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Zdzisław RAABE

Ordo *Thigmotricha* (*Ciliata — Holotricha*)
V
Familiae *Hysterocinetidae* et *Protoanoplophryidae*

I am concerned in the fifth part of my monographic study with two strictly defined families, namely the family *Hysterocinetidae* Diesing, 1866 and fam. *Protoanoplophryidae* Miyashita, 1929 (pro subfam.). Both these families are connected by the fact that their representatives are not living in the mantle cavity or on the gills of their hosts but in their intestine; they reveal a strong adaptation to the life in this medium. Nevertheless both these families evaluated in different directions and they are not connected by closed relations or affinity.

Familia *Hysterocinetidae* Diesing, 1866

syn.: *Ladidae* Cépède, 1910; *Ptychostomidae* Cheissin, 1932.

Investigations on the representatives of the family *Hysterocinetidae* Diesing date from 1860, when Stein described the first species from the intestinal tract of *Tubifex tubifex* Müller (=*Saenuris variegata* Hoffm.) under the name of *Ptychostomum saenuridis*. Next year (1861) the same author gave a description of another ciliate taken from the intestinal tract of *Paludina impura* Drap. and *Paludina similis* Drap. [=*Bithynia tentaculata* (L.) and *Bithynia leachi* (Shepp.) on the terrain examined by Stein] which he included in his genus *Ptychostomum* as *Ptychostomum paludinarum*. The latter species was excluded from the genus *Ptychostomum* by Diesing 1866 and transmitted to a new genus *Hysterocineta* as *H. paludinarum* (Stein). At the same time Diesing established a new family *Hysterocinetinea*, including this genus.

Quite independent of the above investigations, Vejdovsky 1882 gave description of a new species of ciliates from the intestinal tract of *Phraetothrix pragensis* (Vejd.), named *Lada wrzesniowskii*. The description of Vejdovsky shows that he had not an clear idea of the morphology of this ciliate and his drawings represent *Lada* rather in fantastic way. Thus the data given by him could not indicate its systematic position.

Bütschli mentions the described species in his monograph (1887–1889), treating them, however, quite separately. He includes the genus *Ptychostomum* in the family *Microthoracina* Wrześn., suborder *Aspirotricha* Bütschli. As regards the species *Hysterocineta paludinarum* (Stein) he suggests that it belongs to the genus *Ancistrum* Maupas and locates it in the same family. Regarding both these genera, Bütschli makes clear the mistake of Stein, who treats the sucker of these forms as the cytostome and the cytostome as the cytopyge (Bütschli, p. 1709). Bütschli places the species *Lada wrzesniowskii* Vejd. quite separately and far in the system including it, provisionally however, in the family *Opalinina* Stein, suborder *Aspirotricha* Bütschli, together with *Opalina*, *Opalinopsis*, *Anoplophrya*, *Hoplitophrya* and *Discophrya*!

The genus *Lada* was treated similarly by Cépède 1910 who placed it amongst *Astomata* in a separate family *Ladidae* Cépède comprising this genus only, next to the family *Discophryidae* (Cépède, p. 568). It is obvious that the systematic position of *Lada*, as given by these two authors, was a mistake which resulted from the description of Vejdovsky, who regarded the cytostome of the ciliate as the cytopyge, but did not consider, according to Stein, the sucker to be the cytostome. Hence would result the conclusion that *Lada* is a species lacking a cytostome.

During the years 1925–1935 many species of ciliates belonging to the family were described. In connection however with the fact that the genus *Hysterocineta* Diesing had been entirely forgotten and that the synonyms *Ptychostomum* Stein and *Lada* Vejdovsky missed the attention of the authors there arose many systematic divergencies. The species were included in the genus *Lada* Vejdovsky (after explaining mistakes in the description) or in the genus *Ladopsis*, created by Cheissin 1928.

Thus the following species from the genus *Lada* were described as: *L. pygostoma* Rossolimo, 1925, *L. issa* Kijenskij, 1925, *L. vejdovskyi* Kijenskij, 1925, *L. tanishi* Miyashita, 1927, as well as *L. assymetrica*, *L. baicalensis* and *L. elongata*, described by Cheissin 1928. Rossolimo 1925, Studitsky 1930 and Cheissin 1932 stated that the genera *Ptychostomum* Stein and *Lada* Vejdovsky were identical and all species described as *Lada* were included in the genus *Ptychostomum*. Subsequently several other species as: *P. chattoni* Rossolimo, 1925/6, *P. rossolimo* Studitsky, 1930, *P. limnodrili* Cheissin, 1932 and *P. bacteriophilum* Miyashita, 1933 were described.

From the genus *Ladopsis* Cheissin the following were taken under consideration: *L. benedictiae* Cheissin, 1928 and *L. bithyniae* Nikolajeva, 1929. The latter, according to Jarocki 1939 is identical with *Hysterocineta paludinarum* (Stein). Thus, as both species correspond to one genus, the name *Ladopsis* Cheissin should be considered as a synonym of *Hysterocineta* Diesing. Jarocki also restored the former name *Hysterocinetidae* (= *Hysterocinetinea* Diesing) to denote the family instead of *Ladidae* Cépède or *Ptychostomidae* Cheissin.

Heidenreich 1935 made an attempt to bring order to the nomenclature of the genus *Ptychostomum* Stein. He gave the correct definitions of the species: *P. saenuridis*

Stein, *P. issum* Kijenskij and *P. chattoni* Rossolimo = *P. vejdovskyi* Kijenskij. He described a new species *P. rhynhelmis* and excluded as a new species *P. iliodrili*, the forms described by Maupas and Rossolimo as *P. saenuridis*. In other words he described the species *P. lumbriculi* and included the ciliate described by André 1915 as *Anoplophrya simplex* in the genus *Ptychostomum*.

Before the II World War was issued the work of Beers 1938 describing *Hysterocineta eiseniae* from *Eisenia lonnbergi* and the note of Wichterman 1939 concerning the new species of *Hysterocineta* from *Pontodrilus bermudensis* — so both species from *Oligochaeta* (!). Finally Raabe 1939 on the basis of the examinations of two species namely *Hysterocineta paludinarum* (Stein, 1860) and *Ptychostomum saenuridis* Stein, 1860 give the morphologic, comparative description of both compared species in the trophic stage and during the divisional period drawing a special attenton to their argyrophilic system which appeared as a very essential morphologic characteristic.

On the basis of these considerations Raabe 1949 published an attempt of a revision of the family *Hysterocinetidae* (the work was finished in 1939, but its print was stopped by the war and the occupation of Poland). The definition of the family *Hysterocinetidae* was stated in this revision as well as of some genera belonging to it, also the place of the family in the *Thigmotricha* system. Beside the genera existing formerly: *Ptychostomum* Stein (=*Lada* Vejdovsky) and *Hysterocineta* Diesing (=*Ladopsis* Cheissin), Raabe 1949 separated 3 new genera: *Protoptychostonum*, *Cotylothigma* and *Kysthothigma*. The genera *Protoptychostomum* and *Cotylathigma* were approved by the latter authors, *Kysthothigma* was not examined afterwards.

Over the last two decennies three new genera have been included to the family *Hysterocinetidae* namely *Elliptothigma* Meier, 1954, *Craticuloscuta* Kozloff, 1965 and *Epicharocotyle* Kozloff, 1965.

The research of the Puytorac 1957 a, b, 1968 a, c, d on *Hysterocinetidae* were revealed as crucial for the problem. He enriched the list of species of *Hysterocinetidae* with 5 species from *Oligochaeta* from the Ohrid Lake (in the genera *Ptychostomum* and *Cotylothigma*) and 14 species from Gabon (in genera *Hysterocineta*, *Ptychostomum*, *Epicharacotyle*, *Craticuloscuta*, *Protoptychostonum* and in new genera *Thurstonia*, *Preptychostomum* and *Kozloffia*), besides it he based the taxonomy on new elements of the structure of the sucker and on many other morphologic characters. De Puytorac 1968 d in the achieved revision of the family created new genera: *Coelothigma* for *P. canalis* Katashima, 1952, *Thurstonia* for *T. kaczanowskii* de Puytorac, 1968, *Preptychostomum* with *P. almae* de Puytorac, 1968 and *Kozloffia* with *K. catenula* de Puytorac, 1968. In this study I would like to separate three new genera namely: *Puytoracia* g. n. for 3 species described by de Puytorac 1968 in the genus *Epicharocotyle*, *Drilocineta* g. n. for *Hysterocineta libyodrili* de Puytorac, 1968 and *H. pontodrila* Wicht., 1939, and *Taeniocineta* g. n. for *Hysterocineta eiseniae* Beers, 1938.

Many authors were concerned on the problems of the systematic situation of the family *Hysterocinetidae* Diesing; they ranged this group in different ways even including it to *Peritricha* (as Miyashita 1927) or *Astomata* (as Cépède 1910). However many authors compared *Hysterocinetidae* to the particular representatives or groups of *Thigmotricha* even on the basis of the outward similarity. A more motivated point of view represents Rossolimo 1925 writing:

"Je suppose que les *Thigmotricha* Ch. et Lw. et nos *Ptychostomum* présentent deux séries évolutives paralleles qui émanent d'un seul groupe d'organismes libres. Les premiers rattachèrent leur existence aux mollusques en donnant une série de commensaux et de parasites; les derniers s'adaptèrent au parasitisme dans l'intestin des Oligochètes, en s'y développent des organes fixateurs compliques et variés en structure" (p. 230). This point of view accepted also Raabe 1949 comparing the proper orientation of the body of *Hysterocinetidae*. He recognized the flattening of their body as latteral, the side with the thigmotactic apparatus that is the sucker as the left side, the mouth as moved to the ventro-posterior or posterior margin; the cytostome is directed in result to the dorsal margin of the body. This orientation was recognized by many authors, mainly by de Puytorac 1957, 1968.

Considering the systematic position of *Hysterocinetidae* many authors indicated and still indicate their multiform reference to *Astomata*, for the reason of the similarity of the primitive forms of the adhesive apparatus of the both groups (i.e., *Protoptychostomum*) or for the reason of the general shape of the body, especially its elongation or for the reason of the polymerization of the contractile vacuoles, or finally for the reason of the catenulare multiplication (which occurs, i.e., in *Elliptothigma* or *Kozloffia*). These characters may be virtually considered as arguments for the affinity of both groups but may be also considered as a manifestation of the convergention in view of similar life conditions and similar ways of adaptation. I intend to discuss once more this problem in the further parts of this paper when I will refer the systematic affinities of *Thigmotricha*.

Hysterocinetidae constitute a very cohesive and well outlined group therefore its general description could be relatively exact and exhausting (Fig. 1).

The body of *Hysterocinetidae* according to the accepted orientation is strongly flatened laterally, especially in the anterior part, where, at the left side, a peculiar thigmotactic apparatus is situated in the form of a fairly or strongly developed sucker. This apparatus in the plesiomorphic forms in this respect consists of an elongated zone in the form of invented V or U arranged along the anterior margin of the body, embracing by its arms the anterior segments of the kineties of the left part of the body. However the naked zone increases intensively, and especially its arms with a backwards orientation are somewhat enlarged. Its ends tending to each other cutting and closing a more or less great number of segments of kineties which form the ciliature of the inner groove of the sucker. In the posterior part of the sucker, or rather outside of it additional flanges develop in some genera intensifying its activity.

The surface of the sucker is covered by a thinner or thicker, more or less regular net apparent when treated with $AgNO_3$ or by similar reagents. The sucker is reinforced by a system of fibres formed in various ways, running subpellicularly, in one layer, parallely to the anterior margin of the sucker (*Hysterocineta*), but in other cases occurs a complex system of fibres running at many levels and in different directions.

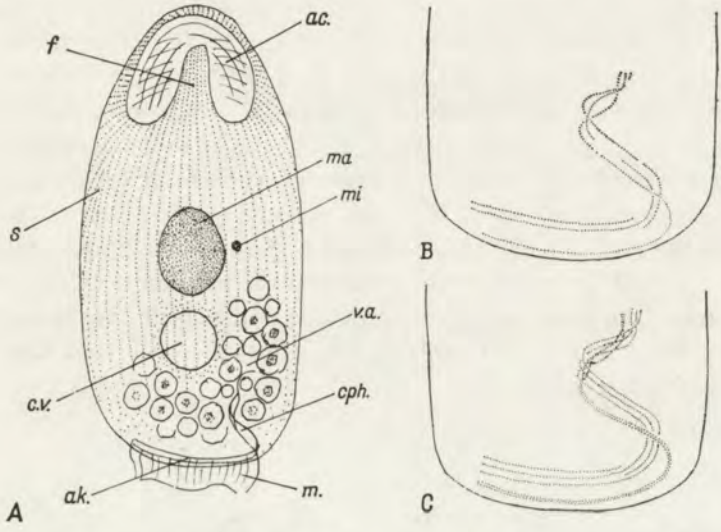

Fig. 1. The general characteristic features of *Hysterocinetidae*: A — scheme of the body: ac — sucker, f — the ciliated furrow of the sucker, k — kineties, s — système sécant on the ventral margin, ps — peristome, cph — cytopharynx, ma — macronucleus, mi — micronucleus, c.v. — contractile vacuole, v. a. — vacuolized area, a. k. — adoral kineties, m — membranelles (from Raabe, modified); B — scheme of the structure of the buccal apparatus based on the data of light microscope investigations; C — the same on the basis of electron microscope investigations (from de Puytorac)

These problems were examined by many authors but recently de Puytorac did it especially exactly presenting in his papers some very interesting pictures of these arrangements in various species of the genus *Ptychostomum*, *Epicharocotyle* and others. Finally, as it is, i.e., in *Cotylothigma*, the skeletal apparatus could take the form of a coherent construction of a ring with two long thorns oriented backwards.

The ciliature of the whole body of the representatives of the family *Hysterocinetidae* is markedly dense but delicate. The number of kineties on both sides of the body ranged from 80 to 250 generally depending on the size of the body. The both parts of the ciliary system, the left and the right one, separates the anterior suture in the anterior part of the body, the anterior margin of the sucker constitutes the margin of the sucker which is reached by the kineties of the right side of the body. Along the ventral margin the kineties of both sides converge somewhat concurrently that means some of them snap, forming in some sense a unipolar "Système sécant". The kineties of the left and right part of the body run rather consistently and parallely

to one another. The regular kineties arrangement disappears usually in the posterior part of the body, the kinetosomes here distinctly more rare are arranged within an irregular argyrophilic net. The adoral kineties run on this area working as a posterior suture. The adoral kineties are therefore not parallel to the kineties of the general ciliature, they are rather perpendicular to then and in the state of rest they do not show any relationship to the general kineties arrangement.

The number and the structure of the adoral kineties in *Hysterocinetidae* was and still is the subject of different interpretation. At first after the researches of Raabe 1939, 1949 it seems clear that in all *Hysterocinetidae* occur three adoral kineties: two of them are limiting the peristomal gutter from the left posterior side, one kinety — from the right. These kineties enter to the peristomal funnel — the cytopharynx and run spirally over its walls. The adoral apparatus of *Hysterocinetidae* constructed in this way is placed as a rule on the posterior margin of the body so that the cystostome is oriented dorsally. The divisional stages could explanate the original ventral situation of the mouth and the peristome. During these stages the peristome of the proter and presumably also of the opisthe are set on the ventral margin and are oriented backwards by the cytostome and then take a definite posterior position (Fig. 2).

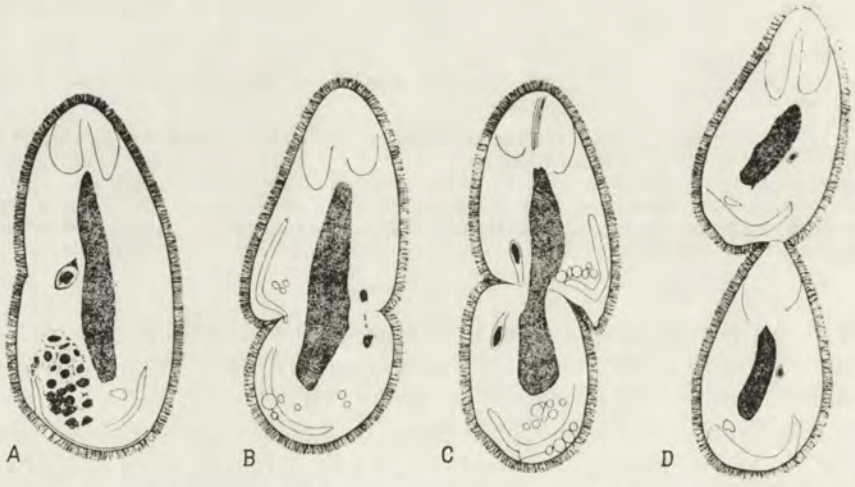

Fig. 2. Division and stomatogenesis in *Hysterocinetidae*: A–D — the phases of division and stomatogenesis in *Drilothigma pontodrila* (from Wichterman)

Kozloff 1960 added additional elements to the studies on the adoral apparatus of *Hysterocinetidae* and several authors followed him suggesting that the first adoral kinety from the left does not invade inside the cytopharynx but snap on its margin where it is replaced by the additional kinety interlocking with other kineties in a spiral inside the cytopharynx. Finally de Puytorac 1968 denies also this picture; he stated that at the left side of the peristome run not two but three kineties, and

after the exchange discovered by Kozloff 1960 also three kineties, invade the cytopharynx. The right, anterior kinety corresponding to U. M. (or to kinety 1 according to Chatton et Lwoff see part I p. 121, part II p. 22) is a diplokinety. De Puytorac 1968 based his results on the examination of the preparations in the electronic microscope and he proved it on the photographs (Fig. 1B, C).

Therefore the adoral kineties of *Hysterocinetidae* constitute a quite peculiar adoral apparatus preserving as it seems a similar character in the whole family. The differences may concern the difference in the length and width of the outside peristomal gutter and of the depth of the inside funnel — cytopharynx. I intend in one of the final parts of my study to refer once more the detailed structure of the adoral kineties in *Hysterocinetidae* and their stomatogenesis, finally I would like to compare the relations among the *Thigmotricha*.

The nuclear apparatus of *Hysterocinetidae* is not highly differentiated, the macronucleus is elipsoidal or elongated, the elipsoidal Ma is arranged perpendicularly or parallely by its longer axis to the body axis, the elongated Ma — always parallelly. It occurs 1 or 2 Mi next to Ma, dorsally as a rule but sometimes in its cavity. In the catenulitive forms (*Elliptothigma*) Mi is arranged in the posterior part of Ma and in the posterior part of the body.

The osmoregulative excretory system constitutes the more often one C. V. arranged in the back of the ciliate's body, sometimes there are two of them (*Protoptychostomum*), and even a larger number; they are arranged in one or two rows at the both sides of Ma (*Elliptothigma*, *Craticuloscuta*, *Epicharocotyle*).

In most species occurs a large, distinctly outlined sphere of food vacuoles in the posterior part of the body. It has an elipsoid shape or it protrudes to the anterior part of the body, one (the dorsal one) or two arms embracing the nuclear apparatus and the contractile vacuole.

The plasma of *Hysterocinetidae* is transparent in general and slightly granulated, the pellicule seems thin and delicate — these features allow for plasticity and elasticity of the body and changes of the shape of the sucker. In some species in which the sucker is specially strong and stiff some plasmatic folds occur behind it and therefore the adhesion of the animal to the base is easy.

The subpellicular grains with an eventually secretive character are only slightly investigated. It seems that this role may be played by the granulations arranged on the sucker, observed and signalized by some authors. De Puytorac 1963 determines them by a not obligatory name of "protrichocystes"; de Puytorac 1968 b supposed that these are probably mucocysts and prepares another paper on this topic. These bodies are sometimes very regularly arranged on the naked field of the sucker and in some way typical of the particular species.

The multiplication of the *Hysterocinetidae* has the most often the shape of a transversal division; its result is the arising of two equal individuals. This fact was observed by many authors who rather unanimously agree and indicate that at first this division seems unequal because the dividing fissure runs at the ventral

side, in the back of the dividing individual. Only in *Elliptothigma* and *Kozloffia* a distinctly unequal division has been observed, virtually a gemmation of the smaller opisthe from the body of a larger proter. This division leads often to catenulation when the counting of the opisthes (tomits, satelits) occurs faster than their detachment. Then arises a chain of tomits drawn by a large proter (primite).

In the divisional processes attention must be paid on the processes of arising of the sucker of the opisthe produced in the body of the ancestral individual completely at the base of neoformation. Very good results, concerning the cortical system, are achieved by Klein's silver method applied mainly by Raabe 1939, 1949.

The originating of the naked surface of the sucker is initiated by a gradual backwards regression retrogradation of kineties of the left side of the body and behind next to the divisional groove and the development of the net with the more or less regular meshes as in *Hysterocineta* (Fig. 3). In the species having a closed sucker (i.e., *Ptychostomum*) the arms of the sucker oriented backwards develop strongly and finally they reach one another, closing the cut segments of kineties inside the sucker — Fig. 3. De Puytorac 1957 observed similar processes in *Ptychostomum chattoni* Rossolimo.

However almost quite unknown are the processes of the origin of the skeletal system of the sucker, its composed system of layers of the fibers or of the compact skeleton. Only de Puytorac 1957 (p. 243–245) reports that anyway some of the fibres in *Ptychostomum chattoni* Rossolimo arise in connection with the kineties on which the kinetosomes disappear; the basic fibre is strengthened. This continuation of kinetodesmata by the fibres is suggested also by Kaczanowski 1963 in *Hysterocineta*. However this suggestion seems acceptable for some fibres only in the skeletal system of the sucker similarly as it is in many *Astomata* perhaps only in the more superficial ones. Quite unknown seems the origin of these skeletal forms which occur in the sucker of *Cotylothigma*.

The oral and adoral apparatus of the *Hysterocinetidae* undergoes during the division some interesting transformative processes, but mainly neoformative ones. There is no doubt as concerns the buccal apparatus of the proter setting at rather long distance from the old peristome of the dividing individual. It seems that two forms appear independently and parallelly: the subpellicular strip which would give the inner buccal apparatus and mainly its cytopharynx, and over it a ribbon-like, superficial organization field on which arise the adoral kineties. The way of connection of these parts and of immersion in the cytopharynx and finally of twisting of adoral kineties is still quite unknown.

Also the genesis of the adoral apparatus of the opisthe is not elucidated!

Wichterman 1942 describes these processes in *Drilocineta pontodrila* unprecisely and unadequately "The cytopharynx at the posterior region becomes completely dedifferentiated and is lost from view. A newly differentiated cytopharynx makes its appearance as a tubular structure in the posterior region of the dividing animal on the side opposite the micronucleus (Fig. 6). Later a somewhat similar tubular

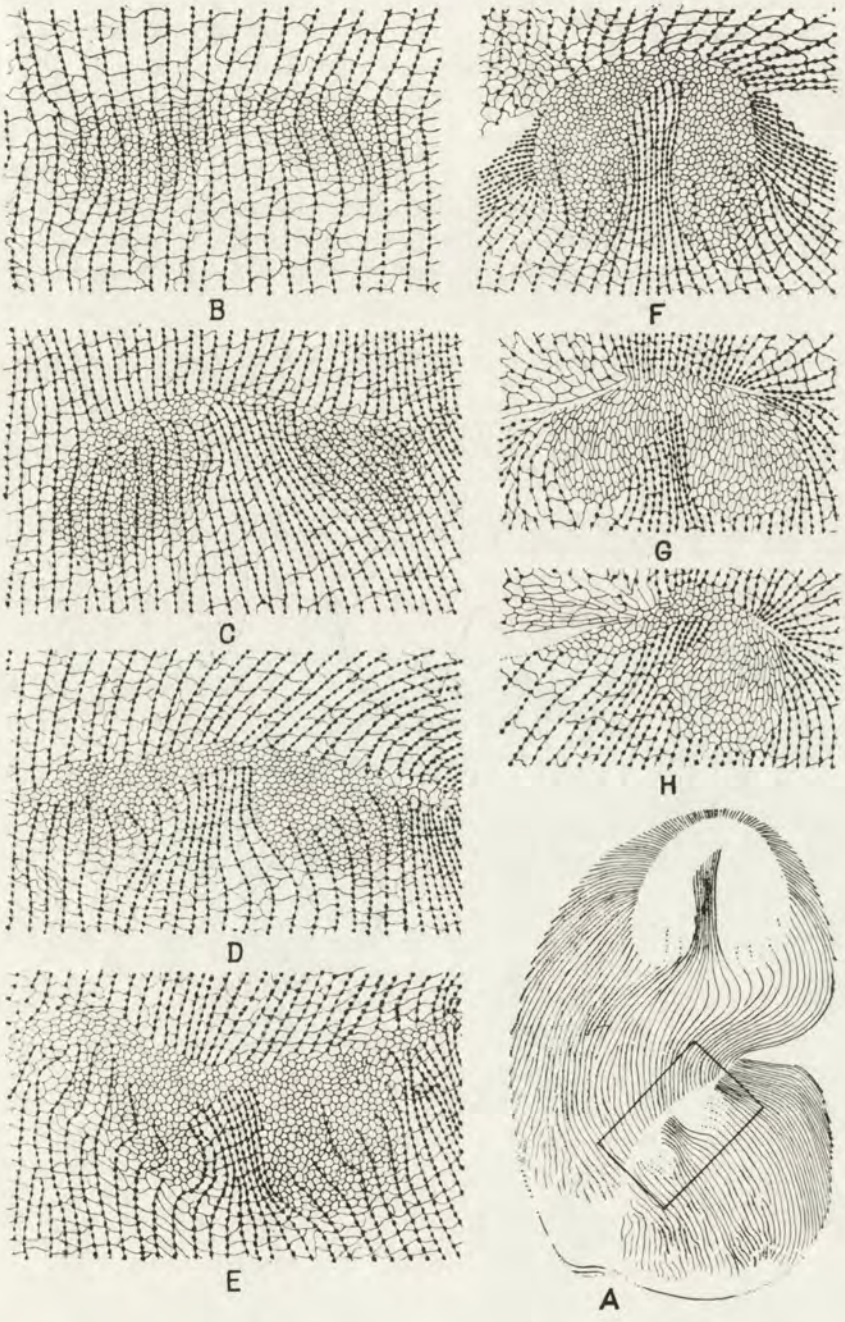

Fig. 3. The formation of a new sucker in the opisthe during fission of: A–F — *Hysterocineta paludinarum*, G–H — *Ptychostomum saenuridis* (from Raabe) ×1000

structure arises de novo in the anterior daughter (Figs 7, 8, 9). Both new cytopharynx structures then shift their position gradually toward the side opposite where they were originally, and eventually they are found on the same side with the micronucleus".

It seems that at least in many species the adoral and oral apparatus of the opisthe arises also de novo using nothing for its construction of former apparatus of the dividing animal. The phenomenon of the stomatogenesis de novo in the descendent with the former peristome falling to him does not occur in any other *Thigmotricha*, however it may occur in the ciliates in general. I intend to discuss

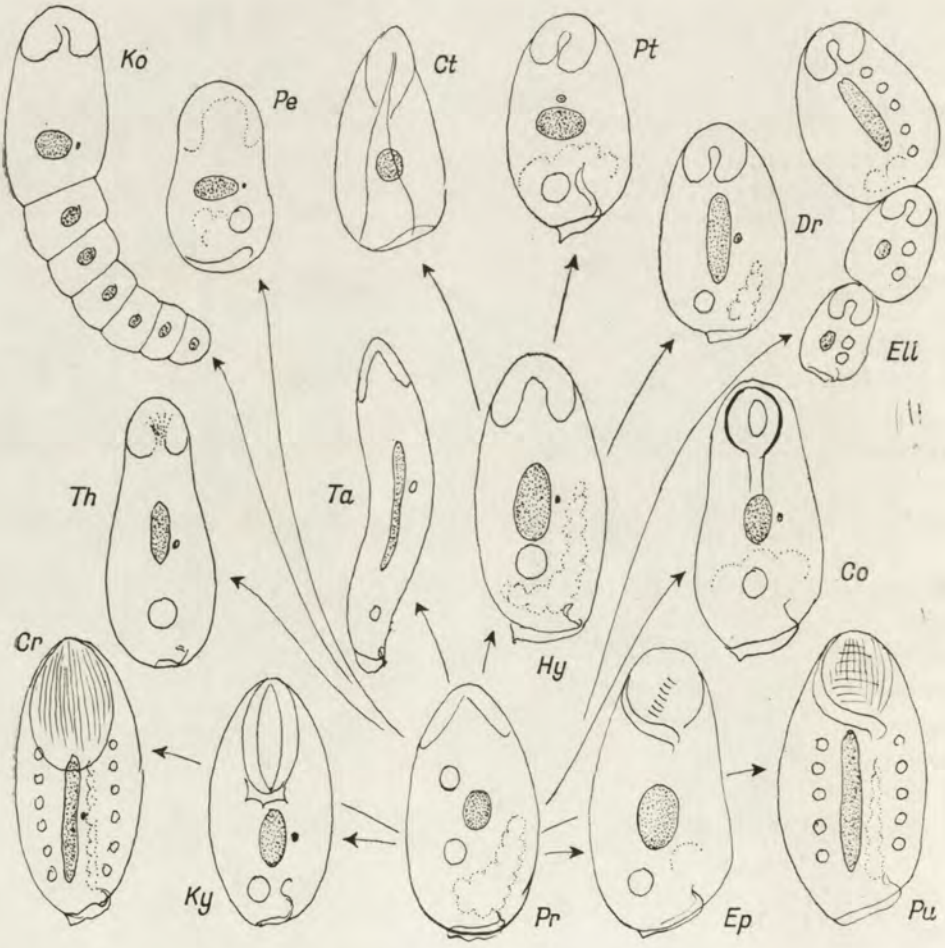

Fig. 4. The reviev of the genera of the family *Hysterocinetidae*: Pr — *Protoptychostomum*, Hy — *Hysterocineta*, Pt — *Ptychostomum*, Ell — *Elliptothigma*, Co — *Cotylothigma*, Pu — *Puytoracia*, Ep — *Epicharocotyle*, Ky — *Kysthothigma*, Cr — *Craticuloscuta*, Pe — *Preptychostomum*, Ko — *Kozloffia*, Th — *Thurstonia*, D — *Drilocineta*, Ta — *Taeniocineta*, Ct — *Coelothigma*

once more this matter in the further part of my study for it seems necessary to present it in a broader background.

The conjugation in *Hysterocinetidae* where it was seen and described occurs among individuals of rather unequal size (Kaczanowski 1961, Miyashita 1927). The smaller individual clings by its anterior end to the posterior side of the body of the larger individual. Miyashita 1927 observed a triple conjugation in *Ptychostomum tanishi* (comp. part III, p. 442 in *Goniocoma* and p. 457 in *Sphenophrya*).

The nuclear processes during the division and conjugation of *Hysterocinetidae* were not the topic of special studies as well as in general other karyologic problems in this group.

Hysterocinetidae are parasites or symbionts of *Oligochaeta* and *Gastropoda–Prosobranchia*. Many authors agree that they occur only in the posterior segment of intestine of their hosts, therefore in this segment where there are no more digestive processes but rather the absorption or the formation of excrements. Their adaptation is of morphologic character — the opposition to the current environment; physiological adaptation has not been studied till now.

Hysterocinetidae, as it has been mentioned many times, and accordingly to the reported morphologic description and to the approximate vital medium are a very compact and uniform group. However they reveal many interesting, adaptive evolutional tendencies (Fig. 4).

Protoptychostomum with the species *P. simplex* (André, 1915) would be recognized, according to Raabe 1949, as a more plesiomorphic form among *Hysterocinetidae* originally described as *Anoplophrya simplex* therefore a representative of *Astomata*. As a matter of fact the sucker in this species is slightly outlined, it consists of a narrow naked strip, stretching along the anterior body margin at its left side. Kaczanowski 1961 compares this strip with similar bald strips in some *Astomata*, as i.e., *Radiophrya olivieri* de Puytorac, 1954 and others. But other characters of *Hysterocinetidae* are already formed in *Protoptychostomum*, as adoral appartus. Two C. V. occur what constitutes an apomorphic feature.

If *Protoptychostomum* would be recognized as a plesiomorphic form, at least concerning the structure of the sucker then some tendencies are seen in the evolutionary sequence of *Hysterocinetidae*:

(1) Tendency towards the enlargement of the arms of the naked field of the sucker and of closing between them the anterior segments of the kineties of the left body side.

(2) Tendency to the producing and complication of the skeletal apparatus of the sucker in the form of fibres with a reinforced contracting character and other skeletal shapes.

(3) Tendency to the increase of the number of contractile vacuoles.

On the basis of the above statements the definition of the family *Hysterocinetidae* may be set as follows:

Familia *Hysterocinetidae* Diesing, 1866
syn.: *Ladidae* Cépède, 1910; *Ptychostomatidae* Cheissin, 1932

Thigmotricha of medium or large size (40–350 μ) of the body covered with a dense and almost equal ciliature. In the front part of the left flattened side is situated the thigmotactic area. It consists of the anterior sectors of the kineties surrounded frontally and laterally, and finally on all sides by horse-shoe shaped naked field forming a sucker. The sucker may be strengthened by a number of fibres or other skeletal structures. The front margin of the sucker makes the anterior suture of the ciliary system; the posterior suture forms an irregular net in the posterior part of the body. The buccal apparatus lies on or alongside the posterior body margin; 3 adoral kineties run along the naked peristomal field: 2 from the left, one from the right side, entering infundibulum. The nuclear apparatus: ovoidal or elongated Ma, 1 or 2 Mi. One or more C. V.-es. In the posterior body part there occurs vacuolized area, often strongly limited. The division is equal, in particular cases there occurs posterior budding or even catenulation.

Hysterocinetidae are parasites of the intestine (posterior part) of *Oligocheta* and *Gastropoda-Prosobranchia.*

Typus familiae: genus *Hysterocineta* Diesing, 1866.

De Puytorac 1968 d proposed a division of the family *Hysterocinetidae* on 4 subfamilies, namely (the original characteristics of de Puytorac 1968 d):

Hysterocinetinae. "Ventouse nottement circonscrite, pourvue d'une armature squelettique dans l'aire non ciliée antérieure. Chez les Mollusques, les Limnicoles et les quatre grands groupes de Terricoles". Genres: *Hysterocineta, Ptychostomum, Elliptothigma, Coelothigma, Cotylothigma.*

Epicharocotylinae: "Ventouse bien délimitée avec concavité centrale trés marquée, pouvant se prolonger en une gouttière, l'ensemble pourvu d'un cytosquelette complexe. Dans *Eudrilidae* et *Glossoscolecidae*". Genres: *Epicharocotyle, Kysthothigma.*

Craticuloscutinae: "Toute la partie antérieure de la cellule déprimée en une concavité très développée, pourvue de trames squelettiques. Chez les *Eudrilidae* et les *Glossoscolecidae*". Genre: *Craticuloscuta.*

Protoptychostominae: "Pas de cytosquelette dans l'aire non ciliée antérieure. Uniquement chez les Terricoles". Genres: *Protoptychostomum, Thurstonia, Kozloffia, Preptychostomum.*

De Puytorac 1968 d considers *Protoptychostominae* as the most primitive forms, the most approximate to other *Thigmotricha* as *Thigmophryidae* or *Hemispeiri-*

dae (also plesiomorphic forms). They occur in *Lumbricidae*, *Eudrilidae* and *Megascolecidae*. He writes: "Les genres que nous considerons précédemment commes les plus primitifs des *Hysterocinetidae* sont donc infeodes aux seuls Oligochaetes les plus evolués".

In my opinion, *Protoptychostomum* may be recognized as a primitive or rather a plesiomorphic form. However *Preptychostomum* the more so *Thurstonia* and *Kozloffia* in spite of the fact that they do not produce the skeletal apparatus of the sucker are not plesiomorphic in respect of other characters as mainly the development of the naked surface of the sucker, the reduction of the number of kineties which invade its groove. Simply: among the three evolutional tendencies mentioned above these species and genera preserved the tendency of the increase of the naked field of the sucker they do not realize the tendency to skeletization of the sucker and to the increase in the number of the pulsating vacuoles. In this sense they represent an individual specific evolutional way among *Hysterocinetidae*.

I am always convinced that the genus *Protoptychostomum*, presently embracing 3 species, represents the more plesiomorphic form among the *Hysterocinetidae*. We are able to deduce from this form all the subfamilies proposed by de Puytorac: Among the *Protoptychostominae* the development was oriented to the increase of the sucker but without producing the skeleton; among the *Hysterocinetidae* — towards the development of the sucker, the complication of its skeleton and the withdrawing of kineties from the sucker, among *Craticuloscutinae* towards a strong increase of the sucker which have a skeletal system, but without withdrawing the ciliature from its area, finally among *Epicharocotylinae* towards the strong development of the skeleton of the sucker and the "goutiere" assisting it. Poorly described, derived as genus *Kysthothigma*, the species *K. bacteriophila* Miy. is in my opinion connected rather with *Craticuloscuta*, not with *Epicharocotyle*; for the time it is rather the genus incerte sedis (Fig. 4).

Subfamilia *Protoptychostominae* de Puytorac, 1968

This subfamily has been created by de Puytorac 1968 d for the differentiation of *Hysterocinetidae* which does not have the skeletal apparatus of the sucker in spite of its different development.

Subfamilia *Protoptychostominae* de Puytorac, 1968

Thigmotricha — Hysterocinetidae of a moderately flattened body. The V-shaped or rounded sucker do not possess any skeletal elements. Ma oviform or elongated, one or two C. V. Parasites in the intestine of *Oligochaeta — Terricola*.

Typus subfamiliae: genus *Protoptychostomum* Raabe, 1949.

Four genera may be ranged to this subfamily, embracing presently 7 species, namely: *Protoptychostomum* Raabe, *Preptychostomum* de Puyt., *Thurstonia* de Puyt. and *Kozloffia* de Puyt. It seems worth to mention that *Kozloffia* represents here a apomorphic feature that is the catenulative multiplication similarly as *Elliptothigma* Meyer from the subfamily *Hysterocinetinae*.

Genus *Protoptychostomum* Raabe, 1949

This genus has been created (Raabe 1949) for the species *P. simplex* (André, 1915) differentiated from the genus *Ptychostomum* Stein both for the reason of its plesiomorphic and specific characters. The latter examinations, among the others of Meier 1954, Kaczanowski 1961 and de Puytorac 1963 confirmed the specificity of the species and the validity of the preservation of a separate genus for it.

The genus *Protoptychostomum* would be considered as the more plesiomorphic of all *Hysterocinetidae* concerning the development of the sucker, this is revealed in its complete aperture to the back, its narrowness and the lack of more distinct skeletal fibers. According to these characters *Protoptychostomum* is not only far from *Ptychostomum* but also gives way to *Hysterocineta*. However the presence of two C. V. must be recognized rather as an apomorphic character not strange to the evolutional tendencies among the *Hysterocinetidae*.

Hysterocineta davidis Rees, 1962 corresponds also to these two characters having also a sucker opened to the back and two sets of C. V. Rees described his species knowing only two genera of the family *Hysterocinetidae* namely *Ptychostomum* and *Hysterocineta* being not oriented in the real size of the family. This species should be assigned to the genus *Protoptychostomum*. So did de Puytorac 1968 b describing at the same time the third species in this genus, namely *P. tertium*.

Protoptychostomum Raabe, 1949

Hysterocinetidae of a strongly flattened body of an oval outline. The naked, feebly developed sucker has the form of a widened inverted V. To the middle part of the sucker enter numerous kineties of the general ciliature of the left body side, acting as a thigmotactic area. The peristome occupies the posterior body margin; infundibulum well developed. Ma ellipsoidal, located in the middle of the body; 1 or 2 Mi. Two C. V.-es are located before and behind the Ma, towards the ventral body margin. An irregular area occupied by food vacuoles extends behind the Ma. Parasites in the intestine of *Oligochaeta*.

Typus generis: *Protoptychostomum simplex* (André, 1915), Raabe, 1949.

The genus *Protoptychostomum* embraces presently 3 species:

Protoptychostomum simplex (André, 1915)

syn.: *Anoplophrya simplex* André, 1915; *Ptychostomum simplex* (André) Heidenreich, 1935.

Body strongly flattened with an ovoid outline; length 90–198 μ (according to Meier), width 50–80 μ. The sucker has a form of the letter V oriented backwards with widely arranged arms. The ciliature is abundant, uniform, composed of cilia

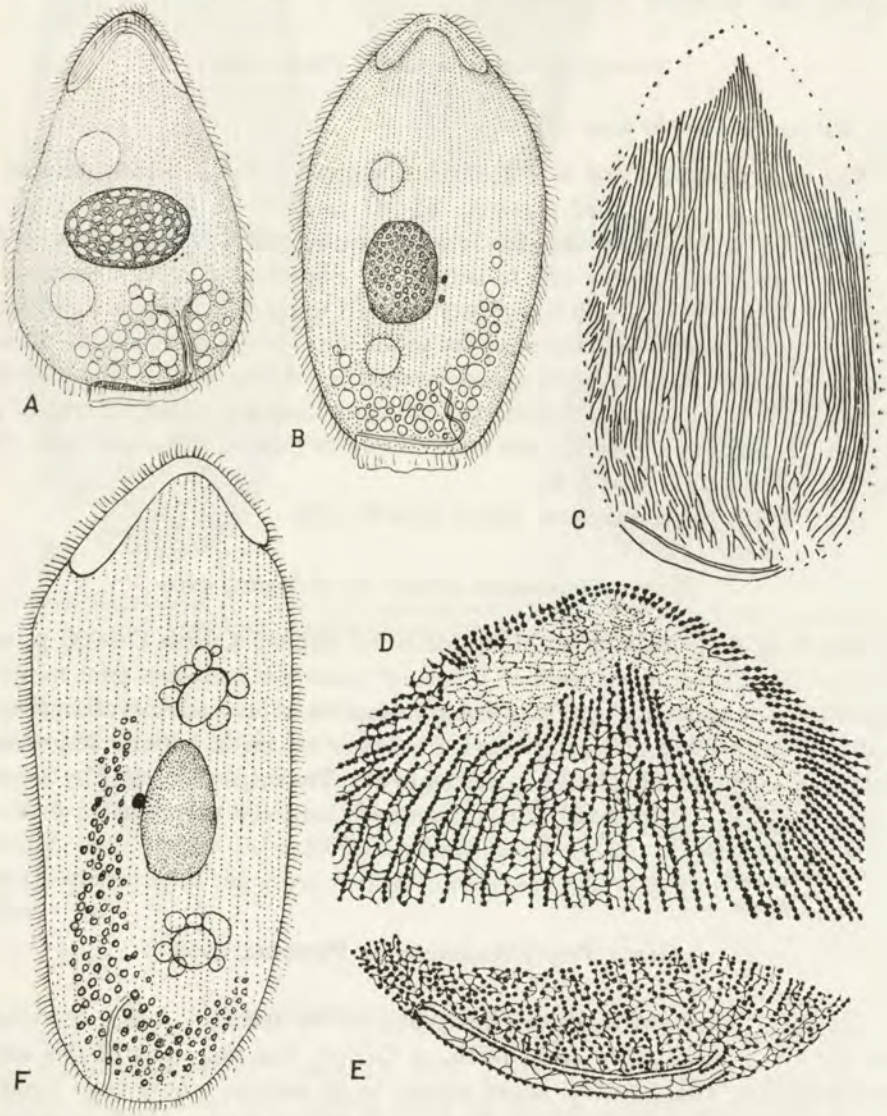

Fig. 5. *Protoptychostomum*: A–E — *P. simplex*: A — general view (after Heidenreich), B — general view (after Kaczanowski), C, D — the cortical system (a. Kaczanowski), E — the peristomal region (from de Puytorac); F — *P. davidis* (a. Rees) × 500 resp. 1000

6–7 μ long arranged in over 100 kineties. Ma ovoid, 50–60 μ, lies in smaller individuals with its longer axis crosswise of the body, in bigger ones along the body (according to Meier); 1–2 Mi. Two big C. V.-es arranged anteriorly and behind Ma towards the ventral margin. The area of food vacuoles occupies the posterior body margin, in general distinctly cut (Fig. 5 A–E).

Host: *Eiseniella tetraedra* Sav. Central Europe, also in brack-waters (Raabe — Wiślana Bay, S. Baltic Sea, Poland).

Protoptychostomum davidis (Rees, 1962)

syn.: *Hysterocineta davidis* Rees, 1962.

The body elongated and oval in shape, the anterior end is bluntly pointed, the posterior margin is rounded. Length 165–245 μ width 75–150 μ, thickness not exceeding 15 μ. The sucker has the form of an inverted V: the arms are to 50 μ (the right) and to 30 μ (the left) in length. Ma ellipsoidal, 50×30 μ, occupies the centre of the body; the 3–5 μ lies closely adposed to the Ma. There are two groups of C. V.-es, the anterior and the posterior to the Ma. These groups consist of usually one main vacuole surrounded by accessory vacuoles of varying size. The peristomal groove is 35–50 μ in length, cytopharynx 25–30 μ; there is a U-shaped area of food vacuoles, ranging on the dorsal side more forewards than on the ventral side. There are 150–160 kineties (Fig. 5 F).

Host: *Allobophora caliginosa* (Sav.) Cardiff, U.K.

Protoptychostomum tertium de Puytorac, 1968

The body flattened with an elongated ovoid outline. Length 170–230 μ, width 65–80 μ. The sucker in the form of V opened backwards has equal arms measuring 19 μ×7–9 μ. Any skeletal system is visible only as the transversal rows of argyrophilic bodies. There are 90 kineties in general; 25 of them are on the left side. Ma rounded, 30–35 μ in diameter, a big Mi lies posterior to Ma. The single C. V. leaks by 3–6 pores at the right side of the body. The peristome is long and narrow (Fig. 6 A).

Host: *Eminoscolex* sp.? — Boué, Gabon, Africa.

Genus *Preptychostomum* de Puytorac, 1968

De Puytorac 1968 d created this genus for the species *P. almae* described at the same time from *Alma emini* Mich. of Gabon. The characters of this species, and genus are: a large, wide, naked sucker, to its medium groove enter numerous kineties, there is a lack of skeletal fibres of the sucker, a wide and deep peristome, arranged perpendicularly to the body axis, a large Mi. De Puytorac describes in the same study another species within this genus namely *P. katashimae* (it ought

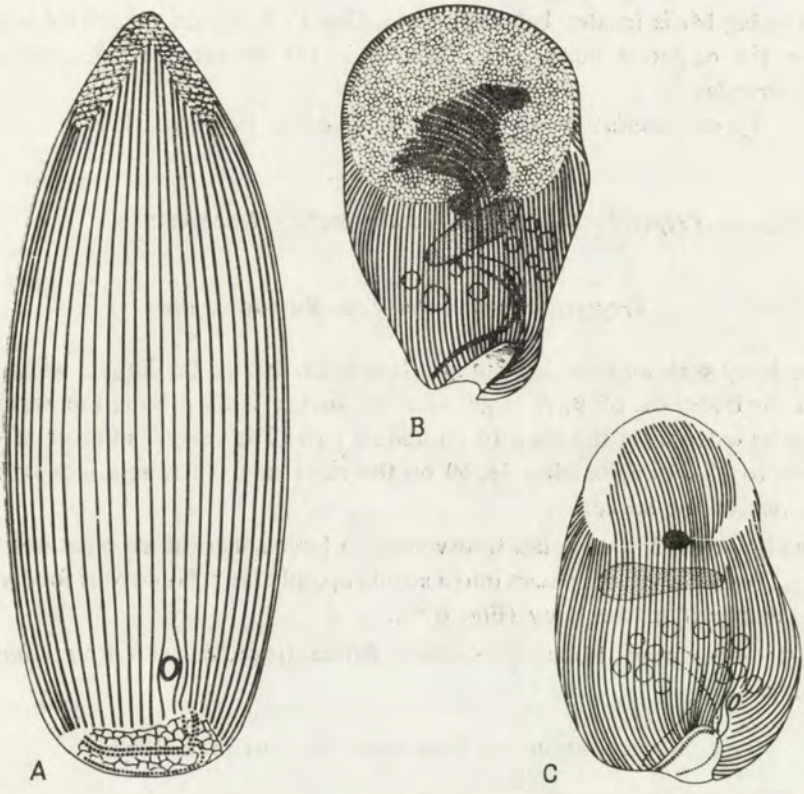

Fig. 6. *Protoptychostominae*: A — *Protoptychostomum tertium*; B — *Preptychostomum katashimai*; C — *Preptychostomum almae* (all from de Puytorac)

to be called *katashimai* — Z. R.) of the same host. The arrangement of kineties entering to the groove of the sucker is rather different in both of those species: in the first one they seem to be the continuation of kineties of the left body side, in the second one they constitute separated segments. Nevertheless at least for the time being, both species should be included in one genus.

The characteristic of the genus *Preptychostomum* would after de Puytorac run as follows:

Preptychostomum de Puytorac, 1968

Hysterocinetidae of a rounded, court body. The big, oval, naked sucker does not has any skeletal elements. In the middle part of the sucker enter numerous kineties. The peristome forms a distinct depression. Ma elongated, directed perpendicularly to the body axis;

the big Mi is located before the Ma. One C. V. in the vacuolized area in the posterior body part. Parasites in the intestine of *Oligochaeta–Terricola.*

Typus generis: *Preptychostomum almae* de Puytorac, 1968.

The genus *Preptychostomum* embraces presently 2 species:

Preptychostomum almae de Puytorac, 1968

The body with an rounded outline; length ca. 100 μ, the largest width (in the half of the body) ca. 65 μ. A large rounded sucker is 25 μ long, the segments of 25 kineties enter from the back to its middle part. The general number of kineties amounts to 25–29 on its left side, 60 on the right side. There is a lack or skeletal formations of the sucker.

Ma elongated, 35×15 μ lies transversaly, a large spherical Mi measures 9–10 μ. A large, deeped peristome passes into a strong cytopharynx. Numerous food vacuoles in the hind part of the body (Fig. 6 C).

Host: *Alma emini* Mich. — Gabon, Africa (together with *Preptychostomum katashimai*).

Preptychostomum katashimai[1] de Puytorac, 1968

The body strongly enlarged in its anterior part (90 μ), narrowed in the back (20 μ), length 120–150 μ). The large sucker occupies nearly a half of the surface of the left side of the body. The middle part of the sucker complete 24–26 kineties, rather distinctly separated from their continuation of the left side of the body. There are 42–45 kineties on the left side, ca. 85 on the right.

Ma elongated obliquely arranged; a large Mi. The large peristome is deeped. The posterior body part is occupied by food vacuoles (Fig. 6 B).

Host: *Alma emini* Mich. — Gabon, Africa (together with *Preptychostomum almae*).

Genus *Thurstonia* de Puytorac, 1968

This genus was created by de Puytorac 1968 d for the species *T. kaczanowskii* from the *Alma emini* Mich. of Gabon, described at the same time. De Puytorac mentions some characteristic features which make possible the creation of a new genus: a reniform, symmetric sucker provided at its back by two "lips" without

[1] I change the original name of "*P. katashimai*" according to the rule of the Code of Zoological Nomenclature.

skeletal elements, Ma elongated, one C. V. A drawing and a photograph made by de Puytorac provide better evidence of the individuality of the species.

The diagnosis of the genus may be stated as follows:

Thurstonia de Puytorac, 1968

Hysterocinetidae of a pyriform body. The reniform sucker occupies the front part of the body and has two mobile lips on the posterior margin. Lack of any skeletal armature of the sucker. On the naked field of the sucker there exist radially directed rows of argyrophilic bodies. Ma elongated, directed parallely to the body axis, the big Mi, single C. V. Parasites in the intestine of *Oligochaeta-Terricola*.

Typus generis: *Thurstonia kaczanowskii* de Puytorac, 1968.

The genus *Thurstonia* embraces only one species:

Thurstonia kaczanowskii de Puytorac, 1968

The body elongated, pearshaped, measures: length 150–260 μ the maximum width 70–80 μ. The sucker is symmetric, small 17 μ with a reniform outline. There are radially arranged rows with argyrophilic bodies (mucocysts) on the naked surface of the sucker, there is a lack of any skeletal elements of the sucker. Number of kineties — ca. 50 (? Z. R.), 28 of them on the left body side.

Ma elongated, 9–10 μ in diameter, Mi large, one C. V. in the posterior part of the body. The peristome is wide but short, lies on the posterior pole of the body (Fig. 7 E).

Host: *Alma emini* Mich. — Gabon, Africa (together with *Ptychostomum commune* and *Kozloffia catenula*).

Genus *Kozloffia* de Puytorac, 1968

The genus was created by de Puytorac, 1968 d for the species *K. catenula* from *Alma emini* Mich. of Gabon described at the same time. *K. catenula* is characterized by a multiplication in the form of catenulation; de Puytorac observed steadily the catenulae composed of several (up to 10) individuals. *K. catenula* has a wide sucker which has no (as far as the author was able to observe it) skeletal elements and has an elongated Ma arranged across the body. It seems quite obvious that the catenulative division in *Kozloffia* and *Elliptothigma* appears quite independently and that both of these species are not in a close connection.

The diagnosis of the genus may be stated as follows:

Kozloffia de Puytorac, 1968

Hysterocinetidae of a moderately elongated, truncated body, appearing usually in a form of long catenulae, consisting of 5–10 individuals. The sucker is large, reniform and does not has any skeletal armature. Into the furrow of the sucker enter few kineties. Ma elongated, directed perpendicularly to the body axis; Mi is big. One C. V. Parasites in the intestine of *Oligochaeta–Terricola.*

Typus generis: *Kozloffia catenula* de Puytorac, 1968.

The genus *Kozloffia* embraces presently only one species:

Kozloffia catenula de Puytorac, 1968

The body moderately elongated, the anterior part bluntly ended. The size of proter (primite): length 150–165 μ, width over 100 μ. The divisional chains consist of 5–10 individuals and reach in total 690 μ of length. The sucker is wide, reniform; several (4) kineties enter to its groove. No skeletal elements of the sucker. Number of kineties reaching the margin of the sucker: 40 at the left side and 50 at the right side of the body.

The elipsoid Ma is arranged perpendicularly to the body axis it measures in proter 28×20 μ. Two Mi follow the Ma measuring 1.5–2 μ in diameter. One C. V. (Fig. 7 A–D).

Host: *Alma emini* Mich. — Gabon, Africa (with *Thurstonia kaczanowskii*).

Subfamilia *Hysterocinetinae* Diesing, 1866, de Puytorac, 1968

This subfamily, which range I left as it was determined by de Puytorac 1968 d, may be characterized as follows:

Subfamilia *Hysterocinetinae* Diesing, 1866, de Puytorac, 1968

Thigmotricha-Hysterocinetidae of a rounded or elongated body. The sucker has a skeletal system consisting of fibres lying in one or many layers. Lack of through or flanges. Ma rounded or elongated, one or more C. V. Parasites in the intestine of *Oligochaeta* and *Prosobranchia.*

Typus subfamiliae: genus *Hysterocineta* Diesing, 1866.

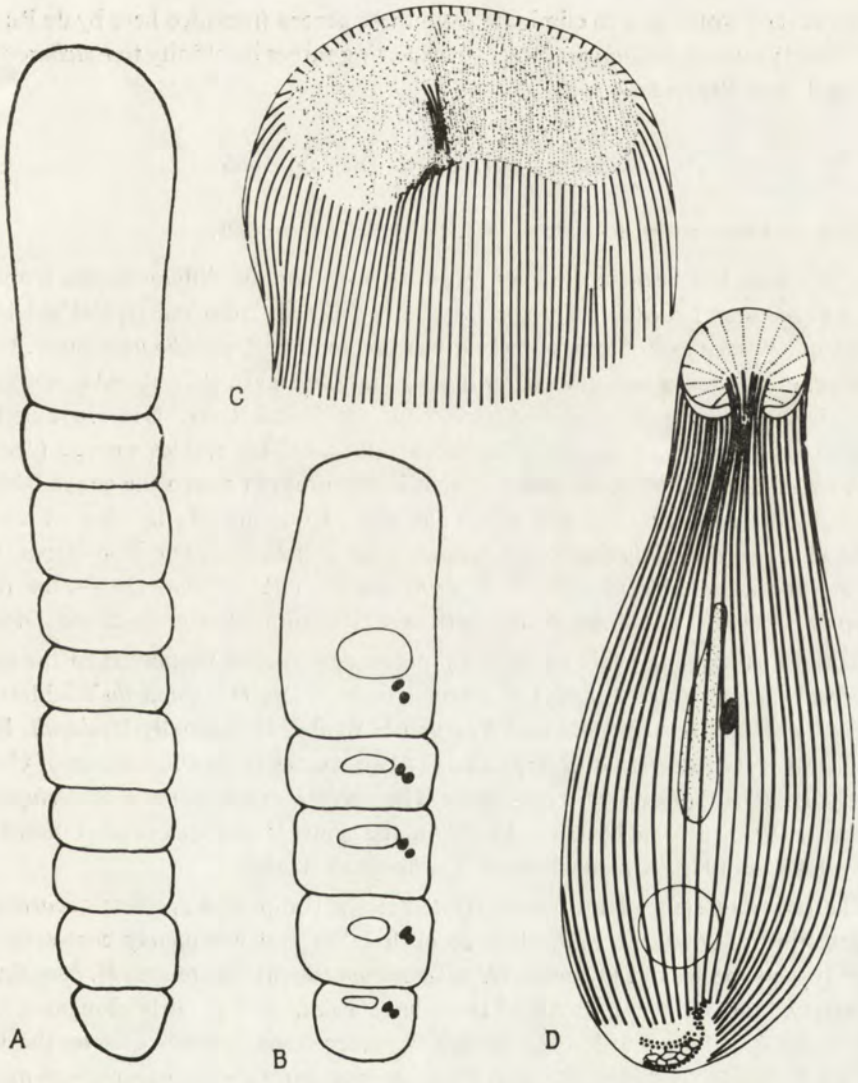

Fig. 7. *Protoptychostominae*: A–C — *Kozloffia catenula*; D — *Thurstonia kaczanowskii* (all from de Puytorac) A–C×300, D×500

I consider also *Protoptychostomum* as a model of an ancestral form leading to this subfamily, besides it the skeletal apparatus of the sucker was produced and its ciliated groove is gradually closed. Five genera may be enclosed here: *Hysterocineta* Diesing, *Taeniocineta* g. n., *Drilocineta* g. n., *Ptychostomum* Stein and *Coelothigma* de Puyt. From these genera *Elliptothigma* represents a catenulative type of division, concurrent, however independent of this type of division revealed by *Kozloffia* de Puyt. of the subfamily *Protoptychostominae*.

However I would like to eliminate from these genera (included here by de Puytorac 1968 d) genus *Cotylothigma* Raabe, suspecting rather its affinity to *Epicharocotyle* Kozloff and *Puytoracia* g. n.

Genus *Hysterocineta* Diesing, 1866

syn.: *Ptychostomum* pro parte — Stein, 1961, *Ladopsis* Cheissin, 1928.

This genus has been created by Diesing 1866 for the differentiation from the genus *Ptychostomum* Stein of a species highly different from the typical species of the genus: *P. saenuridis* Stein, namely it was created for *P. paludinarum* Stein. In the latter years this genus was quite forgotten so that Cheissin 1928 stated a new genus *Ladopsis* for the species *L. benedictiae* from the Baical Lake, Nikolajeva 1929 reports a description of *L. bithyniae* from *Bithynia*. This species proved identical to *H. paludinarum* (Stein), the more so that in the times of Stein the genus *Bithynia* was not differentiated from the genus *Paludina*. Consequently in view of an undoubtful affinity of *L. benedictiae* Cheissin, 1928 and *L. bithyniae* Nikolajeva, 1929 and an unquestionable identity of *L. bithyniae* with *P. paludinarum* — the name *Ladopsis* Cheissin would be recognized as a synonim of *Hysterocineta* Diesing.

In the forthcoming years — descriptions of new species numbered to the genus *Hysterocineta* are issued namely: *H. eiseniae* Beers, 1938, *H. pontodrila* Wichterman, 1939, *H. cheissini* Raabe, 1949 and *H. horvathi* Raabe, 1950, finally *H. davidis* Rees, 1962. De Puytorac 1968 d describes finally two species from *Oligochaeta* of Gabon namely *H. libyodrili* and *H. pheretimae*. The species *Ptychostomum bacteriophilum* Miyashita, 1933 enclosed by Jarocki 1939 to the genus *Hysterocineta* was differentiated by Raabe 1949 in a new genus *Kysthotigma* Raabe.

The coherent stem of the genus *Hysterocineta* compose 4 species; occurring in the intestine of *Prosobranchia*, close to each other and completely corresponding to the typical species of the genus: *H. paludinarum* (Stein). There are: *H. benedictiae*, *H. cheissini* and *H. horvathi*. All of them have a flat, elastic, fairly elongated body (ratio from 2.5: 1 to 4.5: 1). The sucker is rather large opened towards the back and strengthened by fibres running more or less parallely to its anterior margin (the ratio of the sucker to the body are different (from 1.5: 1 up to 11: 1). Ma oval or slightly elongated oriented by its longer axis parallely to the body axis. A distinctly separated area of vacuolized plasma occupies the posterior part of the body beyond the nucleus and C. V. The area is anteriorly elongated in the form of one (dorsal) or two arms, embracing C. V. and the nuclear apparatus.

The species occurring in the intestine of *Oligochaeta* depart from this type of structure in different directions: *H. pontodrila* and *H. libyondrili* distinguish themselves by a strong elongation of Ma (ratio 6: 1) and limitations of the vacuolized area and also as it seems, additional fibres of the sucker, in the case of *H. eiseniae* by the length of the body (5.5 : 1) and of Ma (6 : 1) and a nearly undeveloped

vacuolized area. I exclude these species to new genera: *Drilocineta* g. n. and *Taeniocineta* g. n. The fourth species from *Oligochaeta*, namely *H. pheretime* de Puyt., 1968 corresponds completely to the character of the genus and may be left within it.

Hysterocineta Diesing, 1866

syn.: *Ladopsis* Cheissin, 1928

Hysterocinetidae of a flexible body of an oval or elongated outline. The strong naked sucker has a horse-shoe shape with widened arms. Numerous kineties of the general ciliature enters into the middle part of the sucker. The sucker is provided with certain number of fibres arranged in one layer parallely to the anterior margin. The peristome occupies the posterior margin of the body; infundibulum is well developed. The ovoid or ellipsoid Ma is located centrally, the longer axis being parallel to that of the body, the Mi lies at the dorsal side of the Ma. The single C. V. is located posterior to the Ma. A very distinctly defined posterior vacuolized area extends usually anteriorly along the dorsal or the dorsal and ventral body margins in the form of one or two pointed arms. Parasites of the intestine of *Gastropoda–Prosobranchia* and *Oligochaeta*.

Typus generis: *Hysterocineta paludinarum* Stein, 1861 (= *Ladopsis bithyniae* Nikolajeva, 1929).

I include 4 species to the genus *Hysterocineta*: two from *Presobranchia* and one from *Oligochaeta*.

Hysterocineta paludinarum (Stein, 1861)

syn.: *Lada bithyniae* Nikolajeva, 1929; *Ladopsis bithyniae* Cheissin, 1932; *Ptychostomum paludinarum* Stein, 1861.

The body is flattened and very flexible, its outline being an elongated oval. The anterior end is rounded, the posterior pointed. Length 110–160 μ, width 43–60 μ. The big sucker, ca. 35 μ in diameter, is strengthened by few (6) fibres, more or less parallel to the dorsal portion of the anterior margin. Between the arms of the sucker enter from behind some kineties (ca. 10) forming the ciliation of the furrow. The number of kineties amounts approx. 120, the cilia are 9–15 μ long.

The ovoidal Ma, size ca. 25×18 μ, is pointed anteriorly, the longer axis being parallel to that of the body. The Mi, 1–2 μ is located at the dorsal side of Ma. The area filled by food vacuoles is well limited and occupies the posterior portion of the body extending along the dorsal and the ventral margins towards the anterior

of two projecting tips, the dorsal being longer than the ventral one. C. V. lies behind the Ma, between the arms of the vacuolized area.

The peristome lies distinctly in the posterior of the ventral margin, reaching the sharpened end of the body which is pointed somewhat dorsally. In the posterior of the ventral margin lies the cytopharynx whose location is postero-terminal (Fig. 8 A–D).

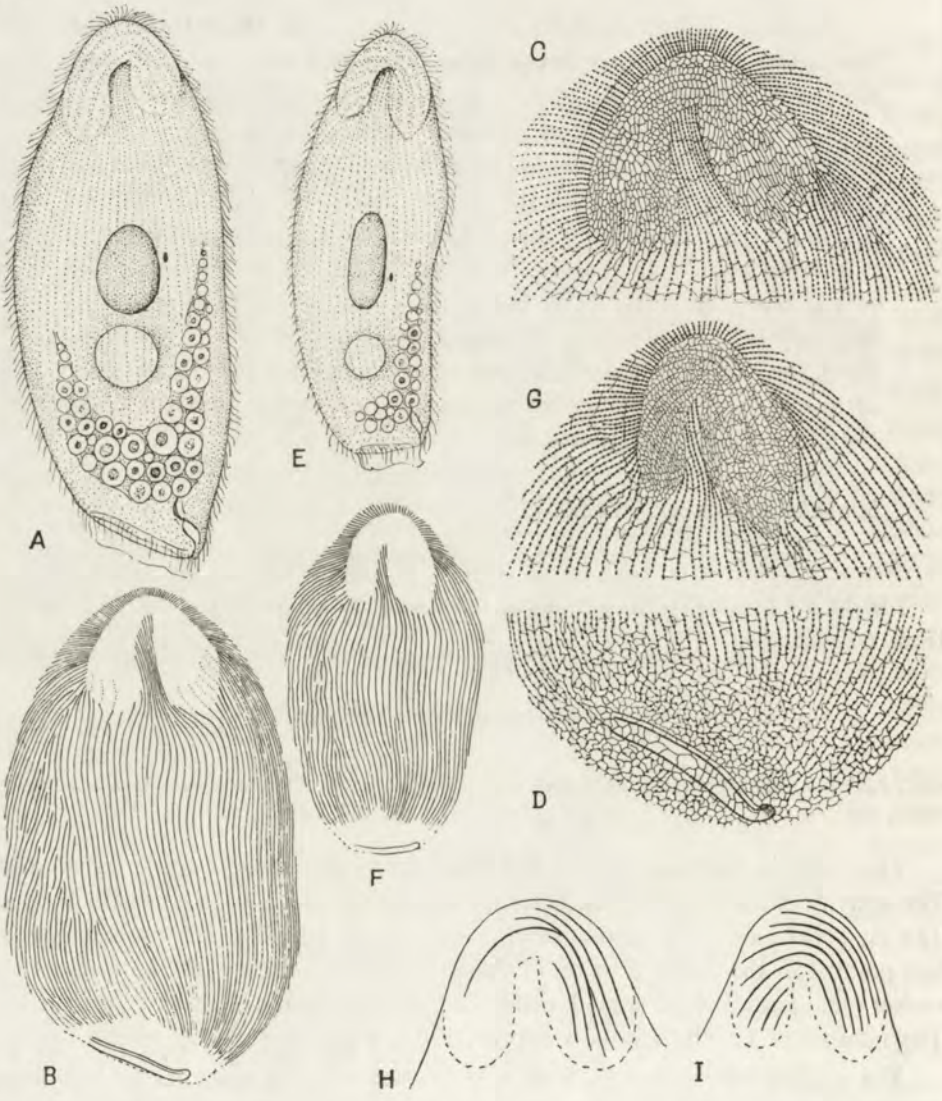

Fig. 8. *Hysterocineta*: A, B, C, D — *H. paludinarum*, general view, scheme of the ciliature, sucker and peristome regions; E, F, G — *H. cheissini* — the same figures; H, I — fibrils of the sucker in *H. paludinarum* and *H. cheissini* (all from and after Raabe) ×500 resp. 1000

The silverline-system of *H. paludinarum* as well as in the interphase state as in the division and reorganization is well discovered by Raabe 1939, 1949 (Fig. 3 A–F).

Hosts: *Bithynia tentaculata* (L.) (=*Paludina impura* in Stein) and *B. leachi* (Shepp.) (=*P. similis* in Stein) — Europe. I stated *H. paludinarum* in many regions of Poland and Balaton Lake; infection varies, but is in general frequent.

Hysterocineta cheissini Raabe, 1949

The outline of the body is an elongated oval, the anterior end is rounded, the posterior is truncated vertically to the body axis. Length 85–140 μ, width ca. 40 μ. The sucker is proportionally smaller than that of *H. paludinarum*, ca. 25 μ in diameter; the dorsal arm is markedly bigger than the ventral one. The sucker is strengthened by a number of parallely to the anterior margin arranged fibres, ca. 10 in number. Between the arms of the sucker enter ca. 5–6 kineties, forming the ciliation of the furrow. The number of kineties of the general ciliature is 85–90; the cilia are 10 μ long.

The elongated Ma, size ca. 35×20 μ, is located in the middle of the body; Mi 2×1 μ, is at the side of Ma. The area of food vacuoles occupies the middle of the posterior of the body and extends posteriorly along the dorsal margin in the form of the sharp arm, reaching half of the length of the body. C. V. lies behind the Ma, ventrally to the arm of the vacuolized area.

The peristome occupies the posterior, truncated margin of the body; the cytopharynx lies on the dorsal extremity of the margin.

The silver-line system of *H. cheissini* is well described by Raabe 1949 (Fig. 8 E–G).

Hosts: *Bithynia tentaculata* (L) in Poland, but never in association with *H. paludinarum*. I found *H. cheissini* in only two of many biotops examined: shallow draining ditch in the vicinity of Warszawa and the rivulet Płutnica which falls into the Puck Bay (S. Baltic Sea, Poland).

Hysterocineta benedictiae (Cheissin, 1928)

syn.: *Ladopsis benedictiae* Cheissin, 1928.

The elongated body has a length 200–350 μ and the width 40–80 μ in the narrowest and 80–100 μ in the widest part. The sucker is proportionally small and measures 20–35 μ in diameter (the sucker: body ratio is 1 : 7 to 1 : 11). The sucker is strengthened by 14–16 fibres running parallely to the anterior margin of the sucker; after Cheissin there exist the posteriad directed bundle of longitudinal fibres, a retractor.

The elongated Ma is located in the middle of the body, Mi at the side of Ma. The region of food vacuoles extends anteriorly only along the dorsal margin of the body and reaches approximately half its length. C. V. in the midline of the body, behind the Ma.

The peristome lies on the posterior, truncated margin of the body; the entering into the cytopharynx is distinctly moved towards the dorsal margin (Fig. 9 D, E).

Hosts: *Benedictia baicalensis* (Gerstfeldt), *B. limnaeoides* (Schrenck), *Baicalia ciliata* Dyb., *B. carinatocostata* Dyb., *B.* sp. and *Kobeltocochlea* sp. — Baical Lake, Siberia.

Hysterocineta horvathi Raabe, 1950

Body elongated, oval in outline, length 150–200 μ, width ca. 65 μ. The relatively small sucker is ca. 30 μ in diameter (the sucker: body ratio is 1 : 5.5). The anterior end of the body is rounded, the posterior somewhat truncated. The sucker is strengthened by fibres of the non-determined number. Between the arms of the sucker enter ca. 20 kineties. The ciliature is very dense; the total number of kineties being ca. 220.

The ovoid Ma, 50×20 μ, is located in the middle of the body and directed with

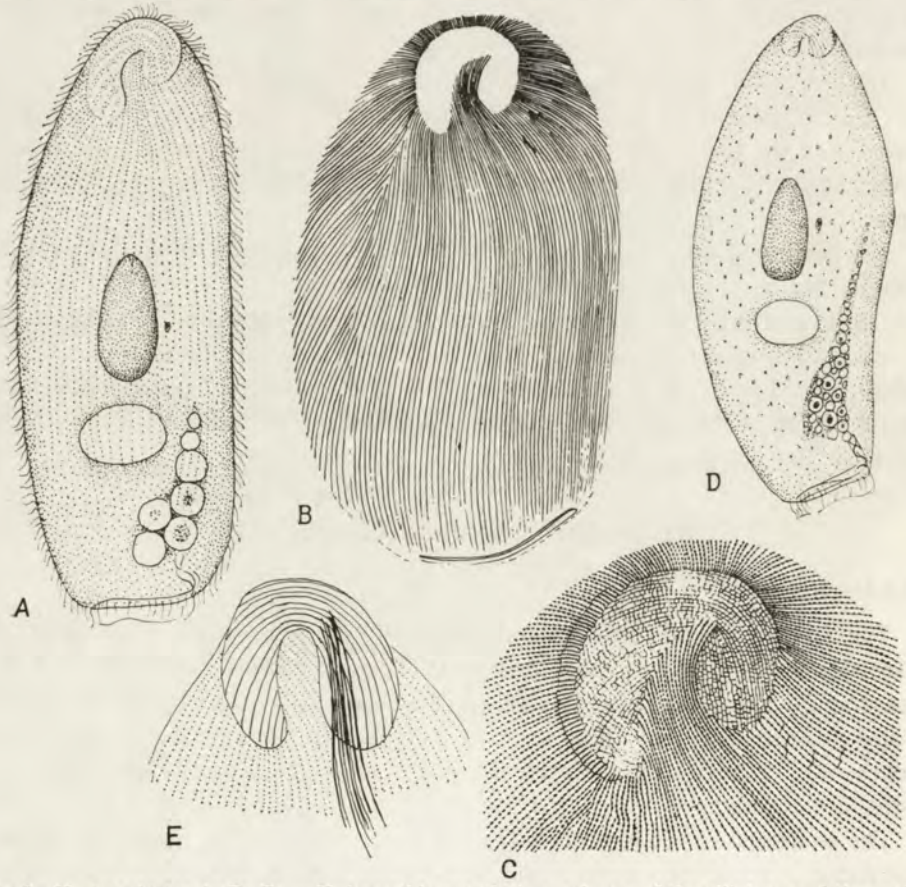

Fig. 9. *Hysterocineta*: A, B, C — *H. horvathi* general view, scheme of the ciliature and the sucker region (from and after Raabe); D, E — *H. bendictiae*, general view (×250) and the sucker (after Cheissin)×500 resp. 1000

the longer axis parallel to the body axis; the 2 μ measuring Mi lies on the side of Ma. The vacuolized area occupies the small, triangular part of the hind part of the body and is prolongated anteriorly in a sharp arm; they measure 20×40 μ.

The peristome, 50 μ long, lies on the rear margin of the body, the cytopharynx is small. The surface of the sucker is on the $AgNO_3$ preparations covered by dense net of fibrills, with the meshes directed parallelly to the front margin of the sucker (Fig. 9 A–C).

Host: *Lithoglyphus naticoides* Pfeiffer — Balaton Lake in Hungary. I have never found *H. horvathi* in *Lithoglyphus* in Poland.

Hysterocineta pheretimae de Puytorac, 1968

Body oval in outline, length 120–160 μ, width 60–80 μ. The inverted V-shaped sucker has the dorsal arm greater than the ventral one. The sucker is strengthened

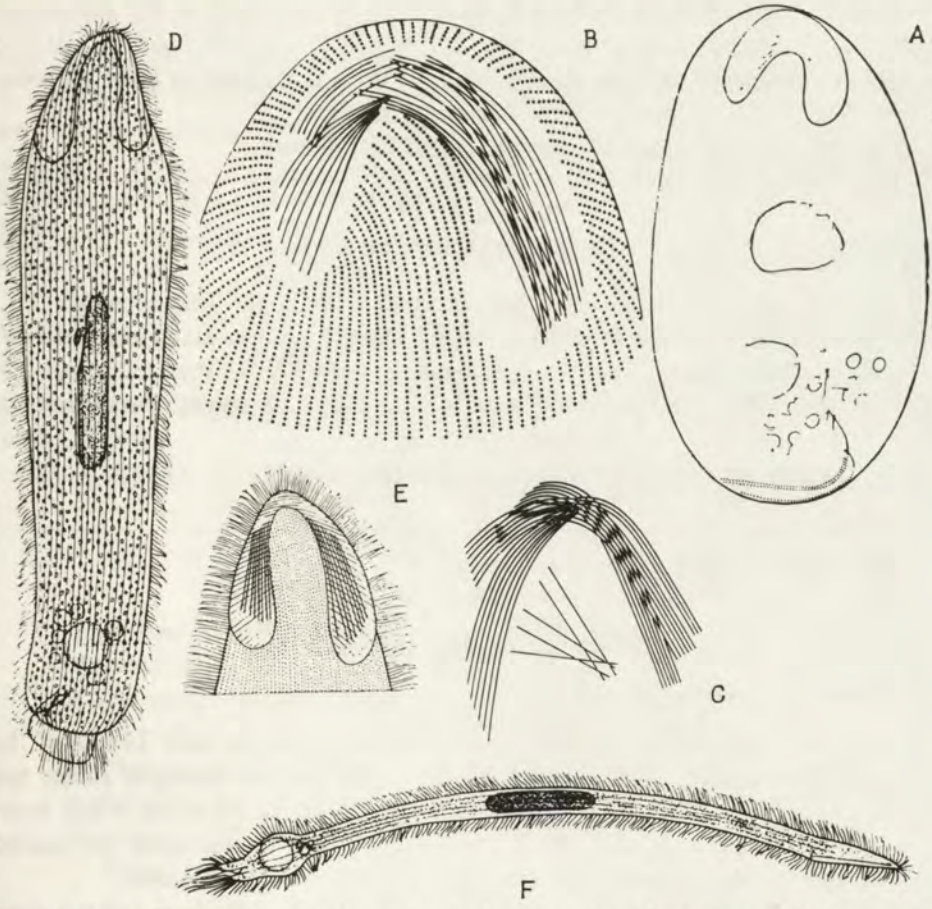

Fig. 10. *Hysterocinetinae*: A–C — *Hysterocineta pheretimae* (from de Puytorac); D–F — *Taeniocineta eiseniae* (from Beers) ×500 resp. 1000

with 3 complexes of fibres, 3–6 fibres each; the fibres run parallely to the front and side margins of the sucker. Between the arms of the sucker enter ca. 20 kineties. The total number of kineties is ca. 40 on the left and 60 on the right body side.

The circulary Ma is located in the middle of the body; Mi is not observed. The C. V. and the vacuolized zone are located in the posterior half of the body (Fig. 10 A–C).

Host: *Pheretina* sp.? (*Megascolecinae*) — Gabon, Africa.

Genus *Taeniocineta* genus novum

I created this genus for the species *Hysterocineta eiseniae* Beers, 1938 from *Eisenia lonnbergi* Mich. (*Lumbricidae*) from North America. This species diverges from all other species of the genus *Hysterocineta* by the proportion of the body. Whereas the ratio of the width length of the body of *Hysterocineta* was maintained within the bounds of 1 : 2 to 1 : 3, here it amounts to 1 : 5! Similarly Ma is also strongly elongated (1 : 5). According to Beers there is no zone of food vacuoles. The sucker is widely open, its arms are rather narrow.

The character of the genus may be stated as follows:

Taeniocineta genus novum

Hysterocinetidae of a strongly elongated body, the length: width ratio being 5 : 1. The inverted V-shaped sucker embraces many kineties is in *Hysterocineta*. The Ma is elongated; one C. V. in the hind part of the body. The vacuolized area is very scarce or absent. Parasites in the intestine of *Oligochaeta–Terricola*.

Typus generis: *Taeniocineta eiseniae* (Beers, 1938)

This genus includes by now 1 species:

Taeniocineta eiseniae (Beers, 1938)

syn.: *Hysterocineta eiseniae* Beers, 1938.

The body elongated and slender, about 5 times as long as wide. Length of the body 190–230 μ, width 35–45 μ, thickness 10 μ. The inverted V-shaped sucker has the ventral arm (21–26 μ) smaller than the dorsal (25–30 μ); the fibres in one layer, transverse the arms obliquely. 15–20 rows of cilia enter uninterruptly the middle ciliated area of the sucker. The total number of kineties is 90–100.

Ma elongated, 45–50 μ by 8 μ (ratio 4 : 1 to 5 : 1). Mi is fusiform and lies close to the Ma. The C.V. lies near the posterior end. "There are no obvious food vacuoles

in *H. eiseniae* and attempts by Beers to feed particles of Chinese ink to the ciliate were not successfull. However, in protargol preparations the cytoplasm in the region where the C.V.-es forms contains what appear to be ingested cilia probably derived from the intestinal epithelium of the host" — Kozloff 1960.

"The buccal cavity has the form of a narrow funnel; there are transverse rows of cilia: the cilia of the row nearest the superior surface are fused into a hyaline membrane..., the cilia of the other two rows are coherent and very active". — Kozloff 1960 (Fig. 10 D–F).

Host: *Eisenia lonnbergi* Mich. — N. Carolina, USA.

Genus *Drilocineta* genus novum

I create this genus for the differentiation of the species *H. libyodrili* de Puytorac, 1968 from the genus *Hysterocineta*. This species occurs in *Libyodrilus violaceus* Bedd. from Gabon. However it differs from the other *Hysterocineta* by the limitation of the zone of food vacuoles, strongly elongated Ma and mainly by the fact that the skeletal apparatus of the sucker contains many pairs of fibres arrangement, crossing at different levels, finally the segments of kineties lying in the furrow of the sucker are cut (as in *Ptychostomum*) from their continuation on the left side of the body. In several respects this species stands between *Hysterocineta* and *Ptychostomum*.

It seems that *H. pontodrila* Wichterman, 1942 of *Pontodrilus* from the North America may be also included to the genus *Drilocineta* g. n. It corresponds to the typical species of the genus by the character of the vacuolized zone, the Ma and as it seems, by the character of the sucker as it is visible from the drawings of Wichterman 1942.

The diagnosis of the genus *Drilocineta* g. n. may be stated as follows:

Drilocineta genus novum

Hysterocinetidae of an elongated, ovoidal body. The sucker is provided with the skeletal fibres lying in two layers. In the groove of the sucker there are several fragments of kineties, separated from their continuations on the left body side. Ma elongated, directed parallely to the body axis; the C. V. and the small zone of food vacuoles in the hind part of the body. Parasites in the intestine of *Oligochaeta–Terricola*.

Typus generis: *Drilocineta libyodrili* (de Puytorac, 1958)

Two species may be included in the genus *Drilocineta*:

Drilocineta libyodrili de Puytorac, 1968

The body elongated, length 140–200 μ, width 55–60 μ. The sucker heart-shaped with slightly assymmetric arms (the dorsal one is wider) reaches 30 μ of width. The skeleton of the sucker constitute two pairs of sets: the most numerical are arranged more or less parallely to the arms of the sucker and less numerical, oblique to the other ones; the dorsal and the ventral part of the skeleton are not connected. 24–28 kineties are rarely arranged on the left side of the body, ca. 80 kineties on the right one. There are ca. 10 segments of kineties in the furrow of the sucker, they are cut off from the kineties of the left side of the body.

Ma elongated, 60×9 μ with a sharpened anterior and rounded posterior end, is associated by a large Mi. One C. V. A short peristome; near the infundibulum numerous food vacuoles (Fig. 11 A–C).

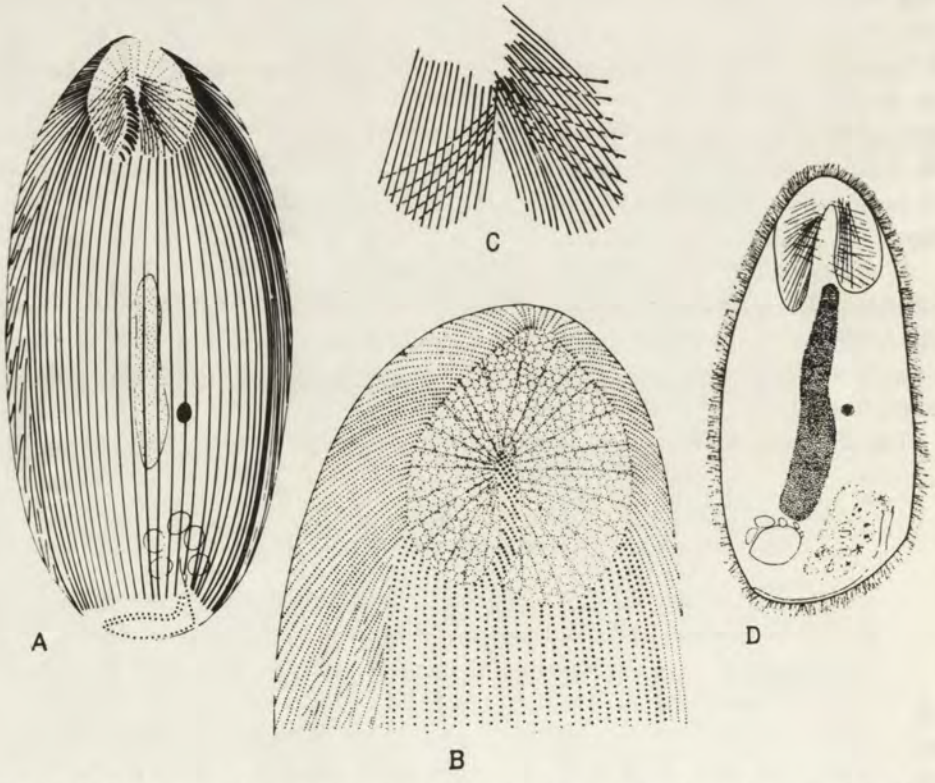

Fig. 11. *Drilocineta*: A–C — *D. libyodrili* (from de Puytorac); D — *D. pontrodrila* (from Wichterman)×500 resp. 1000

Host: *Libyodrilus violaceus* Beddard — Gabon, Africa (together with *Epicharocotyle kozloffi* de Puyt. and *Craticuloscuta gigas* de Puyt).

Drilocineta pontodrila (Wichterman, 1942)

syn.: *Hysterocineta pontodrila* (Wichterman, 1942).

The body is flattened, somewhat pyriform in outline, the anterior end tapers to a blunt point, and the posterior end widens to become truncate. Length 87–157 μ, width 38–70 μ, thickness 29 μ. The characteristically inverted V-shaped sucker has two arms of not the same size: the dorsal arm is shorter and slightly ticker (34×16 μ) than the ventral one (41×14 μ). Long cilia measuring ca. 7 μ are found in the wedge-shaped depression between them.

Ma is very long, centrally located, measuring ca. 65×10 μ Mi, 3×4 μ lies on the dorsal side of the center of Ma. Many small C. V.-es emptying into one main vacuole lie in the hind part of the body. In the posterior part of the body there exists an area of food vacuoles.

The adoral kineties, 30 μ long run along the posterior margin of the body and lieds to the 18 μ long cytopharynx (Fig. 11 D).

Host: *Pontodrilus bermudensis* Beddard — marine earthworm of the intertidal zone — Tortugas, Florida USA.

Genus *Ptychostomum* Stein, 1860

syn.: *Lada* Vejovsky, 1882.

This genus has been created by Stein 1860 for the species found by him in the intestine of *Tubifex tubifex* Müller (synonime used by Stein: *Saenuris variegata* Hoffm.) for which the name of the species *P. saenuridis* gives Stein 1861. Independently of this research Vejdovsky 1882 described *Lada wrześniowskii* of *Trichodrilus pragensis* Vejd. Both of these authors described the found ciliates quite incorrectly: Stein took the sucker for the mouth, the mouth for cytopyge, Vejdovsky also took the mouth for the anus, treated the mouth only as an adhesive organ and consequently recognized *Lada* as an mouthless ciliate (hence its latter place among *Astomata* in Cepède 1910).

Over the years 1925–1930 several species of the genus *Lada* have been described as forms having the buccal apperture: *L. pygostoma* Rossolimo, 1926, *L. issa* Kijenskij, 1925, *L. vejdovskyi* Kijenskij, 1925, *L. tanishi* Miyashita, 1927 and *L. asymmetrica*, *L. baicalensis* and *L. elongata* described by Cheissin 1928 from the Baical Lake. After the statement of Rossolimo 1925, Studitsky 1930 and Cheissin 1932, that the genera *Ptychostomum* Stein, 1860 and *Lada* Vejdovsky, 1882 are synonyms, the species described in the genus *Lada* were verified as belonging to the genus *Ptychostomum*. Heidenreich 1935 achieved the revision of forms approximate to *P. saenuridis* Stein, Raabe 1949 did a general review of these forms, finally Meier 1954 summed up the existing considerations.

The intensification of studies on the representatives of the genus *Ptychostomum* is characteristic for the period 1952–1968; 14 new species of this genus have been described — among them 4 species from the Lake Ohrid in Yugoslavia and four from Gabon — Africa (de Puytorac 1958, 1968). In this way their number increased to 30. From these species *P. bacteriophilum* Miyashita, 1933 was transferred to the genus *Kysthothigma* Raabe, 1949, *P. simplex* (André, 1915) to the genus *Protoptychostomum* Raabe, 1949, *P. rhynchelmis* Heidenreich, 1935 to the genus *Cotylothigma* Raabe, 1949. *P. iliodrili* Heidenreich, 1935 and *P. issum* (Kijenskij, 1925) were recognized as synonyms of *P. saenuridis* Stein, 1861, *P. vejdovskyi* Kijenskij, 1925 as synonym of *P. chattoni* Rossolimo, 1925. De Puytorac 1968 d separates in a new genus *Coelothigma* the species *P. canalis* described by Katashima 1952; however in my opinion the description of Katashima gives no full reasons for such decision, therefore I leave here the genus created by de Puytorac as not quite sure.

It has been accepted as result of this revision that to the genus *Ptychostomum* may be ranged the species having following characters:

(1) The body generally meanly elongated with a strong, round sucker with broad arms cutting off the ends of kineties embraced in a ciliate sucker.

(2) The nucleus is oval ranged by its longer axis perpendicularly to the body axis.

(3) In the back of the body a distinctly outlined zone of food vacuoles embracing also the pulsating vacuole.

(4) A rich skeletal system of the sucker composed of fibres lying at different levels, signallized by many authors, but especially well elaborated for many species by de Puytorac 1957, 1968.

De Puytorac 1957 on the example of *P. chattoni* Rossolimo differentiates (p. 233): "de fibrilles tenues situées dans l'ectoplasme notablement épaissi au fond de la ventouse (1.5 μ) ou à la limite ectoendoplasmatique. Elles sont disposées en six plans principaux repérables, constituant ainsi autant de trames squelettiques superposées, dont l'étude detaillée, peu aisée sur coups, est grandement facilitée par l'examen in toto au contraste de phase. Ces différentes strates se succèdent comme auit de la profondeur vers la surface". De Puytorac 1963 (p. 220–221): "Chez certaines espèces où le cytosquellete est plus imposant (*P. chattoni*, *P. lumbriculi*) nous avons pu distinguer (1957), dans le détail, six plans constitutifs, disposés en antagonisme deux à deux, mais ceux-ci peuvent se remaner essentiellement à trois plans fondamentaux. Les études de coupes ultrafines, observées au microscopes électroniques... nous permettent d'infirmer la nature myoides de ces éléments, de démontrer leur ultrastructure périodique complexe, de prouver l'origine infraciliaire de certains d'entre eux, au moins, de confirmer l'opinion que nous avancions (1957) selon laquelle certains seraient des cinétodesmes, et d'tayer l'idée de leur nature d'éléments de soutien".

Undoubtedly the studies on the architecture of the sucker are a serious background for systematic and phylogenetic considerations. However, attention must be paid

to the problem that this system as it is stressed by de Puytorac, is not only subjected to some individual fluctuations but first of all to distinct ontogenetic changes, from its origin de novo in opisthe as to its complete organization. Hence we have to take into consideration for the comparative purposes the skeletal system of the sucker of a completely developed individual, i.e., an individual beginning the division (proter).

The known species of the genus *Ptychostomum* Stein, left within it after the revision are finally very close to each others both by the general shape of their body and by the arrangement of the nuclear apparatus, the vacuolized zone of food vacuoles, and the character of the sucker. Very often their description differ from one another by not essential characters depending on the question whether the ciliate was examined in vivo or in preparations with a flatten or contracted sucker. In some not very numerous descriptions the silver method has been applied (especially valuable was the dry Klein's silver method) which allowed for a more detailed description of the structure of the sucker, the number of kineties, the range of adoral kineties. There were many profits from the statement of the arrangement of fibres strengthening the sucker what was the masterpiece of de Puytorac. However, it introduced some confusion for the reason that many previous descriptions did not report these details what in turn did not mean that these species were really lacking these details. Generally speaking the lack of uniformity of descriptions makes difficult the comparison therefore the statement of an adequate synonimics of species.

It seems that in many cases the individuality of the species is determined in the opinion of the authors by the individuality of the host; this view is not valid. Also reversely — the identity of the host does not prove the identity of the parasites therefore the cause to synonimization. In one of so well examined host as *Tubifex tubifex* occur at least two species: *P. saenuridis* and *P. magna*, and in *Lumbriculus variegatus* also two: *P. chatoni* and *P. lumbriculi* — both pairs of "good" species.

The two species from the alimentary canal of *Prosobranchia T. tanischi* (Miyashita) and *P. campelomae* Kozloff, do not differ from most of the representatives of the genus *Ptychostomum* living in the intestine of *Oligochaeta*, they are by the way very close to one another. In spite of the basic individuality of the host groups, these species would remain within the genus *Ptychostomum* Stein, similarly as vice versa one species parasitizing in *Oligochaeta*.

On the basic of a schedule of a large material and a definition stated by previous authors and Raabe 1949 — the genus *Ptychostomum* may be characterized as follows:

Ptychostomum Stein, 1860

syn.: *Lada* Vejdovsky, 1882.

Hysterocinetidae with a lateral flattened body of an oval outline. The sucker has the shape of an elongated and rounded horse-shoe

with widened arms joining posteriorly together. Through the middle part of the sucker there runs a furrow with ciliary rows; these rows are separated from those covering the remainder of the body by a non-ciliated area. The peristome occupies a part of the posterior body margin. The ellipsoidal Ma, located in the middle of the body; Mi lies in front of Ma. One C. V. lies in the posterior part of the body, occupied by the vacuolized area. The vacuolized area is distinctly limited and does not project anteriorly. Parasites of the intestine of *Oligochaeta* and *Gastropoda–Prosobranchia.*

Typus generis: *Ptychostomum saenuridis* Stein, 1860, 1861.

I range 24 species to the genus *Ptychostomum* after a revision; 22 among them from *Oligochaeta*, two from *Prosobranchia.*

Ptychostomum saenuridis Stein, 1861

syn.: *Ptychostomum iliodrili* Heidenreich, 1935; *Lada issa* Kijenskij, 1925; *Ptychostomum issum*: Heidenreich 1935, Raabe 1949.

This genus has been described from *Saenuris variegata* Hoffm. therefore undoubtedly from *Tubifex tubifex* Müller by Stein 1861. Maupas 1883 reports from this host (as *Tubifex rivulorum*) from Algeria, Rossolimo 1925 as a parasite of *Ilyodrilus hamoniensis* Mich. from Russia, Kijenskij 1925 describes *Lada issa* from the intestine of *Tubifex tubifex* Müll. and *Ryacodrilus* (=*Taupodrilus coccineus* Vejd.).

Heidenreich 1935 a did a review of the known forms and compared then with his material from *Tubifex tubifex* Müll. and as a result proposed the exclusion of the forms described by Maupas 1883 and Rossolimo 1925 in an individual species of *P. iliodrili.* Raabe 1949 recognized *P. iliodrili* Heidenreich, 1935 as a synonym of *P. saenuridis* Stein, 1861; on the other hand he recognized the possibility of the exclusion of this species of the form described by Heidenreich as *P. saenuridis* and recognizing it as identical with the species *P. issum* (Kijenskij, 1925). Finally Meier 1954 dealing with the parasites of *Tubifex tubifex*, *Ryacodrilus coccineus*, *Tubifex albicola* and *Ilyodrilus hamoniensis* recognized all of the forms from these hosts as *P. saenuridis* Stein and synonimized the diverging names. Her decision may be as a proper one in this confused problem (Fig. 12 A–E).

The comprehensive description of the species may by as follows:

The body slightly elongated, widened in this posterior part, especially in the moment of the contraction of the sucker. Length 40–110 μ, width 26–85 μ (the data of Maupas 1883, Rossolimo 1925, Kijenskij 1925, Heidenreich 1935, Raabe 1949, Meier 1954). The sucker rounded, dimensions ca. 20×15 μ, some-

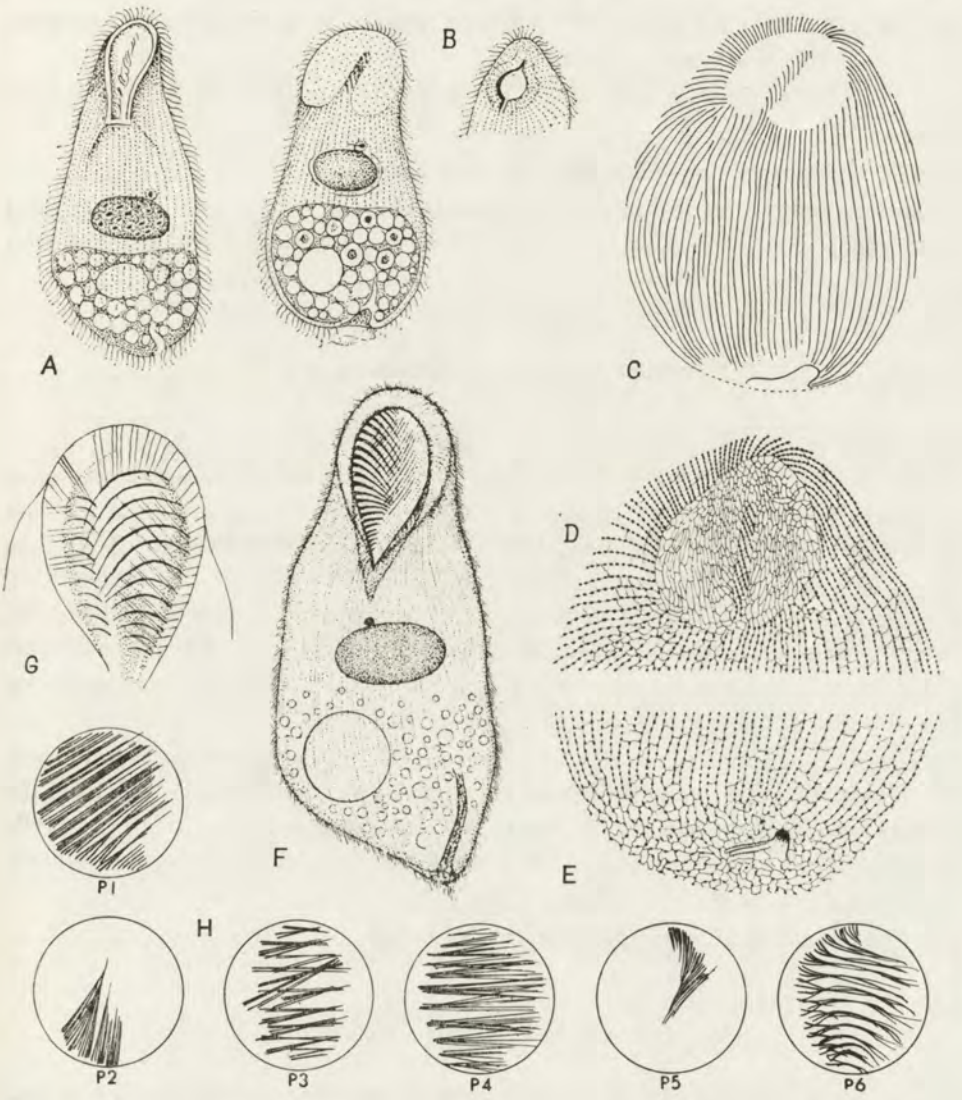

Fig. 12. *Ptychostomum*: A — *P. saenuridis* (=*P. issum*) (after Heidenreich); B, C, D, E — *P. saenuridis*, general aspect, kinetome, argyrophilic structure of the sucker and of the buccal region (after and from Raabe); F — *P. chattoni* (from Rossolimo), G — *P. chattoni*, the sucker (after Heidenreich), H — *P. chattoni*, elements (P1–P6) of the skeletal apparatus of the sucker (from de Puytorac)×500 resp. 1000

what obliquely arranged. Ca. 8 kineties enter to its ciliate gutter, the general number of kineties of the general ciliature — ca. 85, the cilia measure ca. 10 μ.

Ma ovoid measures 10–25×12–15 μ, lies in the middle of the body, transversally to its length, a small Mi, ca. 5 μ, lies in front of Ma. The widened hind part of the

body is occupied by a well defined vacuolized area; C. V. lies in this zone somewhat shifted towards the ventral margin.

The peristome is short and lies on the posterior margin of the body, the cytopharynx is not long.

The skeleton of the sucker has not been examined.

Hosts: *Tubifex tubifex* Müller, *T. albicola* (Mich.), *T. barbatus* (Grube) most often and highly infested (Meier) and *Ryacodrilus coccineus* Vejd. and *Ilyodrilus hamoniensis* Mich. — Europa.

Ptychostomum chattoni Rossolimo, 1925/26

syn.: *Ptychostomum vejdovskyi* Kijenskij, 1925.

The body elongated widened at the back, the anterior end narrowed, the posterior end sharply blunted. Length 120–185 μ width max. 45–70 μ (according to the data of Rossolimo 1925, Kijenskij 1925, Heidenreich 1935, Meier 1954). The sucker narrowed in the posterior end, dimensions 50–80×25 μ (de Puytorac 1957). Ma ovoid 25×15 μ, arranged obliquely more or less in the middle of the body, Mi anterior to Ma. The peristome is short, the cytopharynx long. The posterior part of the body is occupied by a vacuolized zone (30 μ according to Kijenskij) among it C. V. with a diameter to 10 μ).

The skeletal apparatus of the sucker consist of fibres arranged in several layers. The dominating layer consist of thick fibres running arcuately backwards from the ventral margin of the sucker. De Puytorac differentiates 6 layers of them: P_1–P_6 arranged in an individual way — the layer P_6 was the most distinctly outlined, consisting of arcuated fibres (Fig. 12 F–H).

Host: *Lumbriculus variegatus* (Müll.) — Europe.

Ptychostomum lumbriculi Heindenreich, 1935

The body elongated with a rounded anterior end, the posterior one is truncate. Length 60–95 μ, width ca. 40 μ. The sucker is large, narrowed towards the back.

Ovoid Ma lies in the middle of the body, transversally to its axis, Mi anterior to Ma. 1/3 of the body is occupied by the vacuolized zone; a large C. V. among it. The peristome occupies the truncate margin of the body.

The sucker strengthened by fibres, arranged in 3 layers: according to Heidenreich; transversal fibres running obliquely and crossing one another. De Puytorac finds 6 layers; its P_1, P_2 and P_4 correspond to these which has seen Heidenreich (Fig. 13 A–C).

Host: *Lumbriculus variegatus* (Müll.) rarely with *P. chattoni* — Europa.

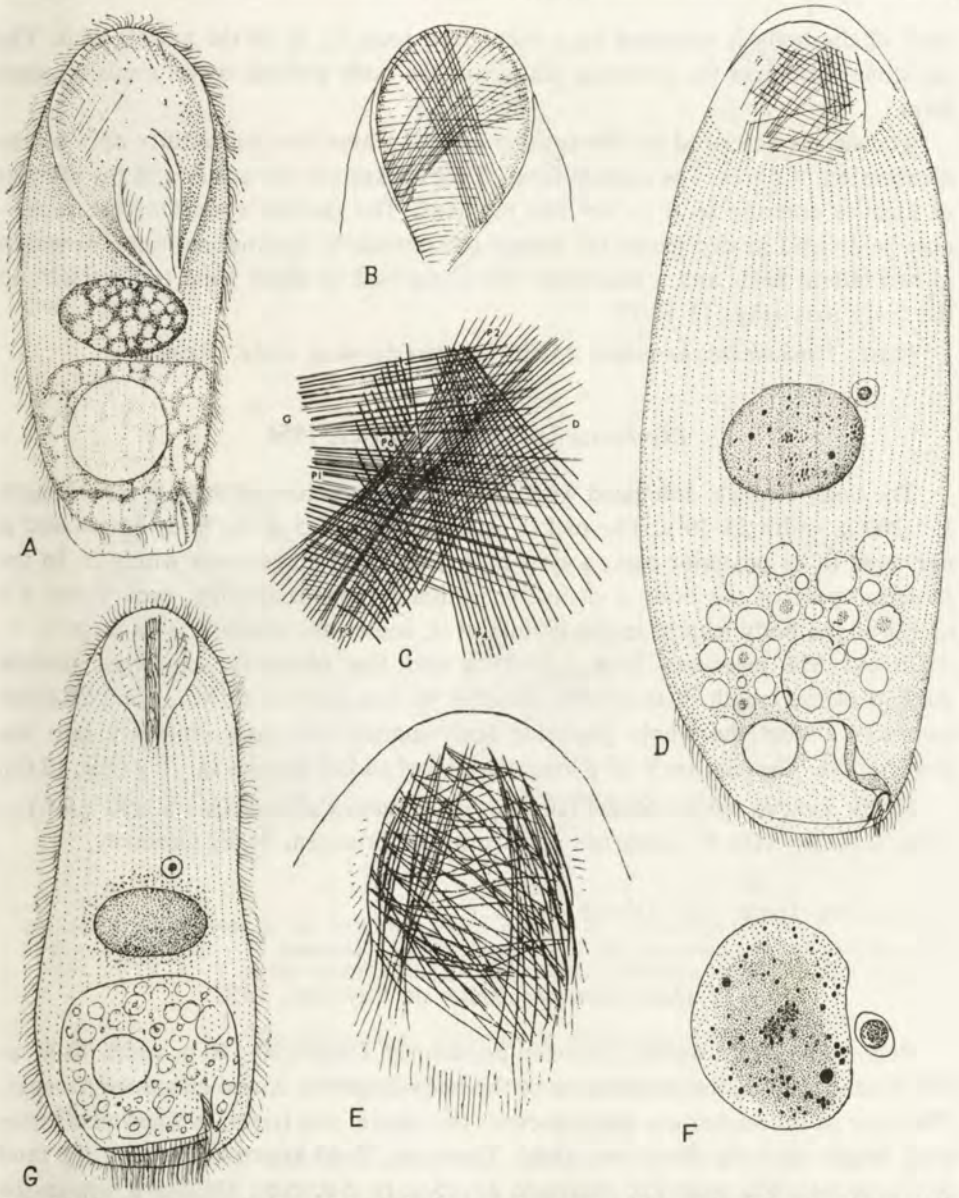

Fig. 13. *Ptychostomum*: A, B — *P. lumbriculi*, general aspect and the sucker (after Heidenreich), C — *P. lumbriculi*, fibrils of the sucker (from de Puytorac); D, E, F — *P. rossolimoi*, general aspect, the sucker and nuclear apparatus (after Studitsky); G — *P. magnum* (a. Meier) × 500 resp. 1000

Ptychostomum rossolimoi Studitsky, 1930

The body in the shape of an elongated oval. Length — 75–85 μ. The sucker, diameter ca, 25 μ, occupies the anterior part of the body. Ma 40–50 μ × 34–39 μ lies obliquely in the middle of the body, Mi 7–9 μ lies in a small cavity of Ma. The

back of the body is occupied by a vacuolized zone, C. V. in the middle of it. The peristome embraces the posterior margin of the body pointed by an arcuately bent form.

The sucker described by the authors as oval whose two parts differ only by the distribution of fibres. The outside form of the sucker nor the gutter and the number of kineties entering to it — are not reported. The skeletal system of the sucker, may be divided in two parts: the deeper one consist of longitudinal fibres arranged in two lateral fields and a shallower one, composed of fibres lying transversally to the body axis (Fig. 13 D–F).

Host: *Limnodrilus nevaensis* Mich. — Pereslavskoe Lake, Russia.

Ptychostomum magnum Meier, 1954

The body strongly elongated with a symmetric structure of the left side. Length 125–200 μ, width 55–70 μ. The sucker occupies 1/4 to 1/5 of the body length and is narrowed in its posterior part; a system of transversal fibres occur within it. In the posterior part of the body a distinctly outlined vacuolized zone, occuppying 1/3 to 1/4 of the body length, in the middle of it, somewhat ventrally — a large C. V. An ovoid Ma measures 35–45×20–25 μ and lies obliquely somewhat outside the half of the length of the body; the oval Mi lies anterior to Ma. The peristome embraces almost the whole posterior body margin and pass obliquely into the cytopharynx. The cilia are 8–10 μ long, the cilia of adoral kineties ca. 15 μ (Fig. 13 G).

Hosts: *Tubifex tubifex* Müller (10–25% inf.), *Tubifex albicola* (Mich.) (30% inf.) — often together with *P. saenuridis* — vicinity of Erlangen, N. E. Germany.

Species from the Ohrid Lake:

Ptychostomum ohridanum de Puytorac, 1958

Body of an ovoid outline, rounded posteriorly. Length 80–140 μ, width 70–80 μ. The sucker is large, the relationship to the body length 1 : 2, and closes 8–9 kineties. The arms of the sucker are dissymmetric: the ventral one (right) is wider and somewhat longer than the dorsal one (left). There are 75–80 kineties reaching the sand of the sucker. Ma spherical measures 21–25 μ in diameter; Mi 5–6 μ lies in the depression of Ma.

The fibres of the sucker are arranged in 3 layers: the deeper one contains 4–5 longitudinal fibres arranged rather in the ventral arm of the sucker, the central layer — 6 fibres somewhat obliquely arranged, the outside, superficial layer — 3 fascicles with 5 thick fibres each of them crossing with the previous ones and with each others (Fig. 14 A, B).

Host: *Ilyodrilus isochaetus* Hr. — Lake Ohrid, S. Yugoslavia.

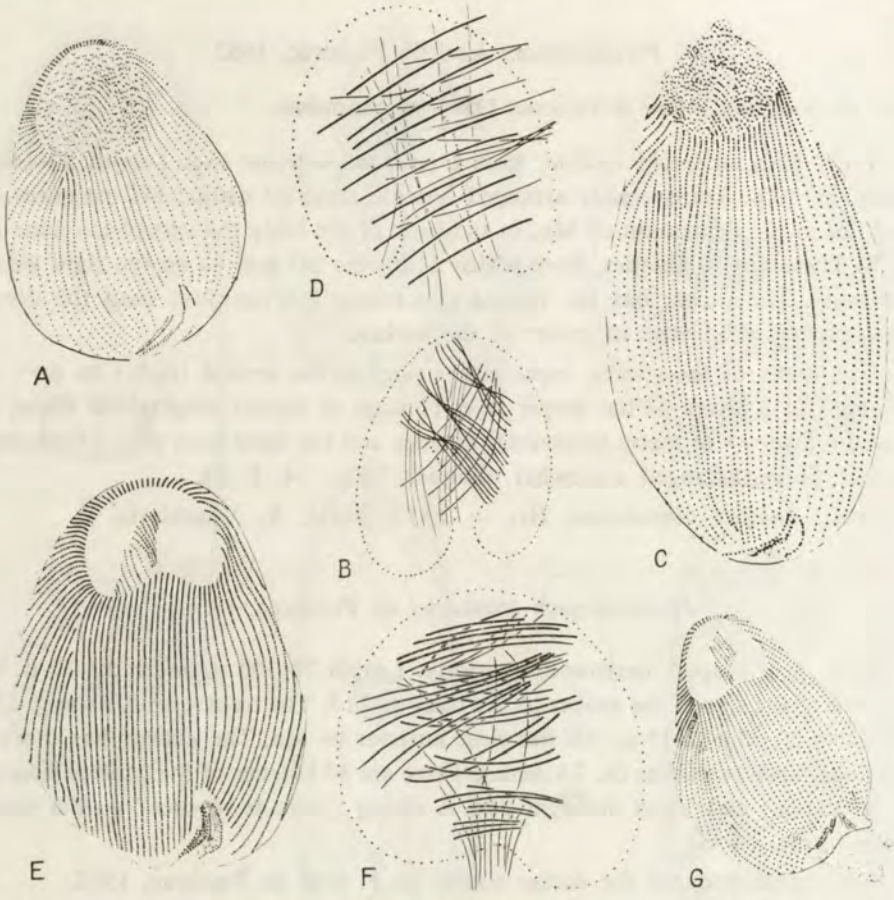

Fig. 14. *Ptychostomum* sp. sp. from the Ohrid Lake: A, B — *P. ohridanum*; C, D — *P. meierae*; E, F — *P. lomi*; G — *P. stankovici* — general aspects of the kinetome and the skeletal systems of the sucker (from de Puytorac) × 500 resp. 1000

Ptychostomum meierae de Puytorac, 1958

syn.: *Ptychostomum meieri* de Puytorac, 1958 — the corrected name according to the Codex of Nomenclature.

Body elongated, narrowed anteriorly. Length 117–190 μ width 57–90 μ. Ma spherical, measures 20–25 μ in diameter; a small Mi. The general shape approximate to *P. elongata* Cheissin from Baical Lake. About 60 kineties, 20 of them at the left side of the body; a strong "zone de sécance" on the ventral margin. The sucker is small, the relationship to the body as 1 : 5, the arms of the sucker are dissymmetric, in their posterior parts distant to one another. The gutter contains 8–10 kineties.

The fibres of the sucker arranged in 3 layers are feeble, straight and scarce (Fig. 14 C, D).

Host: *Stylodrilus leucocephalus* Hr. — Lake Ohrid, S. Yugoslavia.

Ptychostomum lomi de Puytorac, 1962

syn.: *Ptychostomum jirilomi* de Puytorac, 1958 — nomen nudum.

Body with an ovoid outline, with a rounded anterior end. Length 80–140 μ, width 77–80 μ. Transversally arranged Ma measures 50×10 μ; Mi measures 6 μ and lies in the depression of Ma. In the back of the body the vacuolized zone and C. V. There are 70 kineties, from which 24 on the left and 46 on the right side of the body. The sucker has the ventral arm longer but narrower than the dorsal; 14 segments of kineties in gutter of the sucker.

The fibres of the sucker, especially strong in the ventral (right) its part, are arranged in 3 layers — the deeper layer consists of several longitudinal fibres, the medium layer — of many transversal kineties and the third layer of 3–5 fascicles of surface fibres, arranged somewhat obliquely, (Fig. 14, E, F).

Host: *Tubifex oligosetosus* Hr. — Lake Ohrid, S. Yugoslavia.

Ptychostomum stankovici de Puytorac, 1958

Body pear-shaped, narrowed anteriorly. Length 70–100 μ, width 50–70 μ. The relation of the size of the sucker to the body as 1: 3. The transversally arranged Ma measures 20–30×12–15 μ. Mi lies close anterior to Ma. The sucker has more or less equal arms, contains ca. 7 kineties. There are 80 kineties of the general ciliature, 20 on the left and 60 on the right side. A strong "zone de sécance" on the ventral margin (Fig. 14 G).

The cytoskeleton of the sucker similar to *P. lomi* de Puytorac, 1962.

Host: *Tubifex ochridanus* Hr. — Lake Ohrid, S. Yugoslavia.

Species from the Baical Lake — Siberia:

Ptychostomum pygostomum (Rossolimo, 1926)

syn.: *Lada pygostoma* Rossolimo, 1926.

Body elongated and strongly flattened. Length ca. 200 μ, width 75–85 μ. Diameter of the round sucker ca. 50 μ. Ma reniform, Mi in the cavity of Ma. C. V. in the posterior part of the body rather ventrally in the area of food vacuoles. Peristome in the back of the body; the left (dorsal) part strongly developed, forms a sharp posterior process. The strong cilia are in the inside of the periostome turning into membranelles of the cytopharynx (Fig. 15 A).

Hosts: *Peloscolex inflatus* (Mich.) and *Lampodrilus satyriscus* f. *ditheca* Mich. — Baical Lake, Siberia.

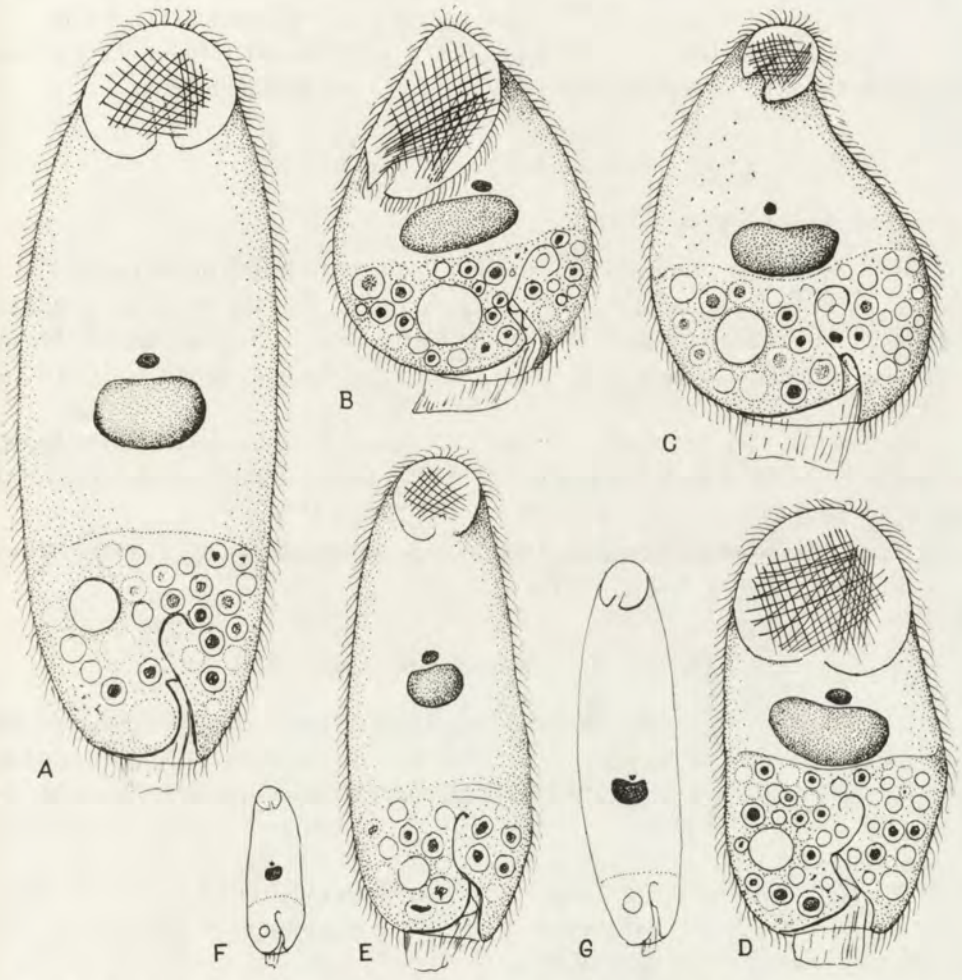

Fig. 15. *Ptychostomum* sp. sp. from the Baical Lake: A — *P. pygostomum*; B — *P. asymmetricum*; C — *P. limnodrili*; D — *P. baicalense*; E — *P. elongatum*, F, G — *P. elongatum* typ. et *P. elongatum* f. *gigas* (all after Cheissin) ×500, F, G ×250

Ptychostomum asymmetricum (Cheissin 1928)

syn.: *Lada asymmetrica* Cheissin, 1928.

Body wide and short with a distinct dissymmetry of the large sucker, arranged clearly obliquely to the body axis. Length ca. 100 μ, width from 25 to 85 μ; the diameter of the sucker ca. 50 μ, the relation of the length of the sucker to the body length as 1 : 2. Ma ovoid, elongated, arranged obliquely anterior to the half of the body length, Mi anterior to Ma. The vacuolized area occupies 1/2 of the body length.

The sucker is strengthened by fibres arranged in 3 directions (Fig. 15 B).

Hosts: *Clitellio korotneffi* (Mich.), *Clitellio multispinosus* (Mich.). *Limnodrilus arenarius* (Müll.), *Limnodrilus dybowski* Grube — Lake Baical, Siberia.

Ptychostomum baicalensis (Cheissin, 1928)

syn.: *Lada baicalensis* Cheissin, 1928.

Body ovoid with a large sucker, the relationship of the length of the sucker to the length of the body as 1 : 3. Length 85–170 μ, width 30–60 μ; the length of the sucker 25–50 μ. Ma reniform somewhat anterior to the half of the body length, Mi in its anterior cavity. The vacuolized area occupies 1/3 of the body length. Cilia 10–12 μ long.

The sucker, with the ventral arm distinctly longer, is strengthened by the fibres, crossing in 3 directions. Its surface on the stained (silvered) preparations is covered by a net with meshes drawn along its anterior margin (Fig. 15 D).

Hosts: *Styloscolex chorioidalis* Isossimo, *S. baicalensis* Mich., *S. assymetricus* Isossimoff — Baical Lake, Siberia.

Ptychostomum elongatum (Cheissin, 1928)

Body strongly elongated with a relatively small sucker — the relationship of the length of the sucker to the body as 1 : 5. Length 120–160 μ, width 35–50 μ, sucker 25–30 μ. Ma reniform lies in the middle of the body, Mi anterior to Ma. The vacuolized zone occupies 1/5 of the body length; C. V. in this area, somewhat ventrally (Fig. 15 E, F).

Cheissin differentiates *P. elongatum* f. *gigas* with following dimensions: length 260–360 μ, width 35–50 μ, the sucker 25–35 μ; the relationship of the sucker to the body length as 1 : 7 up to 1 : 9 (Fig. 15 G).

Hosts: *Lamprodrilus ammophagus* Mich., *Teleuscolex korotneffi* Mich.; *P. e.* f. *gigas* in *Limnodrilus* sp. — Baical Lake, Siberia.

Ptychostomum limnodrili Cheissin, 1932

Body considerably widened in the posterior part. Length 100–170 μ, width in the anterior part 20–25 μ, in the posterior part 60–70 μ. Dissymmetric relatively small sucker measures 20–25 μ, the relationship of the sucker to the body length as 1 : 5. The sucker has a wide groove and is provided by crossing strengthening fibres. Ma ovoidal lies in the middle of the body; Mi anterior to Ma. A vacuolized zone occupies 1/3 of the body length; C. V. in this area. Peristome short, cytopharynx also short, slightly curved (Fig. 15 C).

Host: *Limnodrilus baicalensis* Mich. and *Tubifex* sp. — Baical Lake, Siberia.

Ptychostomum longinuclei Katashima, 1952

The body is oval. Length 173 μ, width 86 μ. A large cup-shaped sucker occupies the anterior part. In the middle of the sucker there is a narrow groove resembling a convex lens in shape. The fibers are regularly arranged in the posterior half of the sucker. A long Ma (56–75 μ in length) accompanying a Mi is present in the center. The peristome is well developed, running from the left corner to the cytostome. It opens to the right corner of the posterior margin and is provided with the ventral and dorsal lips. A single C. V. is surrounded by a distinct area filled with many food vacuoles. (Fig. 16 A).

Host: *Pheretima communissima*, Onomichi, Japan.

Species from Gabon:

Ptychostomum eminoscolecis de Puytorac, 1968

Body moderately elongated, length 130–170 μ, width 78–100 μ. The sucker is round and very large. The skeletal apparatus see Fig. 16. There are 28–30 kineties on the left and ca. 80 kineties on the right body side. In the funnel of the sucker there are ca. 12 segments of kineties. Ma ovoid disposed perpendicularly to the long body axis, Mi before the Ma (Fig. 16 D, E).

Host: *Eminoscolex torentus* (?) Mich. — Gabon, Africa (with *Epicharocotyle raabei* de Puyt., 1968 and *Protoptychostomum tertium* de Puyt., 1968).

Ptychostomum commune de Puytorac, 1968

Body elongated; length 120–130 μ, width 50–55 μ. The skeletal apparatus of the sucker see Fig. 16. There are 35–40 kineties, among them 14 on the left body side. The peristomal apparatus is well developed. In the funnel of the sucker there enter 3–6 segments of the kineties.

The large Ma, 30–35 μ in diameter is situated in the middle of the body and directed obliquely to the long body axis. The Ma, 6–7 μ, on the ventral side of Ma. (Fig. 16 F, G).

Host: *Alma emini* Mich. — Gabon, Africa.

Ptychostomum vulgare de Puytorac, 1968

The young individuals are 260–270 μ in length and 85–90 μ in width. The rounded sucker has an abundant skeletal apparatus — see Fig. 16. Ma large and spherique; C. V. has a number of pores on the right (?) body side. Peristome is long and large (Fig. 16 B, C).

Host: *Eminoscolex* sp.? — Mekambo, Gabon, Africa.

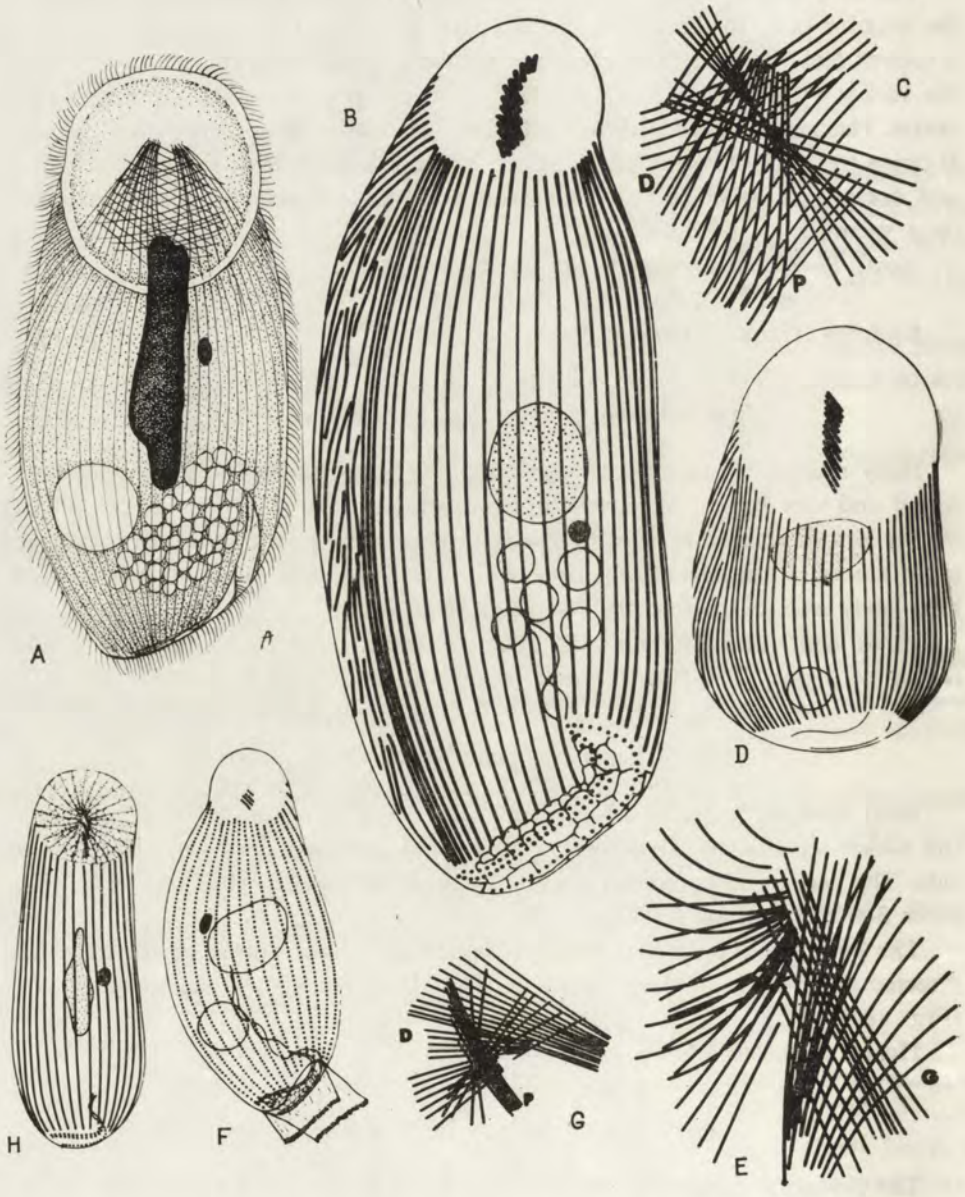

Fig. 16. *Ptychostomum*: A — *P. longinuclei* (after Katashima); B–C — *P. vulgare*; D–E — *P. eminoscolecis*; F–G — *P. commune*; H — *P. almae* (from de Puytorac) ×500 resp. 1000

Ptychostomum almae de Puytorac, 1968

Body elongated, 90–120 μ in length, 30–35 μ in width. The small sucker occupies 1/4 of the body length. There are 12–14 kineties on the left and ca. 20 kineties on the right body side. 2–3 segments of kineties (Z. R.) enter in the groove of the sucker.

Ma elongated, 30×9 μ; Mi is large, 5–6 μ in diameter (Fig. 16 H).

Host: *Alma* sp. — Bolinga, Gabon, Africa.

Ptychostomum wrzesniowski (Vejdovsky, 1882)

syn.: *Lada wrzesniowski* Vejdovsky, 1882.

The description and drawings of this species reported by Vejdovsky 1882 are quite fantastic, and the morphologic interpretation completely false. The description of Vejdovsky reports only: "The anterior end with the sucker; the depression surrounded by a semicircular swelling; in the hollow ranges of cilia; the eliptic nucleus; vacuoles in the left part and endoplasmic granulations (= in my opinion food particles, Z. R.) in the posterior end an aperture, which I consider as anus". (Fig. 17 A).

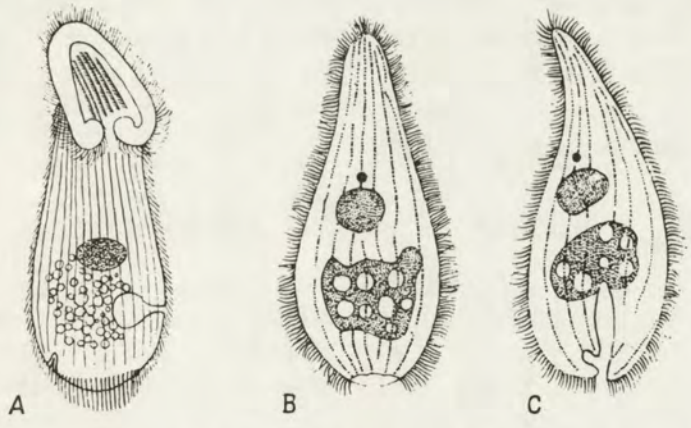

Fig. 17. *Ptychostomum*: A — *P. wrzesniowskii* (after Vejdovsky); B, C — *P. dichogasteri* (A. Tuzet et Zuber-Vogeli)×500

This species has never been seen or examined by anybody — it may be considered as species inquirenda!

Host: *Trichodrilus* (=*Phreatothrix*) *pragensis* Vejd. — spring waters of Praha (Central Europe).

Ptychostomum dichogasteri Tuzet et Zuber-Vogeli, 1955

Species absolutely incompetently described compared by the authors only to *P. lumbriculi*. Body pear-shaped anteriorly narrowed. Length 68–84 μ, width 33–38 μ. Ma and Mi lies in the back of the body, behind them the vacuolized zone of the plasma. This species must be recognized as species inquirenda! (Fig. 17 B, C).

Host: *Dichogaster inermis* Mich. in vesiculae seminales (? ! Z. R.) — Man, Africa.

Species from *Prosobranchia*:

Ptychostomum tanishi (Miyashita, 1927)

syn.: *Lada tanishi* Miyashita, 1927.

Body flattened, rounded anteriorly and truncated posteriorly. Length 60–140 μ, width 30–70 μ. The sucker is sphaerical, 30–50 μ in diameter, and has in the middle a thickly ciliated groove with 20 transverse kineties, 3–10 basal granules each. In the sucker numerous "myonemes".

The Ma is ellipsoidal or sometimes reniform in outline, its longer axis measures 20–30 μ. Mi is located at the anterior side of Ma and is 7–10 μ in length. A large well outlined area of food vacuoles in the hind part of the body. C. V. lies in this area.

The peristome at the posterior end of the body, the cytostome at its posterior left corner (Fig. 18 A, B).

The division and the heterogamic conjugation are observed.

Host: *Viviparus japonicus* — Japan.

Ptychostomum campelomae Kozloff, 1960

Very closely related to *P. tanishi* Miy. Body rounded anteriorly and truncated posteriorly. The size and shape varies a great deal: relatively small forms in which the sucker often occupies nearly all the width of the anterior part of the body, and larger individuals in which the sucker is usually only about one-half the width. The body is very plastic. Length 57–184 μ, width 30–102 μ, the thickness is 2/3 of the width.

The Ma is ovoid, ellipsoidal, or sometimes reniform in outline, its long axis being perpendicular to the long axis of the body; the relatively large Mi lies close to the Ma, on its anterior side. Posterior to Ma, there are numerous small food vacuoles: C. V. in this area. The number of kineties is 95–115; the cilia are about 9 μ long. The buccal cavity is a rather narrow canal at the posterior end of the body.

The disc-shaped sucker is approximately circular in outline; the number of kineties in the median furrow is 11 or 12 (or even more), some of them consisting

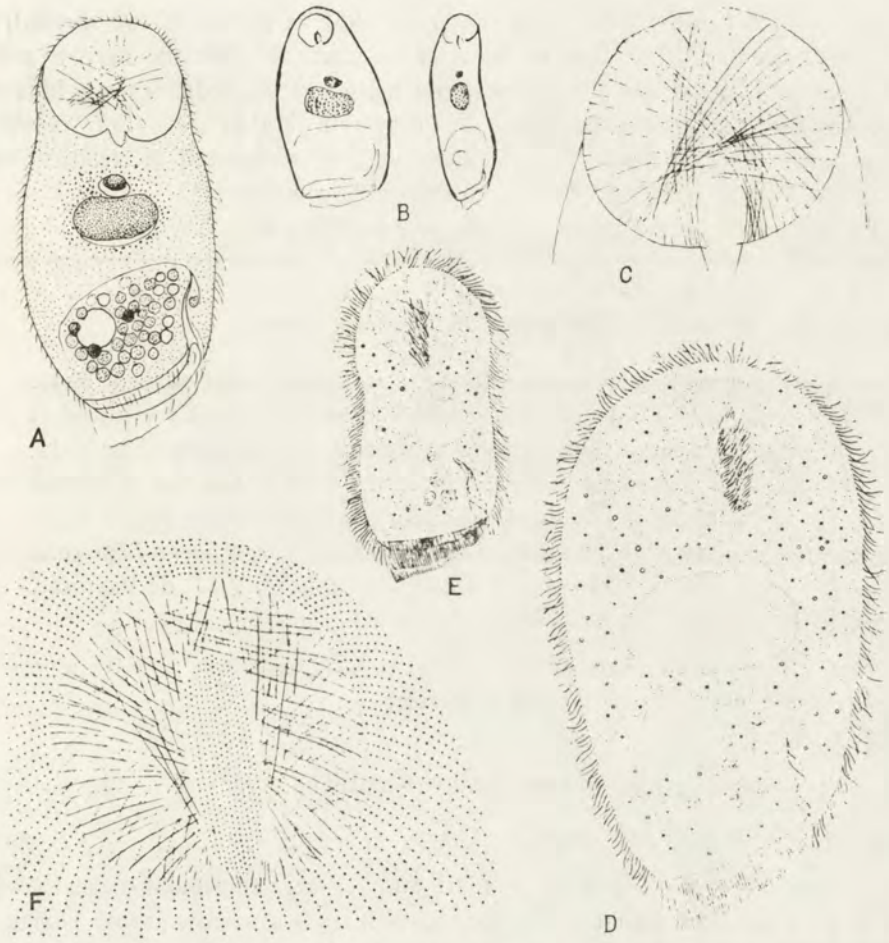

Fig. 18. *Ptychostomum* sp. sp. from *Prosobranchia*: A, B, C — *P. tanishi*, general aspect and two various types (after Miyashita); D, E, F — *P. campelomae*, small and great form and the structure of the sucker (after Kozloff) × 500 resp. 1000

of only few cilia. The fibres in the sucker appear to fall into three principal groups (Fig. 18 D, E).

Host: *Campeloma geniculum* (Antony), *Viviparidae* — N. Carolina, USA. Of 46 snails, 31 were infected; to 100 ind. or more in one snail (a number of snails were infected also by a species of *Protoanoplophrya*).

Genus *Coelothigma* de Puytorac, 1968

This genus created de Puytorac 1968 d for the differentiation of the species *P. canalis* Katashima, 1952 from the genus *Ptychostomum* [n.b. de Puytorac

reports incorrectly (p. 264, 274) Miyashita, 1951 as author of the species!] De Puytorac 1968 d recognized as an essential character that the narrow groove of the sucker is prolongated to the posterior margin of the body. Some doubts may arise whether the groove described and drawn by Katashima 1952 is not an artefact, for the time being this character may be recognized as really virtual. Therefore I leave here the genus *Coelothigma* as motivated.

The diagnosis of the genus may be stated as follows:

Coelothigma de Puytorac, 1968

Hysterocinetidae of a moderately elongated, pear-shaped body. The front part of the body occupies the sucker, its ciliary funnel is prolongated posteriorly, enlarged and reaches the hind part of the body. No data on the skeletal apparatus of the sucker. Ma lies in central part of the body, the C. V. and the area of the vacuolized plasm in the posterior body part. Parasites in the intestine of *Oligochaeta–Limnicola*.

Typus generis: *Coelothigma canalis* (Katashima, 1952) de Puytorac, 1968.

The genus embraces at present 1 species:

Coelothigma canalis (Katashima, 1952)

syn.: *Ptychostomum canalis* Katashima, 1952.

The body is oval. Length 137 μ; width 50 μ. A large ellipsoidal sucker occupies the anterior part. In the middle of the sucker there is a narrow groove having the outline of a convex lens. The groove runs to the posterior margin of the body. An oval Ma and Mi are present in the center. The peristome is well developed running from the right corner of the cytostome, with aperture at the left (? Z. R.) corner of the posterior margin. It is provided with the ventral and dorsal lips. A single C. V. is found near the food vacuoles (Fig. 19).

Host: *Limnodrilus* sp. in the vicinity of Hiroshima, Japan.

Genus *Elliptothigma* Maier, 1954

This genus was created by Marie Meier 1954 in order to stress the individuality of the species *E. limnodrili* Meier, 1954 with a following motivation: "Die Einordnung dieses Ciliates in die bestehenden Gattungen stöst schon hinsichtlich der lenggestreckten Macronucleus und den Zahlreichen kontractilen Vacuolen auf Schwierigkeiten. Vor allem unterscheidet sich aber der Saugnapf zunächst von

dem für die Gattung *Ptychostomum* typischen durch das Fehlen einer Wimperrinne". The last character mentioned by the author depended clearly on the inexactitude of observation. De Puytorac 1957 presented a rediscription of the same species writing, that there exist here a ciliated groove completely corresponding to the relations in *Ptychostomum*. However de Puytorac emphasizes the distinct elongation

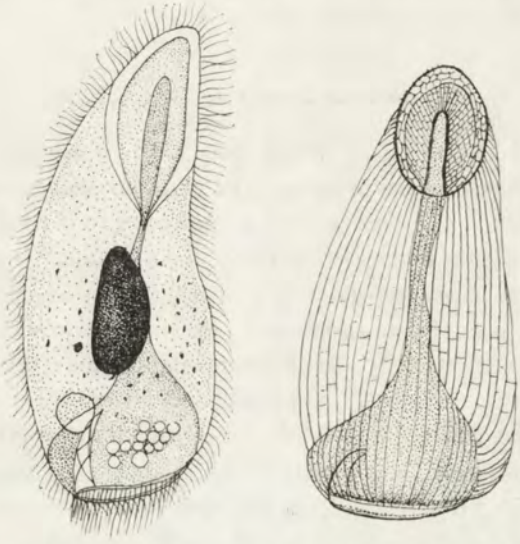

Fig. 19. *Coelothigma canalis* (after Katashima)×500

of Ma, a large number of C. V.-es and the multiplication by catenulative gemmation. This justifies the maintaining of the individuality of the genus.

Ptychostomum filiferum Katashima, 1952 accomplishes also the characters of the genus; it is so approximate to *E. limnodrili* that some suspection may arise concerning their identity. If it proved to be adequate, the species could be named: *Elliptothigma filiferum* (Katashima, 1952), syn.: *E. limnodrili* Meier, 1954, as wanted de Puytorac 1968 d.

The diagnosis of the genus *Elliptothigma* may run as follows:

Elliptothigma Meier, 1954

Hysterocinetidae of a middle elongated and flattened body, of a pear-shaped outline. The sucker rounded, open posteriorly, many kineties of the general ciliature enter to its furrow. The sucker is strengthened by many fibres disposed in several levels. The peristome occupies the posterior margin of the body; the adoral kineties and the infundibulum are short. The posterior vacuolized area is well outlined.

Ma is elongated, Mi is located on the side of the posterior part of Ma. There are numerous (ca. 10) C. V.-es. There occurs posterior budding and catenulation. Parasites of the intestine of *Oligochaeta*.

Typus generis: *Elliptothigma limnodrili* Meier, 1954.

The genus *Elliptothigma* embraces 2 (or 1) species:

Elliptothigma limnodrili Meier, 1954

Body large, strongly elongated; length 200–300 μ (together with the tomits it even amounts to 580 μ), width 50–90 μ. The ellipsoid sucker occupies 1/3 to 1/4 of the body. According to Meier kineties do not enter in it what was denied by de Puytorac. Kineties run somewhat spirally; their number is not reported.

The elongated Ma measures 100–150 μ of length and ca. 15 μ of width in the anterior end, 20–25 μ at the posterior end.; Mi in the posterior part of the body close to Ma, or in its small cavity. The posterior part of the body is occupied by the food vacuolized plasma. C. V. occur in the number of 10 and are arranged unregularly, ventrally to Ma. The peristome is small, the cytopharynx short (Fig. 20 A).

The sucker is provided in a system of fibres in which many layers may be distinguished according to Meier: lying the most lower, obliquely arranged fibres, then the longer fibres, diagonally arranged along the sucker.

De Puytorac 1957 describes in details the tomit of *E. limnodrili* and reports the drawning of its kinetom. The sucker embraces here 16 kineties entering from the back to its groove; it seems possible that over the maturation of the tomit the sucker shuts and cut the kineties as in *Ptychostomum*. The Ma of the tomit is oval or reniform, the C. V. occur anteriorly. De Puytorac distinguishes in the skeleton of the sucker "six trames principales superposées" (Fig. 20 B, C).

The multiplication of *E. limnodrili* is achieved by a posterior gemnation of tomits, measuring 60–100 μ of length and 40–50 μ of width. This in turn leads to catenulation; Meier observed the chains composed of a large protor and 4 opisthes — tomits (Fig. 20 D).

Hosts: *Limnodrilus udekemianus* Clap. (40% of inf.), *L. hoffmeisteri* Clap. and *L. claparedeanus* Ratz. — North Germany (Meier) *L. udekemianus* Clap.— Ohrid Lake, S. Yugoslavia (de Puytorac).

Elliptothigma filiferum (Katashima, 1952)

syn.: *Ptychostomum filiferum* Katashima, 1952.

The body is oval in shape. Length 230 μ, width 114 μ. A large ellipsoidal sucker occupies the anterior region. The sucker is provided with a narrow groove and four

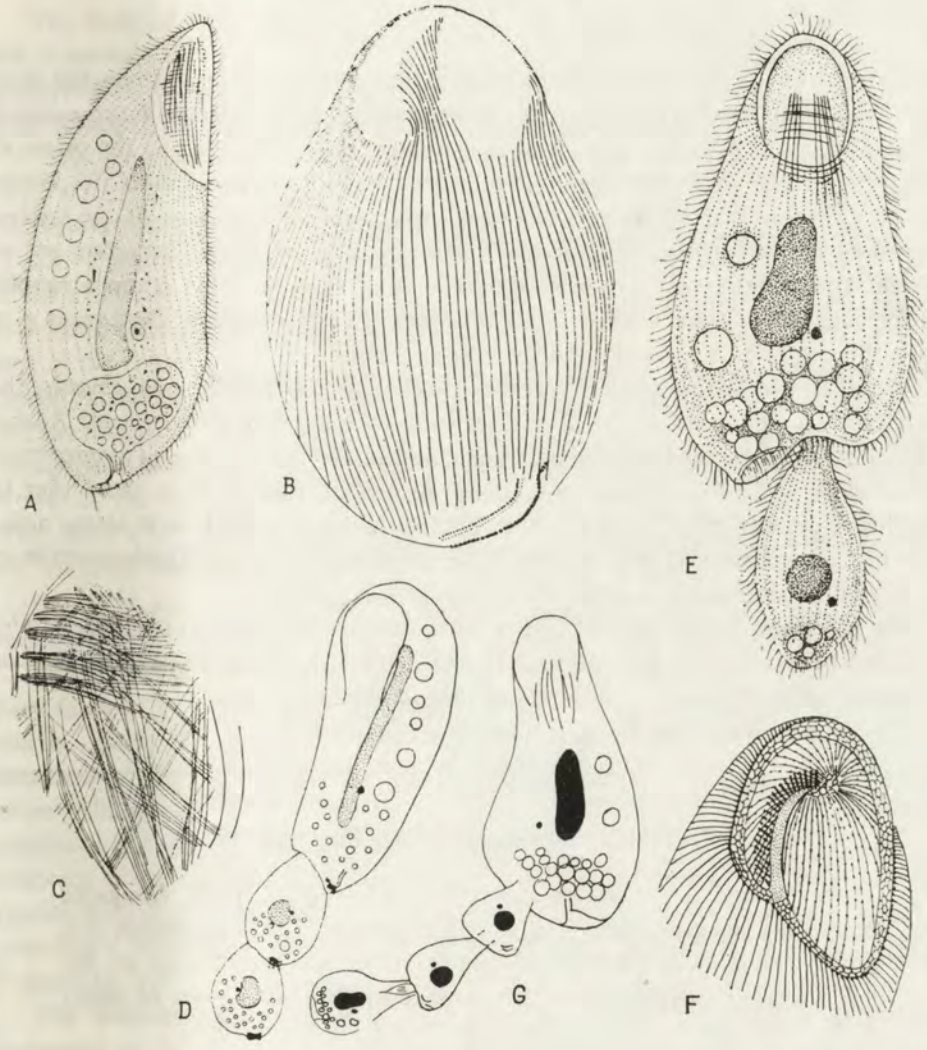

Fig. 20. *Elliptothigma*: A — *E. limnodrili* (after Meier); B — kinetome of a tomite of *E. limnodrili*, C — general aspect of the fibrils system, D — budding in *E. limnodrili* (all from de Puytorac); E, F, G — *E. filiferum* (after Katashima) A, B, F×500, C×1000, D, E, G×300

systems of the fibers. There are an ellipsoidal Ma and a Mi in the central region. In the left side of the posterior margin of the body the peristome is surrounded by the ventral and dorsal lips. Three or four C. V.-es are arranged in a row (Fig. 20 E, F).

Host: *Limnodrilus gotoi* — Hiroshima, Japan.

Subfamilia *Epicharocotylinae* de Puytorac, 1968

De Puytorac 1968 d differentiated this subfamily in order to stress the morphological individuality and a different evolutionary path of the genus *Epicharocotyle* Kozloff, 1965 in a wide approach of de Puytorac. This path led to the origin of the trough (la gouttière) elongating the sucker to the back, two flanges (les levres) in the posterior part of the sucker and a strong skeletal apparatus of the sucker, presented in the fair drawings of tde Puytorac 1968 a. These characters are so specific and amazing that they perfectly differentiate from the other *Hysterocinetidae* two genera close to one another: *Epicharocotyle* Kozloff, 1965 and *Puytoracia* g. n. with three species described by de Puytorac 1968 a.

Another evolutional trend perhaps parallel to the former one reveals the development which led to the forms assigned to the genus *Cotylothigma* Raabe, 1949. Both these trends has some common characters, namely: a strong development of the skeleton of the sucker, the existence of a canal running from the sucker to the posterior, finally the flanges in the back of the sucker, which as it seems occur also in *Cotylothigma*. For this reason I include to the subfamily *Epicharocotylinae*, at least provisionally, the genus *Cotylothigma* Raabe, 1949.

On the other hand I do not share the opinion of the Puytorac 1968 d, who included here the genus *Kysthothigma* Raabe, 1949. I think that this genus could be assigned rather to the subfamily *Craticuloscutinae* de Puytorac.

This subfamily could be characterized as follows:

Subfamilia *Epicharocotylinae* de Puytorac, 1968

Thigmotricha-Hysterocinetidae of a great (130–250 μ) elongated body. The sucker is provided with a complicated skeletal system and pass into a short through. There exist or not two flanges on the posterior part of the sucker. Ma rounded or elongated, one or many C. V. Parasites in the intestine of *Oligochaeta–Terricola*.

Typus subfamiliae: genus *Epicharocotyle* Kozloff, 1965.

I include to this subfamily 3 genera: *Epicharocotyle* Kozloff, *Puytoracia* g. n. and *Cotylothigma* Raabe.

Genus *Epicharocotyle* Kozloff, 1965

Kozloff 1965 established this genus in order to stress the high individuality of the species *E. kyburzi* found by himself and described at the same time. Virtually this species reveals many characters differentiating it among all *Hysterocinetidae*.

The body of *Epicharocotyle* is large (up to 200 μ) strongly elongated. Similarly Ma is strongly elongated as well as the zone of vacuolized plasma along it. There are numerous C. V.-es arranged in two rows. The sucker described by Kozloff is the most amazing form:

"The principal part of the sucker is approximately oval in outline and widest in its posterior half. Behind this and continuous with it, there is a trough — like excavation which extends posteriorly and curves toward the left. The deeper portion of this trough and a small area of the more expansive part of the sucker just anterior to the trough are ciliated. On either side of the sucker arising from the margin there is a delicate flange. These flanges are widest in the posterior portion of the principal part of the sucker, then diminish as they follow the trough to its posterior end. The flange on the right side is more extensively developed then the one on the left. In life, the flanges usually appear as veil-like coverings which have the effect of reducing the aperture of the sucker, and which overlap to cover the trough almost completely. However, the flanges may be thrown back and the portions covering the trough often show a vibratile response to the activity of the cilia beneath them. The flanges are bordered by a fringe of thick, inactive cilia".

Kozloff reports further some data concerning the structures strengthening the sucker and rod-like bodies arranged on its surface.

De Puytorac 1965 a described 3 species of *Hysterocinetidae* in *Oligochaeta* from Gabon; he enclosed them to the genus *Epicharocotyle* as *E. kozloffi*, *E. grassei* and *E. raabei*. Virtually they seem to correspond to *Epicharocotyle kyburzi* by the structure of the sucker and the presence of flanges, however they have an ovoid or only slightly elongated Ma and, what is more important, only one C. V. I consider especially this difference as very important for the problems of evolution; the same concerns *Elliptothigma* and *Craticuloscuta*. For these reasons I consider all of 3 species described by de Puytorac 1968 a as very approximate to each others and revealing a similar structure; in my opinion it would be correct to assign them to a separate, new genus, giving to it the name of *Puytoracia* g. n.

The definition of the genus *Epicharocotyle* narrowed in this way would be expressed as follows:

Epicharocotyle Kozloff, 1965

Hysterocinetidae of an elongated body, longit.: latit. ratio 2.5–3 : 1. The anterior third of the left side is occupied by a deep and complex sucker, continuous with a short through which extend posteriorly. Veil-like flanges arise from the margins of the sucker on either side, the ventral one being stronger. The long vacuolized area extends parallely to the elongated Ma. The numerous C. V.-es are arranged in two series. Parasites of the intestine of *Oligochaeta*.

Typus generis: *Epicharocotyle kyburzi* Kozloff, 1965.

Epicharocotyle kyburzi Kozloff, 1965

The body is 2.5 to 3 times as long as wide: length 130–203 μ, width 50–77 μ, the thickness is 1/3 of the width. The anterior end is rounded, the posterior — truncated. The deep and complex sucker occupies the anterior third of the left side of the body; the trough-like excavation curves ventral wards. The ventral flange is great.

The elongated Ma is approximately 2/3 the length of the body; Mi lies close to the Ma near the anterior end of Ma. C. V.-es are arranged in two groups, one on each side of Ma; there may be as many as 10 vacuoles in each group. The number of kineties 220–260.

The adoral kineties as in other genera. There is a long column of vacuolized, finely – granular ingested material extending anteriorly from the cytostome (Fig. 21A).

Host: *Driocrius breymanni* (Mich.) — Departamento del Valle, Colombia.

Genus *Puytoracia* genus novum

I form this genus for 3 species described by de Puytorac 1968 a from *Oligochaeta* from Gabon and assigned by him to the genus *Epicharocotyle* Kozloff, 1965. Undoubtedly the decision of de Puytorac has serious basis and mainly due to the fact that the structure of the sucker with its flanges and trough are completely similar. However, as I mentioned it above, the character of Ma and above all the presence of only one C. V. (instead of two rows of them) is considered by me as evolutionary very important. Just these characters opposed to *Epicharocotyle kyburzi* Kozloff, are common for these three species. De Puytorac 1968 beautifuly described and presented on slides (1968 d) and drawings (1968 a) the amazing skeletal apparatus of these ciliata (Fig. 12, 13).

The diagnosis of the genus may be presented a follows:

Puytoracia genus novum

Hysterocinetidae of a great, elongated body; the length 130–250 μ. The front part of the body is occupied by a large, deep sucker, continuous with a short through which extend ventralwards posteriorly. Veil-like flanges in the posterior part of the sucker. Several kineties in the middle part of the sucker are separated from its continuation on the left body side. There exists a complicate skeletal apparatus of the sucker and through. Ma elongated, one C. V. Parasites in the intestine of *Oligochaeta–Terricola.*

Typus generis: *Puytoracia kozloffi* (de Puytorac, 1968)

Three proximate species would be included to the genus *Puytoracia* g. n.:

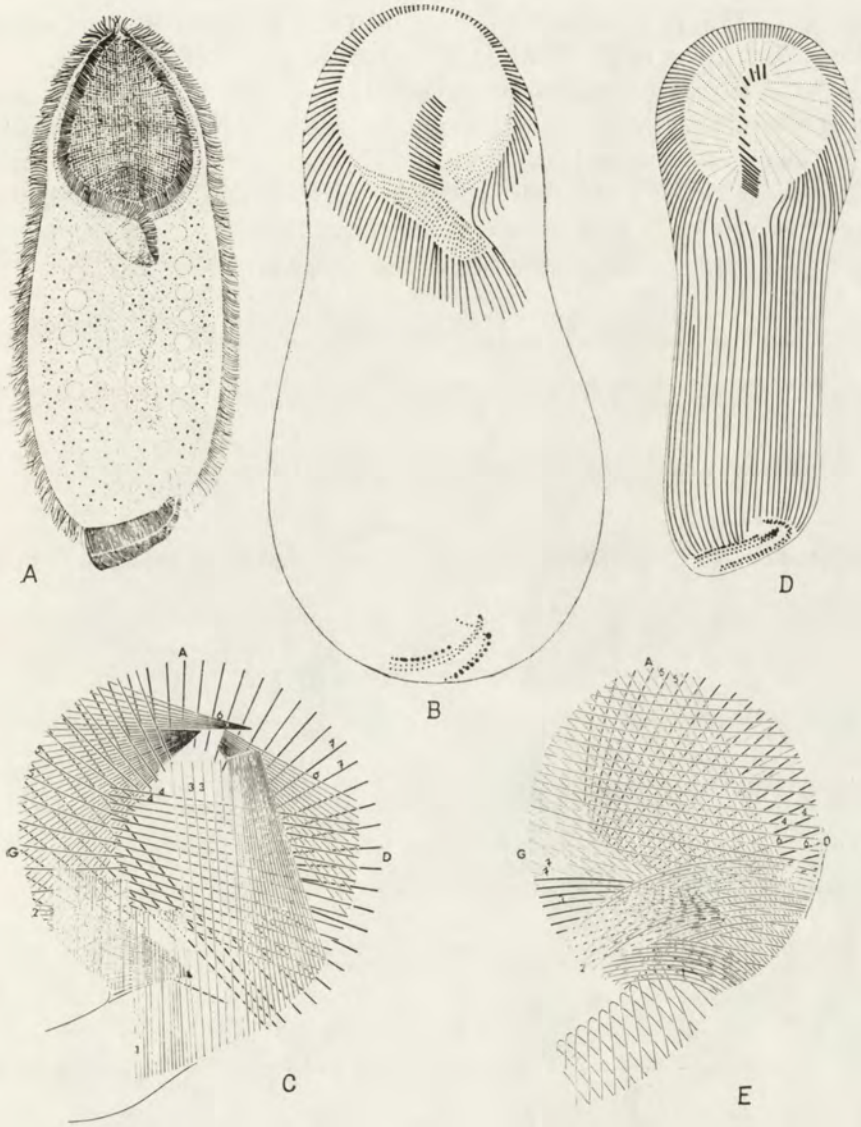

Fig. 21. A — *Epicharocotyle kyburzi* (after Kozloff); B, C — *Puytoracia kozloffi* (from de Puytorac); D, E — *Puytoracia raabei* (from de Puytorac) × 500 resp. 1000

Puytoracia kozloffi (de Puytorac, 1968)

syn.: *Epicharocotyle kozloffi* de Puytorac, 1968.

Body strongly elongated, rounded anteriorly, narrowed at the back. Size: length 130–140 μ, width 30–50 μ, thickness 27 μ. The sucker rounded, 30–35 μ in diameter; it is elongated by the gutter (la goutière) oriented to the right and

measuring 10–15 μ of length and 5–7 μ width. There are about 20 short segments of kineties (according to the drawing) in the ciliated groove of the sucker.

Ma ovoid, large, 15–20 μ in diameter, lies in the middle of the body; Mi measures 5–6 μ and lies next to Ma. V. P. posteriorly, has many excretory pores (Fig. 21 B).

The ciliature fairly abundant; 90–95 kineties reach the sucker, 45 of them on the left side (inferiéure). The skeletal structure of the sucker and of the gutter is highly complicated — it is presented on the (Fig. 22).

Host: *Libyodrilus violaceus* Beddard — Gabon, Africa.

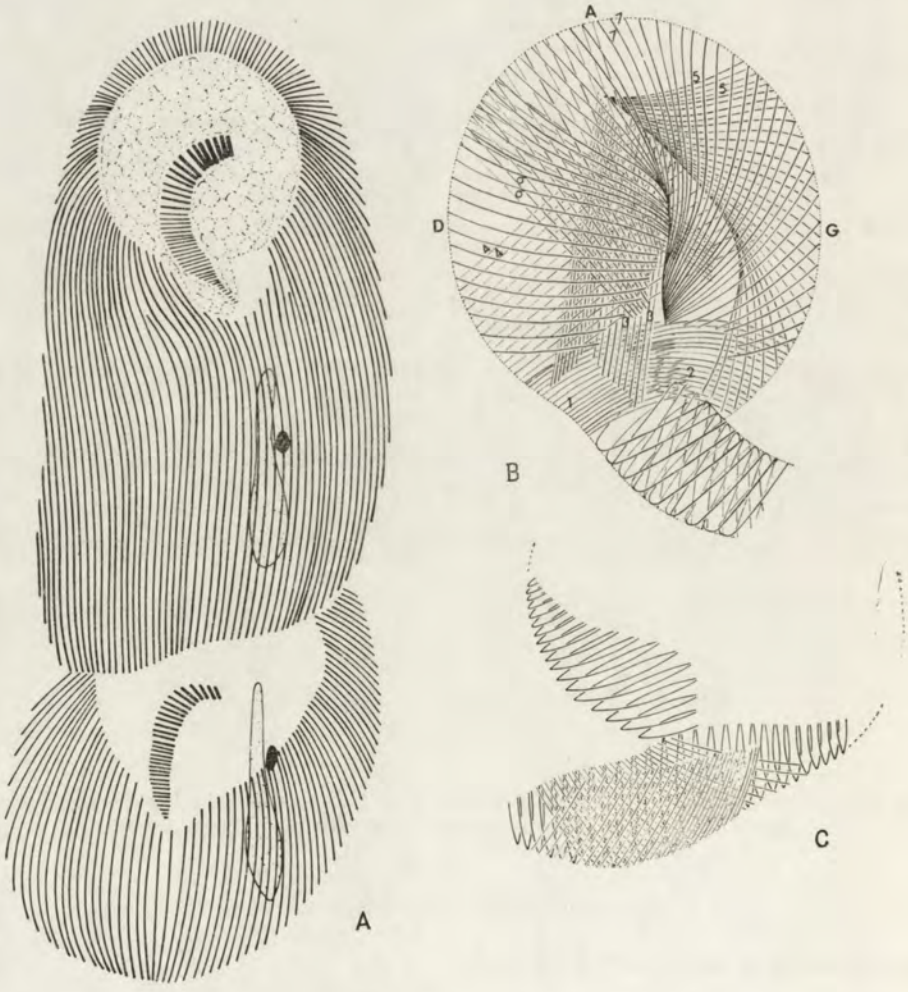

Fig. 22. *Puytoracia*: A, B — *P. grassei*; C — *P. raabei* — the funnel (all from de Puytorac) × 500 resp. 1000

Puytoracia raabei (de Puytorac, 1968)

syn.: *Epicharocotyle raabei* de Puytorac, 1968.

Body fairly elongated, wider in its posterior part than in the anterior. Length 160–180 μ, maximum width posteriorly 70–80 μ. The rounded sucker has in its back two plasmatic folds; the ventral one (right) is more developed. La goutière runs to the right from the sucker. There are ca. 17 segments of kineties in the ciliate groove of the sucker (according to the Figure). Ma ovoid, one V. P. in the back of the body.

The general ciliature has ca. 200 kineties reaching to the sucker (Fig. 21 D, E, Fig. 22 C).

Host: *Eminoscolex torentus* (?), Mich. — Gabon, Africa.

Puytoracia grassei (de Puytorac, 1968)

syn.: *Epicharocotyle grassei* de Puytorac, 1968.

Body elongated, more or less uniformly wide. Length 210–250 μ, width 80–90 μ. Rounded sucker, with the lips slightly formed. Ca. 30 segments of kineties lie in the ciliate groove. La goutière oriented to the right.

Ma slightly elongated lie posteriorly. One V. P.

The general ciliature numbers ca. 160 kineties, 25–30 of them disappear in their run from the posterior to the anterior on the ventral margin (Fig. 22 A, B).

Host: *Buttneriodrilus congicus* Mich. — Gabon, Africa.

Genus *Cotylothigma* Raabe, 1949

This genus has been established by Raabe 1949 for the species *Ptychostomum rhynchelmis* Heidenreich, 1935, differentiated from the genus *Ptychostomum* Stein on the basis of an individual structure of the sucker and a parallel arrangement of the macronucleus axis and the axis of the body (this character has not been confirmed by de Puytorac 1958).

The separateness of the structure of the sucker is the existence of a separate skeletal form namely a ring unclosed from the back, from where run backwards two long and sharp thorns. The fact that this peculiar structure of the sucker has been properly described by Heidenreich 1935 confirmed the data of de Puytorac 1958 concerning the second species found by him, that is *C. heidenreichi* and also my own observations on the last species.

On the basis of these distinct characters the definition of the genus may be presented as follows:

Cotylothigma Raabe, 1949

Hysterocinetidae of a flattened body and oval outline. The sucker is supplied with a skeletal apparatus, having the form of a ring opened in the hind part; from the break they run posteriorly two big, parallel thorns. Peristome is located on the posterior body margin. Ma is oval and lies in the middle portion of the body; Mi at the side of Ma. Distinctly outlined vacuolized area occupies the posterior part of the body. One C. V. is located in the area, as in *Ptychostomum*. Parasites of the intestine of *Oligochaeta*.

Typus generis: *Cotylothigma rhynhelmis* (Heidenreich, 1935), Raabe, 1949.

Cotylothigma rhynchelmis (Heidenreich, 1935)

The body with an ovoid outline, narrowed anteriorly, widened posteriorly, length 110–160 μ, width 40–65 μ. The sucker is rounded and strengthened by a characteristic skeletal apparatus, having a form of a ring unclosed from the back from where run backwards two big and long parallel thorns. In the anterior part in their base the thorns are connected and according to Heidenreich brought up by transversal short fibres (myonems according to Heidenreich). The canal

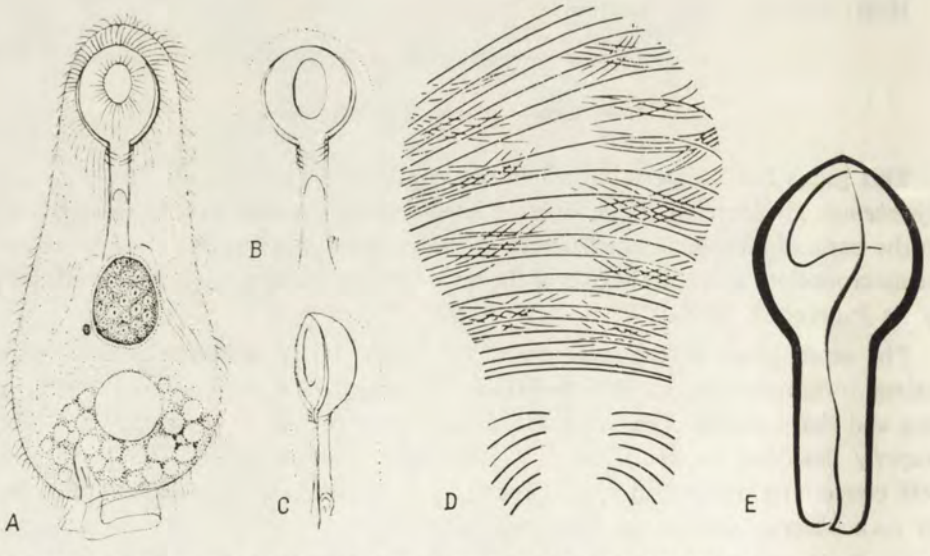

Fig. 23: *Cotylothigma*: A, B, C — *C rhynchelmis* (after Heidenreich); D, E — *C. heidenreichi*, skeletal ring and the fibrils (from de Puytorac) × 500 resp. 1000

leading from the back to the inside of the sucker is ciliated, the sucker itself is strongly depressed and serves for a strong adhesion of the animal to the base.

Ma ovoid, lies in the middle of the body and is oriented by its longer axis parallely to the body axis; Mi lies next to Ma. The posterior part of the body is occupied by a vacuolized plasma with not so distinct outline as in *Ptychostomum*, among it is a large C. V. The peristome lies in the back of the body, is small but provided in long and strong cilia forming membranellae (Fig. 23 A–C).

Host: *Rhynchelmis limosella* Hoffmeister — region of Wrocław, Poland.

Cotylothigma heidenreichi de Puytorac, 1958

The body has an outline of elongated oval; length 140–170 μ, width 90–100 μ. The sucker is relatively small; very strong adoral kineties with cilia 10–13 μ long. Ma ovoid lies in the middle of the body and is oriented by its longer axis perpendicularly to the axis of the body. Mi lies at the side of Ma C. V. in the back of the body. The ciliature is dense; ca. 100 kineties reach the sucker from both sides of the body.

The skeletal apparatus of the sucker consists of "bourrelet squelettique" and a system of fibres. The main part specific for *Cotylothigma*, consist of a semicircular form oriented backwards by its opened arms. Two elongations run from the arms towards the back; the ventral one (right) is elongated in a strong thread again oriented anteriorly (Fig. 23 E). The system of fibres is composed of a deeper layer of transversal fibres and of surface layer consisting 5 to 7 pairs of bunches of fibres running from the margin of the sucker to its centre (Fig. 23 D). The surface of the sucker covers in its central part the argyrophilic net with big meshes and in the peripherical parts — the net with slight meshes.

I would like to suggest on the basis of my own observations achieved also on the material from Ohrid Lake that besides the sucker there are two plasmatic folds (the dorsal one seems larger), overlaping one another as flaps of the coat. They look likely as described in the representatives of the genus *Epicharocotyle* (p. 167). It seems that these folds slightly compensate the stiffness of the sucker given by a solid skeletal apparatus and help in its closure and in the fastening of the animal to the base.

Host: *Rhynchelmis komarecki* f. *typica* Hr. — Lake Ohrid, S. Yugoslavia.

Subfamilia *Craticuloscutinae* de Puytorac, 1968

This subfamily has been created by de Puytorac 1968 d for one genus *Craticuloscuta* Kozloff, 1965 with two species. This genus diverges virtually of others *Hysterocinetidae* by the structure of its sucker: it consists presently a depression of the anterior part of the body, covered nearly completely by cilia and provided

in a skeletal system composed of several layers of crossing fibres. This structure reveals virtually a completely specific evolutionary tendencies.

I enclose quite provisionally the genus *Kysthothigma* Raabe, 1949 to this subfamily; it has been assigned by de Puytorac 1968 d to subfamily *Epicharocotylinae*. I suppose that this ciliate so akwardly described and schematicaly recognized would find his place.

The characteristic of the subfamily may be presented as follows:

Subfamilia *Craticuloscutinae* de Puytorac, 1968

Thigmotricha–Hysterocinetidae of an elongated body. The great ellipsoidal sucker is provided with a system of fibres lying in several layers and in the great part covered with ciliature. Ma rounded or elongated, one or more C. V. Parasites in the intestine of *Oligochaeta–Terricola*.

Typus subfamiliae: genus *Craticuloscuta* Kozloff, 1965

Genus *Craticuloscuta* Kozloff, 1965

This genus has been created by Kozloff 1965 for the differentiation of the species. *C. escobari* described at the same time for the reason of a distinct structure of the body, the elongated nucleus numerous C. V.-es, the shape of the zone of food vacuoles and especially of specific construction of the sucker. The sucker is oval, elongated and strengthened by long, thick fibres running parallely along the sucker. Their number amounts to 30–40; there are transversal fibres under them. According to Kozloff 1965 the surface of the sucker is covered by the points arranged among the fibres and similar to kinetosomes. It is presumably ciliated in its posterior part.

The data of Kozloff 1965 finely completed de Puytorac 1968 by his observations concerning the second species of the genus *Craticuloscuta*, namely *C. gigas* de Puytorac, 1968. Here they are: the sucker constitutes virtually an oval somewhat depressed field, strengthened by fibres arranged in several systems, running obliquely through the sucker, transversally to its length and in the complementary directions. The sucker is nearly completely covered by the cilia; only the small arcuated field in its anterior end is free of them.

Virtually two characters: a strongly arranged, uniform system of sucker's fibres and its near complete ciliature constitute sufficiently distinct features of the genus *Craticuloscuta*.

On this basis the characteristic of the genus *Craticuloscuta* may be stated as follows:

Craticuloscuta Kozloff, 1965

Hysterocinetidae of a flattened the elongated body; longit.: latit. ratio 2.5–3 : 1. The sucker, which occupies about 1/4 to 1/3 of the left body side, is a shallow oval concavity, strengthened with strong longitudinal fibres and transverse supporting elements. The sucker in its posterior portion is ciliated. The peristome is small. The long vacuolized area extend parallely to the elongated Ma. One or numerous C. V.-es arranged in two series. Parasites of the intestine of *Oligochaeta*.

Typus generis: *Craticuloscuta escobari* Kozloff, 1965

Craticuloscuta escobari Kozloff, 1965

Body flattened and elongated, about 2.5 to 3 times as long as wide: Length 74–192 μ, width 32–64 μ; the thickness 1/3 the width. The sucker which occupies about 1/3 or 1/4 of the left surface, is oval in outline and is strengthened by 30–40 strong-longitudinal fibres and transverse supporting elements. The number of kineties of the left side is 70–90, of the right side 120–145, the total number being 190–235.

The Ma is elongated, about 2/3 the length of the body. Mi lies close to Ma, near the middle of Ma. The numerous C. V.-es are arranged in two series on either side of Ma: there may be up to 9 or 10 vacuoles in each series.

The buccal apparatus occupies the hind margin of the body; the long vacuolized area extends parallely to the Ma (Fig. 24) A, B).

Host: *Drilocrilus breymanni* (Mich.) — Departamento del Valle, Colombia.

Craticuloscuta gigas de Puytorac, 1968

Body flattened, rounded in the front, truncated in the hind part. Length 450–630 μ, width 180–200 μ. The front part of the body forms an oval depression 130–140 μ long and 75–81 μ wide. The sucker is strengthened by about 150 transverse fibres, 50 fibres running meridionally and accessory fibres in the front part of the sucker. There are 120–140 kineties ranging the margin of the sucker on the left (inférieure) side of the body.

Ma "en boudin". The cilia in the front part of the body are 6–8 μ long, in the hind part 7–9 μ long. 2 V.P.-es in the hind part of the body (Fig. 24 C, D).

Host: *Libyodrilus violaceus* Beddard — Gabon, Africa.

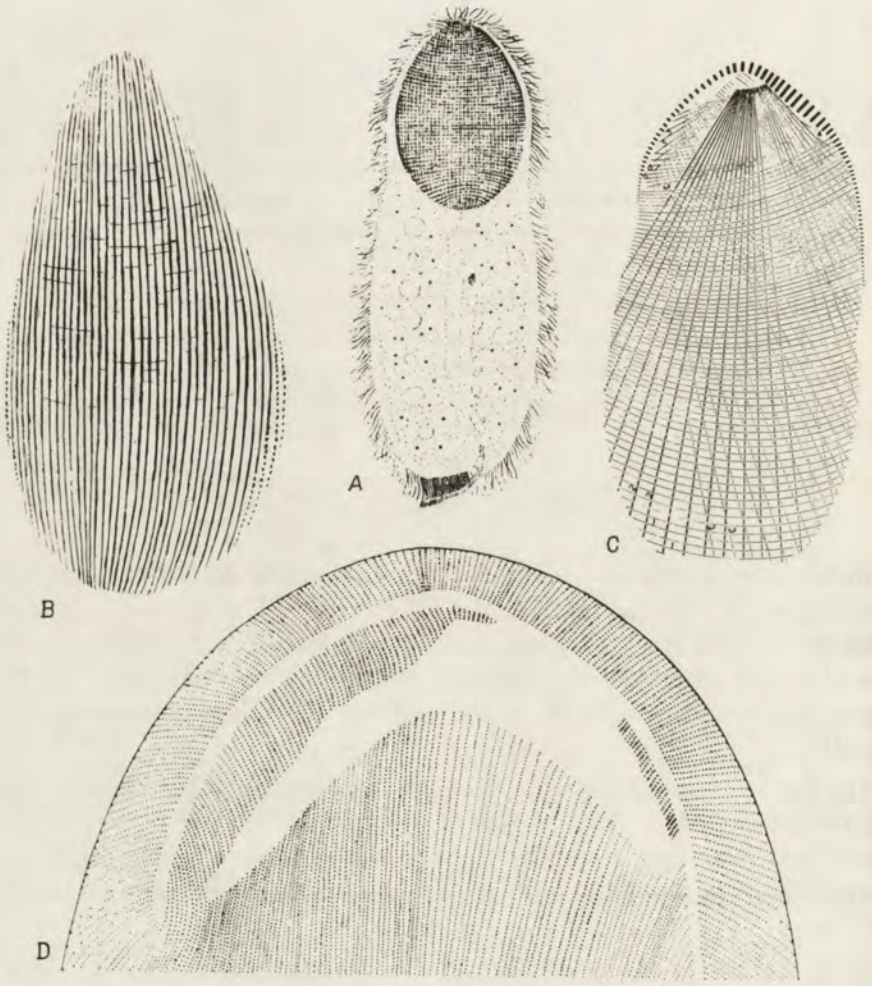

Fig. 24: *Craticuloscuta*: A, B — *C. escobari* (after Kozloff); C, D — *C. gigas* (from de Puytorac) ×500 resp. 1000

Genus *Kysthothigma* Raabe, 1949

This genus has been created by Raabe 1949 for the differentiation of the species *P. bacteriophilum* Miyashita, 1933 from the genus *Ptychostomum* Stein. This species has a very different structure of its sucker and the arrangement of the nucleus, it distinguishes oneself from other species of the genus *Ptychostomum*. Jarocki 1939 transfered this species to the genus *Hysterocineta* Diesing on the basis of these characters, but this position could not be preserved for the reason of the closure

of the sucker. In the description and above all on the figure of Miyashita 1933 the distinctly schematic presentation of its body and its sucker with a complementary trifid fold. Especially this kind of symmetry seems very exaggerated.

The examinations of Miyashita have never been confirmed, and the structure of the sucker has never been analysed by other methods. Therefore it must be accepted that both the species *P. bacteriophilum* Miyashita, 1933 must be recognized as species inquirenda, as well as the genus *Kysthothigma* as rather provisional. It may be characterized as follows:

Kysthothigma Raabe, 1949

Hysterocinetidae of a laterally flattened body, of an elliptical outline. The longitudinal, inner, ciliated furrow of the oval sucker is closed; the sucker is supplied with a plasmatic fold directed posteriorly. The ovoid Ma lies in the middle of the body and is directed with its long axis parallely to that of the body, the Mi lies at the side of Ma. The peristome is moved somewhat to the dorsal margin of the body. C. V. is located in the posterior part of the body in the vacuolized area. Parasites of the intestine of *Oligochaeta*.

Typus generis: *Kysthothigma bacteriophila* (Miyashita, 1933), Raabe, 1949.

Kysthothigma bacteriophila (Miyashita, 1933)

syn.: *Ptychostomum bacteriophilum* Miyashita, 1933; *Hysterocineta bacteriophila*: Jarocki 1939.

Body in the shape of an elongated oval, length 70–130 μ width 30–45 μ. The sucker measuring 30 μ is according to the description and the figure of Miyashita of an regularly oval form contains in the middle a narrow ciliated fissure, and is provided at its back in a trifid fold. On the side fields of the sucker there are visible fibres running from the margin of the sucker to its middle and somewhat anteriorly.

Ma ovoid, lies in the middle of the body, Mi next to Ma. (?). In the back of the body the zone of vacuolized plasma with faint outline; C. V. in this area. Peristome in the posterior end of the body so that its origin (outset) lies in the end of the body slipped out backwards, however, the end of the peristome together with the cytopharynx — more anteriorly on the dorsal (left) body margin (Fig. 25).

Host: *Criodrilus* sp. — Japan.

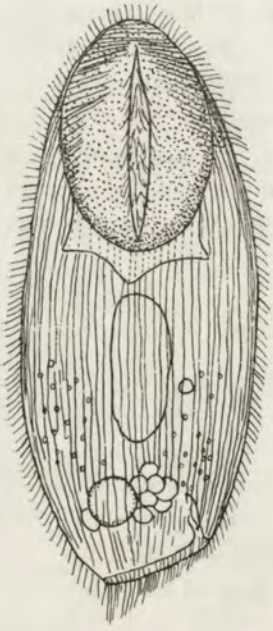

Fig. 25. *Kysthothigma bacteriophila* (after Miyashita) × 500

Familia *Protoanoplophryidae* Miyashita, 1929, Raabe 1967

The studies on the representatives of this interesting family, including, for the time being, only one genus with two species, starts from the year 1929 when Miyashita describes a ciliate *Protoanoplophrya stomata* from the intestine of snails from the genus *Viviparus* from Japan. This ciliate reveals according to this author all characters of *Astomata*, and even more exactly — of the genus *Anoplophrya*. However, Miyashita discovered, applying Klein's silver method, the existence of a reduced but distinct buccal apparatus in the form of two kineties lying on the naked peristomal field, moreover as he believed he found the existence of trichocysts. In spite of this Miyashita left his species within the family *Anoplophryidae*, as a primitive form in comparison to other forms of described *Astomata* and believed that he would find more of these primitive forms. He even mentioned an unadequately described *Anoplophrya vermicularis* Leidy, 1877 which probably corresponds to the characters of the genus *Protoanoplophrya*.

Miyashita 1929 as a result of his considerations proposed the enlargement of the scope of the family *Anoplophryidae* and the creation within it a separate subfamily *Protoanoplophryinae*!

Several years later, Raabe 1933 described the new species included by him to the genus *Protoanoplophrya* from the intestine of *Bitynia tentaculata* (L.) from Poland and named it *P. bithyniae*. Virtually this species completely corresponds to the character of the genus and of the family which may be stated on the basis

of the description of Miyashita, although it differs distinctly in details from the species discovered by Japanese author.

The literature as far as I am concerned does not include any description of another species which would correspond to the discussed genus, there is also no mention of its known species. Only Kozloff 1960 mentioned that in some specimen of *Campeloma geniculatum* Antony (*Prosobranchia*) he found besides *Ptychostomum campelomae* Kozloff, 1960 also "a species of *Protoanoplophrya*".

On the basis of the description of these two species, Corliss 1961 includes the genus *Protoanoplophrya* to the family *Conchophthiridae* within the *Thigmotricha*. This proposal seems to me unacceptable, because the genus *Protoanoplophrya* does not correspond to the family *Conchophthiridae* even in the broad approach of Corliss. The more so it does nor correspond to the subfamily *Conchophthirinae* in my version (vide part IV p. 143) and even to the family *Thigmopryidae* in this form as I presented it before (vide part I, p. 32 and part IV, p. 121).

Nevertheless it should be recognized that the genus *Protoanoplophrya* corresponds in general to a not highly developed order of *Thigmotricha* as a representative of a highly specialized and adapted group to the parasitizing way of life in the intestine of its hosts, but revealing many convergent characters with other *Thigmotricha* mainly these ones which reveal a polymerization of kineties of the general ciliature and a certain reduction of adoral kineties. Such characters as a strong elongation of the body, a strong elongation of Ma, the presence of numerous V. C.-es and (at least in *P. stomata*) a gammation and even a catenulation — are the characters of intestine's parasites, which may occur in some *Hysterocinetidae*.

For these reasons I formerly suggested (part I, p. 32), the creation of a separate family for the genus *Protoanoplophrya* Miyashita, 1929 which would be named: *Protoanoplophryidae* Miyashita, 1929.

Therefore it seems clear that the position of the family *Protoanoplophryidae* within the *Thigmotricha* is not fundamentally motivated. However it may be recognized that they originate of the most primitive forms of *Thigmotricha* and that the evolutionary sequence aims for the adaptation to the life in the intestine of the hosts leading consequently to the appearance of characters approximate to *Anoplophryidae*. Their peristome is however preserved, it undergoes the anterogradation, two adoral kineties run through the naked peristomal field and lead backwards to the rather unknown cytopharynx. In spite of a certain reduction of the buccal apperture it is able to take food. Miyashita proved that besides the peristome there is an accumulation of food vacuoles, Raabe thinks that there are no formed food vacuoles in then, both authors suppose that the food uptake is achieved in the way of osmosis, therefore as we would like to determine presently — on the way of pinocytosis.

Other characters really resemble *Astomata*. Here there are: a dense, uniform ciliature, the elongation of the body and of the Ma, the occurrence of a large number

of C. V.-es arranged along the body in two rows and finally (at least in *P. stomata*) — the posterior gammation and even a catenulation.

Miyashita 1929 ascribes to *P. stomata* the occurrence of trichocists; Raabe 1933 did not find then in *P. bithyniae*. The occurrence of trichocysts in such intestinal parasites seems rather unexpected; it seems possible that Miyashita was concerned with other structures, yet in that period not separated from trichocysts. The fact that Miyashita locates them in a cavity in the anterior part of the body, to which he ascribes a thigmotactic role, may suggest that these forms are some mucogenic bodies (vide *Conchophtirus* part IV, p. 144 and *Hysterocinetidae* — this part p. 115).

The fact that the family *Protoanoplophryidae* includes only one genus with two species, makes certainly difficult the statement of its definition so that the attempt presented below must be recognized as provisional:

Familia *Protoanoplophryidae* Miyashita, 1929

Thigmotricha of a lateral compressed and strongly elongated *Astomata*-shaped body. The dense and uniform ciliature arranged in parallel, longitunal rows. The naked peristomal field lies in the anterior part of the body on its ventral margin; two parallel adoral kineties run along through it; the cytostome lies on the hind end of the peristomal field. Ma strongly elongated, 1 Mi. Numerous C. V.-es are distributed in two rows along the body. Multiplication in the form of equal division or budding. Parasites of the intestine of *Gastropoda–Prosobranchia*.

Typus familiae: genus *Protoanoplophrya* Miyashita, 1929

The unic genus of the family would be also characterized in a provisional shape:

Protoanoplophrya Miyashita, 1929

Protoanoplophryidae bearing the characteristics of the family. Body flattened and strongly elongated — length up to 1500 μ, width 50–70 μ. Parasites of the hind part of the intestinae of *Gastropoda–Prosobranchia*.

Typus generis: *Protoanoplophrya stomata* Miyashita, 1929.

Protoanoplophrya stomata Miyashita, 1929

Body very strongly elongated, reaches up to 1500 μ of length ca. 70 μ of width. The anterior end flattened laterally and somewhat depressed on the ventral side

(thigmotactisms?). A thick pellicle, distinctly separated from the plasma; cilia equal, ca. 5 μ long.

Ma has a form of a long band and is drawn along the whole body; Mi spindle-shaped, big, ca. 20 μ long, lies parallely to Ma. The C. V.-es in the number of 60, lie along the whole body; the ectoplasm is clear, contains many corpuscles strongly refracting the light, the peristomal field is a narrow tract, up to 70 μ long with two adoral kineties. In the posterior end of the peristomal field presumably lies the cytopharynx. Behind the peristome numerous food vacuoles (Fig. 26 A, B). The

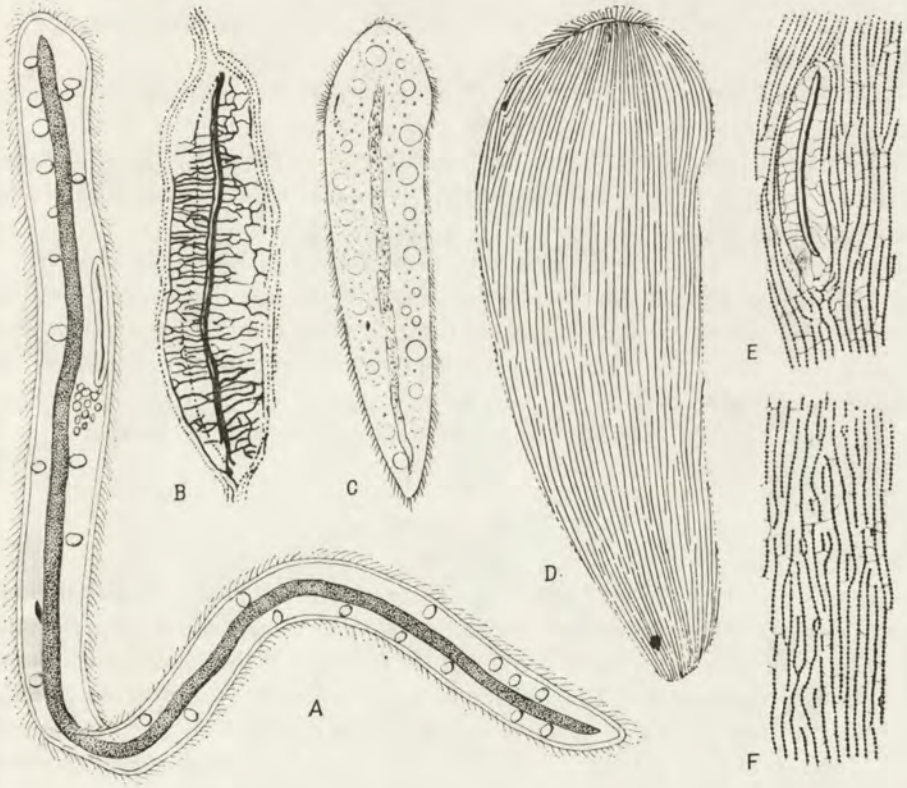

Fig. 26. *Protoanoplophrya*: A, B — *P. stomata*, the general aspect and the oral field (after Miyashita); C, D — *P. bithyniae*, the general aspect and ciliary system, E, F — the oral field and the fragment of the ciliature after $AgNO_3$ preparations (from Raabe) ×250 (A), 500 resp. 1000

multiplication of smaller individuals is achieved by an equal division, of the larger ones — by gemmation; sometimes three buds (tomits) are cut off at once. The size of a young individual is ca. 200 μ. A slow movement — with circles around the body axis.

Hosts: *Viviparus japonicus* and *Viviparus malleatus* — Japan.

Protoanoplophrya bithyniae Raabe, 1933

The body flattened lateraly, elongated; length 130–260 μ, width 50–60 μ. The ventral margin somewhat convex in the anterior body part at the level of the peristome, the dorsal one on the same level somewhat concave; the back of the body sharpened. The pellicle thick, cilia ca. 6 μ long, uniformly arranged on the whole body.

Ma is strongly elongated and is drawn along the body; it is ca. 200 μ long and ca. 7 μ wide. Mi spindle-shaped, ca. 7 μ, lies besides Ma in the posterior half of the body. C. V.-es in the number of 20–30 are arranged in two rows along the body and measure up to 10 μ in diameter; the pulsation is irregular.

The buccal apparatus lies in the anterior part of the body, 20–40 μ from its anterior pole, it consists a naked field, measuring ca. 32 μ×5–6 μ, along which run two parallel adoral kineties; in the posterior part of the peristome — a coniform cavity (cytopharynx). Behind the peristome, in the plasma, numerous food vacuoles, not containing more food particles (Fig. 26 C, D).

The multiplication by division, the gemmation has not been observed.

P. bithyniae occur in the posterior segment of the intestine of its hosts, often (in some biotops up to 50%) but not in great numbers (up to 20 individuals in one host). A stronger infestation has been observed in the absence of *Hysterocineta paludinarum* (Stein, 1861).

Host: *Bithynia tentaculata* (L.) — in different regions of Poland.

Summary

The fifth part of the monograph on *Thigmotricha* comprises the elaboration of two families, *Hysterocinetidae* and *Protoanoplophryidae*, whose representatives live not on the respiratory surfaces but in the intestine of their hosts. However, the evolutionary development of these families proceeded in two different directions and they are not directly related to each other. The characteristics of both families, and of subfamilies of the family *Hysterocinetidae*, are given, as well as the descriptions and definitions of the genera and the diagnoses of species. Three new genera are erected: *Drilocineta* g. n. for *Hysterocineta libyodrili* de Puyt., *Taeniocineta* g. n. for *Hysterocineta eiseniae* Beers, and *Puytoracia* g. n. for three species described by de Puytorac within the genus *Epicharocotyle*.

STRESZCZENIE

Piąta część monografii *Thigmotricha* zawiera opracowanie dwu rodzin, a mianowicie *Hystrocinetidae* i *Protoanoplophryidae*, których przedstawiciele pasożytują nie na powierzchniach oddechowych, lecz w tylnej części jelita swych żywicieli i wykazują wybitne adaptacje do takiego trybu

życia. Rozwój ewolucyjny tych rodzin poszedł jednak w odrębnych kierunkach i nie łączy ich żadne bezpośrednie powinowactwo. Podano charakterystykę obu rodzin, zachowując podział rodziny *Hysterocinetidae* za de Puytoraciem na podrodziny *Hysterocinetinae*, *Craticuloscutinae* i *Epicharocotylinae*. Podano opisy i diagnozy rodzajów i gatunków. W obrębie rodziny *Hysterocinetidae* wyodrębniono następujące nowe rodzaje: *Dricolineta* g. n. dla *Hysterocinaeta libyodrili* de Puyt., *Taeniocineta* g. n. dla *Hysterocineta eiseniae* Beers oraz *Puytoracia* g. n. dla trzech gatunków opisanych przez de Puytorac w rodzaju *Epicharocotyle*.

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Studies on encystation and excystation in *Opalina ranarum*

Badania nad encystacją i ekscystacją u *Opalina ranarum*

The life-cycle of *Opalina* has been already described by many authors. It is well known that during the spring the cysts of *Opalina* are formed and they are defecated outside frogs. These cysts are eaten by young tadpoles and they hatched inside the tadpoles intestinum. The period of encystation can be considered as a good starting point for investigation of *Opalina* development because it can be expected that these cells are naturally synchronized in many respects. However, cysts differ in its size, shape and number of nuclei.

Hatching of young opalinids in the tadpole's intestinum is followed by gametogenesis. During few days very easy distinguishable microgametes and the copulation followed by the next generation of cysts (zygocysts) are observed. Gametogenesis is proceeded by meiosis (Kaczanowski 1971). So encystation and excystation are related to the moment of meiosis.

For the all reasons listed above, some observations and experiments on encystation, the characteristic of living cysts and the study on excystation, including some analysis of mechanism involved in these processes could be important for further study on development of *Opalina*.

Material and methods

Opalina ranarum (Ehrbg.) from *Rana temporaria* collected in the vinicity of Warsaw was used for all observations and experiments. During winter, frogs were kept in temperatures about 4–8°C. The opalinids removed from frogs intestinum were washed and maintained in salt buffer medium of Holftretter or Yang's medium. (Yang and Bamberger 1953, Yang 1960). For in vitro culturing of *Opalina*, the Yang's slant medium with antibiotics (streptomycin, penicillin and neomycin) was used. Unfortunately, we were unable to obtain the permanent culture of young opalinids even in the presence of antibiotics. During some days the medium was overgrowing with bacteria introduced from frog faeces resistant to the antibiotics or fungi. In some cases, the rapid uncontrolled growth of fungi inhibited the growth of bacteria, but in every case the culture of opalinids was finally dead during 7–12 days. So, all results can be referred only to the first days of culturing.

But even such very limited results, in uncontrolled conditions give an opportunity to look into the same initial developmental processes which are followed by excystation.

For caryological studies, opalinids were stained by Feulgen method followed by additional toluidin blue staining after Kaczanowski 1968.

For morphological comparison of various stages of *Opalina* some Protargol stained slides were prepared.

Results

Palintomy and encystation

Palintomy in *Opalina ranarum* (Ehrbg.) is usually observed during the breeding season of hosts. However, palintomy can be obtained starting from the end of December, if previously hibernated frogs were placed into the room temperature for some days (6–10 days). During the following months there is gradually more and more easy to get palintomy, as it was reported by Sukhanova 1963.

It is remarkable, that there was no visible sexual activation of frogs in experimentally induced palintomy.

There was the another way of receiving the palintomy, which was followed by encystation. In this case the following procedure was employed. During March, opalinids from hibernated frogs in which palintomy was not observed were placed into the test tubes with slant egg medium of Yang. These test tubes were kept in the room temperature. (18–23°C). Very quickly, during 24 h of in vitro culture the palintomy was observed. There was a great number of dividers and very narrow and elongated specimens (postdividers after longitudinal division) and relatively small, but proportional cells, which subsequently divided. After two days of experiment, the numerous, little, fusiform cells, just before encystation as well as cysts were observed. Since the fourth day there was a majority of cysts, some little cells and only few large opalinids.

According to the supposition that the change of temperature is triggering factor inducing palintomy in one experiment the sample of opalinids was divided in two test tubes and kept in different temperature, both in the same Yang's medium. One of them were kept in 4°C while another in room temperature. In the both temperatures the cyst formation and palintomy were followed in the same time, but in the lower temperature they were not so numerous. The effect of temperature in this case was not crucial for inducing these developmental processes. It can be added, that the rate of palintomy and induction of encystation in opalinids which were transferred to Yang's medium in vitro was higher in all experiments than those in experiments and observations which were performed with infected frogs in all seasons including the spring season. For instance if frogs were placed from temperature of 4°C into the room temperature in April, many stages of palintomy and numerous cysts were observed after four days while similar effects in vitro cultures was obtained in March after two days only.

In the field conditions, opalinids taken from frogs *Rana temporaria*, just in the

period of copulation, were not in palintomy if ice on the pool was still present. Mass palintomy and encystation in these conditions was found approximately after a week. This observation was confirmed in two spring seasons.

Observations on cysts of *Opalina*

The cysts of *Opalina* are round or a little elongated. They differ in their size from 25 μ until to 45 μ in diameter and number of nuclei which can vary from 1 nucleus per cyst (in rare cases) to about 25 of nuclei with average number 4–8 nuclei per cyst. These nuclei are relatively small with the condensed chromatin. They keep an interphasal character.

The cyst wall is transparent and it rests unstained after toluidin blue or Protargol staining made after routine fixation used in both methods. It was particularly clear after Protargol staining that *Opalina* enclosed inside the cyst wall is twisted and folded (Fig. 1). Clearly visible folding of pellicle indicates

Fig. 1. Cyst of *Opalina ranarum*

that there is no contraction of the pellicle during encystation. In living cysts, the movements of cilia are observed. Small opalinids even rotate inside the cyst during first stage of encystation. In further stages, the cyst wall becomes more solid, the space between the wall and the cell diminishes and the rotation is reduced. However, even in such cysts, in contrast phase microscope, the slow cilia beating was observed. It never stops, if the cysts are alive. According to Wessenberg 1961 and Sukhanova 1960 such cysts are alive in pond water up to 3–4 weeks.

The artificial excystation

Two methods of artificial excystation were used in our experiments:

(1) The cysts were treated with a bile of frogs, according to Sukhanova 1960. For this purpose, the content of frog's intestinum was washed up in the buffer salt solution and centrifugated. After washing up, the fresh bile of frogs (taken from 1–2 frogs) was dropped into the test tube with cysts for a period of 1–2 h. After this, the cysts were resuspended in buffer salt solution and centrifugated. This procedure was repeated 8–10 times in order to remove a bile. During the bile treatment and after it, the intensive movement of cells inside the cyst wall was observed. The first free swimming opalinids were observed at about 1 h after the end of treatment. These results, however, can vary probably because of the different activity of a fresh bile taken from the different specimens of frogs.

(2) A content of frogs hindgut with many cysts was filtered using a glass filter funnel held in vacuum flask. The diameter of pores of glass filter was 5–15 μ. The cysts were collected directly from the surface of filter, while the smaller particles were filtered. If the filtering was combined with a previous rough filtration with removing of large particles of faeces, the relatively pure fraction of cysts can be obtained. The most of bacteria were removed and the fraction of cysts were less contaminated by them, however the exact purification of cysts was never received. The germs of fungi have the similar diameter as the cysts of opalinids, therefore they were not at all eliminated during filtering.

The pumping force during filtration (water pomp) was sufficient to bring out the excystation of some opalinids during this procedure if filtering lasted about 30 min–1 h. The excystation of some opalinids was also observed just after the end of filtering or some minutes later and free swimming opalinids always some minutes after collecting of sample from the surface of filter. Free swimming opalinids excysted during filtering were lost because they are elongated and more flexible than cysts and they pass through the filter.

Independently to the method of excystation used the rotation of opalinids inside the cyst wall and faster ciliary movement were the first symptoms of approaching excystation. However, it is not sure if true physiological activation is involved in this case. The rotation and more rapid ciliary movement could be results of the decreasing of pressure exerted on twisted opalinid while the cyst wall becomes weaker.

In the moment of hatching, a cyst wall was broken out and little *Opalina* emerges by the rupture and it empties the cyst envelope. It seem that the cyst's wall is not dissolved before the hatching (Fig. 2).

Two morphological forms of newly excysted opalinids can be described:

Fig. 2. Hatching of opalinid cell

Form a (Fig. 3 a) with shortened and rounded posterior part of the body as the result of twisting of the pellicle, and form b (Fig. 3 b) with conical posterior end, thickened central region and distinct folding of the pellicle. Forms a and b differ one from another by pulling in or stretching out the posterior conical end.

Newly excysted opalinids swim rather slowly and rotate. After few hours they become a typical "young" opalinids called gamonts by Wessenberg 1961. They

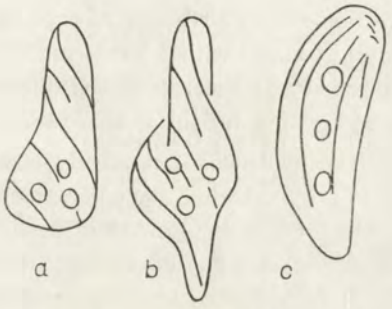

Fig. 3. Excysted opalinid cells, a and b — just after excystation, c — two hours later

are flat, elongated, but broader in its anterior part with smooth pellicle surface (Fig. 3 c). They swim more vigorously than just after excystation in a typical opalinid manner, i.e., with rare rotation. So, newly excysted cells are clearly distinguishable in comparison with the older ones.

Early development of artificially excysted opalinids

In order to examine a viability of excysted opalinids and their early development, they were placed into the test tubes containing salt buffer solution, or slant egg medium of Yang. Excystation in all experiment was not synchronous. In pure buffer salt solution opalinids were alive during 1–2 days after the end of a treatment with bile. During these two days the new excysted cells, living cysts and opalinids in the moment of excystation were recorded.

In Yang's medium these stages were present during about 4 days after Dile treatment or after filtration. They were always mixed with the various stages of development of the gamonts cells. In this medium, opalinids were cultivated during

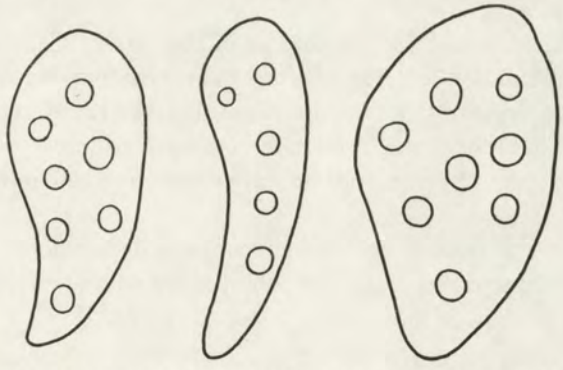

Fig. 4. Growing opalinid cells with larger nuclei since four days after excystation

7–10 days and cell divisions were observed in this time, but in all cases the medium was overgrown by bacteria and fungi. Nevertheless, some observations during the first days seems to be valuable.

Since the fourth day of experiment, the wide, flat specimens with two or three rows of nuclei with 2,3 or 4 nuclei per row were observed. In the same time, nuclei enlarged and became well visible in vivo. The large, wide specimens (Fig. 4) were accompanied by little, narrow and uninuclear cells.

The evidence of relatively large and wide specimens indicate that some cells were grow up while the enlargement of nuclei suggest the nuclear activity.

In fact, in Feulgen stained slides some cases of meiosis were found if smears were made 4–7 day of experiment. Conforming the previous report (Kaczanowski 1971) meiosis was found in multinuclear cells. As a rule in such cells only one nucleus entered meiosis while the others kept their interphasal character. The meiosis was found in 3–8 and even 12 nuclear cells. Only in one experiment starting from the 7 up to 10 day of culturing, the microgametes were observed. In other experiments they were not observed, but the decaying character of cultures at that time (7–10) makes an answer this question impossible.

Discussion

The problem of the induction of a palintomy in frog's intestinum is still open. According to Sukhanova 1963 and our data, the action of pituitory hormones on frogs and their sexual activation is not obligatory for inducing palintomy of *Opalina*. The hibernation of frogs followed by the shock of the higher temperature gave the positive results in inducing palintomy.

Experiments on activation by pituitory hormones the frogs reported by Rugh 1962 cannot resolve the question if according to this procedure pituitary injection and transfer of hibernated frogs the 24°C temperature are requested at once for breeding induction.

On the other hand, in the soft climate as in the case of California there is no long and deep hibernation of frogs before their breeding season and opalinids palintomy. So, in contrast to our data and suggestion, Wessenberg in 1961 working in San Francisco described the artificially induced palintomy in frogs during September without any hibernation but after injection of pituitary hormones extract.

In all the above mentioned cases the temperature or hormones act on the host, but only indirectly on protozoans. The interactions between frogs and opalinids are quite obscure and it is difficult to conclude about it.

It was observed that the transfer of opalinids from hibernated frogs directly into the rich Yang's medium even in law temperature of 4°C was sufficiently for triggering the palintomy and the encystation. It is possible that palintomy was induced by the transfer of protozoans from condition of hindgut of starved frogs into the rich food supplied medium. In such case, the palintomy in naturally infected frogs could be consider as an affect of a change of feeding conditions inside the

intestinum. The changes in a content of hindgut of frogs can be a result of host physiological activity and active feeding after the stimulus of hormones injection or temperature treatment, or both. This questions needed further investigation.

If one observes the phenomena occurring during encystation and compares them with excystation, he has an impression that the same stages are observed in opposite suquences. For instance, the visible rotation of cell inside the cyst wall is observed for the first time during encystation and second time during their excystment. Similarly, the twisting and folding of specimens accompanied the encystment, while the opposite processes are observed after the excystment.

On the basis of the presented data, the following mechanism of encystation is proposed. A little opalinid cell called progamont or tomite (Wessenberg 1961) produces a viscous substance which is secreted outside the cell and this substance reduces its motility in mechanical way, leading up to complete arrest of effective progressive movement. Starting from this moment the cell rotates in the same place because its ciliary movement is not inhibited. Viscous substance, which has been produced by a cell, gradually is transformed into the solid and rigid cyst wall. All changes of the shape, i.e., twisting and folding are only pure mechanical result of enclosing in the cyst wall and can not be treated as a true morphogenetical reorganization.

In such a way, the question about the induction of encystation can be replaced by the question about the factor inducing synthesis and/or the extrusion of a cyst wall material. There is no information about the chemical composition of a cyst wall. Probably the most important question concerns homogenity or heterogenity of substances in the cyst wall. If the cyst wall would be composed of only one chemical substance one could expect that one simple metabolic pathway is crucial for the encystation.

Observations of excystment proved that after some initial changes of the shape the further development progresses. This development concerns: the growth of the cell surface, the enlargement of nuclei and meiosis of some of them.

The successful purely mechanical way for artficial excystation, and further development in vitro, suggests that no essentially special chemical treatment is requested for the initiation of the further development in this stage. It does not mean, however, that some chemical substances do not play a regulatory function during the very early development of *Opalina* or that they are not obligatory for development in other stages (protrophonts? young and mature trophonts?).

It arises further questions, why the growth of a cell surface, the enlargement of nuclei, entering of mitosis and meiosis are so sharply inhibited in the cysts, if there is no obligatory action of special external chemical stimulus for putting on the further development of excysted cells. One can wonder if the development of *Opalina* can be induced by a start of feeding, or by the suppression of some inhibition. Such inhibition could be a result of the mechanical pression on cells of enclosed opalinids or of the accumulation of metabolites inside the cyst.

According to the presented results and considerations, at least three phenomena can be recognized as the possible triggers for entering some nuclei in meiosis. These are:

(1) The start of palintomy;

(2) The induction of synthesis and/or secretion of the cyst wall material;

(3) Excystation.

However, it is not clear if the points second and third are obligatory for the initiation of meiosis. It is not known if any inactive phase of life cycle, inside the cyst wall is necessary for gametogenesis. Next question is if the synthesis of the cyst wall material switch the metabolism and it enables the entering of meiosis stage.

Summary

Encystation was observed after transfer of hibernated frogs to room temperature and after transfer of opalinids from hibernated frogs to Yang's medium.

The artificial excystation was obtained in two different ways: (a) after bile treatment, (b) in pure mechanical way after pumping force treatment using water vacuum pump joined with glass filter, while opalinids were put on the filter surface.

Some growth and meiosis were observed in vitro in Yang's medium after artificial excystation, however, medium was overgrowing with bacteria and fungi introduced from frog faeces.

STRESZCZENIE

Ekscystacja opalin była indukowana bądź na drodze przenoszenia hibernowanych żab do temperatury pokojowej, bądź na drodze przenoszenia opalin z hibernowanych żab do pożywki Yanga.

Uzyskiwano ekscystację opalin in vitro stosując dwie różne metody: (a) w wyniku działania żółci, (b) w wyniku mechanicznego działania podciśnienia podczas filtrowania zawiesiny cyst na sączku szklanym.

Obserwowano pewien wzrost i meiozy ekscystowanych opalin in vitro, pomimo że pożywka Yanga szybko przerastała bakteriami i grzybami pochodzącymi z treści jelita tylnego żaby.

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The position and the number of contractile vacuole pores (CVPs) in conjugants and exconjugants of *Chilodonella cucullulus* (O.F.M.)

Położenie i liczba por wodniczek tętniących u koniugantów i ekskoniugantów *Chilodonella cucullulus* (O. F. M.)

The morphogenesis during division in *Chilodonella cucullulus* (O. F. M.) is a complex process. The divisional processes were described by Faurè-Fremiet 1950, Radzikowski 1966 and Kaczanowska et Kowalska 1969. During division, in this ciliate, the adoral kineties — the new mouth and the kinety X for the opisthe are formed on the ventral side. On the dorsal side the cytopyge for the proter and the opisthe appear.

Kaczanowska and Kowalska 1969 have shown, that during division the contractile vacuole pores (CVPs) of the proter and the opisthe are formed in the constant pattern, different for each of the offsprings. As a result two classes of individuals differ in CVP pattern, exist in the population. In the proters there are three CVPs. The space between CVP-1 and 2, situated in the anterior part of the cell, is covered by 5 kineties, whereas there is one kinety between CVP-2 and the CVP-3 situated in the posterior part of the body (Fig. 1 a). In the opisthes usually 4 CVPs appear. The additional CVP appear on the same interkinetal space as CVP-2, but more posteriorly. Between CVP-1 and 2 there are 6 kineties, and between CVP-2 and 3 — two kineties (Fig. 1 b).

Kaczanowska and Kowalska 1969 stated also, that in the logarithmic culture besides cells with typical distribution of CVPs for proter and opisthe, appear about 6% cells with atypical pattern of CVP, and that percentage of those cells increase in starved cultures.

Radzikowski 1966 showed that in *Chilodonella cucullulus* during morphogenesis of conjugation the process of resorption of old mouth and formation of new one take place in the same mode as in opisthe during division. However new kinety X, situated on the dorsal side, is not formed.

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The process of formation of new CVPs during conjugation in *Chilodonella cucullulus* has not been studied till now. The aim of the present studies was to elucidate the position of CVPs and number of kineties in conjugating cells of *Chilodonella cucullulus*. The sequential stages of morphogenesis during conjugation

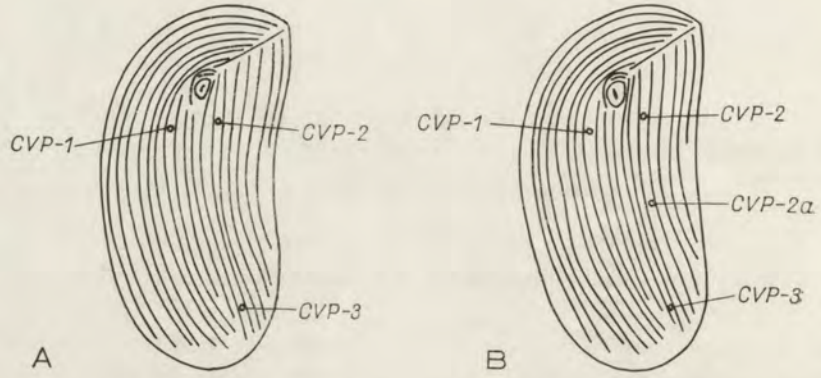

Fig. 1. Pattern of position of the CVPs in *Chilodonella cucullulus* (after Kaczanowska and Kowalska 1969). A — disposition of CVPs in proter, B — disposition of CVPs in opisthe

and during the first division after conjugation was followed in order to determine where new CVPs are formed. The role of the conjugation process in the regulation of the number and distribution of cortical organelles in *Chilodonella cucullulus* will be discussed.

Materials and methods

Studies were carried out on stock B2 of *Chilodonella cucullulus* (O. F. M.). It was the following clone of stock B, which was isolated by dr Radzikowski from the sawage treatment centre near Otwock in 1965. Protozoans were maintained in Petri dishes at room temperature in small amount of tap water to which a drop of fresh baker yeast suspension was added every second day. The clon B_2 was induced to intraclonal conjugation by starvation. Conjugating cells were fixed at different stages of conjugation and afterwards silvered after Chatton-Lwoff method in Corliss 1953 modification. Observations were also made on ciliates stained with nigrosine and impregnated by Protargol according to Jerka-Dziadosz and Frankel 1969.

Results

Cortical morphology in early conjugants

The observations were carried out on 301 conjugating protozoans, fixed at early stage of conjugation (Table 1). The purpose of the observation was to describe the pattern of CVPs of the animals which enter conjugation. The specimens in

Table 1

Number of CVPs and their position in conjugants B_2 of *Chilodonella cucullulus*

Number of CVPs*	Distance between CVPs**											
	8–3	8–2	7–3	7–2	7–1	6–2	6–1	5–2	5–1	5–0	4–1	Total
3			6	8	1	43	22		97	5	3	185
4		1	17	8		77	9					112
5	1					2		1				4
Total	1	1	23	16	1	122	13	1	97	5	3	301

* Ciliates with two CVP — 8. Ciliates with two CVP (always CVP-1 and 2) discussed in the text are mentioned only in notes to Tables 1–3.

** Explanation see text.

Table 1 were classified on the basis of the number of CVP and their distribution in relation to the kineties. The results presented in Table 1 indicate, that in the studied population most numerous were the typical proters, in which the CVPs-1 and 2 were spaced by 5 kineties and CVPs-2 and 3 by 1 kinety (designated as 5–1 in Table 1) and the typical opisthes, in which the CVPs-1 and 2 were spaced by 6 kineties, and CVPs-2 and 3 by 2 kineties (designated as 6–2). They form about 71% of the population. Frequent were also the conjugants with the patterns of CVPs 6–1 and 7–3. In the studied population the range of variability of the CVPs pattern is relatively high. The atypical number of the kineties between vacuoles, that mean, other than 5–1 and 6–2, appeared in about 29% of specimens whereas in the clone B_1 maintained in good conditions, according to Kowalska and Kaczanowska 1970, the atypical pattern appeared only in 6% of specimens.

In the population attract attention the large number of specimens with decreased number of vacuoles. In the group of protozoans with the CVPs pattern 6–2, 35% of the specimens possessed less vacuoles than typical 4. It is possible, that during the starvation of the culture which was led to the conjugation, in the same cases the reduction in the number of CVPs took place, which is in agreement with the data of Kowalska and Kaczanowska 1970.

From above follows that the defections from the typical pattern of CVPs in the conjugants of *Chilodonella cucullulus* consists of the changes in distance between vacuoles measured in kineties, and of the reduction of the number of vacuoles.

Among 104 pairs of the conjugants, in which every specimen was well stained, so, that the number and position of vacuoles was easily recognizable, only 31 pairs possessed the typical pattern of CVPs. The pairs were classified into three groups on respect of the CVP pattern. Among the 31 pairs there were 6 pairs of "proter × proter" type, 9 pairs of "opisthe × opisthe" type, and 16 pairs of "proter × opisthe" type (the probability of appearance of the pairs "proter × opisthe" is twice that high, that the probability of appearance of other types of pairs). From the data presented

above follows, that in the process of the formation of the mating pairs, the interclonal dimorphism (Kaczanowska and Kowalska 1969) does not play any role.

The diagram on Fig. 2 A shows the number of kineties in ciliates from stock B2. In the studied population most frequent were the ciliates with 18 kineties (43%). Fairly frequent were also the kinetoms 19 (24%) and 17 (22%). The small number

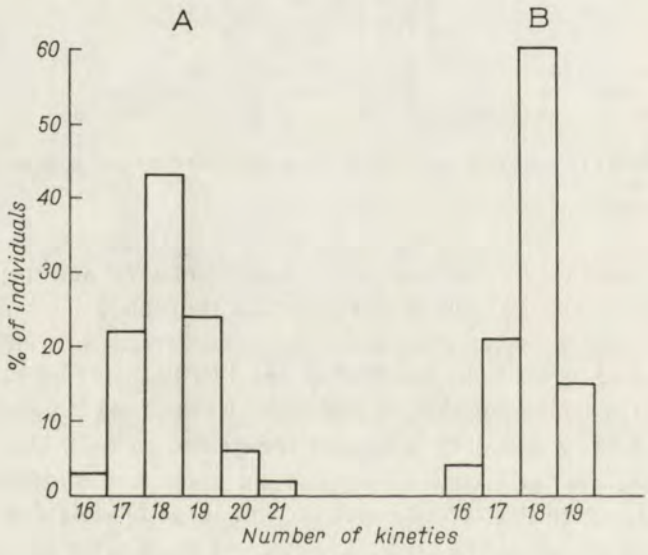

Fig. 2. Total number of kineties in stock B2 of *Chilodonella cucullulus*. A — number of kineties in conjugants. B — number of kineties in specimens after the first post-conjugation division

of vacuoles and the wide range of the kinetome variability of the conjugating specimens from the stock B_2, remind the starved population B_1.

Morphogenesis during conjugation

The cortical development during division in *Chilodonella cucullulus* described Fauré-Fremiet 1950, Radzikowski 1966 and Kaczanowska and Kowalska 1969. The first sign of the beginning of division is the appearance of kinety X on the right margin of the ciliate. Later it will move to the dorsal side of the opisthe. On the ventral side, the formation of adoral and preoral kineties of the opisthe starts from fragmentation of three kineties in the central region of the cell. The kineties which surround the oral basket in the proter remain intact during division. All kineties, except one short kinety K, are divided by the division furrow among the proter and opisthe. The kinety K remain in proter. The new contractile vacuoles are formed in the proter and the opisthe (see introduction). The new CVP can be observed during the movement of the fragments of kineties. On the dorsal side in both off springs the new cytopyge are formed.

The morphogenetic movements connected with the cortical morphogenesis during conjugation was described by Radzikowski 1966. At the beginning the adoral and preoral kineties are resorbed, then the fragments of three kineties in the middle of the cell appear. From those fragments the new adoral and preoral kineties will be formed as well as the additional kinety of the middle region — like it usually does during divisional morphogenesis in the opisthe. Only new kinety X is not formed.

Observations performed on the conjugating pairs of stock B_2 are consist with the observations of Radzikowski 1966. The process of morphogenesis has been followed in order to find out if and when the new CVP are being formed. It came out, that the new contractile vacuole pores are not formed anew during the conjugation (Pl. I 1–5). Only the distance between the old CVP increased, since as a result of cortical reorganization, the additional kinety was formed between CVP-1 and 2. Typical specimens after conjugation usually possessed the following CVPs pattern: 6–1 and 7–2. In the exconjugants till the first division after the conjugation no new CVPs were formed. Also the cytopyge on the dorsal side did not exhibit any visible changes. (Pl. I 6).

Cortical morphology in exconjugants

Among the exconjugants two groups of ciliates can be distinguished. One group with the CVP pattern 7–2, which had originate from the cells with the CVP pattern 6–2, the other group with the CVP pattern 6–1, which have had the pattern 5–1 before the conjugation. Among the animals fixed between 24–72 h after the conjugation (Table 2) no cell was found with the different CVPs pattern (even in

Table 2

Pattern of CVPs in *Chilodonella cucullulus*
24–72 h after conjugation

Number of CVPs*	Distance between CVPs		
	7–2	6–1	Total
3	17	15	32
4	8	1	9
5	—	—	—
Total	25	16	41

* Number of cells with two CVPs – 5.

the cells which possessed only two CVPs, the vacuoles were separated by 6 or 7 kineties). Among the ciliates fixed about 100 h after conjugation (Table 3) only 5% of specimens exhibited atypical pattern of CVPs.

Table 3

Pattern of CVPs in *Chilodonella cucullulus* about 100 h after conjugation

Number of CVPs*	Distance between CVPs								
	7–3	7–2	6–3	6–2	6–1	5–2	5–1	5–0	Total
3	—	12	—	1	22	—	18	3	56
4	1	15	1	24	2	1	1	—	45
5	—	—	—	1	—	—	—	—	1
Total	1	27	1	26	24	1	19	3	102

* Number of cells with two CVPs — 12.

Since till first division after conjugation the contractile vacuole pores remain unchanged, it seems, that most of the ciliates with atypical distribution of vacuoles dyed after conjugation (the mortality of exconjugants in studied stock B_2 reached about 20%).

Likewise among the conjugants, also within the exconjugants, a large number of cells with smaller than typical number of vacuoles have been found. The reduction of the number of vacuoles went even further than in the conjugants, since the two-vacuole exconjugants made up of 10% of the population, whereas in the conjugants population only 3% of such cells were find. It is possible, that the reduction of the number of vacuoles was caused by the bad conditions of culture, i.e. non sufficient amount of food. Starvation was not employed, but perhaps, the requirements of food is greater when the cells undergo internal reorganization and growth. Table 3 shows the exconjugants fixed about 100 h after the conjugation. They are divided into four groups: the ciliates which were before division with CVPs pattern 7–2 and 6–1, and 6–2 and 5–1, which were after the postconjugation division.

First division after conjugation

About 100 h after the end of conjugation, the exconjugants were usually much larger than the non-conjugating cells. In that time the first division occurred. During morphogenesis the new CVPs were induced to form in such distances that the typical pattern of the proter and the opisthe was reconstructed. The number of kineties between CVPs-1 and 2 decreased. The new CVP-1 and 2 for the proter and the opisthe, appeared in distance of 5 kineties, no matter what CVPs pattern had possessed the maternal cell (Fig. 3). The new CVP-3 was formed in the distance of one kinety from the new CVP-2 in the proter and in the distance of two kineties in the opisthe, since in opisthe an additional kinety is formed in the middle region. That means that the new CVPs originate in the same way as in any division process. During the first division after conjugation also new kinety X and cytopyge for the proter and the opisthe was formed. After division the ciliates exhibited normal and

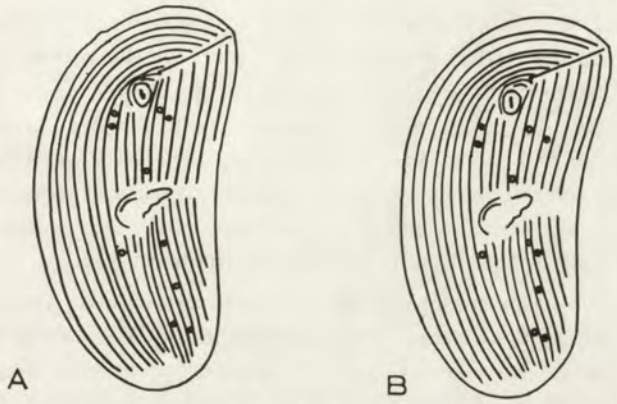

Fig. 3. Pattern of disposition of CVPs in dividing exconjugants of *Chilodonella cucullulus*. A — exconjugant with proter-pattern. B — exconjugant with opisthe-pattern, open circles — new CVPs, black circles — old CVPs

typical pattern of CVPs distribution. Among the population of exconjugants the most frequent were the ciliates with small number of kineties (18), but the range of variability of the kinety number was narrower (see diagram Fig. 2 B). That means that the conjugation would cause leading of variability of studied features to the standard value.

Discussion

The population of *Chilodonella cucullulus* which enters the conjugation shows a considerable variability of CVPs pattern and of the number of kineties. That would indicate that in respect of those features the population begun to deviate from the "stability sink" (Nanney 1968), which is characteristic for the logarithmic phase of growth.

The atypical pattern of CVPs could arise during the division which preceded the conjugation as a result of a disturbances in the processess which regulate the distribution of organelles on the ciliate cortex. The increase of the number of cells, which show the atypical pattern of CVPs can be considered as a symptom of the growing old (senility) of a population. This process is accelerated by the maintenance of the culture in semi-starved conditions.

The processess, which cause growing old (senility) of a clone are very complicated and are not elucidated yet. Many investigations have proved, however, Maupas 1889, Calkins 1919, Woodruff and Spencer 1924, Jennings 1944, 1945, Sonneborn 1954, that the sexual processess such as the conjugation and the autogamy support the physiological processess and cause rejuvenation of the clone.

In *Chilodonella cucullulus* the "rejuvenation" of the clone after conjugation would be expressed in returning to the typical pattern of CVPs distribution and in the increase of the division index. The low percentage of the deviation from the

standard corticotype indicates that, the most of the atypical cells die after conjugation. One may suppose that lethal genes could show up, or the process of growing old went to far (Faurè-Fremiet 1953, Sonneborn 1954).

During the morphogenesis of the conjugation the normal stomatogenesis take place, whereas the process of the formation of new CVPs and cytopyge and the resorption of the old ones does not occur. The entire cortical reorganization is then constituted from at least two parallel processes, which do not have to always accompany each other. During normal divisions the processes occur simultaneously.

Similarly as in *Chilodonella cucullulus* the process of the uncomplete cortical reorganization during conjugation was described in *Euplotes patella* by Hammond 1937 and *Euplotes green* by Diller 1966. In *Euplotes* occur two cortical reorganization which correspond to the reorganization of nuclear apparatus. During the first cortical reorganization the incomplete peristom and the incomplete set of cirri are formed. During the second reorganization the oral apparatus is completed and a new, full set of cirri is formed. The contractile vacuoles in *Euplotes* are formed together with each generation of the cirri (Diller and Maloney 1968).

The cortical reorganization in *Chilodonella cucullulus* occurs, likewise described by Katashima 1959 in *Euplotes*, in the presence of the old macronucleus, whereas the new macronuclear anlage is in stage of the syncarion (Radzikowski 1971). During the morphogenesis of the first post-conjugation division old Ma already does not exist (Radzikowski 1971), that means that the process of the reconstruction of the typical pattern of CVPs is under control of the new macronucleus.

Summary

In the cultures of *Chilodonella cucullulus* (O. F. M.) in which the process of conjugation take place the range of variability of such features as the number of kineties, the number of CVPs and the loci of CVPs formation increases. The process of conjugation reduces the variability of the studied features to the standard value.

The regulation of the distribution of CVPs and the return to the typical number of the cortical organelles take place during morphogenesis of the first division after conjugation. The conjugation morphogenesis occurs in the presence of old Ma and is a process of incomplete cortical reorganization. The post-conjugation division does not differ from any other division.

STRESZCZENIE

W kulturach *Chilodonella cucullulus*, (O. F. M.) w których zachodzi koniugacja, obserwuje się rozszerzenie zakresu zmienności takich cech morfologicznych, jak liczba kinet, CVPs, miejsca indukcji wodniczek. Koniugacja odgrywa tu rolę procesu sprowadzającego różnorodność badanych cech do standardowej wartości. Regulacja położenia wodniczek i powrót do typowej liczby po-

wierzchniowych organelli odbywa się w czasie morfogenezy pierwszego podziału ekskoniugantów. Morfogeneza koniugacyjna przebiega w obecności starego makronukleusa i jest procesem niepełnej reorganizacji powierzchniowej. Podział postkoniugacyjny natomiast nie różni się przebiegiem zmian morfogenetycznych od innych podziałów.

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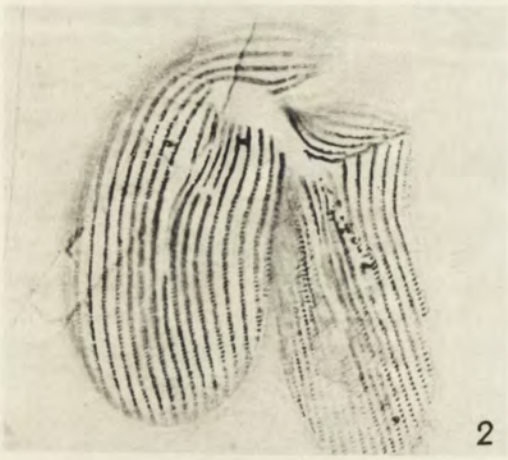
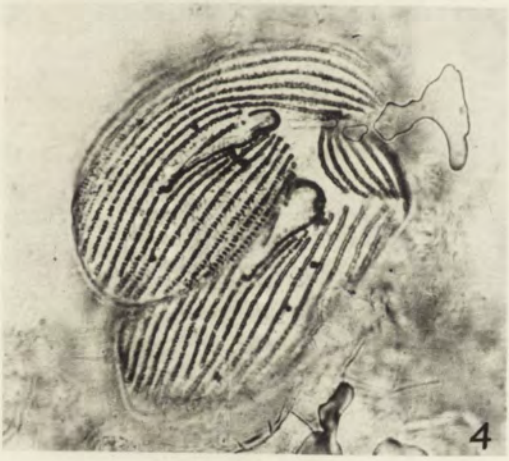
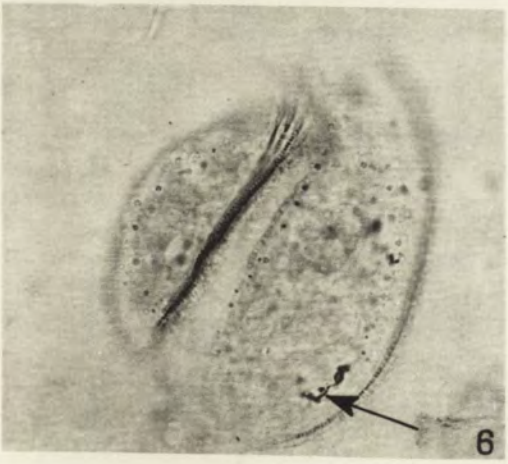
EXPLANATIONS OF PLATE I

1–5: The ciliature of ventral surface in conjugating specimens of *Chilodonella cucullulus*. (O. F. M.). The photographs represent the successive stages of morphogenesis during conjugation. The CVPs remain unchanged

6: Dorsal side of *Chilodonella cucullulus* in late stage of conjugation. The unchanged cytopyge is clearly seen. All photographs are taken from specimens impregnated with silver after Chatton and Lwoff method







I. Janus auctor phot.

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Jolanta KINK

Observations on morphology and oral morphogenesis during regeneration of ciliate *Lacrymaria olor* (O. F. M. 1786) (*Holotricha, Gymnostomatida*)

Obserwacje nad morfologią i morfogenezą oralną w czasie regeneracji orzęska *Lacrymaria olor* (O. F. M. 1786) (*Holotricha, Gymnostomatida*)

The holotrichous ciliates from genus *Lacrymaria* (*Enchelyidae–Gymnostomatida*) possess an apically situated mouth, and the cells are covered by uniform ciliature — that means they represent the simplest type of composition. The study on morphology and development of the oral structures in *Lacrymaria*, may have a significance for the recognition of developmental patterns of primitive ciliates.

Didier and Bohatier 1970 and Bohatier 1970 described the ultrastructural morphology of *Lacrymaria olor* (O. F. M.).

The results of my observations on ultrastructure of *Lacrymaria olor* differ in some points from the results of above authors. The differences will be discussed later on in this paper. This report contains also the preliminary observations on regeneration of oral apparatus of *Lacrymaria olor*.

Material and methods

The organismes used in this investigation have been collected from pond on Sadyba in Warsaw. They were maintained on small Petri dishes in Pringsheim solution as a medium and fed daily with *Colpidium* sp. maintained separately in the same medium with oat seeds.

In the light microscope observations the preparations stained with iron hermatoxylin after Parducz 1952 and protargol impregnation after Dragesco 1962 have been used.

The specimens for observations in electron microscope were fixed in 2% OsO_4 in phosphate buffer at pH 7.2 for 30–45 min. They were then embedded in Epon 812, sectioned on Reichert's ultramicrotome and stained with uranyl acetate. The material was examined with an JEM 7A electron microscope.

In the study on regeneration of posterior fragments following procedure has been employed. Groups of about 30–50 morphostatic cells were picked up from the culture and transferred to the one depression slide. The anterior parts of the animals containing the neck and mouth were then cut off. Operations were carried out by hand under a stereoscopic dissecting microscope at 60× magnification. Cells were cut with a glass micro-needle. Posterior fragments were then isolated to

another depression slide and fixed at various time after the operations. The whole procedure: isolation of animals from the culture, operation and isolation of fragments took about 15 min. In series of experiments the regenerating opimers were fixed at 0–15, 15–30, 30–45, 45–60, 60–75 min after operations.

Results

Morphology of non-dividing animal

Lacrymaria olor has the shape of a bottle with the posterior part tapering into the caudal pole and the anterior end elongated as a neck (Fig. 1). It is 150–200 μ long and 40 μ wide in the widest part of the body. At the anterior end of the neck the snout is situated. The snout consists of two parts: the anterior-one unciliated, situated apically and posterior-one, covered by short, oblique kineties. (Pl. I 4). The inside of the snout is filled up by toxicysts, which have indentical appearance after hematoxylin or protargol staining as the toxicysts present in the rest of body. The toxicysts in the snout are attached concentrically around the unpermanent cytostome, to the apex of the unciliated dome of the snout (Pl. I 1–3).

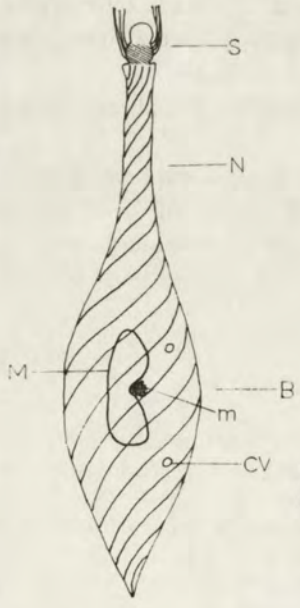

Fig. 1. *Lacrymaria olor* (O. F. M.), general view. Oblique rows represent the kineties S — snout N — neck, B — body, M — macronucleus, m — micronucleus, CV — contractile vacuole

The body of the ciliate is entirely covered by about 12 longitudinal, spiralled kineties which meet at the posterior pole. The kineties are situated in deep grooves which are deeper at the neck than at the rest of the body. Some of the kineties are shorter, they start at the different levels of the cell and end at the posterior pole. The cilia on the neck and body are shorter than the snout's cilia.

The nuclear apparatus of *Lacrymaria olor* is localized in the wide part of the body. It consists of lobe-shaped macronucleus and small, spherical micronucleus.

Inside the body of the ciliate, a long, thin trichocysts can be seen, which are arranged in bundles.

Two contractile vacuoles are situated in one row at the body of the animal.

The study of ultrastructure of the body showed, that very flattened alveole under the pellicle can be seen. The ciliary unit contains kinetosome connected with three kinds of fibrils: (1) transverse fiber, which appears as a row of tubules, running perpendicular to the kinety, (2) postciliary fiber, which appears as a bundle of tubular fibrils running posteriorly under the pellicle parallel to the kinety, and (3) kinetodesma as a fiber running lato-anteriorly at the base of the kinety (Pl. III 8, Pl IV 10).

The interkinetal folds of the body are wide and flat. They are underlayed by a discontinual, microfibrillar layer of varying width (Pl. IV 11, 12, V 14). This microfibrills are not connected with kinetosomes. Oval mucocysts are attached to the pellicle of the body (Pl. IV 11, Pl. V 14). They are filled up with a dense material. The toxic trichocysts possess a very long external tube and distinctly shorter internal tube. All toxicysts are of one kind (Pl. VI 16). They are arranged in random bundles surrounding the nuclear apparatus. In one of the studied strains of *Lacrymaria olor* an extensive bacterial infection have been observed. Bacteria were present in macronucleus and endoplasm as well (Pl. III 8, Pl. VI 17). Micronucleus always was free from the infection.

The neck of *Lacrymaria olor*. The pellicle system and the ciliary units are same as on the body, but kineties are spaced by high and narrow pellicular folds. Each kinetosome is accompanyied by baggy depression in pellicle, which remind the parasomal sac. (Pl. IV 10). To the left from kineties and at the base of interkinetal folds run the microfibrillar bands (Pl. III 8, 9, IV 10, V 13). They are situated as the prolongations of the microfibrillar layer underlaying pellicular folds in the body. These bands do not contact with kinetosomes nor subpellicular fibers. On the course of the microfibrillar bands irregular aggregations of dense material can be seen. Likewise in the body, mucocysts are attached to the pellicle of the neck. In the endoplasm are situated the bundles of toxic trichocysts.

In the ultrastructure of the somatic part of *Lacrymaria olor* a uniform arrangement of reticulum canals in the epiplasm of the pellicular folds can be seen. (Pl. V 15) There are also large, with regular crists mitochondria accompanying the infraciliature.

The oral apparatus is situated on the apex of the neck of *Lacrymaria olor*. It is surrounded by a special oral ciliature, which differs significantly from the somatic one (Fig. 2). The ciliated part of the snout bears oblique kineties, which are not connected with ciliature of the neck. The bases of kinetosomes in their kineties are connected by a microfibrillar material. This material is regularly striated. The kinetosomes bear the same kind of fibrils as in the somatic kineties. In front of the

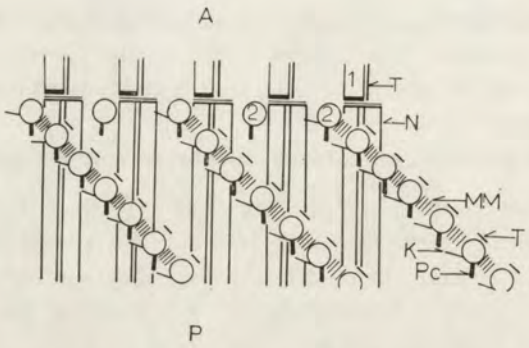

Fig. 2. The schematic drawing of the arrangement of the oral ciliature on the snout of *Lacrymaria olor*. A and P — anterior and posterior parts of the body, 1 — unciliated kinetosome, 2 — ciliated kinetosome, T — transverse fiber, Pc — postciliary fiber, K — kinetodesma, N — nemadesma, MM — microfibrillar material between kinetosomes

oblique kineties a short, two-kinetosomal kineties are localized. They are almost perpendicular to the oblique kineties. The number of two-basal body kineties is twice as large as the one of the oblique kineties. They are situated at different plane than the rest of kinetosomes. The system of two-basal body kineties is directly connected with the cytopharyngeal complex. Each kinety possesses one ciliated and one unciliated kinetosome. That last ones bears double nemadesma, whereas the transverse fibers of those kinetosomes run anteriorly towards the apex of the snout. The unciliated basal bodies are situated under the angle to the ciliated ones, which do not possess the transverse fibers (Pl. I 5, II 6, 7).

Encircling the border between ciliated and unciliated parts of the snout there is a microfibrillar ring, situated close to the proximal end of the unciliated kinetosomes (Pl. I 5, II 6, 7). This ring is separated from kinetosomes and nemadesma. On the level of each pair of basal bodies it forms stripes running perpendicularly toward the pellicle. The naked part of the snout has a shape of dome, which scaffolding is made from transverse fibers forming so-called cytopharyngeal ribbons (Pitelka 1969) (Pl. I 5). The ribbons divide the top of the snout into segments which are filled up with the toxic trichocysts attached to the pellicle. Between them mucocysts are situated. They, however, are spherical and smaller than the ones of the body.

Observations on regeneration of oral apparatus in *Lacrymaria olor*

Observations on regeneration process performed under the light microscope, made possible to determine the time required for reconstruction of the mouth in the posterior fragments of *Lacrymaria olor*. The process lasts about 1 h and 15 min. It has been noted, that the regeneration of the snout get ahead of regrowth of the neck of the animal. The grow and elongation of neck occur later.

Opimers at 0–15 min after operation

At the place, where the operation has been made, the displacement in the subpellicular system occurs. The process of cilia resorption takes place (Pl. VII 18). Cilia can be seen both — under the pellicle and inside the cytoplasm. The cilia lack of the external membrane, however, the arrangement of tubules remain unchanged. The precipitation membrane has not been observed, nor the aggregation of trichocysts, opposite to the situation which takes place in *Dileptus cygnus* (Golińska et Grain 1969). Wound healing in *Lacrymaria* is probably limited to the sealing of the pellicle under the pressure of the operation needle. The cytoplasm at the region of future field of the oral primordium appear to be uniform.

Opimers 15–30 min after operation

Within the cytoplasm still resorbed cilia can be seen (Pl. VII 19). In the same time the proliferation of new kinetosomes in the vicinity of old, ciliated basal bodies is observed (Pl. VII 20). (cf. Dippell 1968, Golińska et Grain 1969, Paulin and Bussey 1971).

Opimers 30–45 min after operation

In this stage of development of new mouth, the new double-basal body kineties are formed (Pl. VII 21). The localization of these pairs of kinetosomes indicates, that they have proliferated simultaneously around the primordium of the snout (Pl. VIII 22). On the base of the unciliated kinetosomes the nemadesma begin to form. They are single at the beginning. The cytopharyngeal ribbons can be observed, they run from the unciliated basal bodies toward the surface of the primordium of a snout. At the same time proliferation of new basal bodies occurs, they form the oblique kineties (Pl. VIII 23, 24). In the cytoplasm of primordium region numerous mitochondria can be seen, but there are no toxic trichocysts.

Opimers 45–60 min after operation

In this fragments the nemadesma become thicker. The double nemadesma are visible, but the outer one in each pair is distinctly thiner (Pl. IX 26). The proliferation of new kinetosomes in the oblique rows continue. The cytoplasm of the primordium exhibits the feature of phagoplasm. Inside the endoplasm the rudiments of young toxicysts are seen (Pl. IX 25). They appear to be similar to the ones described in different ciliates by other authors (Dragesco et al. 1965 and Nilsson 1969).

Opimers 75 min after operation

At this stage of stomatogenesis all elements of oral apparatus are formed and arranged in pattern typical for an adult snout (Pl. IX 27).

Discussion

The specimens of *Lacrymaria olor* used in this investigation possessed two contractile vacuoles, localized in the body. The form studied by Didier et Bohatier 1970 and Bohatier 1970 possessed only one contractile vacuole situated at the posterior terminus of the body. This may indicate, that *Lacrymaria olor* collected around Warsaw represents other strain of the species. The differences found in the ultrastructure seem to support the idea. They apply to the ultrastructure of the microfibrillar layer and the arrangement of the oral ciliature. It is interesting to discuss the differences on account of the localization and degree of complexity of the oral apparatus of *Lacrymaria* and also on account of the relations between the genus *Lacrymaria* and other ciliates, which belong to *Rhabdophorina*.

The depressions in the pellicle, which accompany the kinetosomes, have been stated. They remind the parasomal sacs described by Ehret and Powers 1959 in *Paramecium*. In *Lacrymaria* they seem to represent elements, increasing the surface of the cell. This feature could have a significance in the process of body elongation.

The microfibrillar material, which is localized as a bands on the neck and form discontinuous layer on the body, reminds M-bands described by Randall and Jackson 1958 or microfibrillar system described by Grain 1968 in *Stentor*. These authors basing on differences in morphological pictures of microfibrillar system in contracting and elongating ciliates consider the system as a contractile structure. Both, the M-bands in *Stentor* and the microfibrillar system in *Lacrymaria* are autonomic in that sense, that they lack connections with kinetosomes. The character of contraction in *Lacrymaria*, however is probably different from the one in *Stentor*. In *Lacrymaria* the most contractile part of the cell is the neck, which elongates and contracts equally fast. The microfibrillar material at the neck is arranged in bands. The irregular aggregates of dense material in the bands, which are not always well seen, could be the morphological pictures of different stages of neck contractions.

The arrangement of the microfibrillar structure in studied specimens of *Lacrymaria*, which appears as a bands in the neck and posteriorly spread on as a discontinuous layer reminds the microfibrillar layer, situated between ecto-and endoplasm in many rhabdophorin ciliates. Probably it is a modified form of it.

Didier et Bohatier 1970 observed in their form of *Lacrymaria*, that microfibrillar bands are present in the neck and the body as well. On the bands they noted loci of dense material, interpreted as a periodity of the structure, with the period from 1500 to 2500 Å. In my study the periodity of structure exhibits only that microfibrillar material, which connect the bases of kinetosomes in the oblique kineties. This is in agreement with Bohatier 1970. The structure of this material reminds microfibrillar connections in the polykinety of *Opisthonecta hennegui*

described by Bradbury 1965 or in the peniculus of *Paramecium aurelia* described by Schneider 1964.

My study of the structure and arrangement of oral ciliature in *Lacrymaria* supported the observations of Bohatier 1970, that it consists of oblique rows of cilia and short, double-basal body kineties. The last ones are directly connected with the cytopharyngeal complex. However, in contrary to the observation of Bohatier 1970, it has been stated, that the short kineties of the snout are not situated on the prolongation of the axis of the oblique rows of cilia. The pairs of the basal bodies show different direction than the oblique kineties.

The orientation of oral ciliature of *Lacrymaria* is very similar to the one of *Lagynophrya fusidens* (Grain 1970) and to the ciliature of the left side of proboscis in *Dileptus cygnus* (Grain et Golińska 1969). The structure of cytopharyngeal complex of *Lacrymaria olor* is very similar to that in *Didinium*, described by Yagiu and Shigenaka 1965 and Wessenberg and Antipa 1968, and also *Lagynophrya fusidens* described by Grain 1970. In all three species the inside of the snout "proboscis" is filled up by toxicysts attached to the apex and surrounded by a basket made from double nemadesma. Pairs of nemadesma similar to the ones in *Lacrymaria* described also Puytorac 1965 in *Holophrya* and Puytorac 1964 in *Prorodon*. But in these ciliates the nemadesma are attached to the oral ciliature in different manner than in *Lacrymaria*. The naked dome of the snout of *Lacrymaria* seems very like so as the ventral band of the proboscis in *Dileptus* (Grain et Golińska 1969). The morphological similarities of oral structures in *Lacrymaria* and *Dileptus* has gained a new support from observations on development of oral apparatus during regeneration.

The whole process of reconstruction of the mouth in *Lacrymaria* lasts twice times shorter than in *Dileptus*. It can be explained by the simpler structure of the mouth in *Lacrymaria olor* than in *Dileptus cygnus*. The process of wound healing in *Lacrymaria* is limited only to the sealing of the edges of wound. No precipitation membrane and displacements of toxicysts in endoplasm has been observed.

During stomatogenesis in *Lacrymaria* 15–30 min after operation the first unciliated kinetosomes appear. During next 15 min the organization of nemadesma and cytopharyngeal ribbons takes place. The development of the basket of nemadesma occurs in two steps and this respect is very similar to the formation of inner and outer baskets of nemadesma in *Dileptus*. About 45 min after operation in *Lacrymaria* the basket made up from single nemadesma is well developed. Later, the nemadesma shift apart from the base of unciliated kinetosomes and in that place the new nemadesma are organized. Simultaneously with the development of cytopharyngeal complex the proliferation and ordering of cilia in oblique kineties on the snout take place.

In summary, the process of regeneration in *Lacrymaria* contains following steps: first, the pairs of basal body are formed, next the oblique kineties are

organized and later the process of growth and elongation of the neck occur. That means, that reconstruction of somatic part of the cell is antecedent by the formation of the oral parts. The process of stomatogenesis starts from the apex and in this respect reminds the pattern of development of oral apparatus in *Dileptus*.

It can be stated, that the simple ciliate *Lacrymaria olor* possesses specialized oral ciliature differentiated into double-basal body kineties connected with the cytopharyngeal complex and the oblique kineties of the snout. The oral ciliature is formed as the first and fundamental in the process of stomatogenesis in *Lacrymaria*.

The excellent technical assistance of Mrs. U. Kujawska is thankfully acknowledged.

Summary

The paper contains the study on morphology and oral development of the ciliate *Lacrymaria olor* (O. F. M.). The oral ciliature is constructed from oblique kineties and pairs of kinetosomes. Each pair consists of one ciliated and one unciliated kinetosome. The unciliated kinetosome bear double nemadesma. Inside the cytopharyngeal basket there are toxicysts, attached to the naked apex of the snout.

The regeneration of oral apparatus lasts about 75 min. Formation of the oral primordium starts from its apex. The first unciliated kinetosomes appear 15–30 min after operation. During next 15 min pairs of kinetosomes are formed and accompanying them cytopharyngeal ribbon. The organization of nemadesma starts close to the unciliated kinetosomes.

STRESZCZENIE

Praca dotyczy badań nad morfologią i morfogenezą oralną orzęska *Lacrymaria olor* (O. F. M.). Orzęsienie ryjka stanowią ukośne kinety i pary kinetosomów. W skład każdej pary kinetosomów wchodzi kinetosom bezrzęsy i orzęsiony. Kinetosom bezrzęsy jest przesunięty względem orzęsionego co sprawia, że pary kinetosomów mają inny kierunek uporządkowania od kinet ukośnych. Kinetosomy bezrzęse niosą podwójne nemadesmy. Koszyk nemadesm otacza toxicysty, które są podwieszone do nagiego apexu ryjka.

Regeneracja aparatu gębowego trwa ok. 75 minut. Odtwarzanie zawiązka gębowego rozpoczyna się od jego apexu. Pierwsze bezrzęse kinetosomy pojawiają się w 15–30 min. po operacji. W ciągu następnych 15 min. rozwoju zawiązka gębowego pojawiają się pary kinetosomów i towarzyszące im cytopharyngeal ribbon. Przy kinetosomach bezrzęsych rozpoczyna się organizacja nemadesm.

Dyskutuje się pozycję orzęska *Lacrymaria* wśród *Gymnostomatida*.

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EXPLANATION OF PLATES I–IX

1–4: Morphology of *Lacrymaria olor* (O. F. M.)

1 and 2 — The snout of *Lacrymaria olor*. Iron hematoxylin staining after Parducz. Inside the snout the toxicysts can be seen
3 — The neck of *Lacrymaria* stained after Parducz. The bundle of toxicysts is seen inside
4 — The oblique kineties on the snout stained with protargol.

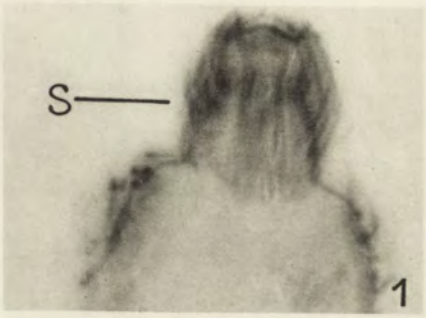
5–17: Ultrastructure of *Lacrymaria olor*

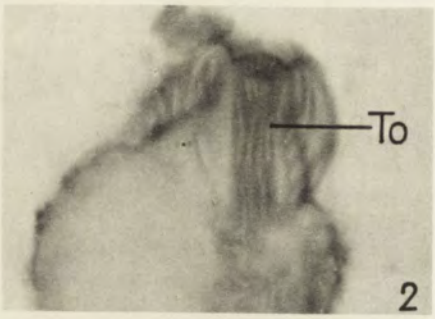
5 — The naked and ciliated parts of the snout. Oblique section ×14 000
6 — Fragment of the naked and ciliated parts of the snout. Oblique section. ×39 000
7 — Arrangement of the oral elements in the ciliated part of the snout. Oblique section. ×10 000
8 — Fragment of the neck. Longitudinal section. ×19 000
9 — The microfibrillar band in the neck. Transverse section. ×30 000
10 — Pelicular fold of the neck. Tangential section. ×21 000
11 — Longitudinal section of the body. ×30 000
12 — The microfibrillar layer in the pellicular fold of the body. Transverse section. ×35 000
13 — The microfibrillar band in the neck. Tangential section. ×26 000
14 — The microfibrillar layer in the body. Tangential section. ×20 000
15 — Canals of reticulum in epiplasm of the body. Longitudinal section. ×30 000
16 — Bundle of trichocysts in the body. Transverse section. ×27 000
17 — Macronucleus with nucleoli and bacteria. ×50 000

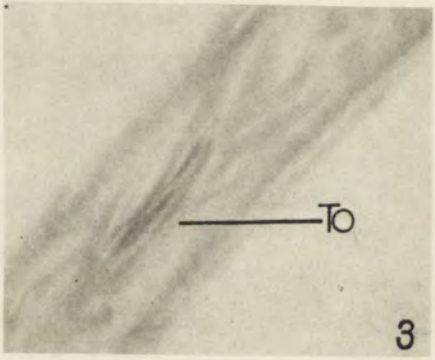
18–27: Ultrastructure of regenerating fragments of *Lacrymaria olor*.

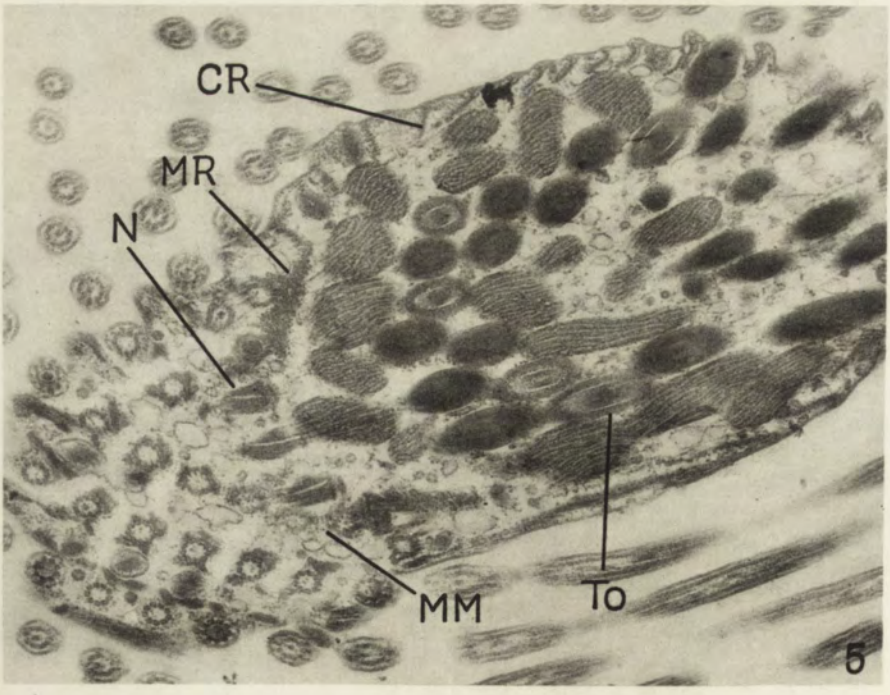
Fragment fixed between 0–15 min after operation
18 — Resorbed cilia (RC) close to the wound can be seen. ×14 000
Fragments fixed between 15–30 min after operation
19 — Resorbed cilia (RC) deep in the endoplasm. ×15 000
20 — The unciliated kinetosome (Kn) close to the ciliated one (Kc). ×22 000
Fragments fixed between 30–45 min after operation
21 — The pair of kinetosomes from the oral primordium. Close to the unciliated kinetosome (Kn) nemadesma is organized (N). Cytopharyngeal ribbon (CR) is seen. ×25 000
22 — Oral primordium with the cytopharyngeal ribbon (CR). Nemadesma is more wide (N). ×21 000
23 — The kinetosome (Kn) is formed in the oblique kinety of the snout. × 12 000.
Fragments fixed 45–60 min after operation
24 — The proliferation of new kinetosome (Kn) in the oblique kinety of the snout. × 15 000
25 — The formation of the toxicyst (To) in the endoplasm. × 23 000
26 — The doubling of nemadesma (N). ×27 000
Fragment fixed after operation 75 min
27 — Primordium of oral apparatus with all elements of the adult mouth. ×22 000

Abbreviations. CR — cytopharyngeal ribbon, K — kinetodesma, Kc — ciliated kinetosome, Kn — unciliated kinetosome, M — mucocyst, MB — microfibrillar band, ML — microfibrillar layer, MM — microfibrillar material, MR — microfibrillar ring between kinetosomes, N — nemadesma, Nu — nucleoli, Pc — postciliary fiber, Ps — pellicular sac, R — canals of reticulum, T — transverse fiber, To — toxicyst, X — bacteria.









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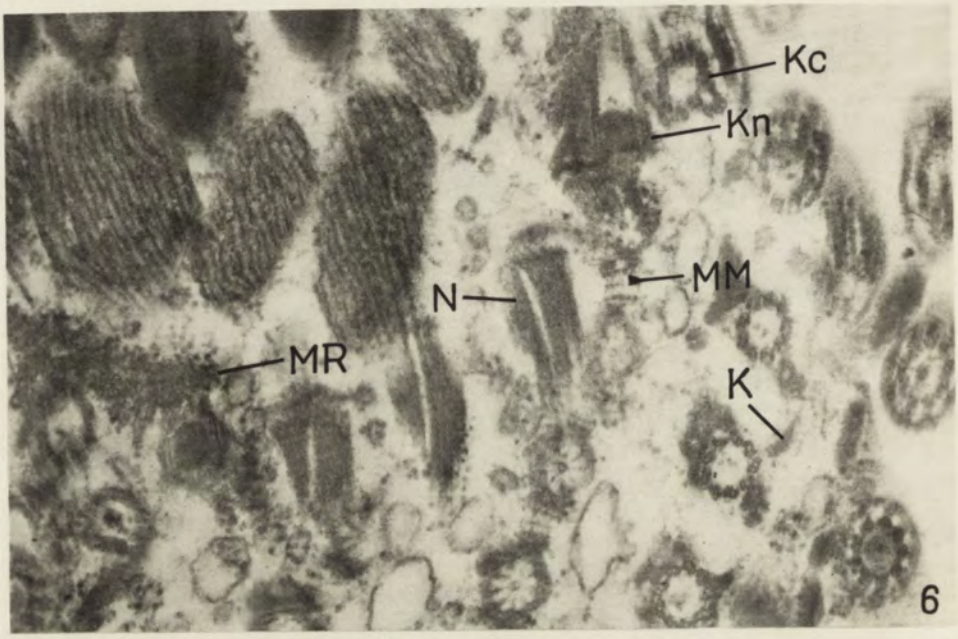

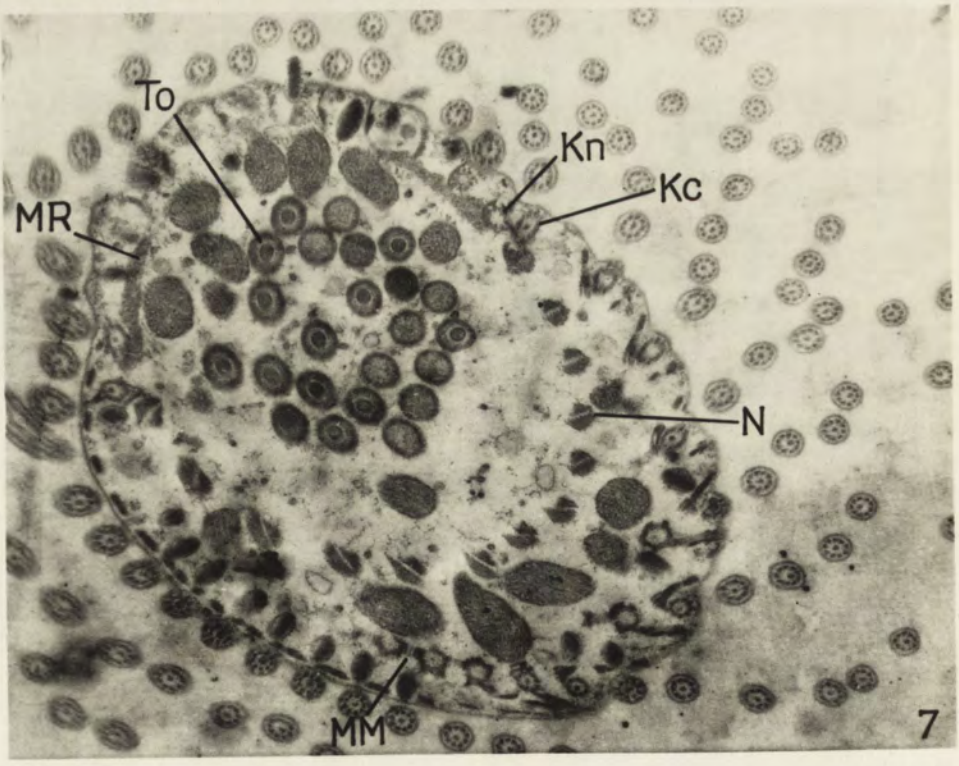

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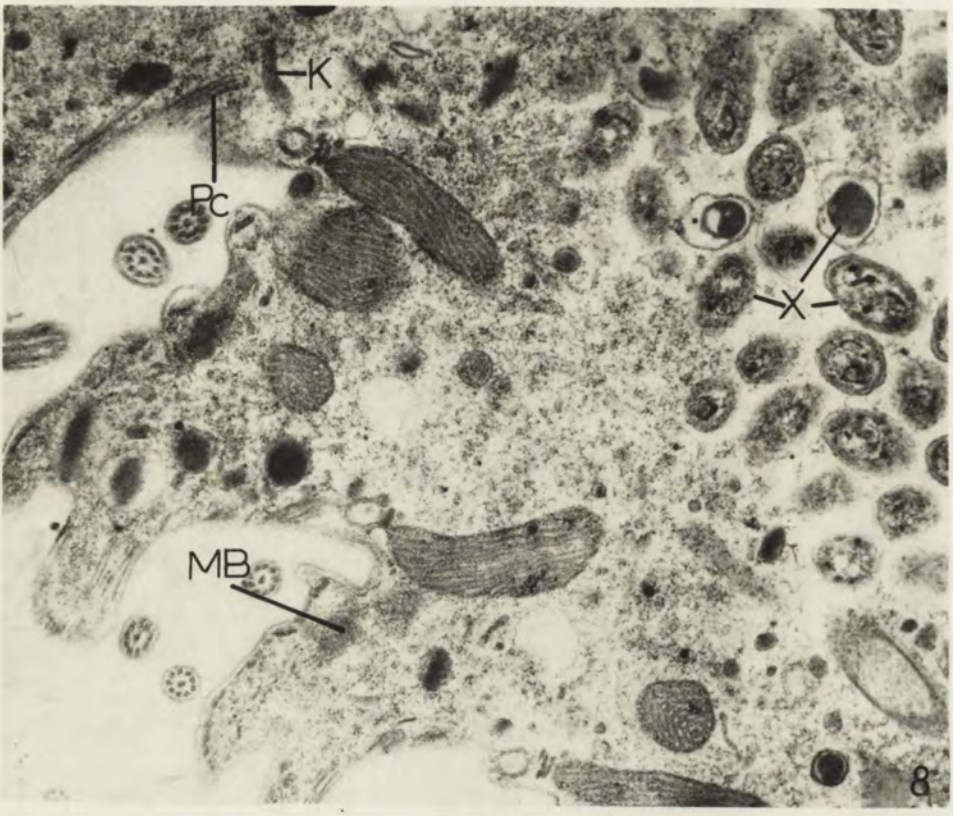

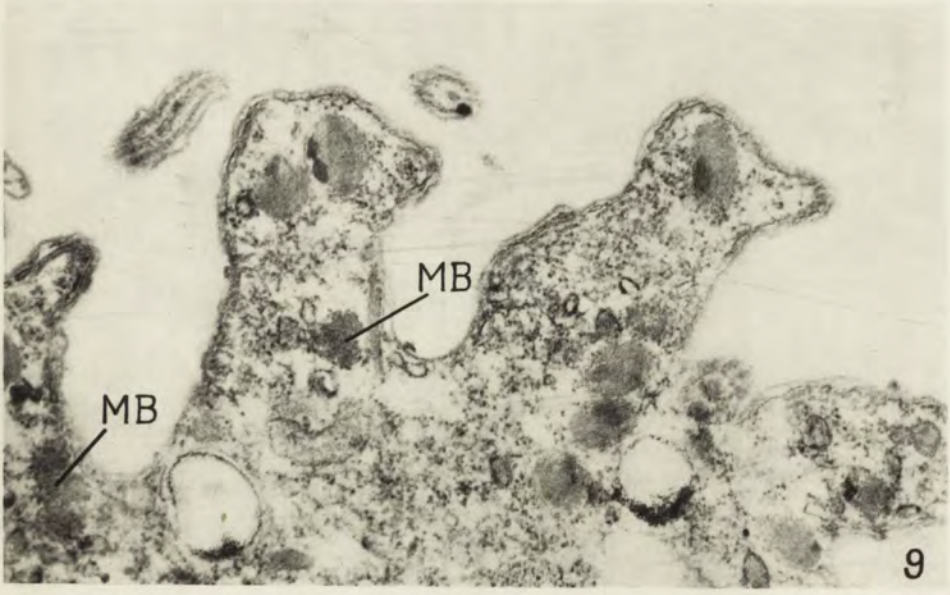

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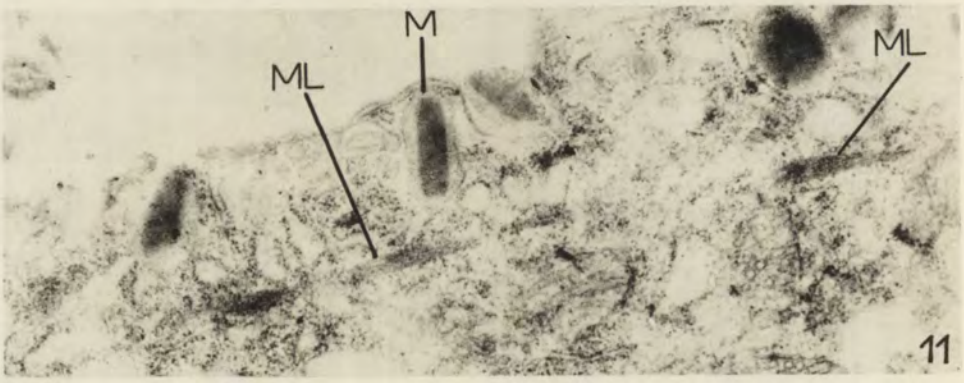

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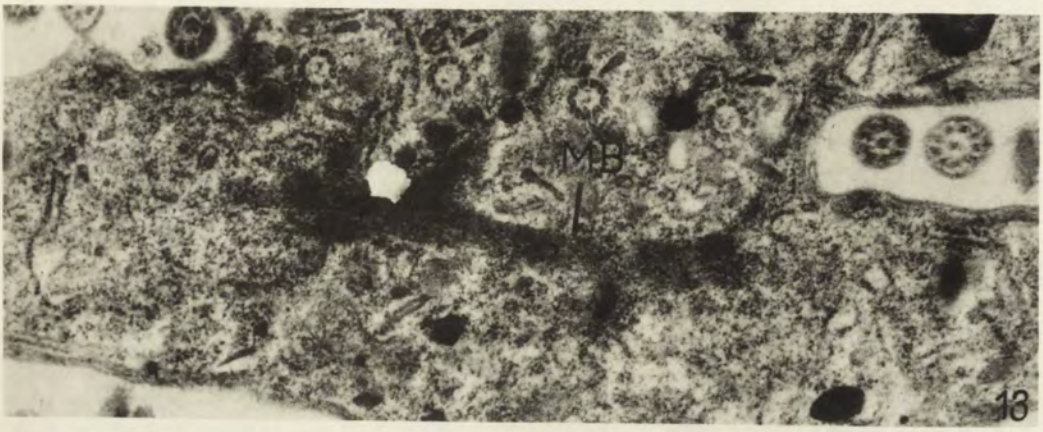

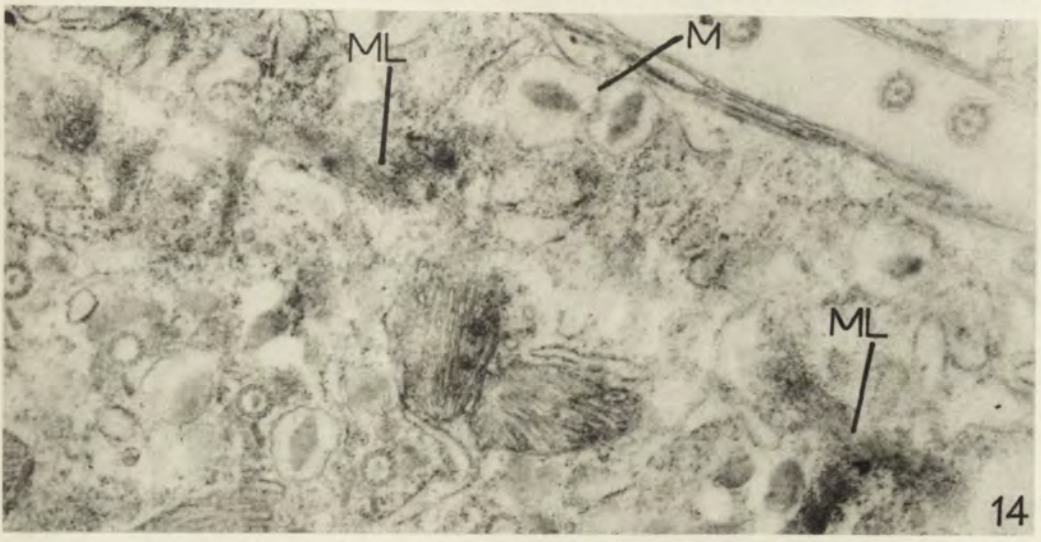

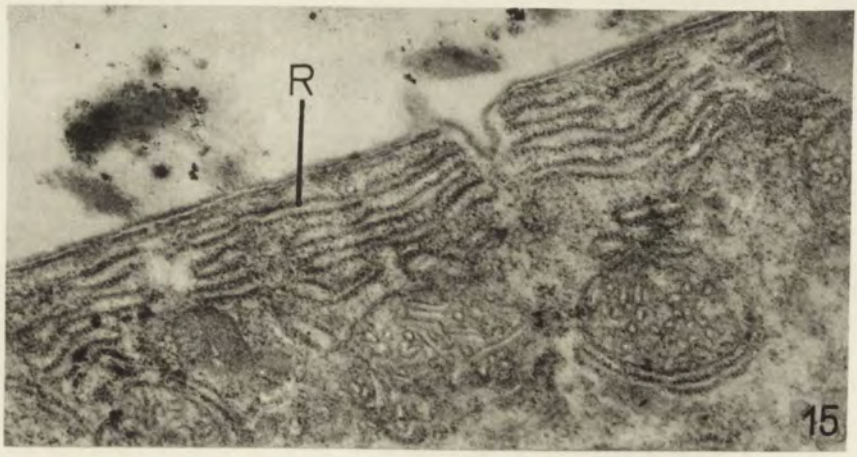

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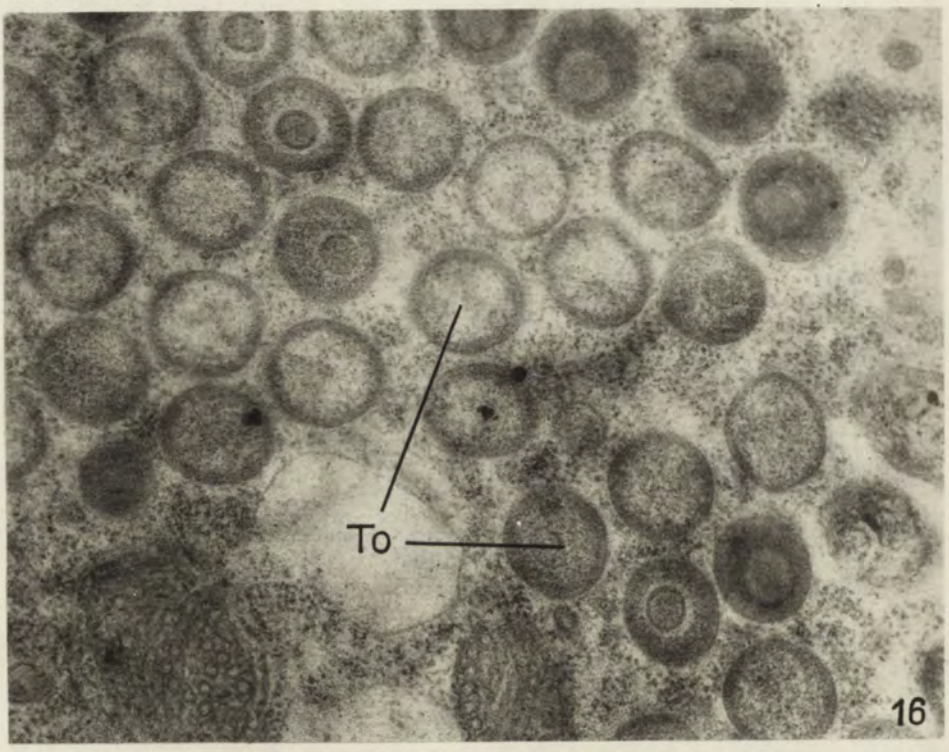

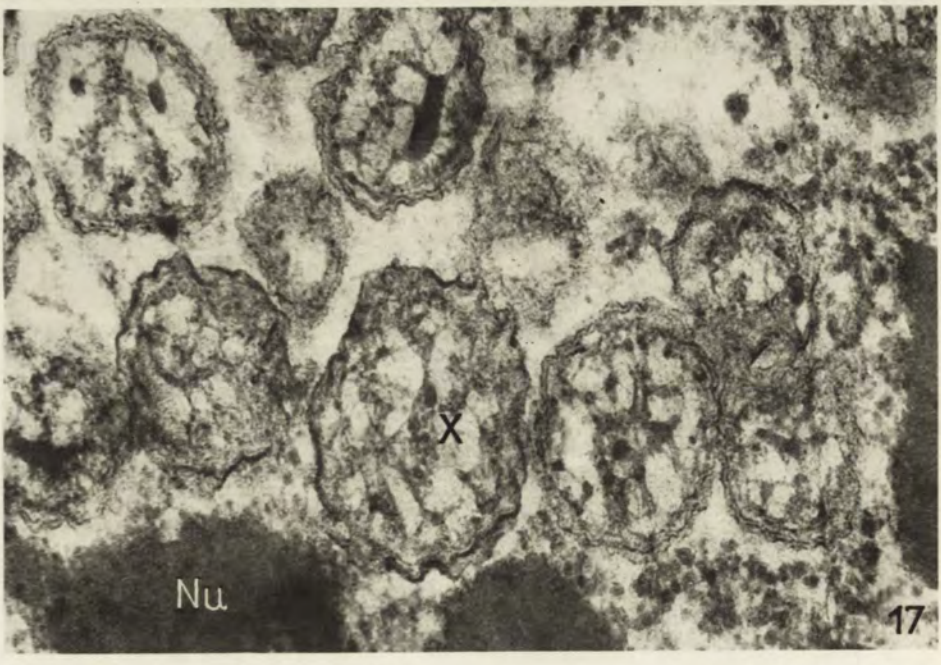

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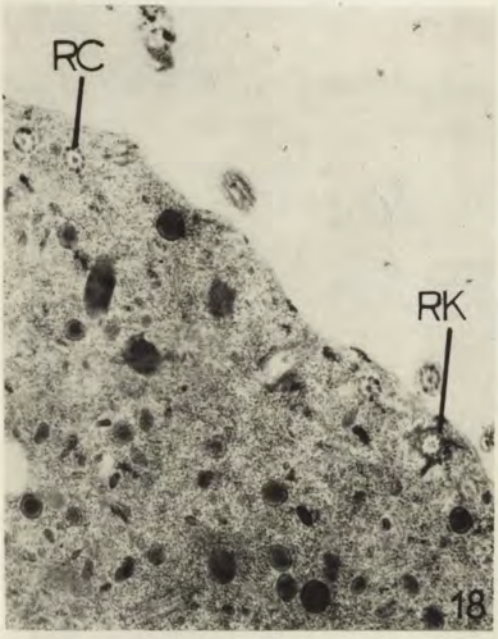

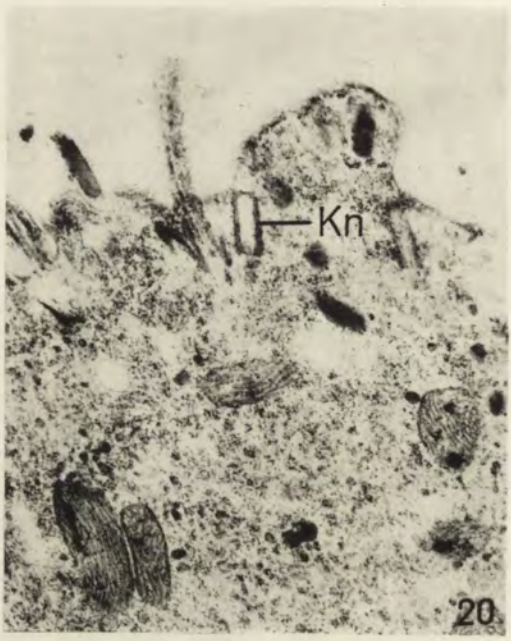

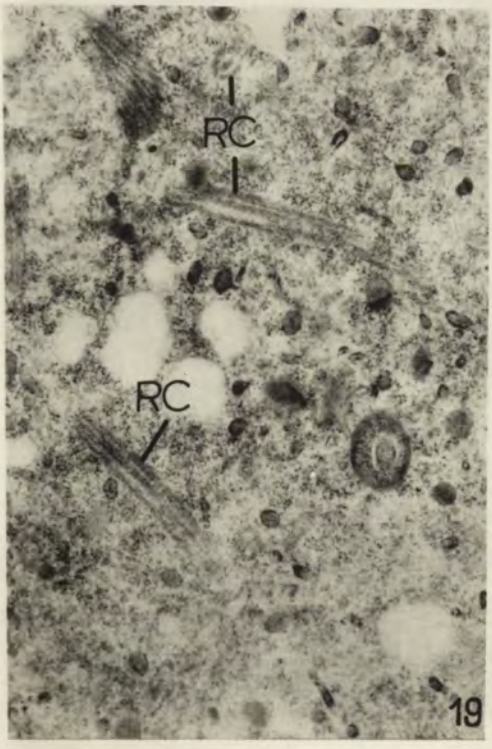

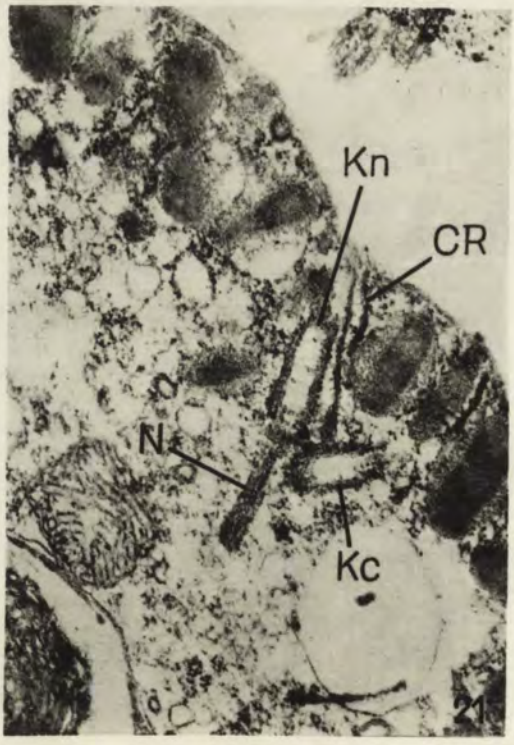

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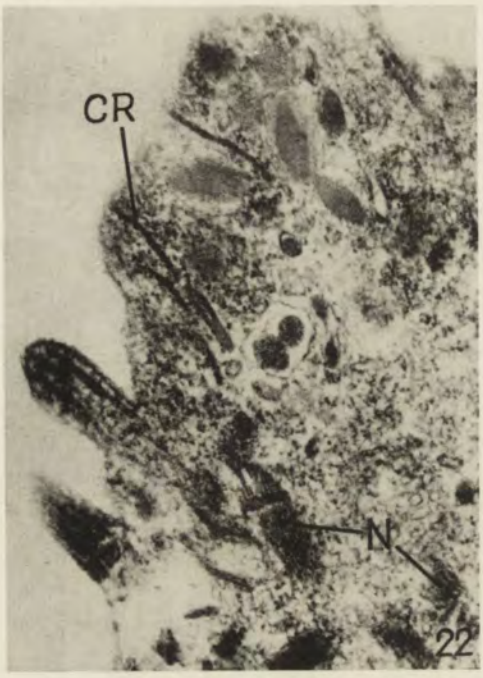

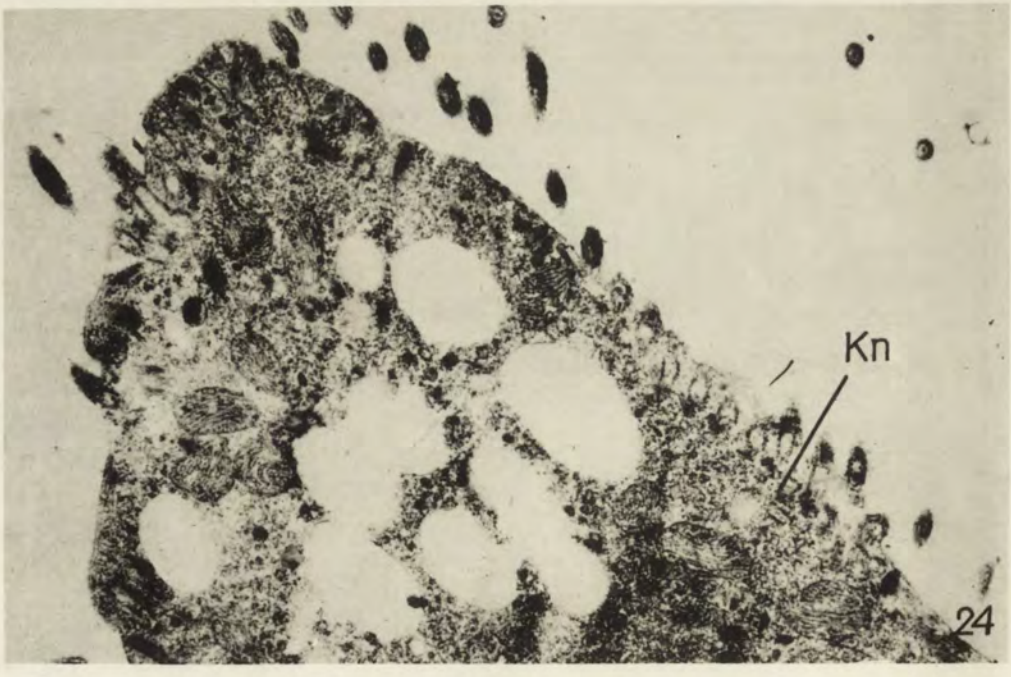

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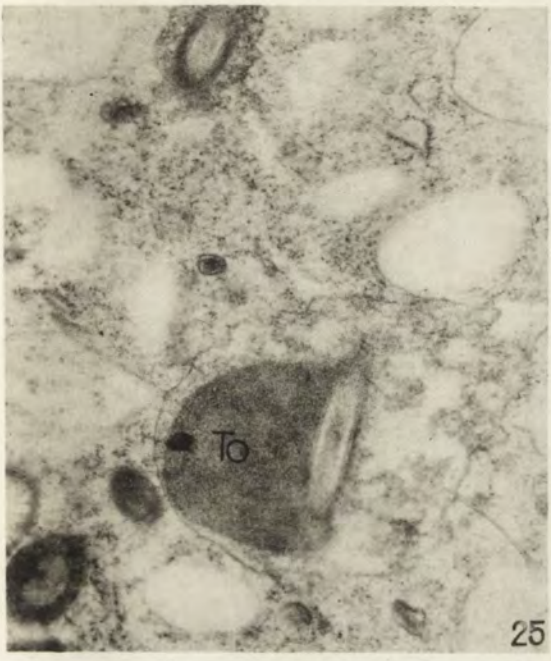

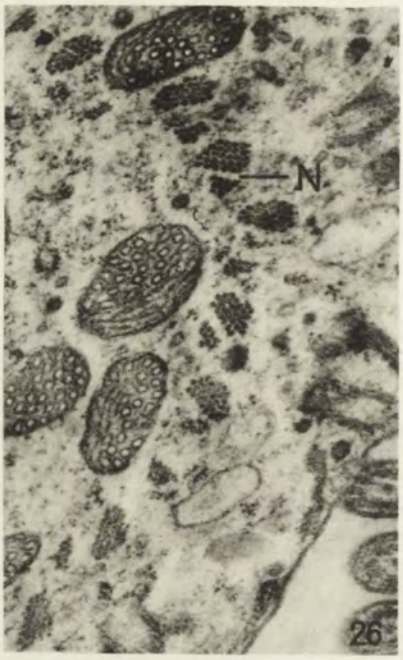

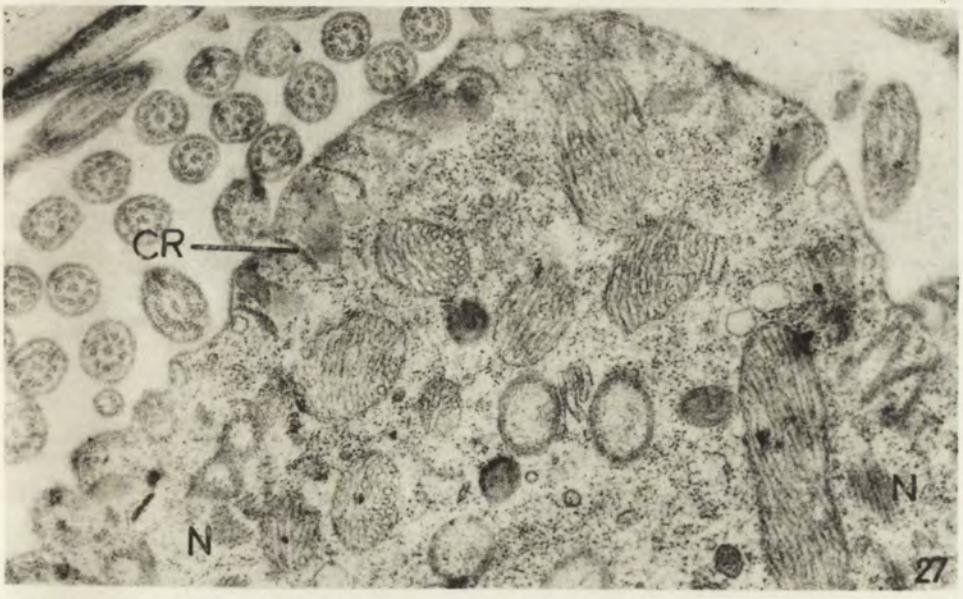

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Bogna SKOCZYLAS

Deoxyribonuclease in *Paramecium aurelia* syngen 4 strain 51

Dezoksyrybonukleaza *Paramecium aurelia* 4 szczep 51

The studies of DN-ase in protozoa are scarce. It has been investigated only in flagellates by Walther et al. 1970, by Carell et al. 1970 and in ciliates by Holm who gave a characteristic of DN-ase in *Tetrahymena pyriformis* suggesting the presence of two enzymes (1969 b, 1971). Holm studied the dependence of the enzyme activity on the course of DNA biosynthesis in the interdivision cycle of cultures synchronized by thermic shock (1966, 1969 a).

The aim of the present study has been to follow the properties of DN-ase in *Paramecium aurelia* syngen 4, strain 51. The similitude and differences of the enzyme studied in *Paramecium* and in *Tetrahymena* will be discussed.

Material and methods

Culture

Paramecium aurelia syngen 4, strain 51 was cultivated according to Sonneborn 1950 on the lettuce medium inoculated with *Enterobacter aerogenes* No. 408 from the Copenhagen collection. In every case the mass culture was initiated from autogamic microcultures by inoculating single cells (2–200) into 400 ml of medium placed in 1 l Roux bottle. The growing culture was kept in temp. 27°C.

Condensation of mass cultures and gaining the "crude extract" of DN-ase

The culture was condensated when the population density attained 1000–2000 cells/ml. About 30 l of culture was filtered through nylon sieves with 30–60 μ diameter meshes. The preliminary condensation was performed on α Laval milk centrifuge of one liter volume. The cell suspension was filtered through cotton wool (absorbent cotton wool B.P.C. qual. — BDH) and centrifuged in 100 ml conical test tubes for 5 min not exceeding 600×g. Cells were rinsed twice with sterile medium of pH 5.5 and diluted with 20–40 ml of H_2O. Samples were taken for count, and the remaining frozen in ethanol at temp. —20°C. Cells killed in this way were homogenized by three times repeated freezing and thawing. The homogenate was centrifuged in MSE centrifuge for 20 min at 3000×g and subsequently dialized against deionized water for 36 h. The "crude extract" gained after the above procedure was stored at the temp. —18°C.

Determination of DN-ase activity

The measure of the enzyme activity was the rise of absorption in 260 nm involved by the liberation of acid soluble compounds from DNA used as substrate. Composition of the incubation mixture was as follows:

0.5 ml DNA (1 mg/ml),
0.125 ml acetate buffer pH 5.3 — ionic strength 0.6 (acting ion strength — 0.025) or
1.0 ml acetate buffer — as above — (acting ionic strength 0.2),
0.1–0.2 ml enzyme solution to 3.0 ml H_2O.

The mixture was incubated for 120 min at 37°C being permanently shaken. Reaction was interrupted by addition of 3 ml of freezing 4% perchloric acid with 0.01 M uranyl acetate which caused a simultaneous precipitation of the non-hydrolized substrate. The samples were cooled for 15 min at —15°C, then centrifuged at 2000 g and the adsorption of supernatant was measured by spectrophotometer Unicam 500 λ = 260 nm. The control samples were incubated without substrate, and DNA was added after the interruption of reaction with perchloric acid. As substrate, the highly polymerized DNA was applied, produced from calf thymus by the Schwander–Singer method 1950.

The DNA-se activity was expressed by the quantity of reaction products released within a unit of time (Δ absorption at 260 nm) 120 min of incubation.

Control determination of DN-ase in bacteria

For excluding the interference of bacterial DN-ase, the activity of this enzyme was controlled in bacteria *Enterobacter aerogenes* cultivated from the culture fluid remaining after centrifugation of protozoa. Bacteria were disseminated on the 3% solid agar medium in Roux bottles. After 24 h they were rinsed out from the agar surface by means of sterile lettuce medium, centrifuged for 30 min at 3000 × g and the cellular mass gained was treated similarly as the cellular mass of paramecia. After many repeated experiments — in the conditions of analysis as described above — no nucleolytic activity has been ascertained in bacteria. Consequently it may be assumed that the entire enzymatic activity revealed in the *Paramecium* culture should be ascribed to protozoa.

Results and discussion

Results of the preliminary purification of DN-ase

Fractionation by ammonium sulphate of the aqueous extracts gained from the *Paramecium* homogenates leads to obtaining several fractions depending on the concentration of ammonium sulphate applied. Out of the four fractions obtained, the most active is this precipitated at 50 to 75% saturation of ammonium sulphate. A certain enzymatic activity appears also in the fractions precipitated between 25% and 50%, as well as 75 and 100% of saturation with ammonium sulphate. The distribution of enzymic activity is represented in Fig. 1. It may suggest the presence of more than one enzyme. Considerable loss of enzymatic activity in the course of preliminary purification, as well as low amount of the exit material (about 5–25 mg of protein was accessible every time for purification) make presently the further purification of the enzyme impossible and — consequently also the determination of its homo- or heterogenity — in this way. However, the subsequent studies of the

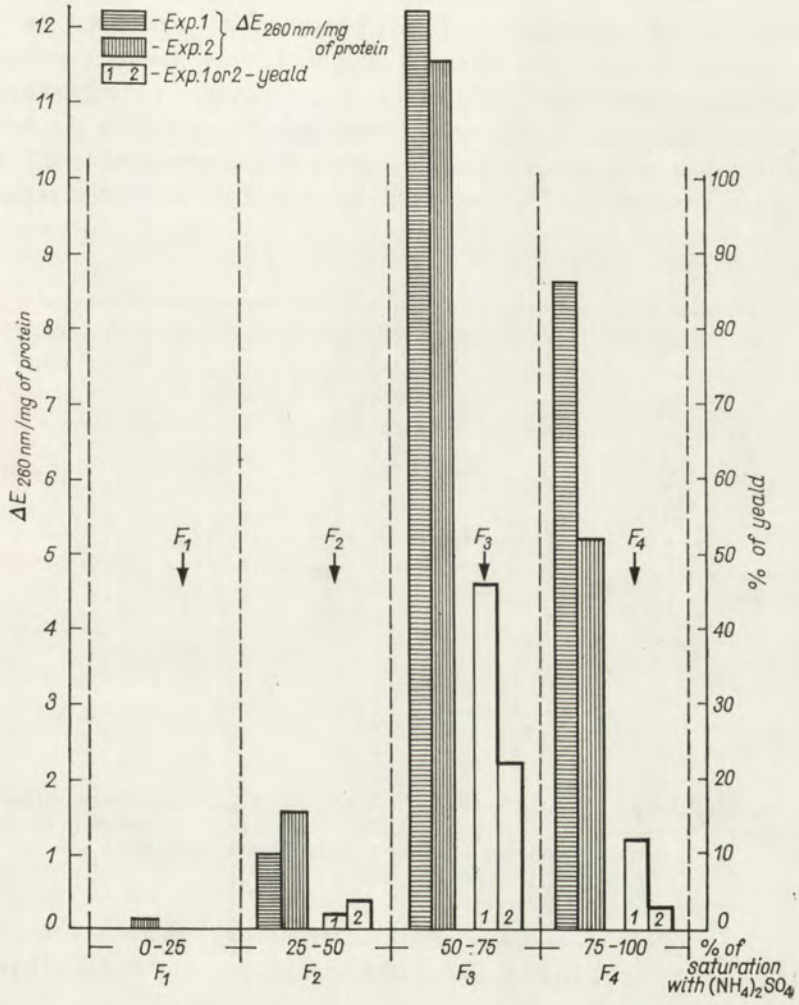

Fig. 1. Two typical experiments of fractionation with ammonium sulphate of crude extract of *Paramecium aurelia* DN-ase. Ammonium sulphate was added in amounts corresponding to saturation: I fraction — 25%, II fr. — 50%, III fr. — 75%, IV fr. — 100%. The consecutive precipitates were centrifuged and dissolved in H_2O dest. in about 1/5 of it's former volume. The DN-ase activity was determined in acetic buffer pH 5.3, I=0.2. The protein content was determined by the Lowry (1951) method. The yeald was related to the exit activity of preparation prior to fractionation

dependence of the enzyme activity on pH, and of the susceptibility of this activity to the action of magnesium ions — provide also evidence of existence of more than one enzyme responsible for DNA splitting in *Paramecium*.

Study of the enzyme activity in dependence on pH

In this series of experiments the following array of buffers was applied: acetic acid-sodium acetate according to Boyd 1956, at pH range 4.5 to 6.0, potassium

dihydrogen phosphate-disodium hydrogen phosphate pH 6.0 to 8.0 and glycine-sodium glycinate pH 6.0 to 9.0 (Long 1961). It has been ascertained that the acetate-veronal buffer recommended by Holm 1966 lowers twice the enzymatic activity as related to the acetate buffer and therefore was not applied in the subsequent experiments. The ionic strength of all the buffers applied amounted I=0.2. Figure 2 presents the dependence of DN-ase activity on pH in the above experimental

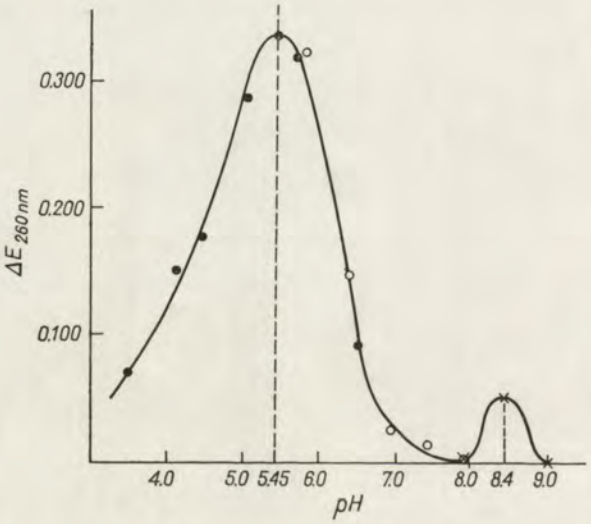

Fig. 2. The dependence of DN-ase activity on pH was determined in the array of three buffers: ● — acetate, ○ — phosphate, × — glycinate. The ionic strength of buffers in the incubation medium amounted 0.2. Magnesium ions were not added

conditions. In all the cases studied two recurrent activity maxima were obtained: the first — high at pH 5.1–5.4, the second one, much lower at the pH range 7.8 and 8.4.

It is characteristic, that the nucleolytic activity, determinable in the acid pH range persists for a number of months without considerable changes in the material preserved at —18°C. The nucleolytic activity determinable in the alkaline pH range may be studied only in fresh material preserved no longer than a few days.

It is commonly known that the addition of magnesium ions stimulates the activity of alkaline DN-ase. Similarly in our experiments, the activity of DN-ase determinable at pH 7.8–8.4 was stimulated more than twice by magnesium ions. The influence of bivalent ions upon the DN-ase activity in the acid pH range will be discussed separately.

In the subsequent experiments, the ionic strength of buffers was lowered down to the final value =0.025. Then, after addition of magnesium ions at concentration 10^{-2} M and 10^{-4} M, the maximal activity was observed at pH 6.6, whereas at

pH 8.0 the nucleolytic activity fell down to 0 (Fig. 3.). A similar shift of pH optimum accompanying the change of ionic strength has been observed by many authors (Kurnick and Sandeen 1959, Rosenbluth and Shan-Ching Sung 1969). The considerable difference in localization of activity peaks may also suggest the presence of more than one enzyme.

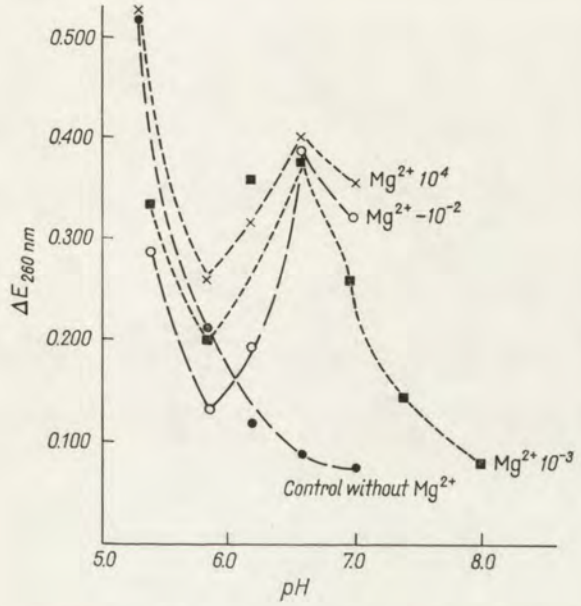

Fig. 3. Dependence of DN-ase activity on the concentration of magnesium ions within the pH range 5.4–8.0. The DN-ase activity was determined in the buffer array as in the experiment presented in Fig. 2. The ionic strength of buffers applied in the incubation mixture amounted 0.025

Summarizing the above results, it may be therefore postulated that in *Paramecium aurelia* more than one DN-ase is present. These enzymes differ in activity optimum in dependence on pH, in susceptibility to the action of magnesium ions and in stability. The enzyme (or enzymes) activated by the magnesium ions which is characterized by its activity peaks at pH 6.6 or 8.4, is very labile which involves serious difficulties in its exact analysis.

All the following studies presented in this article concern exclusively the enzyme active at pH 5.3, i.e., the so-called DN-ase II.

The influence of ionic strength on DN-ase II activity

The rather insignificant changes of the ionic strength in the course of incubation exert considerable influence on the enzyme activity which is presented in Fig. 4. The cases of dependence ascertained here are in conformity with the findings of the other authors (Kurnick and Sandeen 1959, Schack 1959, Holm 1966).

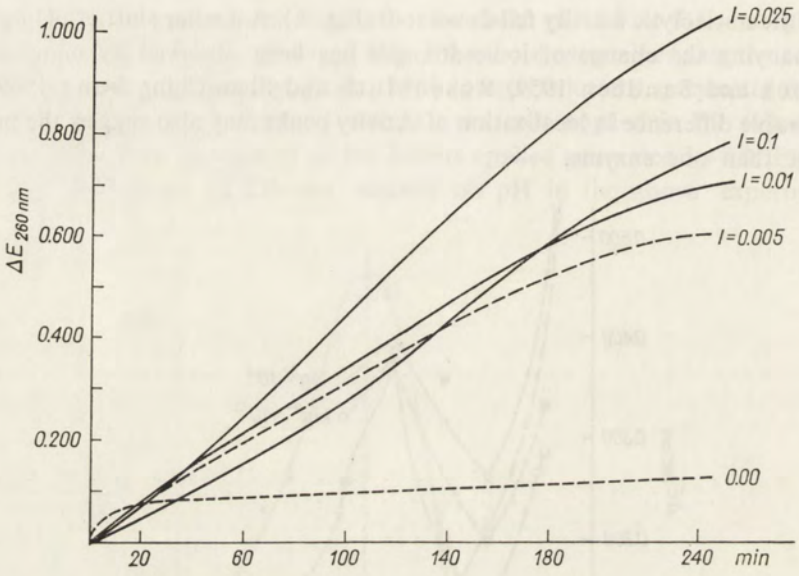

Fig. 4. Dependence of DN-ase II activity on the ionic strength. Activity of DN-ase was determined at pH 5.3 in acetate buffer

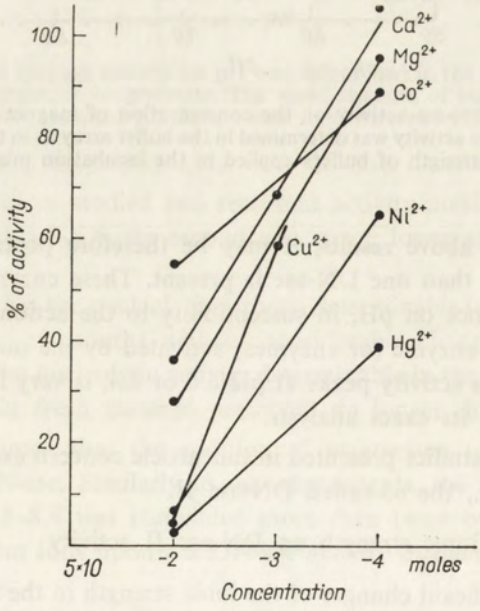

Fig. 5. The influence of bivalent ions on DN-ase II activity. In this experiment the acetate buffer of pH 5.3 was applied, of the ionic strength 0.2. As 100%, the DN-ase II activity without addition of bivalent ions was assumed

They support the importance of a precise determination of ionic strength in the study of enzymatic activity.

The influence of bivalent ions on DN-ase II activity

The bivalent ions as: Mg^{2+}, Ca^{2+}, Co^{2+} at concentration 5×10^{-2} M, at pH 5.3 decrease the enzyme activity to 50%. In the same conditions the Ni^{2+}, Cu^{2+} and Hg^{2+} ions decrease the enzyme activity more than 90% (Fig. 5).

The action of bivalent ions lowering the DN-ase II activity in *Tetrahymena* has been extensively discussed by Holm (1966). Basing on his studies the author suggests that the inhibiting action of bivalent ions is non-specific, being evoked only by the changes of ionic strength. The last statement — as already stressed above — is very essential and cannot be omitted. An attempt was made to determine whether the action of bivalent ions on DN-ase in *Paramecium* is specific or not. We selected the further conditions of experiments in such a way that the changes of ionic strength involved by addition of magnesium ions, was compensated by the simultaneous change of the ionic strength of buffer. So in the six consecutive experiments, three different concentrations of magnesium ions were used: 5×10^{-2} M, 5×10^{-3} M, 5×10^{-4} M, in two concentrations of acetate buffer in which the ionic strength amounted 0.2 and 0.025. On the Table 1, the values of ionic strength are presented which act in the incubation mixture of successive experiments. The changes of activity obtained in this experiment were insignificant when the ionic strength of the buffer had been changed, being very distinct after the change of magnesium ions concentration. This is presented in Fig. 6. It should be stressed that the differences of enzymatic activity which is involved by the action of magnesium ions at conc. 10^{-4} M and 10^{-3} M (compare the column 1 and 2, 4 and in Fig. 6) cannot be accounted for by the action of the ionic strength only, since the differences in the latter are rather insignificant (comp. the Table).

Influence of natural polyanion — heparine upon activity of DN-ase II

Tunis and Regelson (1963) ascertained that the high-molecular acid polyelectrolytes such as heparine, inhibit the action of spleen DN-ase II. Holm (1966) studying the action of heparine on the activity of DN-ase II in *Tetrahymena* during the interdivision cycle and has discussed the hypothetic possibility of occurrence of acid polymers as natural inhibitor of DN-ase in the cell. In our experiments, like in those of Holm, heparine in concentration 0.1 mg/ml reduced the activity of DN-ase II in *Paramecium* for about 20%, and at concentration 1 mg/ml even for 90%.

Influence of temperature on the activity of DN-ase II

DN-ase II of *Paramecium aurelia* is a thermolabile enzyme. Its activity falls down to 0 after boiling for a few minutes.

Table 1

The scheme of ionic strength of compounds used to incubation medium in experiments presented on Fig. 6

No. of exp.	I of $MgCl_2$ + I of buffer = I of $MgCl_2$+buffer
1	0.0015 + 0.2 = 0.2015
2	0.015 + 0.2 = 0.215
3	0.15 + 0.2 = 0.35
4	0.0015 + 0.025 = 0.0265
5	0.015 + 0.025 = 0.04
6	0.15 + 0.025 = 0.175

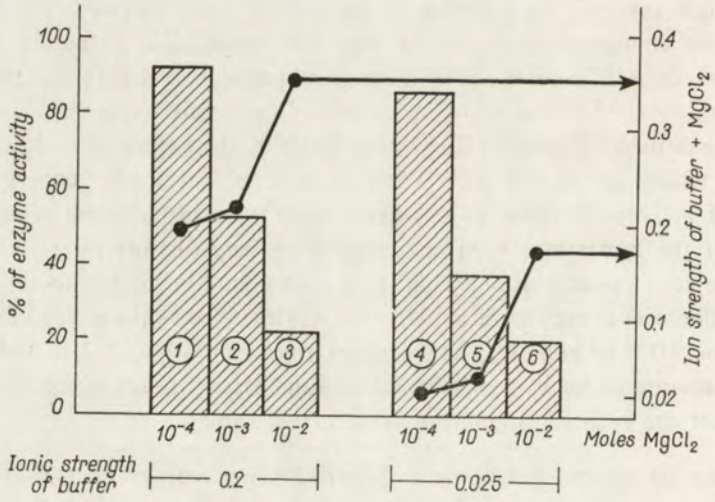

Fig. 6. The influence of different magnesium ion concentrations upon the activity of DN-ase II incubated in the media of different ionic strength. In the experiments 1, 2, 3, the acetate buffer of pH 5.3 and of ionic strength 0.2, was applied, and in the experiments 4, 5, 6 — of the ionic strength — 0.025. As 100% of enzyme activity, its value measured without the addition of magnesium ions was assumed

Summarizing the achieved results it may be stated that in *Paramecium aurelia* acid DN-ase occurs before all. The optimum of its activity is at the range of pH between 5.1–5.4 at the ionic strength = 0.2. This enzyme has similar properties as DN-ase II described by Holm (1966) in *Tetrahymena pyriformis*. Both enzymes are soluble in water, may be dialysed and precipitated by ammonium sulphate at 50–75% of saturation. The activity of those enzymes is susceptible to the changes of ionic strength of medium and to the action of bivalent ions. The rise of their

concentration lowers the activity of the enzyme. In *Paramecium*, this effect seems to be associated not only with the rise of ionic strength because in two buffers differing considerably by the ionic strength the same concentrations of magnesium ions evoke a similar percent of fall of activity which rises in proportion as the concentration of magnesium increases. The DN-ase II activity in *Paramecium* — as well as in *Tetrahymena* — is inhibited by heparine which may be a natural inhibitor of this enzyme, (Holm 1966, 1960 a), similarly as the other polyanions occurring in the cell. Both DN-ase described differ slightly in pH optimum which in *Tetrahymena* is nearer the neutral medium range of pH. This difference may be a result of slightly different conditions of incubation (ionic strength, kind of buffer), as well as by the specific differences of the two of ciliates species.

As stated above, in *Paramecium* besides the acid DN-ase, nucleolytic activity is observed which is stimulated by addition of magnesium ions. Its optimum occurs at the range of pH 6.6 and 8.4 depending on the ionic strength applied. It is difficult to decide presently whether we have to do with one or with two different enzymes because the enzymatic activity observed in the alkaline range is very susceptible and cannot be preserved, which makes difficult the further study on such a small amount of accessible material. Holm (1969 b, 1971) ascertained in his studies the existence of more than one enzyme in *Tetrahymena*, however both enzymes described by him have a character of acid DN-ases. It seems possible that DN-ase which is active in the alkaline pH range in *Paramecium* is an enzyme appearing only periodically, in association with the stage of cellular or life cycle of the cell.

Summary

The properties of DN-ase were investigated in *Paramecium aurelia* strain 51, syng. 4. Nucleolytic activity was ascertained at the pH range 5.1–5.4 and in the pH range 7.8–8.3. The enzymatic activity, the optimum of which is in the alkaline pH range, is stimulated by magnesium ions. In contrast to this, the stimulating action of magnesium upon the nucleolytic activity at pH 5.1–5.3 has not been stated. At this pH range, the bivalent ions at concentrations 5×10^{-2} M and 5×10^{-3} M distinctly lower the enzyme activity. The changes of the ionic strength of incubation medium exert influence upon the DN-ase activity and cause presumably a shift of the optium activity peak. The obtained results suggest the existence of more than one DN-ase in *Paramecium*.

STRESZCZENIE

Zbadano właściwości DN-azy u *Paramecium aurelia* syngn. 4, szczep 51. Stwierdzono aktywność nukleolityczną w zakresie pH 5,1–5,4 i w zakresie pH 7,8–8,3. Aktywność enzymatyczna, której optimum znajduje się w alkalicznym zakresie pH stymulowana jest jonami magnezu. Przeciwnie, nie stwierdzono stymulującego działania magnezu na aktywność nukleolityczną w pH 5,1–5,3.

W tym zakresie pH jony dwuwartościowe w stężeniach 5×10^{-2} M i 5×10^{-3} M wyraźnie obniżają aktywność enzymu. Zmiany siły jonowej środowiska inkubacyjnego nie pozwalają bez wpływu na aktywność DN-azy, a w pH alkalicznym powodują prawdopodobnie przesunięcie szczytu optimum aktywności. Uzyskane wyniki sugerują istnienie u *Paramecium* więcej niż jednej DN-azy.

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