions Ca^{++}

Concentration $CaCl_2$ in mM/l	C m σ	T m σ	% nR	Significance of difference between T and C
1.5	47.9+3.5	49.7+3.1	109.7	non-sign.
2.0	34.5+2.8	26.3+1.4	76.2	sign.
3.0	27.3+2.6	18.6+1.6	68.1	sign.
5.0	59.0+3.0	32.7+2.6	55.4	sign.
7.0	43.1+2.8	21.3+1.9	49.4	sign.
17.0	25.0+1.5	9.5+1.2	38.0	sign.
33.0	25.6+2.0	5.9+0.9	23.0	sign.
65.0	46.1+5.0	3.6+0.9	7.8	sign.

Explanations – see Table 1.

Mg^{++} ions

Mg^{++} ions evoke the negative chemotaxis beginning with the concentration 0.25 mM/l. At this concentration the statistic difference between T and C is not significant. Therefore the area of the chemotactic sensitivity threshold of S. *coeruleus*

Table 5

Chemotactic sensitivity of *S. coeruleus* to ions Mg^{++}

Concentration $MgCl_2$ in mM/l	K m σ	T m σ	% nR	Significance of difference between T and K
0.25	39.3+4.2	38.5+3.1	98.5	non-sign.
0.5	46.6+3.1	29.4+3.0	63.1	sign.
1.0	41.5+2.0	24.6+2.4	59.3	sign.
2.0	30.9+1.9	17.9+2.5	57.9	sign.
4.0	31.4+2.2	12.4+1.6	49.2	sign.
8.0	48.8+2.8	12.1+2.5	24.8	sign.
16.0	31.4+2.2	6.2+1.1	19.8	sign.
32.0	42.2+3.3	6.7+1.5	15.8	sign.
64.0	42.4+2.8	4.0+0.8	9.4	sign.

Explanations – see Table 1.

for $MgCl_2$ is in the concentrations 0.25–0.5 mM/l (Table 5). Addition of $MgCl_2$ solution in high concentration to the medium containing protozoa does not evoke movement disturbances which would complicate the observation and evaluation of the chemotaxis phenomenon.

Summary of results and discussion

The results gained by the study of chemotactic sensitivity to the changes of pH in *Stentor coeruleus* are summarized in the diagram (Fig. 1). The typical negative chemotactic reaction to the highly acid medium changes its character in dependence

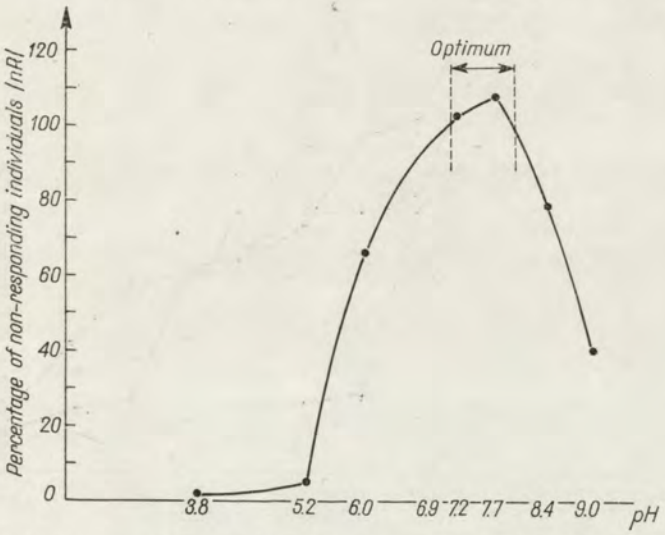

Fig. 1. Chemotactic sensitivity of *Stentor coeruleus* to changes of pH

on the value of pH. The range of pH 6.9–7.7 proved to be optimal for *S. coeruleus*. This follows from the quantitative data which indicate the occurrence of positive chemotaxis in these conditions.

It is worth being mentioned that the observations of Strom 1926 indicate the medium of pH 6.2–8.0 as the most advantageous for the growth of *Stentor* sp. whereas Sleigh observed that the optimum pH for *Stentor polymorphus* is 6.8. Chambers and Kao 1952 ascertained that the cytoplasm of *Stentor* sp. is of pH 6.8 and of its macronucleus — 7.6. All those data concern the pH values between 6.2–8.0 — i.e. near the optimum pH for chemotaxis in *Stentor coeruleus*.

The different sensitivity to the various pH of medium may presumably be a characteristic feature of species but surely not of the genus. This is also indicated by the distinct divergence of results concerning the optimum of pH for *Stentor coeruleus* and those ascertained by Dryl 1952 a, 1961 b for another ciliate —

Paramecium caudatum in which the optimal chemotactic pH is embraced by the limits 5.4–6.4.

The results of experiments with the chlorides K^+, Na^+, Ca^{++}, Mg^{++} are summarized on the diagram Fig. 2.

A distinct tendency to intensification of the negative chemotactic reaction is observed when the concentration of the ions rises. The threshold areas marked on the diagram occur at the concentrations where the indifferent chemotactic reaction passes to the negative one.

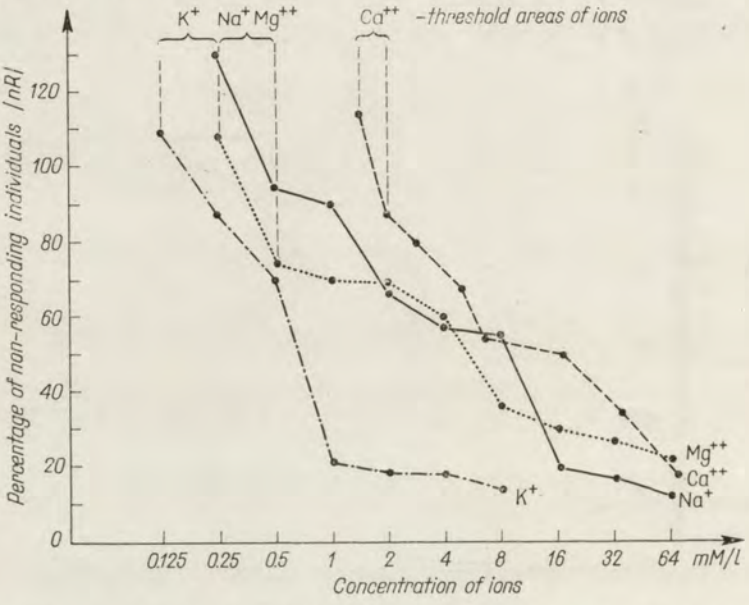

Fig. 2. Chemotactic sensitivity of *Stentor coeruleus* to K^+, Na^+, Ca^{++}, Mg^{++}

Potassium ions at high concentrations involve strong disturbances in the movement which make the observation of chemotaxis phenomena impossible. The other ions do not evoke changes of this type. Considering the power of the negative chemotactic activity on *S. coeruleus*, the ions may be arranged into a row — from those of a strongest to a weakest action: $K^+ > Na^+ > Mg^{++} > Ca^{++}$.

The comparison of the results achieved in the present study on *S. coeruleus* with similar research of Dryl (1959 a, b, 1961, 1963) on chemotaxis in *Paramecium caudatum* proved to be interesting. The range of sensitivity for *Paramecium* as stated by Dryl was: K^+, $Mg^{++} > Ca^{++} > Na^+$.

As follows from the above comparison, the essential differences concern the position occupied by Ca^{++} and Na^+. This may be the result of application of different buffers. In the case of *Paramecium* it was sodium phosphates and citrate. A more prolonged adaptation to the medium containing sodium ions could cause the change

of sensitivity threshold of this ion. This may act on the postulated system of ionic equilibrium in the membrane of protozoan which presumably determines its reactions to definite stimuli. The application of Tris/HCl buffer seems to be more adequate in this respect.

Summary

The phenomena of chemotaxis have been studied by quantitative method and the following range of negative chemotactic sensitivity of *Stentor coeruleus* to the cations of biological importance was ascertained: $K^+ > Na^+ > Mg^{++} > Ca^{++}$.

This array indicates a very similar manner of reaction to cations in *Stentor* and in *Paramecium*. Essential difference concerns only the position of Na^+ to which *Stentor* is much more sensitive than *Paramecium*.

The optimum pH for the positive chemotactic reaction of *Stentor coeruleus* was also determined. It is comprised in the neutral range of (pH 6.9–7.7), in contrast to *Paramecium* in which the chemotactic optimum is in the acid pH zone. The obtained results indicate the existence of essential differences in the reaction of those ciliates to the changes of pH of the surrounding medium. Presumably the optimal conditions of adsorption on the cell membrane concern in *S. coeruleus* the neutral zone and in *Paramecium* — the zone of acidic pH.

STRESZCZENIE

Stosując metodę ilościowego badania zjawiska chemotaksji, ustalono następujący szereg ujemnej wrażliwości chemotaktycznej *Stentor coeruleus* na kationy o znaczeniu biologicznym: $K^+ > Na^+ Mg^{++} > Ca^{++}$.

Uszeregowanie to wskazuje na bardzo podobny sposób reagowania na kationy *Stentora* i *Paramecium*, a istotna różnica dotyczy jedynie pozycji Na^+, na którym *Stentor* jest znacznie bardziej wrażliwy niż *Paramecium*.

Określono również optimum pH dla reakcji chemotaksji dodatniej *Stentor coeruleus*, mieści się ono w przedziale obojętnym (pH 6.9–7.7) w odróżnieniu od *Paramecium*, u którego optimum chemotaksji mieści się w strefie kwaśnej pH. Wyniki te wskazują na istnienie istotnych różnic w reagowaniu tych orzęsków na zmiany pH środowiska otaczającego. Prawdopodobnie optymalne warunki adsorpcji na błonie komórkowej *S. coeruleus* dotyczą strefy obojętnej, zaś u *Paramecium* strefy kwaśnej pH.

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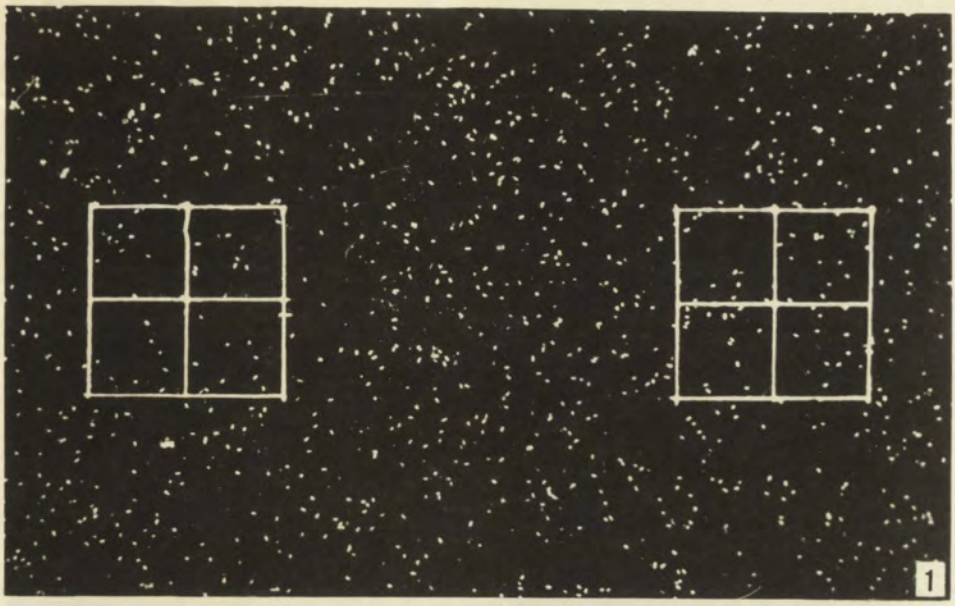
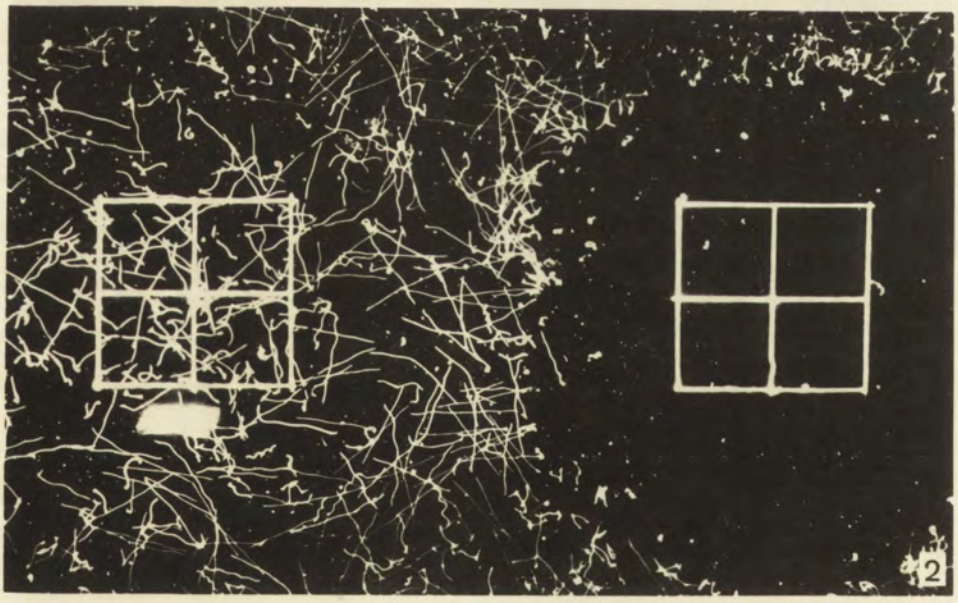
EXPLANATION OF PLATE I

1: Experimental plate with dropped protozoa material — initial state. ×3
2: Registration of movement during typical negative chemotaxis in *Stentor coeruleus*
Control square K — left, test square T — right





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