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Department of Parasitology. Polish Academy of Sciences Warszawa 22, Pasteura 3, Poland

Włodzimierz MICHAJŁOW

Euglenoidina (*Flagellata*)—parasites of *Cyclopidae* (*Copepoda*)

Euglenoidina (*Flagellata*) — pasożyty *Cyclopidae* (*Copepoda*)

Parasitism is a phenomenon often encountered among *Protozoa*. According to Dogiel et al. 1962, out of nearly 19 000—20 000 species of protozoans known, 3 500—4 000 (i.e. about 16 per cent) are parasites. *Sporozoa* and *Endosporidia* consist exclusively of parasites. Among *Mastigophora* (the number of parasites in this group reaches 25.7 per cent) several big groups are known consisting exclusively of parasites (*Polymastigina, Hypermastigina* and *Opalinata*). *Entodiniomorpha* form such a group among *Ciliata*. The existence of these compact and large systematic groups that can be easily differentiated and consisting exclusively of parasites can be an example of the influence of parasitism on megaevolutionary processes leading to the development of taxons of a higher order. Other groups can be found among *Mastigophora* (*Dinoflagellata, Euglenoidina, Protomonadina*) in which, however, parasites occur together with free living species.

The existence of individual species (or genus) among protozoans adapted to parasitism and probably existing thanks to it, is interresting as the result of macroevolutionary processes which in turn are the result of speciation. According to Dogiel et al. 1962 about 10 parasitic species are known among *Euglenoidina*, one of them parasitizes in *Copepoda*. These data are out of date at present, as numerous (over 50) species of *Euglenoidina* are known to be parasites exclusively in *Copepoda*. They are distinguished by various and interesting adaptations to parasitism.

That is why it seems useful to review the species of *Euglenoidina* known at present as parasites of *Copepoda* and to analyse the adaptations of their representatives to the sort of life they live. Such a review and analysis concerning parasitic *Ciliata* has been made by Raabe 1947. This approach should be, however, preceeded by several introductory remarks.

All the material elaborated so far is restricted in character and this results from the following reasons. Samples of the plankton were taken from a fairly great number of geographical regions (Poland, Germany, France, England, Norway, Finland, Hungary, Yougoslavia, Bulgaria, Rumania, Italy, Switzerland, Egipt, Ghana and Viet-Nam), but only in some places and from a small number of chosen reservoirs (2-3), mainly eutrophic. Moreover, the samples were taken mainly at one season of the year in each place. The species described so far differ between themselves not only in morphological features, but also — and here the differences are especially striking — in biological

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characteristics and mainly in the way of reproduction and the course of the life cycle. Together with the species described similar forms were often observed. The differentiation of these forms as separate species would, however, require the elaboration of a larger material, and, where morphological features are concerned — the use of more detailed methods of analysis, karyological methods among others. Lack of data concerning the range of occurence of some of the described species and especially the relations between the host and the parasite should be reported. As an example only part of the data characterising the extensiveness and intensity of infection can be applied to natural conditions. Studies on the relations between the host and parasite were not always conducted at the same place, immediately after the collection of plankton had been made, but were often carried out in Warsaw, basing on individual cultures of host derived from cultures maintained in the laboratory for a longer period of time, so that conditions became experimental in some way.

In spite of that it seems that the material is representative enough for certain conclusions concerning parasitology to be drawn and for future studies to be indicated.

The restriction to only one group hosts, namely to *Copepoda,* is the result of the following methodical assumption: to reduce as much as possible the diversity and fluctuations of the conditions of the first range environment: the species of *Cyclopoida.*

Systematical review

This systematical review will include the genus to which belong the parasitic species, the belonging to taxons of a higher order will be discussed and in some case the peculiarities of genus will be characterized. Also the differences within the genus and the features which should be taken into consideration for description of species — will be discussed.

Astasia Dujardin

The representatives of the genus *Astasia* Dujardin form a fairly compact group of parasites of the intestine of different species of *Copepoda.* The borders of this genus are not as yet established. Its existence is questioned by Hollande 1952 because the change from green species from the genus *Euglena* Ehrenberg into colourless forms described as *Astasia* (e.g. *Euglena gracilis* var. *longa* into *Astasia longa*) has been proved to occur. The change of coloured forms into colourless, its causes and symptoms were studied in detail by Pringsheim 1936, the relations between them — by Pringsheim et Hovasse 1936, the biochemical changes accompanying them — by Neff 1960. The students of zooplankton, however, and especially phytoplankton, use the generic name *Astasia* for about fifty described species of free-living and colourless *Euglenoidina.* Skuja 1948 basing exclusively on morphological features together with other authors e.g. Skvortzow 1958 classifies them as *Euglenaceae.* Christen 1958, 1962 who has made especially detailed studies on *Flagellata,* particularly from the region of Winterthur in Switzerland, in his later papers classes the previously described free-living species of the genus *Astasia* (together with several other genera) to

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the family *Astasiaceae*. Christen divided the free-living species of *Astasia* into 5 groups basing mainly on their morphological features (Christen 1963). Also Kudo 1954 differentiates the genus *Astasia* Dujardin and classes within the family *Astasiidae* Butschli.

Several so far described parasitic species from *Copepoda* were temporarily classed in the genus *Astasia* as their features correspond to the very general diagnosis of this genus. The complete distinctness of their way of living and development cycle shall probably require in the future the differentiation of these species in a separate genus. The creation and detailed diagnosis of this genus should be however postponed till the further members of this group are known. This diagnosis will certainly have to include the existence of two forms of protozoans: a large one, strongly metabolic and parasitic trophic form filled with grains of paramylum and free-living, small form provided with a flagellum, devoid of means of feeding and taking energy from the paramylum resources that had been accumulated at the previous stage of development.

The species of *Astasia parasitica* differ among themselves by the dimensions of both forms — the trophic one living in the intestine of the host, and the flagellate free-living form. Moreover, where the parasitic form is concerned — the specific differences are related to the number and shape of the paramylum grains, the presence or absence of a vestigial flagellum in the reservoir and the stigma, the patern of metabolic movements (in one, two or three "phases"). When the flagellate form is considered the following features are taken into account: its shape, the length of the flagellum, the number, shape and size of the paramylum grains, the presence or lack of the "stalk" and "foot", the pattern of movements, the length of the life period. If it is assumed that the flagellate forms of *Astasia parasitica* are comparable to the free living species of the genus *Astasia* the same differences in their morphological features could be the basis of differentiating these species and probably of a new genus as well.

Important specific differences within *Astasia parasitica* can be noticed in the course of the life cycle, especially in the way of reproduction. From this point of view the known species can be divided into 3 groups (subgenera): 1. reproducing by means of palintomy, 2. reproducing by means of syntomy and then by means of palintomy, 3. reproducing exclusively by syntomy. Probably *Astasia mobilis* (Rehberg) Alexeieff 1912 can be included in the first group. Its morphology and life cycle are not known in detail, but Penard (fide Grasse 1952) reported having seen 8 divisions during 48 hrs which give 256 daughter specimens. Also species with the well known life cycles can be included in this group, namely *Astasia parvicula* Mich. (Michajłow 1964 b, 1965 m)[1], *A. norvegica* Mich. (1964 d), *A. fennica* Mich. (1961 l) *A. hanoiensis* Mich. (1964 a) and *A. coelomae* Mich. (1967 e).

The following species can be included in the second group: *Astasia cyclopis* Mich. (1956), *A. sophiensis* Mich. and *A. bulgarica* Mich. (1965 d) where the forms resulting from the syntomic division divide once more by palintomy. *A. ovorum* Mich. (1965 a) reproduces exclusively by syntomy. The morphological features of the flagellate form of the latter species — the only parasite of the

[1] When this author is cited further on, only the dates of the publications will be mentioned.

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eggs of *Copepoda* of this sort generally fall within the limits of the wide diagnosis of the genus *Astasia*.

If the description of further species of *Astasia parasitica* leads to the differentiation of a separate genus the problem of the belonging of this group to the family *Astasiidae* will still remain. The available data (e.g. the presence of the stigma in *A. hanoiensis*, the secondary euglenoid-shaped forms that can be observed in the atypical course of the life cycle in this species and in *A. sophiensis)* suggest the direct descendance of *Astasia parasitica* from *Euglenidae* or from ancestors common to them both and their formation parallel to the free living *Astasiidae* and not through this intermediate stage. If this hypothesis is justified, its logical consequence would be the formation of a separate family for *Astasia parasitica*.

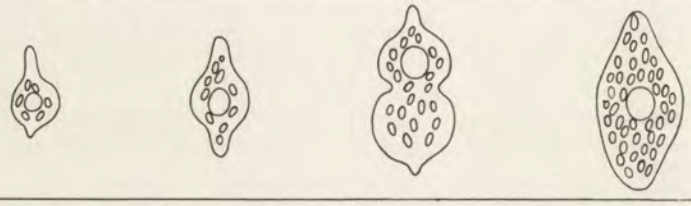
Parastasiella Michajłow

The diagnosis of the genus *Parastasiella* Mich. (1964 c, 1965 m) after appropriate corrections (1966 k) is the following. This genus includes parasites of eggs of *Copepoda* which reproduce by syntomy or palintomy. Small flagellate forms usually highly motile emerge into the water. These organisms are capable in certain circumstances (e.g. during penetration to the eggs of the host) of weak metabolic movements. They do not feed in the water and when they do not invade a host, they lose the flagellum, change into motionless forms and perish.

T a

The life cycles of *Euglenidina*

a. parasites of intestine

	parasitic stages
Astasia parvicula	
Astasia norvegica	
Astasia hanoiensis	

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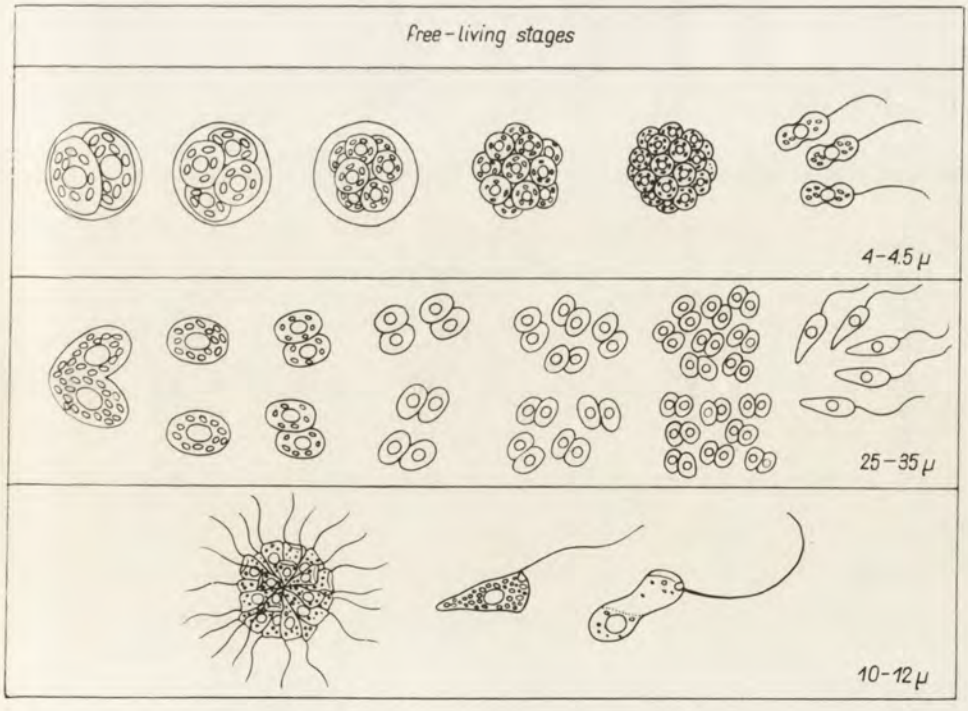
The individual species of this genus differ between themselves by such morphological features as: the shape and size of the flagellate form, the number, size and shape of the paramylum grains, the size of the cellular nucleus, the pattern of movements and the course of the life cycle. *Parastasiella vastans* Mich. (1966 k) and *P. helvetica* Mich. (1967 f) are the species reproducing by palintomy. The following species reproduce by syntomy in the eggs of *Copepoda*: *Parastasiella parva* (Mich.) (1965 g), *P. ovorum* (Mich.) and *P. velox* (Mich.) (1964 c). The morphological features of the genus *Parastasiella* generally fall within the general characteristic of the family *Astasiidae*. Taking into account the lack of ancestral features suggesting descendance from *Euglenidae* so far, this genus should be left within this family. In the future perhaps a separate family could be formed in view of the considerable difference of the biological characteristics.

Anisonema Dujardin

The only parasitic representative of the genus *Anisonema* Dujardin known at present — *A. parasiticum* Mich. (1965 b) has morphological features that agree with the characteristic of the genus (discussed and compared to *Dinema* among others by Christen 1963, who differentiates the family *Anisonemidae*) and can be included in the family *Anisonemidae* Schewiakoff.

ble 1
parasitizing in *Copepoda*



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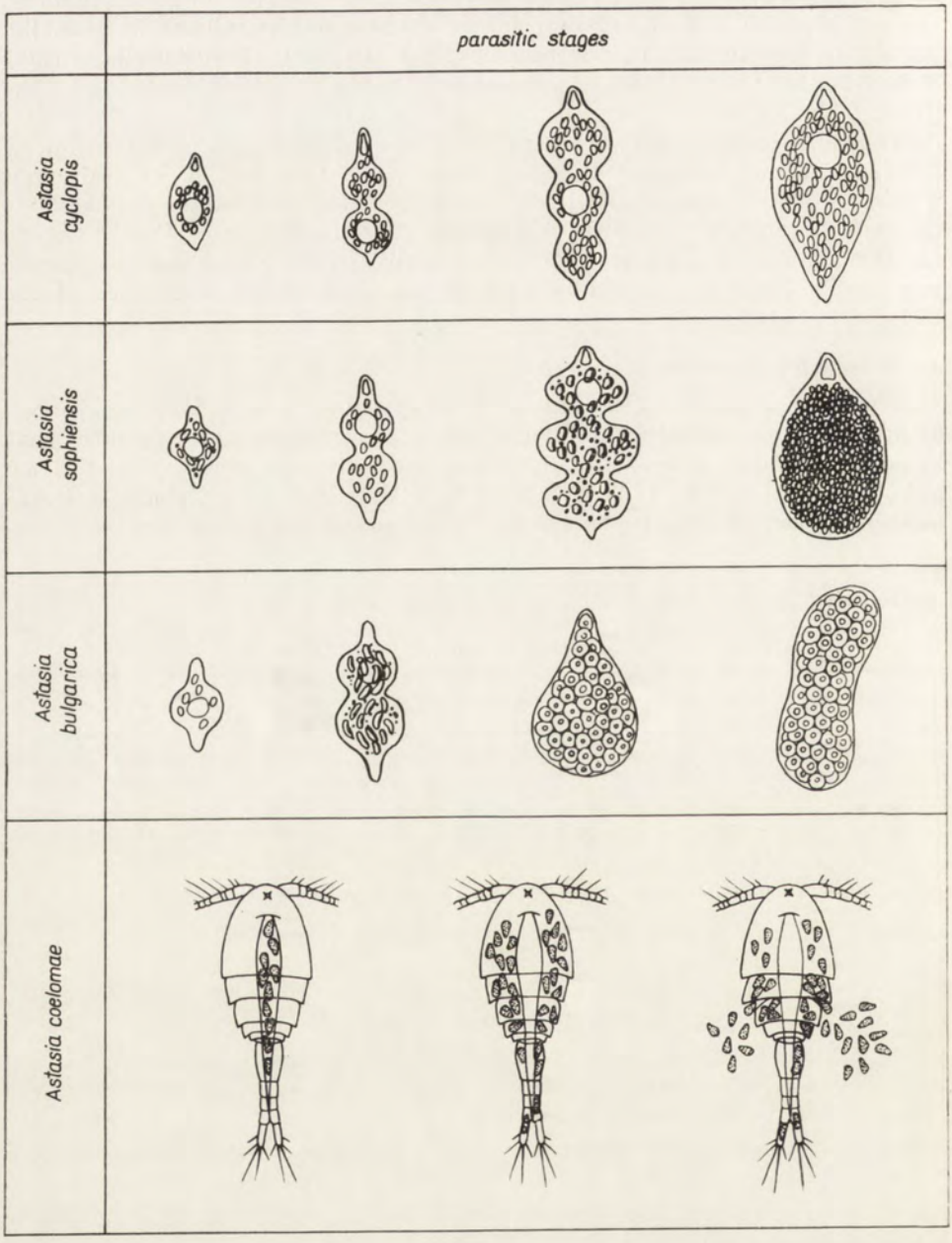
parasites of intestine (continued)



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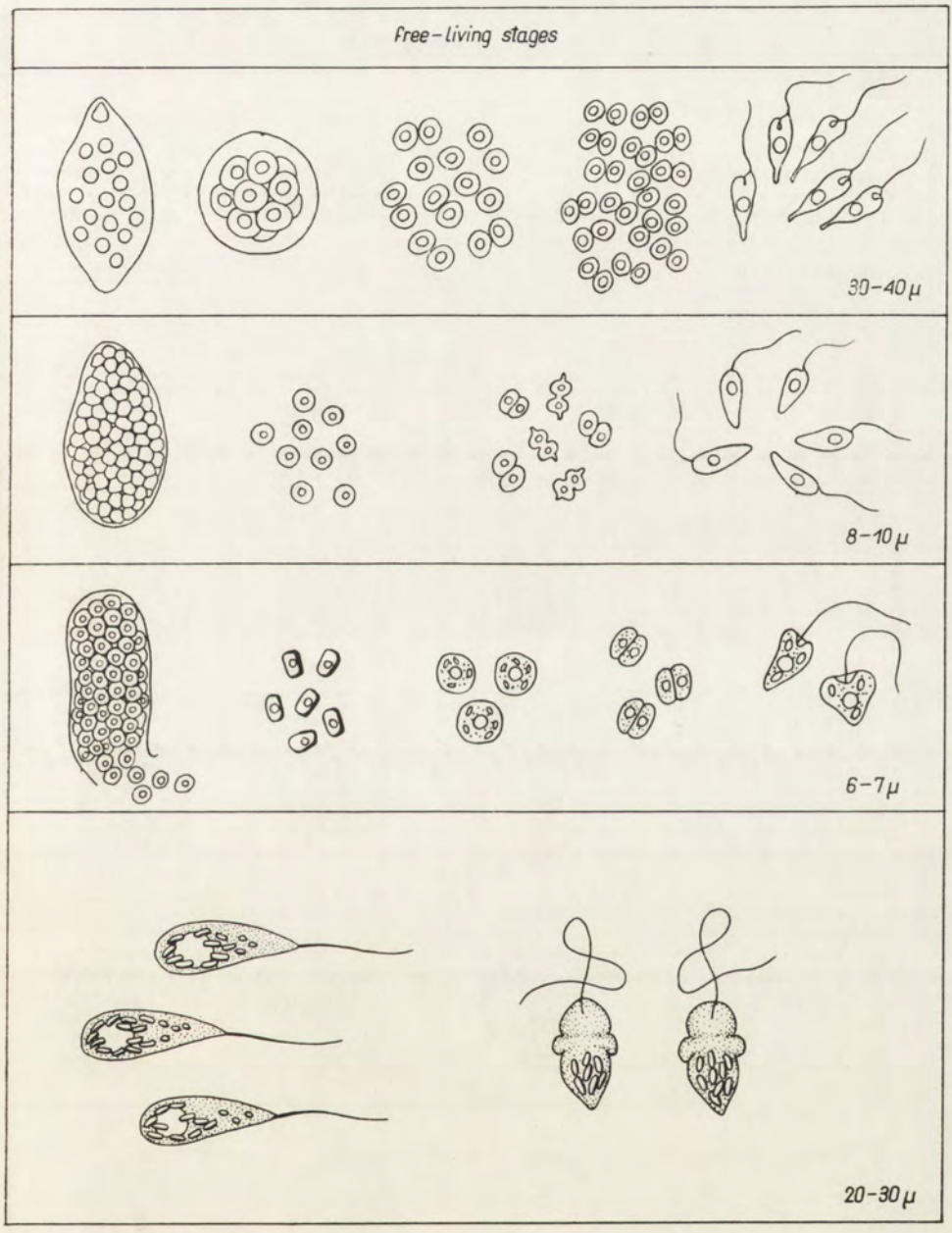

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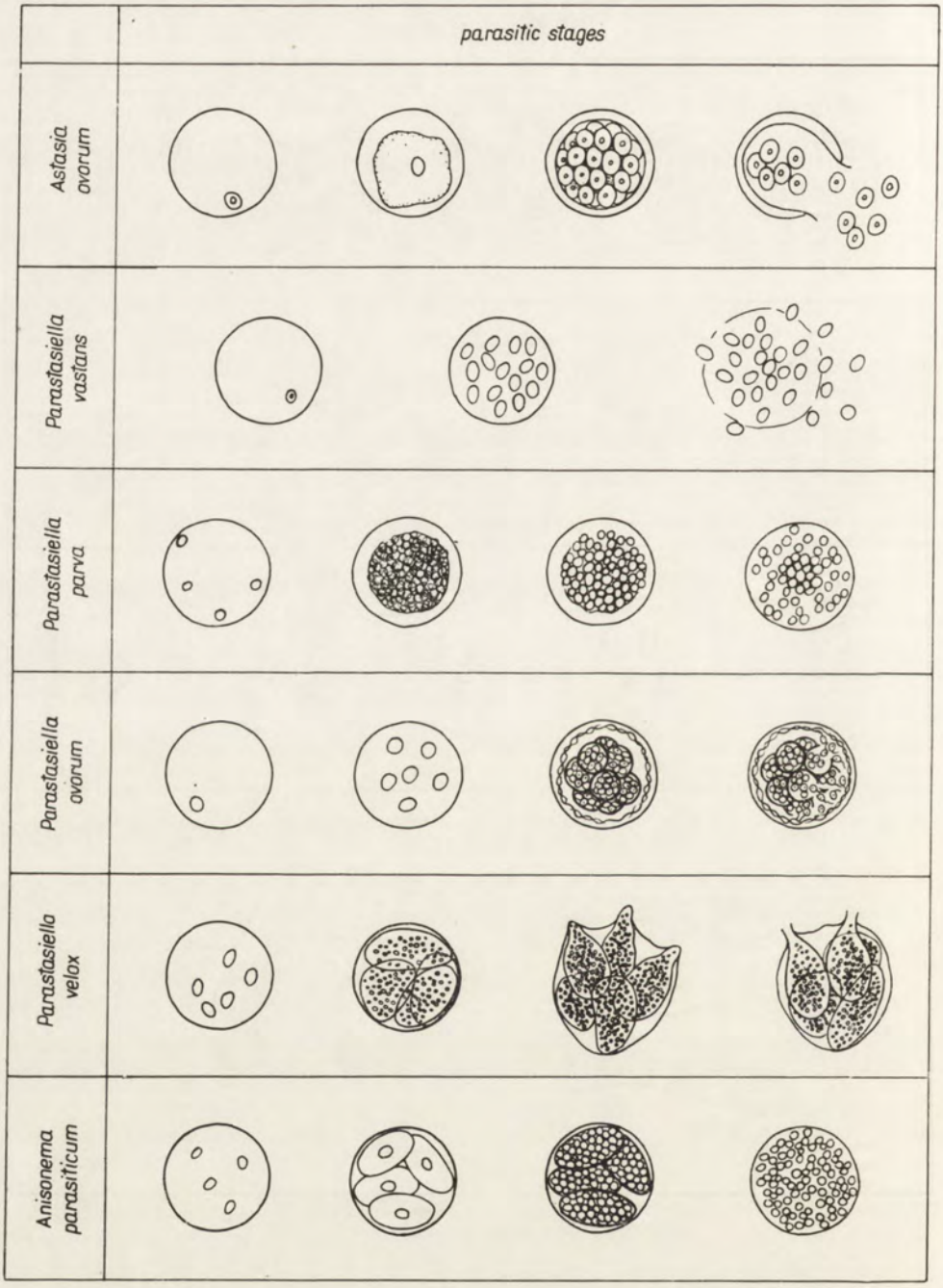
b. parasites of eggs



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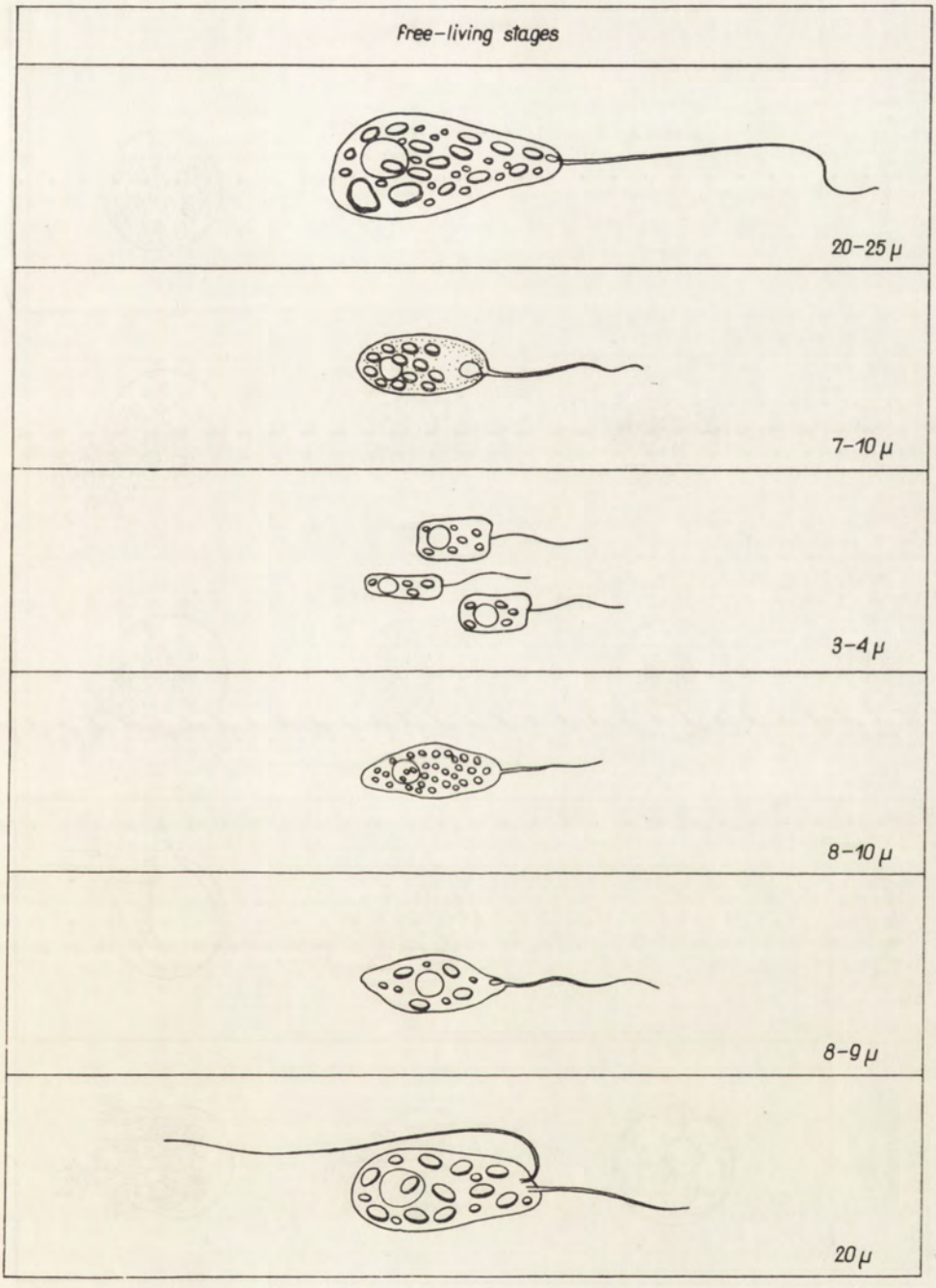

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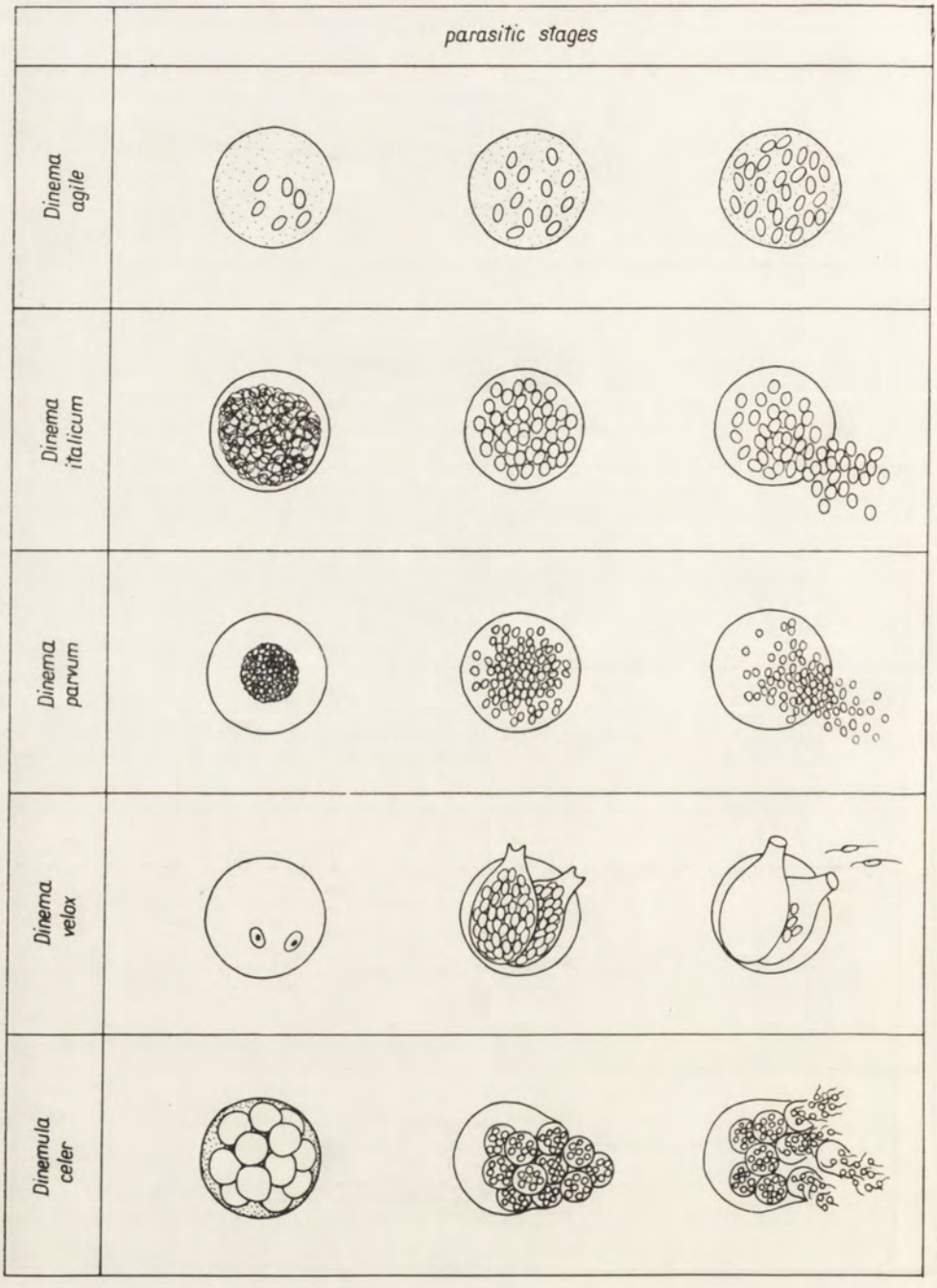
parasites of eggs (continued)



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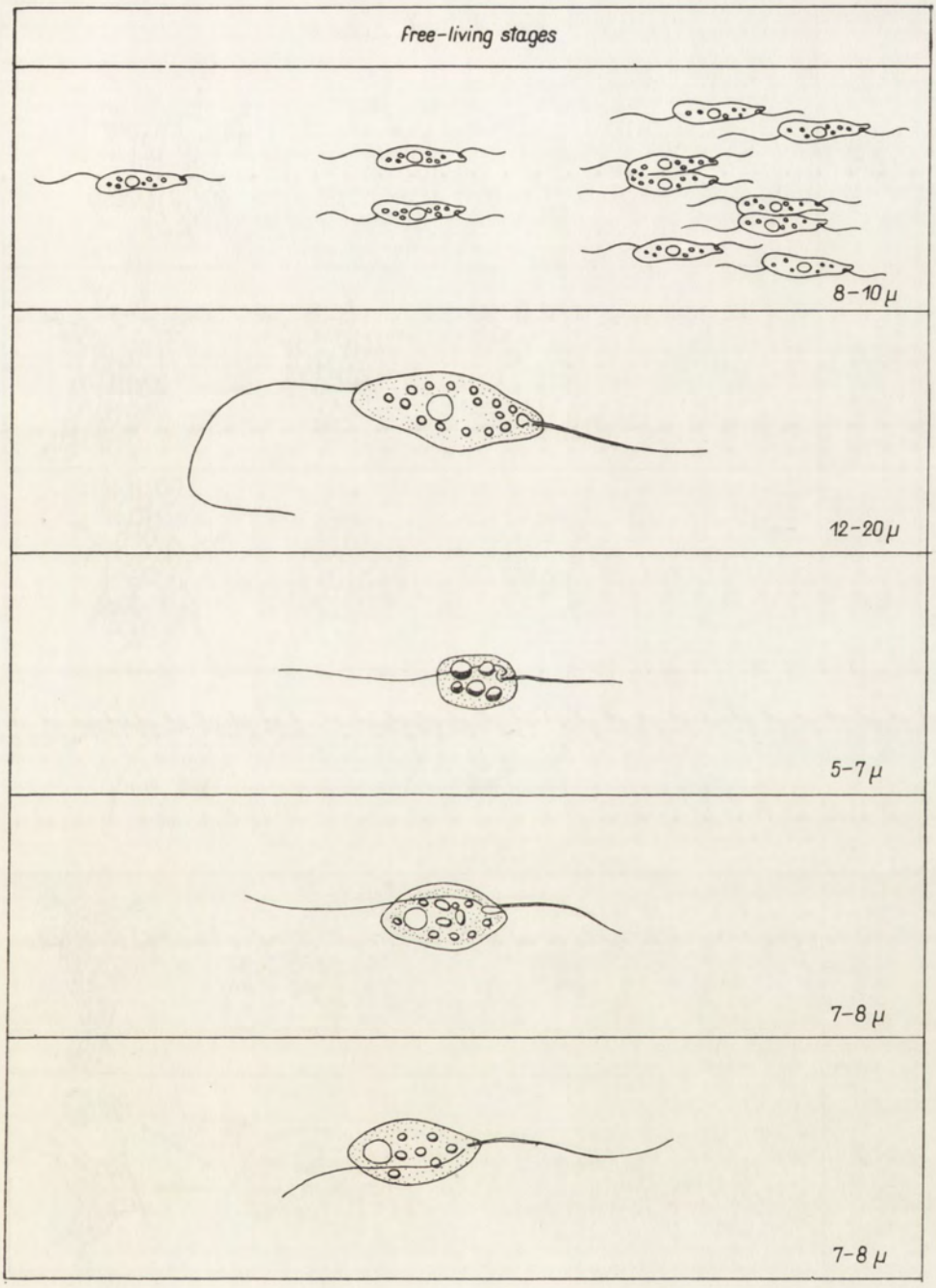

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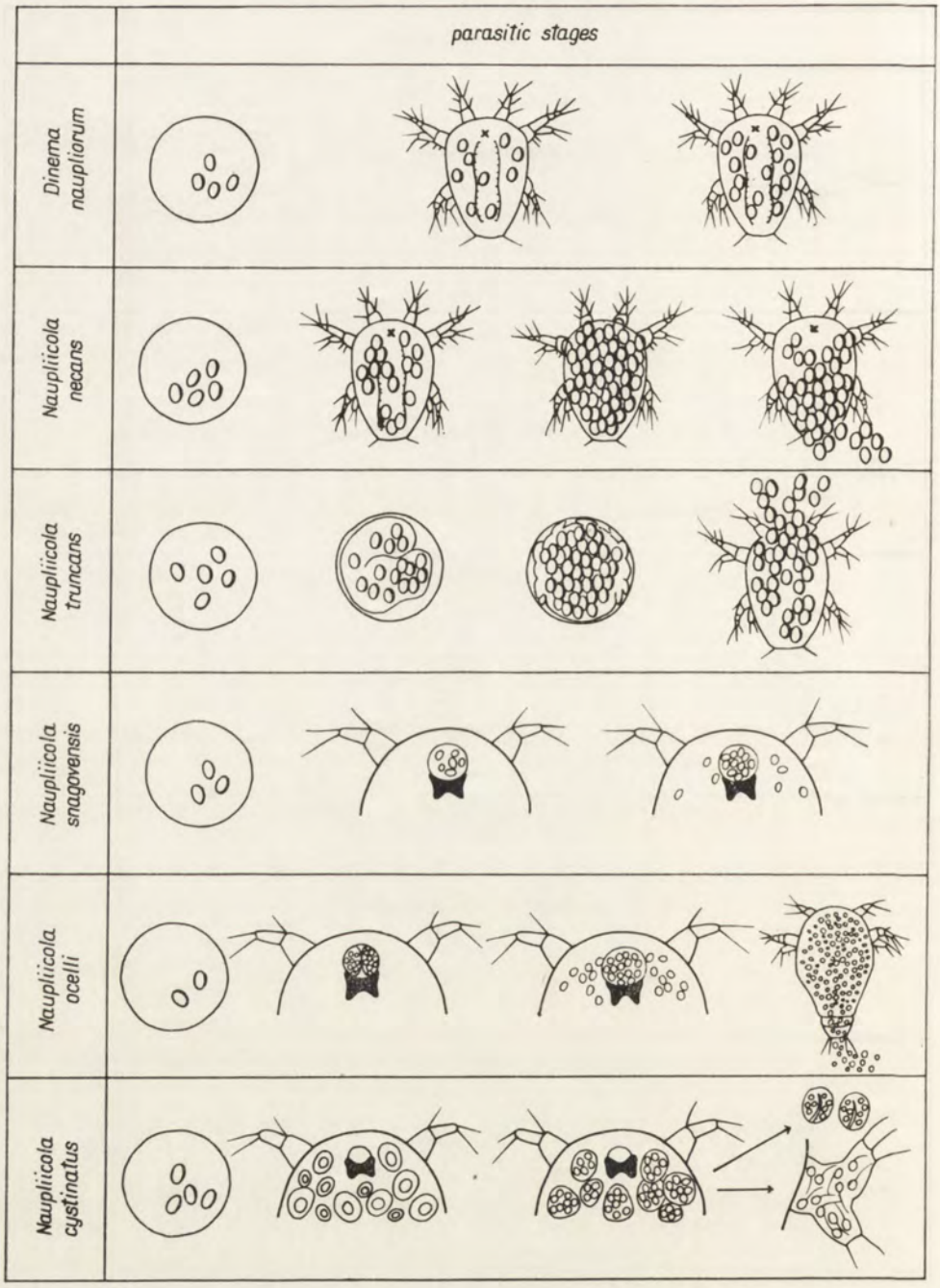
c. parasites of larvae



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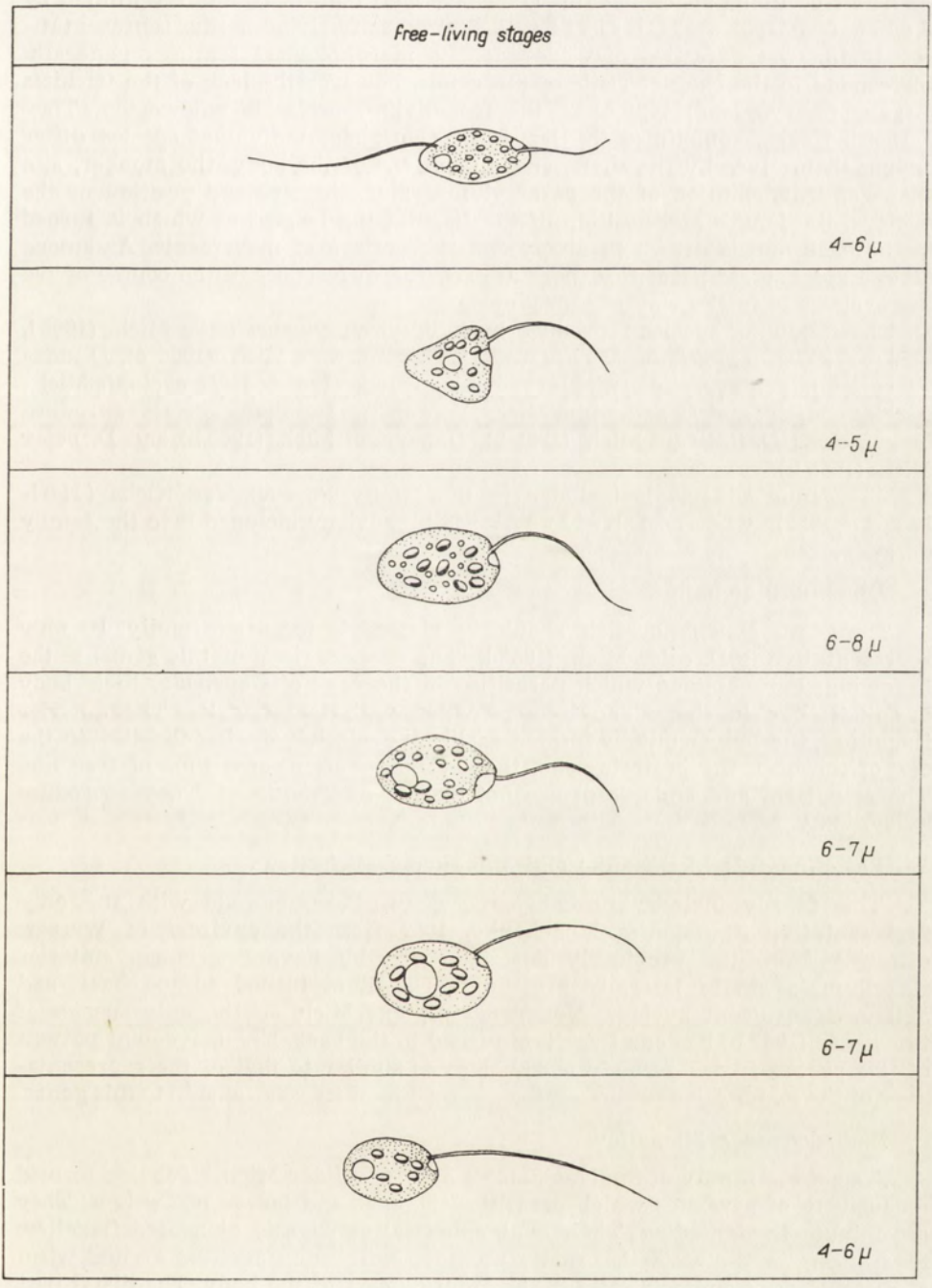

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Dinema Perty

The free living species of this genus are well known. It is differentiated by Hollande 1952 and Christen 1963 and has fairly numerous representatives among the parasites of *Copepoda*. Its morphological features generally correspond to the characteristic of the genus, however the lack of the trichites (organe pharyngiene) typical of the free living species is noteworthy. They parasitize eggs, sometimes the larvae of *Copepoda*. Individual species differ among themselves by the shape and dimensions of the body, the number, size shape and distribution of the paramylum grains, the size and position of the nucleus, the length and position of the flagella the longest of which is turned to the back and is drawn passively and the pattern of movements. As among other *Euglenoidina parasitica* there are specific differences in the course of the life cycles and in the way of reproducing.

The following species reproduce by palintomy: *Dinema agile* Mich. (1965 i, 1966 c, g, k), *D. aegypticum* Mich. (1966 a), *D. penetrans* Mich. (1966 e), *D. naupliorum* Mich., a parasite of the larvae of *Copepoda* (1966 b, f), *D. cyclopis* Mich., *D. rotans* Mich., and *D. rotundum* Mich. (1967 a). The following species reproduce by syntomy: *D. italicum* Mich. (1965 h), *D. parvum* Mich. (1965 h) and *D. velox* Mich. (1965 k).

The genus *Dinema* is included in the family *Peranemidae* Klebs (Hollande 1952); whereas Christen 1963 by analogy included it to the family *Peranemaceae*.

Dinemula Michajłow

The genus *Dinemula* Mich. should be classed to the same family. Its only representative is *D. celer* Mich. (1965 l). The characteristic of this genus is the following: *Peranemidae* which parasitize in the eggs of *Copepoda;* their body is spindle-like in shape and it is provided with two flagella. The anterior flagellum, the longer one, is formed earlier, i.e. already during the stay in the egg of the host, the posterior one, the shorter — after some time of free life. The ectoplasm and endoplasm are not clearly differentiated. They reproduce by syntomy.

Paradinemula Monchenko and *Mononema* Michajłow

This family includes probably the genus *Paradinemula* with the only representative *P. polonica* Monchenko, 1967 from the environs of Warsaw differing from the previously described one by having a longer anterior flagellum, a stiffly laterally protruding flagellum turned to the back and a large translucent nucleus. *Mononema reptans* Mich. — the only species of this genus (1967 b) has one flagellum turned to the back. The movement pattern of this protozoan whose body morphology is similar to that of the representatives of the genus *Dinema*, allows the suggestion of its relationship to this genus.

Embryocolidae Michajłow

A separate family of *Euglenoidina* — *Embryocolidae* Mich. (1965 l) is formed exclusively of parasites which parasitize the eggs and larvae of *Cyclops*. They are minute *Eugleniodina*. Their oval, spherical or slightly elongated flagellate form living in the water is usually provided with one flagellum arched when at rest and set asymmetrically in the anterior part of the body. The distal part of the flagellum is more mobile. A small number of paramylum grains, generally

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small, are scattered in the cytoplasm. In the life cycle there is the trophic--reproductive form (the parasitic one) and motile-penetrative (free).

Two genus belong to this family — *Ovicola* Mich. and *Naupliicola* Mich. The genus *Ovicola* is represented by one species — *O. abyssinicus* Mich., a parasite of the eggs of *Copepoda* (1965 l). The egg-shaped flagellate forms invade the eggs of cyclops where they reproduce by syntomy. In the flagellate form during its formation there is one larger paramylum grain, which disappears later and several smaller ones. A fairly thick and arched flagellum makes rowing movements with the distal part exclusively. Several widely distributed species belong to the genus *Naupliicola* Mich. The representatives of these species invade the eggs of cyclops, but then parasitize their larvae and leave them after their death. Morphologically they represent the above mentioned features of the family. The individual species differ among themselves by the following features: the shape and size of the body, the presence and size of the reservoir, the number, shape and size of the paramylum grains, the size and position of the nucleus, the length and position of the flagellum and the pattern of movements following from this.

Among the species of the genus *Naupliicola* the following species can be mentioned, the representatives of which reproduce by palintomy: *Nauplicola necans* Mich. (1965 f, 1966 a, e, f, g, h, k), *N. truncans* Mich. (1965 f, 1966 e, f, g, h); *N. magnus* Mich. (1966 b), *N. parvus* Mich. (1966 b, f), *N. elongatus* Mich. (1966 a), *N. fusiformis* Mich. (1966 h), *N. snagovensis* Mich. (1966 d), *N. burdigalensis* Mich. (1966 g), *N. ghanensis* (Mich. 1966 h), *N. fennicus* Mich. (1966 m), *N. celer* Mich., *N. cyclopis* Mich. (1967 d), *N. copepoditis* Mich. (1967 c), and *N. vastans* Mich. (1967 f).

The following species reproduce first by palintomy then by syntomy: *N. cystinatus* Mich. (1966 c) and *N. cystifactor* Mich. (1967 c). In *N. ocelli* Mich. (1966 e, f) reproduction by syntomy precedes reproduction by palintomy.

The data given above allow the following statements: *Euglenoidina* — the parasites of *Copepoda* are undoubtedly a biological group that aroused polyphyletically. This concerns also the forms that have invaded the same first order environment e.g. the eggs of *Copepoda* where the representatives of the genus *Parastasiella* occur together with *Anisonema, Dinema, Ovicola, Dinemula, Paradinemula* and *Mononema*. The invasion of hosts and changement to parasitism was the cause of "evolutionary steps" of different dimensions — beginning with the formation of separate species within the known genus, formed during free life (e.g. *Dinema, Anisonema*), then the formation of genus composed exclusively of parasites (*Parastasiella, Dinemula, Ovicola, Naupliicola, Paradinemula, Mononema*) which form sometimes systematical units of a higher order (e.g. the family *Embroocolidae*). The general impression is that among *Euglenoidina parasitica* the are older forms, differentiated by evolution since a long time (e.g. *Embryocolidae* and genera *Mononema* and *Paradinemula*) and younger forms (e.g. species of the genus *Astasia*) and quite young, not differentiated to a great extent (e.g. the species of the genus *Anisonema, Dinema*).

Palingenetic (ancestral) and cenogenetic features

When trying to analyse the philogenetical links among *Euglenoidina*, as it has been done above, the present author aimed to distinguish the ancestral (palingenetic) features and properties of the individual species and genus. In

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most of the known species such features can be distinguished from the very characteristic cenogenetic features.

Ancestral features

In relation to the species belonging to the genus which also include free living representatives the ancestral features would be considered as generic features widely understood, e.g. the presence of two flagella appropriately arranged in the representatives of *Dinema*, the structure and activity of the flagellum turned to the posterior part of the body in the genus *Anisonema*.

The occurence of ancestral features indicating the belonging to unities of a higher order (ancestral features s. str.) can be reported within the genus *Astasia*. They can be divided into features occuring as a rule in forms developing normally (ancestral normal features) and the ones that occur within the limits of individual variability, i.e. also in the case of deviations from the normal course of the life cycle (ancestral features deviating from normality). The ancestral features s. str. include: 1. the occurence of a vestigial flagellum in the reservoir in the parasitic form of *Astasia norvegica* and in *A. fennica* Mich. (1966 l); 2. the occurence of the stigma in the same forms of *A. sophiensis* and *A. hanoiensis;* 3. the occurence of a motile flagellum in all the species of the genus during the period of free life; 4. the fact that all the species of *Astasia* undergo at least one division by palintomy, and in some (*A. parvicula, A. norvegica, A. fennica, A. hanoiensis, A. coelomae)* the occurence of reproduction exclusively of this type. The ancestral abortive features s. str. include: 1. the presence of flagellate forms together with parasitic forms in *A. mobilis* (Alexeieff 1912); 2. the occurence of immature parasites in the euglena-shaped free living form in the case of their liberation from the intestine of the host in *A. hanoiensis*. This immature parasite is provided with a short flagellum and a stigma and can continue its development in the host if it is swallowed by it (1964 a); 3. the occurence of similar euglena-shaped forms in *A. sophiensis* which are capable also of reproduction by palintomy in the water (1965 d); 4. the reproduction by means of palintomy of immature parasitic specimens that leave the intestine before time (or squeezed out of it) in *A. cyclops* (1956), *A. sophiensis* (1965 d) without changing into the euglena-shaped form.

Cenogenetical features

Numerous cenogenetical features occur in all the species of *Euglenoidina parasitica*. They are correlated mainly with their peculiar way of life. From the evolutionary point of view these features can be classified in the following way.

Features carrelated to the fact of parasitism and widely distributed among zooparasites. These include first of all the tendency to simplify the structure and in its essence to the reduction of features and proprieties that are of importance exclusively under conditions of free life. As an example of the "parasitic reduction" the loss of the flaggellum as the organellum of movement in the parasitic forms of the genus *Astasia* and the loss of the stigma in the majority of the species in this genus should be mentioned. A parasitic way of life often leads to a change of function of certain organs in animals. The vestigial flagellum in the reservoir of *A. norvegica* and *A. fennica* takes probably part in the excretory function of the reservoir instead of the motory one. The flagellum of the free form of the majority of the species of the genus *Astasia* ceases to be an organellum of movement that makes progressive mo-

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vement possible, but plays an important part in the "alluring" of the host. An increased reproductivity, characteristic of parasites in general, can be observed nearly in all *Euglenoidina parasitica*. Moreover, the change to the parasitic way of life is accompanied by deep physiological changes in most animals. In the groups discussed this is reflected not only in the change of feeding (organic compounds that are taken from the intestin of the hoste, the reserve substances in the eggs of *Copepoda* or the contents of the body of their larvae) but also in the fact than the life cycle lasts longer and, where the parasites of the intestin are concerned, in the acquiring of immunity to the action of digestive juices of determined species of *Copepoda* (specificity). As it has been demonstrated the general tendencies observed in all parasites can be also shown to be present in the group discussed.

As concerns the features probably related to the fact that this group belongs to Protozoa parasitica they are considered to be the ability to reproduce by syntomy of many species that are included in all genera with no exceptions. This way of reproduction is widespread among *Sporozoa* (*Telosporidia*) and *Amoebosporidia*. Thus it is impossible not to correlate this exceptional for *Euglenoidina* feature with their belonging to parasitic protozoans. The question of describing the development cycles of *Euglenoidina* as single ontogeneses or metageneses (Michajłow 1956) should be discused. An argument in favour of treating the changes in the development of *Astasia parasitica* as ontogeneses is the ability to take up and store food exclusively by the forms belonging to one of these links (the trophic or the trophically generative form). This is true of *Astasia parasitica* in which separate trophic and generative links of the cycle can be discerned as well as of the parasites of the eggs and larvae of *Copepoda* in which the trophic--generative link and the motile-penetrating form can be observed. The transitory forms of the obligatory parasites utilize the reserves accumulated during parasitism and in this sense are transitory, dependent forms. A further argument in favour is the fact that there is no sexual reproduction followed by asexual reproduction in the next generation but reproduction occurs exclusively by palintomy or syntomy. If this argumentation is right then the following analogy can be suggested, the analogy between multinucleate specimens, arising temporarily in some species — representing all genera and the schizonts of *Telosporidia* and specimens arising as a result of syntomy and merozoites. The fact that complex life cycles so characteristic of e.g. *Haemosporidia* arise in parasitic *Euglenoidina* is also noteworthy. However, the fact that in the genera *Astasia, Dinema* and *Naupliicola* there are species reproducing exclusively by palintomy rather shakes credit of the hypothesis of Alexeieff 1912 of the phylogenetic linkage of some *Telosporidia* (especially *Coccidiida* and *Gregarinida*) with *Flagellata*, a problem argued about, among others, by Dogiel and all. 1962 from another point of view. It is possible that the occurence of complex life cycles and the presence of a specific schizogony (which is accompanied by the formation of "cysts", sometimes very specific) in *Euglenoidina parasitica* should be treated as a certain trend occuring in groups of parasitic protozoans very distant from one another. This would be the expression of a cenogenesis resulting from a specific way of life. Finally, it should be stressed that the wide plasticity and adaptivity that can be observed also among *Euglenoidina* supports somehow the thesis represented among others by Raabe 1964 about the necessity of treating the *Protozoa* not only as unicel-

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lular organisms but also as highly complex organisms with great possibilities which are fullfilled in a specific way for this stage of organization.

As the "material" from which the discussed parasitic forms arised was represented in all cases by protozoans belonging to *Euglenoidina,* numerous features of this group, appropriately transformed, in the course of cenogenesis, can be noticed. As an example the metabolic functions, which characterize *Euglenoidina* in general, are enhanced to a great extent in *Astasia parasitica* as parasites of the intestine. The continuous movement of the protozoan has the result that is "swims" somehow in the intestine of the host in the opposite direction to the movement of the food, and the peristaltic movements of the intestine staying thus in the same place. In this case this movement takes place of numerous clinging adaptions occuring among other parasites (R a a b e 1947). In parasites which begin their life cycle in the eggs of *Copepoda* a very intense metabolism occurs only during penetration into the membrane of the eggs and — in some species — during the getting out of the cysts that have arized in the eggs or the larvae of the host. The paramylum resources accumulated during the parasitic period of life in *Astasia parasitica* quantitatively exceed those occuring in the free living species. It should be noted, that it is the number of single grains that increases, and not their size which could be an obstacle in the maintenance of the necessary plasticity of the body. In the case of the parasites of the eggs and larvae of *Copepoda* these small dimensions can be correlated with the general diminution of the dimensions of their body which in turn is probably related to the necessity of perforating of the membrane of the eggs when invading the host.

The change of the functions of the flagellum in *Astasia parasitica* has already been discussed above. In the representatives of other genera they conserve generally the morphology and motile functions characteristic of free living *Flagellata*, but they can play an important part in the invasion of the eggs of the host. It is interresting to note that in *A. ovorum*, the only species of the genus *Astasia* being a parasite of the eggs, the patern of the function of the flagellum typical of the free living *Euglenoidina* is basically maintained.

Besides the above mentioned adaptations to parasitism which arise from their belonging and descendance *Euglenoidina parasitica* posses also specific adaptations that develop on their background and in correlation with them. These adaptations should be discussed separately.

Biological adaptations

The polygenic character of the group of *Euglenoidina* — parasites of *Copepoda* is expressed in the fact that these organisms can be classified not only from the point of view of their supposed phyletical relations but also taking into account the way of invading the host and the location in the organism. Consequently the biological adaptations observed are undoubtedly related to the provenance of the parasite and — on the other hand — to the way of parasitizing. That is why the latter criterion should be applied when grouping the parasites to be discussed (M i c h a j ł o w 1966 i).

Euglenoidinae parasitizing the intestine of *Copepoda*

In the majority of these parasites the trophic link of their life cycle which closed during parasitism in the intestine of the host and the generative link

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taking place in the water during the transitory period of free life can be discerned. The differences in biological adaptations among parasites concern mainly the generative link. Thus we deal with a group of species in which reproduction consists in palintomical divisions taking place more or less rapidly. In *Astasia hanoiensis* these divisions do not lead to an immediate seperation of flagellate forms, which are agglomerated during some time in the form of a bundle swimming in the water and sometimes are eaten by the host in this form. In other species the division of the specimens leads as a rule to their separation from each other. In *Astasia parvicula,* probably less advanced from the evolutionary point of view, the flagellate forms preserve the ability to move forwards. The flagellate forms of *A. norvegica* and *A. fennica* are sedentary and vibrate, staying at the same place "baiting" the host. The number of daughter specimens (of the fifth order, as a rule after the fifth division) in this group of species is generally 32 though deviations in both sides were noted. The occurence of reproduction by syntomy in some species after which there is one division by palintomy should be considered as a step forward towards parasitism. In *Astasia cyclopis* a small number (32) of the specimens of the second order are sedentary, in *Astasia sophiensis* they are much more numerous, however they retain the ability to free swiming to a certain extent they are rotating often without changing place.

The differences between the trophic and generative link of the cycle begin to dissapear in *Astasia bulgarica* in which very numerous daughter specimens arise already in the intestine of the host, they leave the intestine encapsulated in "cysts" and undergo one division by palintomy in the water.

The development cycle of *A. coelomae,* a species morphologically very close to others belonging to this genus is quite different. The form parasitizing the intestinal tract does not reach dimensions exceeding the primary dimensions twice, but reproduces by means of simple divisions. The daughter individuals reach the body cavity through the walls of the intestine. Then they continue reproduction by palintomy. After the death of the cyclops and having got out into the water they form directly flagellate forms.

When observing the directions of adaptation of the parasites of the intestine of *Copepoda* one should expect that during further studies species with the flagellate forms firmly united should be found or species reproducing exclusively by syntomy, or species in which the formation of the flagellate or at least the preflagellate forms occurs already in the intestine of the host.

Euglenodina — parasites of the eggs of *Copepoda*

The invasion of these parasites into the eggs of the host prevents the embriogenesis and leads to the destruction of the egg. The periodically obligatory parasite *Dinema agile* should be mentioned as noteworthy in this group. This parasite reproduces by palintomy in the egg, after having left is, reproduces similarly during several generations, probably feeding in the same way as the free living *Dinema* (Michajłow 1965 i). Taking into account the observed "gradation" of parasitism the finding of species of accidental parasites belonging to the same genus.

The obligatory parasites of the eggs differ mainly by the way of reproduction. The following species reproduce by palintomy in the egg of the host: *Dinema aegypticum, D. penetrans, D. rotans, D. rotundum, Parastasiella helvetica and P. vastans.* The latter species when leaving the integument of the

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egg causes their complete destruction in a way that is not known exactly whereas other species cause usually only their tearing in one or several places. In all the other parasites of the eggs reproduction occurs exclusively by syntomy and the specimens growing in the egg form some kind of cysts. Usually final cysts are then formed at once. The only exception is *Parastasiella ovorum* in which two "generations of cysts" can be observed — one is a large mother cyst, the second — several (usually 8) daughter cysts inside the large one (Michajłow 1965 c). The remaining species differ mainly by the number of the cysts that are formed inside the egg. In a certain group of parasites (*Astasia ovorum, Parastasiella parva, Dinema italicum, D. parvum*) only one cyst is formed in the egg of the parasite. It is not known yet how this can occur in the conditions that the egg is attacked (e.g. during an experiment) by numerous flagellate forms. In the case of all the other parasites several cysts are formed in parallel if the egg is invaded by several parasites. The parasites that arose by syntomy often leave the egg passively — singly (*Anisonema parasitimum*) or together with the cyst (*Dinemula celer, Ovicola abyssinicus*). There are species, however, the cysts of which are provided with special apparatus that makes possible a rapid and active leaving of the empty integuments of the eggs by the flagellate forms of the parasite. This is the case of *Parastasiella velox* and *Dinema velox*.

Taking into account the diversity of biological adaptations to parasitism in the eggs of *Copepoda* formed in the course of the evolution, it could be expected to find in this group parasites that also form more than 2 "generations" of cysts or parasites in which syntomy and palintomy occur alternatively.

Euglenoidina — the parasites of the larvae of *Copepoda*

The life cycle of these parasites begins similarly as in the above discussed ones. However, they do not cause a complete arrest of embryogenesis, only a restrain to a certain extent. The representatives of some species (*Dinema naupliorum, Naupliicola truncans, N. parvus, N. vastans* and *N. celer*), however, reproduce by palintomy already at the stage of eggs so that as they fill in completely an already formed larva causing its death immediately after its hatching and sometimes even during the leaving of the egg. Then they rapidly leave the decomposing integuments the larva passively, so that this could be considered a specific adaptation to the leaving of the eggs.

In the case of other species yet known the reproduction in the egg either does not occur at all, either it is very slow so that the larvae of the host hatch from the eggs alive and move in the water for at least a few days. Among these species of parasites the ones that do not show a determined tissue specificity and reproduce directly in the body cavity of the larva worth mentioning. This reproduction can be exclusively palintomical as e.g. in *Naupliicola necans, N. magnus, N. elongatus* and *N. fusiformis* or after division by syntomy the formation of cysts occurs as in *Naupliicola cystinatus* and *N. cystifactor*. In some species the reproduction of parasites goes on slowly and the death of their hosts occurs only after having reached the copepodite form (*N. copepoditis*) or the form of a sexually mature cyclops (*N. cyclopis*).

The second group includes the species of the genus *Naupliicola* that show a determined specificity of the tissue, during embriogenesis they invade the translucent part of the eye of the embrion of the nauplius. Some of these species reproduce in the eye by palintomy, leaving the swollen and destroyed

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eye once (*Naupliicola burdigalensis, N. fennicus,* and *N. snagovensis*) or reproduce further on in the remnants of the eye (*Naupliicola ghanensis*). In both cases the further reproduction in the body of the larva occurs by palintomy till its death.

In the case of one species, as yet, namely *Naupliicola ocelli* reproduction has been found to occur by syntomy in the eye, then when the daughter specimens have left the cysts — by palintomy in the body cavity.

The diversity of the biological adaptations of the known species parasitizing the larvae and the analysis of the directions of these adaptations allow to expect the finding of further species e.g. reproducing by syntomy and causing the destruction of the larvae during hatching, or the destruction of the larvae in later stages of their development (e.g. as copepodites) or even allowing the formation of the mature *Copepoda* in the body cavity of which they could then parasitize.

A common biological adaptation for the parasites of the eggs and larvae of *Copepoda* is, of course the ability of directed reaction to the presence of fresh eggs of the host. The nature of this taxis its conditions and role within the individual species require further studies. The fact that similar adaptations occur in species belonging to different genera and even families can be correlated with the influence of the way of parasitizing and these adaptation can be treated as the expression of a specific biological convergence.

The physiological and biochemical side of the above described biological adaptations is still to be studied as it is still unknown. The specificity of the parasites towards their hosts is undoubtedly an important biological adaptation. It will be discussed later.

The directions of biological adaptations in relation to individual variability

The previously mentioned ancestral features different from the normal ones and being sometimes also essentially individual features showed the phyletical correlations of the parasites. At present the deviations from normality shall be discussed as being also the expression of individual variability, but, possibly the showing directions of further changes of the species on the background of the known types of biological adaptations of *Eugleniodina parasitica*. It is not important to study the individual variability that can be observed when hundreds of specimens of the parasites are tested, similarly as in other organisms, but the symptoms of variability that can have an importance from the evolutionary point of view. This variability can also concern morphological features.

As an example the flagellate forms of *Astasia norvegica,* that arose after five divisions by palintomy of the mother specimen are twice as large as the sometimes formed in 6 divisions 64 flagellate specimens. It is noteworthy that although they differ among themselves not only by the dimensions but also by the shape flagellum has in both cases an identical absolute length (1964 d). The tendency to increase the number of divisions by palintomy occurs also in *Astasia fennica* in which a division into 128 daughter specimens has been once observed (1966 l).

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Another tendency in the development of the species of the genus *Astasia* consists in the fact that the specimens formed in the divisions by palintomy conserve for some time links between one another. In the case of *A. norvegica* agglomerations of 32 spherical daughter specimens, not covered with a membrane, were observed several times. These specimens separated only after the change into flagellate forms had occured so that they formed during a certain period a bundle similar to that observed in *A. hanoiensis* (1964 a), though not motile. In *A. fennica* this tendency occurs more often and flagellate forms in 1 or 2 agglomeration have been reported or tightly set in the ground within the circular remnant of the parent form (1966 l). This direction of variability could be correlated with the occurence of species reproducing by syntomy within the genus. Among the latter the increase of the number of specimens arising by means of syntomy to 32 and their decrease to 8 with the simultaneous increase of palintomic divisions to the number of 3 can be observed in relation to *A. cyclopis*. In both cases 64 daughter specimens are formed (1956, 1957). In the case of *A. bulgarica* which forms a great number of daughter specimens by syntomy (sometimes more than 500) direct formation of the flagellate forms was sometimes observed. In these cases even a single division by palintomy occuring as a rule in *A. cyclopis, A. sophiensis* and *A. bulgarica* is completely eliminated from the cycle.

The morphological variability of the flagellate forms of the "saltatory" type occurs also in the parasites of the eggs. As an example it can be said that in *Anisonema parasiticum* the occurence of flagellate forms twice as small than the ones observed more often and treated as normal has been reported. Smaller specimens are formed in cysts that contain not about 30 but about 60 nuclei after the division by syntomy (1965 b). Attempts to ascertain whether the diminution of the dimensions of the body and the increas of the number of the progeny, which are part of a general tendency of adaptation among parasites of the eggs are genetical in character were unsuccessful. It is noteworthy that the "minute" forms of *A. parasiticum* are nearly six times smaller that e.g. the free living *Anisonema costatum* Christen, 1962 whereas the normal forms — only 3 times smaller.

Among other parasites of the eggs of *Copepoda* a biological adaptation has been reported in *Parastasiella vastans* consisting in complete destruction of the integuments of the egg of the host after having reached the maximal number of specimens in the egg. It has been noticed that in other species (*Dinema aegypticum, D. agile*) the extent of destruction of these integuments can be different and the variability observed can be an indication of an adaptation of the parasites that is being formed.

Among the parasites of the larvae of *Copepoda* the most striking adaptation occurs sometimes in the development cycle of *Naupliicola ocelli*. In this species the development in the larvae lasts often till the moment of formation of older metanauplional forms of the host. In 25% an interresting adaptation was observed consisting in the complete destruction of the larva formed after the molt and thus all the parasites with no exception reached the water at the same time (1966 f). In the remaining cases — part of the daughter specimens leaves the remnants of the larva through the conjunction of the abdomenal segments that are formed — others perish in the remnants of the host. It seems that in this case an adaptation in statu nascendi is dealt with. This adaption consits in the physiological synchronization of the maximum moment

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of the palintomy of the parasite when the progeny reaches the number of hundreds of specimens with the moment of the molt.

The duration of the whole life cycle or of its individual stages of the parasites undergoes considerable fluctuations. The fluctuations in the duration of the whole development cycle in many species exceed 100% and even 150%. The differences in the duration of the reproduction of the parasites from the genus *Naupliicola* during their stay in the nauplional eye of the larva (e.g. in *N. burdigalensis* (1966 g) or contrariwise, during their further reproduction in the body cavity of the larva (as in *N. ghanensis* 1966 h) is especially striking. It is difficult, however, to find any definite development tendencies, taking into account that this variability depends on the environmental conditions (e.g. temperature) as well as on the species of the host. As an example *N. ghanensis* from *Microcyclops varicans* (Sars) destroyed the host only after it had reached the form of the copepodite I whereas the same parasite leaded in *Afrocyclops deryphorus* (Kiefer) to a much more rapid destruction of metanauplius II, III, rarely IV.

The specificity of the "parasite-host" system

Many facts prove the existence of parasitic specificity in the systems "*Euglenoidina-Copepoda*". This problem has been studied in detail in relation to *Astasia parasitica*. Where *Astasia cyclopis, A. norvegica* and *A. fennica* are concerned *Copepoda* can be divided into 3 groups basing on the extensiveness and intensity of natural and experimental invasion (as well as their comparison). 1. Copepoda in which the intestinal juices destroy completely the flagellate specimes of *Astasia* swallowed by them. In relation to *Astasia cyclopis* these are *Diaptomus gracilis* Sars, *Diaptomus vulgaris* Schmeil, *Marcrocyclops fuscus (Jur.)* and *Mesocyclops leuckarti* (Claus); in relation to *Astasia norvegica — Ectocyclops phaleratus* (Koch), *Mesocyclops leuckarti* (Claus), *Cyclops strenuus* (Fischer) Koźmiński and *Heterocope appendiculata* Sars; in relation to *Astasia fennica — Megacyclops gigus* (Claus), *Diacyclops bicuspidatus* (Claus), *Cyclops strenuus* and *C. scutifer* (Claus). 2. The *Copepoda* in which the intenstinal juices destroy part of the parasites; while the ones that survive develop normally (auxiliary system). In relation to *Astasia cyclopis* these are: *Macrocyclops albidus, Megacyclops viridis* (Jur.), *Cyclops vicinus* Uljan., *C. furcifer* Claus, *C. insignis* Claus and *C. strenuus*; in relation to *Astasia norvegica — Megacyclops viridis;* in relation to *Astasia fennica — Acanthocyclops vernalis* (Fischer). 3. *Copepoda* in which the intestinal juices do not destroy the parasite, its development goes on normally (obligatory system). In relation to *Astasia cyclopis* these are: *Eucyclops serratulus* (Fischer), *E. macruroides* (Lill.), *E. macrurus* (Sars); in relation to *Astasia norvegica — Eucyclops serrulatus, E. macrurus, Macrocyclops fuscus, M. albidus;* in relation to *Astasia norvegica — Eucyclops macruroides, Megacyclops viridis, Macrocyclops albidus* (1956, 1957 b, 1964 d, 1966 e).

A. coelomae reproduces in the intestine as well as in the body cavity of the host, it is specific for *Microcyclops planus* (Gurney). The specimens swallowed by *M. varicans* (Sars) are digested in the intestine. However normal development in the body cavity of *M. planus* takes place only in the copepodites, whereas in the specimens of mature cyclops a degeneration of the

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present parasites can be observed most often. It is probable that specificity towards determined conditions in the body cavity of the copepodites is involved.

The fact that 30% of the eggs of *Eucyclops serrulatus* has been found to be invaded by *Naupliicola parvus* the specimens of which formed in developing larvae cannot leave their integuments and extracted artificially from them after 8—10 days do not form flagellate forms in the water and die (systema accidentalis) (Michajłow 1966 b) suggests the existence of analogical groups of hosts and systems among the egg parasites of *Copepoda*.

Numerous other data point to the existence of specificity of the studied systems. Although each of the 32 studied *Copepoda* was the host of at least one species of *Euglenoidina* the number of potential parasites differed widely. Six species of *Copepoda* (*Ectocyclops phaleratus, Cyclops furcifer, C. insignus, Diacyclops crassicaudis* (Sars), *D. languidus* (Sars) and *Macrocycylops rubellus* (Lill.) were so far reported to be the hosts of one species of *Euglenoidina parasitica*, five — of 2 species, two — of 3 species, three — of 5 species, two — of 7 species, one (*Macrocyclops albidus*) — of 8 species, one (*Acanthocyclops vernalis*) — of 9 species, one (*Megacyclops viridis*) — of 10 species and one (*Eucyclops macrurus*) — of 13 species (1965 n and others).

Among *Copepoda* mainly the representatives of the family *Cyclopidae* are the hosts of *Euglenoidina*. Only one parasite of *Calanoida* — *Naupliicola eudiaptomi* Mich., has been reported so far from the larvae of *Eudiaptomus* sp.

Among *Cyclopidae* the *Eucyclopinae* and *Cyclopinae* do not show essential differences in this respect. Among the genera of *Eucyclopinae* the dispersion of the proper hosts in not uniform; the representatives of the genera *Afrocyclops* and *Ectocyclops* have less parasites, the representatives of the genera *Macrocyclops* and *Eucyclops* are invaded to a greater extent. Among *Cyclopinae* the species of the genera *Megacyclops, Acanthocyclops* and *Mesocyclops* have more parasites, *Cyclops, Dicyclops, Thermocyclops* and *Metacyclops* — less. In many cases the presence of *Copepoda* invaded to a great extent as completely immune was reported in the same reservoirs. This is the case of well as species e.g. *Naupliicola burdigalensis* from Bordeaux, where 6 species of *Copepoda* invaded to different extents by this parasite were reported together with two (*Mesocyclops leuckarti* and *Macrocyclops albidus*) species completely free from infection (1966 g).

The problem of the specificity of the systems "*Euglenoidina — Copepoda*" can be viewed from the parasite viewpoint by studying the number of hosts invaded by them and determining the extensivness and intensity of infection. However the latter attributes cannot be compared where parasites of the alimentary tract are taken into account and the ones that begin their life cycle in the egg of the host. In relation to the parasites of the alimentary tract of *Copepoda* the notion of extensiveness and intensity can be used in the general parasitological meaning. In relation to parasites of the eggs and larvae they can correspond to the occurence in the integumentum of the egg of one female of at least one infected egg as well as to the number of eggs in the same case thus having a completely different meaning.

Consequently some data illustrating the specificity of systems of the egg alimentary tract and larvae parasites of *Copepoda* should be given separately. Among *Astasia parasitica* there are species of "wide" specificity as well as of a "narrow" specificity. One host was reported for 2 species (*A. hanoiensis*,

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A. bulgarica), two for one (*A. parvicula*), three — for two (*A. fennica* and *A. sophiensis*), five — for one (*A. norvegica*) and nine for one (*A. cyclopis*). After summarizing, 14 species of *Cyclopidae* belonging to 5 genera can be the host of the species of the genus *Astasia*. The genus *Eucyclops* (*Eucyclopinae*) stands out here as its species can be the hosts of 5 species of the genus *Astasia*. Among the egg and larvae parasites of *Copepoda* the species of the genus *Parastasiella* occur together in 7 species of hosts belonging to 4 genera of both subfamilies of *Cyclopidae;* the represetatives of *Cyclopinae,* however, predominate. The species of the genus *Dinema* parasitize 11 species of *Cyclopidae* belonging to 7 genera of both subfamilies; a fairly narrow specificity predominates. Four species (*D. italicum, D. parvum, D. velox, D. aegypticum*) have each one species of host. *D. naupliorum* has been encountered in 2 species of cyclops. Only *D. agile* has been encountered in three species of hosts and *D. penetrans* — in six.

The case of the genus *Naupliicola* is different. The hosts of this genus can belong to 18 species of *Cyclopidae* from the 11 genera belonging to both subfamilies. The "wide" type of specificity predominates. Only 2 species (*N. fusiformis* and *N. elongatus*) have been encountered in one species of host. Two species (*N. cystinatus* and *N. magnus*) have each 2 hosts, 2 species (*N. ocelli* and *N. snagovensis*) have each 5 hosts, 3 species (*N. truncans, N. fennicus* and *N. burdigalensis*) — 6 hosts each and *N. necans* has 10 species of hosts. The following genera of *Copepoda* are invaded by numerous species of the genus *Naupliicola:* among *Eucyclopinae* — *Macrocyclops* (3 species parasites), *Eucyclops* (8 species of parasites), among *Cyclopinae* — *Megacyclops* (4 species of parasites), *Acanthocyclops* (5 species of parasites), *Diacyclops* and *Mesocyclops* (3 species of parasites each).

The above given data are not, of course, complete considering the different extent of accuracy of the studies on the materials from different geographical regions (among others data concerning exclusively breeding of the plankton from Ghana, Ethiopia and Viet-Nam). From these data, however, conclusions can be drawn concerning not only the fact of the existence of the specificity of the *Euglenoidina-Cyclopidae* systems, but also the gradual evolutionary formation (obligatory and auxiliary systems) and the species of hosts invaded to the greatest extent by parasites can be differentiated. The following species belong to the latter: the genera *Macrocyclops* and *Eucyclops* among *Eucyclopinae* and *Megacyclops, Acanthocyclops, Mesocyclops* and *Diacyclops* among *Cyclopinae.*

The "parasite-host" systems and the environment of the second order

Basing on the model "*Euglenoidina parasitica-Copepoda*" not only the relations between the parasites and their hosts can be observed on the stage of the links of two species between one another as well as by the summing of individual data as it has been done in the preceeding chapter. This material allows the analysis of the accumulated data also from the point of view of the role of the 2nd order environment in the formation of parasitic systems. This environment acting on the organism of the host has an indirect influence on the fate of the parasite. As in all *Euglenoidina parasitica* one chain of the cycle takes place in the water, the external environment has also a direct

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influence during some time. Several facts showing the existence of such an influence and divided conventionally into ecological and geographical factors shall be reviewed.

The influence of ecological factors on the "*Euglenoidina-Copepoda*" systems

There are data showing the marked influence of the biotope on the formation of systems. They concern a few species of hosts and parasites. In relation to *Astasia norvegica* the presence of which has been reported in 3 of the studied 13 water bodies, mainly in the region of Oslo the following facts are characteristic in this respect. *Macrocyclops albidus* was invaded in the lake Kijemsjöen (100% extensiveness of invasion), however in 5 other water bodies it was free from the parasite. Thus on account of some factors, probably, ecological in character, the parasite, though distributed fairly widely does not accompany its appropriate host and occurs in a sort of mosaic in one region. In two other small water bodies where *M. albidus* occured together with other species of cyclops invaded by *A. norvegica* — it was however free from the parasite. It is difficult to relate this fact to the direct action of the biotope factors on the parasite. This yet unknown phenomenon can be determined as the ecological vicariate. Two of its variants should be reported consisting in the replecement of one by another in the case of the lack of the first or even in its presence.

From the point of view of interest the considerable changes in the extensiveness of invasion of *Eucyclops serrulatus* in three water bodies not far from one another reaching from 20% to 82% are characteristic. In cultures from the lake Sognsvannet where the extensity of natural infection by *Astasia norvegica* was 20% and the mean intensity 1.5 the experimentally obtained extensiveness was 100% and the mean intensity was 9.2. The data concerning *Eucyclops macrurus* from the same reservoir are still more striking. The extensivenes of natural infection was 0 and the experimental one 100% with the mean intensity 11 (1964 d).

In Finland the extensiveness and intensity of infection of the host *Eucyclops macruroides* by *Astasia fennica* increased also in experimental cultures. Moreover, the 60% extensiveness of experimental invasion has been reported in *Macrocyclops albidus* from a reservoir where this species was not infected by *Astasia fennica* at all. The phenomenon described as the ecological vicariate was also observed in 5 reservoirs studies near Helsinki. It consisted in the "replacement" of hosts or in the change of the role of the main hosts into auxiliary hosts (1966 e).

Where the egg and larvae parasites are concerned similar phenomenon was sometimes observed. Different species of *Copepoda* were the hosts of *Naupliicola* in different lakes near Helsinki. *Macrocyclops viridis* was infected in the lake Pitkäjärvi and was not in the reservoir Pitkäjärvi Nuuksion, in spite of the presence of the parasite in other species of hosts. The decrease of extensiveness of infection of *E. macruroides* from 80% reported in the natural conditions of the Pitkäjärvi plankton to 26% in cultures (1966 e) is noteworthy. Generally in the aquarium cultures in the conditions of a considerable density of hosts and relatively rapid reproduction of parasites the increase of intensity and extensiveness of infection is observed. The above mentioned case is so far an exception.

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The relation of the host-parasite systems and zoogeographical factors

Although the geographical distribution of *Euglenoidina parasitica* are yet poorly known (the available data concern mainly Palearctic) the data present can be arranged in order to obtain a general idea of the role of the geographical factors in the formation of the biological group of *Euglenoidina* parasitizing *Copepoda*.

First of all it has to be said that there are species of a very narrow area. All the species of *Astasia parasitica* described so far belong to this group of species which can be found only in one country. Where egg parasites are concerned these are: *Parastasiella parva* (Italy), *P. ovorum* (Hungary), *P. velox* (Hungary), *Anisonema parasiticum* (Hungary), *Dinema italicum* (Italy), *D. parvum* (Italy), *D. aegypticum* (Egipt), *D. velox* (Poland), *D. penetrans* (Finland), *Dinemula celer* (Ethiopia) and *Ovicola abyssinicus* (Ethiopia). Among larva parasites this group includes: *Naupliicola magnus* (Bulgaria), *N. elongatus* (Egypt), *N. snagoviensis* (Rumania), *N. burdigalensis* (France), *N. ghanensis* (Ghana), *N. fusiformis* (Ghana), *N. copepoditis, N. cyclopis, N. cystifactor, N. celer* and *Mononema reptans* (Yugoslavia). Even if we suppose that the finding of further places of occurence of the above mentioned species shall change this image to some extent, there is still no doubt concerning the existence of species of a small area (or even local ones). Among these such are found that have been collected from only one species of host. These are: *Astasia hanoiensis, A. ovorum, Parastasiella ovorum, P. velox, Anisonema parasiticum, Dinemula celer, D. italicum, D. aegypticum, Ovicola abyssinicus* and *Naupliicola fusiformis*. Their presence in hosts of a much wider, sometimes cosmopolitic range should be noted (the case of *Astasia hanoiensis* and *Mesocyclops leuckarti, Naupliicola elongatus* and *Mesocyclops leuckarti*). The remaining species of parasite have been found in several (at least two) hosts and the greatest number of hosts was reported in the case of *Naupliicola snagoviensis* (5), *N. burdigalensis* (6), *N. fennicus* (6) and *Astasia cyclopis* (9).

The rare examples of a wide geographical distribution can be found among egg parasites (*Dinema agile* in Finland, England, France and Italy), these examples are fairly numerous among parasite of the larvae of *Copepoda*. As an example *Dinema naupliorum* has been found in Germany and Bulgaria, *Naupliicola ocelli* in 3 countries (Germany, Egypt, Ethiopia), *Naupliicola truncans* — in 5 (Finland, Germany, France, Yugoslavia, Ghana), *Naupliicola necans* — in 8 countries (Ghana, Finland, England, Germany, France, Hungary, Italy and Egypt). Widely distributed species which include first of all representatives of the genus *Naupliicola* usually have a fairly large host range.

Where the geography of the "parasite-host" systems as certain biological units is concerned the following fact should be noted. In some cases the areas of occurence of the host and parasite are identical, at least where the countries studied yet are concerned. *Anisonema parasiticum* has been found in Hungary in *Ectocyclops phaleratus* exclusively, *Naupliicola ghanensis* and *N. fusiformis* in Ghana in *Afrocyclops doryphorus* exclusively.

More often the parasite has an area more narow than that of only one or of one out of his several hosts. *Astasia hanoiensis* has been found in *Mesocyclops leuckarti* only in Viet-Nam, although it had been studied in many countries. *Naupliicola snagovensis* has been found in *Macrocyclops fuscus, M. albidus, Eucyclops macrurus, E. macruroides, Megacyclops viridis* exclusi-

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vely in Rumania although theses species of *Cyclopidae* are widely distributed and in other countries have other numerous parasites. This concerns also *Naupliicola burdigalensis* and *N. fennicus*. As the studies are at this stage it is difficult to determine these species as endemic ones, though this cannot be excluded.

Table 2
The occurence of *Dinema agila* in different species of hosts depending on their region of occurence

Country	Hosts
Finland	*Cyclops strenuus*
England	*Diacyclops languidus*
France	*Acanthocyclops vernalis*
Italy	*Mesocyclops leuckarti*

Table 3
The occurence of *Naupliicola necans* in different species of hosts depending on their region of occurence

Country	Hosts
Finland	*Macrocyclops albidus* *Acanthocyclops vernalis*
England	*Acanthocyclops vernalis*
Poland	*Cyclops vicinus*
Germany	*Eucyclops serrulatus* *Acanthocyclops vernalis* *Diacyclops bicuspidatus* *Diacyclops bisetosus*
Hungary	*Eucyclops serrulatus*
Italy	*Megacyclops viridis* *Megacyclops gigas* *Mesocyclops leuckarti*
Egipt	*Mesocyclops leuckarti*
Ghana	*Afrocyclops doryphorus*

A more wide range of occurence than one of its (yet known) hosts of a parasite can be observed e.g. in the case of *Afrocyclops doryphorus*, which is among others in Ghana the host of *Naupliicola necans*, a species widely distributed also in Europe. The phenomenon of the identity, partial overlapping, or exclusion of ranges of potential parasites and hosts show the

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geographical variability of the "host-parasite" systems in which one partner is a determined stable species.

The above mentioned data are interesting from the viewpoint of the "replacement" of parasites and hosts and the known phenomenon of the parasitological vicariate. The most striking data can be put together in several tables showing the mutual relations of parasites and hosts in the geographical aspect.

The variability of the systems "*Dinema-Cyclopidae*" is a classical example of the parasitological vicariate or the replacement of hosts of the same parasite in different geographical regions (Table 2). This phenomenon is also encountered among parasites of the larvae of *Copepoda* (Table 3).

In the case of *Naupliicola necans* not only the replacement of hosts in different geographical regions is noteworthy but also a different extent of infection of individual species, i.e. their replacement in the role of the principal and auxiliary host. In Hungary *Eucyclops serrulatus* was the principal host of the parasite, whereas in France — rather the auxiliary one.

A complete lack of infection was also observed in determined geographical regions in species which were infected to a great extent in other regions. As an example *Eucyclops serrulatus* was the host of *Naupliicola necans* in Hungary, while in Italy althought it occured in the same reservoir as *Mesocyclops leuckarti* and *Megacyclops gigas* (both susceptible) *Eucyclops serrulatus* was not the host of this parasite neither in natural conditions nor experimentally. However it was infected by *Naupliicola truncans* (1965 f). This concerns also *Megacyclops viridis* from Italy where it was infected by *Naupliicola necans* and from Finland where in spite of the presence of the parasite in the environment it was immune to infection. It is possible that in this case there are "physiological varieties" of parasites or hosts in different regions and certainly this is an indication of the role of the geographical factor in the formation of parasitic systems, the study of experimental parasitological hybrids of geographically distant identical species of hosts and parsites could clarify that matter to some extent.

Table 4 illustrates a specific parasitological vicariate à rebours. This consists in the replacement of parasites of different related species in the same host in different geographical regions.

Table 4

The replacement of parasites of the genus *Astasia* in *Megacyclops viridis* depending on the region of occurence

Country	Finland	Norway	Poland	Bulgaria
Parasite	*A. fennica*	*A. norvegica*	*A. cyclopis*	*A. sophiensis*

As it can be seen, different species of the genus *Astasia* the geographical range of which seems to be quite narrow occur in the same (together with some others species) host with a wide geographical range.

This phenomenon can be also observed in the larva parasites, as it can be seen from the table concerning *Mesocyclops leuckarti* and some species from the genus *Naupliicola* (Table 5).

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Table 5
The replacement of parasites of the genus *Naupliicola* in the *Mesocyclops leuckarti* depending on the region of occurence

Country	Italy	Egipt	Ghana
Parasite	*N. necans*	*N. elongatus*	*N. cystinatus*

Taking into account all the above mentioned data and some others not mentioned (among others Michajłow 1965 n) the undoubtful influence of the 2nd order environment on the formation of separate species of *Euglenoidina parasitica* should be stressed. On the other hand the formation of analogical biological adaptations (e.g. the infection of the nauplional eye of the host by *Naupliicola ghanensis* and *N. fennicus*) in species geographically distant is an especially striking phenomenon. It can be said that this is the expression of geographical biological homology occuring in inhabitants of different geographical regions, similarly as the similarities shown by representatives of more distant systematical entities were determined by the expression of biological homology.

Problems concerning the evolution of *Euglenoidina parasitica*

The data collected at present concerning the systematics, morphology, and biology of *Euglenoidina* — parasites of *Copepoda* make possible the study of some problems of the evolutionary development of this polyphyletic and polygenic group of parasitic protozoans. When inspecting great series of specimens and biological observations related to them, the undoubted fact of the occurence of microevolutionary process within individual species becomes apparent. In many cases the above discussed variability of morphology and life cycles of parasites exceeds beyond the continous individual variability and suggests the formation of smaller differentiated groups within a species. This phenomenon can be related to geographical factors as it is e.g. in the case of distinctness of certain features in *Astasia norvegica* of Kjemsjöen from the features of the population from the region of Oslo (1964 d). Sometimes ecological factors are involved as it is in the case of the population of *Astasia fennica* from a small reservoir near Nupuri — different from the form occuring in other lakes of the region of Helsinki (1966 l).

It can be speculated that the process of speciation has resulted in the formation in several distant geographical regions of several parallel but separate species of parasites in the intestinal tract of *Copepoda* these parasites belonging to the genus *Astasia*. In this case it seems that there are several places where species are formed. The macroevolutionary process probably influenced the formation of the parasitic genus *Anisonema* and *Dinema* differently as the above mentioned genera do not differ essentially from free living species in this environment. It is noteworthy that the general pattern of the course of speciation shown when comparing data concerning protozoans with undoubtfully different degrees of adaption to parasitism corresponds

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essentially to the classical description of the course of the evolution and formation of species made by C. Darwin. The different degree of adaptation to parasitism and the different scale of "distances" from the free living species related to the studies species suggest that within *Euglenoidina parasitica* there are younger forms from the evolutionary point of view and forms that are much older. The transition from free life to parasitism can be shown taking as an example the course of the life cycle of the periodically obligatory parasite *Dinema agile* (1965 i). The existence of the species and genera (*Naupliicola, Ovicola*) which had to be differentiated as a separate family *Embryocolidae* shows evolution in such an advanced stage (also considering its duration time) that in the case of these organisms a megaevolutionary process is dealt with.

Where factors causing speciaton and evolutionary development are concerned conclusions from such an inadequate amount of material can be rather very restricted in character and are highly hypothethical. Nonetheless the fact that similar biological adaptations are formed (e.g. syntomy) among species of different origin (from the genera *Astasia, Parastasiella, Dinema, Anisonema, Naupliicola* and *Ovicola*) and in different geographical regions is in favour not only of a great plasticity of these protozoans but also suggests the existence of common conditions of development which depend on internal characteristics. Moreover, numerous data show that these potential possibilities restricted in some directions can appear to some extent under the influence of the 1-st order environment and are somewhat modelled by it. This can be demonstrated by the differences in the course of the life cycle between *Astasia ovorum* and all the parasites of the alimentary tract belonging to this genus and the observed differences in specificity, variability of the course of the life cycle correlated to the species of the host, etc. The influence of the 2nd order environment is also easily discernible. This influence causes ecological and geographical variability within species.

In the theory of the evolution an important role is ascribed to the interspecific and intraspecific relations (struggle for life). *Euglenoidina parasitica* furnish some data concerning this problem.

Where the interspecific relations are concerned — they were studied in the case of *Astasia cyclopis* (1956). Basing on mass and individual cultures of hosts and parasites it has been ascertained that in populations with a small number of specimens of *A. cyclopis* their development goes normally. In populations overcrowded with more than 14—15 specimens the development of the whole populations is slower (sometimes twice as slow), the growth of some specimens is arrested, they grow slower, and only a part of them leave the intestine of the host as they mature, their maturation being not synchronous. Finally all the parasites mature and leave the host. Only seldom when the population is very dense (up to 99 specimens) was the formation of the mature forms of the parasite observed. These formes leaved the instestinal tract of the host twice as small as the normal forms. These phenomenons occur in the same way in the case of populations arising from simultaneous infection as well as in those formed by several infections one following another.

In the egg and larvae parasites of *Copepoda* the phenomenon of intraspecific competition between specimens of parasites which could cause death of a part of the population is not observed. Unknown mechanisms ensure in some

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species the penetration of an egg of the host by only one specimen and in the case of the parasites of the nauplional eye — the penetration of a very restricted number (1—4) of specimens. It is not known whether only these specimens infect this egg or whether those that do not reach the eye for some reason perish.

The lack of data concerning the interspecific relations among *Astasia parasitica* is caused by the fact that individual species do not meet in the same geographical regions. The supposed coinfections of the egg and larvae parasites consisting in the fact that in the egg integuments of the same specimen of host there are eggs infected by different species of parasites (e.g. *Naupliicola burdigalensis* and *N. necans* in *Eucyclops serrulatus*, *Parastasiella vastans* and *Dinema agile* in *Diacyclops languidus* 1966 g) have litlle in common with the discussel problem.

The real coinfections of parasites are encountered fairly rarely. They consist in the occurence in the same egg or in the same larva of the host of different species of parasites. In experimental cultures of proper hosts of *Naupliicola necans* and *N. truncans* the population of *N. necans* gradually diminished. This can be explained by the fact that in the case of coinfection of both species the population of *Naupliicola truncans* develops more rapidly causing the death of the larvae of the host before the second parasite can increase in number. In the case of coinfection of *Naupliicola cystinatus* and *N. necans* in the larvae of *Cyclops vicinus* the latter parasite increased in number near the cysts of *Naupliicola cystinatus*, the integuments of the dead larva bursted and in this manner the way was made free for the cysts of the partner, the cysts being abandoned afterwards by the flagellate forms (1966 c). During coinfection by *Naupliicola ocelli* and *Dinema naupliorum* in *Eucyclops serrulatus* as a rule the first parasite perished as the one that developed slower and a similar situation could be observed in the case of coinfection by *Naupliicola ocelli* and *N. parvus* (1966 f).

As it can be seen, a great variability of situations is observed, and it is possible that relations which could be described as antagonistic occur together with neutral ones.

The biology of *Euglenoidina parasitica* shows interesting problems from the point of view of the strugle for existance.

Observations of parasites of the intestinal tract favour the thesis that the natural selection acts on the whole "parasite-host" system as a biological entity, causing mutual adaptation of both partners (Michajłow 1960). The problem is different, however, where the parasites of the eggs and larvae causing their death are concerned. The specific transition from the semi-predatory way of life of periodically obligatory parasites (*Dinema agile*) to the endoembrional obligatory parasitism should be noted. It is characteristic of this kind of parasitism that the only food of the parasite is the material of the egg or the larva of the host. If a female of a cyclops is considered as a host, then she remains alive, of course, althoug part of her progeny dies (in experimental cultures maintained longer, often the whole populations of hosts perish out). This situation should be studied more closely from the viewpoint of the natural selection, considering the statistical data which would be possibly accumulated in the future experiments. There is no doubt even at present, however, that *Euglenoidina* — specific endoembrional parasites of *Cyclopidae* — are an important factor regulating the number of

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populations of these hosts in the neutral environment. Thus it is difficult to consider the mutual adaptations of parasites and hosts within the system and under the influence of the natural selection acting in this case each partner of the system separately (e.g. *Euglenoidina* during their free life in the water and on the cyclops the egg pouches of which are sometimes for a longer period of time filled with eggs containing parasites that develop more slowly than the larvae the life cycle of which is thus indubitably disturbed). It is difficult to assume the possibility of the simultaneous action of selection in a determined direction on the system as an entity. However the fact that the development of parasites in the body cavity of some species lasts longer than the mean time is noteworthy. This lasts sometimes till the formation of the metanauplius IV and V or oven copepodite II. This is the case of e.g. *Naupliicola burdigalensis* in *Acanthocyclops vernalis* (1966 g), *Naupliicola fennicus* in *Megacyclops viridis* (1966 e), *Naupliicola snagovensis* in the nauplional eye of *Megacyclops viridis* (1966 d), *Naupliicola ghanensis* in the nauplional eye of *Microcyclops varicans* (1966 h). It is probable that this indicates the possibility of transition from so specific form of parasitism as the endoembrional parasitism to the parasitism proper in the body cavity of the mature form of the cyclops, conserving the ability of reproduction and perishing only after having born progeny, i.e. to qualitatively different way of parasitism from the evolutionary point of view. The presence of a small number of species (*Dinema cyclopis, Naupliicola cyclopis*) which cause the death of the host after it had reached sexual maturity shows in a way this direction of the progress of evolution.

Possibilities of further studies

Above all it is desirable to continue the studies in the same countries where the existence of different species of *Euglenoidina* — parasites of *Copepoda* has been reported. It is important not only to study further typical water bodies including also the mesotrophic ones, but also to gain knowledge of the seasonal dynamics of the occurence and variability of systems, and, if possible, to study the phenological vicariate of hosts. The studies conducted till the present have not revealed the presence of spores in any species. As a rule the flagellate forms perish in the water after some time even in the case of the periodically — obligatory parasites. However, the presence of species of *Euglenoidina* possessing resting forms in aquatic environment is not excluded, especially when parasites specific towards species of *Cyclopidae* appearing periodically are concerned.

It seems desirable to couple geographical studies to experimental ones as these two forms complement one another. This has been used with good results in the studies concerning *A. cyclopis* (1956, 1957) and *A. norvegica* (1964 d). This renders possible observation of some phenomena (e.g. ecological vicariate, the influence of the 2nd order environment) which would be difficult for studies when using one method only.

By means of experimental cultures together with histochemical and biochemical studies it would be possible, perhaps, to ascertain the deeper essence of the relations between the parasites and hosts and specificity of systems, to make clear the mechanism of penetration of the flagellate forms

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into the hosts, etc. Some knowledge on the specificity of systems can be gained by the method of forming experimental systems composed of partners having different areas including the ones that are geographically very distant.

Similar methods can also give good results and lead among others to the description of new species of *Euglenoidina parasitica* in geographical regions that have not been studied in this respect. The ascertaining of a larger and not only more accurate map of the distribution of the systems "*Euglenoidina-Cyclopidae*" can play an important role in the solution of general problems concernign the arising and development of parasitism or oven organic evolution.

The results of the studies conducted up to data show that among *Copepoda* the representatives of the family *Cyclopidae* succumb to the infection of numerous species of *Euglenoidina*. The occasionally studied species *Diaptomidae* and *Temoridae* (*Heterocope appendiculata* Sars) did not succumb to infection (1964 d). It seems desirable, however, to extend studies on other *Calanoida* and on *Harpaticoida* as the possible negative result would confirm the specificity of *Euglenoidina parasitica* towards *Copepoda-Cyclopoida*.

Data concerning *Euglenoidina* parasitizing other hosts than *Copepoda* are very scarce till the present. They are restricted to reports concerning the occurence of *Astasia captiva* in *Catenula lemane* (Beauchamp 1911), *Astasia* sp. in *Trilobus gracilis* (Nieschultz 1922), *Astasia chaetogastris* from *Chaetogaster diastrophus* (M. et R. Codreanu 1928), *Astasia* sp. in *Stentor coerulens* (Schonfeld 1959), *Hegneria leptodactyli* and *Euglenomorpha hegneri* from the intestine of tadpoles (according to Dogiel et al. 1962).

A more detailed study of these species from further hosts belonging to different groups of the animal kingdom and their comparison with the species parasitizing *Copepoda* could make possible a more detailed knowledge of the role of the 1-st order environment in the process of speciation of the parasites from the same systematic group.

Parasites of *Copepoda* not belonging to *Euglenoidina* are known. They include among others a representative of *Dinoflagellata* — a parasite of the eggs of *Copepoda* — *Chytriodinium parasiticum* (Dogiel). The present author has encountered in the eggs of *Copepoda* among others the unidentified protozoans forming one flagellum and numerous "pseudopods" after having left the host (1966 c). This is an indication of the presence of other species of parasitic protozoans than those known and described. A more detailed study of parasites of *Copepoda* belonging to other protozoans than *Euglenoidina* could reveal valuable indications concerning the role and changes of the palingenetic features and properties of protozoans of different origin and invading the same first order environment, thus making the role of the external environment in the process of the organic evolution more clear.

Summary

In the monograph concerning the *Euglenoidina* parasitizing *Copepoda*, the author after some introductory remarks reviews the systematics of the 50 species known at present belonging to the genera *Astasia, Parastasiella, Anisonema, Dinema, Dinemula, Mononema, Paradinemula, Naupliicola* and *Ovicola*. Further on the palingenetic (ancestral) and cenogenetic features of

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the parasites are discussed. Their adaptations to parasitism are reviewed separately for the parasites of the intestinal tract, and for the egg and larvae parasites of *Copepoda*. The outstanding biological adaptations in this polyphyletic and polygenic group are analysed also from the viewpoint of their general tendency on the background of individual variability observed within the species. A separate chapter is devoted to the specificity of the "parasite--host" systems, further the dependence of these systems on the second order environment in its ecological and zoogeographical aspect is discussed. In the part devoted to the problems of evolution of *Euglenoidina parasitica* the up to date conclusions are put together. This is ended by the chapter in which the desirable further studies are indicated.

STRESZCZENIE

W szkicu monograficznym dotyczącym *Euglenoidina* — pasożytów *Copepoda* autor, po uwagach wprowadzających zajmuje się przede wszystkim systematyką dotąd poznanych 50 gatunków, należących do rodzajów *Astasia, Parastasiella, Anisonema, Dinema, Dinemula, Mononema, Naupliicola, Paradinemula, Ovicola*. W dalszym ciągu omówione są cechy palingenetyczne (ancestralne) oraz cenogenetyczne tych pasożytów. Ich adaptacje biologiczne do pasożytniczego trybu życia rozpatrywane są oddzielnie dla pasożytów jelit, jaj i larw *Copepoda*. Uwydatniające się w tej polifiletycznej i poligenicznej grupie adaptacje biologiczne są przeanalizowane także z punktu widzenia ich ogólnego kierunku na tle zmienności osobniczej obserwowanej w obrębie gatunków. Osobny rozdział poświęcony jest specyficzności układów „pasożyt-żywiciel", następnie zaś rozpatrywana jest zależność tych układów od środowiska drugiego rzędu w aspekcie ekologicznym i zoogeograficznym. W rozdziale dotyczącym problemów ewolucji *Euglenoidina parasitica* zebrane są dotychczasowe wnioski w tym zakresie. Pracę zamyka rozdział, w którym naszkicowane są pożądane kierunki dalszych badań.

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Department of Systematic Zoology, University of Łódź, Łódź, Narutowicza 68, Poland

Maria WOLSKA

Study on the family *Blepharocorythidae* Hsiung. IV. *Pararaabena dentata* gen. n., sp. n. from the intestine of Indian elephant

Badania nad rodziną *Blepharocorythidae* Hsiung. IV. *Pararaabena dentata* gen. n., sp. n. z jelita słonia indyjskiego

The ciliate which has been the subject of the present study resembles in its general outline and in some structural details to that one which has been described in my previous publication (Wolska 1967 b) — *Raabena bella* Wolska. Some of its features however are so specific that I found it adequate to distinguish this ciliate as a new genus *Pararaabena* gen.n.

Material and methods

As material, a sample of excrements of the Indian elephant from Łódź zoological garden and sample of the Indian elephant from the Warszawa zoological garden was used. The ciliate under study occurs in samples less frequently than *Raabena bella* and undergoes decomposition more quickly. Only the body shape and the ciliature could be characterized in some individuals found in this condition.

The silver solution of Bielszowski was applied for impregnation. The method is described in the previous publication (Wolska 1967 b). Some details were observed in the material fixed with formalin without staining.

Pararaabena dentata g. n., sp. n.

The ciliate has an ovoid outline and is flattened laterally (Fig. 1 A, B, Pl. I 1). Dimensions: lenght 50—60 μ, thickness 28—36 μ. In the anterior body part, on the left side, near the dorsal margin, a lobular frontal process is protruding. On the ventral anterior body wall — similarly as in the other *Blepharocorythidae* studied by me — is an elevation produced by the vacuole. The posterior part of body is equiped with processes (Pl. I 1). The right margin of the buccal overture runs along the body margin, sinks backwards on the left side approx. down to the level of the posterior margin of the vacuole. The buccal overture leads to the buccal cavity producing a funnel which reaches up to the middle of the body length or further, and bends towards the ventral side. Near the ventral body margin, one or two vacuoles (possibly

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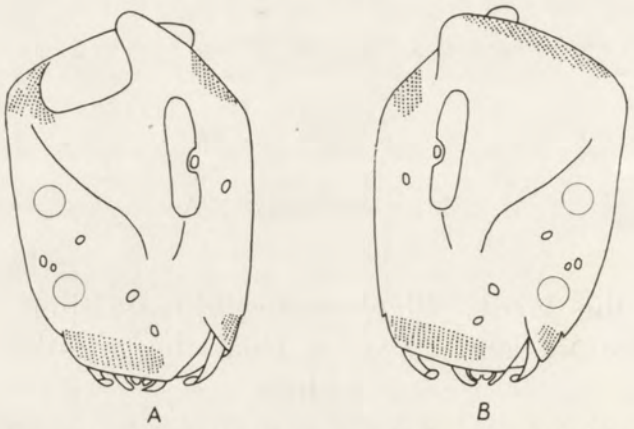

Fig. 1. *Pararaabena dentata* gen. n., sp. n.; general view; A — left side, B — right side

contractile) are present. Numerous refringent oval or spherical bodies are scattered all over the cytoplasm. Cytopyge occupies a terminal position on the posterior pole. The elongated macronucleus lies in the middle of the body length nearer the dorsal margin. The small ovoid micronucleus is shifted into the depression of the macronucleus.

The somatic ciliature consists of four zones, out of them two lie in the anterior body part, and two in the posterior one. One of the anterior zones

Fig. 2. *Pararaabena dentata* gen. n., sp. n.; the buccal ciliature

runs along the margin of the buccal overture on its right and ventral side and partly on the left side. This zone corresponds to the ciliature of the ventral lip in the other *Blepharocorythidae*. On the ventral side, several short kineties, being the prolongation of this zone, encroach upon the vacuole

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(Pl. I 2). Another anterior zone lies at the base of the frontal process, partly on the left and partly on the right body side. The posterior zones lie on the lobular processes (Fig. 1 A, B). The ventral zone lies on the ventral lobe which encroaches slightly upon the right side and extends broadly on the left side (Pl. I 3). The dorsal zone lies on the dorsal lobe, which covers mostly the right side of the posterior body pole, and passes slightly to the left side (Pl. I 4). Out of those lobes protrude two thin leaf-like processes, the ventral and the dorsal one. Two smaller — ventral and dorsal — processes lie more centrally.

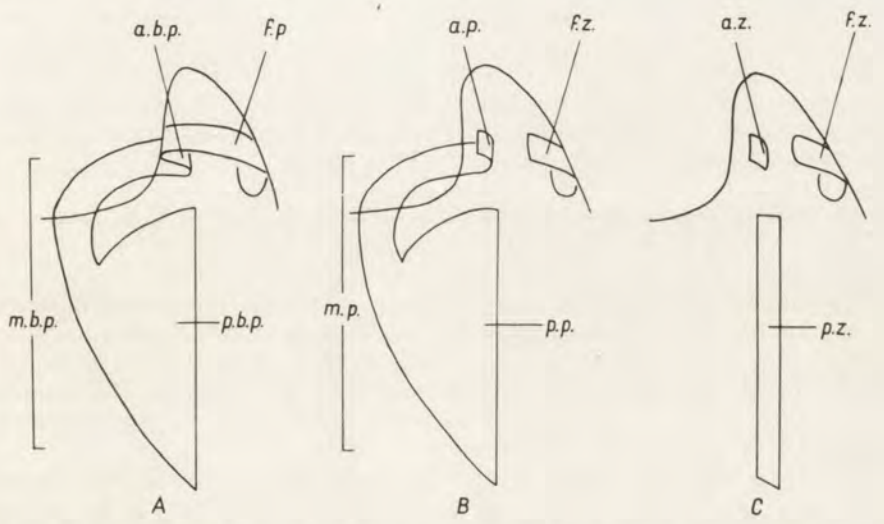

Fig. 3. Scheme of buccal ciliature *Raabena, Pararaabena, Blepharocorys.* A. Fronto--buccal zone of *Raabena.* Frontal part (*f.p.*), anterior buccal part (*a.b.p.*), middle buccal part (*m.b.p.*), posterior buccal part (*p.b.p.*). B. Frontal zone and buccal zone of *Pararaabena.* Frontal zone (*f.z.*), anterior part of buccal zone (*a.p.*), middle part of buccal zone (*m.p.*), posterior part of buccal zone (*p.p.*). C. Frontal zone, and two buccal zones of *Blepharocorys.* Frontal zone (*f.z.*), anterior buccal zone (*a.z.*), posterior buccal zone (*p.z.*)

The buccal apparatus of *Pararaabena dentata* resembles to that of *Raabena bella,* however in *P. dentata* the buccal ciliature is differentiated from the somatic one whereas in *R. bella* exists only one fronto-buccal zone. In the photogram (Pl. I 5) a part of the frontal zone and the anterior segment of the buccal zone are seen. The buccal ciliary zone of *P. dentata* (Fig. 2, Pl I 6) has in its anterior part the shape of a band constituted of oblique kineties which penetrates into the posterior part of the buccal cavity and extends abruptly owing to elongation of kineties. The buccal kineties elongate as well forwards as backwards coating the major part of the right buccal cavity wall. Initial segment of the buccal kineties band lies on the internal surface of the flat frontal process. At the base of the process, the band passes upon the right wall of the buccal cavity, describes an arch on it running backwards and towards the ventral wall, and then becomes broader. Consequently this part

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of the right buccal cavity wall which is covered with kineties, lies nearer the ventral wall. Semicircular fibers describing a part of the right wall as well as the dorsal (Pl. I 6) and left wall, leave vertically the long buccal kinety which is the nearest to the dorsal wall. On the left wall, those fibers form anastomoses (Pl. I 5) and terminate at the ventral wall. The central (arched) part of the buccal zone is surrounded by fibers of the same disposition as in *R. bella*. Forwards of this framing, on the right wall of the buccal cavity, the pattern of fibers is also similar to those in *R. bella*, however those fibers run separated along their whole course and are very thin.

Only one individual in division was found. This specimen proved that all the ciliary zones (the anterior, posterior and the buccal) arise independently one of another and of the parental ciliature. Consequently the morphogenesis of division shows the same character as in the other representatives of *Blepharocorythidae* (W o l s k a 1966, 1967 a and 1967 b). This specimen clearly indicates also that the semicircular part of buccal zone constitutes an integrity with the long posterior buccal kineties (Pl. I 7).

Discussion

The ciliate just described has all the characters of the representatives of the family *Blepharocorythidae*. Owing to the disposition of its ciliary zones as well as to the presence of the frontal process, as to the character of its division morphogenesis it fits to the frame of this family. The enigmatic vacuole with special kinetosomes which was found by me in some species of *Blepharocorys*, occurs also in *P. dentata*.

Is there a sufficient reason to form a new genus for this ciliate? It seems that there is one. *P. dentata* differs from the genus *Blepharocorys* by its buccal ciliature not differentiated into two parts and by the presence of two posterior somatic zones. It differs of the genus *Raabena* by the independent buccal ciliature which is not associated with the somatic zone lying at the base of the frontal process. An independent buccal ciliature and two posterior zones occur also in the genus *Charonina* (W o l s k a 1967 a) however the characteristic processes at the posterior body end are absent in this genus. The posterior processes distinguish however the genus *Pararaabena* of all the genera of this family.

Considering the buccal ciliature of the genera: *Blepharocorys, Raabena, Pararaabena* it appears that *Pararaabena* should be placed between the more primitive genus *Raabena* and genus *Blepharocorys* which is more advanced in its evolution. The genus *Charonina* has been placed by me at the same position as *Pararaabena* on account of its independent but not differentiated buccal ciliture (W o l s k a 1967 a). Nevertheless the juxtaposition: *Raabena — Pararaabena — Blepharocorys* is more interesting than in the case of *Raabena — Charonina — Blepharocorys*. It may be clearly indicated in the first case how could the phylogenetic development lead to formation of the anterior buccal zone and of the long buccal kineties in *Blepharocorys*. This may be the result of a gradual disappearing of some parts of the somato-buccal (fronto-buccal) exit zone in the primitive form — *Raabena*.

The first phase of reduction of a part of ciliature on the surface of the frontal process would transform the uniform fronto-buccal zone into two

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indepedent ones: the frontal and the buccal zone (*Pararaabena*). The second phase of reduction could concern the middle part of the buccal zone leaving only its anterior part in a form of a small zone, and its posterior one as several long terminal kineties (*Blepharocorys*). Fig. 3 A represents schematically the shape of the fronto-buccal zone in the genus *Raabena*. After elimination out of this scheme of a part of ciliature on the frontal process, the scheme 3 B is left which presents the frontal and buccal zones of the genus *Pararaabena*. Cancelling the central part of the buccal zone of *Pararaabena* leaves in the scheme the buccal ciliature divided into two parts (Fig. 3 C) as it really is the case in *Blepharocorys*. Proportions of zones and their localization are of course in each of those genera somewhat different. The scheme illustrates those realtions in a general manner without respecting the changes of the body shape. If the development really followed this course, the directions of the long buccal kineties in *Blepharocorys* should be inverted. This is evident in the genera *Raabena* and *Pararaabena* — as the drawing clearly shows. In *Blepharocorys*, only the position of kinetodesmes might solve the problem but my material provides no evidences. Nevertheless the possibility of those transformations appears to be very probable. The schemes are very suggestive. The presence of processes at the posterior end of the body in *Pararaabena* proves a great possibility of differentiation in this group of ciliates.

Diagnosis of the genus *Pararaabena* gen. nov.

The ciliate is flattened laterally. The extensive buccal overture on the left body side. Four zones of somatic ciliature. One of the anterior zones on the so called ventral lip, the second one at the base of the frontal process. Two remaining somatic zones on the lobular processes embracing the posterior body pole. The buccal ciliature initiates as a band of kineties on the interior side of the frontal process, passes subsequently upon the right wall of the buccal cavity, here describes an arch, broadens in the posterior part of the buccal cavity, coating a considerable part of its area. One or two contractile (?) vacuoles at the ventral body wall. At the posterior pole, besides the ciliated lobes, two pairs of leaf-like processes.

Type of the genus: *Pararaabena dentata* gen. nov., sp. nov.

Intestine parasite of the Indian elephant.

Summary

A new species and a new genus of the family *Blepharocorythidae* of the intestine of the Indian elephant has been described. The relationship between the genera *Raabena, Pararaabena* and *Blepharocorys* is discussed.

STRESZCZENIE

Autorka opisuje nowy gatunek i nowy rodzaj orzęska z rodziny *Blepharocorythidae* z jelita słonia indyjskiego. Autorka rozważa stosunki pokrewieństwa jakie zachodzą między rodzajem *Raabena, Pararaabena* i *Blepharocorys*.

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EXPLANATION OF PLATE I

Pararaabena dentata gen. nov., sp. nov.

1: General view
2: Part of the anterior somatic ciliary zone and kineties on vacuole
3: Posterior body end, left side
4: Posterior body end, right side
5: Anterior body end, frontal zone and anterior part of buccal zone
6: Buccal ciliary zone, optical section
7: Buccal ciliary zone on the territory of the opisthe

Microphotograms of silver impregnated preparations. Magnification: 1—1000×; 2—7 — 2000×

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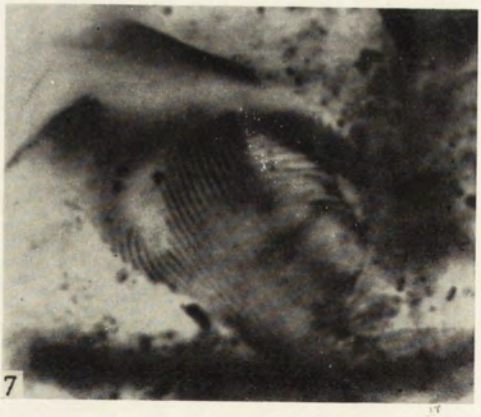

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Institut Zoologique de l'Université Jagellonne, Kraków, Krupnicza 50, Pologne
Collège de France, 11 Place M. Bertholet, Paris 5, France

Anna CZAPIK

La morphologie de *Uronema elegans* Maupas et de *Uronema parva* sp.n.

Morfologia *Uronema elegans* Maupas i *Uronema parva* sp. n.

Le genre *Uronema* Dujardin a attiré l'attention de plusiers chercheurs anciens ainsi que contemporains. Les études sur sa morphologie et sa morphologenèse ont indiqué son importance théorique comme le lien entre les *Tetrahymenina* et les *Pleuronematina*. D'autre part, une des espèces *Uronema marinum* intéresse l'hydrobiologie appliquée comme organisme index des eaux poluées (Liebmann 1954). Parmi les neuf espèces (dont quatre sont incertaines) citées dans la monographie de Kahl trois ont été décrites de nouveau à l'aide des techniques modernes, à savoir: *Uronema marinum* Dujardin (Thompson 1964), *U. acutum* Buddenbrock et *U. filificum* Kahl (Borror 1963, 1965). Récemment Thompson 1964 a créé la nouvelle famille *Uronematidae* comprenant deux genres: *Uronema* et *Paranophrys*.

Je voudrais ajouter ici la déscription de deux autres espèces que j'ai trouvé pendant mon séjour à la Station Biologique de Roscoff; j'ai identifié la première à *Uronema elegans* Maupas et je décris la seconde sous le nom de *U. parva* n.sp. (Fig. 1).

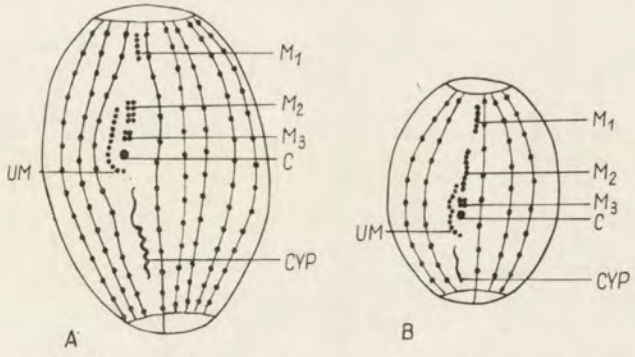

Fig. 1. A — *Uronema elegans* Maupas, B — *Uronema parva* n. sp., UM — la membrane parorale (ondulante), M_1, M_2, M_3 — les membranelles orales, C — le cytostome, CYP — cytoprocte

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Les préparations imprégnées à l'argent ont été exécutées d'après la méthode de Chatton, modifiée par Corliss. Pour *Uronema parva,* très difficile à imprégner, la concetration du nitrate d'argent a dû être augmentée jusqu'à 10% et les préparations sont restées durant une heure dans le réactif. Après avoir introduit ces modifications, j'ai obtenu des préparations très claires et bien détaillées. J'ai trouvé *Uronema elegans* dans un aquarium rempli d'eau marine courante, mais dont le fond était couvert d'une couche épaisse de vase avec des morceaux de bois pourri. Les animaux se ramassaient autour du bois où se développait une riche flore bactérienne.

Le corps de ce Cilié est régulièrement ovoïde; il mesure environ 40 μ de longueur. Ce Cilié possède 21 à 22 méridiens parallèles à l'axe longitudinal du corps; chaque cinétie contient environ 16 cinétosomes qui sont plus serrés dans la partie antérieure du corps. Le péristome est typique du genre *Uronema;* la membranelle M_1 mince et courte, est située vers le haut, près du pôle antérieure; M_2 située plus bas, également courte, est assez large; M_3 est presque ronde; au-dessous d'elle se trouve le cytostome. La membrane parorale UM, qui entoure la cavité buccale, se prolonge jusqu à la moitié de M_2. La cavité buccale atteint à peu près l'équateur du corps. Le cil caudal a comme base deux cinétosomes.

Uronema parva n.sp. a été trouvée également dans le milieu marin à Roscoff. J'ai réussi à la cultiver avec des grains de riz cru. Cette espèce se développait fort bien dans ces conditions et donnait après quelques jours, une culture dense. Le corps de ce cilié est largement ovoïde, le coté ventral aplati; c'est une petite espèce mesurant environ 20 à 30 μ de longueur; les cils sont rangés sur 13 cinéties parallèles contenant chacune 14 à 18 cinétosomes. Le cytostome est situé ici plus bas que chez les autres espèces du genre *Uronema,* au-dessous de l'équateur du corps. La partie postérieure du corps est aplatie par la dépression de la cavité buccale. La membranelle M_1 commence tout

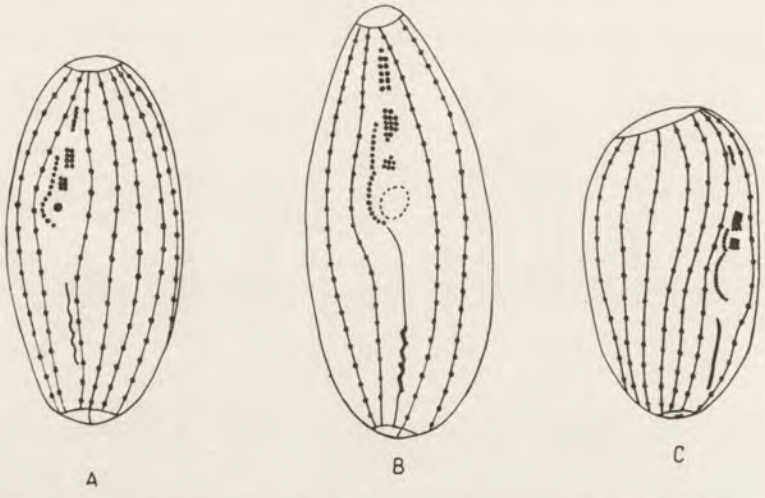

Fig. 2. A — *Uronema marinum* Dujardi n (13—16 cinéties), B — *U. acutum* Buddenbrock (9—12 cinéties), C — *U. filificum* Kahl (16—17 cinéties) B et C d'après Borror

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près du pôle antérieur ainsi que M_2 qui la suit; l'une et l'autre sont minces et plus longues que chez les autres espèces. Il parait qu'elles consistent en une simple rangée de cinétosomes. La membranelle M_3 est très courte et large; la membrane parorale UM, dont la forme est typique et ressemble à celle de *U. filificum*, commence beaucoup plus bas que chez les autres espèces.

En resumant les suivants caractères distinguent *Uronema parva* n.sp. des autres espèces de ce genre: 1. la petite taille (20—30 μ), 2. la forme du corps non allongée, plus large que chez les autres espèces, 3. les cils rangés sur 13 cinéties, 4. le cytostome situé au-dessous de l'équateur du corps.

Chez le genre *Uronema*, le péristome est une structure adaptée à la nourriture bactérienne. Les membranelles, rangées en long l'une après l'autre comme des rames, provoquent un courant d'eau qui est orienté vers le cytostome par la dépression allongée de la cavite buccale. Ce type de structure, qui atteint la perfection dans le groupe *Pleuronematina* est réalisé chez *Uronema* d'une façon plus simple. Les membranelles sont séparées ici l'une de l'autre par des distances plus ou moins grandes. La membrane parorale UM parallèlle au grand axe du corps forme souvent, au milieu de son parcours une petite courbure dont l'angle est orienté vers la cavité buccale. Pourtant, elle n'entoure pas le cytostome comme chez les *Pleuronematina*. Pour faciliter la distinction des espèces on se refera aux trois figures (Fig. 2) représentant les formes décrites à nouveau d'après les indications données par les techniques modernes.

Resumé

On a étudié la morphologie de deux ciliés marins trouvés à Roscoff dont un a été identifié à *Uronema elegans* Maupas et l'autre décrit sous le nom de *Uronema parva* n.sp. Les préparations argentées ont été effectuées d'après la méthode de Chatton à l'aide d'une solution 10% de $AgNO_3$. *Uronema parva* n.sp. possède les suivants caractères qui la distinguent des autres espèces de ce genre: la petite taille (20—30 μ), la forme du corps largement ovoïde, 13 cinéties, le cytostome situé bas, au-dessous de l'équateur du corps.

STRESZCZENIE

Opisano morfologię dwóch morskich orzęsków znalezionych w Roscoff, z których jeden został zidentyfikowany jako *Uronema elegans* Maupas a drugi opisany jako *Uronema parva* n. sp. Preparaty srebrowe sporządzano podług metody Chattona, stosując jednak silniejszy roztwór $AgNO_3$ (10%). *Uronema parva* n. sp. posiada następujące cechy, które odróżniają ją od pozostałych gatunków tego rodzaju: małe rozmiary (20—30 μ), ciało szeroko owoidalne, 13 kinet, cytostom położony nisko, poniżej równika ciała.

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EXPLICATION DE PLANCHE I

1: *Uronema elegans* Maupas
2—5: *Uronema parva* n. sp., 2 — l'aspect général, 3 — le pôle postérieur du corps, 4 — le côté ventral, 5 — la division

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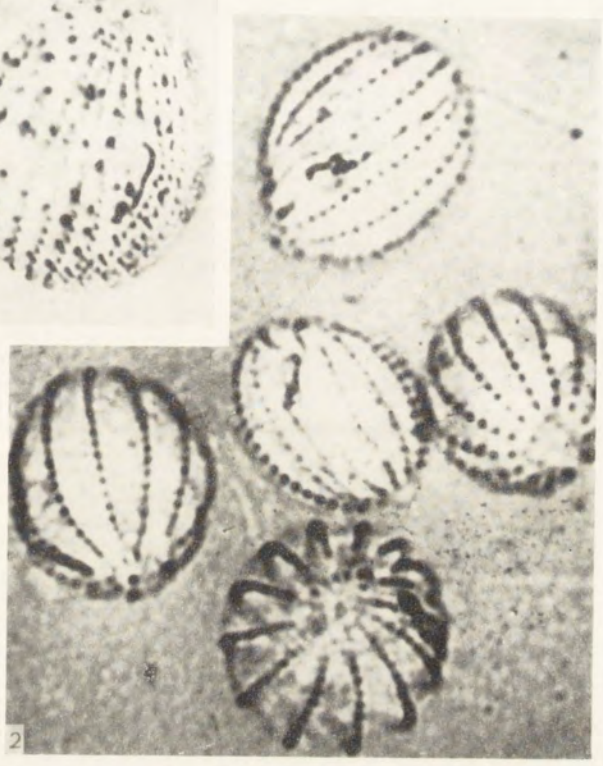

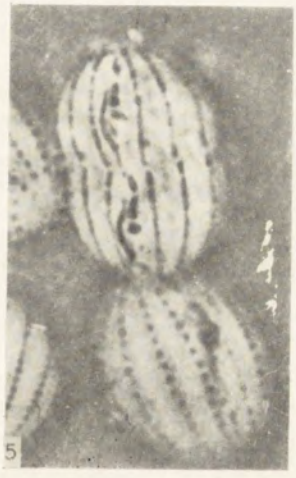

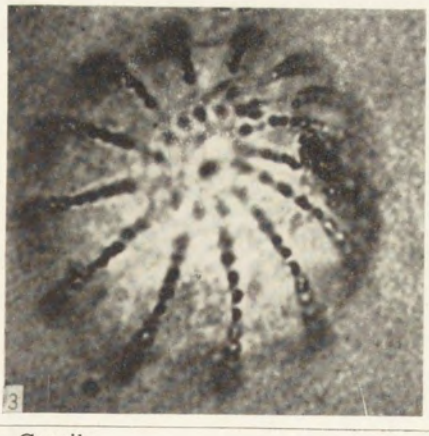

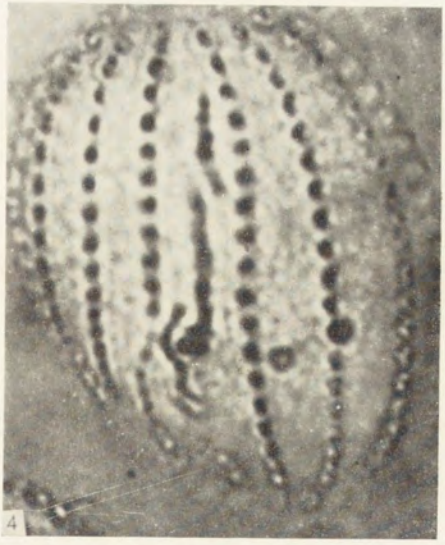

A. Czapik auctor phot.

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Лаборатория цитологии одноклеточных организмов, Институт цитологии Академии Наук СССР, Ленинград Ф-121, Проспект Маклина 32, СССР

Г. А. ШТЕЙН

G. A. STEIN

Паразитические инфузории (*Peritricha, Urceolariidae*) рыб бассейна Амура

Parasitic ciliates (*Peritricha, Urceolariidae*) of fishes of the Amur basin

В 1957—1959 гг. комплексная экспедиция Зоологического института АН СССР и Государственного научно-исследовательского института озерного и речного рыбного хозяйства проводила паразитологические исследования в бассейне реки Амур. В результате многолетних работ был собран богатый материал по паразитам амурских рыб, в том числе и по паразитическим простейшим. С 1961 г. эту работу продолжает сотрудник Амурского отделения ТИНРО С. С. Юхименко. Часть материала по инфузориям *Urceolariidae* была обработана и опубликована Чан Сын Маном в 1961 г. Остальной материал был передан нам. Настоящая статья представляет результаты его обработки.

В нашем распоряжении были сухие мазки с инфузориями, импрегнированными азотнокислым серебром по Клейну, и мазки, дифференцированные пикриновой кислотой после фиксации жидкостью Шаудинна и окраски железным гематоксилином Гейденгайна.

Мазки собраны с представителей 21 вида рыб, принадлежащих к 8 семействам.

Сем. *Salmonidae* — лососевые

Ленок — *Brachymystax lenok* (Pallas)

Сем. *Esocidae* — щуковые

Амурская щука — *Esox reicherti* Dybowski

Сем. *Cyprinidae* — карповые

Чебак, амурский язь — *Leuciscus waleckii* (Dybowski)

Озерный гольян — *Phoxinus percnurus* (Pallas)

Краснопер, амурский плоскоголовый жерех — *Pseudaspius leptocephalus* (Pallas)

Желтопер, подуст-чернобрюшка — *Xenocypris macrolepis* Bleck

Чебачек — *Pseudorasbora parva* (Schlegel)

Амурский белоперый пескарь — *Gobio albipinnatus tenuicorpus* Mori

Пескарь-губач Черского — *Chilogobio czerskii* Berg

Пескарь-лень — *Sacrochilichthys sinensis lacustris* (Dybowski)

Колючий горчак — *Acanthorhodeus asmussi* (Dybowski)

Серебряный карась — *Carassius auratus gibelio* (Bloch)

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Амурский сазан — *Cyprinus carpio haematopterus* Temm. et Schleg.
Белый амур — *Ctenopharyngodon idella* (Val.)
Толстолоб — *Hypophthalmichthys molitrix* (Val.)

Сем. *Cobitidae* — вьюновые

Амурский вьюн — *Misgurnus fossilis anguillicaudatus* (Cantor)

Сем. *Siluridae* — сомовые

Амурский сом — *Parasilurus asotus* (L.)

Сем. *Bagridae* — косатки

Косатка-скрипун — *Pseudobagrus fulvidraco* (Richardson)
Косатка уссурийская — *Liocassis ussuriensis* (Dybowski)

Сем. *Gadidae* — тресковые

Налим — *Lota lota* (L.)

Сем. *Eleotridae* — головешковые

Головешка-ротан — *Perccottus glehni* Dybowski.

На мазках с ленка и пескаря-губача Черского инфузорий не оказалось.

Как было показано Стрелковым и Шульманом 1964, для Амура характерен исключительно неоднородный состав паразитофауны рыб. Наряду с паразитами, типичными для южных и северных водоемов Голарктической области, большое количество видов, обнаруженных в Амуре, отсутствуют в Голарктике, но известны из водоемов Китая и Индии. Такая гетерогенность свойственна не только паразитофауне рыб, но и ихтиофауне и гидрофауне в целом. Последняя характеризуется смещением форм, свойственных ледовитоморской зоогеографической провинции, с некоторыми формами, свойственными сино-индийской области (Берг 1949). Богатство и гетерогенный состав гидрофауны Амура, согласно гипотезам Линдберга и Таранца (по Шульману 1958, Стрелкову и Шульману 1964), явились результатом сложного геологического прошлого Амура, обусловившего его связь с двумя зоогеографическими областями — Голарктической и Сино-индийской. Поэтому было интересно не только уточнить фаунистический состав, но и попытаться дать зоогеографическую характеристику урцеоляриид, относящихся к трем родам: 7 видов *Trichodina*, 2 вида *Trichodinella* и 1 вид *Tripartiella*.

Trichodina nobilis Chen, 1963 (Рис. 1, 2, Pl. I 1, 2)

Довольно крупные инфузории с венчиком зубцов очень характерной формы. Наружные отростки зубцов слабо изогнуты и состоят из широкой треугольной лопасти, которая соединяется с центральной конической частью зубца тонким стеблем. Внутренние отростки тонкие, сужающиеся к концу и изогнуты в том же направлении, что и наружные. Макронуклеус обычной подковообразной формы. Небольшой удлиненный микронуклеус расположен сбоку от макронуклеуса. На импрегнированных препаратах центральная часть прикрепительного диска темная.

Диаметр тела 57.0—91.5, прикрепительного диска 34.5—67.5, венчика 30.0—64.5 μ. Длина наружного отростка 6.0—10.5, внутреннего 6.0—13.5 μ. Число зубцов в венчике варьирует от 18 до 25, чаще всего 21—23 зубца. На каждый зубец приходится 8—14 полос прикрепительного диска. Диаметр макронуклеуса 33.0—58,5, отрезок „x" 7.5—28.5 μ. Длина микронуклеуса 3.0—4.5 μ. Последний лежит сбоку от макронуклеуса на расстоянии 3.0—10.5 μ от его конца (отрезок „y"

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имеет положительное значение). Отношение диаметров тела и прикрепительного диска 1.08—1.53, тела и венчика 1,20—1.69, прикрепительного диска и венчика 1.02—1.28, тела и макронуклеуса 1.31—1.81. Отношение длины наружного и внутреннего отростков 0.44—1.00.

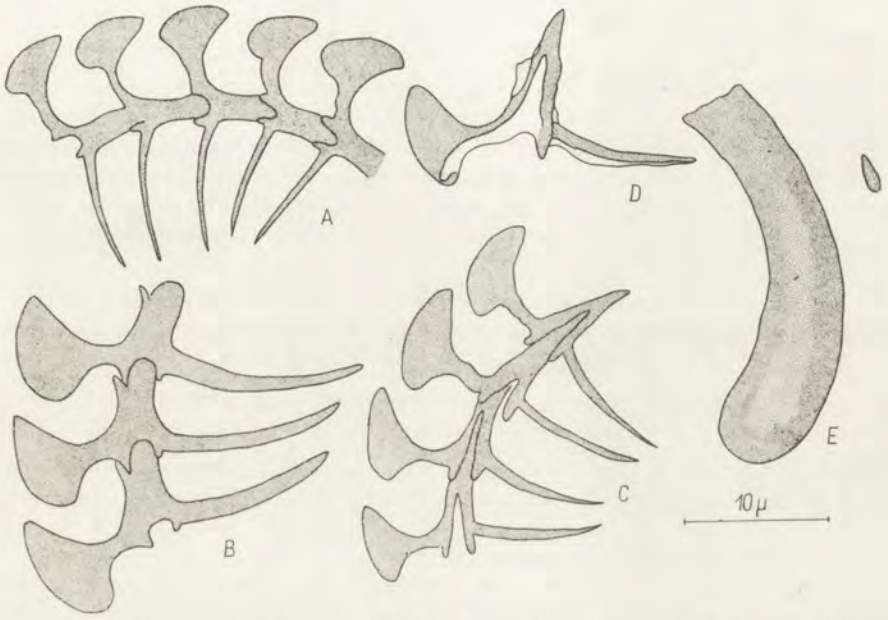

Рис. 1. *Trichodina nobilis* Chen, 1963 с A. B. — *Leuciscus waleckii*, C — *Carassius auratus gibelio*, D — *Hypophthalmichthys molitrix*, E — *Cyprinus carpio haematopterus*. A—D — Разрозненные зубцы, E — ядерный аппарат (фиксация жидкостью Шаудинна, окраска железным гематоксилином)

Fig. 1. *Trichodina nobilis* Chen, 1963 from A, B — *Leuciscus waleckii*, C — *Carassius auratus gibelio*. D — *Hypophthalmichthys molitrix*, E — *Cyprinus carpio haematopterus*. A—D — separated denticles, E — nuclear apparatus (Schaudinn fixation, iron heamatoxylin)

У мелких экземпляров *T. nobilis* тонкие участки лопастей при фиксации и окраске ломаются и могут приобретать причудливую форму. Такие лопасти изображены на рисунке 2. На некоторых препаратах, окрашенных гематоксилином, у основания наружных и внутренних отростков иногда заметны небольшие направленные вперед выросты. По-видимому, они становятся видны в тех случаях, когда при дифференцировке зубцов от них сильно оттягивается краска и окрашенными остаются лишь наиболее плотные части зубцов. Значительно реже эти выросты заметны на препаратах, импрегнированных серебром (Рис. 1). Считая этот признак непостоянным и недостаточно отчетливо выраженным, мы относим описываемых инфузорий все же к роду *Trichodina*, а не *Tripartiella*. Строение адоральной спирали рассмотреть не удалось.

Хозяева: *Leuciscus waleckii, Carassius auratus gibelio, Cyprinus carpio haematopterus, Hypophthalmichthys molitrix, Ctenopharyngodon idella* (?).

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Рис. 2. *Trichodina nobilis* Chen, 1963 с *Leuciscus waleckii* с зубцами причудливой формы (фиксация жидкостью Шаудинна, окраска железным гематоксилином)
Fig. 2. *Trichodina nobilis* Chen, 1963 from *Leuciscus waleckii*, specimen with the denticles of the strange shape (Schaudinn fixation, iron haematoxylin)

Локализация: поверхность тела, плавники, носовые ямки, жабры.
Местообитание: река Амур (Головино), Зея (Черемхово), озеро Болонь. В бассейне Амура и в пределах СССР отмечаются впервые.

Trichodina reticulata Hirschmann et Partsch, 1955. (Рис. 3. Pl. I 3, 4)
(Син. *T. domerguei* f. *megamicronucleata* Dogiel 1940).

Вид широко распространен у пресноводных рыб Европы и многократно описан. Характеризуется своеобразным строением центральной части прикрепительного диска на препаратах, импрегнированных серебром, и наличием очень крупного удлиненного микронуклеуса.

Диаметр тела 37.5—91.5, прикрепительного диска 24.0—61.5, венчика 21.0—54.0 μ. Длина наружных отростков 4.5—9.0, внутренних 4.5—10.5 μ. Венчик состоит из 20—32 зубцов, на каждый зубец приходится 8—10 (у одного экземпляра 12) полос прикрепительного диска. Диаметр макронуклеуса 25.5—40.5, отрезок „x" 7.5—24.0, длина микронуклеуса 7.5—15.0 μ. Микронуклеус чаще всего расположен сбоку от макронуклеуса на расстоянии 6.0—10.5 μ („+y"), реже против его конца (отрезок „y" равен нулю) (Рис. 3). Отношение диаметром тела и прикрепительного диска 1.22—2.12, тела и венчика 1.38—2.42, диска и венчика 1.03—1.40, тела и макронуклеуса 1.39—1.59, венчика и макронуклеуса

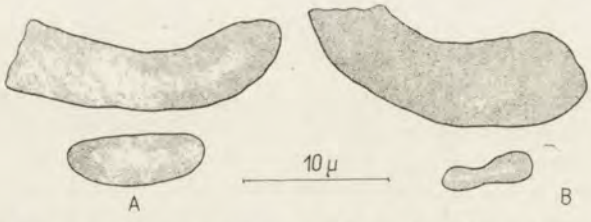

Рис. 3. *Trichodina reticulata* Hirschmann et Partsch, 1955 с A — *Cyprinus carpio haematopterus*, B — *Ctenopharyngodon idella*. Различная форма микронуклеуса (фиксация жидкостью Шаудинна, окраска железным гематоксилином)
Fig. 3. *Trichodina reticulata* Hirschmann et Partsch, 1955 from A — *Cyprinus carpio haematopterus*, B — *Ctenopharyngodon idella*. Various form of macronucleus (Schaudinn fixation, iron haematoxylin)

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0.61—1.12, длины наружного и внутреннего отростков 0.75—1.33. Адоральная спираль образует дугу, немного превышающую 360°. Ширина краевой мембраны 3.0—7.5 μ. Центральная ось внутреннего отростка несколько смещена назад по сравнению с наружным.

Триходины с белого амура из озера Болонь, по сравнению с триходинами с карася и сазана, отличались меньшими размерами и меньшим числом зубцов в венчике. В связи с этим возникает предположение, что в бассейне Амура условия обитания *T. reticulata* на белом амуре менее благоприятны, по сравнению с условиями обитания на карасе и сазане, а сам белый амур для *T. reticulata* является дополнительным хозяином.

Хозяин: *Carassius auratus gibelio, Cyprinus carpio haematopterus, Ctenopharyngodon idella.*

Локализация: поверхность тела, плавники, носовые ямки, жабры.

Местообитание: река Амур (Головино, Хабаровск, Чумка), Зея (Черемхово), озеро Болонь. В бассейне Амура отмечается впервые.

Trichodina strelkovi Chan, 1961 (Рис. 4. Pl. II 5)

Довольно крупные инфузории с хорошо развитым венчиком. Наружные отростки зубцов в виде широких лопастей с закругленной передней стороной и слегка заостренной вершиной. Внутренние отростки прямые или слабо изогнутые, длинные, сужающиеся к концу. Прикрепляются на некотором расстоянии от внутренней стороны центральной части зубцов. Для этого вида характерны массивные центральные конуса зубцов с толстыми стенками и гребневидным выростом на наружной стороне. Наружный и внутренний отростки расположены на одной вертикали. На препаратах, импрегнированных серебром, центральная часть прикрепительного диска темная (Pl. II 5).

Подковообразный макронуклеус, микронуклеус небольшой, округлый или слабо вытянутый, расположен чаще всего сбоку от макронуклеуса. Адоральная спираль характерная для представителей рода *Trichodina*. Суммарные результаты измерений инфузорий с разных хозяев следующие. Диаметр тела 38.6—90.0, прикрепительного диска 31.5—67.2, венчика 24.3—61.5 μ. Венчик состоит из 18—32 зубцов на каждый зубец приходится 8—14 полос прикрепительного диска. Длина наружного отростка 2.9—10.5, внутреннего 5.7—15.0 μ. Диаметр макронуклеуса 25.7—57.2 отрезок „x" 4.3—21.5, длина микронуклеуса 1.4—4.3, отрезок „y" 2.9—34.3 μ и имеет положительное значение.

Отношение диаметров тела и прикрепительного диска 1.04—1.82, тела и венчика 1.22—2.18, прикрепительного диска и венчика 1.0—1.68, тела и макронуклеуса 1.26—2.04, венчика и макронуклеуса 0.77—1.54. Отношение длины наружного и внутреннего отростков 0.50—1.00.

Хозяин: *Parasilurus asotus, Esox reicherti, Carassius auratus gibelio, Percottus glehni,* моногенетический сосальщик *Ancylodiscoides asoti* с жабер *Parasilurus asotus.*

Локализация: жабры, плавники, поверхность тела.

Местообитание: река Амур (Головино), Зея (Черемхово), озеро Гусь.

Единичные экземпляры триходин, отнесенных нами к *T. strelkovi*, были также найдены в районе Черемхово на жабрах *Pseudobagrus fulvidraco,* на жабрах *Misgurnus fossilis anguillicaudatus* и на поверхности тела *Pseudaspius leptocephalus.*

По строению зубцов венчика и по общим размерам *T. strelkovi* обнаруживает наибольшее сходство с *T. reticulata.* На препаратах, импрегнированных

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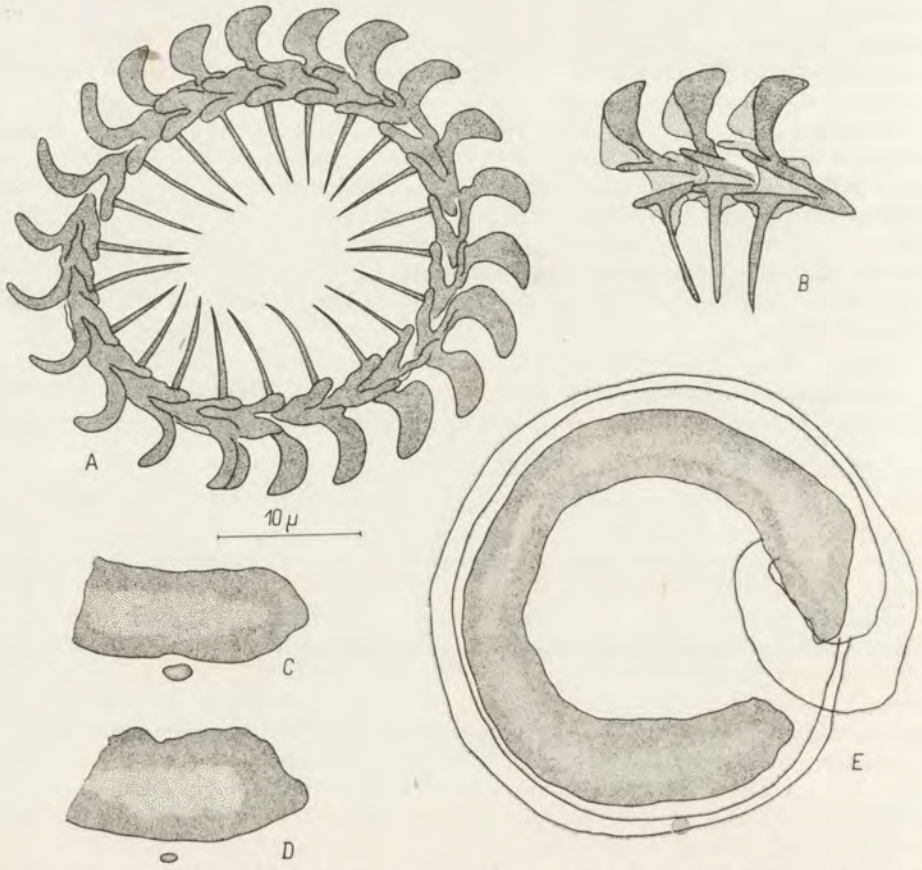

Рис. 4. *Trichodina strelkovi* Chan, 1961 с A — *Perccottus glehni*, B — *Carassius auratus gibelio*, C—E — *Parasilurus asotus*. A — венчик, B — строение отдельных зубцов, C — ядерный аппарат, E — адоральная спираль (фиксация жидкостью Шаудинна, окраска железным гематоксилином)

Fig. 4. *Trichodina strelkovi* Chan, 1961 from A — *Perccottus glehni*, B — *Carassius auratus gibelio*, C—E — *Parasilurus asotus*. A — skeletal ring, B — structure of separated denticles, C — E — nuclear apparatus, E — adorale zone (Schaudinn fixation, iron haematoxylin)

серебром, эти виды четко различаются по строению центральной части прикрепительного диска („сетчатое" у *T. reticulata* — импрегнируемое у *T. strelkovi*). На препаратах, окрашенных гематоксилином Гейденгайна, *T. strelkovi* отличается от *T. reticulata* размерами микронуклеусов, соотношением длины наружного и внутреннего отростков, положением внутреннего отростка по отношению к наружному и т. д. (Таблица 1).

T. strelkovi впервые была описана Чан Сын Маном 1961 с поверхности тела моногенетических сосальщиков, паразитирующих на жабрах *Siniperca chuatsi*, *Parasilurus asotus* и *Esox reicherti*. Особенностью этого вида автор считал положение внутреннего отростка, сильно сдвинутого назад, по срав-

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Таблица 1

Сравнение *Trichodina strelkovi* и *T. reticulata* по биометрическим данным
Comparison of biometrical data of *Trichodina strelkovi* and *T. reticulata*

	T. strelkovi	*T. reticulata*
Длина микронуклеса в μ Lenght of micronucleus in μ	1.4—4.3	7.5—10.5
Отношение диаметров тела и диска Body/adhesive disc diameters ratio	1.04—1.82	1.22—2.12
Отношение диаметров тела и венчика Body/skeletal ring diameters ratio	1.22—2.18	1.38—2.42
Отношение диаметров диска и венчика Adhesive disc/skeletal ring diameters ratio	1.00—1.68	1.03—1.40
Отношение диаметров тела и макронуклеуса Body/macronucleus diameters ratio	1.26—2.04	1.39—1.59
Отношение диаметров венчика и макронуклеуса Skeletal ring/macronucleus diameters ratio	0.77—1.54	0.61—1.12
Отношение длины наружн. и внутр. отростков зубца Denticle blade/ray lenghts ratio	0.56—1.00	0.75—1.33

нению с наружным. Как мы уже отмечали (Штейн 1962), это наблюдение оказалось неверным. По сравнению с данными Чан Сын Мана, в нашем материале *T. strelkovi* имели более крупные размеры.

Trichodina leucisci (Suzuki, 1950). (Pl. II 6)
(Син. *Cyclochaeta leucisci* Suzuki, 1950, *Trichodina ovaliformis* Chen, 1955).

Инфузории среднего размера с диаметром тела 22.9—42.0, диаметром прикрепительного диска 18.6—30.0, венчика 15.7—24.0 μ. Венчик состоит из 22—30 зубцов, наиболее часто 26 и 25 зубцов. Наружные отростки зубцов в виде узких лопастей с почти параллельными краями, расширены на концах и закруглены, в 1.5—4 раза длиннее внутренних шиповидных отростков. Длина наружных отростков 2.9—6.0, внутренних 1.4—3.0 μ. Центральные части зубцов имеют вид тонких узких конусов. Наружные лопасти расположены под небольшим углом к окружности венчика. На каждый зубец приходится 4—6 полос прикрепительного диска. Отношение диаметров тела и диска 1.12—1.79, тела и венчика 1.43—1.79, диска и венчика 1.07—1.43.

Хозяин: *Xenocypris macrolepis, Sarcochilichthys sinensis lacustris, Ctenopharyngodon idella, Pseudorasbora parva.*

Локализация: жабры.

Местообитание: река Амур (Головино), озеро Хиванда, река Зея (Черемхово), Будунда.

С некоторым сомнением к этому виду были отнесены также инфузории с жабер *Lota lota* и *Phoxinus percnurus* из озера Хиванда.

Suzuki 1950 описал *T. leucisci* в Японии с поверхности тела, плавников и жабер *Leuciscus hacuensis* Günter, а Чен Чи-лю (Chen Chih-leu 1955) с *Ctenopharyngodon idella* из прудов Цзянсу, Чжэцзян и Кантон в Китайской Народной Республике.

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Основанием для сближения амурских инфузорий с инфузориями из Японии и Китая в первую очередь послужили форма и число зубцов в венчике. Вместе с тем следует отметить, что инфузории из бассейна Амура имели более крупные размеры и иное соотношение диаметров тела и венчика, чем у инфузорий, описанных Чен Чи-лю. Возможно, что различия в размерах связаны с обитанием в естественных (бассейн Амура) и искусственных (пруды) водоемах.

При сравнении с другими видами следует отметить наибольшее сходство *T. leucisci* с *Tripartiella bulbosa* (Davis 1947). Это сходство выражается в абсолютных размерах и в соотношении наружных и внутренних отростков зубцов. Вместе с тем, наружные отростки зубцов *T. bulbosa* имеют вид лопастей, равномерно широких на всем протяжении, с закругленными концами и расположены перпендикулярно к окружности венчика. У основания наружного отростка имеется выступающий вперед вырост наружной стороны центральной части зубца, на основании чего (а также длины адоральной спирали — 180°—270°) эти инфузории были отнесены к роду *Tripartiella*. Среднее число зубцов в венчике *T. bulbosa* меньше, чем у *Trichodina leucisci*. В бассейне Амура *T. leucisci* отмечается впервые.

Trichodina amurensis Chan, 1961 (Рис. 5)

Сравнительно мелкие инфузории с диаметром тела 22.9—50.5, прикрепительного диска 17.2—30.0, венчика 14.3—22.9 μ. Венчик состоит из 20—25 зубцов, на каждый зубец приходится 6—8—10 полос прикрепительного диска. Наружный отросток зубца в виде треугольной лопасти с плотной прямой осевой частью. При дифференцировке после гематоксилина Гейденгайна краска легко оттягивается и сама лопасть обесцвечивается, так что наружный отросток на таких препаратах имеет вид прямой палочки. Внутренний отросток расположен на одной вертикали с наружным, прямой и шиповидный, отстающий от

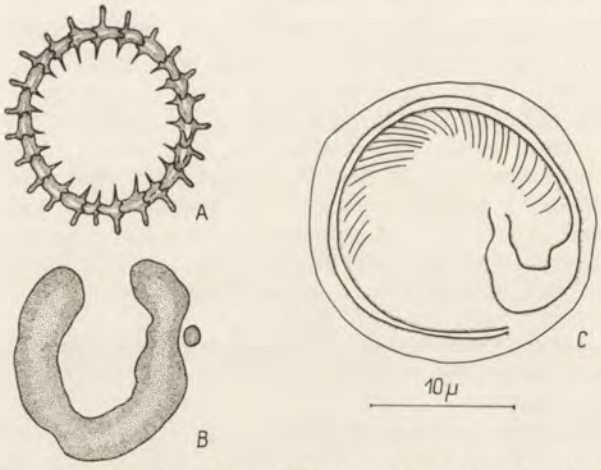

Рис. 5. *Trichodina amurensis* Chan, 1961 с жабер *Pseudaspius leptocephalus*. A — венчик, B — ядерный аппарат, C — адоральная спираль (фиксация жидкостью Шаудинна, окраска железным гематоксилином)

Fig. 5. *Trichodina amurensis* Chan, 1961 from the gills of *Pseudaspius leptocephalus*. A — skeletal ring, B — nuclear apparatus, C — adoral zone (Schaudinn fixation, iron haematoxylin)

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края внутренней стороны центральной части зубца. Длина наружного отростка 2.9—4.3, внутреннего 1.4—4.3, в обоих случаях преобладало 2.9 μ.

Ядерный аппарат состоит из подковообразного макронуклеуса и довольно крупного округлого микронуклеуса. Последний лежит сбоку от макронуклеуса, чаще против выемки, на довольно большом расстоянии от его конца. Диаметр макронуклеуса 12.9—40.0, микронуклеуса 1.4—2.9, отрезок „x" 2.9—15.7, отрезок „y" 4.3—18.6 μ и имеет положительное значение. В одном случае микронуклеус лежал между ветвями макронуклеуса („−y" = 2.9 μ). Адоральная спираль чуть меньше полной окружности (310°—355°).

Отношение диаметров тела и прикрепительного диска 1.06—1.46, тела и венчика 1.33—1.90, диска и венчика 1.10—1.58, тела и макронуклеуса 0.81—2.10, венчика и макронуклеуса 0.72—1.33. Отношение длины наружного и внутреннего отростков 0.67—2.00, однако эти данные следует принимать с некоторым сомнением, так как из-за небольших размеров зубцов их очень трудно измерить.

Хозяин: *Pseudaspius leptocephalus, Liocassis ussuriensis.*

Локализация: жабры.

Местообитания: река Зея (Черемхово).

В Определителе паразитов пресноводных рыб СССР (1962) *T. amurensis* была ошибочно отнесена нами к роду *Tripartiella*. Переисследование показало, что у этого вида зубцы венчика лишены типичного для представителей рода *Tripartiella* направленного вперед выроста наружной стенки центральной части зубца.

При сравнении наших данных с описанием Чан Сын Мана близкими оказались абсолютные размеры инфузорий, тогда как отношение диаметров тела и венчика, тела и макронуклеуса существенно отличались. Сравнить форму зубцов оказалось невозможно из-за неудачных иллюстраций в работе Чан Сын Мана. Однако, учитывая сходство в размерах тела и ядер, в соотношении наружных и внутренних отростков зубцов, в числе зубцов, а также паразитирование на одном и том же хозяине (*Pseudaspius leptocephalus*) мы отнесли описываемых инфузорий к *Trichodina amurensis Chan*, 1961.

Trichodina domerguei f. *acuta* Lom, 1961 (Pl. II 7)

Вид описан Ломом с пресноводных рыб в Чехословакии. В бассейне Амура отмечается впервые. Обнаружен на поверхности тела, плавниках и жабрах *Carassius auratus gibelio* из Амура (Головино).

В центре прикрепительного диска светлая неимпрегнированная зона сравнительно небольшого диаметра (приблизительно 10μ). Зубцы, образующие венчик, с широкими массивными центральными конусами. Лопасти сравнительно короткие, с заостренными или слабо закругленными концами. Передний край лопасти более или менее зазубрен. Внутренние отростки мощные, конусовидно сужающиеся к концу. Весь венчик в целом производит впечатление очень массивного. Удалось промерить один и сосчитать число зубцов в венчике у трех экземпляров.

Диаметр тела 72.0, прикрепительного диска 46.5, венчика 42.0μ. Венчик состоял из 18, 18, 25 зубцов, на каждый зубец приходилось 12 полос прикрепительного диска. Длина наружного отростка 7.5, внутреннего 9.0μ. Ширина краевой мембраны 4.5μ. Отношение диаметров тела и прикрепительного диска 1.55, тела и венчика 1,71, прикрепительного диска и венчика 1,11, длины наружного и внутреннего отростков 0.83.

Инфузории с карася из Амура и с рыб Чехословакии оказались очень

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близки по размерам. Незначительные различия в диаметре венчика, длине отростков, максимальном числе зубцов, числе полос могут быть результатом индивидуальной изменчивости.

Trichodina nigra Lom, 1960 (Pl. II 8)

Триходины с диаметром тела 55.5—67.5, прикрепительного диска 30.0—42.0, венчика 27.0—33.0μ. Венчик из 18—24 зубцов, на каждый зубец приходится 8—10 полос прикрепительного диска. Наружные отростки лопастевидные, с закругленной, как правило, вершиной. Передний край также закруглен, а задний вогнут. Внутренние отростки прикрепляются на одной вертикали с наружными, прямые, палочковидные или шиповидные. Длина наружных и внутренних отростков 4.5—6.0 (6.0)μ. Ширина краевой мембраны 3.0—4.5μ. Отношения диаметра тела и прикрепительного диска 1.81—2.02, тела и венчика 1.85—2.12, прикрепительного диска и венчика 1.0—1.27. Отношение длины наружного и внутреннего отростков 0,75—1,33, чаще 1.0. На препаратах, импрегнированных серебром, центральная часть прикрепительного диска темная. Строение ядерного аппарата осталось невыясненным.

По строению прикрепительного диска и по биометрическим данным триходины с поверхности тела толстолоба почти полностью совпадают с типичными *T. nigra*, описанными Ломом с поверхности тела многих пресноводных рыб Чехословакии (L o m 1961).

Хозяин: *Hypophthalmichthys molitrix*

Локализация: поверхность тела.

Местообитание: река Амур (Головино).

В бассейне Амура *T. nigra* отмечается впервые.

Trichodina sp.

На жабрах *Gobio albipinnatus tenuicorpus* был обнаружен один плохо сохранившийся экземпляр инфузории, венчик которой состоял из тесно сближенных зубцов с широкими лопастями, напоминающими *T. domerguei* f. *latispina*. Обращает на себя внимание большее число зубцов и венчике (34).

Диаметр тела 65.8, прикрепительного диска 55.8, макронуклеуса 28.6, отрезок „x" 4.3, отрезок „y" 12.9μ и имеет положительное значение. Микронуклеус овальный, его длина 2.9μ.

Одновременно с *Trichodina* sp. на жабрах пескаря обнаружены инфузории, которых мы отнесли к роду *Tripartiella*.

К роду *Trichodina* без определения видовой принадлежности были отнесены также единичные экземпляры инфузорий, обнаруженных у *Perccottus glehni*, *Acanthorhodeus asmussi*, *Pseudobagrus fulvidraco*.

Tripartiella sp. (Рис. 6).

На жабрах *Gobio albipinnatus tenuicorpus* из реки Зеи (Черемхово). Отдельные зубцы, сохранившиеся на препарате, по своему строению напоминают зубцы *Tripartiella copiosa*, описанной L o m 1959 с жабер горчака *Rhodeus sericeus* из водоемов Чехословакии.

На препаратах, окрашенных гематоксилином Гейденгайна, наружные отростки зубцов короткие, прямоугольной формы, внутренние отростки шиповидные, также короткие и слабо изогнутые. Наружная сторона центральной части зубца вытянута вперед в виде небольшого округлого выроста. На каждый зубец приходится 5 полос прикрепительного диска. Зубцы, как и полосы, удалось просчитать лишь у одного экземпляра (21). Макронуклеус утолщен-

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Таблица 2

Сравнение *Trichodinella epizootica* и *T. longispira* (измерения в μ)

Comparison of *Trichodinella epizootica* and *T. longispira* (measurements in μ)

Паразит Parasite	*T. longispira*	*Trichodinella epizootica*		
Хозяин Host	*Esox reicherti*	*Carassius auratus gibelio* (II)	*Lota lota*	*Carassius auratus gibelio* (*I*)
Диаметр тела Diameter of body	28.6—50.1	21.5—31.5	27.2—45.8	18.0—30.0
Диаметр прикрепительного диска Diameter of adhesive disc	24.3—35.8	15.7—22.9	18.6—30.0	15.0—21.0
Диаметр венчика Diameter of skeletal ring	21.5—28.0	14.3—17.2	15.7—20.0	10.5—18.0
Длина наружного отростка зубца Lengh of outer blade of denticle	2.9—5.7	2.9	1.4—2.9	1.5—3.0
Число зубцов в венчике Number of denticles	22—28	18—21	19—27	19—23
Число полос прикрепительного диска Number of radial pins	8—12	4—6(? 8—12)	6—10	4—5(8—10)
Диаметр макронуклеуса Diameter of macronucleus	20.0—30.0	12.9—20.0	10.0—27.2	12.0—21.0
Отрезок „x" Section „x"	7.2—18.6	2.9—11.4	1.4—15.7	3.0—9.0
Диаметр микронуклеуса Diameter of micronucles	2.9—4.3	1.4—2.9	2.9—4.3	3.0
Отрезок „y" Section „y"	+5.7—15.7	0, +4.3—8.6	0, +2.9—8.6	+4.5—9.0
Адоральная спираль Adoral zone	315°	180°	180°	—
Отношение диаметров тела и прикрепительного диска Body/adhesive disc diameters ratio	1.05—1.57	1.31—1.69	1.18—1.80	1.08—1.50
Отношение диаметров тела и венчика Body/skeletal ring diameters ratio	1.25—1.93	1.74—2.00	1.36—2.42	1.40—2.00
Отношение диаметров диска и венчика Adhesive disc/skeletal ring diameter ratio	1.10—1.33	1.10—1.46	1.08—1.67	1.11—1.57
Отношение диаметров тела и макронуклеуса Body/macronucleus diameters ratio	1.20—1.79	1.31—1.91	1.58—2.72	1.00—1.90
Отношение диаметров венчика и макронуклеуса Skeletal ring/marcronucleus diameter ratio	0.76—1.00	0.80—0.92	0.67—1.44	0.67—1.12

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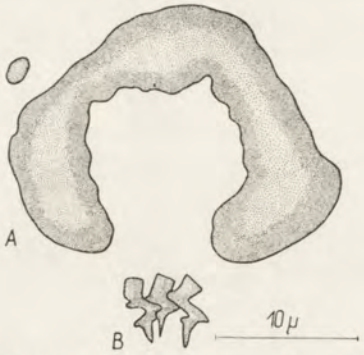

Рис. 6. *Tripartiella* sp. с *Gobio albipinnatus tenuicorpus*. А — ядерный аппарат, В — зубцы (фиксация жидкостью Шаудинна, окраска железным гематоксилином)
Fig. 6. *Tripartiella* sp. from *Gobio albipinnatus tenuicorpus*. A — nuclear apparatus, B — denticles (Schaudinn fixation, iron haematoxylin)

ный, подковообразный, микронуклеус сравнительно крупный, овальный или почти округлый.

Диаметр тела 18.6—34.3, прикрепительного диска 17.2—18.6, венчика (1 экз.) — 15.7μ. Диаметр макронуклеуса 12,9—25.7, отрезок „x" 2.9—10.0, диаметр микронуклеуса 1.4—2.9, отрезок „y" 1.4—12.9μ и имеет положительное значение.

Отношение диаметров тела и прикрепительного диска 1.25—1.84, тела и венчика 2,18, диска и венчика 1.18, тела и макронуклеуса 1.0—1.44, венчика и макронуклеуса 0.65.

Плохая сохранность прикрепительных дисков и недостаток материала (7 экз.) не позволили более точно определить видовую принадлежность инфузорий с пескаря.

Инфузории рода *Trichodinella* были обнаружены у амурской щуки, серебряного карася и налима и представлены двумя видами — *Trichodinella lon-*

Рис. 7. *Trichodinella epizootica* (Raabe, 1950) с жабер карася *Carassius auratus gibelio*, ядерный аппарат и адоральная спираль (фиксация жидкостью Шаудинна, окраска железным гематоксилином)
Fig. 7. *Trichodinella epizootica* (Raabe, 1950) from the gills of *Carassius auratus gibelio*. Nuclear apparatus and adoral zone (Schaudinn fixation, iron haematoxylin)

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gispira и *T. epizootica*, причем *T. epizootica* с карася и налима отличались по своим размерам и отнесены к разным формам. Результаты измерений представителей рода *Trichodinella* приведены в таблице 2.

Trichodinella epizootica (Raabe, 1950) (Рис. 7)

Обнаружены на жабрах *Carassius auratus gibelio* из озера в районе Черемхово и из озера Болонь. Следует отместить, что по биометрическим данным триходинеллы из озера Болонь и из района Черемхово несколько отличались друг от друга.

Trichodinella epizootica f. *lotae* (Chan, 1961)

(Син. *T. percarum* f. *lotae* Chan, 1961).

Обнаружены на жабрах налима из озера Хиванда. От типичных *T. epizootica* инфузиории с налима отличаются увеличением абсолютных и относительных размеров. Это и дало Чан Сын Ману повод выделить инфузорий с налима в виде самостоятельной группы. От описания Чана инфузории из нашего материала отличались меньшими размерами.

Trichidinella longispira G. Stein, 1962 (Рис. 8)

Вид был описан с жабер *Esox reicherti* из реки Зеи (Черемхово). Как уже отмечалось Штейн 1962, от других представителей рода *Trichodinella longispira* отличаются длиной адоральной спирали (около 315°). Зубцы, образующие венчик, с удлиненными конусами. Выступ наружной стенки центрального конуса отчетливо выражен.

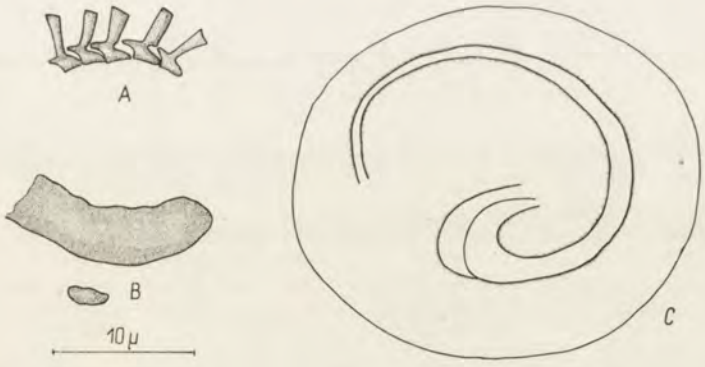

Рис. 8. *Trichodinella longispira* G. Stein, 1962 с *Esox reicherti*. A — зубцы, B — ядерный аппарат, C — адоральная спираль (фиксация жидкостью Шаудинна, окраска железным гематоксилином)

Fig. 8. *Trichodinella longispira*, G. Stein, 1962 from *Esox reicherti*. A — denticles, B — nuclear apparatus, C — adoral zone (Schaudinn fixation, iron haematoxylin)

Триходинеллы, обнаруженные на рыбах Амура, по длине адоральной спирали могут быть разделены на две группы: инфузории, спираль которых образует дугу примерно в 180° (*T. epizootica* и *T. epizootica* f. *lotae*) и инфузории с более длинной, около 315°, спиралью (*T. longispira*). Обе группы обна-

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руживают большое сходство в строении зубцов венчика: центральные части зубцов конусовидные, лопасти плоские, перпендикулярны к окружности венчика, их концы тупо срезаны. Все различия сводятся к количественным признакам: число зубцов в венчике, диаметр тела, прикрепительного диска, венчика и т. д. (Таблица 2). Поэтому в настоящее время триходинеллы представляют наибольшую трудность при определении.

Наряду с другими инфузориями, единичные экземпляры триходинелл были обнаружены на жабрах сазана, вьюна и белого амура. Установить их видовую принадлежность не удалось.

Как уже упоминалось в начале статьи, для Амурской зоогеографической провинции свойственна большая гетерогенность всей гидрофауны в целом, в том числе и паразитофауны рыб. Неоднородной оказалась в бассейне Амура и фауна урцеолярий, насчитывающая в настоящее время по нашим данным и данным Чан Сын Мана 1961 13 видов. Предварительно урцеолярий с амурских рыб можно разделить на следующие группы:

1. Виды широко распространенные. Кроме Амура известны в водоемах Голарктики. К этой группе мы относим *Trichodina domerguei* f. *acuta*, *Trichodinella epizootica*, *Tripartiella* (*? T. copiosa*).

2. Виды кроме водоемов Голарктической области и Амура описаны в Китае — *Trichodina reticulata*, *T. nigra*.

.3. Виды кроме Амура зарегистрированны в Китае и Японии, — *Trichodina leucisci*, а *T. nobilis* — в Китае. Есть основания предполагать, что вместе с акклиматизируемыми китайскими растительноядными рыбами (белый амур, толстолоб) в прудовые хозяйства Советского Союза были завезены и их паразиты — *T. leucisci* и *T. nobilis*, которые могут быть обнаружены при соответствующих исследованиях.

4. Виды зарегистрированны пока только в Амуре — *Trichodina strelkovi*, *T. amurensis*, *T. poljanskyi*, *T. bychowskyi*, *T. soldatovi*, *Trichodinella longispira*.

Возможно, что перечисленные виды являются амускими эндемиками. В пользу этого предположения говорит тот факт, что большинство видов рыб — хозяев перечисленных видов инфузорий (*Parasilurus asotus*, *Esox reicherti*, *Pseudaspius leptocephalus*, *Silurus soldatovi*) также эндемичны для Амура. Однако, все высказанные здесь предположения нуждаются в проверке. Поэтому продолжение и расширение фаунистических работ не только у нас в стране, но и за ее пределами, представляет большой интерес для решения ряда интересных вопросов в области зоогеографии и в других разделах паразитологии.

Резюме

На мазках с 21 вида рыб, принадлежащих к 8 семействам, обнаружено 10 видов инфузорий. Впервые в бассейне Амура отмечаются *Trichodina nobilis*, *T. reticulata*, *T. leucisci*, *T. domerguei* f. *acuta*, *T. nigra*. Подобно всей гидрофауне, урцеолярииды рыб Амура оказались неоднородными по своему составу. Предположительно всех известных для Амура урцеолярий разделяют на следующие четыре группы: 1. Виды широко распространенные. Кроме Амура известны из водоемов Голарктической области (*Trichodina domerguei* f. *acuta*, *Trichodinella epizootica*, *Tripartiella*). 2. Виды кроме водоемов Голарктики и Амура описаны в Китае — *Trichodina reticulata* и *T. nigra*. 3. Виды кроме Амура зарегистрированные в Китае и Японии — *Trichodina leucisci*, и в Китае — *T. no-*

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bilis. 4. Вероятно амурские эндемики — виды, зарегистрированные только в Амуре — *Trichodina strelkovi, T. amurensis, T. poljanskyi, T. bychowskyi, T. soldatovi, Trichodinella longispira.*

SUMMARY

In the smears taken from 21 species of fishes belonging to 8 families, the presence of 10 ciliate species was ascertained. The species *Trichodina nobilis, T. reticulata, T. leucisci, T. domerguei* f. *acuta, T. nigra* have been sygnalized for the first time in the fauna of the Amur basin. The composition of the *Urceolariidae* fauna of this region is not uniform, similarly as its whole hydrofauna.

The division of the Amur *Urceolariidae* into 4 groups is suggested. 1. Species of a wide distribution. Besides Amur, known from the waters of the Holarctic region (*Trichodina domerquei* f. *acuta, Trichodinella epizootica, Tripartiella*). 2. Species, besides the waters of Holarctic and Amur, described from China (*Trichodina reticulata, T. nigra*). 3. Species sygnalized — besides the Amur waters — in China and Japan: *Trichodina leucisci* and in China: *T. nobilis*. 4. Possibily the Amur endemic species, sygnalized from Amur only: *Trichodina strelkovi, T. amurensis, T. polianskyi, T. bychovskyi, T. soldatovi, Trichodinella longispira.*

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ПОДПИСИ К ТАБЛИЦАМ I—II

1—2: *Trichodina nobilis* Chen, 1963. 1 — прикрепительный диск, 2 — адоральная спираль
3—4: *Trichodina reticulata* Hirschmann et Partsch, 1955. 3 — прикрепительный диск, 4 — адоральная спираль
5: *Trichodina strelkovi* Chan, 1961, прикрепительный диск
6: *Trichodina leucisci* (Suzuki, 1950) прикрепительный диск
7: *Trichodina domerguei* f. *acuta* Lom, 1961, прикрепительный диск
8: *Trichodina nigra* Lom, 1960, прикрепительный диск
1, 3, 5—8 — импрегнация азотнокислым серебром; 2 и 4 — фиксация жидкостью Шаудинна, окраска железным гематоксилином

EXPLANATION OF PLATES I—II

1—2: *Trichodina nobilis* Chen, 1963. 1 — adhesive disc, 2 — adorale zone
3—4: *Trichodina reticulata* Hirschmann et Partsch, 1955 3 — adhesive disc, 4 — adorale zone
5: *Trichodina strelkovi* Chan, 1961, adhesive disc
6: *Trichodina leucisci* (Suzuki, 1950) adhesive disc
7: *Trichodina domerguei* f. *acuta* Lom, 1961, adhesive disc
8: *Trichodina nigra* Lom, 1960, adhesive disc
1, 3, 5—8 — silver impregnation, 2 and 4 — Schaudinn fixation, iron haematoxylin

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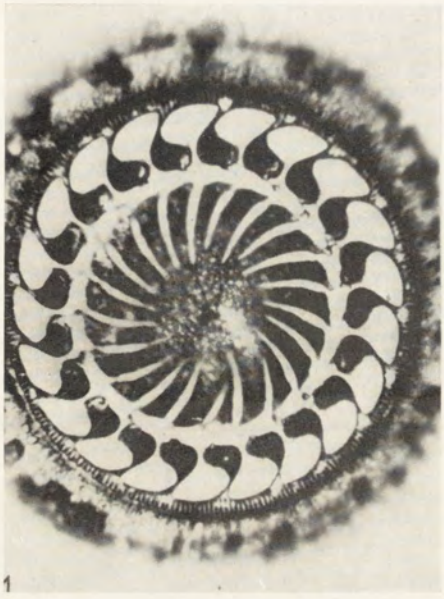
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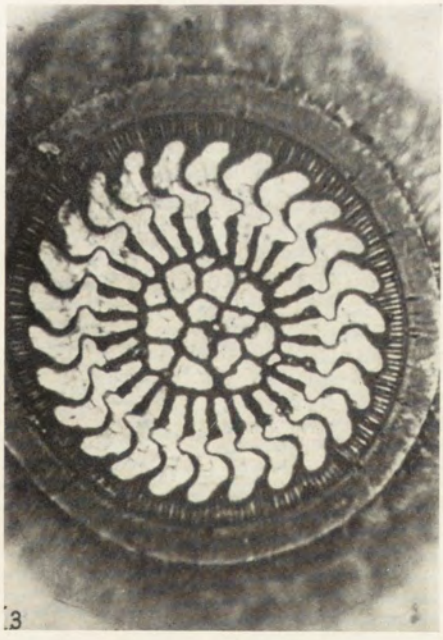
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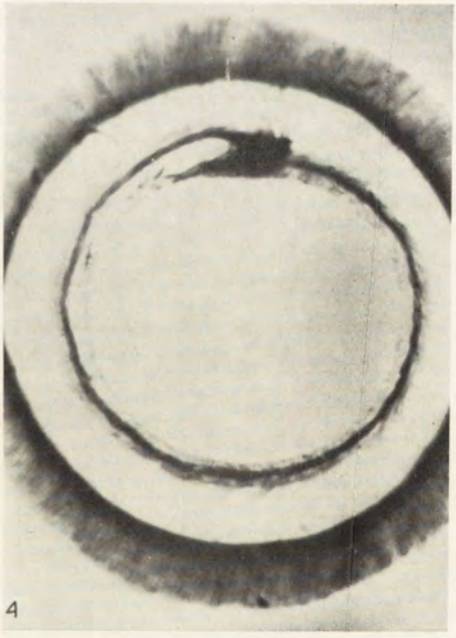
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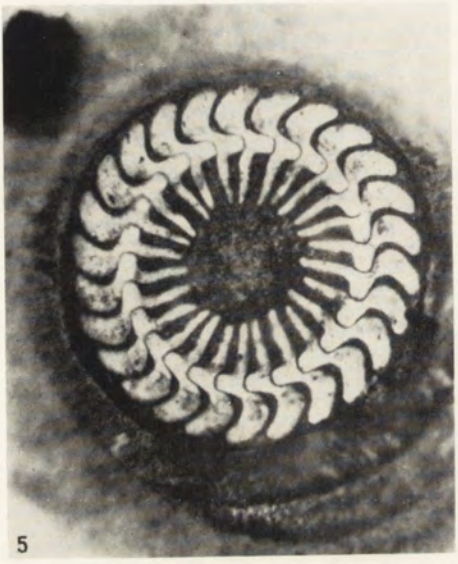
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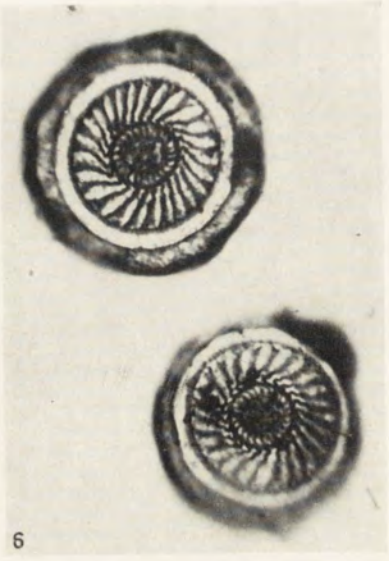
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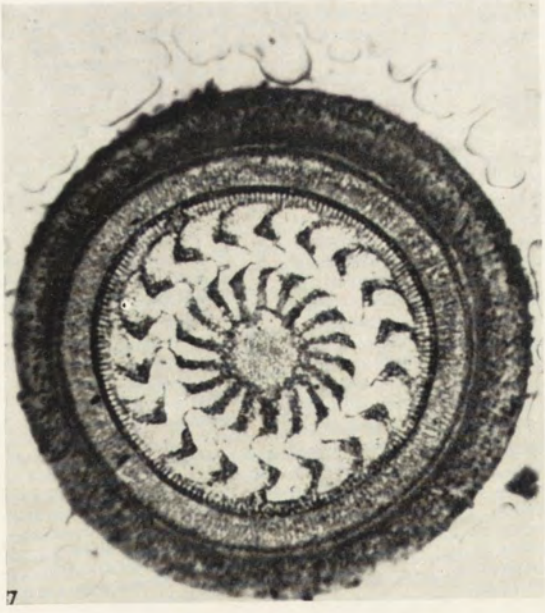
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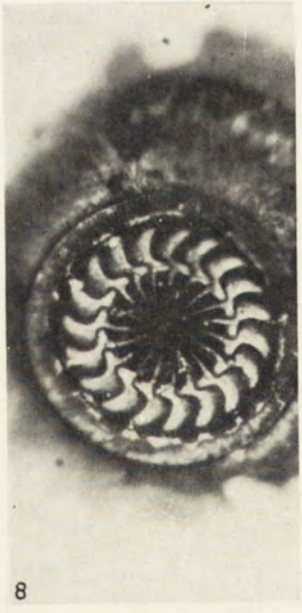
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Department of Zoology, Chelsea College of Science and Technology, Manresa Road, Chelsea, London S.W.3, England

Tim J. BROWN*

A reconsideration of the nomenclature and taxonomy of *Aspidisca costata* (Dujardin, 1842), *(Ciliata)*

Neue Erwägungen über die Nomenklatur und Taxonomie von *Aspidisca costata* (Dujardin, 1842), *(Ciliata)*

The correct naming and systematic position of the species *Aspidisca costata* (Duj.) have been vexatious questions. Researches into the early literature have shown that the commonly used name "*A. costata* (Duj.)" is a junior synonym of *A. cicada* (Müller). Since the first description of the species by Müller 1786, a number of different names have been proposed for what is recognisably the same organism, while at the same time certain workers have clearly confused several other related species with the original *A. costata*. In addition there have been a number of changes in generic status.

Materials and methods

During a quantitative survey of the protozoan fauna of an activated sludge tank at the North Surrey Joint Sewerage Board purification plant, *Aspidisca costata* (Duj.) was found to be abundant throughout the year. Single individuals of this organism were separated from the sludge with micropipettes and introduced into petri dishes containing 15 ml. of medium. The subsequent asexual division of the Protozoa produced clonal cultures. The medium consisted of 50% 0.025% Oxoid liver broth and 50% 0.03% egg extract in distilled water. The observations of earlier workers were compared with the 'type organisms' from the clonal cultures, and are discussed in the light of modern protozoological methods.

Discussion

Aspidisca costata (Duj.) was first observed and named by Müller 1786, as *Trichoda cicada* Müller. Müller defined *Trichoda cicada* as follows: "*Trichoda* ovalis, marginibus obscuris, antice et subtus crinita, postice mutica." [Oval *Trichoda* with obscure margins, hairy (ciliated) anteriorly and ventrally, truncated posteriorly].

One of his diagrams, all of which are very crude, does indicate that the organism possesses dorsal longitudinal ridges (Fig. 1). This first diagnosis of

* Present address: Department of Zoology, Massey University, Palmerston North, New Zealand.

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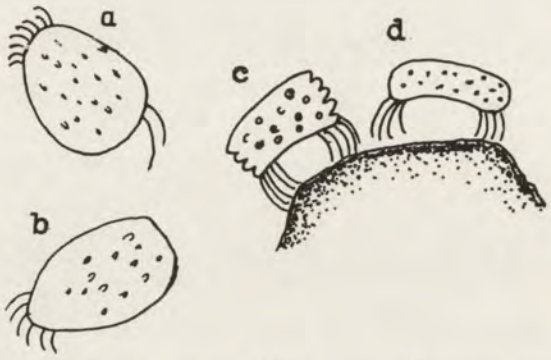

Fig. 1. *Trichoda cicada* Müller (after Müller 1786)

Trichoda cicada with its reference to the truncated posterior aspect leads the author to conclude that this species is identical with the form now commonly known as *Aspidisca costata* (Duj.). Schrank 1803 lists *T. cicada* Müller in a comprehensive work on ciliates and other "animalicules" which were at that time grouped with the Protozoa. He refers directly to the work of Müller 1786. Bory 1824, who also refers to Müller's work, transferred *Trichoda cicada* to the genus *Coccudina* as *Coccudina cicada*.

Bory defined his new genus *Coccudina* as follows:

"Genre *Coccudine. Coccudina;* N. (new genus)"

"Molecular body attached beneath a crystalline test which is hollow and free at the edges, and which is in the form of a small hood; the cirri are used principally for walking. The convex side of the body is always dorsal. No tract of heart or ovaries can be distinguished." (Translation)

In this genus he included a number of ciliates previously referred to other genera and even species; for example, *Kerona patella* (Müller) became *Coccudina Keronia* N. and is now recognised as *Eutoples patella* (Müller). In his description of *Coccudina cicada* he includes Müller's diagrams and a definition of *Trichoda cicada* Müller.

In 1838, Ehrenberg redefined the genus *Oxytricha*, which is recognised today as containing number of hypotrich species. Ehrenberg states that the organisms in this genus are "...sans styles et crochets, dépourvu de cones."

Ehrenberg then proceeds to define a species *O. cicada* in the following terms: "*O.* corpore albo, ovato, fere hemispherico, ventro plano, sulcao crenatque." [*Oxytricha* with white oval body, sub-hemispherical, flat ventrally ridged and notched].

Ehrenberg also includes the illustrations reproduced in Fig. 2. In the detailed decription of this species of hypotrich Ehrenberg stated that *O. cicada* possesses 8—13 dorsal ridges which are crenated or notched. This species therefore is clearly not identical with *T. cicada* Müller or *C. cicada* (Müller), as described by Bory, because in spite of the very crude illustrations and descriptions of all these authors, it is clear that *C. cicada* (syn. *T. cicada*) does not possess ribs which are crenated or notched. This feature is recognised as an important systematic character by more modern workers. Ehrenberg's species *O. cicada* is clearly a different organism from the animal generally

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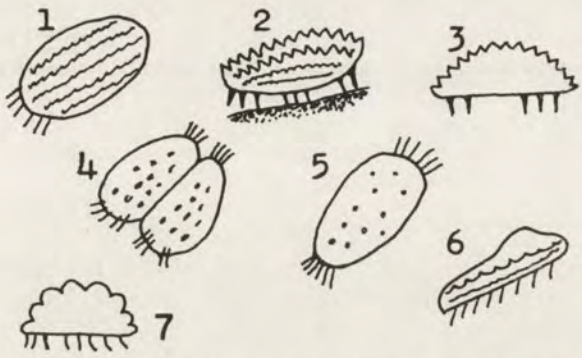

Fig. 2. *Oxytricha cicada* Ehrenberg (after Ehrenberg 1838)

recognised as *Aspidisca costata* (Duj.), on account of the number and form of the ridges (Fig. 2); but Müller's *T. cicada* (syn. Bory's *C. cicada*) does appear to be identical with *A. costata* (Duj.), for reasons which will be subsequently set down.

Ehrenberg 1830 defined the genus *Aspidisca* and created the family *Aspidiscidae;* he transferred to this genus, as its type, the species *Trichoda lynceus* Müller (as *Aspidisca lynceus*). Ehrenberg defined the genus *Aspidisca* as follows (in translation):

"Polygastric animals which have a carapace and a distinct intestinal tract with two openings of which only the anus is terminal."

It is therefore evident that the original descriptions of the genera *Oxytricha* and *Aspidisca* as given by Ehrenberg are inadequate as they are not based on the number and arrangement of the cilia (cirri), the criteria adopted by modern workers, or any type of external configuration which is distinctive. Hence it is not always easy to recognise the identity of species defined by Ehrenberg, and this applies especially to *O. cicada* Ehr., whose specific name is the same as that of *T. cicada* Müller, with which it is clearly not identical. *Oxytricha cicada* Ehr., would not today be recognised as a species of *Oxytricha* but rather as a species of *Aspidisca.* However, it could not become *Aspidisca cicada,* as that specific name is already preoccupied (*A. cicada,* syn. *T. cicada* Müller).

Dujardin 1842 is the first authority to describe a hypotrichous ciliate as *Coccudina costata* Duj.; this species is recognised by the possession of five to six markedly projecting, tuberculated dorsal ribs. Dujardin regards *C. costata* Duj. as identical with *O. cicada* Ehr. Dujardin 1842 also described a species which he named *C. cicada,* but unlike Müller, Shrank and Bory, he does not indicate that this species *C. cicada* is identical with *T. cicada* Müller, although, as already indicated, Müller shows *T. cicada* as having some form of dorsal rib structure.

At this point it is suggested that whereas the difference between teeth and tubercles on the ribs is only one of degree, the difference between plain and crenated ribs is more absolute and important as a specific character. Claparède et Lachmann 1861 transferred certain species of *Coccudina* and *Oxytricha* to Ehrenberg's genus *Aspidisca,* which they redefine as follows:

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"Les *Aspidisca* se distinguent facilment de tous les autres genres de la famille par l'absences des cirrhes frontaux." The frontal cirri of Claparède et Lachmann are not those recognised as frontal cirri by modern workers, but they appear to represent the extreme anterior region of the adoral zone of membranellae which is well developed in all other families of hypotrichs except the *Aspidiscina*. Therefore their diagnosis is fundamentally in line with that of modern workers.

Claparède et Lachmann discuss briefly the classification of the genera contained in the Family *Oxytrichina*. Their original key showing the diagnostic features of each genus is given in Table 1. It should be noticed that they are primarily concerned with the position and form of the cirri for their classification, while Ehrenberg in his definition of the genus *Aspidisca* does not mention the cirri. Claparède et Lachmann also criticise Ehrenberg's classification of his Family *Aspidiscidae*. They say that their Family *Oxytrichina* corresponds almost exactly to the three Families *Aspidiscidae, Oxytrichidae* and *Euplotidae* of Ehrenberg. While Ehrenberg rightly classified the *Euplotidae* and the *Oxytrichidae* together in his Order *Catotreda,* because the "mouth and anus" of both families open on the ventral surface, he then assigned the *Aspidiscidae* to another order on the mistaken assumption that the "anus" was terminal. Claparède et Lachmann have shown that the "anus" of the genus *Aspidisca* is in fact on the ventral surface, and therefore this genus should be classified with *Euplotes* and *Oxytricha*. The reason for this digression on the classification

Table 1

Tableau des genres de la famille de Oxytrichiens*

<table>
<tr><td rowspan="8">*Oxytrichina*</td><td rowspan="3">Des cirrhes marginaux</td><td rowspan="2">Des pieds-cirrhes en rangées reguliers longitudinale ou oblique</td><td></td><td>Partie anterieure non prolongé en forme de col perisse de soies</td><td>Oxytricha</td></tr>
<tr><td></td><td>Partie anterieure prolongé en forme de col perisse de soies</td><td>Stichochaeta</td></tr>
<tr><td colspan="2">Des pieds-cirrhes non rangées reguliers longitudinale ou oblique</td><td></td><td>Stylonichia</td></tr>
<tr><td rowspan="5">Pas de cirrhes marginaux</td><td rowspan="3">Des cirrhes frontaux</td><td rowspan="2">Des pieds crochet</td><td>Pas de pieds dorsaux</td><td>Euplotes</td></tr>
<tr><td>Pieds dorsaux</td><td>Schizopus</td></tr>
<tr><td>Pas de pieds crochet</td><td></td><td>Campylous</td></tr>
<tr><td colspan="2">Pas de cirrhes frontaux</td><td colspan="2">Aspidisca</td></tr>
</table>

* After Claperède et Lachmann 1961

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of the higher taxa is two-fold. Firstly, it shows that Ehrenberg's systematic treatment of *Aspidisca* and related forms has been under criticism by other authors; secondly, that the features which Ehrenberg regarded as important in identification are more applicable to the classification of the *Metazoa* than the *Protozoa*. The topography of the cirri and diversity of external form now used by modern workes as essential features in species identification were first applied in this field by Claparède et Lachmann. The characters that these workers use for identification are much more significant in determining a protozoan species than those selected by Ehrenberg.

Claparède et Lachmann describe a species within the genus *Aspidisca* which they call *Aspidisca cicada*. This is the first time that the organism which is the subject of this discussion has been referred to the genus *Aspidisca*. These workers recognised the presence of ribs in this species, but clearly indicate that these ribs are not crenated, stating that this species is: "*Aspidisca* à carapace non épineuse mais avec de 6 ou 8 côtés longitudinales très marquées."

There can be little doubt from the diagrams and descriptions that their *A. cicada* is identical with the *A. costata* of Stein 1803, Saville-Kent 1880—1882, and Kahl 1930—1935. Claparède et Lachmann suggest that their *A. cicada* is not identical with the *O. cicada* Ehr., which is defined as having dorsal crenated ribs (Fig. 2). While admitting the possibility that *O. cicada* Ehr. might be transferred to the genus *Aspidisca*, they still think that the toothed ribs make *O. cicada* Ehr., "Specificement différent de notre *A. cicada*." This seems to be a valid observation. Claparède et Lachmann have made no attempt to homologise any of Dujardin's species with any of theirs. The reason for this is plainly shown in their discussion of the genus *Aspidisca*. "Mais cet auteur (Dujardin) a donné de ses *Coccudines* une caracteristique tout aussi imparfait que la diagnose générique des *Aspidisca* Ehr. En effet, le principal caractère que doit servir à distinguer les *Coccudines* des autres *Ploesconiens*, c'est l'absence de la bouche. Or un *Oxytrichien* astome est déjà, a priori, quelque chose de fort invraisemblable, et il n'y a, pour nous, aucune espèce de doute que les *Coccudines* sont toutes fournies d'un orifice buccal, mais que M. Dujardin n'a su le voir." Further Claparède et Lachmann summarised Dujardin's treatment of certain species of Aspidisca in these words: "Du reste s'il est incontestable que M. Dujardin a observé en général ses *Ploesconiens* d'une manière très--imparfaite, cela est vrai surtout de ses *Coccudines* et ce serait un travail inutile et presque dérisoire que de s'arrèter aux diagnoses spécifiques qu'il a donné de ses Infusoires. Tout que nous pouvons dire à cet sujet, c'est que la *Coccudina costata* Duj., et la *C. polypoda* Duj., sont probablement des *Aspidisca;* encore est-ce plus que douteux pour la seconde espèce, qui, en juger paraîtrait avoir des cirrhes frontaux. Quant à la *C. crassa* Duj., c'est probablement un *Euplotes*, et pour ce que conclure la *C. cicada* Duj., nous aventurer à emettre opinion quelconque." I think that this shows clearly that Claparède et Lachmann had little or no regard for the work of Dujardin, at least in this matter, and thus they would not wish to recognise any of his species of *Coccudina* as identical with any of theirs.

Perty 1852 added to the confusion by identifying a species *Coccudina cicada* with the *Oxytricha cicada* of Ehrenberg, while stating that his

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C. cicada was "ohne Rippen" when Ehrenberg 1830 had clearly shown that *O. cicada* Ehr., had ribs bearing teeth. Perty does, however, refer to a *C. cicada* (sic), which has four or five heavy dorsal ribs, but he does not liken it to any other species of *Aspidisca, Coccudina* or *Oxytricha*. Perty's species is probably identical with *A. cicada* Müller, recognised by Claparède et Lachmann.

Stein 1859, who was the first to use the name *Aspidisca costata,* does not regard this species as identical with *O. cicada* Ehr., on account of the difference in the number of dorsal ribs. He states that *A. costata* has six dorsal ribs whereas Ehrenberg 1838 stated that *O. cicada* Ehr., had 8—13 dorsal ribs. He does not mention the presence or absence of teeth on the ribs at all. Stain states that this species *A. costata* is identical with *Coccudina costata* Duj.

However, to recapitulate briefly, we have seen that Dujardin has described his *C. costata* as having five to six, markedly projecting, tuberculated dorsal ribs. Stein states that his *A. costata* has six blunt dorsal longitudinal ribs, without mention of teeth or tubercles. Stein's diagrams indicated that *A. costata* has plain ribs and the organism he figured is the same as the one called *A. costata* by Saville-Kent 1880—1882 and Kahl 1930—1935. I cannot agree, therefore, that the *A. costata* of Stein is identical with the *C. costata* of Dujardin. Stein's *A. costata* is considered to be identical with the *C. costata* of Perty 1852 and the *A. cicada of* Claparède et Lachmann 1861. From this argument and "by priority" (Corliss 1961) it would seem reasonable to suppose that the name of the animal which is the subject of this discussion should be *A. cicada* (Müller) and not *A. costata* (Duj.), by which name it is commonly known.

Saville-Kent 1880—1882 dentifies *A costata* with the *A. cicada* of Claparède et Lachmann, but at the same time likens it to the *C. costata* of Dujardin, even though the latter states specifically that his *C. costata* has five to six tuberculated dorsal ribs.

Kahl's analysis of the identity and relationships of *A. costata* will be considered. Kahl 1930—1935 indicates that Stein's *A. costata,* a generic synonym of *C. costata,* is defined as having toothed ridges, whereas the species *A. costata* recognised by Stein, Saville-Kent and Kahl is described as having plain ridges. Presence or absence and their form, i.e. crenated or plain, are recognised as specifically significant characters. This is in agreement with Claparède et Lachmann 1861. It is considered that Kahl is correct in regarding *A. costata* (Duj.) of Stein, but not the *C. costata* Duj., as a synonym of *A. cicada* of Claparède et Lachmann, for reasons already stated. The names of Müller and Ehrenberg are also mentioned by Kahl in connection with *A. costata,* but it is difficult to be sure what his statement with regard to synonyms really implies. Kahl's actual taxonomic summary is as follows: "*Aspidisca* (*Coccudina*) *costata* (Dujardin, 1842) [*A. cicada* (Müller — Ehr.) Clap. et Lachmann, 1859]." It can be seen that Kahl recognises *A. cicada* as a synonym of *A. costata,* but the bracketting and hyphening of Müller and Ehrenberg are confusing. It seems that even if the name *A. costata* is retained, then the senior synonym *T. cicada* Müller or *C. cicada* (Müller) should be mentioned. Ehrenberg should not be mentioned either alone or in connection with Müller. The only reason for discarding *A. cicada* as the final name for the species under consideration would be as a nomen oblitum. However, it would seem that the application of the specific name *costa-*

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ta of Dujardin to this species is based on an error of observation and that there does exist another species of the genus *Aspidisca* (syn. *Coccudina*) with the characters given by Dujardin to the species *C. costata,* and that this organism is probably known today by an entirely new specific name. It is possible that *C. costata* Duj., also *O. cicada* Ehr., could be identical with the modern species *A. tuberosa* Kahl. Whatever the name, the organism here discussed is not identical on grounds either of taxonomy or morphology with *C. costata* Duj. Corliss 1961 said that, "Unfortunately Kahl, whose authorative taxonomic monographs are justly held in high regard, was guilty of a rather large number of minor nomenclatural mistakes."

The identification of many of these apparently well established species go back a long way, and the data on which they are based are few. In most cases there are no type specimens, yet a number of these older ill-defined species are recognisably identical with well known modern species. Now that we know much more about their morphology, more accurate diagnoses can be made. One can recognise quite clearly the modern *A. costata* as the *Trichoda cicada* of Müller and as the *A. cicada* of Claparède et Lachmann. A large number of errors and confusions have resulted in a multiplicity of names being applied to the same animal or, conversely, the same name being applied to different organisms. The only satisfactory method of identification today is that which is based on clonal culture thus a stock of identical animals of the species under consideration is available for exhaustive study.

In the light of the foregoing discussion it is suggested that *Aspidisca cicada* (Müller) be recognized as the proper name for "*A. costata* (Duj.)".

Emended diagnosis of *Aspidisca cicada* (Müller, 1786)

Aspidisca with oval body, anterior rounded, posterior border truncated. 30—34 μ long × 25—30 μ broad. Maximum height 15—20 μ. Stiff pellicle with 6 dorsal longitudinal plain ribs, without teeth or tubercles (Brown 1966). No dorsal cilia. Ventral ciliature composed of cirri four frontals, three ventrals, five anals, and membranellae. Adoral zone of membranellae represented only by a much reduced zone median to the ventral keel. Meganucleus horse-shoe shaped, open posteriorly with the right arm slightly shorter than the left and with a swelling at its anterior end. Micronucleus single, oval, applied to the left anterior surface of the meganucleus.

Summary

The hypotrich *Aspidisca costata* (Dujardin, 1842), was recorded from the protozoan fauna in the activated sludge tanks of the North Surrey Joint Sewerage Board purification plant. *A. costata* was found to compete with species of the genus *Vorticella* for the numerically dominant position in the protozoan population. The first clonal cultures of *A. costata* were successfully produced in the laboratory and used as a supply of 'type organisms'. An extensive study was made of the earlier observations on *A. costata,* most of which were unfortunately made on an inadequate number of organism. The naming and systematic position of *Aspidisca costata* (Dujardin, 1842) is discussed and it is concluded that the name should be changed to *Aspidisca cicada* (Müller, 1786). An emended definition of *Aspidisca cicada* is given.

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ZUSAMMENFASSUNG

Der Hypotriche *Aspidisca costata* (Dujardin, 1842) wurde schon als Bestandteil der Protozooenfauna der aktiven Kläranlagen in der North Surrey Joint Sewerage Board Purification Plant beschrieben. Es wurde festgestellt, dass er im Bereiche der quantitativen Dominanz der Protozooen-Population als Konkurrenzart zu den Arten aus der Gattung Vorticella hervortritt.

Mit Erfolg wurden im Laboratorium die ersten klonalen Kulturen des *Aspidisca costata* erreicht und als Lieferant der „typischen Organismen" ausgenutzt.

Zu den früheren Beobachtungen über *A. costata* wurden ausführliche Forschungen unternommen; leider war die Mehrzahl davon an einer unausreichender Zahl der Organismen durchgeführt. Die systematische Stellung sowie die Bezeichnung des *Aspidisca costata* (Dujardin, 1842) wurde besprochen, wobei festgestellt wurde, dass sie auf *Aspidisca cicada* (Müller, 1786) zu ändern ist. Eine gebesserte Diagnose des *Aspidisca cicada* wurde gegeben.

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ACTA PROTOZOOLOGICA

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Laboratory of Biological Control, Institute of Plant Protection, Grunwaldzka 189, Poznań, Poland

Jerzy J. LIPA

Herpetomonas chatoni (Paillot) comb. nov., a flagellate parasite of adult moth of *Agrotis segetum* Schiff. (*Lepidoptera, Noctuidae*[1])

Herpetomonas chatoni (Paillot) comb. nov., wiciowiec pasożytujący w motylu *Agrotis segetum* Schiff. (*Lepidoptera, Noctuidae*)

While conducting studies on pathogens of cutworms an extensive survey of microorganisms associated with several species of *Agrotis* genus has been undertaken. On November 29, 1966, one dead moth from our laboratory culture of *Agrotis segetum* Schiff. was found to be very heavily infected by a flagellate belonging to the *Trypanosomatidae* family. At further investigations it was stated that this species appeared to be identic with *Leptomonas chatoni* Paillot described from a larvae of *Agrotis pronuba* L. (= *Agrotis pronubana* L.) (Paillot 1923).

The morphology and the life cycle of the flagellate were studied on fresh and stained preparations. Smear preparations were fixed in methanol and stained with 0.5% Giemsa's solution for 16 to 24 hours.

Herpetomonas chatoni (Paillot) comb. nov.

Synonyms: *Leptomonas chatoni* Paillot, 1923; *L. chattoni* (Paillot, 1933); *L. chattoni* auct.
Host insects: Larvae of *Agrotis pronuba* L. (Paillot 1923); female moth of *Agrotis segetum* Schiff.
Habitat: Intestine and body cavity of the hosts.
Distribution: France and Poland, in laboratory cultures of the hosts.

Morphology

A number of morphological forms are recognized in the development of insect flagellates. In the investigated species leptomonad, herpetomonad and crithidial forms were observed.

The herpetomonad form is the predominant form in the insect gut and in the hemolymph. The herpetomonad form is characterized by the kinetoplast located posterior or laterally to the nucleus (Pl. II 5, 6, III 7, 8, IV 13). The size of the herpetomonad form varies from 17 to 24 μ in length and from

[1] This investigation was supported by the research grant FG-Po-194 from the United States Department of Agriculture.

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2 to 3 μ in width. The length of the free flagellum is from 6 to 14 μ and it passes through a long reservoir (Table 1).

The leptomonad form is less frequently observed than the herpetomonad form. The leptomonads are longer than other forms and are stained much deeper with Giemsa's solution. Their length varies from 27 to 39.5 μ and their width from 2.5 to 3 μ. In typical leptomonad forms the kinetoplast is located closely to the anterior end and the flagellum passes through a short reservoir (Pl. III 9).

Table 1

Results of measurements of 10 flagellates *Herpetomonas chatoni* of each form in microns

Form		1	2	3	4	5	6	7	8	9	10
Herpetomonad	pe-mn	5	3.5	5	3.8	3	5	4	4	3	4
	mn-k	—1.5	—1	—4	±1.5	±1	—2	±1	—2	±1	—2
	k-tff	20	21	22	20	17	14	20	21	22	19
	lff	13	13	14	14	10	6	14	13	14	10
	gb	3	2.5	3.5	3	3	2.6	3	3	3	2
	oal	23.5	23.5	23	23.8	20	17	24	23	25	21
Leptomonad	pe-mn	6	19	4	6	3	6.5				
	mn-k	4	3.5	4	4	4	4				
	k-tff	21	17	19	21	24	21				
	lff	17	15	15	16	21	16				
	gb	2.5	3	3	3	3	2.8				
	oal	31	39.5	27	31	31	31.5				
Crithidial	pe-mn	3	3.5	3	2	4	4	2	2	2	3
	mn-k	3	2	3.5	3.5	2	3	2	1	2.2	+3
	k-tff	21	21	26	21	12	22	23	15	20	21
	lff	18	19	23	19	8	19	21	13	17	18
	gb	6	6	6	6	6	6	6	6	5	7
	oal	27	26.5	32.5	26.5	18	29	26	18	25	24

Legend:

pe-mn — posterior extremity to middle of nucleus
mn-k — middle of nucelus to kinetoplast
k-tff — kinetoplast to tip of free flagellum
lff — lenght of free flagellum
gb — greatest breadth
oal — over-all length
(—) or (±) — indicate that kinetoplast is located posterior (—) or laterally (±) to the nucleus.

The crithidial form is round or oval in shape and its free flagellum is longer than in other forms and is from 8 to 21 μ (Pl. IV 11—14). The flagellum passes through a short and widely opened reservoir. The diameter of crithidial forms is from 5 to 7 μ.

Multiplication

The life cycle of the flagellate was not completely worked out. However, several important data on this subject have been collected. Binary fission is the main kind of multiplication (Pl. IV 11), multiple fission was not observed. At the binary fission the sequence of division of various organellas varies among individual flagellates. Some flagellates had already flagella and only a single

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kinetoplast and nucleus. In others the division began from the nucleus and kinetoplast.

The site of infection

In a single infected adult moth of *Agrotis segetum* the flagellate inhabited the intestine and body cavity of the host (Pl. I 1—3, II 4). Paillot 1923 in the original description of this species observed that this flagellate developed in the intestine and body cavity of larvae of *Agrotis pronuba*.

Taxonomic position

The most prevalent morphological form in the development of the flagellate under investigation was the herpetomonad form. Although crithidial and leptomonad forms occur in the life cycle of this species the typical and most prevalent in its development is the herpetomonad form. Therefore, according to the present concept of taxonomy of insect trypanosomes this species belongs to the genus *Herpetomonas*.

The morphological features of this *Herpetomonas* species from *Agrotis segetum* make it very closely related to *Leptomonas chatoni* Paillot described from *Agrotis pronuba* as leptomonad, crithidial and herpetomonad forms also occur in its development. I consider, therefore that both species can be identified as one species and I propose to transfer *Leptomonas chatoni* to the genus *Herpetomonas* as *Herpetomonas chatoni* (Paillot) comb. nov.

It is interesting to notice that in the original description of this flagellate Paillot 1923 named it *Leptomonas chatoni*. In his later paper Paillot 1933 changed the spelling of the specific name to "*chattoni*" without any explanation. This second name has been incorrectly used throughout the literature (Doflein and Reichenow 1953, Grasse 1953, Weiser 1966). In accordance with the Code (Article 33(b)) I propose to reject the form "*chattoni*" and to use the name *Herpetomonas chatoni* according to the original spelling in the first Paillot's paper and present taxonomic position.

Discussion

The concept of the taxonomy of insect trypanosomes has greatly changed (Wallace 1963, Lipa 1963, 1965) and is quite different from that of Paillot 1923, 1933, who believed that all members of the *Herpetomonas* genus must have a double-parallel flagellum. As the flagellate from *Agrotis pronuba* did not have such a feature Paillot placed it among the genus *Leptomonas*.

Wallace 1963 proposed to differentiate both genera according to the position of the kinetoplast. In *Leptomonas* the kinetoplast is near the anterior end of the body and the flagellum passes through a short reservoir. The *Herpetomonas* genus resembles *Leptomonas* but the kinetoplast is located posterior to the nucleus and the flagellum passes through the long reservoir extending from the kinetoplast to the anterior end.

When the drawings in the papers of Paillot 1923, 1933 are examined one can see that there are some forms with the kinetoplast located posterior or lateral to the nucleus as in typical *Herpetomonas* species. Such forms were observed by the author in *Herpetomonas muscarum* (Leidy) but not in the development of *Leptomonas pyrrhocoris* (Zotta) (Lipa, unpublished results).

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For the reasons outlined above I consider that the transference of *Leptomonas chatoni* to the genus *Herpetomonas* as *Herpetomonas chatoni* (Paillot) comb. nov. is justified.

Summary

The intestine and body cavity of the adult moth of *Agrotis segetum* Schiff. were found to be heavily infected with a flagellate of the genus *Herpetomonas*. The herpetomonad, leptomonad and crithidial forms occurring in the life cycle of the investigated species are described. Due to similar morphology this species is identified as *Herpetomonas chatoni* (Paillot) originally described as *Leptomonas chatoni* Paillot from larvae of *Agrotis pronuba* L. The reasons of transference of the investigated species to the genus *Herpetomonas* are discussed.

STRESZCZENIE

Stwierdzono silne zarażenie jelita i jamy ciała motyla *Agrotis segetum* Schiff. przez wiciowca z rodzaju *Herpetomonas*. Opisano herpetomonalne, leptomonalne i kritidialne postacie występujące w cyklu rozwojowym pasożyta. Z uwagi na podobieństwo badany gatunek identyfikowany jest jako *Herpetomonas chatoni* (Paillot) opisany początkowo jako *Leptomonas chatoni* Paillot z larw *Agrotis pronuba*. L. Omówiono powody przeniesienia tego gatunku do rodzaju *Herpetomonas*.

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EXPLANATIONS OF PLATES I—IV

Herpetomonas chatoni (Paillot) comb. nov.

1—2: Living flagellates in water preparations of the gut and body cavity contents (phase contrast)
3—4: A huge number of flagellates in smear-stained preparation at a low (3) and higher (4) magnifications
5—8: Herpetomonad forms with kinetoplast located laterally or posterior to the nucleus: N — nucleus, K — kinetoplast, R — reservoir, F — front end of the flagellate from which flagellum arises
9—10: Leptomonad forms
11—14: Crithidial forms and one herpetomonad form in Fig. 13.

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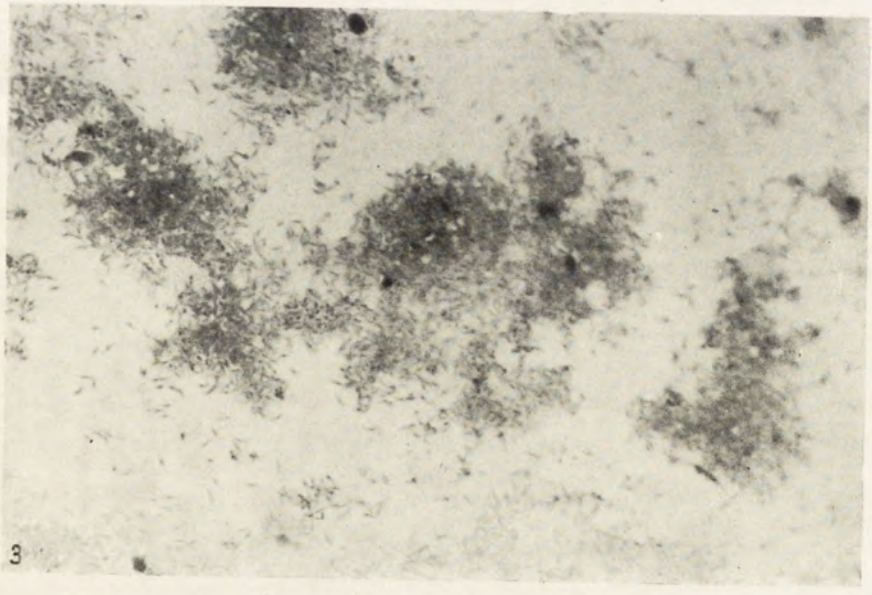

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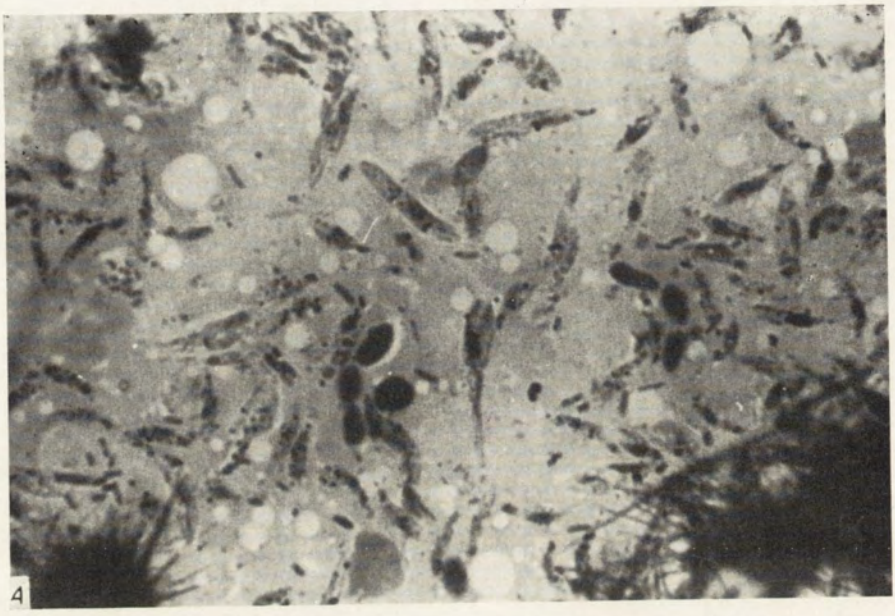

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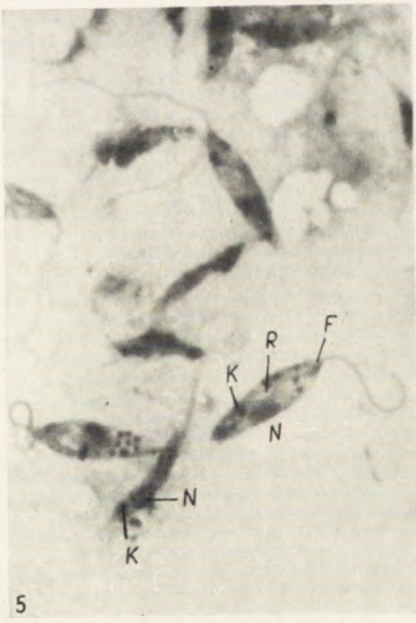

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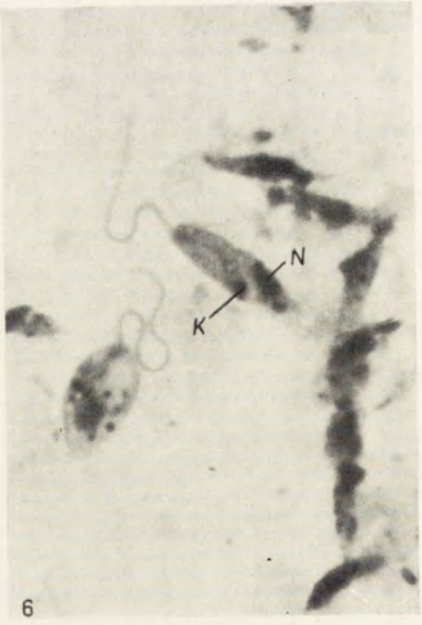

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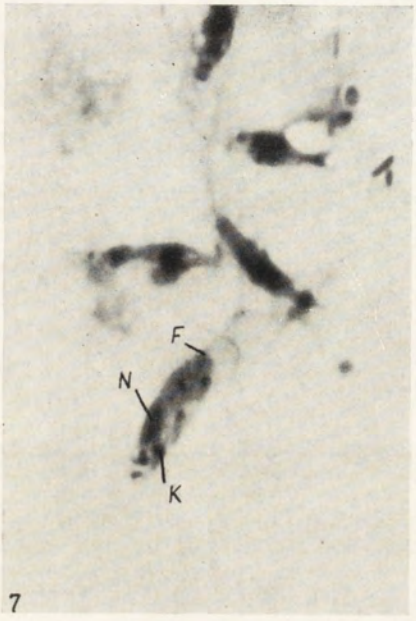

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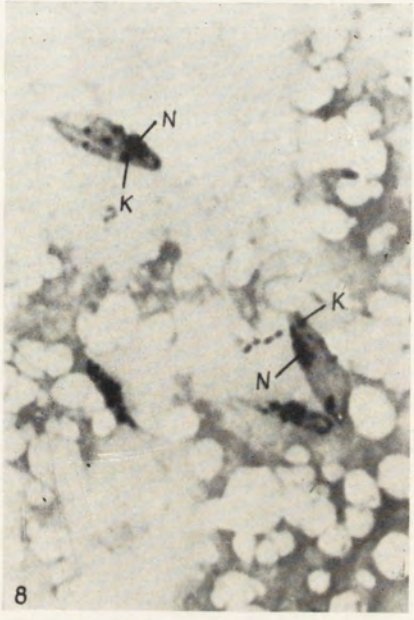

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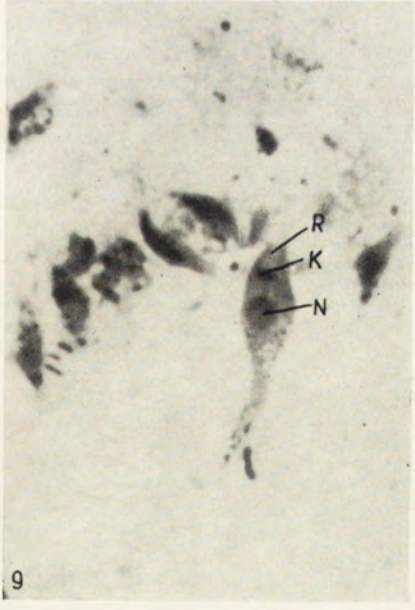

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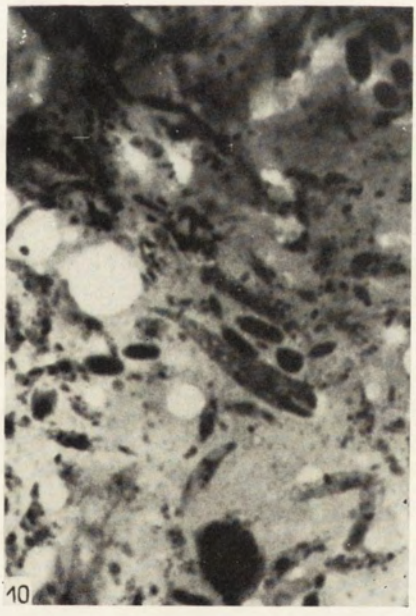

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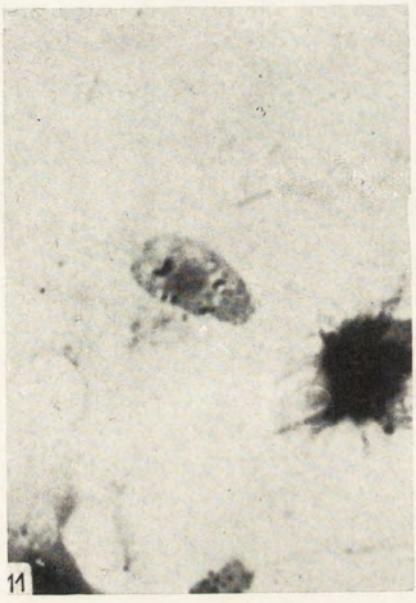

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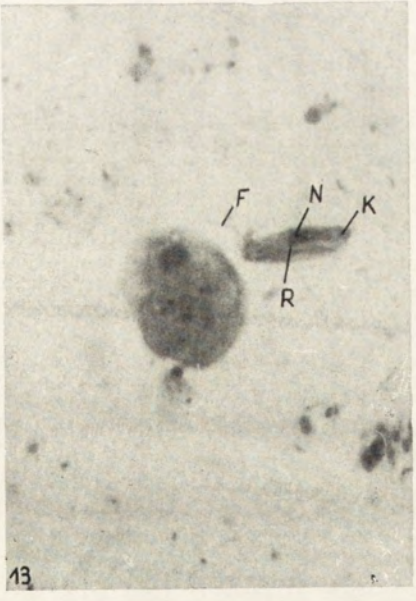

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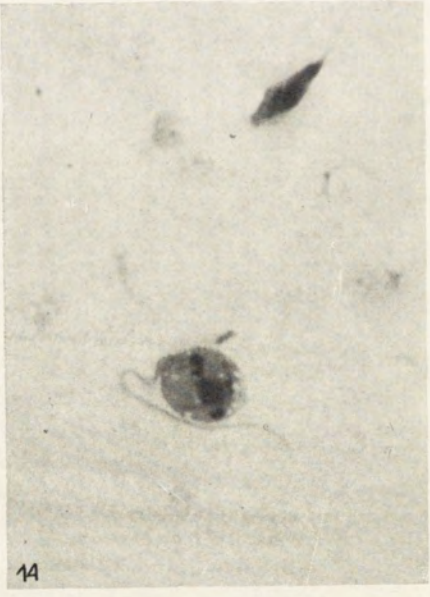

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Laboratory of Biological Control, Institute of Plant Protection, Grunwaldzka 189, Poznań, Poland

Jerzy J. LIPA

Observations on gregarines of *Gammaridae (Crustacea)* in the Baikal Lake

Obserwacje nad gregarynami *Gammaridae* (*Crustacea*) jeziora Bajkał

The gregarine fauna of the Baikal Lake is uncompletely known. The only published works on this group are Zvetkov's 1928, describing two species of *Gregarina* from gammarids and Swarczewski's 1910 a,b on gregarine *Lankesteria* from turbellarians.

In the spring of 1966, I had the opportunity to work in the Biological Station of the Irkutsk State University at Bolshiye Koty at the Baikal Lake and to examine various arthropods on protozoan infections. In the course of this work a number of gammarids and one trichopteran were found infected by gregarines and one microsporidian. This paper gives only results of studies on gregarines parasitizing gammarids; results of studies on other protozoans are published elsewhere (Lipa 1967 a,b).

Material and methods

From April 6 to 8, 1966, 142 specimens belonging to nine species of gammarids were examined for gregarines. Larvae and adults of each host species were collected at the shore of the Baikal Lake, close to buildings of the Biological Station at Bolshiye Koty. As the Baikal Lake was covered, at that time, with ice about 60 to 80 centimeters thick air holes were made and the sea diver Mr. Nikolay S. Rezinkov dived for them to the bottom.

The collected gammarids were immediately taken to the laboratory, identified by Mrs. Galina S. Kaplin, and later the author examined them microscopically. The gammarids were dissected and their gut and other tissues were used for microscopic preparations. Photographs were taken by Mr. Vitali Kaplin and the author.

I wish to express my acknowledgements to Professor M. M. Kozhov, Head of the Chair of Invertebrate Zoology of the Irkutsk State University, who put every facility at my disposal and to various members of the Staff of the Biological Station at Bolshiye Koty, especially to Mrs. Galina S. Kaplin for the identification of gammarids, to Mr. Nikolay Rezinkov for collecting the material and to Mr. Vitali Kaplin for taking the photographs. The joint travel grant of Polish Academy of Sciences and the USSR Academy of Sciences is greatly appreciated.

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Results

All nine collected and examined gammarid species were found to be infected with gregarines. The number of examined and parasitized specimens of each species is given in Table 1.

Table 1
Infection level of nine species of gammarids from the Baikal Lake by gregarines

Gammarids	Total number of examined specimens	Number of infected specimens
Bacialogammarus pullus (*Dyb.*)	67	7
Brandtia lata lata (Dyb.)	21	3
Eulimnogammarus cruentus (Dor.)	7	2
Eulimnogammarus viridis (Dyb.)	1	1
Gmelinoides fasciatus (Stebb.)	26	5
Microrupus vortex vortex (Dyb.)	14	2
Pallasea cancellus (Pall.)	3	1
Pallasea kessleri (Dyb.)	9	2
Pallasea viridis (Garj.)	4	4

Mrs. Galina L. Vasilieva of the Biological Station at Bolshiye Koty informed me that when studying some gregarines for various purposes she observed unidentified gregarines in *Brandtia lata lata* (Dyb.), *Pallasea cancellus* (Pall.), *P. cancelloides* (Gerstt.), *P. verrucousus* (Gerstt.) and in other species she could not recall.

1. *Rotundula dybowskii* sp. n.

Host: *Brandtia lata lata* (Dyb.), *Gmelinoides fasciatus* (*Stebb.*), *Pallasea cancellus* (Pall.), *Pallasea kessleri* (Dyb.) and *Pallasea viridis* (Garj.).
Habitat: Intestine.
Locality record: Baikal Lake, close to Bolshiye Koty, April 6 to 8, 1966.

Morphology

Sporonts biassociative (Pl. I 1). In many associations the primite much longer than the satellite Pl. I 2). Length of mature sporonts observed in the studied material was up to 175 and width up to 82 μ. Ratio LP:TL = 1:3.2 to 10; ratio WP:WD = 1:1 to 4.

Protomerite wider than long. Septum and constriction between protomerite and deutomerite well seen. Endoplasm of protomerite is granular, not transparent, and brown.

Deutomerite cylindrical, widest in front, and narrowing toward the end. Endoplasm granular, dense and brown but not dark (Pl. I 1, 2).

Cysts oval up to 150 μ in diameter (Pl. I 3) without spore ducts, dehiscence of spores by simple rupture.

Parasitism

Intensity of infection was not very great and usually 6 to 12 gregarines were observed in one host.

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Table 2
Maesurements of sporonts of *Rotundula dybowskii* sp. n. (in microns)

	LP	LD	WP	WD	TL	TLA	Ratio	
							LP:TL	WP:WD
Prim.	31	118	49	54	149	278	1:4.7	1:1.1
Sat.	26	103	46	41	129		1:5	1:1
Prim.	20	100	35	40	120	177	1:6	1:1.1
Sat.	7	50	15	20	57		1:7.7	1:1.3
Prim.	45	130	65	75	175	268	1:3.8	1:1.1
Sat.	8	85	42	55	93		1:5.1	1:1.2
Prim.	41	134	67	82	175	294	1:4.2	1:1.2
Sat.	21	98	41	54	119		1:5.7	1:1.2
Prim.	31	139	42	62	170	304	1:5.5	1:1.5
Sat.	21	113	32	42	134		1:6.5	1:1.3
Prim.	22	133	31	41	155	249	1:8.3	1:1.3
Sat.	11	83	22	87	94		1:8.4	1:4
Prim.	31	154	51	72	185	241	1:6	1:1.4
Sat.	5	51	10	15	56		1:10	1:1.1
Prim.	8	17	10	12	25	47	1:3.2	1:1.1
Sat.	5	17	8	10	22		1:4.3	1:1.2
Prim.	23	139	41	51	162	272	1:7.2	1:1.2
Sat.	15	95	26	31	110		1:7.2	1:1.2
Prim.	20	102	35	42	122	179	1:6.1	1:1.2
Sat.	7	50	15	20	57		1:7.6	1:1.6

Legends:
LP—length of protomerite, LD—length of deutomerite, WP—width of protomerite WD—width of deutomerite, TL—total length of sporont, TLA—total length of association of sporonts, Prim.—primite, Sat.—satellite.

Taxonomic position

Due to its features this gregarine is placed to the genus *Rotundula* as defined by Goodrich 1949. It differs from *Rotundula baicalensis* (Zvetkov) by its smaller size of sporonts which in case of *R. baicalensis* reach the length of 350—400 μ and by lack of radical ribs on protomerites.

Due to these differences I assume that the gregarine being described here is a new species and propose the name *Rotundula dybowskii* sp. n. for it. The specific name is given in honour of the late Professor Benedykt Dybowski, who contributed greatly to the knowledge of fauna especially of *Gammaridae* of the Baikal Lake.

2. *Rotundula godlewskii* sp. n.

Host: *Brandia lata lata* (Dyb.), *Pallasea kessleri* (Dyb.) and *Pallassea viridis* (Garj.).
Habitat: Intestine.
Locality record: Baikal Lake close to Bolshiye Koty, April 6 to 8, 1966.

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Morphology

Sporont biassociative (Pl. II 4, 5). Satellites and primites usually of the same length, but slight differences are observed. Maximum length of sporonts 235 μ. Ratio LP:TL = 1:6.3 to 11.7; ratio WP:WD = 1:1.1 to 1.6.

Table 3
Measurements of sporonts of *Rotundula godlewskii* sp. n. (in microns)

	LP	LD	WP	WD	TL	TLA	Ratio	
							LP:TL	WP:WD
Prim.	36	190	52	77	226	397	1:6.3	1:1.4
Sat.	22	149	46	62	171		1:7.9	1:1.3
Prim.	30	120	42	55	150	385	1:5	1:1.3
Sat.	20	215	35	50	235		1:11.7	1:1.6
Prim.	28	165	67	77	193	388	1:6.9	1:1.1
Sat.	31	165	51	72	196		1:6.3	1:1.4

Protomerite large, wider than long. Septum and constriction well seen in primite but in the satellite constriction may be absent (Pl. II 4, 5). Endoplasm of protomerite in primites and satellites is granular and dark.

Deutomerite widest at the shoulder, narrowing toward the posterior end. Endoplasm is granular, dense and very dark. Nucles is rarely seen in dark endoplasm.

Cysts are oval up to 250 μ in diameter with thick gelatinous layer (Pl. II 6). Dehiscence of spores by simple rupture.

Parasitism

Intensity of infection was not very high and up to 30 gregarines in the gut of the hosts were observed. This species frequently parasitizes gammarids together with *Rotundula dybowskii* sp. n.

Taxonomic position

This gregarine is determined as belonging to the genus *Rotundula* Goodrich. It differs from *Rotundula baicalensis* by its smaller size and lack of radial ribs on protomerites. From *Rotundula dybowskii* sp. n. it differs by dark endoplasm, larger size of sporonts and presence of gelatinous layer surrounding the cysts.

Due to these differences I assume that this is a new species and I propose the name *Rotundula godlewskii* sp. n. for it. The specific name is in honour of the late Wiktor Godlewski, who working together with the late Benedykt Dybowski, contributed to the knowledge of the Baikal Lake.

3. *Heliospora acanthogammari* (Zvetkov) comb. nov.

Synonym: *Gregarina acanthogammari* Zvetkov, 1928.
Host: *Eulimnogammarus cruentus* (Dor.), and *Eulimnogammarus viridis* (Dyb.), Zvetkov 1928 described this species from *Acanthogammarus godlevskii* var. *victori* (Dyb.).

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Habitat: Intestine.
Locality record: Baikal Lake close to Bolshiye Koty, April 6 to 8, 1966. Zvetkov 1928 found this species close to Marituj at the Baikal Lake.

Morphology

Sporonts biassociative and very elongate (Pl. III 6, 7). Maximum length of sporonts 1099 μ. Ratio LP:TL = 1:58 to 126, ratio WP:WD = 1:1.4 to 2.3. Sporonts with epimerites were not observed.

Table 4
Measurements of sporonts of *Heliospora acanthogammari* (Zvetkov) comb. nov. (in microns)

	LP	LD	WP	WD	TL	TLA	Ratio	
							LP:TL	WP:WD
Prim.	11	1088	21	47	1099	1743	1:97	1:2.3
Sat.	5	639	15	33	644		1:126	1:2.1
Prim.	8	757	10	41	765	1617	1:93	1:1.4
Sat.	7	845	21	33	852		1:118	1:1.6
Prim.	12	711	18	41	723	1753	1:58	1:2.2
Sat.	13	1017	21	41	1030		1:78	1:2

Protomerite globular and very small as compared with the length of deutomerite (Pl. III 8, 9). Endoplasm homogenous and transparent. Septum well seen; constriction at septum absent. Deutomerite very elongated being equally wide over the whole length (Pl. III 8). Endoplasm of the deutomerite granular and not translucent.

All features of the satellites are the same as those of primites, except the very small size of protomerite of satellites (Pl. III 9). Nucleus about 30 μ in diameter located in the middle of the deutomerite.

Cysts and spores unknown.

Parasitism

Intensity of infection in the specimens of *Eulimnogammarus cruentus* was very high and in one case more than two hundred of gamonts were observed in the intestine of the host (Pl. III 7). There is no doubt that in such cases the gregarine has harmful effect on its hosts. Zvetkov 1928 also recorded high intensity of infection of *Acanthogammarus godlevskii* var. *victori*.

Taxonomic position

The gregarine under investigation was described as *Gregarina acanthogammari* Zvetkov from *Acanthogammarus godlevskii* var. *victori* (Zvetkov 1928). Detailed measurements of *G. acanthogammari* were not given by Zvetkov and he mentioned only that single gamonts are up to 500—600 μ long while those in associations are 1 to 2 milimeter long. Value of ratios was LP:TL = 1:20 to 27.5 and WP:WD — 1:2 to 2.3.

The sporonts of the gregarines from *Eulimnogammarus* spp. are up to 1099 μ long and the maximum width is 47 μ. The ratio value LP:TL — 1:58

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to 126. There are also some differences in size and structure of the protomerites of sporonts observed by Zvetkov and the author. Zvetkov observed that the epimerite remains even in sporonts in associations, and protomerite in satellites is well seen. On the other hand, in the studied gregarine the epimerite was not observed and the protomerite of the satellites is very small (Pl. III 9).

In spite of differences in structure of the body between *G. acanthogammari* and the species discovered in *Eulimnogammarus* spp. it is assumed that they are both the same species. It is evident that Zvetkov's description of presence of epimerite in satellite being in association is not correct as it never can happen. Due to this reason also ratio value will change. Accordingly the size and ratio values of *G. acanthogammari* will be the same as of the studied species. However, according to Goodrich's paper (1949) on gregarines of *Gammaridae, Gregarina acanthogammari* is transfered to the genus *Heliospora* as *Heliospora acanthogammari* (Zvetkov) comb. nov.

4. *Cephaloidophora poltevi* sp. n.

Host: *Baicalogammarus pullus* (Dyb.), *Gmelinoides fasciatus* (Stebb.), *Microrupus vortex vortex* (Dyb.).
Habitat: Intestine.
Locality record: Baikal Lake close to Bolshiye Koty, April 6 to 8, 1966.

Morphology

Associations of two or three sporonts (Pl. IV 10, 11). Maximum length of sporonts up to 211 μ; maximum width 37 μ. Ratio LP:TL = 1:15 to 116 (on an average 1:24); ratio WP:WD = 1:1 to 3.5.

Protomerite of primite small, wider than long. Endoplasm dense but not translucent. Septum and constriction well seen. Deutomerite elongated, widest in the mid-region where the nucleus is located (Pl. IV 10, 11). In some gamonts a shoulder is present. Endoplasm of the deutomerite is slightly granular and

Table 5
Measurements of sporonts of *Cephaloidophora poltevi* sp. n. (in microns)

	LP	LD	WP	WD	TL	TLA	Ratio	
							LP:TL	WP:WD
Prim.	6	165	12	37	171		1:26	1:3
I Sat.	5	130	10	20	135	391	1:27	1:2
II Sat.	5	80	8	12	85		1:17	1:1.5
Prim.	6	125	10	20	131	291	1:22	1:1.2
Sat.	5	155	10	20	160		1:31	1:2
Prim.	6	205	10	35	211	327	1:35	1:3.5
Sat.	1	115	10	15	116		1:116	1:1.5
Prim.	5	125	10	18	130	219	1:26	1:1.8
Sat.	4	85	10	13	89		1:22	1:1.3
Prim.	5	100	10	19	105	193	1:21	1:1.9
Sat.	3	85	10	15	88		1:29	1:1.5
Trophoz.	5	106	9	14	121		1:24	1:1.5

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not translucent. Nucleus without clearly seen karyosomes. The morphological features of the first and second satellites are the same as of primites except that their protomerites are small.

Cysts and spores unknown.

Parasitism

Hosts were very strongly invaded and frequently some hundreds of parasites were observed in the gut of the host (Pl. IV 11). This indicated that the gregarine can be harmful to the hosts.

Taxonomic position

Due to its morphological features this gregarine is placed to the genus *Cephaloidophora* Mawrodiadi, 1908 and is considered to be a new species, as it cannot be identified with any other species known from gammarids. Therefore I propose the name *Cephaloidophora poltevi* sp. n. for it. The specific name is in honour of Professor Vasil I.Poltev of the Biological Institute of the Siberian Branch USSR Academy of Sciences at Novosibirsk.

5. *Cephaloidophora gershensoni* sp. n.

Host: *Gmelinoides fasciatus* (Stebb.)
Habitat: Intestine.
Locality record: Baikal Lake close to Bolshiye Koty, April 7, 1966.

Morphology

Sporonts biassociative (Pl. IV 12). Maximum length 150 μ, maximum width 21 μ. Ratio LP:TL — 1:18 to 19; ratio WP:WD = 1.1 to 1.5.

Table 6
Measurements of sporonts of *Cephaloidophora gershensoni* sp. n. (in microns)

	LP	LD	WP	WD	TL	TLA	Ratio	
							LP:TL	WP:WD
Prim.	8	142	14	21	150	291	1:17.7	1.1.5
Sat.	3	138	6	159	141		1:40	1:2.8
Prim	6	103	13	14	109	233	1:18.8	1:1.1
Sat.	3	121	11	14	124		1:35	1:1.3

Protomerite wider than long with granular and not transparent endoplasm. Constriction and septum well seen. Deutomerite cylindrical with granular and dense endoplasm. The satellite has similar features as primite except that it has very small protomerite. Nucleus well seen as light area in the middle of deutomerite. Due to well developed myonemes the sporonts show great ability of contracting their bodies (Pl. IV 12).

Taxonomic position

Morphological features indicate that this species belong to the genus *Cephaloidophora*. It differs from other *Cephaloidophora* species being described

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here by its different size and high motility of body. Therefore I consider it as a new species and propose the name *Cephaloidophora gershensoni* sp. n. for it. The specific name is in honour of Professor Sergey M. Gershenson of the Zabalotny's Institute of Microbiology and Virology at Kiev, Soviet Union.

7. *Rotundula baicalensis* (Zvetkov) comb. nov.

Synonym: *Gregarina baicalensis* Zvetkov, 1928.
Host: *Pallasea brandti* Dyb. (Zvetkov 1928).

This species was not recorded during this study, however, it seems necessary to correct its taxonomic position.

Zvetkov 1928 placed the gregarine from *Pallasea brandti* to the genus *Gregarina* as *Gregarina baicalensis* Zvetkov. However, according to a later Goodrich's paper (Goodrich 1949) on gregarines from *Gammaridae* it is necessary to transfer this species to the genus *Rotundula* as *Rotundula baicalensis* (Zvetkov) comb. nov.

Diagnostic characteristics of *Rotundula baicalensis* are as follows: Sporonts biassociative. The body length 300—400 μ. Ratio LP:TL — 1:5 to 7; ratio WP:WD = 1:1.1 to 1.7. Protomerite oval, constriction and septum well seen. Several radial ribs present on protomerites. Deutomerites broadest at shoulder and slightly narrowing toward the end. Known as parasite of *Pallasea brandti* Dyb. from the Baikal Lake.

Discussion

The results of this study indicate that gammarids of the Baikal Lake are commonly infected with gregarines, as all nine examined species were found to harbour parasites. The level of infection was different in various species but rather high as even single specimens of some species were found to be infected. Out of 142 examined gammarids belonging to nine species, 26 were infected with gregarines that is 17.1% of total number.

All studied gregarines show a rather wide range of host specificity (Table 7) e. g. *Rotundula dybowskii* sp. n. parasitizes six species of *Gammaridae*. This can be understood as hosts of these gregarines inhabit the same biotopes and the parasites can easily be transmitted from one host to another.

Those that have worked on gregarines of *Crustacea* generally agree that there is a great deal of confusion in the taxonomy of gregarines of *Gammaridae* and of other *Crustacea*. Several ill-defined genera were described and therefore it is difficult to determine correctly the taxonomic status of many gregarines parasitizing in crustaceans. There is, therefore, an urgent need to revise all gregarine genera and species described from *Crustacea*.

The fauna of *Gammaridae* of the Baikal Lake could give a good opportunity to study the taxonomy of gregarines associated with these arthropods as they occur there in great number of species and in high densities. As fauna of *Gammaridae* of the Baikal Lake is almost fully endemic their relationships with gregarines have to be of unic character and excellent for such a study.

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Table 7
Host range of gregarines recorded from gammarids in the Baikal Lake

Host	Gregarine species					
	Cephaloidophora gershensoni sp.n.	*Cephaloidophora poltevi* sp.n.	*Heliospora acanthogammari* (Zvetkov)	*Rotundula baicalensis* (Zvetkov)	*Roundula dybowskii* sp.n.	*Rotundula godlewskii* sp.n.
Acanthogammarus godlewskii var. *victori* (Dyb.)			+			
Baicalogammarus pullus (Dyb.)		+				
Brandtia lata lata (Dyb.)					+	+
Eulimnogammarus cruentus (Dor.)			+			
Eulimnogammarus viridis (Dyb.)			+			
Gmelinoides fasciatus (*Stebb.*)	+	+			+	
Microrupus vortex vortex (Dyb.)		+				
Pallasea brandti (Dyb.)			+			
Pallasea cancellus (Pall.)					+	
Pallasea kessleri (Dyb.)					+	+
Pallasea viridis (Garj.)					+	+

Summary

In April, 1966, nine species of gammarids from the Baikal Lake were examined for gregarine infections. The following five species of gregarines were found: *Rotundula dybowskii* sp. n., *R. godlewskii* sp. n., *Heliospora acanthogammari* (Zvetkov) comb. nov., *Cephaloidophora poltevi* sp. n. and *C. gershensoni* sp. n. These gregarines parasitized various gammarids listed as follows: *Baicalogammarus pullus* (Dyb.), *Brandtia lata lata* (Dyb.), *Eulimnogammarus cruentus* (Dor.), *E. viridis* (Dyb.), *Gmelinoides fasciatus* (Stebb.), *Microrupus vortex* (Dyb.), *Pallasea cancellus* (Pall.), *P. kessleri* (Dyb.), *P. viridis* (Gar.). Out of 142 specimens of those nine species 26 were infected i.e. 17% of the total number of gammarids. The results of these studies indicate that gammarids of the Baikal Lake are strongly infected by gregarines and can be an excellent object to make the revision of the taxonomy of gregarines of *Gammaridae*, what is urgently needed. The previously known *Gregarina baicalensis* Zvetkov is transferred to the genus *Rotundula* as *R. baicalensis* (Zvetkov) comb. nov.

STRESZCZENIE

W kwietniu 1966 roku autor przeprowadził badania nad gregarynami pasożytującymi w kiełżach jeziora Bajkał. Badaniami objęto dziewięć gatunków kiełży: *Baicalogammarus pullus* (Dyb.), *Brandtia lata lata* (Dyb.), *Eulimnogammarus cruentus* (Dor.), *E. viridis* (Dyb.), *Gmelinoides fasciatus* (Stebb.), *Microrupus vortex*

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vortex (Dyb.), *Pallasea cancellus* (Pall.), *P. kessleri* (Dyb.) i *P. viridis* (Gar.). Wszystkie gatunki okazały się żywicielami dla różnych gatunków gregaryn. Stwierdzono następujące gatunki gregaryn: *Rotundula dybowskii* sp. n., *R. godlewskii* sp. n., *Heliospora acanthogammari* (Zvetkov) comb. nov., *Cephaloidophora poltevi* sp. n. i *C. gershensoni* sp. n. Na 142 okazy badanych dziewięciu gatunków kiełży 26 (17.1%) było zarażonych przez gregaryny. Wyniki badań wskazują, że gregaryny są pospolitymi pasożytami kiełży Bajkału i byłyby dobrym obiektem badań nad rewizją gregaryn kiełży, które to badania są bardzo pożądane. *Gregarina baicalensis* Zvetkov zostaje przesunięta do rodzaju *Rotundula* jako *R. baicalensis* (Zvetkov) comb. nov.

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EXPLANATION OF PLATES I—IV

Rotundula dybowskii sp. n.

1—2: Sporonts in the association
3: A cyst

Rotundula godlewskii sp. n.

4—5: Sporonts in the association
6: A cyst with gelatinous layer

Heliospora acanthogammari (Zvetkov) comb. nov.

7: A group of sporonts in the associations
8: Front of sporont with the visible small protomerite
9: Primite (P) and satellite (S) in the association

Cephaloidophora poltevi sp. n.

10: A group of sporonts in the association
11: Sporonts inside the gut of host

Cephaloidophora gershensoni sp. n.

12: Sporonts in the association

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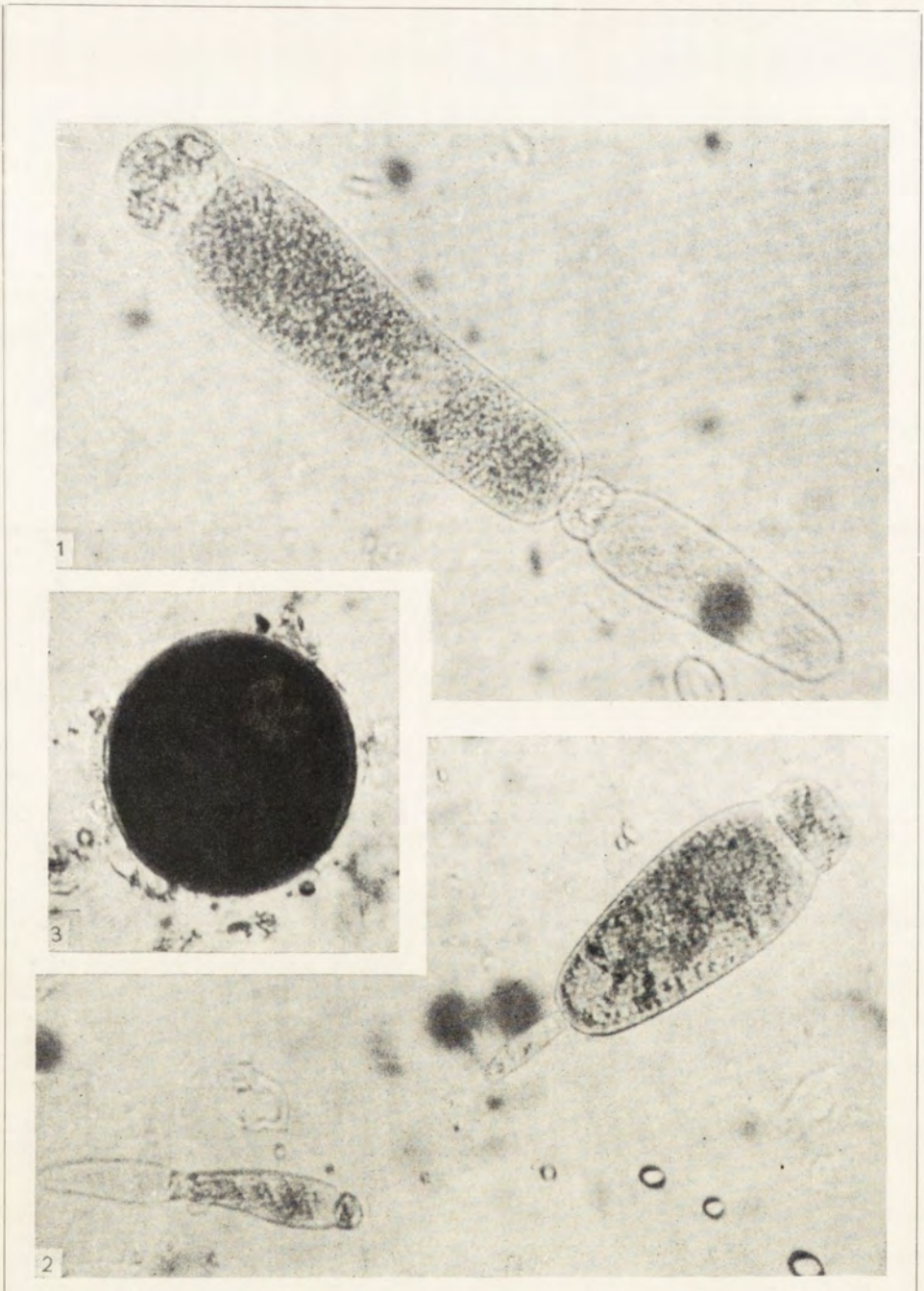

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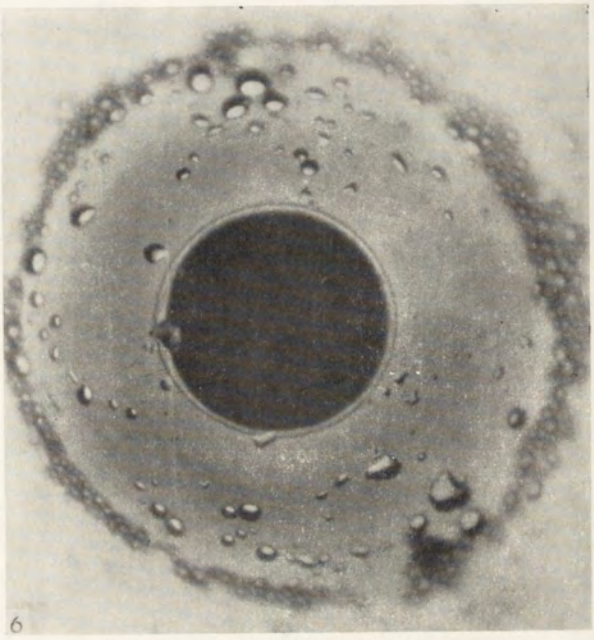

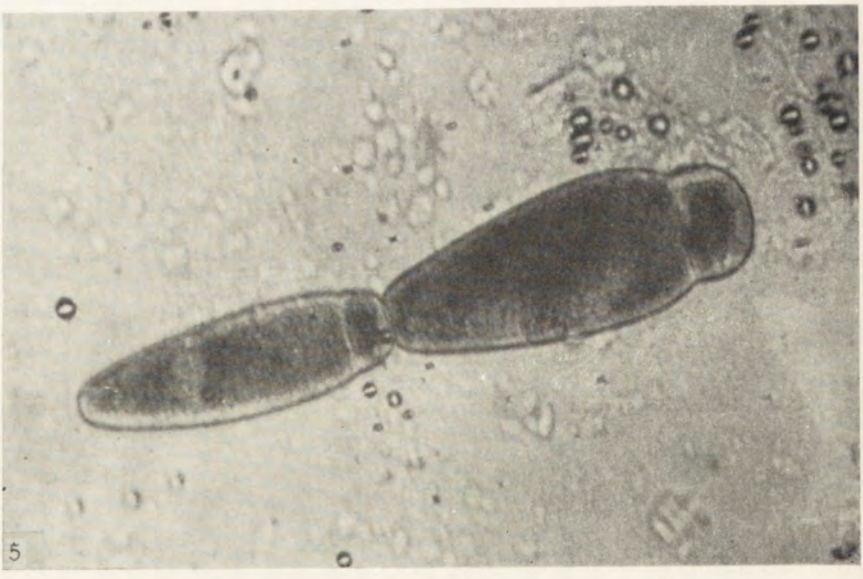

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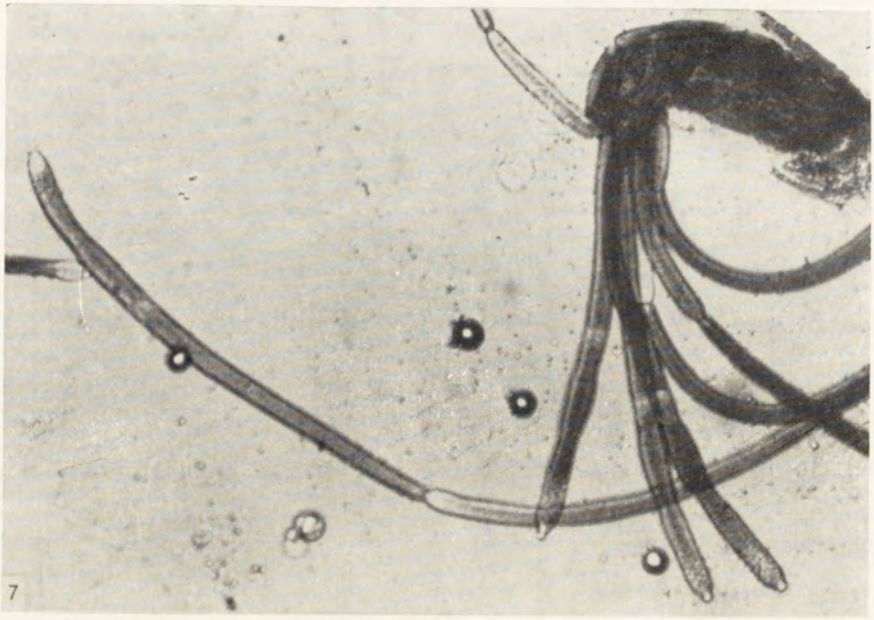

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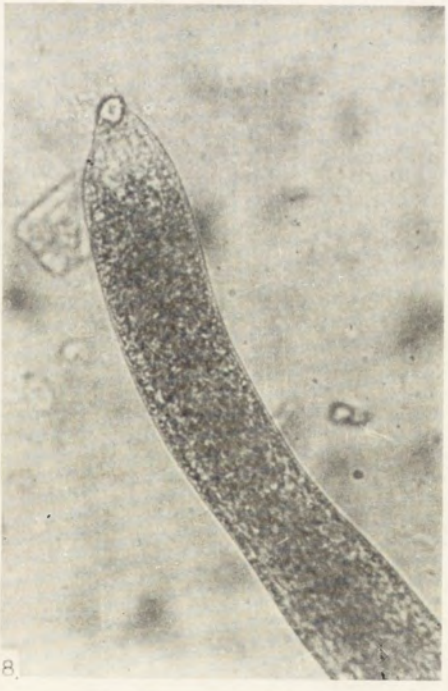

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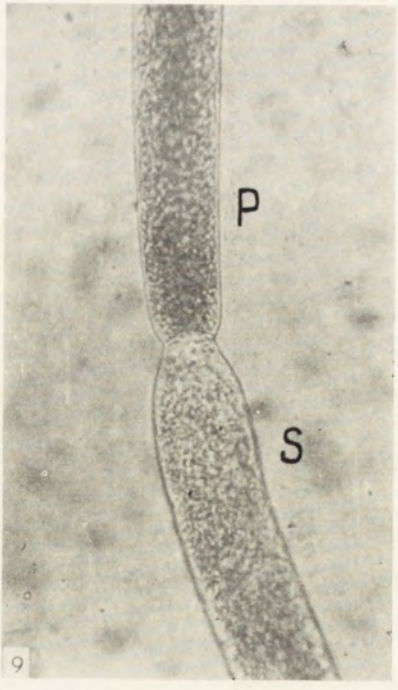

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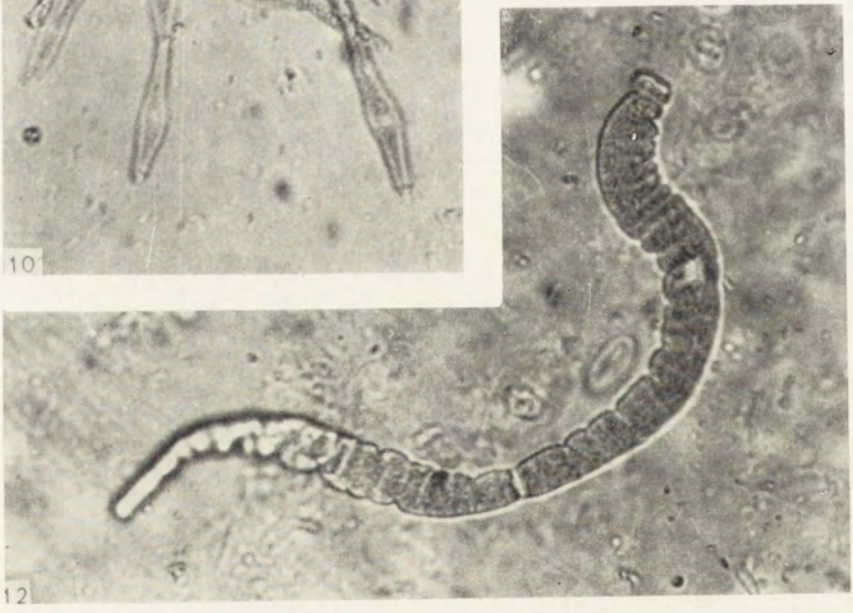

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ACTA PROTOZOOLOGICA

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Laboratory of Biological Control, Institute of Plant Protection, Grunwaldzka 189, Poznań, Poland

Jerzy J. LIPA

Nosema lepturae sp. n., a new microsporidian parasite of *Leptura rubra* L. *(Coleoptera, Cerambycidae)*[1]

Nosema lepturae sp. n., nowy gatunek mikrosporidia pasożytujący w *Leptura rubra* L. (*Coleoptera, Cerambycidae*)

During our studies on infection processes caused by protozoans in insects a number of insects, belonging to various orders were collected and examined on protozoan infections. The longhorned beetle *Leptura rubra* L. was one of studied insects that have been found to be infected with a microsporidian.

Adult beetles of *Leptura rubra* L. were collected on various plants in the Białowieża National Park. The collected insects were dissected and various tissues were examined on the presence of parasites. Then, smears were prepared, fixed in methanol and stained with 0.25% Giemsa's solution for 16—24 hours.

Nosema lepturae sp. n.

Host insect: *Leptura rubra* L.
Infected tissue: General infection, but especially tracheal matrix, fat body, and mid-gut epithelium were destroyed.
Locality record: Białowieża National Park, July 13, 1965.
Infection level: Out of six examined adult beetles one of them was infected.

Schizogony and sporogony

Schizont are small and spherical, and measure 2 to 5 μ in diameter. When treated with Giemsa's solution one or two nuclei appear deep red against a blue cytoplasm.

Sporogonic stages stain weaker than schizonts. Sporonts are elongated and are 3 to 6 μ long. From each sporont on spore is formed and it characterizes the genus *Nosema*.

The spore

Spores of the *Nosema* being described are oval or ellipsoidal and rather uniform in size (Pl. I 1, 2). Fresh spores are 4.6 to 6.1 μ long and 2.2 to 3.5 μ

[1] This investigation was supported by the research grant FG-Po-112 from the United States Department of Agriculture.

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wide; fixed and stained spores are slightly smaller and measure from 3.1 to 5.6 by 1.6 to 3 μ (Pl. II 6). A very few macrospores were observed having up to 6.9 μ in length (Pl. II 6). Results of measuring of two samples of 50 spores each of fresh and stained spores are given in Table 1.

Table 1

Frequency distribution of length of two samples of 50 spores ench of *Nosema lepturae* sp. n. (in microns

	3.0—3.4	3.5—3.9	4.0—4.4	4.5—4.9	5.0—5.4	5.5—5.9	6.0—6.4	6.5—6.9
Fresh spores	—	—	1	6	26	8	8	1
Stained spores	3	5	15	14	12	1	—	—

Polar filament is easily extruded (Pl. I 2, II 4, 5) by application of pressure on a cover glass. The maximum length of the polar filament is 117 μ.

Pathogenicity

In an examined adult beetle a general infection was observed. Tracheal matrix (Pl. I 3) fat body and midgut intestine were heavily infected and destroyed.

Taxonomic position

On the basis of sporogony this species should be placed to the genus *Nosema* as one spore is produced from each sporont. There is no previous record of microsporidian infection in *Leptura rubra* as well as in whole family of *Cerambycidae* (W e i s e r 1961). Due to various significant differences this species cannot be identified with other microsporidians known from *Coleoptera*. Therefore I consider that the microsporidian found in *Leptura rubra* is a new species and I propose for it name *Nosema lepturae* sp. n.

The holotype slide, stained with Giemsa, from an adult host collected on July 13, 1965, in the Białowieża National Park, is in the author's collection.

Summary

A new microsporidian species *Nosema lepturae* sp. n. parasitizing in a long-horned beetle *Leptura rubra* L. is described. Spores are oval to ellipsoidal; fresh spores are 4.6—6.1 by 2.2—3.5 μ; fixed and stained spores are 3.1—5.6 by 1.6—3.0 μ. The length of polar filament is up to 117 μ. The parasite infects tracheal matrix, gut, fat body and other tissues of the host insect.

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STRESZCZENIE

Opisano nowy gatunek mikrosporidia *Nosema lepturae* sp. n. pasożytującego w kózce *Leptura rubra* L. Spory pasożyta są owalne lub elipsoidalne; świeże spory mierzą 4.6—6.1 μ długości i 2.2—3.5 μ szerokości; spory utrwalone i barwione mierzą 3.1—5.6 μ długości i 1.6—3.0 μ szerokości. Maksymalna długość nici biegunowej wynosiła 117 μ. Pasożyt zaraża błonę podstawową tchawek, jelito, ciało tłuszczowe i inne tkanki.

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EXPLANATION OF PLATES I—II

Nosema lepturae sp. n.

1: Spores seen under phase contrast.
2: Spores after application of pressure; notice the extruded polar filament and broken spore walls (1200×)
3: Spores as seen in tracheal matrix and fat body (550×)
4—5: Spores with extruded polar filaments as seen at a darkfield illumination (1200×)
6: Several spores and one macrospore in smear preparation stained with Giemsa's solution (1200×)

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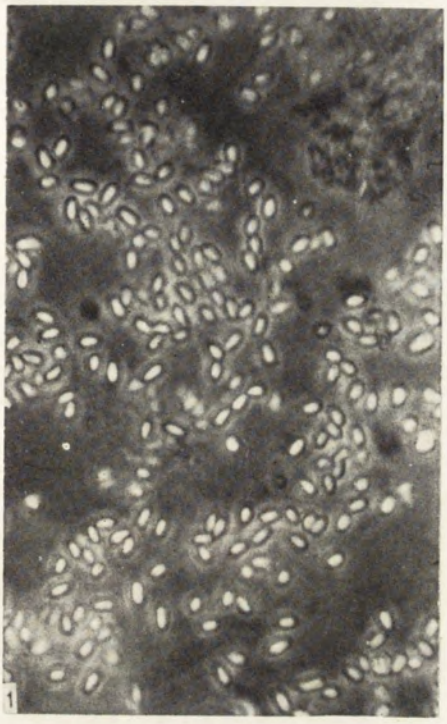

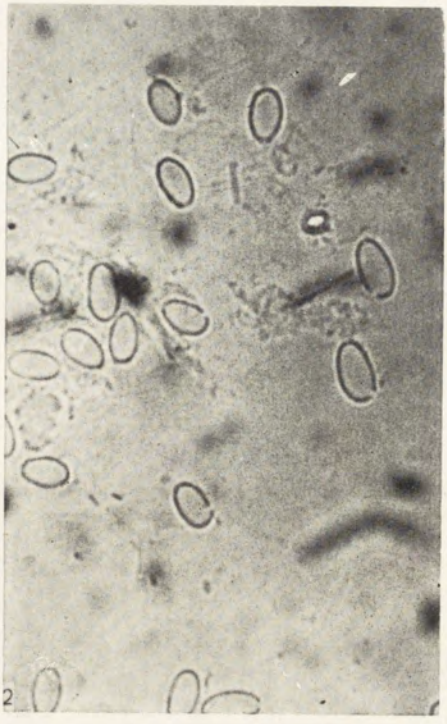

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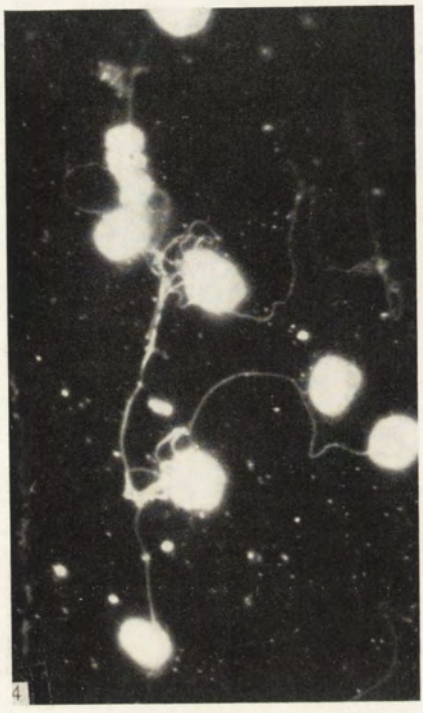

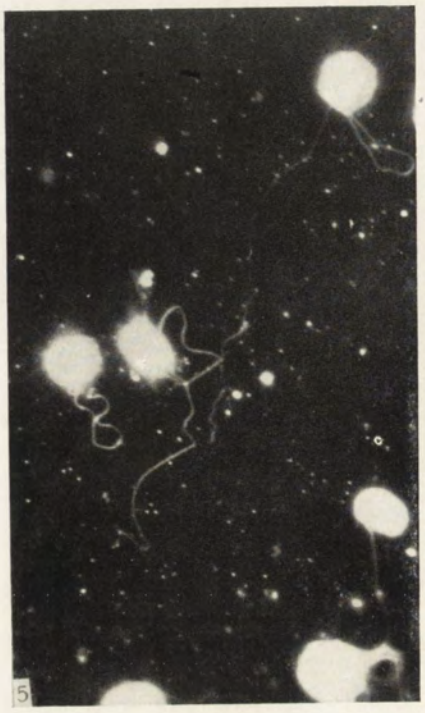

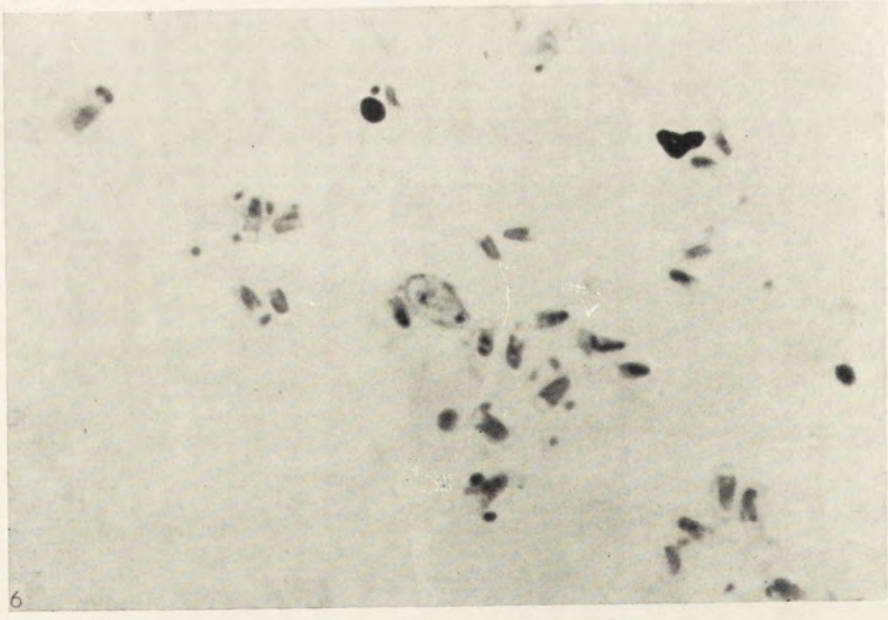

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Laboratory of Invertebrate Animals, Biological Research Institute of Leningrad State University, Leningrad B-164, USSR

I. I. SKOBLO and D. V. OSSIPOV

The autogamy during conjugation in *Paramecium caudatum* Ehrbg. I. Study on the nuclear reorganization up to stage of the third synkaryon division

Автогамия при конъюгации *Paramecium caudatum* Ehrb. I. Изучение ядерной реорганизации до стадии III деления синкариона

In ciliates a great variety of types of sexual processes occurs: conjugation, autogamy, cytogamy, apomixis and others (Wichterman 1953, Dogiel and others 1962, Jankowski 1960, 1962). Out of those phenomena, conjugation has been studied most exactly whereas the others which occur less frequently, have been described only in a limited number of ciliate species.

The cytological picture of the autogamy process is the same as that of conjugation except that the nuclear reorganization occurs in single individuals without any even temporary pairing (Diller 1936). In the autogamont, two haploid pronuclei which are identical genetically, fuse together producing a synkaryon homozygotic in all genes (Sonneborn 1942, 1947). This accounts for the great methodical advantages the genetic study of those ciliate species in which the natural autogamy has been observed. This process however has been described in 4 ciliate species only: *Paramecium aurelia* (Diller 1936), *P. polycarium* (Diller 1954), *P. jenningsi* (Mitchell 1962), *Tetrahymena rostrata* (Corliss 1952).

The problem of autogamy in *P. caudatum* is somewhat complicated. Some authors found autogamy in this species (Erdmann and Woodruff 1916, Giese and Arkoosh 1939). However the more recent and precise investigations failed to support the earlier results (Gilman 1939, 1959). The controversies observed gave ground to Sonneborn for the postulation that autogamy occurs in some syngenes of *P. caudatum* being absent in the others (Sonneborn 1957). Gilman after having investigated 10 syngenes of *P. caudatum* ascertained that the natural autogamy is absent in this species (Gilman 1959). The attemtps to evoke autogamy in single cells of *P. caudatum* by means of different chemical agents gave no positive results (Hiwatashi 1959). Besides the normal extraconjugation autogamy, for some ciliates, an autogamy during the conjugation has been described (Chen 1940, Hiwatashi 1949, 1955 a, Vivier 1960, Miyake 1960, 1961, Poliansky 1938, Jankowski 1960, 1965, Ossipov 1966). In all those cases autogamy occurs as a fortuitous process (coinciding with the normal conjugation) when : 1. the position of conjugants interferes with the exchange of pronuclei or 2. the

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pair members diverge or are artificially separated prior to the exchange of pronuclei, 3. the migrating pronuclei lose the capability of active movement. For inducing the nuclear reorganization in the conjugation autogamy, either an initial contact of cells of the complementary mating types is absolutely necessary (Hiwatashi 1949, Metz 1954) or the activation of the mating substances on the surface of the cilia (Metz 1954, Miyake 1960, 1961). The conjugation autogamy in *P. caudatum* has been described by a number of investigators (Hiwatashi 1949, Miyake 1960, 1961, Vivier 1960, Ossipov 1966), however the cytological changes of nuclei in this process have remained until recently not investigated.

In the present study, a great number of crossing combinations of *P. caudatum* clones of complementary mating types were investigated. The results revealed that in some of them the conjugating pairs separate after 7—8 hrs. In the process of normal conjugation of *P. caudatum* at room temperature the individuals remain paired for 24 hrs. The nuclear reorganization in some cells of the precociously separated pairs, follows the autogamy type. The aim of the present study is a cytological analysis of conjugation autogamy in *P. caudatum*.

Material and methods

The investigation was carried out on two clones of *P. caudatum* of complementary mating types: M-13 and M-17. Paramecia of the M-17 clone have a micronucleus (Mi) usual for *P. caudatum*, the clone M-13 is amicronuclear. Clones originated of one natural population — Staryj Peterhof. Ciliates were cultivated in test tubes at 25°C, in lettuce medium, fed with *Aerobacter aerogenes* (Ossipov 1966). Crossing of clones was executed in microaquaria and micro-test tubes at room temperature. The study of the nuclear reorganization was carried out as well on temporary preparations stained after Dippell 1955 as on fixed whole-mount ones stained after Feulgen and light green. Ciliates were fixed with the Bouin fluid at definite time intervals since the moment of the conjugation onset of clones M-13 and M-17.

The cytological study of autogamy prior to the first synkaryon division was carried out on preparations consisting of a mixture of clones M-13 and M-17, in which — besides the autogamonts — a certain number of vegetative cells had occured which failed to produce pairs, as well as an insignificant percentage of homotypic pairs (M-17 × M-17). At more advanced stages of the nuclear reorganization, ex-autogamont and ex-conjugant cannot be distinguished because the pair members of normal conjugation separate at the stage of the first synkaryon divison (Pl. I 1).

The following technique was applied for securing a great number of autogamonts for examination of nuclear processes after the first synkaryon division. As known, the conjugating ciliates in the moment of pairing stop forming new food vacuoles because the peristome and the mouth cilia become resorbed at that time (Hiwatashi 1955 b). Towards the prophase of the first progamic division of Mi the ciliate cytoplasm is practically free of food vacuoles. If a suspension of China ink has been added to the mixture of clones M-13 and M-17 10 hrs. after the onset of conjugation (pairs M-13 × M-17 separate already about that time) then phagocyting cells are easily distingui-

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shable from those which participate in the sexual process and fail to produce new food vacuoles. The cells not involved in pair formation proved to be phagocytic as well as those of pairs separated precociously in which the process of nuclear reorganization has not as yet begun. The individuals with quite clear cytoplasm, not phagocyting China ink, are autogamonts. Those were fished out of the mixture and used for the further work, after fixation at definite time intervals. In the course of investigation about 1000 autogamonts were studied and drawn with a drawing apparatus (ob. 40×, eye piece 7×). Photomicrographs were executed by means of MFN-3 apparatus with the microscope MBI-3.

Results

Mixing clones M-13 with M-17 in one microaquarium evokes an active agglutination reaction in ciliates and 1—2 hrs. later a mass formation of conjugating pairs. In contrast to the normal conjugation pairs, in the case of crossing clones M-13 × M-17 the conjugants separate already after 7—8 hrs. It was shown in the preliminary experiments that the nuclear reorganization occurs not in all the precociously separated cells of conjugating pairs. In order to ascertain the percentage of cells out of all participating in formation of conjugating pairs which subsequently are to undergo the nuclear reorganization, 100 conjugating pairs were placed in separate containers (1 pair in an container). After 48 hrs. temporary preparations of each single cell were executed to prove the occurrence of the nuclear reorganization. The results of this experiment are presented in Fig. 1. Formation of a certain number of homotypic pairs is not unexpected since it takes place in the normal conjugation of ciliates (Hiwatashi 1951). The partners of the conjugating pairs M-13× ×M-13 separate after 7—8 hrs. and by the 48-th hours undergo up to two cell divisions. The nuclear reorganization has not been observed in any of such cell pairs. The conjugants in the pairs M-17×M-17 remain joined for a normal period of time and the nuclear reorganization proceeds quite normally as in the usual conjugation. The fate of M-13×M-17 pairs is not the same. In 42% of those pairs, both partners divide twice within 48 hrs. and no nuclear reorganization occurs. In 40% of M-13×M-17 pairs, the amicronuclear partner (M-13) divides twice after 48 hrs. and in the normal partner (M-17) the nuclear reorganization of the autogamy takes place.

Normal course of nuclear reorganization

Owing to the fact that in the clone M-17 the authors succeded to obtain self-conjugation, there was a possibility to compare the cytological picture and subtle changes in nuclei in autogamy and in conjugation of the same clone M-17. As follows from the facts revealed in this study, the nuclear processes in the autogamont of *P. caudatum* proceede in the same sequence as in conjugation (Calkins and Cull 1907, Wichterman 1953). After two meiotic divisions of Mi, 3 nuclei are resorbed and the fourth one undergoes the third equation division which results in formation of pronuclei. Those fuse together giving origin to a diploid synkaryon which subsequently divides three times. Out of the 8 nuclei, 4 become the anlagen of macronucleus and are distributed to the products of the first two metagamic cell divisions, one nucleus becomes Mi, whereas the last three products of synkaryon division become resorbed.

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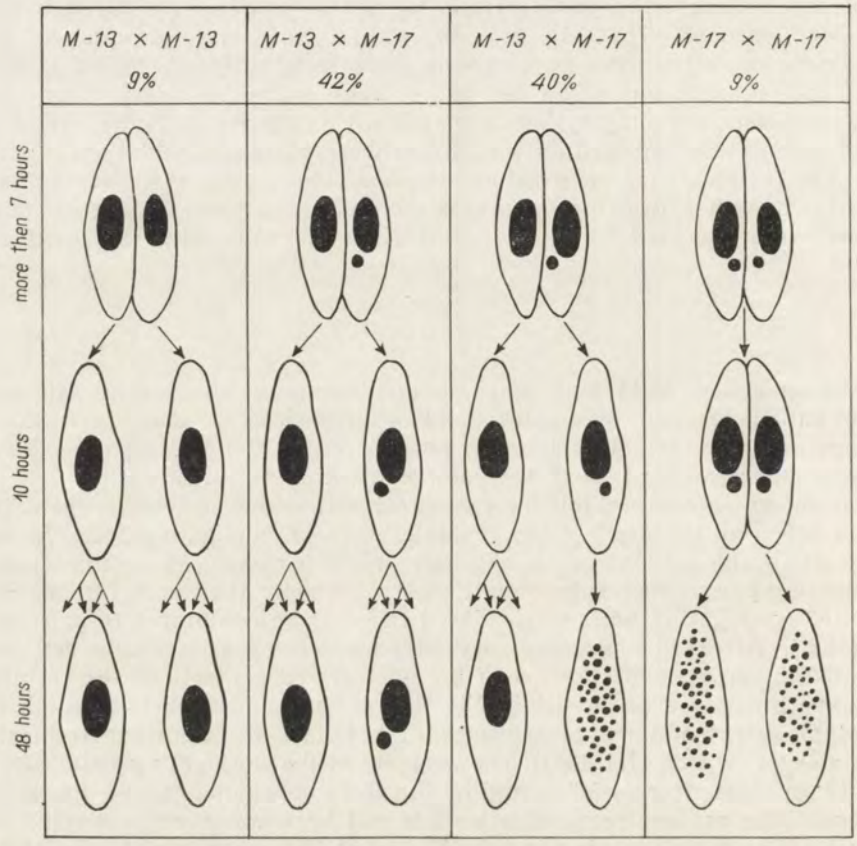

Fig. 1. Types of the conjugating pairs during crossing the clones M-13 and M-17 of *Paramecium caudatum* and their subsequent fate. 100 conjugating pairs were examined

The old macronucleus begins to fragmentate at the stage of the third synkaryon division.

Examination of ciliates which have been fixed at definite periods of time from the moment of the onset of sexual process allowed to ascertain the duration of separate stages of the nuclear reorganization in autogamy and in conjugation of *P. caudatum* (Fig. 2). Attention should be paid to the fact that the process of nuclear reorganization in autogamonts proceeds much more slowly. This concerns mostly the prophase of the first progamic division of Mi. The first metagamic division of the ex-autogamont is also much delayed and occurs not earlier than after 4 days, whereas the ex-conjugants start dividing usually after 24 hrs.

Since the moment of fusion of the partners into a conjugating pair, the dimensions of Mi begin to increase gradually, inside the nucleus occurs the rearrangment of the chromatin elements and Mi enters its first meiotic division (Pl. I 2, 3). Prophase of the first Mi division last about 18 hrs. In the prophase of the first division when the cells are still joined in pairs,

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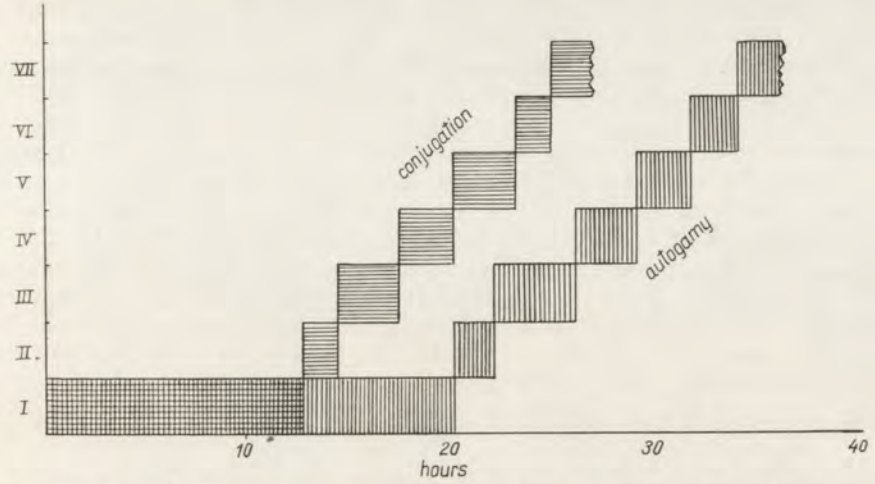

Fig. 2. Duration of the nuclear reorganization stages in autogamy and conjugating of *Paramecium caudatum*. I — First division of micronucleus, II — Second division of micronucles, III — Third division of micronucles, IV — Formation of synkaryon, V — First metagamic division of synkaryon, VI — Second metagamic division of synkaryon, VII — Third metagamic division of synkaryon

Mi undergoes the characteristic crescent stage (Pl. I 4). At the moment of this stage, the pair members separate and the subsequent reorganization occurs in single cells (Pl. I 5, 6). In the late prophase of the first division, Mi presents a swollen nucleus with discernible filamentous chromosomes (Pl. I 7). Migration of chromosomes to the poles occurs non-synchronically, a distinct metaphase was not observed. The axis of the first Mi division is oriented along the body, so that one of the arising nuclei lies on the anterior and another on the posterior end of the ciliate body (Pl. I 8, 9).

The interphase between the first and second Mi division lasts no longer than one hour. The spindle axes of the second division are oriented so that one of the arising nuclei lies in the paroral zone whereas the 3 other nuclei are out this region (Pl. II 10, 11). Towards the moment of conclusion of the second Mi division, the paroral cone becomes well discernable. This structure was observed in living paramecia and consequently is not an artifact evoked by fixation or by pressure on the autogamont in technique. The nuclei which lie out of cone become pycnotic while the nucleus in the cone remains active (Pl. II 12). About one hour later this nucleus undergoes the third progamic division (Pl. II 14), the resultant nuclei differentiate as a stationary and a migrating pronuclei (Pl. II 14—16). At the stage following the third division of Mi, the paroral cone appears especially distinctly (Pl. II 13—18). It seems quite possible that the exchange of the migrating pronuclei in the normal conjugation of *P. caudatum* occurs just across the paroral cone (Pl. III 19). The study of the formation mechanism of the paroral cone, of its connection with the buccal apparatus in paramecia may present the aim of a special investigation.

Over one hour elapses from the moment of the pronuclei formation till their fusion. Pronuclei fuse inside the body, near the paroral cone which

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becomes scarcely discernable about that time (Pl. III 20, 21). Determination of the chromosome number in the diploid and haploid stage of meiosis was as yet not carried out by the authors because in *P. caudatum* chromosomes stain faintly, are filamentous and their count is connected with serious methodical difficulties. Synkaryon undergoes three consecutive metagamic divisions. The spindles of the first two divisions are oriented along the longitudinal axis of the autogamonts so that products of the second synkaryon division lie on one line on the marconucleus (Pl. III 22, 23). The third synkaryon division coincides with the moment of onset of macronucleus fragmentation (Pl. III 24, 25). The first stages of this process are observable a long time before the formation of the fragments themselves. Macronucleus fragmentates into long sausage-shaped segments, and subsequently into spherical bodies in a number of 30 — 70 in each ex-conjugant. The examination of further alterations in the nuclear apparatus of ex-conjugant will be the subject of a special communication.

Aberrations in the behaviour of Mi

The normal type of the nuclear reorganization has been examined (Fig. 3 A — E). It does not essentially differ from the picture of nuclear changes in conjugants except — of course — for the absence of the pronucleus migration. Now it is necessary to consider the variety of anomalies of nuclei which were found in 49% of autogamonts as well in the progamic as in metagamic part of the nuclear reorganization cycle (Fig. 3). Fig. 3 presents the result of examination of 463 autogamonts at the stages up to the third synkaryon division. It should be remarked in the first place that in the conjugation M-17 × M-17 the nuclear anomalies occur very rarely and as a rule at the stage of formation and migration of pronuclei: 1. loss of capability of passage in the migrating pronucleus of one conjugant; as result of this, the development of a new nuclear apparatus in one of the conjugants from hemikaryon (Pl. VII 61), 2. the fusion of pronucleus arrested in the paroral cone with one of the products of the first synkaryon division.

The anomalies observed by the authors in the autogamonts of *P. caudatum* are exceptionally various and as a rule were not sygnalized before in conjugants. Some of those anomalies were observed for the first time in ciliates.

A. Abnormal behaviour of nuclei in the prophase of the first progamic division of Mi occurs rarely and leads to formation of the pycnotic crescent (Pl. IV 26, Fig. 3 F). It should be stressed that in such cells the fragmentation of macronucleus fails to occur subsequently. Possibly the process of stomatogenesis had been impaired in them. This view was suggested by the absence of formation of new food vacuoles in 30—40 hrs. after the onset of sexual process. The cells with the degenerated Mi which failed to conclude their first progamic division perish within 6—10 days.

B. In the four autogamonts at the anaphase stage of the first progamic division of Mi, pycnotic nuclei were observed (Pl. IV 27, Fig. 3 G). The authors did not succeed to follow the subsequent fate of cells with this anomaly.

C. Anomaly occurring most frequently (in 29% of autogamonts) is the disturbance of the course of the second progamic Mi division (Pl. IV 28—34, V 35—37, Fig. 3 H — N). Normally after the first Mi division, nuclei are spherical and stain very faintly (Pl. I 7), the interphase of the second division

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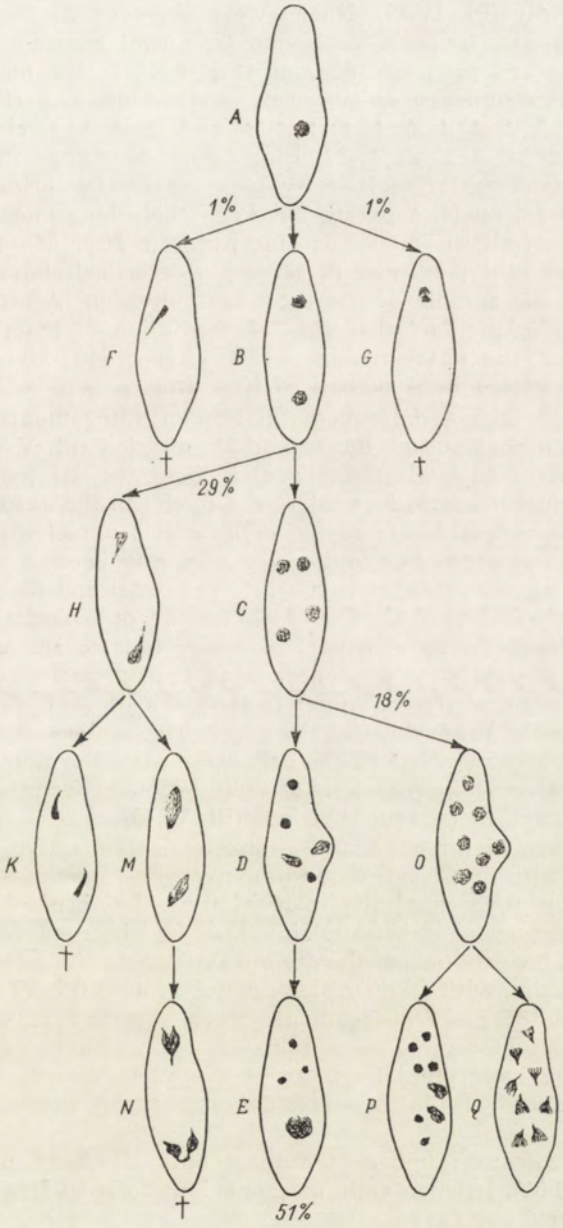

Fig. 3. Appearence of the fundamental aberrations of nuclear behaviour in course of autogamy process *Paramecium caudatum*. Only micronuclei and their derivates are shown. 463 autogamonts were examined. † — all the autogamonts died

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lasts no longer than one hour. In the case of the anomaly observed, the interphase of the second division lasts for a much more prolonged time. A "spout" from chromosomes is detached of the nuclei (Pl. IV 28, Fig. 3 H). The quantity of chromatine gradually augments in the "spout" and nuclei begin to stain much more vividly (Pl. IV 29). Nuclei were observed in this condition up to the stage of 28 hrs. In some autogamonts, nuclei become pycnotic without beginning the second progamic division (Fig. 3 K). In the majority of autogamonts, the nuclei undergo an abnormal second division (Pl. IV 30, 31), its peculiar character is an unusual manner of separation of nuclei which lie at an angle to each other (Pl. IV 32-34, Fig. 3 N). Sometimes the nuclei fail to separate at all and remain joined by long chromatine bridges (Pl. V 35). In a number of cases, nuclei separate but keep their long "noses" (Pl. V 36). In the final outcome, all the nuclei become pycnotic after 35—40 hrs. (Pl. V 37). All the course and the picture of the anomaly described above suggests serious disturbances in the spindle of the second Mi division. A striking peculiarity of this anomaly is the fact that the fragmentation of macronucleus fails to occur at all and the autogamonts perish after 6—10 days (Pl. V 38). This anomaly in ciliates has been sygnalized first time.

D. A very peculiar and frequent (in 18% of autogamonts) anomaly is the lack of nuclear pycnosis after the second Mi division (Pl. V 39—43, VI 44—51, Fig. 3 O,P,Q). As result, all the 4 nuclei begin the III maturation division (Pl. V 39, 40), which leads to formation of 8 nuclei in the autogamont (Pl. V 41, Fig. 3 O). The subsequent fate of the cells with 8 nuclei may be various. In the majority of autogamonts, 6 out of 8 nuclei may become pycnotic, whereas the two remaining active differentiate into pronuclei and fuse together producing the synkaryon (Pl. V 42, Fig. 3 P). In the other cells, the mechanism involving the degeneration of a part of nuclei fails to act at all or concerns only a part of nuclei. In consequence, more than 2 nuclei remain active. An interesting fact is, that their number is always even (4, 6, 8) (Pl. V 43, VI 44, Fig. 3 Q). As a rule, pronuclei fuse by pairs. The authors succeded in finding several autogamonts with 2, and one cell even with 3 synkaryons. In the case of formation of two synkaryons one of them becomes pycnotic and the second one begins a normal metagamic divisions (Pl. VI 45).

In the cells in which more than 2 pronuclei remain active, the nuclei which failed to participate in the formation of synkaryon sometimes neither undergo pycnosis nor loose their capability to division i.e. they become hemikaryons. In result, the phenomenon of heteroploidy may be observed when in the autogamont, besides the products of the diploid synkaryon division, the products of hemikaryon division with a lower plody may be found (Pl. VI 46—48).

In a number of cases autogamonts were observed in which more than 2 pronuclei participated in formation of synkaryon which was suggested by the dimensions of the hyperploidal synkaryon (Pl. VI 49—50). The subsequent fate of the autogamont with a hyperploidal synkaryon has not been as yet elucidated.

In an insignificant number of autogamonts all the 8 pronuclei become pycnotic (Pl. VI 51). In such cells macronucleus fails to fragmentate and the cells perish after 6—10 days.

In many autogamonts with the above anomaly, synkaryon and hemikaryon just formed or the products of their division become pycnotic (Pl. VI 52, VII 53—56). In such cells, the pycnotic bodies differ considerably in size from

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one another which evidently reflects the degree of ploidy of the degenerated nuclei. A normal fragmentation of macronucleus does not occur although it may asume a form and structure which is characteristic for the stage preceding fragmentation or even produce irregular streaks (Pl. VII 54—56). Not rarely at the stage of 48—50 hrs. occurs elongation of the autogamont or even budding of a small fragment of the cell (Pl. VII 55, 56). Nevertheless any normal metagamic division were not observed and the autogamonts of this aberration type perish after 6—10 days.

E. A synkaryon formed as a result of a normal type of nuclear reorganization (Fig. 3 A — E) rarely loses its capability to metagamic divisions and becomes pycnotic. In this case similarly as in the anomalies described above, macronucleus does not begin a normal fragmentation (Pl. VII 57).

F. In some autogamonts at the stage of onset of fragmentation, more than 8 nuclei are seen in macronucleus (Pl. VII 58). Evidently this may be accounted for by the fact that at least a part of the products of the third metagamic synkaryon division is capable to the fourth division. The postulation that all the autogamont nuclei originate of only one synkaryon is based on following indirect evidences. Firstly, the same dimensions of nuclei and their size speak in favour of the fact that they present products of division of synkaryon only and not of hemikaryon and synkaryon simultaneously. Secondly, as mentioned above, in the case of formation of several synkaryons in an autogamont, only one of them is capable to subsequent development.

G. Finally, the last type of anomaly which we would discuss in this communication — is the abnormal fragmentation of macronucleus. In some autogamonts hemixis is observable i.e. fragmentation of macronucleus into 2—3 uneven parts (Pl. VII 59—60). Hemixis in ciliates occurs rarely and is usually looked upon as a peculiar manner of nuclear reorganization (Fauré-Fremiet 1953). For the autogamonts of *P. caudatum* with a hemictic fragmentation of macronucleus, small dimensions of the body and absence of normal active nuclei are characteristic. It may be ascertained in a number of cases that hemixis arose as result of nuclei degeneration at the prophase stage of the first progamic division of Mi (Pl. VII 59).

In the present communication we do not intend to examine the cytological picture of the normal ex-autogamont cycle but to stress that despite the numerous anomalies of the nuclear reorganization in autogamy, the percentage of viable ex-autogamont clones reaches 22.8% (of the total number of cells M-17 beginning autogamy), whereas after conjugation of paramecia M-17×M-17, 52.5% of viable ex-conjugant clones are formed.

It should be remarked in conclusion that the nuclear reorganization of the autogamy type was observed by us later when crossing clone M-13 with clones MG-10 a (karyonid with a normal Mi of ex-conjugant M-17) and KT-20 (clone with a normal Mi from another population than clones M-13 and M-17). Although a detailed study of the nuclear reorganization has not been carried out, some interesting facts should be stressed: similarly as after crossing M-13×M-17, the normal type of nuclear reorganization was stated in the micronuclear partner, as well as the principal types of aberration (the multiple formation of pronuclei, blockade of the second progamic division of Mi and others).

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Discussion

In quite a number of ciliates, autogamy has been described as a casual process during conjugation occurring simultaneously with the normal conjugation (review of Jankowski 1965). For induction of this process, a contact of cells of complementary mating types or of activating of the mating substances on the surface of cilia are absolutely necessary (Metz 1954, Miyake 1960). As a rule, the completion of the nuclear reorganization in autogamy depends on the loss of capability of pronuclei to reciprocal migration. Sonneborn stated that in *P. aurelia* the capability of pronuclei to migration is suppressed by raised temperature or by Ca^{++} ions (Sonneborn 1941). In some cases, the inability of pronuclei to reciprocal migration may be evoked by an abnormal position of partners in the conjugating pair. E.g. when normal cells are crossed with those of complementary mating type fixed with formalin, pairs arise composed of viable cells only (in *P. aurelia* — Metz 1947, in *P. caudatum* — Hiwatashi 1949). Pairing however concerns only the anterior end of the cell. It was observed that in such pairs, the nuclear reorganization is of the autogamy type. Unfortunately the cytological details of nuclear reorganization has not been reported by the authors. Another — very rare — type of abnormal union of ciliates in conjugation had been observed in triplets, when a third cell joins a normally conjugating pair. As a rule this "surplus" cell becomes attached with its anterior body end to the posterior one of the normal conjugant. In this "surplus" partner of abnormal position the nuclear reorganization proceeds according to the autogamy type (in *P. bursaria* — Chen 1940, in *P. caudatum* — Vivier 1960).

In the conjugating ciliate pairs when separated experimentally, the nuclear reorganization concludes also in autogamy (in *Bursaria truncatella* — Poliansky 1938, in *Euplotes eurystomus* — Katashima 1959, in *P. caudatum* — Ossipov 1966).

Wichterman described a sexual process in *P. caudatum* — cytogamy occurring in normally conjugating pairs (Wichterman 1940). This process agrees in details with the usual conjugation except for the regular absence of reciprocal pronuclei migration. Some authors however (Diller 1950, Jankowski 1965) consider the finding of Wichterman with caution because he studied the case of cytogamy in living material kept in a "micro-compression chamber" and therefore a slight pressing exerted on the conjugating pairs might eventually impede the migration of pronuclei.

The above review indicates that till present time the conjugation autogamy in *P. caudatum* was considered either as a fortuitous phenomenon parallel to the normal conjugation or as a result of experimental interfering with the normal course of the sexual process. In the case of crossing the clones M-13× M-17 autogamy is evoked by the precocious separation of the partners in the conjugating pairs. The separation occurs in a moment when nearly 50% of pairs have been involved in the mechanism which switches on the meiosis (Fig. 1). No doubt, that the precocious separation of pairs is associated with impairing the intracellular reactions which control the stability of binding the partners and the moment of separation. In this case such an insignificant disturbance of the sexual process as the precocious separation of the partners is, leads to essential karyological consequence — the autogamy.

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The precocious separation of conjugating pairs has been sygnalized for several species of ciliates (in *P. bursaria* — Chen 1946, Bomford 1965, in *P. caudatum* — Gilman 1939), the nuclear reorganization however fails to occur in these cases and the separated conjugants behave like vegetative cells and soon begin a normal division. It has been stated recently that in *P. bursaria* the precocious separation of pairs involves a stable rise of Mi ploidy (Bomford 1967). It will be interesting to carry out similar studies on conjugants M-17 out of the pairs: M-13×M-17 in which the nuclear reorganization does not occur (Fig. 1).

In Fig. 1 data of the fate of the conjugating pairs are presented which arise after crossing the clones M-13 and M-17. An interesting fact is the absence of nuclear reorganization in all the pairs M-13×M-13, and in the amicronuclear partner (M-13) in the pairs M-13×M-17. At present it seems difficult to discuss decidedly the factors involving the behaviour of amicro nuclear partner deviating from the normal one. It seems however possible to consider two postulations. Firstly, absence of fragmentation of macronucleus may be accounted for blocking the process which induces directly the nuclear reorganization. We came previuosly to the conclusion that in *P. caudatum*, for the onset of reorganization not only the paraoral union of the partners of the conjugating pair is indispensable but also a certain period of duration of this condition. In favour of existence of blockade at a definite stage of the sexual process in the cells M-13, speaks this fact that in all the pairs M-13×M-13 the nuclear reorganization does not occur. Conjugants separate and behave subsequently as normal vegetative cells.

Secondly, it seems possible that in the amicronuclear conjugants (M-13), the mechanism which induces the nuclear reorganization becomes activated more slowly or occurs in a chain of reactions which evoke the normal course of sexual process later than in the normal conjugants (M-17). It may be assumed that the conjugating pairs M-13×M-17 separate exactly at this moment when the nuclear reorganization has been already induced in paramecia of the clone M-17, and not yet induced in the conjugants M-13. A similar postulation was put forward by Larison and Siegel 1961 for explaining the rise of occurrence frequency (up to 13%) of homotypic conjugating pairs Wu-67×Wu-67 in the course of crossing the clone Wu-67 with other clones of complementary mating types in *P. bursaria*. Evidences in favour of the first postulation might be gained only in this case if such a partner to the clone M-13 could be found in which the normal nuclear reorganization would occur in both pair members. We could not succeed as yet to obtain such a combination of clone crossing.

The comparative study of autogamy and of conjugation of the same clone of *P. caudatum* leads us to conclusion that the course of nuclear reorganization and subtle cytological changes are identical in many details in those two processes. The fundamental differences concern the rate of passing of some stages, the number and character of nuclear aberrations and viability of cells after the nuclear reorganization.

As to the duration of stages of the nuclear reorganization process, we found a delay of prophase of the first progamic Mi division (up to 20—22 hrs.) in autogamy, whereas in the normal conjugation of M-17×M-17 the first Mi division is accomplished within 13—14 hrs. It may be assumed that a permanent contact of conjugants is necessary up to a certain moment (normally

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till the first metagamic synkaryon division) for supporting the normal speed of single links of the nuclear reorganization in *P. caudatum*. An essential inhibition (up to 4 days) was observed by us for the first metagamic division of ex-autogamonts as well. It occurs in the ex-conjugants M-17 after 48 hrs. Possibly this reflects also the essential disturbances of the regulatory mechanisms which are evoked by the precocious separation of conjugants of the pair M-13×M-17.

The most significant differences between the conjugation autogamy and normal conjugation concern the frequency of occurrence and character of the nuclear aberrations. It is quite obvious that when ignoring the fact of the field of pathology of the nuclear processes in autogamy it is impossible to understand the mechanism controlling the separate links of the normal conjugation. We stated in this study that the nuclear aberrations are observed in 49% of autogamonts. If the anomalies in normal conjugation of clone M-17 concerned essentially the metagamic part of the cycle, so in autogamy of the same clone aberrations were observed practically at all the stages. This indicates profound disturbances of mechanisms which are controlling the single links of the nuclear reorganization. An apparently not essential disturbance — the precocious separation of partners of the conjugating pair brings about significant karyological consequences. This concerns not only appearing of a great number of various nuclear anomalies but also results which are very important from the point of view of genetics namely a full homozygotation of surviving ex-autogamonts (Sonneborn 1942, Beale 1954). It is quite possible that the high percentage of the non-viable ex-autogamonts may be explained not only by the fatal nuclear aberrations but also by bringing all the recessive lethals in a homozygotic state. The latter view is supported by conclusions of Sonneborn 1957 that the system of mating types and the particularities of the life cycle of *P. caudatum* suggest a high degree of heterozygoty in the natural populations of paramecia (clones M-13 and M-17 originate of one natural population).

It has been shown in the present study that in 42% of conjugating pairs of M-13×M-17 after their firm union during 7—8 hrs. the nuclear reorganization fails to occur and the ciliates start dividing very soon as the normal vegetative cells do. In the cells of M-17 in which the process passed a certain critical point, the nuclear reorganization becomes fully accomplished.

The study of numerous anomalies in autogamy provided an important material for understanding the mechanism which controls the process of degeneration of the old macronucleus. The fragmentation of macronucleus in the autogamonts of *P. caudatum* is marked only in those cases when formation of normal synkaryon (or hemikaryon) capable for metagamic division takes place. In all the nuclear aberrations which lead in the conclusive result to the impossibility of a normal development of synkaryon, a loss of capability of the old macronucleus to fragmentation is a characteristic phenomenon. In conclusion, the ex-autogamont perishes within 6—10 days. In *P. aurelia* however, fragmentation sets on much earlier (second Mi division) than in *P. caudatum* and the temporary contact of cells of the complementary mating types at the agglutination reaction (Metz 1954) is fully sufficient for inducing fragmentation.

In prophase of the third progamic Mi division in the autogamont of *P. caudatum*, the paroral cone becomes clearly distinguishable. With this

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regard *P. caudatum* does not differ from the other ciliate species in which autogamy occurs (review of Jankowski 1965). It seems quite possible that the paroral cone is formed in the normal conjugating pairs M-17×M-17 as well and takes a direct part in the process of pronuclei migration. The electron microscopic and light microscopic study of Vivier et André provided some evidences against the possibility of paroral cone formation (Vivier et André 1961). Those authors consider that the only argument known till now in favour of existence of the cone in *P. caudatum* is its formation in autogamy of *P. aurelia* (Diller 1936). The study of the cytological details of the autogamy process in *P. caudatum* as revealed by us allowed to ascertain directly the existence of the paroral cone in this species.

One of the essential moments of the nuclear reorganization in paramecium is the degeneration of a part of nuclei prior to the third progamic Mi division. Normally in autogamy and in conjugation after the second progamic Mi division, three nuclei out of four become pycnotic and those which remain active undergo the third maturation division. According to the generally accepted view, the mechanism of nuclear degeneration sets on after the second Mi division, and keeping the active properties in one of the nuclei may be explained by the fact that this nucleus is usually located in the zone of the paroral cone, which is endowed with "defensive" properties (Sonneborn 1954).

Our findings concerning the process of nuclear pycnosis after the second Mi division in autogamy, allow to postulate that in *P. caudatum* the process which evokes the degeneration of nuclei sets on normally earlier namely at the stage after the first Mi division. As a rule, in the autogamont two nuclei are at the same degeneration stage simultaneously, whereas in the third one being — no doubt — a sister nucleus of the active one, this process is much delayed. If the degeneration mechanism was engaged already at the stage following the second Mi division when in the cytoplasm of autogamont four nuclei are present then all the three pycnotic bodies would be at the same stage of degeneration which is not the case indeed.

In the case of anomaly when all the four nuclei conclude their third division and 8 pronuclei are formed, it may be stated with certainty that the mechanism evoking the degeneration of nuclei fails to set on in the usual moment. Pycnosis occurring in the anomaly in question, proceeds with regularities observed by us in the normal nuclear reorganization, namely the nuclei undergo the pycnotic stages by pairs. Such a picture of nuclear pycnosis seems to prove that the mechanism of nuclear degeneration in this anomaly begins to act at the stage following the second Mi division.

The above confrontation of pictures of the nuclear degeneration in the progamic part of the autogamont reorganization in the normal course and in anomalies, indicates a considerable lability of single stages of the sexual process. The same mechanism may be engaged at different stages of nuclear reorganization of ciliates. In one case the mechanism evoking degeneration of nuclei begins to act after the first Mi division, in another case — after the second Mi division and, at last, it may fail to be engaged at all.

Anomalies interesting for some reasons appear as result of lability of nuclear pycnosis in the progamic part of the nuclear cycle in *P. caudatum*. They concern the multiple formation of pronuclei and of synkaryons. For some lower ciliates this phenomenon is normal (Raikov 1958) whereas in

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P. caudatum and in *P. putrinum* (J a n k o w s k i 1965) similar deviations are the result of disturbance of interrelation between the links of the sexual process.

In the last months of 1965, when the main part of this study was ready, the article of Jankowski 1965 appeared with the description of autogamy in conjugation of *P. putrinum*. Jankowski succeeded to evoke autogamy in single individuals by an ingenius method of multiagglutination. Anomalies observed by this author in *P. putrinum* strikingly resemble to those described by us in *P. caudatum*. It appears that as well the fact of revealing autogamy in *P. caudatum* as the resemblence of anomalies are a clear support of the theory of N. I. V a v i l o v 1935 concerning the homological orders in *Protozoa*.

In conclusion we find necessary to stress the importance of the cytogenetic studies of the nuclear reorganization in any genetic studies on ciliates. This is necessary because in ciliates, individuals of a much more complex organization participate in the sexual process than the gamets are in the majority of animals. As result of this cemplexity, even an insignificant disturbance of the interconnection of stages of this process, may bring about serious aberrations of the nuclear reorganization.

Summary

Autogamy was found during the conjugation in *Paramecium caudatum* and a cytological analysis of this process was carried out. Conjugating pairs were formed when clones M-13 (amicronuclear) with M-17 (normal) had been mixed. Pairs formed after mixing, became separated precociously into single individuals. The clones M-17 undergo autogamy while in the amicronuclear partners the nuclear reorganizaton fails to set on. In the autogamont, the sequence of the nuclear processes is the same as during conjugation. Four nuclei are formed as result of the two first divisions of Mi, out of them 3 degenerate and the fourth one undergoes its third division. The migrating pronucleus enters the clearly distinguished paroral cone. Pronuclei fuse to form the synkaryon which divides 3 times producing 8 nuclei.

In 49% of autogamonts, aberrations in the nuclear behaviour were ascertained. They were not observed in conjugation of the clone M-17. The aberrations concerned: 1. inhibition of the second Mi division; 2. conclusion of the third progamic Mi division in all the four nuclei and as result — formation of 8 pronuclei, and later on of several synkaryons; pronuclei may give origin to hemikaryons; 3. heteroploidy of nuclei; 4. degeneration of synkaryon or of the products of its division; 5. atypical fragmentation of macronucleus.

A postulation has been put forward as to the interaction and regulation of stages of the sexual process in *Paramecium caudatum*.

РЕЗЮМЕ

Обнаружена и цитологически исследована автогамия при конъгации *Paramecium caudatum* Ehrbg. При смешивании парамеций клонов М-13 (амикронуклеарный) и М-17 (нормальный) образуется большое количество конъюгирующих пар. Образовавшиеся пары преждевременно распадаются. Клетки клона

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M-17 претерпевают автогамию, в амикронуклеарном партнере ядерная реорганизация не запускается. Ядерные процессы при автогамии протекают в той же последовательности, что и при конъюгации. В результате первых двух мейотических делений Ми образуется 4 ядра, 3 ядра дегенерируют, оставшееся делится третий раз. Мигрирующий пронуклеус заходит в ясно различимый околоротовой конус. Пронуклеусы сливаются, образуя синкарион. Синкарион делится 3 раза, образуя 8 ядер. У 49% автогамонтов отмечены аберрации в поведении ядер, которые не встречаются при конъюгации клона M-17: блок II деления Ми; завершение III програмного деления Ми всеми 4 ядрами и, как следствие, образование 8 пронуклеусов и позднее нескольких синкарионов; пронуклеусы могут давать начало гемикарионам; гетероплоидия ядер; дегенерация синкариона или продуктов его деления; ненормальная фрагментация макронуклеуса. Высказывается предположение о взаимосвязи и регуляции отдельных этапов полового процесса у *P. caudatum*.

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EXPLANATION OF PLATES I—VII

Course of nuclear reorganization during autogamy in *Paramecium caudatum* Ehrbg. Total preparations. Feulgen staining. Photographs 12—16, 19—21 were made with obj. 60×, eye piece 15×, all another — obj. 20×, eye piece 20×.

Normal course of nuclear reorganization

1: Divergence of conjugants of M-17×M-17 pair, stage after the first division of synkaryon; there are two nuclei (synkaryons I) in each conjugant (arrows)
2—3: M-13×M-17 conjugating pairs; left partner (M-17) with normal micronucleus, right (M-13) — amicronucleate; early prophase of the first division of micronucleus (Mi is indicated)
4: M-13×M-17 conjugating pair; there is stage of crescent (arrow) in M-17 partner
5: Autogamont; crescent stage
6—7: Autogamonts; late prophase of the first division of Mi
8: Autogamont; anaphase of the first division of Mi
9: Autogamont; two nuclei after the first division of Mi (indicated)
10: Autogamont; late anaphase of the second division of Mi (arrows)
11: Autogamont; four nuclei after the second division of Mi
12: Autogamont; stage after the second division of Mi, one nucleus is functional (arrow), the three others are degenerative (double arrows)
13: Autogamont; formation of the paroral cone (c), Mi are indicated by arrows
14—16: Autogamonts; stage after the third division of Mi; succesive stages of differentiation of pronuclei (*p*); paroral cone (c); degenerating Mi II are indicated by arrows
17—18: Autogamonts: stage after the third division of Mi; paroral cone
19: M-17×M-17 conjugating pair (paroral region of pair) exchange of pronuclei (*p*); one migratory pronucleus lies under the other one; two stationary pronuclei; two pycnotic bodies (arrows)
20: Autogamont; fusion of pronuclei
21: Autogamont; synkaryon (*sk*) and two pycnotic bodies (arrows)
22: Autogamont; two nuclei after the first division of synkaryon (*sk* II are in-
23: Autogamont; four nuclei after the second division of synkarion (*sk* II are indicated)
24—25: Autogamonts; stage after the third division of synkaryon; begining of macronuclear fragmentation, eight nuclei and macronuclear fragments (*sk* III are indicated)

Aberrations of nuclear reorganization

26: Autogamont; pycnotic crescent
27: Autogamont; pycnotic anaphase of the first division of Mi
28: Autogamont; stage after the first division of Mi; formation of chromatin spout from nuclei, two nuclei
29—38: Autogamonts; succesive stages of nuclear reorganization during blockage of the second division of Mi. 37: pycnotic Mi II are indicated
39: Autogamont; late prophase of third division of Mi in all four nuclei
40: Autogamont; early anaphase of third division of Mi in all four nuclei
41: Autogamont; stage after completion of third division of Mi by four nuclei
42: Autogamont; differentiation of two pronuclei (*p*), six pycnotic nuclei (arrows)
43: Autogamont; differentiation of four pronuclei (*p*), four pycnotic nuclei (arrows)
44: Autogamont; differentiation of eight pronuclei (arrows)
45: Autogamont; two synkaryons, one of them at the stage after first division (*sk* I), the other one is pycnotic (arrow), two pycnotic Mi III (double arrows)
46: Autogamont; stage after first division of synkaryon (sk I) and hemikaryon (*hk* I), nuclear heteroploidy
47—48: Autogamonts; stage after divisions of synkaryon (*sk* I, sk II) and hemikaryon (arrows), nuclear heteroploidy
49—50: Autogamonts; hyperploid synkaryon (*sk*)
51: Autogamont; eight pycnotic pronuclei (arrows)

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52—56: Autogamonts; all products of division of synkaryon (arrows) and hemikaryon (double arrows) are pycnotic
57: Autogamont; pycnotic synkaryon
58: Autogamont; stage after the third division of synkarion, ten nuclei
59—60: Autogamonts; abnormal fragmentation of Ma — hemixis; pycnotic Mi is indicated
61: Just separated ex-conjugants of M-17×M-17 conjugating, on the left — products of the first division of synkaryon (pycnotic synkaryon I — *sk*) and one pycnotic pronuclear (*p*), on the right — products of the first division of hemikaryon (*hk*)

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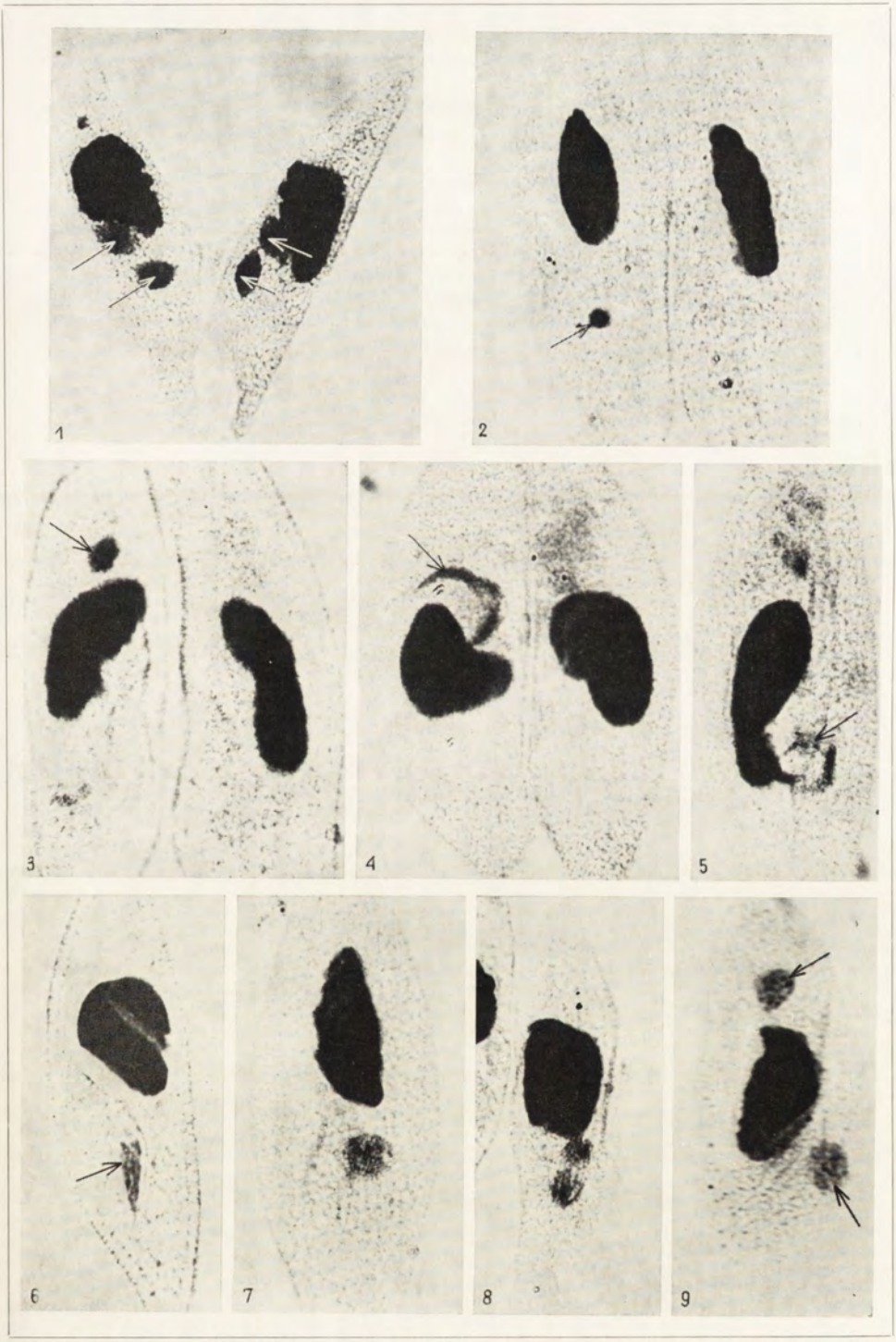

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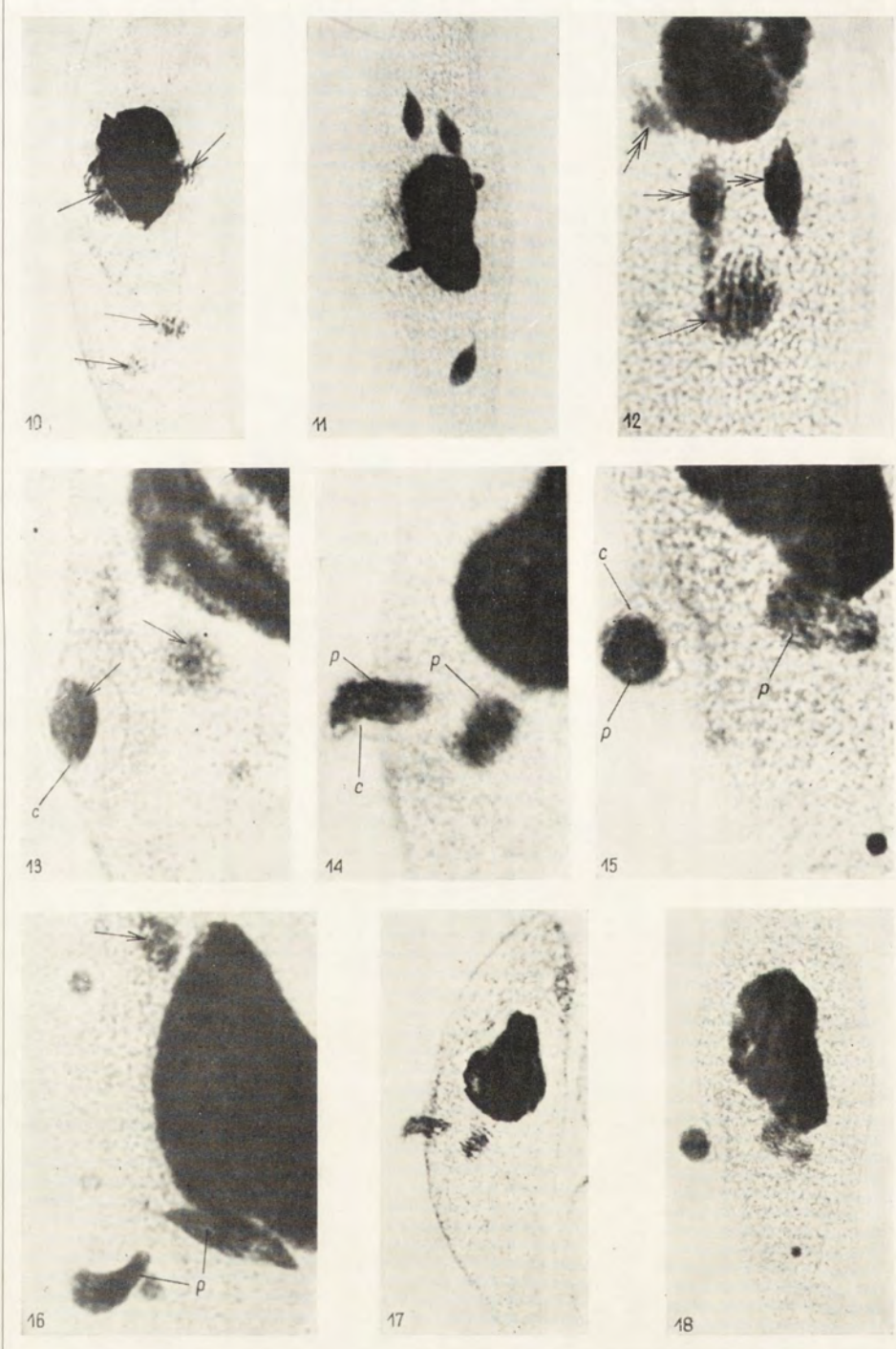

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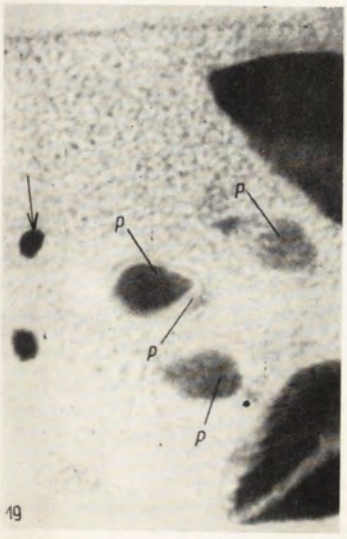

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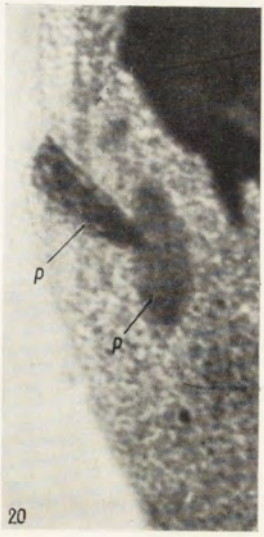

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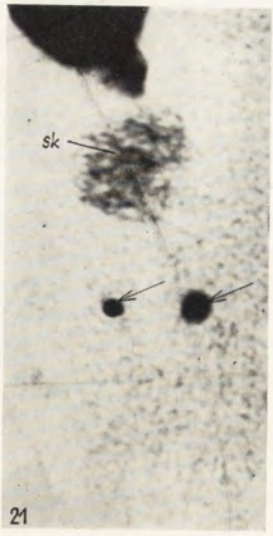

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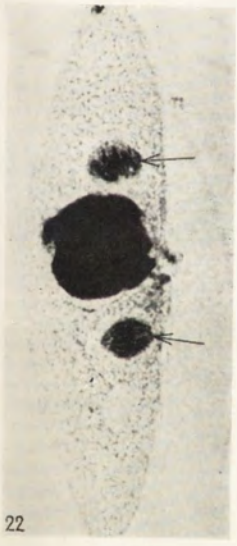
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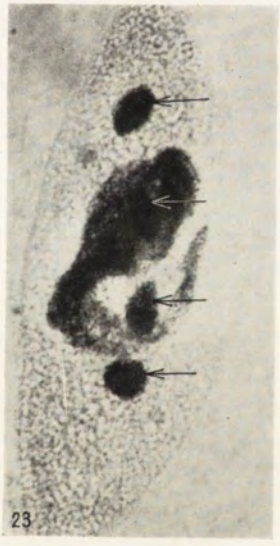
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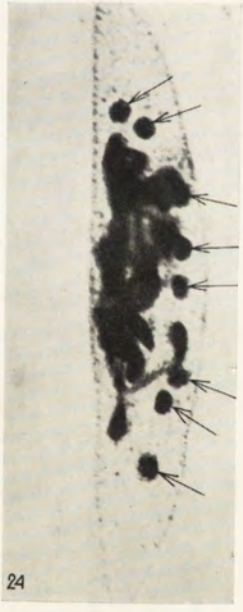
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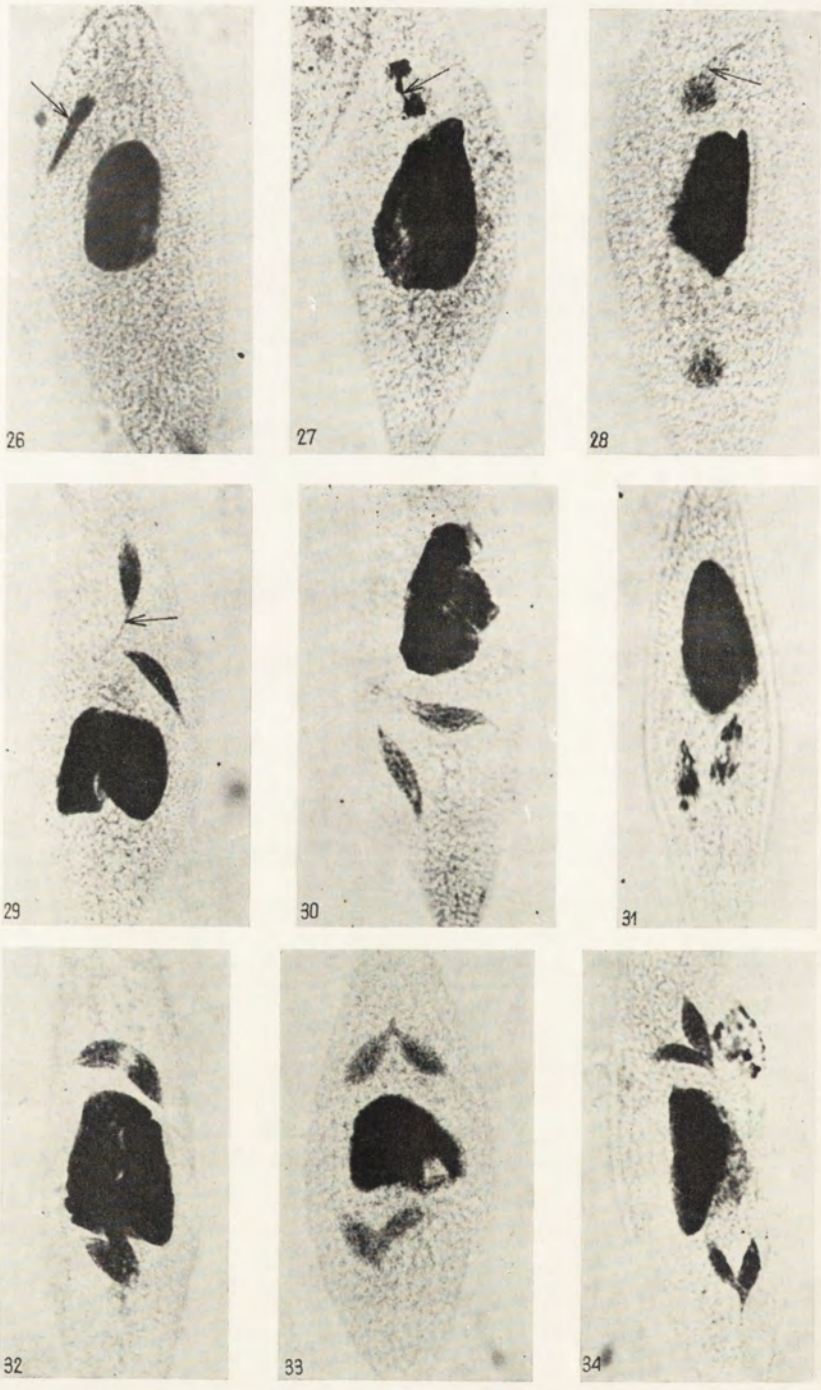

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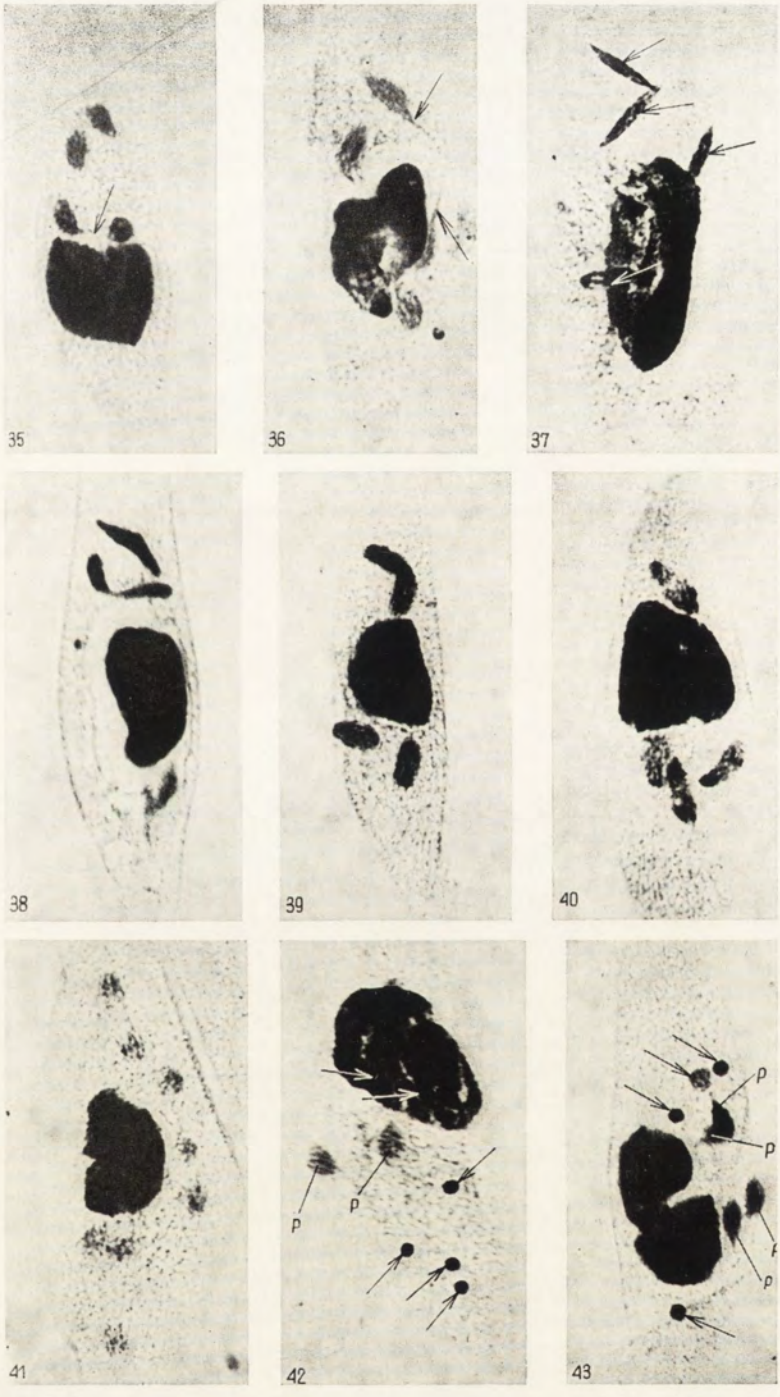

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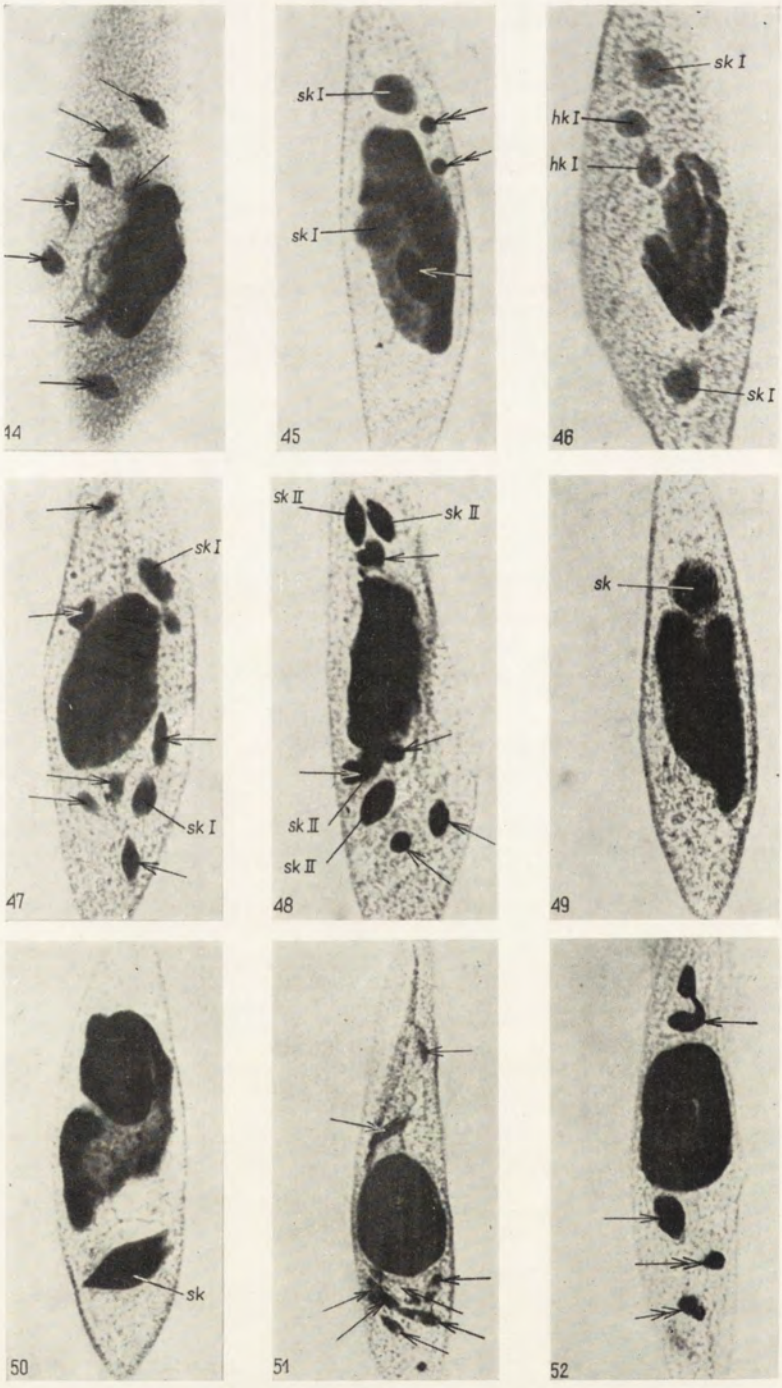

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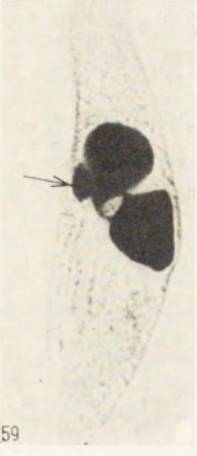
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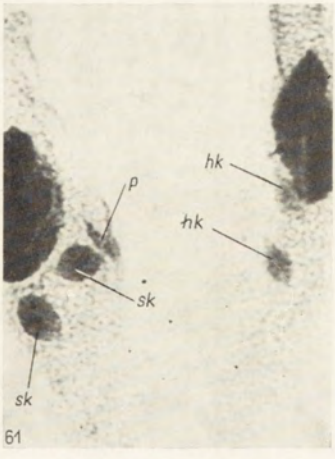

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Department of General Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warszawa 22, Pasteura 3, Poland

Marek DOROSZEWSKI

Responses to shake of water in the course of regeneration in *Dileptus cygnus*

Reakcje *Dileptus cygnus* na wstrząsy wody w przebiegu regeneracji

The aim of the present communication is to add some facts to the problem of the localization of reactions in the ciliate cell. For this purpose, the study of the restitution of motoric functions in the regeneration process was carried out. For ascertaining the changes in responses involved by the lack of the removed cell fragments and for following the restitution of those reactions in the course of regeneration of removed structures, the method of studying the ciliates responses after bisection has bee applied.

The reactions studied have been: response of reversal and that of the forward start. The reaction of reversal in ciliates is classical and was worked out in the litterature in many aspects, whereas the reaction of the forward start has been studied quantitatively for the first time in the present investigation, being mentioned only sporadically in the litterature. Vervorn 1897 was the first who found the reaction of acceleration of movement as a response to the shake of water. An extensive study on the action of water shake up on ciliates was the publication of Kinastowski 1962 a, b who investigated the reactions of contraction in *Spirostomum*. However in general, the ciliate response for this factor are rather little investigated. As known, the water shake may play a certain role in the geotropism of ciliates as one of the factors evoking this reaction. The author of the present article studied the effect of water shake in *Dileptus anser* (Doroszewski 1963 a) however the quantitative examination was then limited only to the reversal reaction. The high mobility of this ciliate species complicated the study of the forward start reaction.

In the present article, the stimulus evoking the reaction under study has been namely the shake of water. The adventage of application of this stimulus is the fact that it — no doubt — occurs in the natural conditions and consequently the adaptation mechanism to its action might have been formed. Besides this, the previous study on the action of the local mechanical stimuli needed being supplemented by the study of the effect of this stimulus acting upon the whole cell. Clark 1946 and Seravin 1962 a ascertained in *Spirostomum* and Seravin in *Dileptus anser*, that the mechanism of stimulation of the anterior body part involves a withdrawal and stimulation of the posterior one brings about in consequence a forward movement. In

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the fragments of *Spirostomum* Clark 1946 and Seravin 1962a found that the stimulation of the posterior fragments evokes a forward movement and of the anterior ones — a withdrawal. The author of the present study examined the reaction of *Dileptus cygnus* to bisection (Doroszewski 1965) and to the local stimulation (Doroszewski 1963 a). It was ascertained in those publications that in this ciliate two regions exist which were determined as "the forward response area" and "the backward response area". The first one is located in the posterior body part of the ciliate and its stimulation evokes a forward movement. The backward response area is in the anterior body part and its stimulation causes a backward movement.

In the course of regeneration, in the posterior fragment — which initially reacts only by the forward movement — in the beginning of regeneration, at the anterior of the fragment a small region appears. Its stimulation evokes a backward movement. The subject of the present study is to investigate the response of this to a mechanical stimulus acting on the whole cell of the ciliate i.e. reaction to the water shake.

The author wishes to express his thanks to doc. dr. S. Dryl for his valuable remarks.

Material and methods

As the experimental material, *Dileptus cygnus* Clap. et Lachm. was used which is characterized by its stationary mode of life. Normally the ciliate lies at the bottom of the vessel and feeds in this position. In experiments, the reversal reaction consists not only in the reversion of movement direction but also in a movement backward starting from the motionless position. A strong forward start reaction is a characteristic of this ciliate.

The culture of *Dileptus* were the same as those on which the study of regeneration was carried out. As food served *Colpidium* and *Tetrahymena* supplied once a day. Cultures were kept in the Pringheim's liquid. Prior to the experiment, ciliates were starved for 24 hrs. for the uniformity of the physiological conditions. Before the experiment ciliates were rinsed and placed into the Pringsheim's fluid, then included into paraffin oil according to the method of Golińska. Paraffin oil was poured into the concavity of the Lindner's slide, a drop of the Pringshaim's fluid with the ciliate was placed at its bottom. The size of the drop was adjusted so that it might be wholly seen under the lens 10× of microscope.

The first part of the experiment was the observation of the unimpaired individual prior to the operation. The shake of water was evoked by pulling away the clip of the microscope stage — which kept the slide — till it resisted, and by letting the clip loose subsequently. After every shock, the reaction of the ciliate or its absence was recorded. When the response had been stated, the individual was bisected with a spear-point needle made of an adjusted steel needle. The bisection was executed under the microscope. The response of the posterior fragment which was separated by bisecting the ciliate in the middle of its body (excluding the proboscis) was examined. This fragment included the nuclear apparatus and regenerated.

Thirty 5-hours long experiments were carried out on the posterior fragment — the opimer — besides the introductory and short-lasting experiments.

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About 15 shakes were applied in the course of 5 minutes. The procedure was repeated every hour. At that time the fragment was photographed. As a control of morphological changes served the studies of Golińska and Doroszewski 1964 and of Golińska (unpublished). Photographs presented the direct documentation for synchronization of images. The quantitative results concern the posterior fragments only.

Results

After stimulation of the unimpaired individuals, 40% of withdrawal responses was obtained for the total number of stimulation cases. 30% of forward start responses and in 30% the absence of reaction to the stimulus was stated (Fig. 1).

0 hours

Immediately after the bisection, the outflow of cytoplasm is seen and subsequently the constriction of the wound (Pl. I 1). As to the responses in this period, a most striking fact occurs that practically all the fragments exhibit a forward start response. Only 0.37% of stimuli are ineffective and no reversal reaction occurs. A considerable rise of reactiveness and its uniformity is observable.

In the anterior fragment, only the withdrawal occurs at this time.

1 hour

The regulation processes had begun in the fragment. Its shape is slightly changed. The dorsal rudiment is seen and the anterior body part is more transparent (Pl. I 2) than before. In 3% the first withdrawals of the opimer occur, and 88% of forward starts, no reaction was observed in 9%. Consequently a raised reactivity is observed with the forward start reaction prevailing considerably.

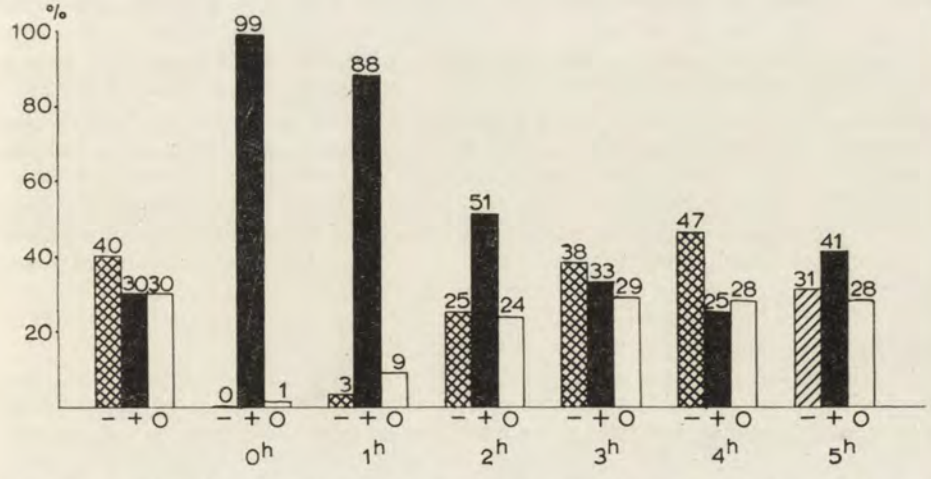

Fig. 1. Responses to the water shake in the posterior fragment of *Dileptus cygnus*. − withrawal response, + foreward start response, ○ no response

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2 hours

A part of mouth is seen which fails to participate in food intake. A small proboscis is already seen (Pl. I 3). In this period, 25% of withdrawals accur, 51% of forward starts and absence of response in 24%. Consequently prevailing of the forward movement persists.

3 hours

Killing and devouring the pray occur for the first time. Proboscis is longer (Pl. I 4). The retreat reaction occurs in 38% of cases, that of forward movement in 33%, no response was recorded in 29%. Comparison with the behaviour of the unimpaired animal proves that the return to the norm has occured in fact.

4 hours

Further growth of the proboscis is manifested (Pl. I 5). The ciliate responses in 47% by retreat, in 25% by forward start and in 28% the stimuli evoked no reaction.

5 hours

The pattern of morphology is similar to that after 4 hours. In the conditions described, without provision of food, regeneration does not proceed and proboscis fails to attain its full length even after several days. As to the responses, 31% of withdrawals, 41% of forward starts occur. In 28% of cases no reaction is observed. These values approach the norm but possibly some fortuitous fluctuations occur.

The anterior fragment (promer) continues reacting with the reversal response only.

Discussion

In the cell of *Dileptus*, tendencies to two opposite responses are manifested. The localization of those tendencies is different and they may be separated experimentally from each other. On the whole body of the ciliate, in the case of a local touch or puncture, the stimulation of the anterior body part evokes a backward movement, and stimulation of the posterior one — the forward movement. This may be of a biological importance as an escape of the stimulus but those responses — as stated in the previous studies are independent of the direction of the stimulus but depend only of its localization (Doroszewski 1963 a). The shake of water acts upon the whole body and consequently both areas of the responses are stimulated. The results of stimulating an unimpaired individual are such ones that approx $^1/_3$ of cases are withdrawals, forward movements and absence of response. This may possibly be a result of competition of opposite tendencies to response: some of them dominate sometimes and annihilate themselves mutually another time.

The bisection produces an experimental separation of both areas, enabling the deviation of opposite tendencies and manifestation of their capabilities. This results in the withdrawal of promer and in forward movement of opimer. A characteristic fact is the rise of reactivity which may be the result of absence of opposite influences or of the operation shock. Nevertheless the deficient areas of response grow up again in the course of regeneration and those tenden-

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cies to response are present on the posterior fragment. It is characteristic that the time of return of the reversion response coincides with the regeneration of proboscis and with the beginning of the normal functioning of mouth. Responses have become various again and the ciliate is an organism, no longer a machine with a uniform functioning. The state of equilibrium returns, the opposite responses tendencies become balanced which is so characteristic for living organism.

It may be considered that the results of this study indicate the role of neuromotorium in *Dileptus* (Vissher 1927) which might play a role in the initiation of the reversal response since neuromotorium is localized near the cytostome. However the more recent publications e.g. that of Okijama and Kinosita 1966 ascertain the lack of a coordinating and conducting role of the neuromotoric systems.

The results of the present study are in conformity with the postulation of Seravin 1962 b about two opposite gradients of regulation mechanisms. Those problems are more extensively discussed in his recent study (Seravin 1967).

It should be emphasized that the results of the present study concern only the mechanical stimuli whereas many authors obtained the reversal reaction in all the fragments as a response to chemical stimuli (in *Paramecium* Alverdes 1922, Horton 1930, in *Spirostomum* Seravin 1967).

As for the reversal reaction is concerned, the results presented in this study would be in harmony with the hypothesis of the stomato-caudal gradient. This theory has been put forward by Grębecki 1963. The buccal region is in *Dileptus* — no doubt — the most sensible as follows from the presented experiments and of the previous ones with the local stimulation. If *Dileptus* and *Paramecium* were compared from this point of view, it could be noticed in the first place that the cytostome of *Paramecium* is located much more posteriorly than in *Dileptus*. *Dileptus* as well as *Spirostomum* are distinguished by a comparatively considerable length of the body and this may be the reason why the delimitation of the area of reactiveness is manifested in it so distinctly.

Summary

Experiments were performed on responses to the water shake in the ciliate *Dileptus cygnus* and on its fragments. Proir to the bisection the ciliate may react with the forward movement, perform the reversal response or not respond at all. Immediately after bisection, on the posterior fragment of the ciliate the response of the forward start occurs only. With the progress of regeneration, the reversal responses appear and 3 hours later the reactiveness of the ciliate returs to the norm.

STRESZCZENIE

Wykonano doświadczenia nad reakcjami na wstrząs wody u wymoczka *Dileptus cygnus* i jego fragmentów. Przed operacją wymoczek może reagować ruchem naprzód, wykazywać reakcje rewersji, lub nie reagować wcale. Bezpośrednio po operacji na tylnym fragmencie wymoczka występuje jedynie reakcja ruszenia naprzód. W miarę postępu regeneracji pojawiają się reakcje rewersji i po upływie trzech godzin reaktywność wymoczka powraca do normy.

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EXPLANATION OF PLATE I

1: Posterior fragment of *Dileptus cygnus* directly after bisection
2: same fragment after 1 hour
3: same fragment after 2 hours
4: same fragment after 3 hours
5: same fragment after 4 hours

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Department of General Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warszawa 22, Pasteura 3, Poland

Andrzej GRĘBECKI and Ewa MIKOŁAJCZYK

Ciliary reversal and re-normalization in *Paramecium caudatum* immobilized by Ni ions

Rewersja rzęskowa i powrót do normy u *Paramecium caudatum* unieruchomionego przez jony Ni

It has been postulated in a former article of the authors (Grębecki, Kuźnicki, Mikołajczyk 1966) that the suppressing action which is exerted by Ni ions on the ciliary apparatus of *Paramecium* is expressed in three successive stages: 1. inversion of the spiralling from left to the right one, 2. physiological immobilization characterized by an ineffective ciliary beat, and 3. traumatic immobilization in which cilia stop their work at all. Furthermore, it has been established in the same study that, during the first two stages, the impaired ciliary apparatus is still able to perform the ciliary reversal which is fully effective, i.e. it results in a backward swimming of the ciliate.

Almost simultaneously appeared the important study of Naitoh 1966 who found — besides determining the electrophysiological parameters of Ni-immobilized paramecia — that some form of ciliary reversal is still possible even under the conditions of traumatic immobilization. Namely, the nonbeating cilia which, according to Naitoh, point to the posterior body end under normal conditions, inverse their position when the cell membrane becomes depolarized and they point towards the anterior direction. This finding seems to be so relevant for the physiology of ciliary reversal that it deserves further study by means of apparently more precise methods of rapid fixation.

On the other hand, the occurrence of ciliary reversal in fully immobilized paramecia offers an excellent possibility to make a new approach to the inconformity of opinions expressed by one of the present authors and Párducz (Párducz 1959, Grębecki 1965 and 1966), as to the question from what end of the body starts the recovery of the normal direction of ciliary beating when the continuous ciliary reversal is gradually disappearing by self-normalization.

Results

Paramecium caudatum grown in milk culture, prior to the experiment, was collected by means of geotaxis and rinsed in a standard solution of 0.5 mM KCl + 0.5 mM $CaCl_2$. Potassium chloride and barium chloride solutions

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applied for inducing the ciliary reversal, were also diluted in the same standard medium. The traumatic immobilization was obtained by exposing the ciliates to the action of 5 mM $NiCl_2$, for 3 min. After this time of incubation they were rinsed again in the standard solution.

In the first experimental series the ciliates previously immobilized by Ni^{2+}, as well as the active ones, were exposed to the action of 32 mM KCl. The ciliary reversal seen in the form of backward swimming lasted in the control specimens 180 secs. During the same time cilia of immobilized specimens were observed in the dark-field illumination and in the phase-contrasting microscope. As a matter of fact, they point to the anterior end of ciliate, as it has been described by N a i t o h 1966. They keep this position nearly for the same period of time, for which the control active specimens swim backwards. Later on, the immobilized cilia loss their anterior orientation and they seem to stick out in the position rather perpendicular to the body surface. Also the cilia of immobilized specimens which have not been exposed to a high $[K^+]$ (or even being exposed to a high $[Ca^{2+}]$) apparently occupy such a position.

For the study of the position of cilia at high power, in the fixed preparations, the Ni-immobilized specimens were fixed by osmium tetroxide, following P á r d u c z 1952, and stained with haematoxyline, following the modified procedure of G r ę b e c k i 1964.

Plate II 3 presents a normal specimen which, after immobilization, was immersed in 2 mM $CaCl_2$, instead of KCl. The nonbeating cilia keeping the position corresponding to the normal forward movement are seen on the contour of body. Their orientation becomes more conspicuous in the Pl. II 4—5 which shows the fragments of the left and right margins of body in the same specimen, at a higher magnification. It may be concluded that immobilized cilia in the position corresponding to the conditions of normal movement stick rather perpendicular to the surface in their proximal parts but the distal parts form arches which bend towards the posterior direction. Two other examples of the normal pattern of nonbeating cilia on the lateral body margins are presented by the Pl. IV 10—11.

Other immobilized specimens were fixed and stained immediately after addition of 32 mM KCl, i.e. in the state of a well pronounced continuous ciliary reversal. The nonbeating cilia change their position, as it may be seen in the whole specimen (Pl. III 6) and in the enlarged fragments of its lateral margins (Pl. III 7—8). They are clearly inclined in the anterior direction as well in the distal as in the proximal parts of their shafts. The difference of this position as compared to the conditions of normal movement (Pl. II) is very distinct. The cilia point rather straight to the anterior, as it has been described by N a i t o h 1966. Two further examples of the typical inversed position of nonbeating cilia on the lateral body margins are given in the Pl. V 15—16.

The difference between the normal and the inversed position of immobilized cilia seems apparently less clear in the frontal field of ciliate because of the curvature of the body surface in this area which might produce the impression that cilia are in both cases reversed. However, examination of the anterior cilia at a high power reveals the difference and enables to distinguish between the normal orientation and the ciliary reversal (Pl. IV 9 and V 14). On the contrary, the difference in question seems to be the

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most conspicuous in the caudal region. Pl. IV 12—13 presents the normal position of immobilized cilia, and Pl. V 17—18 their inversed orientation, in the posterior parts of some specimens.

The reversed position of nonbeating cilia as elicited by $BaCl_2$ solutions (in the first stage of Ba-action corresponding to the continuous ciliary reversal) was identical to that induced by KCl.

In the second series of experiments the orientation of immobilized cilia was investigated during the self-normalization of ciliary reversal, which in the normal active specimens is manifested as a longlasting partial ciliary reversal after K-action, or as repeated quick transitions from the backward phase to the forward phase of periodical ciliary reversal following the Ba-action.

Paramecia immobilized by Ni ions and treated with 32 mM KCl as in the previous experiments, were fixed not immediately after inducing the ciliary reversal but after 3.5, 4.5 or 6 min. of delay. The control active specimens manifest in this period the circling movement which is due to the partial ciliary reversal, that is to say they are in the state of self--normalization which proceeds step-by-step and embraces a part of the ciliary apparatus.

In all specimens fixed at this stage of K-action the anterior regions of the immobilized ciliary cloth show still the inversed position of cilia, whereas in the posterior part of the body the nonbeating cilia recover this orientation which is characteristic of normal conditions. A whole specimen with the persisting symptoms of ciliary reversal in the anterior and middle areas, and with distinct re-normalization in the caudal region, is presented in the Pl. VI 19. The caudal onset of re-normalization is more clearly seen at a higher magnification (Pl. VI 20). The asymmetrical character of the re--normalized caudal field, as seen in this picture, is not an exception but a general rule. Many other examples were found in which the recovery of the normal position of nonbeating cilia, starting in each case from the hinder end, has progressed more or less towards the anterior areas, depending on the time of self-normalization and on the individual variation. The Pl. VI 21 shows the border between the reversed and re-normalized ciliary fields, as seen on the lateral edge of another specimen undergoing the partial ciliary reversal.

The caudal origin of the recovery of normal position by the nonbeating cilia and the stages of gradual extension of the re-normalized field towards the anterior regions of ciliature, were controlled by the observation in vivo of cilia immobilized by Ni ions.

Some samples of Ni-immobilized paramecia were fixed a few minuts after addition of 0.5 mM $BaCl_2$. In this period of Ba-action the control active specimens manifest the periodical ciliary reversal which is characterized by the alternation of forward and backward movement phases occurring at 0.5—2 secs. intervals. In the preparation of immobilized paramecia fixed under such conditions, some specimens may be found in which the position of nonbeating cilia corresponds to the ciliary reversal, some others with the ciliary cloth oriented in the normal direction, but in the major part of individuals the ciliary pattern shows a transitory stage between the reversal and the normal phase. In all cases the position of immobile cilia remains

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inversed in the anterior areas and the normal orientation is reassumed in the posterior ones. The extent of both fields may be very different, depending evidently on the phase of the periodical ciliary reversal exhibited by an individual at the moment of its fixation. A whole specimen with anterior cilia keeping the inversed orientation and the posterior ones reassuming the normal position, is given in the Pl. VII 22. Pl. VII 23 shows in a higher magnification a specimen in which the distinct ciliary reversal is limited only to the frontal parts of ciliary cloth, and the Pl. VII 24 — another individual in which the recovery of the normal position of nonbeating cilia starts from the caudal region and covers a rather scarce posterior area. The asymmetrical character of the re-normalized field is as typical for the case of Ba-action as it has been established for the K-induced ciliary reversal.

In vivo observation of paramecia immobilized by Ni ions, and exposed subsequently to the action of barium chloride, confirms the results reported above. The periodical changes of orientation effected by the nonbeating cilia are rather distinctly seen. In some extent (limited by the short duration of the phenomenon) it is possible to follow the "wave" of re-normalization of ciliary orientation between the backward and the forward phase. On the contrary, the appearance of the ciliary reversal, i.e. the transition from the forward phase to the backward one, seems to be momentaneous, which is fully in line with analogous observations made earlier by different authors in the normal active paramecia. The latter fact strongly suggests that all the transitory patterns of the immobilized ciliary cloth recorded in this study in $BaCl_2$ solutions represent the stages of re-normalization of ciliary reversal, that is to say of its disappearance and not of its re-appearance.

Discussion

The first conclusion which should be drawn from the results reported above is the full confirmation, by means of the rapid fixation technique, of the observation made earlier in vivo by Naitoh 1966 that the cilia completely immobilized by Ni ions retain the ability to perform a reversal response in the form of changing their position in relation to the body surface. A slight difference between the orientation, corresponding to the normal movement, as found in the fixed specimens and as described by Naitoh seems to be of rather little importance.

In the light of both studies it should be pointed out that two separate mechanisms are involved in the ciliary behaviour as well in the forward as in the backward movement. A similar, but little different suggestion has been made earlier, in the case of *Spirostomum ambiguum,* by Seravin 1962. This author came to the conclusion that one separate mechanism controls the forward movement (normal ciliary beating), whereas another mechanism is responsible for the control of backward movement (ciliary reversal). It seems now that the proper definition of both mechanisms should be the following: one mechanism is involved in the ciliary beat itself as well in the forward as in the backward movement, that is irrespectively of the direction of the effective ciliary stroke; the second mechanism, on the contrary, is independent of the ciliary beat itself but it controls only the direction of the effective stroke.

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The evidence of the existence of such separate mechanisms is provided by the experiments in which one of them becomes selectively blocked without impairing the other. In the present study, as well as in the former study of Naitoh 1966, only the mechanism supporting the ciliary beat itself has been blocked, and the mechanism controlling the direction of the effective stroke remained fully efficient. One of the earlier experiments of Párducz 1954 may be re-interpreted now as an example of the opposite selective blocking: this author observed that under some mechanical or chemical interference *Paramecium* may form the "hyaline blisters" and the cilia covering such a blister may exhibit only the normal rotary component of their movement without any effective stroke and without any form of the reversal response. That should mean that in the Párducz's experiment only the mechanism controlling the direction of effective ciliary stroke was inactive but the mechanism of the ciliary beat itself was unimpaired.

The existence of such two separate mechanisms which can become blocked one independently of the other, suggests rather strongly that the beating of a cilium itself and the steering the direction of its work are effected by two different contractile structures (or rather systems) of the ciliate cell. However, this last conclusion is merely an anticipation indicating the line of the further research.

As to the second problem involved in this study, the discrepancy of opinions initiated with the study Párducz 1959. In an earlier paper, the same author (Párducz 1956) established that in the course of the short-distance withdrawal (Jennings's "avoiding reaction") the ciliary reversal of *Paramecium* terminates in a self-normalization process which begins from the posterior part of the body of ciliate. However, in a later study concerning the gradual disappearence of the continuous ciliary reversal, induced chemically, a quite opposite course of self-normalization was postulated by this author, and supported by some pictures of fixed paramecia bearing the inversed metachronal waves all over the body and the "normal" waves on the frontal field (Párducz 1959). The view, that the recovery of the normal direction of ciliary beating starts from the anterior part of ciliate, has been subsequently adopted, in a review article, by Pitelka and Child 1964. On the other hand, the concept of the stomato-caudal gradient of excitability of ciliates developed by one of the present authors (Grębecki 1965, 1966) is distinctly incoherent with the opinion of Párducz 1959. Also the results obtained by Machemer 1965 in *Stylonychia* suggest that the self-normalization of the motory apparatus may start from the posterior end of ciliate, as postulated by the concept of the stomato-caudal gradient of excitability.

The detailed analysis of the metachronal pattern of ciliary reversal and the origin of the quasi-normal waves seen sometimes on the frontal field will be presented elsewhere. In this study it should be only pointed out that there is no competition between the inversed and the normal waves in the self-normalization process, but a complete effacement of the metachronal pattern occurs. The waves typical for the continuous ciliary reversal, as seen on the "dorsal" side of *Paramecium*, are presented in the Pl. I 1. The Pl. I 2 shows the ciliary cloth fixed in the transitory stage between the ciliary reversal and the forward movement; in spite of a very regular and characteristic

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position assumed by cilia, the surface is completely devoid of metachronal waves. This proves that the analysis of metachronal pattern cannot provde any solution of the problem from which end of the body starts the process of recovery.

Such a possibility is yet offerred by the reversal responses manifested by nonbeating cilia in completely immobilized paramecia. In all the cases of self-normalization studied in this research, (as well following the K-induced ciliary reversal as those observed under Ba-action) cilia reassume their normal position first in the caudal region, and the re-normalized field gradually extends further towards the anterior areas of the body surface. This result is contradictory to the conclusion of Párducz 1959, but it corroborates the earlier view of Párducz 1956 and it fullfills the expectations of the concept of the stomato-caudal gradient of excitability (Grębecki 1965). Moreover, it is fully in line of the results obtained by other authors which provide evidence that in ciliates the posterior cilia, and not the anterior ones, are the most sensitive to the stimulation promoting the vigorous ciliary beating in the normal direction. Such a results were reported for *Paramecium* stimulated by anodal current (Kinosita 1936), and by UV-beam (Jensen 1959), as well as under the mechanical stimulation in *Spirostomum* (Seravin 1962) and *Dileptus* (Doroszewski 1963).

The concept of the stomato-caudal grandient of excitability is further supported by this study also in this respect that the posterior fields of re-normalized cilia are in all the cases asymmetrical (cf. Pl. VI 20 and Pl. VII 23). In the case of a simple anterio-posterior gradient the recovery would proceed with an equal velocity from all sides. The pictures provided by the present research give evidence that the progression of recovery is different on the "dorsal" and "ventral" sides of ciliate, as it should be expected if the pole opposed to the cauda is not the apex but the cytostone.

Summary

Paramecia in the traumatic stage of immobilization induced by Ni^{2+} were exposed to the action of ions eliciting the ciliary reversal. The nonbeating cilia are still able to respond by the changes of their position which suggests that the ciliary beat itself and the steering of its direction are effected by different cell structures. Recovery of the normal orientation during the self-normalization of ciliary reversal starts, in all instances, from the posterior regions of ciliary cloth.

STRESZCZENIE

Pantofelki pozostające w traumatycznej fazie immobilizacji wywołanej przez Ni^{2+} były poddawane działaniu jonów powodujących odwrócenie ruchu rzęsek. Unieruchomione rzęski nadal zachowywały zdolność reagowania w postaci zmian swego położenia; sugeruje to, że sam ruch rzęski oraz funkcja sterowania jego kierunkiem są zależne od innych struktur komórkowych. Podczas spontanicznej normalizacji rewersji rzęskowej odzyskiwanie zwykłego położenia rzęsek rozpoczyna się we wszystkich przypadkach od tylnych okolic orzęsienia.

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EXPLANATION OF PLATES I—VII

1: Metachronal waves characteristic of the continuous ciliary reversal as manifested by *Paramecium* immediately after immersion in the solution of 5 mM $BaCl_2$+1 mM $CaCl_2$; 900×
2: Ciliary cloth devoid of metachronal waves, characteristic of the transitory stage between ciliary reversal and normal movement, as manifested a few minutes after immersion in 5 mM $BaCl_2$+1 mM $CaCl_2$ (in the course of periodical ciliary reversal); 700×
3: Position of cilia immobilized by Ni ions, under the conditions corresponding to the normal forward movement; whole specimen; 700×
4: Fragment of the left margin of the same specimen; 2800×
5: Fragment of the right margin of the same specimen; 2800×
6: Position of cilia immobilized by Ni ions, under the conditions corresponding to the continuous ciliary reversal (K-induced); whole specimen; 700×
7: Fragment of the left margin of the same specimen; 2800×
8: Fragment of the right margin of the same specimen; 2800×
9: Anterior cilia immobilized by Ni ions and fixed under the conditions corresponding to the normal movement; 2800×
10—11: Two further examples of the lateral body margin with cilia disposed in the position corresponding to the forward movement; 2800×
12—13: Normal position of the immobilized cilia in the caudal region of body; 1400×
14: Anterior cilia immobilized by Ni ions and fixed under the conditions of continuous ciliary reversal (K-induced); 2800×
15—16: Two pictures of the lateral body margins with immobilized cilia showing the position corresponding to the ciliary reversal; 2800×
17—18: Inversed position of the immobilized cilia in the caudal region of the body, as induced by K^+; 1400×
19: Whole specimen fixed during the re-normalization of the K-induced ciliary reversal (i.e. in the course of partial ciliary reversal); note the inversed position of anterior cilia and the normal pattern of posterior ones; 450×
20: Posterior part of another specimen undergoing re-normalization of the K-induced ciliary reversal; line indicates the caudal area in which cilia have recovered their normal position; 1400×
21: Lateral margin of another animal fixed in the course of re-normalization; line shows the border between the field of ciliary reversal and the field of reappearing normal position of cilia; 2800×
22: Whole specimen fixed in the transitory stage between the ciliary reversal and forward movement (during the Ba-induced periodical ciliary reversal); note the inversed position of anterior cilia and the normal pattern of posterior ones; 450×
23: Anterior part of another specimen fixed during the Ba-induced periodical ciliary reversal, in which the inversed position of cilia is limited to the frontal field (line); 1400×
24: Posterior part of an animal fixed during the Ba-induced periodical ciliary reversal, in which the re-normalization of the position of cilia just starts from the caudal area (line); 1400×

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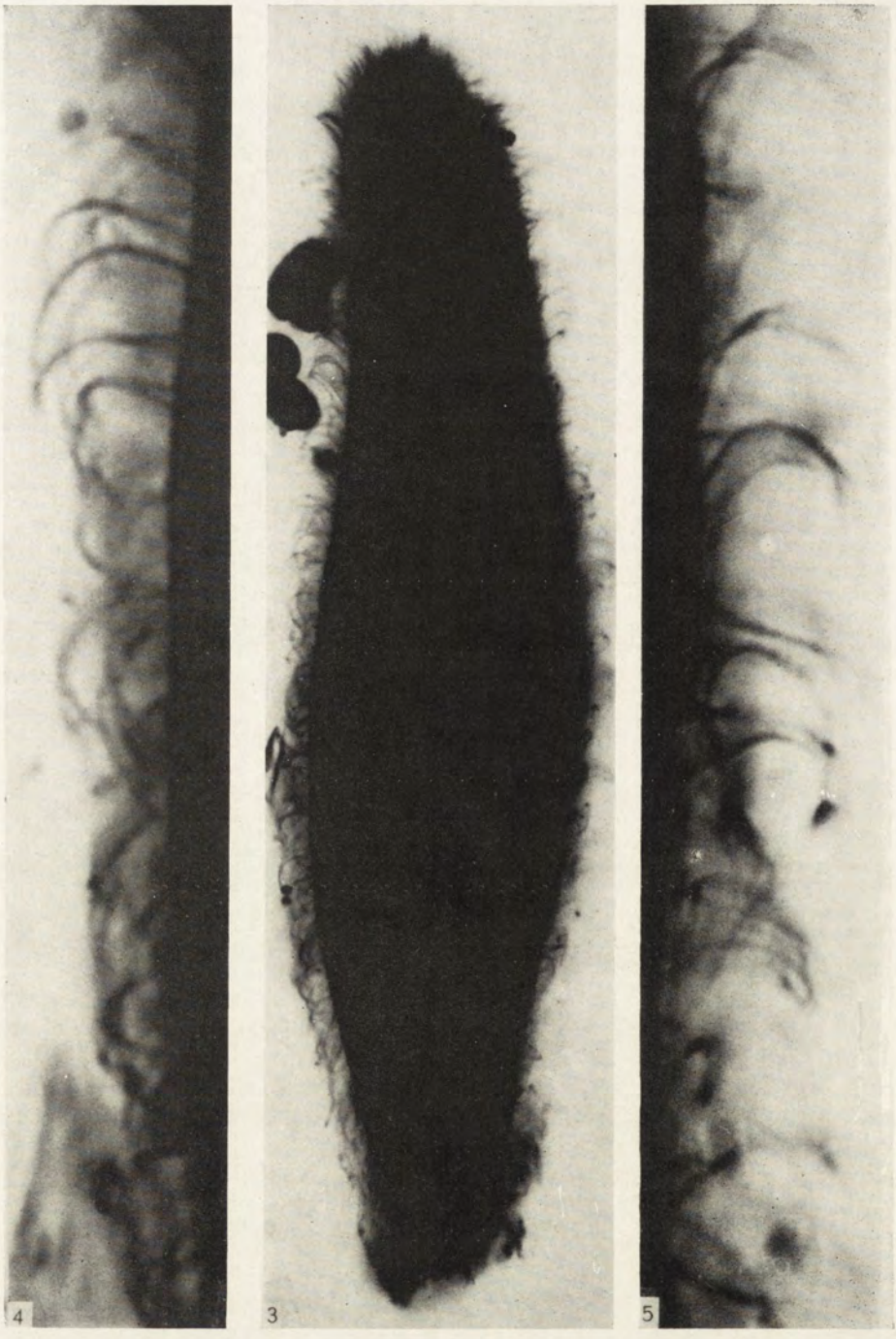

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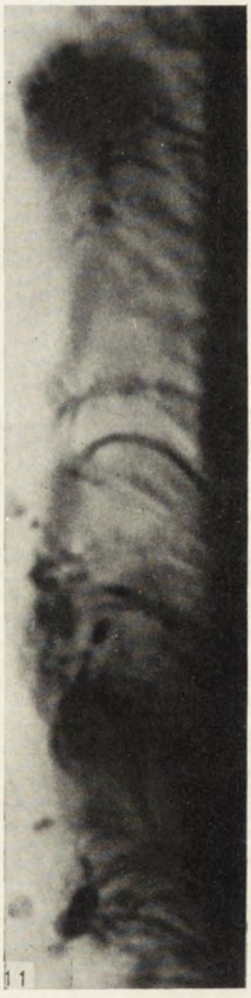

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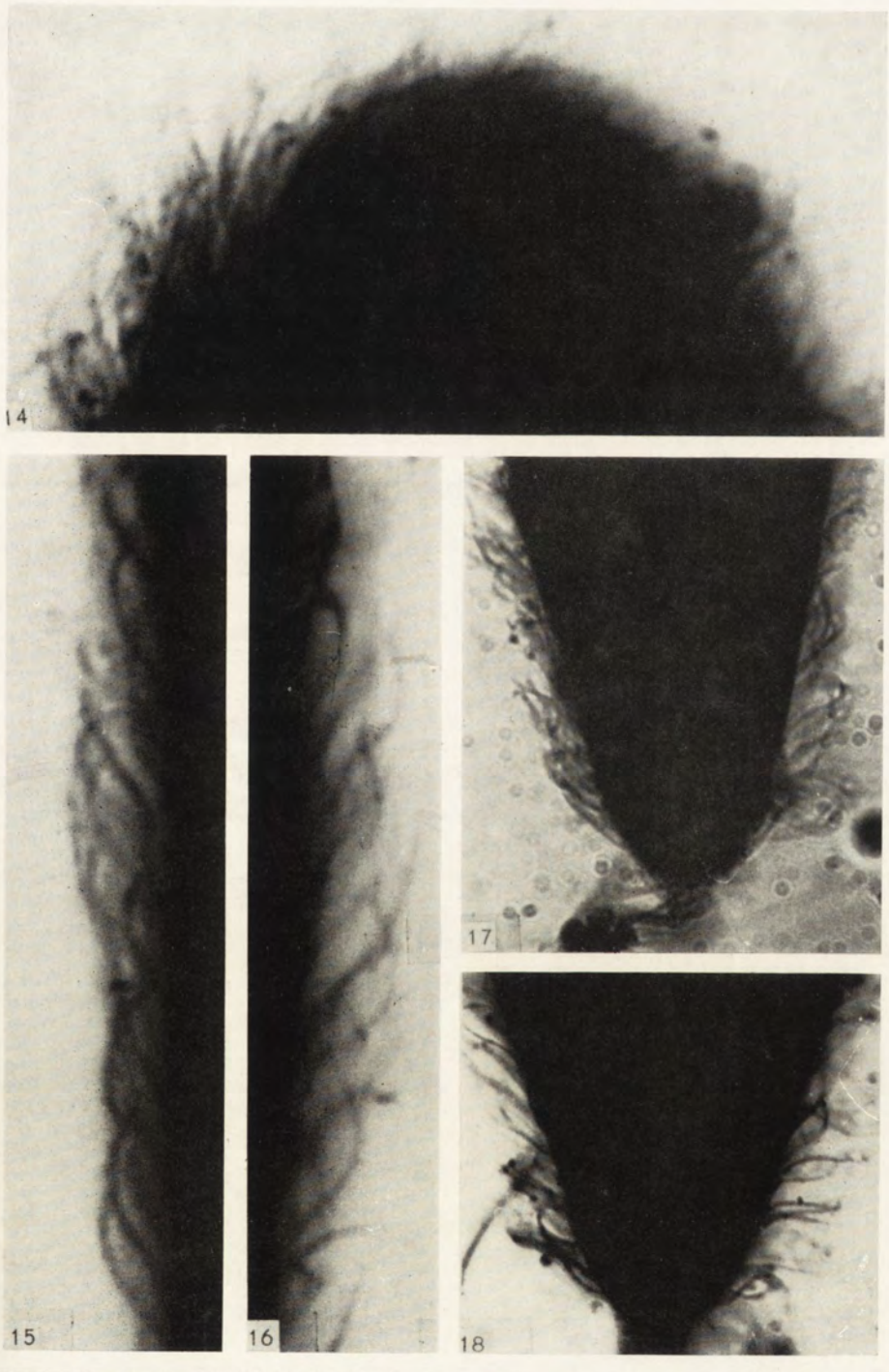

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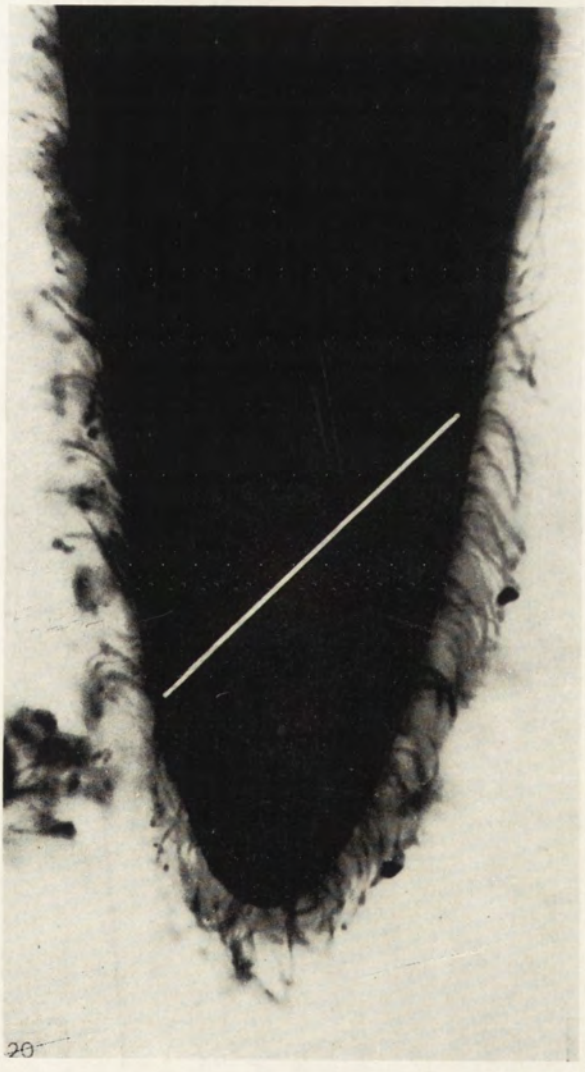

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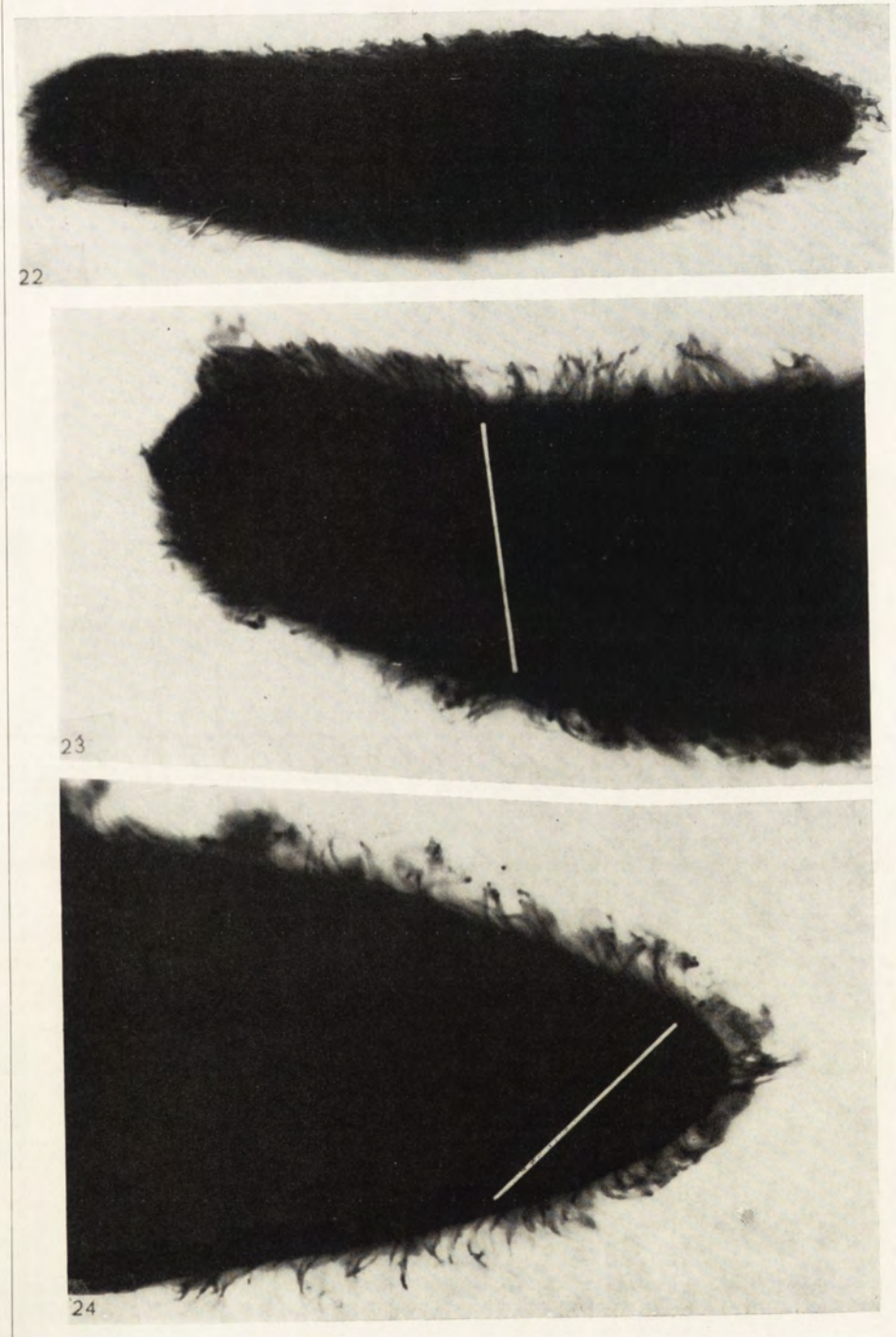

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Department of Biology, Military Medical Academy, Łódź, Pl. 9 Maja 1, Poland

Julia ROSTKOWSKA and Walenty MOSKWA

The influence of magnetic field on susceptibility for toxic compounds in *Spirostomum ambiguum* Ehrb.

Wpływ pola magnetycznego na wrażliwość *Spirostomum ambiguum* Ehrb. na związki toksyczne

Presently a number of observations have been already reported concerning the action of magnetism upon the biological processes as well in vitro as in vivo. Akoyunoglou 1964 applying the magnetic field of a H = 20 000 gausses intensity found a considerable excitation of activity of carboxyldismutase which catalizes the reaction of primary carboxylation in photosynthesis. Cooke and Smith 1964 observed an increase of enzymic activeness of trypsine in the magnetic fields of H = 8 000 — 15 000 gausses. In the experiments of Wiley and all. 1964 under the influence of magnetic exposure in the field of H = 5 000 gausses, occurred a reactivation of enzymic capability of trypsine which had been previously inactivated artificially.

Other enzymes behave in a different manner in the magnetic field showing a fall of catalytic capabilities and sometimes a lack of reaction to magnetization (Beischer 1963), as e.g. a fall of content and of activeness of blood cholinesterase was observed (Kholodov 1965) as well as inhibition of growth and regeneration processes i.e. protein synthesis (Gross, Gottfried, Smith 1961). Particularly the replication of nucleic acids in cellular divisions becomes delayed (M. F. Barnothy 1964).

Reno and Nutini 1963 applying the fields of intensity H = 80 — 7 300 oersteds to the tissue cultures of Carcinoma Ehrlichi and Sarcoma S-37 ascertained a fall of oxygen consumption by the tissues, approx. of 34.4% in the first and of 37.9% in the second case. The normal embryo tissues (e.g. samples of mice embryo kidney) exhibited in the magnetic field a fall of tissue respiration even of 93.5% in younger embryos or an insignification rise of respiration amounting 5.9% in the older ones.

The basal metabolism in mice kept in the magnetic field of 3 000—4 000 oersteds was lowered in average by 14% and their body temperature fell about 1°C (M. F. and J. M. Barnothy 1962).

Gross 1963 ascertained a lower formation of bacterial antibodies as well as those against the grafts of alien tissues of mice and rats kept in the magnetic field of H = 3 000—4 000 oersteds which proves a relaxation of the protein macromolecules synthesis. As observed by Hackel, Smith and Montgomery 1964, the serological reactions e.g. the agglutination

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of erythrocytes occurs more vividly in the magnetic fields of a considerable span of intensity i.e. from 50 Oe up to 16 000 Oe.

Blood coagulation occurs also somewhat more rapidly in the magnetic fields (Duchau 1963 and others). The protein composition of blood becomes also altered (Vjalov iLisičkina 1966).

Druż and Madievskij 1966 demonstrated on surviving rat tissues that the exposure in magnetic fields changed the hydration of tissues and their capability of swelling. At lower intensities (1 000—6 000 Oe) occurred generally an increase of hydration whereas higher intensities (8 000—20 000 Oe) evoked its fall.

The permeability of cell membranes increases for water and non-polar compounds in the magnetic field and falls for those with ionic bounds.

The action of magnetic field evokes atypical distribution of P-32 and J-131 in the animal organs as well as the radioactivity of cobalt Co-60 evokes in the stems and leaves of plants (Gross 1962).

At last, magnetic field lowers or disturbs the chemotactic reactions of *Paramecium* (Kogan i Tichonova 1965).

In our previous communications (Moskwa i Rostkowska 1965) observations have been reported on the action of some poisons upon organisms placed in a strong magnetic field. Namely *Xiphophorus maculatus* v. *aurea* reacts more quickly to narcotization by chloroform water or by urethan solution in the magnetic field. The same phenomenon occurs when ants are narcotized with ether or chloroform vapours in the magnetic field.

The other toxic compounds as atabrine, dehydrochloric acid, ethanol, potassium ferrocyanate, sulphamids etc. (Moskwa i Rostkowska 1965) lower the yeast fermentation in the magnetic field. The normal sugar fermentation is impeded in the magnetic field.

The present communication is a continuation of the previous studies on the magneto-chemical phenomena in living organisms.

Material and methods

As experimental object, the laboratory cultures of *Spirostomum ambiguum* were used exposed to simultaneous action of magnetic field and of chemical compounds. Magnetization of protozoa was performed by using the strong laboratory electromagnet capable to produce fields of intensity up to 15 000 oersteds. Experiments were carried out in the following manner.

One ml. of densified protozoa cultures was placed by a pipette on a watch glass, a suitable quantity of the drug experimented was added, and after a thorough mixing, the liquid was distributed (0.5 ml. portions) into two identical grooved slides; the dimensions of grove being 0.4×7 cm. In this way, each portion of liquid contained approximately an equal number of ciliates — about 100 individuals in 1 ml. of solution with the same concentration of the drug. For evoiding the evaporation of the liquid, both slides with protozoa were placed in test tubes which were covered with lids. Thereafter one of the test tubes was placed (vide Fig. 1) between the poles of the electromagnet on top of a thick layer of cotton wool for securing the thermic and anti-vibration isolation in the case when indirect or pulsating fields were applied. Another test tube was placed far from the magnet and served as

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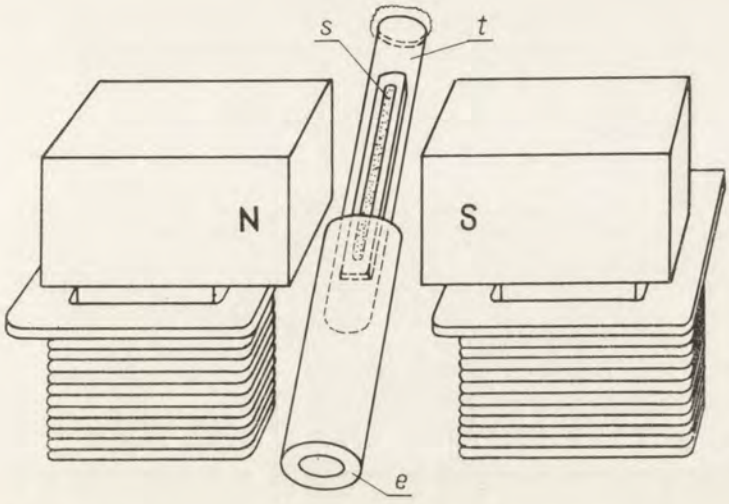

Fig. 1. Set for biomagnetic experiments on Protozoa (schematically) NS — north and south magnet poles; s — grooved slide with protozoa; t — test tube applied as moistening chamber; e — anti-vibration and thermoisolation envelope

a control. At intervals — no longer than 30 min. — both slides were examined under microscope and the changes observed in the feature and in behaviour of ciliates were recorded. The total time of exposure in the magnetic field fluctuated from 1.5 to 3 hrs. The intensity of the magnetic field amounted 5 000 —15 000 oersteds. Mostly the static field was applied but also the alternating and pulsating ones of a frequency 50 — 60 Hz.

Results

Experiments have been carried out on a total number of about 1 300 ciliates. As poisons, protozoa-killing compounds were used as: Entobex, Enteroseptol, Dilombrine and Salol. Four experiments were performed with each of those drugs. The results of observations are summarized in the tables, and diagrams are presented below in which besides the quantitative data, the characteristic is given of the morphological changes occurring under the action of the magnetic field (Fig. 2, Table 1, 2, 3, 4).

As follows from the above material the intensity of cytolysis is always higher in the magnetized samples. However the characteristic differences appear only after a 1—1.5 hrs. long exposure. The essential conformity of quantitative results in all the assays described seems to indicate that the association of the magnetism action with that of the chemical compounds used in the experiments evokes in protozoa more acute symptoms of intoxication than any of those factors applied separately.

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Table 1

Action of Dilombrine (in conc. 0.05 ml of saturated solution in 1 ml of protozoa culture) in constant magnetic field of intensity H = 5 000 0e

Consecutive stages of cytolysis	Protozoa % in different stages of cytolysis						
	0′	30′	60′	90′	120′	150′	180′
Normal	c 100% m 100%	87.1±8.4 64.6±14.7	74.2±3.2 29.4±13.2	49.0±9.0 19.0±6.0			16.1±5.1 2.9±0.3
I	c m	6.5±1.7 23.6±5.8	12.9±2.6 47.1±6.8	22.6±4.1 49.1±7.3			3.2±3.0 0
II	c m						
III	c m	6.4±0.3 11.8±2.6	12.9±2.2 23.5±3.5	29.0±5.0 42.0±6.6			0 0
IV	c m						80.7±13.0 97.1±13.5

c — protozoa in drug solution
m — protozoa in drug solution exposed to the acton of magnetic field

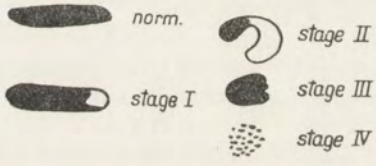

Table 2

Action of Salol (in conc. 0.25 ml. of 0.5% solution in 1 ml. of protozoa culture) in the constant magnetic field of intensity H = 10 000 0e

Consecutive stages of cytolysis	Protozoa % in different stages of cytolysis						
	0′	30′	60′	90′	120′	150′	180′
Normal	c 100% m 100%	87.3±2.5 67.2±3.4	60.3±5.5 46.6±3.0	17.5±1.6 10.3±1.7	9.5±1.6 0	0 0	
I	c m	12.7±1.0 32.8±6.1	31.7±9.5 34.5±1.7	17.5±1.6 5.2±2.7	6.3±1.1 3.4±2.5	7.9±2.5 1.7±1.0	
II	c m		15.9±14.3 19.0±17.0	60.3±21.7 62.1±5.2	71.4±20.7 56.9±7.8	74.6±17.5 41.4±2.5	
III	c m			3.2±2.4 27.0±4.5	7.9±2.4 27.6±4.3	12.7±0.2 41.4±7.6	
IV	c m			1.6±1.0 5.2±1.7	4.8±1.1 12.1±1.2	6.3±1.1 15.5±0.2	

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Table 3

Action of Entobex (in conc. 0.15 ml. of saturated solution in 1 ml. of protozoa culture) in alternating magnetic field of intensity H = 10 000 Oe and of frequency 60 Hz

Consecutive stages of cytolysis	Protozoa % in different stages of cytolysis						
	0′	30′	60′	90′	120′	150′	180′
Normal	c 100% m 100%	43.8±13.0 13.4±8.2	13.8±10.0 0	2.5±1.9 0	0 0	0 0	
I	c m	53.8±2.5 82.1±1.0	77.5±5.5 79.5±4.5	83.8±8.0 79.5±18.0		58.8±5.0 50.0±6.4	
II	c m						
III	c m	2.5±1.8 3.6±2.7	8.8±7.5 20.5±16.0	13.8±8.8 20.5±14.5	16.3±4.3 29.5±8.2	20.0±3.0 3.6±3.0	
IV	c m					21.3±1.9 46.6±3.6	

Table 4

Action of Enteroseptol (in conc. 0.1 ml. of saturated solution in 10 ml. of protozoa culture) in constant magnatic field of intensity H = 15 000 0e

Consecutive stages of cytolysis	Protozoa % in diferent stages of cytolysis						
	0′	30′	60′	90′	120′	150′	180′
Normal	c 100% m 100%	90.4±6.3 67.9±9.2	61.5±14.8 32.1±10.7	48.1±18.0 30.4±12.0			22.1±6.5 4.5±3.0
I	c m	9.6±2.9 17.9±3.6	26.9±19.0 42.9±16.7	46.2±21.0 53.6±25.0			57.7±13.4 38.4±23.0
II	c m						
III	c m	0 14.3±14.0	11.5±9.0 25.0±13.0	5.8±3.8 8.9±5.4			9.6±4.4 33.9±15.0
IV	c m						11.5±8.0 23.2±10.7

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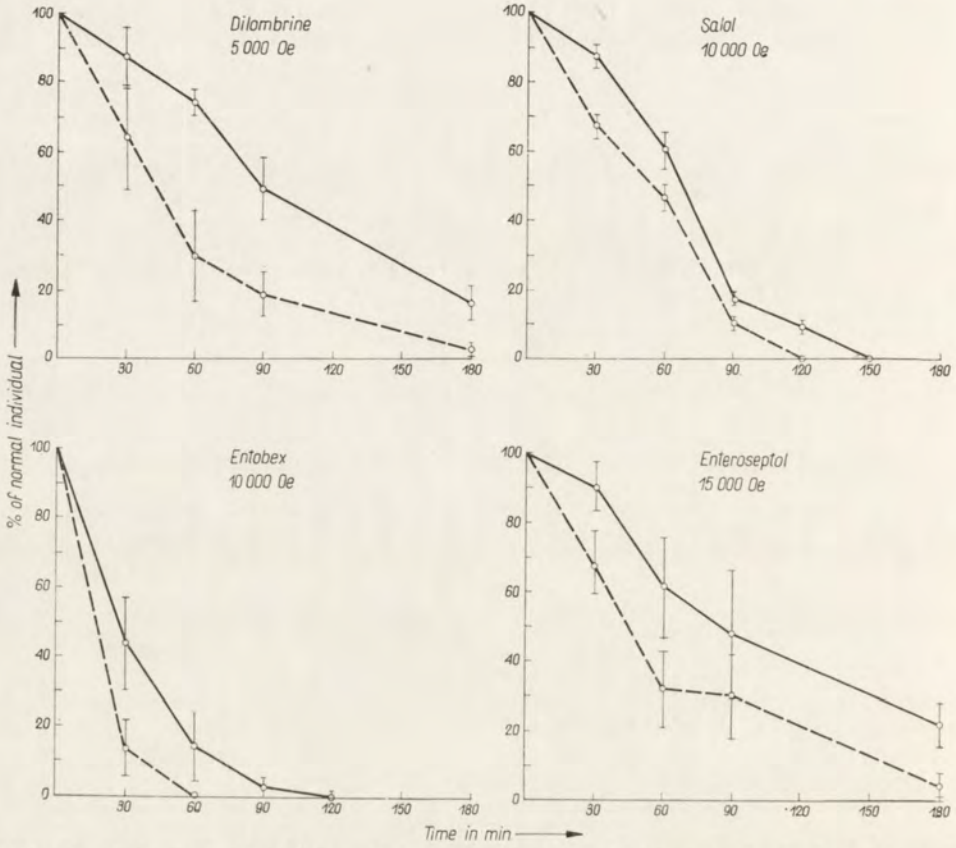

Fig. 2. Action of Dilombrine, Salol, Entobex and Enteroseptol on *Spirostomum ambiguum* in magnetic field, — — — — in magnetic field, ——— control

Discussion

What may be the mechanism of the action of magnetic field upon the living material particularly in the experiments of the type described above?

According to the authors (J. M. Barnothy 1964, Gross 1964, Dorfman 1962, Mulay and Mulay 1964 and Valentinuzzi 1961, 1964) the primary physical phenomena evoked by the action of magnetic field may be segregated into the following categories:

a. Arising of electric vortex currents of Foucault in the case when the organism or its part is moving in the magnetic field or when the circulation of its body liquids occurs.

b. Exertion of electromotoric force upon the motile carriers of electric charges (e.g. inorganic ions diffusing across the cellular membranes, free organic radicals, charged colloidal molecules etc.) which leads to deviation of their actual paths — the so called Hall's effect or akin effects belonging to the same group.

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c. Magnetic polarization of para — and diamagnetic molecules of a non-spherical shape. This concerns in the first place the protein macromolecules because they show a slight thermic movement not desorganizing the forced unidirectional orientation.

d. Forced dislocation of para- and diamagnetic molecules according to the gradient of the magnetic field but in opposite directions i.e. that of the paramagnetic molecules towards the magnetic and of the diamagnetic ones away of it which may lead to their spacial segregation.

e. A temporary deformation of the secondary or tertiary structure of macromolecules as result of a forced alteration of angles between the polypeptide and nuclein chains; action of the hydrogen bridges of DNA in the meaning of modification of the tunnel effects; splitting of the energetic levels in molecules.

The purely physical primary phenomena — as cited above — involve further secondary effects already of a biological character namely the following phenomena: processes (a) of stimulation of excitable elements, processes (b) which would disturb the diffusion across the cellular membranes, (c) (d) processes leading to disturbances in the enzymic reactions and (e) processes which may involve hereditary changes. If we analyse the situation of ciliates in the magnetic field from the point of view of the above postulations, it becomes clear that functional disturbances should arise in the protozoan cell on account of its great motility, vivid osmotic exchange with the medium and its intense metabolism. Those disturbances would be manifested in:

1. the state of increased excitability,
2. disturbances in the osmotic exchange,
3. disturbances in the enzymic processes.

Particularly the two last points suggest that in the magnetic field either a more effective penetration of poisons across the cell membranes might occur, or a rise of their toxic action inside the cell involved by a handicapped course of normal enzymic processes and of a general fall of metbolism. Especially if the magnetic inactivation concerned the assembly of enzymes on which the poison applied acts as well — it is clear that the toxic effect should be intensified.

Consequently in the case of coupled action of magnetism and toxic substances, a rise of the susceptibility to poisons in the case of exposure to magnetism is to be expected as a rule.

One point needs being elucidated: whether the measurable biomagnetic effects may be anticipated after the application of magnetic field of such intensities as those in our experiments. The theoretical calculations carried out by Neurath 1964 indicate that at the intensities of fields of a range of hundreds of oersteds, the magneto-mechanical, magneto-chemical and magneto-electrical effects approach the threshold of excitability of tissues. In fact, the attempts of inducing the electric activity of the rabbit brain cortex by means of magnet as well as the magnetic stimulation of peripheral nerves — among others — in the fishes with electric organs (Lissman, cited after Kolin, Prill and Broberg 1959) ascertained the threshold value for

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the magnetic field of the intensity of a range of 100 oersteds. Those reactions however were preceded by a prolonged period of latency.

So for obtaining distinct biomagnetic reactions, the application of fields of thousends or tenths of thousends gausses is indispensable. This is in full agreement with all the actual practical experience in this problem.

Summary

Protozoa when placed for a prolonged time in a strong magnetic field, exhibit a higher susceptibility to the poisons applied in the experiment: Entobex, Enteroseptol, Dilombrine, Salol. Morphological changes manifested in *Spirostomum* under the influence of the coupled action of magnetism and toxic compounds have been ascertained.

STRESZCZENIE

Pierwotniaki umieszczone na dłuższy czas w silnym polu magnetycznym wykazują większą wrażliwość na badane trucizny: Entobex, Enteroseptol, Dilombrina, Salol. Stwierdzono także zmiany morfologiczne występujące u *Spirostomum* pod wpływem skojarzonego działania magnetyzmu i środków toksycznych.

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Fasciculi praeparati:

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