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The systematic position of *Microsporidia*
О систематическом положении микроспоридии

Since the second half of the last century, the system of *Protozoa* acquired a more or less accomplished and harmonious form. After the time of B ü t-s c h l i 1880—1882 no essential and serious changes have been observed concerning nearly all the classes. The only exception is the clase *Sporozoa* Leuckart. The small dimensions, the extreme specialization involved by the parasitic life, the complexity of the life cycle and the difficulties of observation of those parasitic forms — involved their insufficient investigation.

In result, since the first years after the class *Sporozoa* Leuckart, 1879 had been established, an inclination was observed to include into this class any little known protozoans with a complex life cycle in which spores are formed. Therefore into the class *Sporozoa* which embraced gregarines and coccidia, the following groups were included *Myxosporidia* (B ü t s c h l i 1881, 1882), *Sarcosporidia* (B ü t s c h l i 1882), *Microsporidia* (B a l b i a n i 1882), *Haplosporidia* (L ü h e 1900), *Actinomyxidia* (C a u l l e r y et M e s n i l 1904 a, b, 1905).

Already in those years the compound character of this class was striking, and the differences in morphology and in the life cycle of some of its orders were observable.

All this stimulated S c h a u d i n n 1899, 1900 to divide *Sporozoa* into two different subclasses: *Telosporidia* in which the sporulation occurs at the end of their life cycle and accomplishes it in this way, and *Neospirodia* in which the sporulation may take place in the course of the whole life cycle and consequently is not associated with its completion. Into the first subclass, the rather well investigated *Coccidia, Haemosporidia* and gregarines were included, into the second one — *Myxosporidia, Microsporidia, Sacrosporidia* and later on — *Haplosporidia* and *Actinomyxidia*. Many zoologists adhere to this system nearly to the present time [1].

If the subclass *Telosporidia* presents a more or less natural group characterized by its definite positive features — the subclass *Neosporidia* proved to be compound. It embraced the forms associated only by one negative

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[1] Almost simultaneously an analógical division of *Sporozoa* into subclasses was performed by M e s n i l 1889. The subclass *Ectospora* corresponded in this system to *Telosporidia* and *Endospora* to *Neosporidia*. Somewhat later on, D e l a g e et H e r o u a r d 1896 proposed to divide *Sporozoa* into subclasses: *Rhabdogeniae* (with the sporozoit of a stable form) and *Amoebogeniae* (with an amoeboid sporozoit i.e. with the sporoplasm in our understanding).

feature — the sporulation is not coinciding in them with the conclusion of the life cycle.

D o f l e i n 1901 called attention that the moment of sporulation cannot be recognized as an essential and distinct criterion for separation of those two subclasses because in some *Neosporidia* the sporulation concludes the life cycle as well. A more essential feature of this subclass is — in his opinion — the character of sporulation in which the parasite body is not directly converted into the spore but produces first a definite number of pansporoblasts which desintegrate subsequently producing sporoblasts. The latter ones become transformed into spores after some complex processes.

D o f l e i n divided the subclass *Neosporidia* into two superorders. Into the first one *Cnidosporidia* were included with their multinucleated vegetative stages and with the complex multicellular spores with polar capsules. The second superorder embraced *Sacrosporidia* i.e. the forms with spores deprived of polar capsules. Into this superorder the author included only the carcosporidians.

The subsequent detailed investigations augmented still more the distance between *Telosporidia* and *Neosporidia*. The notion of *Cnidosporidia* became more profound. A u e r b a c h 1910 payed most attention to the structure of spores in his characteristic of *Cnidosporidia*. His diagnosis was as follows:

"Neosporidien, deren Sporen von mehrklappigen Schalen eingeschlossen werden. Die Schalenklappen entstehen im Sporoblasten aus besonderen Zellen. Im Innern der Sporen eine wechselnde Anzahl (je nach Gruppe und Gattung) von Polkapseln, die ebenfalls aus Zellen entstehen und in ihrem Innern einen aufgerollten, durch bestimmte Reagentien ausschnellbaren Polfaden besitzen; daneben weisen sie in ihrem Innern noch einen (Myxo- und Microsporidien) oder mehrere Keime auf (Actinomyxidien)".

D o f l e i n considered *Sporozoa* as a polyphyletic class. He postulated that *Cnidosporidia* arose independently of amoebae.

H a r t m a n n 1907, 1909, 1923 went further on and separated from *Telosporidia* — keeping for them the name of class *Sporozoa* — all the other forms including them all into class *Amoebosporidia*. This class was divided by him into two subclasses: *Cnidosporidia* with the orders *Microsporidia, Myxosporidia, Actinomyxidia,* and the subclass *Acnidosporidia* with the orders *Sarcosporidia,* and *Haplosporidia.*

The names of the above subclasses suggest that the systematic of *Amoebosporidia* is based on the presence or absence of the polar capsule with the filament.

The sublcass *Acnidosporidia* became compound at a certain degree, distinguished by a negative feature. This is especially characteristic for *Haplosporidia*. Their spores are of small dimensions and are deprived of perceptible characteristic details.

W e n y o n 1926 raised the subclass *Cnidosporidia* to the range of a class which was fully justified. The representatives of the subclass *Acnidosporidia* (*Sarcosporidia* and *Haplosporidia*) were looked upon by W e n y o n as parasites with an unclear taxonomic position and were placed by him directly after *Cnidosporidia* with a reservation that this taxonomic position should not prove the relationship of those parasites with *Cnidosporidia*. The majority of the former *Acnidosporidia* should be placed rather among fungi than among *Protozoa*.

Many authors (J i r o v e c 1936, 1953, C h e i s s i n 1956, C h e i s s i n and P o l j a n s k y 1963, P o l j a n s k y i C h e i s s i n 1964) share presently the opinion of W e n y o n.

Finally from the compound class *Sporozoa,* a comparatively distinctly limited class *Sporozoa* and a more or less clearly distinguishable class *Cnidosporidia* appeared. The species with spores deprived of polar filament were excluded from the class and, in the majority of systems placed after *Cnidosporidia* (G r a s s é 1953) as a suplement. The "spores" of *Sarcosporidia* have a definite and characteristic form and account for the taxonomic position of the class whereas *Haplosporidia* became a group characterized by negative criteria only.

This led to a situation vividly defined by C r o n i n 1964 that those protozoa are a "haplosporidian westebasket" for all the insufficiently investigated parasitic protozoa of a life cycle in which spores without polar filament are formed.

The system based on such a criterion is far not natural. Separate representatives of *Haplosporidia* (genera *Haplosporidium, Minchinia* and *Urosporidium*) remind microsporidians and differ from them by the lack of the polar filament in spores.

On the other hand; a more detailed study of *Cnidosporidia* proved that they cannot be considered as a uniform and monophyletic group.

Already D o f l e i n in his diagnosis of *Cnidosporidia* considered their multinuclear vegetative stages and the complex multicellular structure of their spores, as one of the most essential characters of this group. This character found a still more distinct expression in the definition of A u e r b a c h as cited above. Since the multinucleated forms occur in the other *Protozoa* as well A u e r b a c h excluded this feature from the characteristic of *Cnidosporidia* at all.

S h u l m a n 1965, 1966 has fully supported the theory of A u e r b a c h that one of the fundamental features of cnidosporidians are their multicellular spores. But plurality of nuclei of the vegetative stages should not serve as a principal distinction as suggested D o f l e i n. More important for differentiation of this group is the formation in the vegetative stages generative and vegetative nuclei which differ from each other in morphology and in function.

This concept of *Cnidosporidia* which presents a development of the D o f l e i n's and A u e r b a c h's theorie fails however to fit all the groups of *Protozoa* which are included presently into this class.

This definition holds fully only for *Myxosporidia* and *Actinomyxidia.* Their spores are multicellular. The vegetative stages of myxosporidians — as mentioned above — have nuclei of two types. A more detailed study of *Myxosporidia* (G r a s s é 1960, L o m et P u y t o r a c 1965, S h u l m a n 1966) demonstrated that their generative nuclei are embedded into their own cytoplasm i.e. they are separate cells producing multinuclear syncytia. In this way even the vegetative stages or myxosporidians shifted to a certain degree toward formation of muticellular units.

The vegetative stage of actinomyxidians is atrophied. The differentiation however proceeded further on, as far as we have to do with single cells only. The cells of the envelope correspond — according to the general opinion — to the vegetative nuclei of myxosporidians and the progeny cells which in the development become transformed into spores — to the generative cells.

Although the process of spore formation in myxosporidians does not coincide with that in actinomyxidians, nevertheless the general type of spore structure (being multicellular) as well as the diversity of nuclei or of cells at the vegetative stages permit to include both groups into one class.

The position of *Microsporidia* is much more difficult to be defined. Although they had been discovered much earlier than *Myxosporidia,* but the small dimensions of their spores and their highly refractible envelope which complicate their examination, were the reason why a more regular and reliable image of their morphology has been gained only in the recent time.

It should be remarked in the first place that the earliest investigators of this group found the unicellular structure of spores beyond doubt.

As mentioned above, B a l b i a n i 1882 placed them into the class *Sporozoa* considering them more related to *Sarcosporidia*. One of the most prominent investigators of myxosporidians T h é l o h a n 1892 revealed first the polar filament in the spores of microsporidians. This suggested him the idea of a close relationship of *Microsporidia* and *Myxosporidia* and he — followed by G u r l e y 1893 — placed them into the order of *Myxosporidia* as a separate family. This erronous conclusion was supported at a certain degree by two facts.

Firstly, T h é l o h a n 1895 failed to see nuclei of valves in myxosporidians and threfore he did not consider them as derivatives of the cells. He noticed inside the myxosporidian spores only the nuclei of the amoeboid embryos and the nuclei producing the capsule (one or two). It appeared to him that in the spores of microsporidians three nuclei and a vacuole may distinguished after fixation and staining. One of the nuclei was considered as capsulogenic and the vacuole — as the polar capsule.

Secondly T h é l o h a n revealed the spores of the microsporidian *Nosema coris,* a parasite of the plasmodia of myxosporidian *Leptotheca coris*. Since the spores of the myxosporidian-host were not revealed by him, Thélohan mistook the plasmodium of myxosporidian for the vegetative form of microsporidian which supported his view of the relationship between those animals.

In 1904 S t e m p e l l published his first, more or less detailed scheme of the structure of the microsporidian spores in which the polar capsule had not been observed, in cytoplasm however 4 nuclei were demonstrated.

L é g e r   e t   H e s s e 1907 revealed in the gall bladder of a marine fish, a myxosporidian *Coccomyxa morovi* which they recognized as microsporidian. They revealed in it two valves with nuclei, one polar capsule with a nucleus and an amoeboid embryo. They believed possible to find similarity between the spores of *Coccomyxa* and of *Nosema bombycis*. The separate stainable clods in the spores of *Nosema* were recognized by the authors as capsulogenic and valvogenic nuclei. Those facts led to a conclusion about the uniformity of the structure type of spores in microsporidians and myxosporidians.

This point of view was authoritatively supported by a series of publications of M e r c i e r 1906, 1909, in which clear schemes of the spore structure in microsporidians were presented. S c h r ö d e r 1909 agreed with this view as well as S t e m p e l l who in 1909, in contrast to his former views, found not only 4 nuclei but also the polar capsule in the spores of *Nosema bombycis*. This opinion was also shared by A u e r b a c h 1910.

In 1912 and 1914 F a n t h a m and P o r t e r presented a scheme of spore structure in two species of microsporidians. This structure was in conformity

with the type of spores of myxosporidians. The same pattern of spore structure of microsporidians has been presented in the article of M e r c i e r et P o i s- s o n 1926.

Kudo 1916 supported the scheme of M e r c i e r and S t e m p e l l to a certain degree, however he accepted the presence of only 2 nuclei in the sporoplasm of *Nosema bombycis*.

The distinctness of drawings of the multinucleated spores was involved by a permanent inclination to analogize them with the spores of myxosporidians. In conclusion, they acquired a slightly schematical and therefore more comprehensible form.

Unfortunately the schemes of multinucleated spores of microsporidians became a favourable object to text-books owing to their distinctness. They always stressed the affinity of microsporidians and myxosporidians whereas the investigators of those animals became more and more convinced in the lack of identicity of them.

Already in 1910, the article of S c h u b e r g appeared about the structure of the spore in *Plistophora longifilis*. In this extensive and substantial study, the author remarked that in the sporoplasm of the spore only one nucleus is present and the polar capsule does not exist. The polar filament is in a coiled state, located immediately under the envelope mostly in the posterior region of the spore.

Insisting on the uninuclear character of the spore, S c h u b e r g believed that other authors mistook the metachromatic granules for the nuclei of valves and of the polar capsules. These granules have arisen as a fixation and staining artifact. This view on the structure of the spore was accepted by O h m o r i 1912 and fully supported by W e i s s e n b e r g 1913 concerning *Glugea anomala* and *G. hertwigi*.

L é g e r et H e s s e 1916 a studying the microsporidian *Mrazekia argoisi* with the most voluminous spores (length up to 23 µ), revealed only 2 nuclei in sporoplasm. The polar capsule as well as the capsulogenic nuclei were absent. Those observations were carried out on the biggest spores of microsporidians in which the structure details could be most easily discerned. In the smaller spores of *Plistiphora macrospora* L é g e r et H e s s e 1915 b considered as the polar capsule the whole area of the spore not occupied by the posterior wacuole. In this area, two-nucleated sporoplasm is present. It is clear in the picture that this area can scarcely be called the polar capsule.

D e b a i s i e u x 1919 and 1920 accepted the view of S c h u b e r g and O h m o r i concerning *Thelohania varians*. A number of authors revealed 1 or 2 nuclei only in sporoplasm, they accepted however the presence of polar capsule (G e o r g e w i t s c h 1917, P a i l l o t 1918, K u d o 1920, 1921, 1922, 1925, G u y e n o t and N a v i l l e 1922).

Trying to coordinate the controversive and sometimes opposite findings of different authors, K u d o 1924 accepted a compromising point of view postulating that microsporidians occupy an intermediate position between haplosporidians and myxosporidians and may serve as an example of a gradual transition from haplosporidian spores — ot a simple structure — to the more complex spores of myxosporidians.

In the later articles we meet more frequently the indications on the unicellular character of microsporidian spores (Z w ö l f e r 1926, D e b a i s i e u x 1928, R e i c h e n o w 1928). A special attention deserves the series of articles of J i r o v e c summarized in his monography (J i r o v e c 1936). By means

of the Feulgen reaction, he examined spores of 13 species of microsporidians which belonged to 5 genera and ascertained the presence of 1 or 2 nuclei only in sporoplasm, of a unique envelope and a polar filament. He also pointed out the complete absence of the polar capsule. The polar filament was situated inside the spore, directly beneath the envelope. An important statement of J i r o v e c was the documentation of unicellular spores in those species in which the spores were recognized previously as multicellular.

The decisive stroke to the theory of multicellular structure of spores were the results of the electron-microscopic investigations. They all demonstrated that the spore of microsporidians presents only one cell (K r i e g 1955, W e i s e r 1959, H u g e r 1960, P u y t o r a c 1961, K u d o and D a n i e l s 1963, L o m and V a v r a 1961, 1962, 1963, V a v r a 1963, 1965 a, 1965 b).

In this way, only the rather minor part of specialists — investigators from 1892—1926 adhere to the theory of multinuclear nature of microsporidian spores. The majority of authors accepted the unicellular structure of microsporidian spores, especially in the last four tenths of years when the methodics of investigation had made a considerable progress.

Basing on the recent findings, C o r l i s s and L e v i n e 1963 proposed a new diagnosis of microsporidians:

"Class *Microsporidea*: Spore of unicellular origin; single sporoplasm; single valve; one or two long, tubular polar filaments through which sporoplasm escapes; cytozoic in invertebrates, especially arthropods, and lower (rarely higher) vertebrates".

Consequently microsporidians differ distinctly from the other cnidosporidians by the unicellularity of their spores. Another essential difference stressed by L o m and V a v r a 1962 is the absence of multiform nuclei in their vegetative stages.

In this way, the class *Cnidosporidia* — apparently well delimited — had corresponded no longer to the previous theories concerning the fundamental distinctive feature. Realizing this situation, the investigators looked for an exit point of it on different ways.

As mentioned above, K u d o prefered to consider microsporidians as intermediate forms with all their intermediate steps from haplosporidians to myxosporidians. His postulation has not proved to be adequate since the studies of J i r o v e c and other authors demonstrated that microsporidians are an animal group with unicellular spores which are constructed according to a common pattern.

J i r o v e c followed the way of changing the diagnosis of this class in order to retain microsporidians within the class *Cnidosporidia*. The only essential distinguishing diagnostic feature of cnidosporidians is — in his opinion — the presence of the polar filament in spores.

This point of view is in our opinion not acceptable because the polar filament might have arisen convergently in different groups of the animal kingdom, even being situated far from one another.

Among *Protozoa* the polar filament may occur in some flagellates e.g. in the representatives of various families and even of suborders of the order *Dinoflagellata* (*Nematodinium* and *Polykrikos*) and among *Metazoa* in Coelenterata.

All those structures act according to one principle (outshot of a polar filament tightly coiled up) and have one common characteristic peculiarity — the unicellular nature. However this is their only one similitude. In the first

place they considerably differ in function. In coelenterates they serve for attack and defence. In microsporidians — for injection of sporoplasm into the host tissues. In myxosporidians — evidently — for attachment of the spores in a definite region of intestine. In the other animals the function of the filament is not clear.

Those organs have also different types of structure. In coelenterates and in cnidosporidians the polar capsule with the filament are unicellular organs. In microsporidians the whole spore corresponds to the polar capsule. At last in *Nematodinium* and in *Polykrikos* the nematocysts with the polar filament are separate organelles of one cell. The general structure of the polar capsule does not coincide either in different animals. For this reason the attempt of W e i l l 1938 to trace the origin of myxosporidians from the neotenic forms of narcomeduses found no support. The different structure of the polar capsules in microsporidians and myxosporidians presents a special interest for us.

In microsporidians the whole spore performs the function of the whole polar capsule. There is no special vacuole for the polar filament and its flagella are placed directly under the spore envelope. At the base of the filament, a cap is present of a rather simple construction. Sporoplasm is located at the central part of the spore and is in direct touch with the filament. In the process of outshot of the polar filament, an important role is played by the special organelles — the polaroplast and the posterior vacuole.

In myxosporidians, the cytoplasm is in the peripheral part of the capsule and the nucleus is most often pushed out beyond the boundary of the cell. The polar filament is in a special vacuole in the centre of the cell and occupies nearly all its territory. The top of the polar capsule — to which the base of the polar filament is attached from inside — is covered by a cap of a complex structure. In its central part, a substance of another structure — the cork is located. The polaroplast is absent and in the process of outshot of the polar filament the main role is played by the elasticity of the tightly coiled polar filament and possibly the internal turgor inside the vacuole as well.

Of course the presence of the polar filament cannot serve as the only reason for placing into one class animals with different pattern of structure. L o m and V a v r a 1962 suggest quite reasonably to place *Microsporidia* on one side and *Myxosporidia* and *Actinomyxidia* on the other side into separate classes: *Isonucleidea* and *Heteronucleidea*. This view was based on the great importance of diversity of nuclei which was ascribed — quite reasonably — by the authors.

However after this correct recognition, the authors failed to separate fully those classes and united them into one common subphylum *Cnidosporidia*. However formation of a subphylum based on only one feature — which might have arisen as result of convergence — is still less justified than formation of a class.

Nearly all the natural systems of the animal kingdom are based on the structure plan of the living organism. Namely this gives the possibility of evaluation of the relationship of taxones.

The microsporidian spores differ so sharply from the spores of other representatives of *Cnidosporidia* that there is no possibility to homologize their separate parts. Really the valves of myxosporidians or actinomyxidians — which are independent highly specialized cells — cannot be compared with the spore envelope of microsporidians which presents only a part of the cell.

For this reason there is no structure which might be homologized with the polar capsule of *Cnidosporidia* since such a structure does not exist in *Microsporidia*. The polar capsule — which presents only one cell — may be compared to the whole spore of microsporidians but then the full absence of sporoplasm inside the polar capsule interferes with the homology. The sporoplasm of microsporidians (a part of the cell) cannot be compared or homologized with the amoeboid embryo. In another words, it is impossible to compare and homologize (and less so to derive one from the other) single independent cells with the organelles i.e. with specialized cell parts.

Namely this inclined S h u l m a n 1965, 1966 to place *Microsporidia* beyond the limits of the class *Cnidosporidia*. This evoked the question of the situation of microsporidians themselve in the *Protozoa* system.

Too much importance attributed to the polar filament as a fundamental taxonomic feature lead the majority of authors to pay less attention to those protozoa groups which might be compared with microsporidians.

Meanwhile already in 1899 the first haplosporidians (C a u l l e r y et M e-s n i l 1899) had been discovered and in the same year one of the discoverer of this group, M e n s i l 1899 placed them into the subclass *Endospora*[2] in his system, together with microsporidians.

Indeed, the multinucleated vegetative stages, lack of nuclear differentiation, unicellular character of spores, presence of a single envelope, 1—2 nuclear sporoplasm approach haplosporidians to microsporidians, as well as their parasitic character. Both groups are mostly intracellular parasites of invertebrates or of lower vertebrates.

The only essential distinction of haplosporidians from microsporidians — the absence of the polar filament — is recompensed by the possibility to compare the spores and to homologize their separate parts. According the opinion of D e b a i s i e u x 1920 and S p r a g u e 1965, the polar filament of microsporidians may be homologized with the movable lid of the haplosporidian spore. This is also supported by the presence of a structure ("polar cap") at the base the polar filament in the microsporidian spores. This structure reminds the lid of the haplosporidian spores.

However the development of the theories concerning the affinity of microsporidians and haplosporidians was much disturbed by the association of microsporidians with other cnidosporidians.

Already D o f l e i n 1901, 1902 when including haplosporidians into *Neosporidia*, placed them — in contrast to microsporidians — into another superorder, *Acnidosporidia*. His view was supported by the subsequent authors who described haplosporidians — (C a u l l e r y et M e s n i l 1905) and since that time haplosporidians became — after the impressive words of D e b a i-s i e u x 1920:

"Depuis, l'ordre des Haplosporidies a trop souvent servi de refuge aux espèces incomplètement étudiées qui, faute d'un étude civil convenable, ne trouvaient droit de cité dans aucun autre ordre."

In dependence on the vigour of elimination, haplosporidians became gradually a less integrated group which involved their increasing similitude to microsporidians.

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[2] As mentioned above, *Endospora* Mesnil corresponds approximately to *Neosporidia* Schaudinn.

D e b a i s i e u x suggested in 1920 the liquidation of the order *Haplospori-dia* and their inclusion into the order *Microsporidia* as a separate suborder.

K u d o 1924 found possible to approach haplosporidians to the microspo-ridians with spores in which 1 or 2 nuclei are present in sporoplasm and the polar capsule is absent. Just these features provided him reason for conside-ring microsporidians as a transitory group between the primitive structure of haplosporidians to more complex one of myxosporidians.

S p r a g u e 1965, the prominent specialist as well of microsporidians as of haplosporidians performed a most substantiated — in our opinion — revision of the latter leaving only 3 genera in this order: *Haplosporidium* Lühe, 1900, *Minchinia* Labbé, 1896 and *Urosporidium*, Caullery et Mesnil 1905. He also pointed out their great nearness to microsporidians. S p r a g u e remarked before all that at the present level of our knowledge and technique there is no possibility to distinguish the vegetative forms in these parasitic protozoa. Even essential difference in the life cycle could not be revealed. As to the spores, he found a considerably higher similitude of haplosporidians to microsporidians than of microsporidians to myxosporidians.

He was right therefore to put forward two problems: 1. of a comparatively low taxonomic value of such a feature like the presence or absence of the polar filament [3] in protozoa, 2. of the necessity to approach myxosporidians and haplosporidians (S p r a g u e 1965, 1966 a,b).

Unfortunately Sprague himself failed to show a due consistency after having pointed out the difference of myxosporidians from microsporidians and haplosporidians which he raised to the range of class. After having separated them distinctly he ronged them all into one subphlylum. Since the polar filament was no longer, in his opinion, the general property — of the repre-sentatives of this subphylum, he associated them according to another pro-perty — the presence of sporoplasm , suggesting in the same study a new name: *Plasmospora* (S p r a g u e 1965) . In anothed publication (1966) he res-tituted the old term of D e l a g e et H é r o u a r d — *Amoebogena* [4].

It seems clear that the sporoplasm of haplosporidians and microsporidians (a fragment of the cell) and the amoeboid embryos of cnidosporidians (separate cells) cannot be compared or homologized or derived from each other as e.g. the polar capsules of cnidosporidians and polar filaments of microsporidians. In this case as well, we cannot compare the whole cells to their parts — the organelles. There is also no possibility to compare the specialized unicellular spore of microsporidians and haplosporidians with the spores of cnidospori-dians which are constituted of several cells, each of them having reached an extreme specialization.

The highly specialized part of the cell, the organelle, cannot be transfor-med into an independent cell with all the functions of its mother cell. Therefore there is no reason of phylogenetic deriving of myxosporidians from micro-sporidians as it was done by J i r o v e c 1936, or to consider microsporidians as a transitory step from haplosporidians to myxosporidians as it has been

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[3] In some species of *Microsporidia* which infect the host indirectly by parasitic *Hymenoptera,* the reduction of the polar filament occurs.

[4] We think it inadequate to restore the old terms forgotten by nearly all the investigators. The more so as S p r a g u e occasionally restored the term *Rhabdo-gena* for the subphylum *Sporozoa* which is illegal because the earlier name of *Sporozoa* was initially applied only to coccidians and gregarines i.e. it coincided with the present understanding of the content of this group.

suggested by K u d o 1924. There is still less reason to consider microsporidians as a result of a regressive evolution following the line: *Mezozoa* → *Actinomyxidia* → *Myxosporidia* → *Microsporidia* (G o t t s c h a l k 1957),

Every group from those discussed by us: *Haplosporidia, Microsporidia, Myxosporidia* and *Actinomyxidia* represent forms which diverged ever since and are highly specialized due to prolonged evolution. They cannot be derived from one another.

If between *Microsporidia* and *Haplosporidia* on one side and *Myxosporidia* and *Actinomyxidia* on the other side — the elements of similitude of structure are perceived and the possibility of homologization and juxtaposition of their vegetative forms and spores, as well as of separate parts of them are postulated, there is no reason to associate together all the 4 taxons. Possibly we have to do with two groups of *Protozoa* which had arisen at different time periods, independently from each other [5]. A full absence of flagellated forms at all the stages of the life cycle, the amoeboid character of vegetative stages indicate the possibility of their descendence from some parasitic amoebae.

As a result of the above considerations we suggest the following changes in the system of *Protozoa*.

Together with the classes *Sarcodina, Flagellata* and *Sporozoa* all belonging to the subphylum *Plasmodroma*, the two classes: *Cnidosporidia* and *Plasmosporidia* should be included into the same subphylum.

Into the first class, *Myxosporidia* and *Actinomyxidia* are included, into the second one: *Haplosporidia* and *Microsporidia*.

Since the differences between the groups included into each class are sufficiently great — which proves their early divergence — they should be raised to the range of subclasses.

We include the diagnoses of the taxons discussed.

I Class *Plasmosporidia* Sprague, 1965 emend. Issi et Shulman

Parasitic Protozoa with vegetative stages comprising several nuclei of one type, unicellular spores with 1—2 — nucleated sporoplasm and a single envelope. Spores are formed at the conclusion of the life cycle. Intracellular, rarely tissue parasites of invertebrates, rarely of lower vertebrates.

Comprises two subclasses.

Subclass 1. *Haplosporidia* Caullery et Mesnil, 1899. emend. Sprague, 1964

Plasmosporidia with spores; 1—2 nucleated sporoplasm, single envelope and a movable lid — on one of the poles — which lets sporoplasm to flow out. Intracellular or tissue parasites of various invertebrates, rarely of lower vertebrates.

The only order *Haplosporidia* Caullery et Mesnil, 1899 with one family *Haplosporidiidae* Caullery et Mesnil, 1905, comprising 3 genera.

Subclass 2. *Microsporidia* Balbiani, 1892

Plasmosporidia with spores of a 1—2 nucleated sporoplasm, a single envelope and a long tubular polar filament along which sporoplasm gets outside. Intracellular parasites of invertebrates mostly arthropods, and of lower — rarely higher — vertebrates.

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[5] We consciously avoid to take advantage of the character of the sexual process for systematic diagnoses because as yet it has not been investigated with a full reliability in any of the groups discussed by us.

The only order *Microsporidia* with 4 classes comprising 5 families with 18 genera (W e i s e r 1961).

II Class *Cnidosporidia* Doflein, 1901, emend. Shulman

Parasitic *Protozoa* with multinucleated vegetative stages, containing nuclei or cells of two types, and with multicellular spores consisting of a variable number of valves, polar capsules with polar filament, amoeboid embryos and other cells. Spores arising from specialized cells may be formed in the process of plasmodium development or at its conclusion. Parasites of cavities or tissues, rarely intracellular parasites of invertebrates or lower vertebrates [6].

Subclass 1. *Myxosporidia* Bütschli, 1881 emend. Shulman

Cnidosporidia with vegetative forms containing vegetative and generative nuclei, with a capability to asexual reproduction by means of nucleogony followed by division of cytoplasm. Multinuclear spores which arise from generative nuclei, consist of 2, 3, 4 or 6 valves, of a various number of polar capsules (1,2,3,4, or 6) and of one — rarely two — amoeboid embryos.

Parasites of body cavity or tissues of *Teleostei*, rarely of other fishes and aquatic vertebrates.

Comprises two orders: *Bivalvulea* and *Multivalvulea* with 12 families and with 29 genera.

Subclass 2. *Actinomyxidia* Štole, 1899

Cnidosporidia with poorly developed vegetative stages, consisting of 2, rarely 4 somatic and 2 propagation cells. Asexual reproduction has not been observed. After a prolonged process of division and development, complex multicellular spores are formed from the propagation cells. Those spores consist of 3 valves, 3 polar capsules, 1—3 cells of the internal envelope and of various number of amoeboid embryos: of one multinuclear, and of a variable number of 1-, rarely 2-nucleated ones. Body cavity or tissue parasites of annelids.

Comprises one order: *Actinomyxidia* with the characters of the subclass, with 4 families and 9 genera (J a n i s z e w s k a 1955, 1957).

The alteration suggested by us present the following advantages:

1. The principal feature of *Cnidosporidia* is kept: the multiple nuclei which was stressed already by D o f l e i n and A u e r b a c h. Simultaneously the system takes into account: a) the data of S c h u b e r g, D e b a i s i e u x, J i r o v e c, H u g e r, P u y t o r a c, L o m and V a v r a, C o r l i s s and L e v i n e about the unicellularity of the microsporidian spores; b) the view of L o m and V a v r a about the essential difference of vegetative microsporidian forms with even nuclei, and vegetative forms of myxosporidians and actinomyxidians with different nuclei or cells; c) the view of D e b a i s i e u x and S p r a g u e about a greater proximity of microsporidians and haplosporidians.

2. The absence or presence of the polar filament — the quality which might have arisen as a result of convergency — is not put forward as the most important one.

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[6] Our characteristic of *Cnidosporidia* is not contradictory to notion of the early investigators of this animal group. The views of D o f l e i n and A u e r b a c h are somewhat developed and supplemented in it.

3. The base of the system is the plan of structure considering not one but many characters, mostly the positive ones.

4. The system is based not only on differences but on similarity of taxons as well and this makes it nearer the natural system and reflecting — at a certain degree — the phylogenetic interrelations.

Summary

The presence or absence of the polar filament cannot be used as the main diagnostic character of the class as far as the filament itself may originate convergently in different distant groups of animals. The analysis of the structure has shown that *Microsporidia*, in spite of the presence of the polar filament in their spores, must be excluded from the class *Cnidosporidia* and together with *Haplosporidia* placed into the newly created class *Plasmosporidia*.

The class *Cnidosporidia* in the new meaning (subclasses *Myxosporidia* and *Actinomyxidia*) is characterised by multinuclear or multicellular vegetative stages containing nuclei, or cells of two types and multicellular spores consisting of valves, polar capsules, amoeboid embryos and other cells.

The most characteristic features of the class *Plasmosporidia* (with subclasses *Microsporidia* and *Haplosporidia*) are vegetative stages with several unitipical nuclei and unicellular spores posessing 1—2 nuclear sporoplasm and single membrane.

РЕЗЮМЕ

Наличие или отсутствие полярных стрекательных нитей не может быть использовано как основной диагностический признак класса, поскольку полярная нить может возникнуть конвергентно в различных отдалённых группах животных. Анализ строения микроспоридий показал, что они, несмотря на наличие полярных нитей в спорах, должны быть исключены из класса *Cnidosporidia* и вместе с гаплоспоридиями помещены во вновь основанный класс *Plasmosporidia*.

Класс *Cnidosporidia* в новом значении (с подклассами *Myxosporidia* и *Actinomyxidia*) характеризуется многоядерными или многоклеточными вегетативными стадиями с ядрами или клетками 2-х типов и многоклеточными спорами, состоящими из створок, полярных капсул, амебоидных зародышей и других клеток.

Наиболее характерной чертой класса *Plasmosporidia* (с подклассами *Microsporidia* и *Haplosporidia*) являются вегетативные стадии с несколькими однотипными ядрами и одноклеточные споры, имеющие 1—2-х ядерную спороплазму и единую оболочку.

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Urceolariidae from breeding carp — Cyprinus carpio L. in Żabieniec and remarks on the seasonal variability of trichodinids

Urceolariidae karpi hodowlanych — *Cyprinus carpio* L. w Żabieńcu
i uwagi o zmienności sezonowej trichodin

Strangely enough *Urceolariidae* occurring on breading carp, *Cyprinus carpio* L., have not been fully investigated till now. Single species were described in the reports of Raabe 1950, Lom 1960, 1961, 1963 and Chen 1963, however, there was no larger study which would be a detailed review of the fauna of *Urceolariidae* occurring on this fish species. Furthermore, all available data were inadequate and required more detailed corrections. In effect even the recent review papers on the *Urceolariidae* mention different lists of parasites found on carp. Stein 1962 names four species: *Tripartiella carassii* (Dogiel, 1940), *Trichodina domerguei* f. *acuta* Lom, 1961, *T. nigra* Lom, 1961 and *Trichodinella epizootica* (Raabe, 1950). On the other hand Heider 1964 mentions only two species: *Trichodina domerguei* subsp. *domerguei* (Wallengren, 1897) and *Trichodinella carassii* (Dogiel, 1940).

The present paper is an attempt to refer the problem of *Urceolariidae* occurring on carp on the basis of an ample material collected from the ponds of the Department of Fish Culture at Żabieniec near Warsaw. The faunistic and taxonomic problems only will be discussed in this account with consideration of some indispensable data concerning the intensity of infection. One of the authors intends to present in a separate paper the above mentioned questions as well as the problems concerning the ecology of the *Urceolariidae*. Moreover, attempts will be made to examine in details the seasonal variability of the trichodinids on the material of the four discussed species. This problem was previously mentioned (Kazubski 1967) and was then presented in a report (Kazubski and Migała 1967).

Material and methods

Trichodinids were collected from the carp, *Cyprinus carpio* L., since the spawn up to the age of two years. In one case the investigations were carried during a full two years period and in the other one during the second year of their life. The fishes were reared over this period and moved to the

appropriate ponds with a two-years system according to the method of Dubisz accepted in Poland. The material was collected both from the skin and from the gills of fish. The trichodinds were get at random at the interval of one month or somewhat longer according to the schedule of the searched fisheries in the ponds of the Department of Fish Culture at Żabieniec. All water bodies, from which the material was collected, were in a close complex. The fishes of every fish culture came from one spawning. Also the continuity of the material collections was preserved, it was collected from the same population of fishes. The carps were examined immediately when they get the laboratory.

The identification of the species and description of their morphology were based on silver impregnated preparations after Klein as well as on the preparations fixed in Schaudinn's fluid and stained with acid haematoxylin.

The description of the elements of the adhesive disc and the problem of trichodinids variability were elaborated on the basis of the silver impregnated preparations. The samples of trichodinids that were used for comparison were estimated at random. Twenty individuals were arbitrally accepted as maximum number for one sample. In many cases the collection of the indicated number of specimens belonging to one species was impossible because of the unequal occurrence of the particular species of trichodinids in various periods of year. The sizes of the particular samples are indicated in the Tables. The young indivduals were eliminated from comparison according to the criteria of K a z u b s k i 1967 concerning the uncompletely formed inner rays and nonperformed, post-divisional regeneration of the radial pins system.

The diagrams present only two features: the number of denticles and the diameter of the skeletal ring. The high correlation of other diameters of trichodinids, among others between the diameter of the adhesive disc and the diameter of the skeletal ring did not create any doubt and were not discussed separately.

The photographs of the trichodinids were of great help, especially in the examination of the shape of denticles. They were taken at the same magnification.

The nuclear apparatus was examined on the preparations fixed in Schaudinn's fluid and stained with acid haematoxylin. Also in this case the number of individuals in a sample was twenty, all of them were coming from one population. For comparison with the biometrical data presented in Tables 2—5, some other data were also included concerning the body diameter, the diameter of the adhesive disc, the skeletal ring and the number of the denticles. The exclusion of young individuals was impossible in the case of haematoxylin stained specimens.

Results

The occurrence of five trichodinids species was observed as a result of examinations:

Trichodina pediculus Ehrenberg on the skin and the gills,
T. nigra Lom on the skin,
T. domerguei f. acuta Lom on the skin,
T. mutabilis sp. n. on the gills,
Trichodinella (Foliella) subtilis Lom on the gills.

The problem of seasonal variability was studied in four species (all mentioned above, except *T. pediculus* occurring in a small quantity). The results of these studies were presented in a preliminary report for the Twentieth Annual Meeting of the Society of Protozoologists (K a z u b s k i and M i-g a ł a 1967).

The mentioned species will be discussed succesively. It was necessary to introduce several corrections in the descriptions as well as some alterations in the range of the discussed species.

Trichodina pediculus Ehrenberg, 1838 (Pl. I)

Single specimens of this species were found in the early spring on one-year-old fishes just after hibernation. The parasites occurred on the skin of fishes in three cases, and on the gills in one case.

Usually *T. pediculus* parasitizes on hydras (R a a b e 1959), however it may occur and reproduce on fishes (K a z u b s k i 1965). Up to now it has been observed on the fry of *Rutilus rutilus, Alburnus alburnus, Leucaspius delineatus, Coregonus albula*, and on the young specimens of *Carassius carassius*.

Collected specimens of *T. pediculus* are typical. Their denticles have characteristic falcated blades and long, pointed rays, somewhat triangular in shape. In large specimens the longitudinal rib is running along the ray.

For the reason of very scarce data concerning *T. pediculus* found on fishes we present here the measurements of all collected specimens (Table 1). These specimens do not differ virtually from the specimens found on hydras and on other fish species. Furthermore, such comparisons could be of little value because of few existing biometrical data. We do not intend to reveal an attitude towards other findings identified as *T. pediculus* (among the others by M u l l e r 1937 and W e l l b o r n 1967). This discussion requires a more detailed material and will be continued in another report.

Table 1
Measurements of *Trichodina pediculus*

Date	Localization	Diameter of the body	Diameter of the adhesive disc with the border membrane	Diameter of the adhesive disc	Diameter of the denticulate ring	Length of the denticles	Number of the denticles
8 Apr. 1965	skin	103.9	70.0	59.4	38.7	18.0	28
8 Apr. 1965	skin	72.1	57.2	48.8	32.3	15.9	29[1]
21 Apr. 1965	skin	—	67.8	58.3	38.2	18.0	28[2]
8 Apr. 1965	gills	95.6	67.8	58.3	38.2	22.3	29

[1] The rather young specimen

[2] The damaged specimen, the number of denticles was given aproximately

Trichodina nigra Lom, 1960 (Pl. II and III)

The species *T. nigra* was commonly found in high number on the skin of the examined carps.

T. nigra has shape typical of the genus. The adoral zone of cilia forms a spiral of about 400°; its dimensions are $43.5—60.4 \times 37.0—54.1 \mu$ ($49.2 \times 45.6 \mu$).

2*

The adhesive disc is large with a wide (about $5\,\mu$) border membrane. The biometrical data are presented in Table 2. The denticles have big and distinct blades and rays, both these elements have a very similar length. The blades have a falcated shape with a cut and rounded apex. The rays are thick, slightly contracted and bluntly ended. They are thicker when the individual becomes older. The rays are sometimes slightly unequal and they take the shape of fingers or of knotty sticks; distinctly visible in the old, larger specimens (Pl. II 7, III 9,14). The rays are ordinarily directed to the geometric center of the adhesive disc or somewhat to the right side. The inclination of the rays towards the left of the disc center occurs rarely (Pl. II 3,6). There are 9—11 radial pins for one denticle. The center of the disc is silvered regularly according to Klein's method and is dark.

The nuclear apparatus consists of the horseshoe-shaped macronucleus and of a single Mi. The diameter of Ma is 31.2—$42.6\,\mu$ (mean $37.0\,\mu$) and its width is 4.2—$7.3\,\mu$ (mean $5.7\,\mu$). The distance between the ends of Ma (the distance "x" according to D o g i e l) is 7.3—$12.5\,\mu$ ($9.5\,\mu$). Mi is big, elongated, commonly somewhat depressed in its central part, in that way it has a shape of an elongated bean. It has the following size: the length 9.4—$12.5\,\mu$ ($11.0\,\mu$), width 2.1—$4.2\,\mu$ ($3.0\,\mu$) and is situated at the external side of Ma in the distance (the distance "y" according to D o g i e l) of 4.2—$11.4\,\mu$ ($8.1\,\mu$) from one of its ends.

Other biometrical data concerning the individuals of the same sample were as follows: the body diameter 42.6—$68.6\,\mu$ ($55.6\,\mu$), the diameter of the adhesive disc 38.5—$58.2\,\mu$ ($48.8\,\mu$), the diameter of the denticulate ring 25.0—$36.4\,\mu$ ($30.1\,\mu$), the number of the denticles 21—30 (24.5). The measurements were taken from the individuals collected on April 22, 1964.

The species *T. nigra* was described by L o m 1961 from the following species of fishes found in Czechoslovakia: *Cyprinus carpio, Scardinius erythrophthalmus, Rutilus rutilus, Abramis brama, Perca fluviatilis, Tinca tinca, Alburnus alburnus* and *Leuciscus cephalus*. The description of these species was based on the material originating from all hosts and in that way a rather wide range of variability was attained. It seems noteworthy at the same time that L o m 1961 considering the number of denticles of the species described by him gave the general range of its variability as 17—33 and noted two ranges of the most common mean values for the particular populations: 21—23 and 25—29. These data suggested that the material was not homogenous. The assumptions were confirmed when the photographs presented in this work were considered. The photograph 10 (L o m 1961) presents undoubtedly a new species described by the author, however the photograph 11 identified as "*T. nigra* with prolongated »axis« of the thorns extending till the center of the disc" presents the species *Trichodina pediculus* Ehrbg. The last one resembles closely to *T. nigra* but it differs from it, among the others, by the higher number of denticles. The combination of the description of both species in point should give such final effect.

Owing to the kindness of Dr J. L o m we had the opportunity of examining the trichodinids identified by him as *T. nigra* coming from *Cyprinus carpio, Rutilus rutilus, Leucaspius delineatus, Abramis brama* and *Alburnus albrunus*. This comparison revealed a complete morphological coincidence of both materials from Poland and from Czechoslovakia. The size and number of denticles of the individuals presented by Dr J. Lo m correspond completely

Table 2
Measurements of *Trichodina nigra*

Date	Number of specimens	Diameter of the body	Diameter of the adhesive disc with the border membrane	Diameter of the adhesive disc	Diameter of the denticulate ring	Length of the denticles	Number of the denticles
pond fish culture 1963—1964							
20 Apr. 1964	20	73.1—88.0* 77.0**	55.1—69.4 63.3	45.1—60.4 52.1	28.1—38.7 31.8	14.3—17.5 15.6	23—28 25.1
26—29 May 1964	8	53.0—64.7 60.2	43.6—60.4 52.5	35.0—48.7 41.9	21.7—32.3 27.1	11.1—15.9 13.7	21—26 22.6
8 July 1964	7	55.1—70.0 64.0	45.1—55.1 50.6	36.0—44.5 40.5	21.2—26.5 23.5	12.7—15.4 14.0	19—22 20.3
8 Sept. 1964	3	50.9—67.8 58.3	45.6—51.9 49.1	38.2—41.3 39.4	21.2—25.4 23.3	13.8—14.3 14.1	18—20 19.0
2—13 Oct. 1964	4	53.0—58.3 55.6	44.5—67.2 49.3	33.9—45.1 38.3	19.1—29.7 23.2	12.7—14.8 13.6	19—21 20.5
pond fish culture 1964—1965							
3 Aug. 1964	5	53.0—71.0 61.1	45.6—54.6 50.4	38.2—44.0 41.5	22.3—25.4 23.6	12.7—14.8 13.9	18—21 19.6
1—2 Sept. 1964	9	54.1—68.9 60.4	45.1—54.1 50.4	36.0—45.6 41.2	20.7—28.1 24.3	12.7—14.8 13.6	20—22 21.0
1 Oct. 1964	12	57.2—74.2 65.3	46.1—60.4 53.0	36.0—48.8 42.6	21.7—30.2 26.1	13.3—16.4 14.5	20—24 21.3
27 Oct. 1964	20	61.5—91.2 74.6	51.9—68.9 59.5	42.4—57.2 49.3	24.4—33.4 29.2	13.8—15.9 15.0	21—27 22.7
8 Apr. 1965	20	65.7—102.8 81.6	56.2—72.1 64.0	46.6—61.5 54.1	28.6—39.8 34.0	12.2—17.5 15.1	22—27 24.8
20 Apr. 1965	20	67.8—94.3 77.1	54.1—68.9 62.0	44.5—57.2 51.5	27.0—36.6 31.9	13.3—17.0 15.6	22—27 23.8
10 Sept. 1965	5	51.0—56.2 53.9	45.1—56.2 49.3	35.0—45.6 38.6	20.7—25.4 22.6	12.7—14.8 13.9	17—21 18.6
27 Oct. 1965	20	57.2—78.4 69.1	47.7—64.7 55.1	39.2—51.9 45.4	21.7—31.8 26.0	13.8—15.9 14.8	19—23 21.1

* range, ** mean value

with the relevant features of our specimens from the same period of time. On this basis we maintain the conception of the species *T. nigra* with the previously mentioned hosts. However we consider as indispensable to introduce several corrections in the description of the denticles as well as in the biometrical data according to our material presented in this paper.

The species *T. nigra* was also found in the *Hypophthalmichthys molitrix* from the river Amur (S t e i n 1968). In this case both the description and the localization are strictly in line with our own observations. The inserted photograph (S t e i n 1968, Pl. II 8) presents in our opinion a young individual. For this reason it cannot be completely compared with our photographs. On the other hand there are no details on the photograph which may indicate that these individuals are not within the same species.

"*Trichodina nigra*" described by C h e n presents a different problem. The biometrical data revealed by this author exceed the range of variability described in our investigations. This "species" occurs not only on the skin but also on the gills, and the enclosed drawing (C h e n 1963, fig. 4) does not represent in our opinion *T. nigra*. It seems that two species of trichodinids are described together in this work: one of them is *T. nigra* which may occur according to L o m 1961, S t e i n 1968 and our own observations on the *Cyprinus carpio* and *Hypophthalmichthys molitrix,* and the other one presented on the drawing. We will discuss this problem in the further part of our work.

Similarly L j u b a r s k a y a i S t e i n 1967 observed *T. nigra* Lom, 1960 on the fins, on the body surface and, not so commonly, on the gills of young perches and roaches. She found rather considerable morphological differences between them. These differences are shown on the drawings 3 and 4. Unfortunately, the dimensions and other biometrical data were not given for each of these forms apart. On the basis of these drawings we may range the specimens from the perches (L j u b a r s k a y a i S t e i n 1967, fig. 3) to the species *T. nigra*. The specimens collected from the roach we consider as belonging to a separate species (L j u b a r s k a y a i S t e i n 1967, fig. 4).

In our opinion *Trichodina* sp. (? *T. nigra* Lom, 1961): L o m and H o f f-m a n 1964 does not correspond to the species *T. nigra* Lom, 1961 (sensu K a z u b s k i and M i g a ł a in present paper). There are really considerable differences in the aspect of trichodinids, in the shape of denticles and in the biometrical data.

Trichodina domerguei f. *acuta* Lom, 1961 (Pl. IV and V)

This species is very common and occurs sometimes in a great number on the skin of the examined carps.

Trichodina domerguei f. *acuta* has a structure typical of the genus. The adoral zone of cilia forms a spiral of about 400°; its dimensions are 56.2—63.6×56.2—63.6 μ (61.7×61.5 μ). The adhesive disc is large with a wide (about 5 μ) border membrane. The biometrical data are presented in Table 3. The denticles have big blades and distinctly visible rays. The blades have a shape of a very wide sickle (resembling to the moon after the third quarter) with a sharp apex. The length of the rays is close to that one of the blade or somewhat longer, they are relatively wide and sharply pointed. Occasionally the blades and especially the rays may take an odd shape (Pl. V 21). The rays of the denticles are usually directed towards the geometrical center of the adhesive disc but sometimes there are some deviations to the left (more often) (Pl. IV 15,

Table 3
Measurements of *Trichodina domerguei* f. *acuta*

Date	Number of specimens	Diameter of the body	Diameter of the adhesive disc with the border membrane	Diameter of the adhesive disc	Diameter of the denticulate ring	Length of the denticles	Number of the denticles
pond fish culture 1963—1964							
23—24 Apr. 1964	14	75.3—89.0* 81.7**	59.4—70.0 64.6	50.9—59.4 54.4	29.7—36.0 32.2	13.3—17.0 15.0	20—22 21.4
26—29 Apr. 1964	7	56.2—70.0 64.3	47.7—62.5 56.9	39.2—51.9 47.4	26.0—31.3 29.0	12.7—14.8 14.2	18—20 19.0
pond fish culture 1964—1965							
30 June 1964	6	60.4—73.1 65.7	52.5—63.6 55.2	40.8—53.5 44.7	23.8—31.8 26.6	11.7—14.3 12.7	19—20 19.2
3 Aug. 1964	5	58.3—73.1 67.5	49.8—64.7 57.1	40.8—54.1 47.9	21.7—32.3 27.6	12.7—15.4 14.1	17—21 18.4
31 Aug. —2 Sept. 1964	20	64.7—82.7 73.0	51.9—66.8 60.1	43.5—58.3 50.5	22.3—33.9 29.6	12.7—14.8 13.8	18—22 20.5
1 Oct. 1964	20	63.6—89.0 77.7	56.7—70.0 63.8	46.1—57.8 52.6	26.5—35.0 31.8	12.7—15.9 14.6	19—22 20.4
27—28 Oct. 1964	20	77.4—102.3 86.2	65.7—79.0 71.1	56.2—66.8 59.8	31.8—39.7 35.5	14.3—18.0 16.1	19—23 21.4
8—20 Apr. 1965	29	75.3—101.8 86.5	58.3—73.1 65.6	47.7—62.5 54.7	27.6—38.2 32.6	13.8—17.0 15.0	20—22 20.8
1—27 July 1965	6	62.5—89.0 75.3	49.8—71.5 61.2	41.3—60.4 50.7	24.4—36.6 30.6	11.7—16.4 14.2	19—21 20.0

* range, ** mean value

16, V 21) or to the right (Pl. V 25). There are 9—10 radial pins for one denticle. The center of the adhesive disc becomes white when silvered according to Klein's method. The white center is relatively regular and uniform.

The nuclear apparatus is typical of the most *Trichodina*. Ma is horseshoe--shaped with a diameter from 43.7 to 64.5 μ (mean 52.3 μ) and the width from 9.4 to 17.7 μ (12.7 μ). The distance between the two ends of Ma (the distance "x" according to D o g i e l) varies from 4.2—to 18.7 μ (10.0 μ). Mi was invisible in our preparations. The other biometrical data of the individuals of the same sample were as follows: the body diameter 65.5—101.9 μ (79.6 μ), the diameter of the adhesive disc 46.8—65.5 μ (58.3 μ), the diameter of the skeletal ring 29.1—41.6 μ (35.5 μ), the number of denticles 19—23 (21). The measurements were effected on the individuals gathered on October 29, 1964.

T. domerguei f. *acuta* was described by L o m 1961 from the body surface (skin and fins, occasionally gills) of *Cyprinus carpio*, *Perca fluviatilis*, *Lucioperca lucioperca*, *Leucaspius delinatus*, *Rhodeus sericeus*, and on the skin of tadpoles belonging to several species of frogs. The species found by us resembles closely to the species described by L o m. The high seasonal variability only ought to be stressed as observed in our material.

Trichodina mutabilis sp. n. (Pl. VI and VII)

Trinchodinids belonging to this species were commonly present in a relatively large number of specimens on the gills of the carps examined.

The structure of *T. mutabilis* sp. n. is typical. The adoral zone of cilia forms a spiral of about 400°; its dimensions are 47.8—53.0×43.7—51.0 μ (49.7×46.6 μ) in summer, and 59.3—73.8×51.0—67.6 μ (68.2×63.4 μ) in winter specimens. The adhesive disc is large with a wide (about 6 μ) border membrane. The biometrical data are presented in Table 4. The denticles are large with relatively thin centripetal rays. The length of the two elements — the blade and the ray — is very similar. The blade is wide, spade-shaped, slightly curved with a somewhat rounded outward end, rarely falcated. The central part of the denticle is relatively delicate and not very large. The centripetal delicate and pin-shaped rays are slightly curved, directed to the left towards the geometrical center of the adhesive disc. There are 9—10 radial pins for one denticle. The center of the adhesive disc is uniform and dark after the silver impregnation according to Klein.

The nuclear apparatus consits similarly to some other representatives of trichodinids, of the horseshoe-shaped Ma and of a single Mi. The diameter of Ma is 45.0—63.5 μ (mean 55.7 μ), the width is 7.3—13.5 μ (10.5 μ). The distance between the ends of Ma (the distance "x" according D o g i e l) is 8.3—22.9 μ (14.5 μ). Mi is large and fusiform, its length is 9.4—13.5 μ (11.8 μ) and width 2.1—5.2 μ (3.5 μ). Mi is situated outside of Ma in the distance 8.3—25.0 μ (16.7 μ) (the distance "y" according to D o g i e l) from one of its ends. The other biometrical data of the individuals of the same sample are as follows: the body diameter 65.5—105.0 μ (84.8 μ), the diameter of the adhesive disc 52.0—70.7 μ (61.9 μ), the diameter of the skeletal ring 31.2—45.8 μ (37.9 μ), the number of denticles 26—30 (29.15). These measurements were effected on the individuals collected on October 31, 1964.

Table 4
Measurements of *Trichodina mutabilis* sp. n.

Date	Number of specimens	Diameter of the body	Diameter of the adhesive disc with the border membrane	Diameter of the adhesive disc	Diameter of the denticulate ring	Length of the denticles	Number of the denticles
pond fish culture 1963—1964							
26—29 May 1964	20	67.8—86.9* 75.5**	56.2—74.2 66.0	45.6—62.5 53.0	29.7—40.3 34.0	16.4—20.7 18.3	25—30 27.0
11 June 1964	1	—	61.5	47.7	29.7	18.6	25
8 July 1964	20	66.8—82.7 71.2	53.5—68.4 62.5	40.3—54.6 48.9	26.0—33.9 30.4	15.5—19.1 17.8	25—28 26.4
3 Aug. 1964	1	84.8	68.9	56.7	35.0	19.4	26
pond fish culture 1964—1965							
1 July 1964	1	78.4	61.5	49.3	30.2	17.0	24
3 Aug. 1964	20	60.4—79.5 68.8	54.1—66.8 59.3	41.3—54.1 46.4	25.4—33.4 28.8	14.9—18.0 16.2	22—26 24.2
1 Sept. 1964	20	67.8—85.9 75.5	54.1—68.9 60.8	41.3—59.4 48.5	26.0—38.2 29.7	14.8—19.1 16.3	23—27 25.1
1 Oct. 1964	20	71.0—97.5 80.4	62.0—74.2 68.5	48.8—62.5 56.1	29.7—38.7 34.8	17.0—20.1 18.6	27—30 28.8
27 Oct.—2 Nov. 1964	20	68.9—106.0 89.2	59.4—80.6 72.5	46.1—67.8 60.6	29.2—42.9 37.3	17.5—20.1 19.1	27—32 29.6
8—10 Apr. 1965	20	78.4—95.4 85.8	61.5—80.6 71.9	48.8—64.7 58.3	30.7—40.8 36.0	17.0—20.7 18.6	26—33 29.0
23 May 1965	15	65.7—86.9 75.3	58.3—71.0 64.5	45.6—57.8 51.3	28.6—38.2 32.9	15.9—19.1 17.8	25—28 26.9
18 June—1 July	9	63.6—79.5 68.5	57.2—66.8 60.7	43.5—54.1 47.0	27.6—33.4 29.2	15.9—19.1 17.1	24—27 25.5

* range, ** mean value

Fig. 1. *Trichodina mutabilis* sp. n., A — adhesive disc, B — adoral zone of cilia, C and D — nuclear apparatus, E — denticles of the summer specimen and F — denticles of the winter specimen; A — specimen from 3rd August 1964, B — specimen from 27th October 1964, C, D and F — specimens from 31st October 1964, E — specimen from 1st September 1964. Figs. A—D in the same magnification, E and F in the other one

T. mutabilis sp. n. has particularly high seasonal variability. The "winter" specimens (collected in the late autumn or in the early spring) are larger and have more numerous, longer and narrower denticles (Pl. VII) than the summer ones (Pl. VI). This variability gives reason for the name *"mutabilis"* (varied) for the described species.

The problem of the seasonal variability of trichodinids will be discussed in the further parts of this work.

Trichodina mutabilis sp.n. differs distinctly from the other species of *Trichodina* occurring on the carps. First of all it differs from *T. domerguei* f. *acuta* by the absence of the self — non impregnating center of the adhesive disc. It differs from *T. pediculus* and *T. nigra* in the shape of denticles and the size (comp. Pl. I—III, VI, VII and Tabl. 1, 3, 4).

Special attention must be paid to the differentiation of *T. mutabilis* sp.n. and *T. nigra* Lom, 1960 because these two species were confounded (C h e n 1963). The blades of *T. mutabilis* are rather spade-shaped and their position is rather proximal to the direction of the geometrical radius when compared with the falcated and curved blades of *T. nigra*. The rays of *T. mutabilis* are more delicate, have almost the same thickness all over their length and are slightly arched, whereas the rays of *T. nigra* are thicker, contracted towards their end and are ordinarily straight. *T. mutabilis* is larger sized and has higher number of denticles. The diagrams presenting these features from samples, collected at the same time, merely slightly coincide one another. Several differences also occur in the structure of the nuclear apparatus. The macronucleus of *T. mutabilis* is larger and somewhat wider, and the micronucleus lies a little further from one end of Ma as compared with *T. nigra*.

We consider that *T. mutabilis* sp. n. is not identical with any hitherto described species of *Trichodina* occurring on fish. Certainly this statment may be wrong because of the absence of any detailed information concerning many species, moreover the drawings are not precise and give no basis for comparison. It can be stated by now that *T. mutabilis* is the most similar to the form described by L o m 1961 as *T. nigra* f. *cobitis* and fit also the drawing of *T. nigra* in the report of C h e n 1963.

The resemblance of *T. mutabilis* sp. n. to *T. nigra* f. *cobitis* consists mainly on the coincidence of the number of denticles and in a general adequacy of the adhesive disc. Furthermore both these species occur on the gills. However there are some differences between these two species. *T. nigra* f. *cobitis* has somewhat smaller diameter both of the adhesive disc and of the skeletal ring. The dimensions refered by L o m 1961 are smaller than the sizes of our summer population covering the smallest individuals (from the August 3. 1964). The shape of denticles is also inadequate. Comparing our material with the photographs of L o m 1961 it can be stated that *T. mutabilis* has somewhat narrower denticles with a very delicate central part, a thin centripetal ray and a different shape of the centrifugal blade. The blade of *T. mutabilis* is spade-shaped and set along the radius of the adhesive disc when compared to the falciform and strongly curved blade of *T. nigra* f. *cobitis*.

Compared species occur on different species of fishes. *T. mutabilis* has been noted by now merely on *Cyprinus carpio*, however *T. nigra* f. *cobitis* was observed on *Cobitis taenia*. The last one was not present in the experimental ponds at Żabieniec, it occurred rarely in Jeziorka river (R e m b i s z e w s k i 1964).

Taking into account the above mentioned differences we regard both species as distinct ones. Evidently, the definitive solution of this problem would be revealed by the experimental studies based on cross-infections. At the same time probable variability of trichodinids depending on their hosts should be examined.

The problem of *"Trichodina nigra"* was revealed by C h e n 1963 in a different way. The species described by him occurred on the skin and rarely on the gills of *Cyprinus carpio, Hypophthalmichthys molitrix, Ctenopharyngodon idellus* and various frog tadpoles. The range of variability covers, to the present authors knowledge, both species — *T. nigra* and *T. mutabilis* sp. n. It seems more probable that C h e n 1963 considers both species at the same time and that the drawing refers exactly to *T. mutabilis* sp. n. Some features make evident the above suggestion: the shape of denticles (the absence of significant differences when compared with our material), the number of denticles (29) never observed by us in *T. nigra,* but occurring ordinarily in *T. mutabilis* sp. n. This suggestion is confirmed by the incidence of *T. nigra* on the skin of *Hypophthalmichthys molitrix* (S t e i n 1968), corresponding closely in the biometrical respect to our data on this species. However this problem requires additional researches.

The differences among the other species of trichodinids occurring on fishes are more marked. They concern particularly the structure of the denticles but their consideration is not necessary here.

Trichodinella (Foliella) subtilis Lom, 1959 (Pl. VIII and IX)

This species occurs very often and in large quantities on the gills of the examined carps.

The structure of *Trichodinella (Foliella) subtilis* is typical of the genus. The adoral zone of cilia forms a spiral up to 180°. The adhesive disc is small, the border membrane is about 2 μ wide. The biomertical data are presented in Table 5. The denticles are rather small. The blades are straight and falcated. The blade in its proximal part has a triangular outgrowth directed reversely to the clock movement. In the opposite side but somewhat higher a slightly smaller outgrowth is visible. The inner part of the denticle does not occur or is represented by a minute, arched stripling. There are 4—5 radial pins for one denticle. The center of the adhesive disc remains dark in the preparations impregnated according to Klein's method.

The nucelar apparatus is typical. The macronucleus has a horseshoe-shape and the diameter ranges from 14.6 to 23.9 μ (mean 18.8 μ) and its width up to 2.1—5.2 μ (3.9 μ). The distance between the ends of macronucleus (the distance "x" according to D o g i e l) was 3.1—10.4 μ (6.7 μ). Mi was not visible in our preparations.

The other biometrical data for the same sample are as follows: the body diameter 23.9—35.4 μ (28.9 μ), the diameter of the adhesive disc 14.6—21.8 μ (19.2 μ). The skeletal ring in this series of our preparations was slightly visible. When the measurements of the diameter succeeded, it varied between 8.3—12.5 μ (man 10.2 μ), the number of denticles was not determined. The measurements were effected on the individuals collected on October 31, 1967.

Measurements of *Trichodinella subtilis*

Date	Number of specimens	Diameter of the body	Diameter of the adhesive disc with the border membrane	Diameter of the adhesive disc	Diameter of the denticulate ring	Length of the denticles	Number of the denticles
pond fish culture 1963—1964							
23—24 Apr. 1964	20	24.4—36.0* / 30.0**	19.0—27.6 / 23.2	15.9—22.3 / 19.0	9.2—12.7 / 10.9	4.2—6.4 / 4.9	20—24 / 22.2
26 May 1964	20	26.5—37.1 / 32.6	21.2—26.0 / 23.3	17.0—22.3 / 18.9	9.5—12.2 / 11.0	4.2—5.3 / 5.2	19—25 / 21.6
11 June 1964	1	—	21.7	17.5	11.1	4.8	20
8 July 1964	20	26.5—36.0 / 31.5	22.3—27.6 / 25.6	17.0—23.3 / 20.4	9.5—13.8 / 11.6	4.2—5.8 / 5.1	20—25 / 22.8
3 Aug. 1964	20	26.5—38.2 / 31.8	20.1—28.6 / 24.6	15.9—23.3 / 19.5	9.5—13.8 / 11.1	4.2—5.8 / 5.0	19—24 / 21.7
8 Sept. 1964	1	41.3	28.6	24.4	12.7	5.3	22
2—13 Oct. 1964	4	31.8—36.0 / 34.3	21.2—26.5 / 24.4	18.0—22.3 / 20.4	10.1—12.2 / 11.1	4.2—6.4 / 5.4	20—22 / 21.0
pond fish culture 1964—1965							
3 Aug. 1964	20	26.5—36.0 / 30.5	18.5—27.6 / 22.9	15.4—23.3 / 18.9	7.9—14.3 / 10.6	4.2—6.4 / 5.0	19—23 / 21.0
1. Sept. 1964	20	23.3—40.3 / 31.9	19.1—28.6 / 23.8	15.4—23.8 / 19.4	8.5—13.2 / 11.1	4.2—5.8 / 5.2	18—24 / 22.3
1—2 Oct. 1964	20	26.5—39.2 / 33.1	20.1—28.1 / 24.9	15.9—22.8 / 19.9	8.5—13.8 / 11.6	4.2—6.4 / 5.2	21—25 / 23.3
28—31 Oct. 1964	20	27.6—39.8 / 33.2	21.2—28.6 / 24.7	17.0—23.3 / 20.0	10.1—14.8 / 11.7	4.2—5.8 / 5.0	22—25 / 23.4
8 Apr. 1965	20	23.8—36.0 / 31.0	18.5—26.0 / 23.2	14.8—21.2 / 18.8	9.0—12.7 / 10.8	4.2—5.3 / 4.9	19—25 / 21.8
23 May 1965	5	26.5—30.7 / 27.9	21.2—24.9 / 23.4	17.5—22.3 / 19.8	9.5—12.2 / 11.1	4.8—5.8 / 5.3	20—22 / 20.8
18 June 1965	1	32.8	24.4	20.1	10.6	5.3	21
1 July 1965	20	25.4—39.2 / 30.7	19.1—27.6 / 22.4	14.8—23.3 / 18.4	8.5—13.2 / 10.5	4.2—5.8 / 4.8	19—23 / 20.7
11.Aug. 1965	20	25.4—35.0 / 29.3	20.1—27.6 / 23.8	15.2—23.3 / 19.5	9.5—12.7 / 11.1	4.2—5.8 / 5.2	19—23 / 20.9
10 Sept. 1965	20	26.5—37.1 / 31.5	20.1—26.5 / 24.0	13.3—22.3 / 19.5	9.5—12.7 / 11.2	3.7—5.3 / 5.0	19—26 / 21.8
27 Sept. 1965	9	25.4—38.2 / 32.5	21.2—27.6 / 24.7	17.0—23.3 / 20.4	9.5—13.3 / 11.5	4.2—5.8 / 5.1	22—28 / 24.4
23—30 Oct. 1965	20	26.5—42.4 / 33.8	22.3—29.2 / 25.8	17.0—24.4 / 21.2	10.1—14.8 / 12.6	4.2—6.4 / 5.0	21—29 / 24.2

* range, ** mean value

Trichodinella (Foliella) subtilis was described by L o m 1959 from the gills
of the crucian carp (*Carassius carassius*) found in the surroundings of Prague.
In his next work L o m mentions this species from the gills of *Cyprinus
carpio, Carassius carassius, Tinca tinca, Rhodeus sericeus* and *Blicca bjoerkna*,
then he refers further details of the structure of these ciliates and corrects
some details of the description concerning the skeletal ring. In both mentioned
papers, L o m relates also the species *Trichodinella epizootica* which occurs
on *Perca fluviatilis, Acerina cernua, Esox lucius, Lota lota* and other species
of fishes. However in these papers he does not review extensively the work
of R a a b e 1950, in which the species *Trichodinella epizootica* was described
from the gills of *Carassius carassius, Esox lucius* and *Cyprinus carpio* from
the region of Lublin and of *Scardinus erythrophtalmus* from Balaton Lake
(Hungary). It is evident from the above mentioned account that two different
species of the genus *Trichodinella* occur in *Carassius carassius* and *Cyprinus
carpio* on the one hand and in *Esox lucius* on the other one. This statement
gave reason for a view in literature that *Trichodinella epizootica* occurs in
Cyprinus carpio (S t e i n 1962, H a i d e r 1964); this might suggest that on
this species of fish two species of *Trichodinella* occured simultaneously.

The examination of our material from the carps and other species of fishes
led to the conclusion that on the examined carps occured only one species
of *Trichodinella* coresponding to *Trichodinella (Foliella) subtilis* Lom, differing
from the species found on *Perca fluviatilis* and *Esox lucius* — *Trichodinella
epizootica* (Raabe, 1950) sensu L o m. Both species are very similar. However,
if there are some comparable materials they can be easily differentiated. We
confirm Lom's view on this subject.

The discussion concerning *T. carassii* must be postponed to the additional
examination of D o g i e l' s material and eventual studies on *Trichodinella*
from the crucians.

Remarks on the seasonal variability
of trichodinids from carps

During the examination of collected trichodinids the authors paid attention
to the interesting problem of seasonal variability. This phenomenon, previously
reported (K a z u b s k i and M i g a ł a 1967), has not been observed till now
in this group of ciliates.

Significant differences were observed in the size and number of denticles
and even in their shape between the "summer" and "winter" individuals.
This last group covered as well the samples collected in the late autumn and
those collected in early spring, just after the disappearance of ice.

The materials revealing the seasonal variability are presented in Tables
2—5, in which the basic biometrical data are gathered. Moreover the variability
in the diameter of the skeletal ring and the number of denticles are presented
on the Diagrams (Figs. 2—5).

Among three examined species — *Trichodina nigra, T. domerguei* f. *acuta*
and *T. mutabilis* sp.n., the smallest individuals occur in summer, the larger
ones in winter. These differences are considerable as it is distinctly visible on
the Diagrams; they amounted to or even exceed by 1/3 the mean size of the
summer specimens. These dimensions have an intermediate character in the
transition periods.
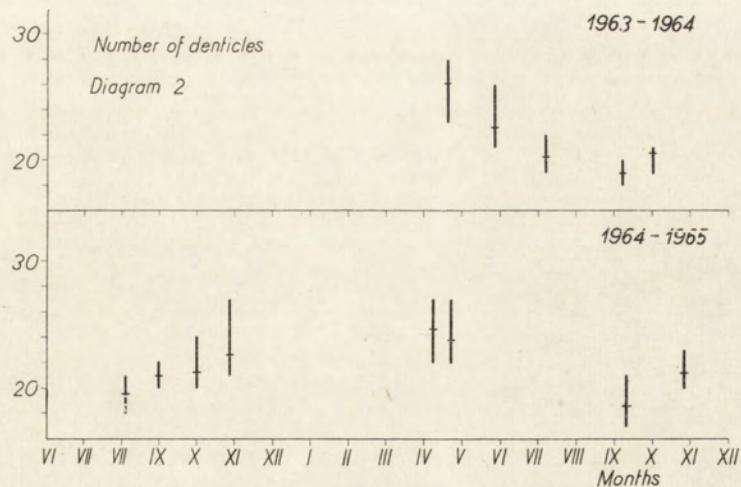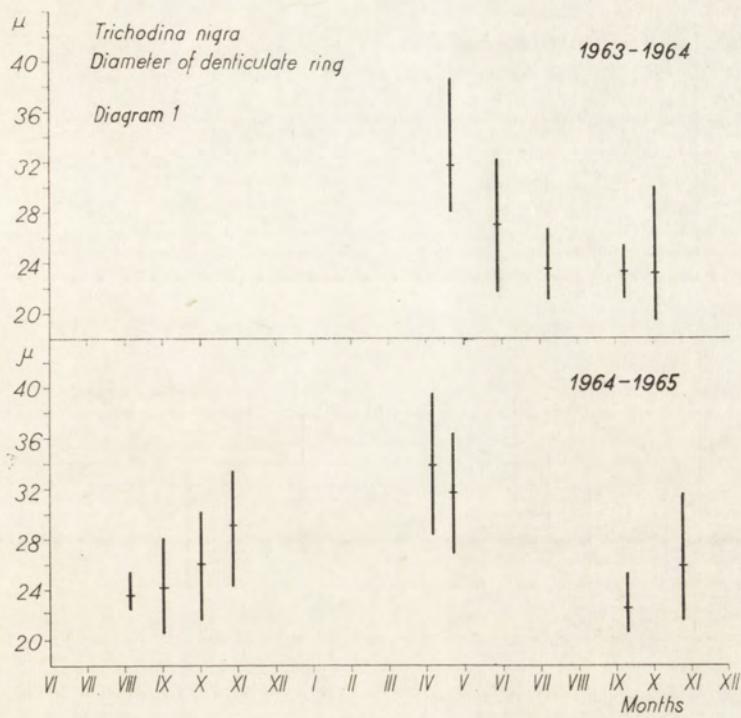
Fig. 2. *Trichodina nigra*, Diagram 1 — variability of the diameter of the denticulate
ring, and Diagram 2 — variability of the number of the denticles
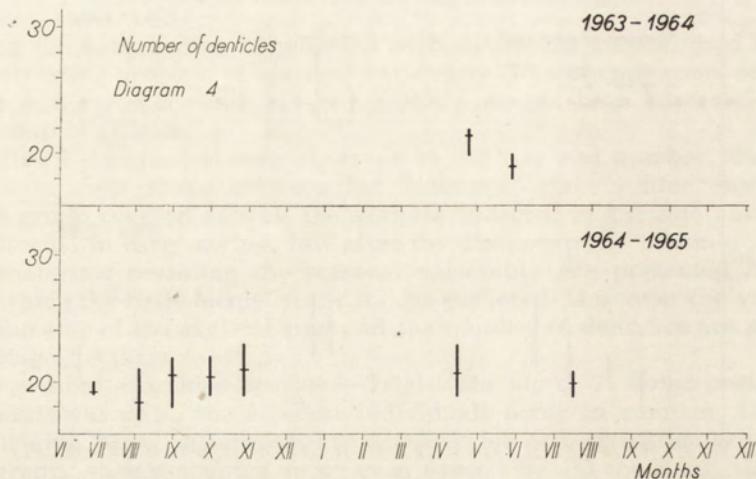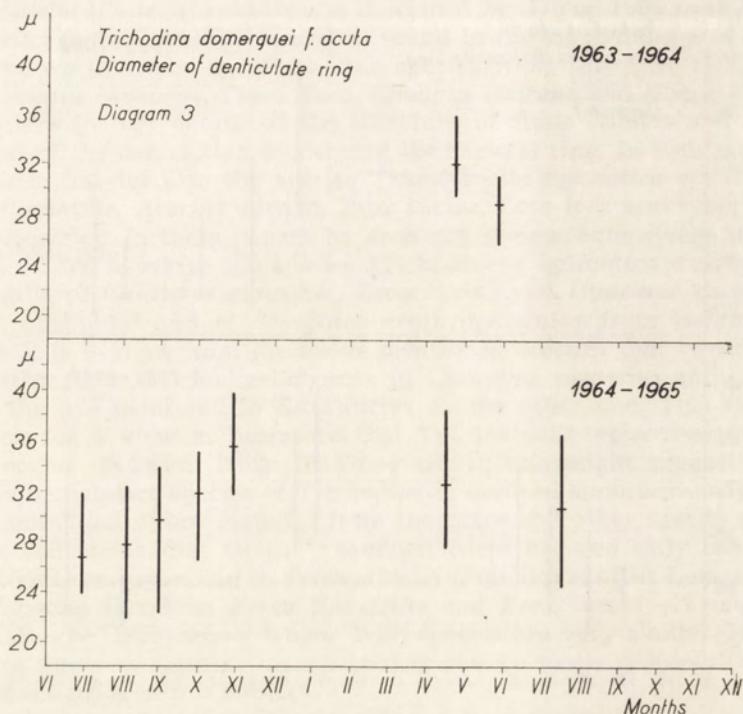
Fig. 3. *Trichodina domerguei* f. *acuta*, Diagram 3 — variability of the diameter of the denticulate ring, and Diagram 4 — variability of the number of the denticles
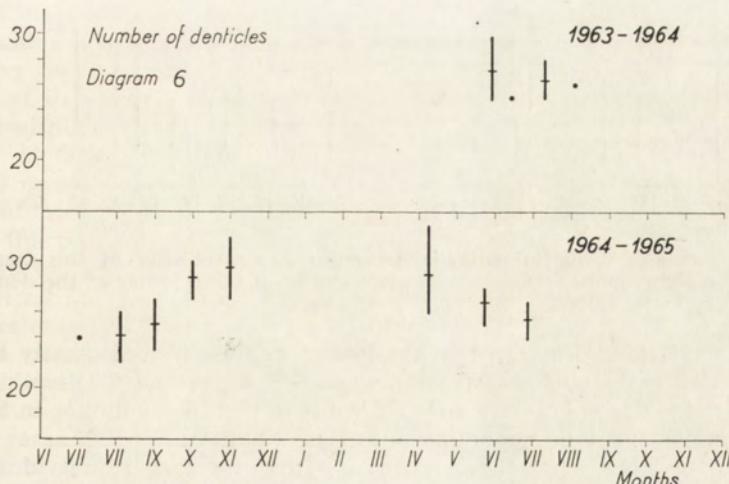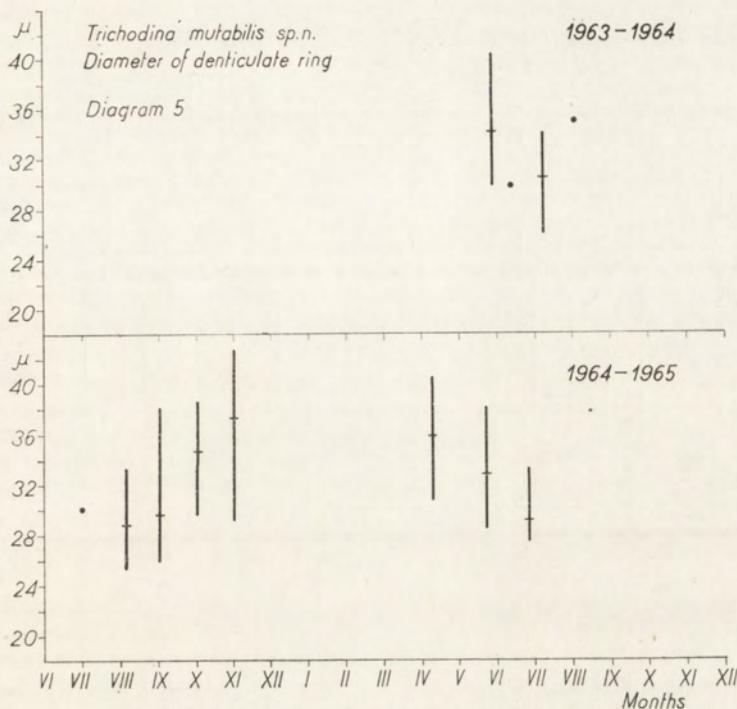
Fig. 4. *Trichodina mutabilis* sp. n., Diagram 5 — variability of the diameter of the denticulate ring, and Diagram 6 — variability of the number of the denticles
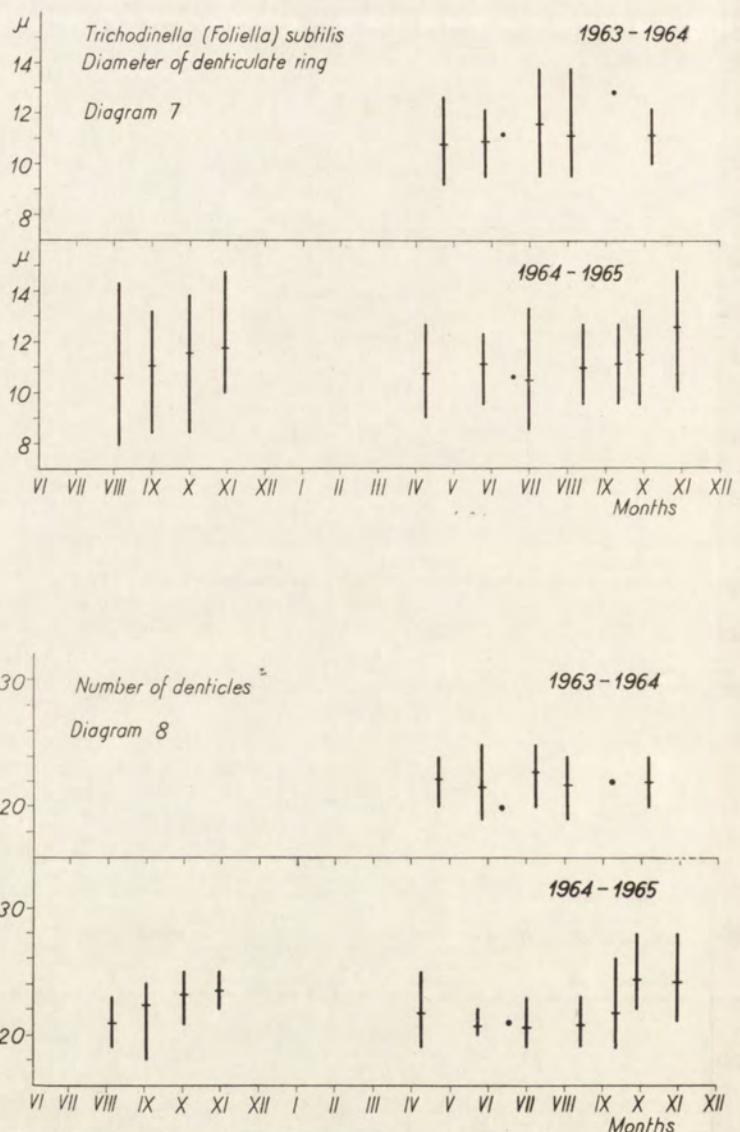
Fig. 5. *Trichodinella* (*Foliella*) *subtilis*, Diagram 7 — variability of the diameter of the denticulate ring, and Diagram 8 — variability of the number of the denticles

Similar variations occur in the number of denticles; occasionally they are even more distinctly outlined. In the case of *T. nigra* and *T. mutabilis* sp.n. there is no coincidence between the Diagrams of denticles number in the most varying summer and winter populations (Figs. 2 and 4).

It should be noted that there is no rule that the larger individuals have always a higher number of denticles than their mean number and that the smaller ones have regularly a smaller number.

Besides the described changes concerning the quantity, several quality changes are observed, particularly the changes in the shape of denticles. The winter individuals have longer and somewhat narrower denticles in comparison with the summer ones. These differences are very significant in *T. mutabilis* sp.n. (Pl. VI, VII) in which there are differences in the shape of blades. These are long and relatively angular in the winter specimens in comparison with arched and a little rounded blades of the summer individuals. Relatively slight differences are also seen in *T. nigra* (Pl. II, III); there are not visible in *T. domerguei* f. *acuta*. The trichodinids of the species in point do not show considerable differences and are very similar to each other concerning their size, shape and number of denticles, besides the difference between the summer and winter individuals in every group.

The fourth of the examined species *Trichodinella (Foliella) subtilis* reveals slight seasonal variability (Fig. 5). The range of this variability concerning the skeletal ring is rather nonuniform, presumably for the reason of an error in the measurements caused by the small size of ciliates. However the increase of mean values, especially in the material collected from the culture during the season 1964—1965, is observed, starting in summer and lasting till the late autumn. On the other hand the increase in the number of denticles is more distinctly outlined.

In this way the seasonal variability is noted to larger or smaller extent in the four species occurring in mass on the carps (*Cyprinus carpio*). Also several provisional data from other *Trichodina* species occurring on other fish reveal the same properties. Several examined populations of *Trichodina pediculus* (K a z u b s k i 1967) show a similar tendency. The possibility of the existence of that seasonal variability is then suggested.

The observations of L a i r d 1953 ought to be mentioned here. This author examined *Trichodina parabranchicola* Laird, 1953 and *T. multidens* Laird, 1953 and he observed a high correlation between the diameter of the skeletal ring and the number of denticles (L a i r d 1953, text-fig. 1). The author stressed that the material used in the investigation was collected over the whole year. The data indicated that in L a i r d' s maerial also occurred the seasonal variability in the diameter of the skeletal ring and in the number of denticles — et least these data did not exclude this variability.

It may be suggested that the seasonal variability concerning the size and number of denticles and sometimes even their shape is very common among the trichodinids.

What is then the mechanism and the reason of this variability? It seems that this mechanism is very simple. Taking into consideration the permanent growth of trichodinids, the fission occurs somewhat later or somewhat earlier than in the paternal individuals. In connection with it the new skeletal ring is formed on a somewhat larger or somewhat smaller circumference than in the paternal individuals. This in turn may cause the increase of the diameter of the skeletal ring and in consequence the increase of the number of denticles. It seems possible that this phenomenon may have a wider ground, because occasionally the purely quantity changes are followed by quality changes, e.g. by the changes in the shape of the denticles in *T. mutabilis* sp.n.

The reason for the occurrence of seasonal variability remain an open question. It seems possible that the temperature may affect the rate of fission. However this statement requires addtional confirmation by means of experimental studies.

3*

Summary

Trichodinids occurring on the breeding carp, *Cyprinus carpio* L., from the ponds of the Department of Fish Culture at Żabieniec near Warsaw were described. Single individuals of *Trichodina pediculus* were found on the body surface and on the gills. *Trichodina nigra* and *T. domerguei* f. *acuta* were found on the skin, and *T. mutabilis* sp. n., and *Trichodinella (Foliella) subtilis* occurred in large quantity on the gills.

The seasonal variability was described as occurring in four examined species of trichodinids. The specimens of *T. nigra, T. domerguei* f. *acuta,* and *T. mutabilis* sp. n. collected in the late autumn or early spring were larger and had higher number of the denticles. A slight variability in the shape of the denticles occurred in *T. mutabilis* sp. n. and *T. nigra.* The seasonal variability in *Trichodinella (Foliella) subtilis* was slightly outlined.

The probable mechanism and the reasons for the described variability of trichodinas were discussed.

STRESZCZENIE

Opisano trichodiny występujące u karpi *Cyprinus carpio* L. w stawach Zakładu Gospodarki Stawowej Żabieniec koło Warszawy. Stwierdzono pojedyńcze okazy *Trichodina pediculus* na powierzchni ciała i na skrzelach, oraz w dużych ilościach *T. nigra* i *T. domerguei* f. *acuta* na skórze oraz *T. mutabilis* sp. n. i *Trichodinella (Foliella) subtilis* na skrzelach.

Opisano zmienność sezonową u czterech badanych gatunków trichodin. U *Trichodina nigra, T. domerguei* f. *acuta* i *T. mutabilis* sp. n. osobniki zbierane późną jesienią lub wczesną wiosną (tzw. zimowe) miały większe wymiary i liczbę haków. U *T. mutabilis* sp. n., a także choć w mniejszym stopniu o *T. nigra,* występowała także zmienność w kształcie haków. U *Trichodinella (Foliella) subtilis* zmiany sezonowe wyrażone były słabo.

Został przedyskutowany prawdopodobny mechanizm i przyczyny opisanej zmienności sezonowej trichodin.

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EXPLANATION OF PLATES I—IX

Trichodina from carp — *Cyprinus carpio* L. from Żabieniec

Trichodina pediculus Ehrbg.

1: Specimen from 8th Apr. 1965 (from skin) with 28 denticles
2: Specimen from 8th Apr. 1965 (from gills) with 29 denticles

Trichodina nigra Lom

The summer specimens

3—5: Specimens from 8th July 1964
3: Minimal diameter of the denticulate ring and minimal number of the denticles
4: Diameter of the denticulate ring near to the maximum and maximal number of the denticles
5: Diameter of the denticulate ring insignificantly bigger than the median and the number of the denticles insignificantly smaller than the median
6—7: Specimens from 3rd Aug. 1964
6: Minimal diameter of the denticulate ring and maximal number of the denticles
7: Diameter of the denticulate ring near to the minimum and number of the denticles near to the median
8: Specimen from 10th Sept. 1965; diameter of the denticulate ring near to the minimum and number of the denticles smaller then the median

The winter specimens

9: Specimen from 20th Apr. 1964; diameter of the denticulate ring near to the maximum and number of the denticles smaller then the median
10—11: Specimens from 27th Oct. 1964
10: Diameter of the denticulate ring bigger then the median and number of the denticles insignificantly bigger then the median
11: Diameter of the denticulate ring insignificantly smaller then the median and number of the denticles bigger then the median
12—14: Specimens from 8th Apr. 1965
12: Diameter of the denticulate ring smaller then the median and number of the denticles bigger then the median
13: Diameter of the denticulate ring equal to the median and maximal number of the denticles
14: Diameter of the denticulate ring near to the maximum and number of the denticles insignificantly bigger then the median

Trichodina domerguei f. *acuta* Lom

The summer specimens

15—16: Specimens from 29th May 1964
15: Minimal diameter of the denticulate ring and minimal number of the denticles
16: Maximal diameter of the denticulate ring and maximal number of the denticles
17—20: Specimens from 30 June 1964
17: Minimal diameter of the denticulate ring and minimal number of the denticles
18: Diameter of the denticulate ring insignificantly bigger then the minimum and minimal number of the denticles
19: Diameter of the denticulate ring a little smaller then the median and maximal number of the denticles
20: Diameter of the denticulate ring a little bigger then the median and minimal number of the denticles

The winter specimens

21—23: Specimens from 27th Oct. 1964
21: Diameter of the denticulate ring smaller then the median and minimal number of the denticles

22: Diameter of the denticulate ring bigger then the median and number of the denticles smaller then the median

23: Maximal diameter of the denticulate ring and maximal number of the denticles

24—26: Specimens from 10th Apr. 1965

24: Diameter of the denticulate ring and number of the denticles approximately in the center between median and minimum

25: Diameter of the denticulate ring bigger then the median and number of the denticles insignificantly bigger then the median

26: Diameter of the denticulate ring and number of the denticles approximately in the center between median and maximum

Trichodina mutabilis sp. n.

The summer specimens

27—30: Specimes from 3rd Aug. 1964

27: Minimal diameter of the denticulate ring and number of the denticles approximately in the center between median and minimum

28: Diameter of the denticulate ring a little bigger then the minimum and number of the denticles approximately in the center between median and minimum

29: Diameter of the denticulate ring a little smaller then the median and number of the denticles approximately in the center between median and minimum

30: Diameter of the denticulate ring and number of the denticles approximately in the center between median and maximum

31—32: Specimens from 1st July 1965

31: Minimal diameter of the denticulate ring and number of the denticles a little smaller then the median

32: Maximal diameter of the denticulate ring and number of the denticles a little bigger then the median

The winter specimens

33—35: Specimens from 27th Oct. 1964

33: Diameter of the denticulate ring smaller then the median and maximal number of the denticles

34: Diameter of the denticulate ring bigger then the median and maximal number of the denticles

35: Diameter of the denticulate ring near to the maximum and number of the denticles approximately in the center between median and maximum

36—38: Specimens from 8th Apr. 1965

36: Minimal diameter of the denticulate ring and minimal number of the denticles

37: Median diameter of the denticulate ring and minimal number of the denticles

38: Diameter of the denticulate ring bigger then the median and number of the denticles approximately in the center between median and maximum

Trichodinella (Foliella) subtilis Lom

The summer specimens

39—40: Specimens from 3rd Aug. 1964 (fishes in the second year of life)

39: Minimal diameter of the denticulate ring and minimal number of the denticles

40: Maximal diameter of the denticulate ring and maximal number of the denticles

41—43: Specimens from 3rd Aug. 1964 (fishes in the first year of life)

41: Diameter of the denticulate ring and number of the denticles approximately in the center between minimum and median

42: Diameter of the denticulate ring a little smaller then the median and minimal number of the denticles

43: Diameter of the denticulate ring approximately in the center between median and maximum; and maximal number of the denticles

44: Specimen from 1st July 1965, diameter of the denticulate ring near to the maximum and maximal number of the denticles

The winter specimens

45—46: Specimens from 23rd Apr. 1964

45: Diameter of the denticulate ring approximately in the center between median and minimum, and maximal number of the denticles

46: Diameter of the denticulate ring near to the maximum and maximal number of the denticles

47—48: Specimens from 8th Apr. 1965

47: Minimal diameter of the denticulate ring and minimal number of the denticles

48: Diameter of the denticulate ring near to the maximum and number of the denticulate ring insignificantly bigger then the median

49—50: Specimens from 30th Oct. 1965

49: Diameter of the denticulate ring insignificantly smaller then the median and number of the denticles smaller then the median

50: Diameter of the denticulate ring near to the maximum and number of the denticles near to the maximum

Microphotographs 1—38 in the magnification 1000×, 39—50 in the magnification 2000×
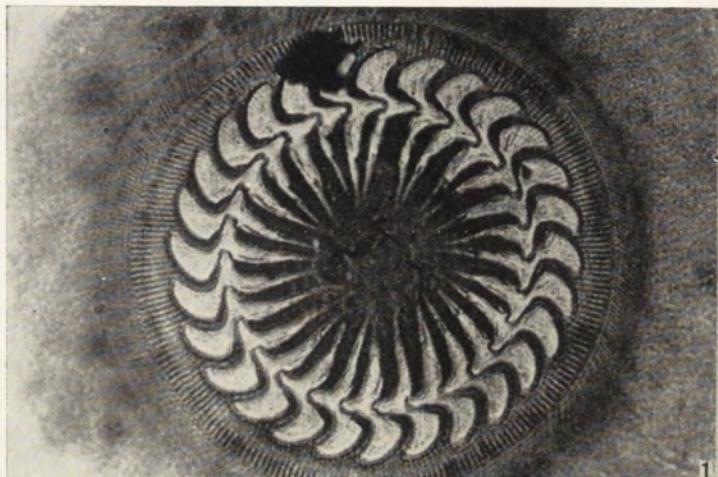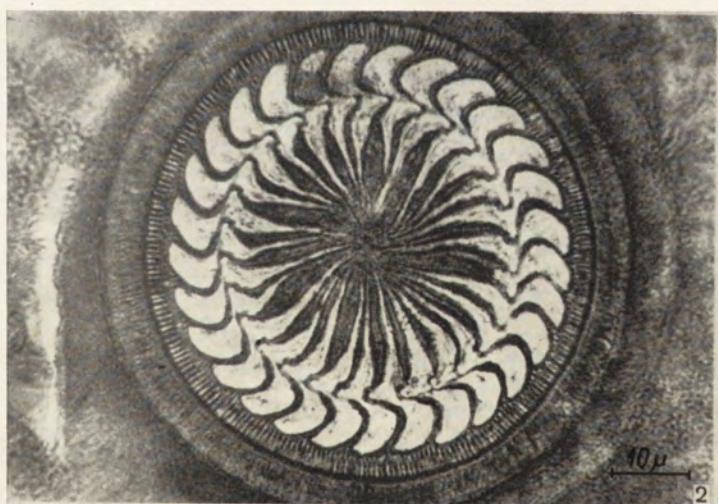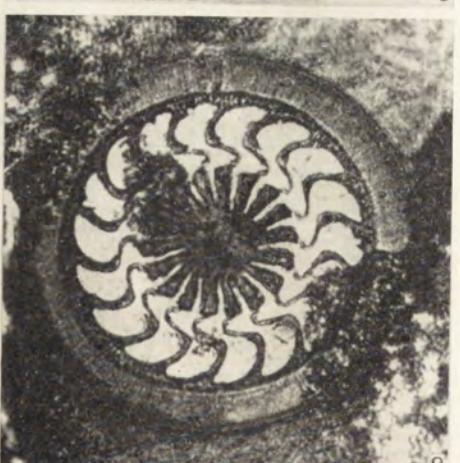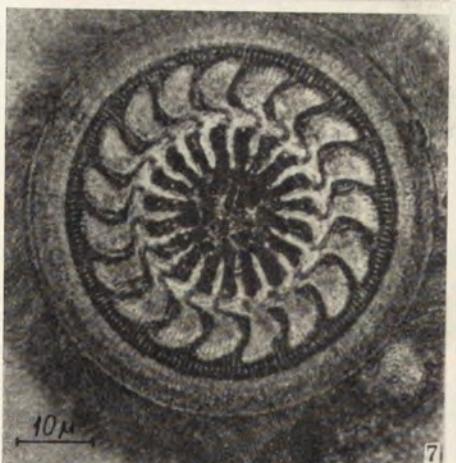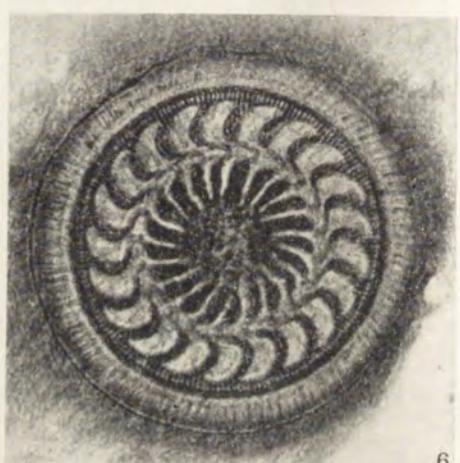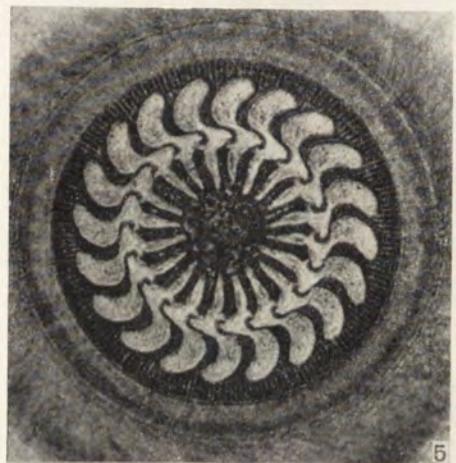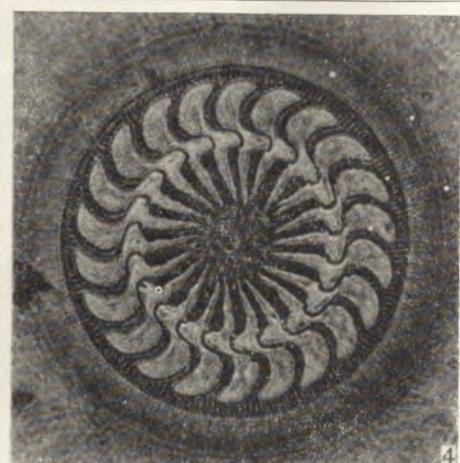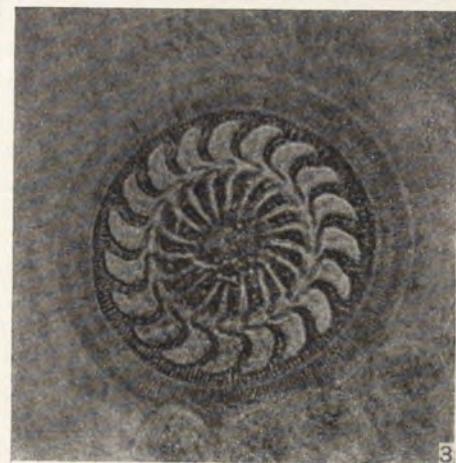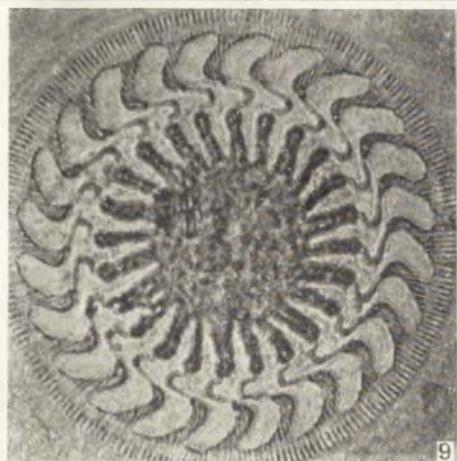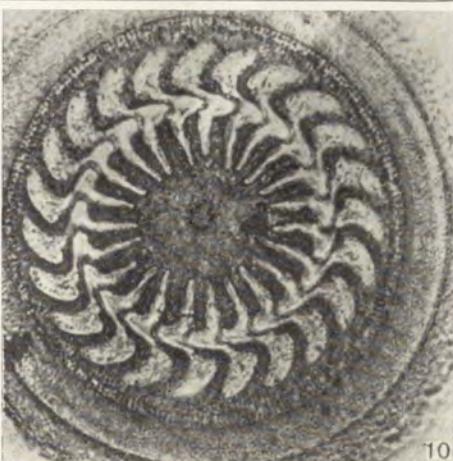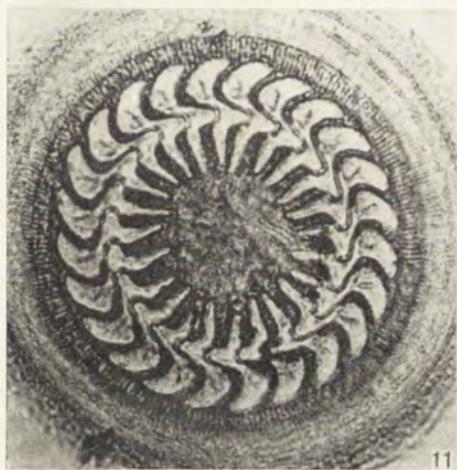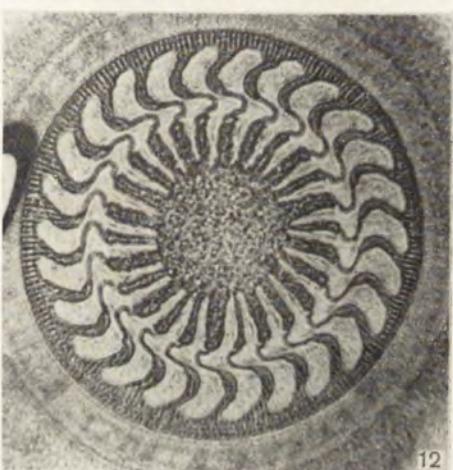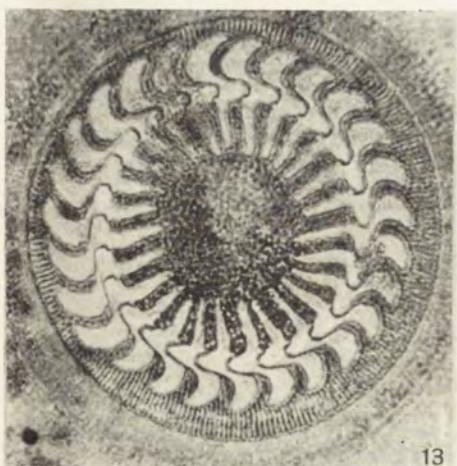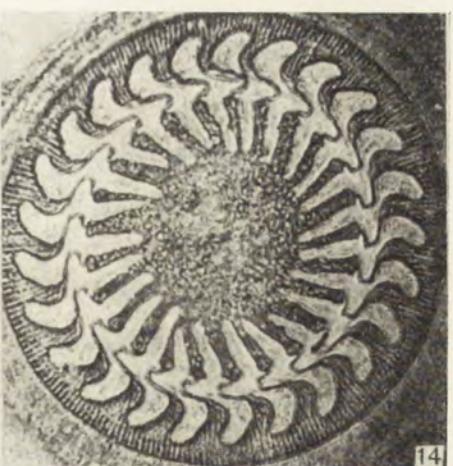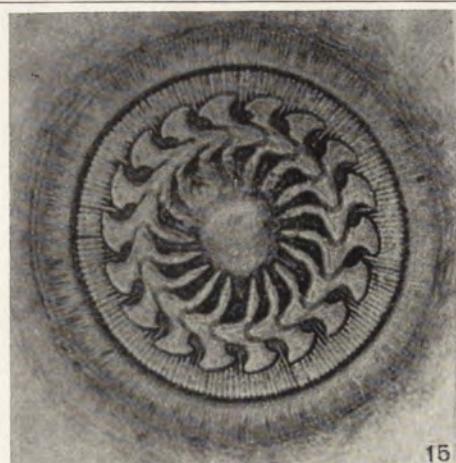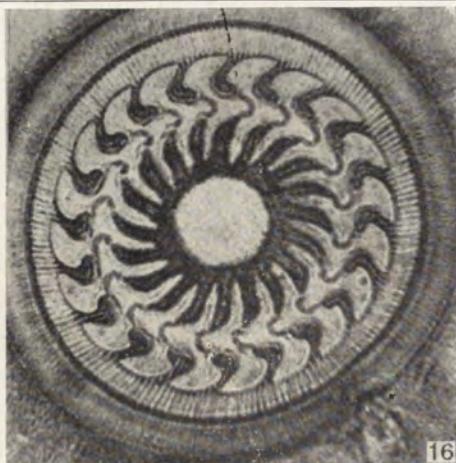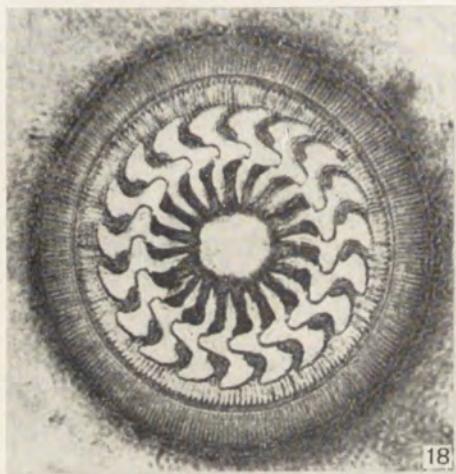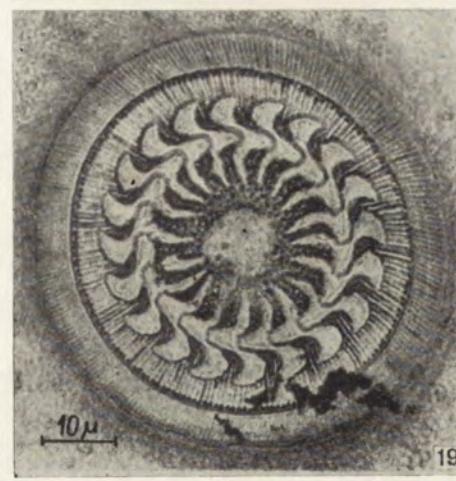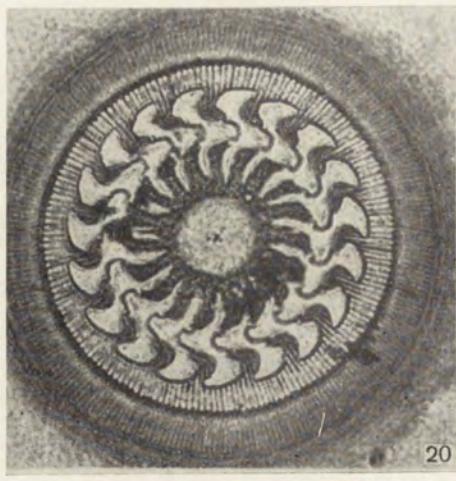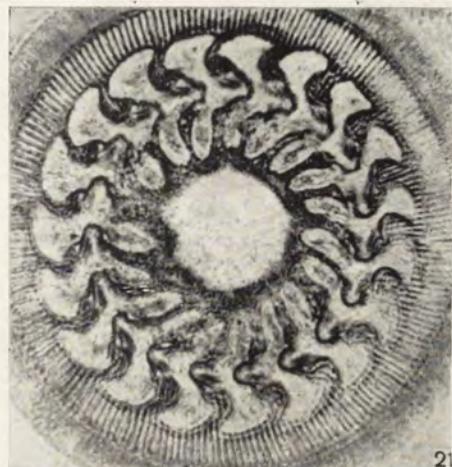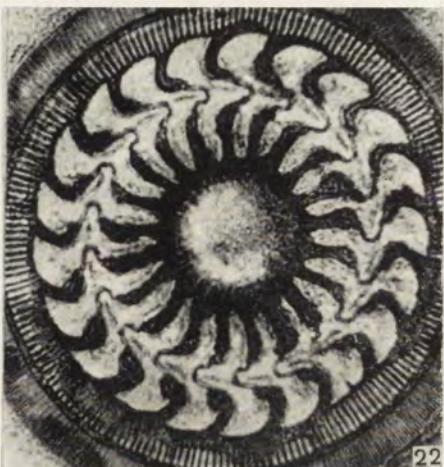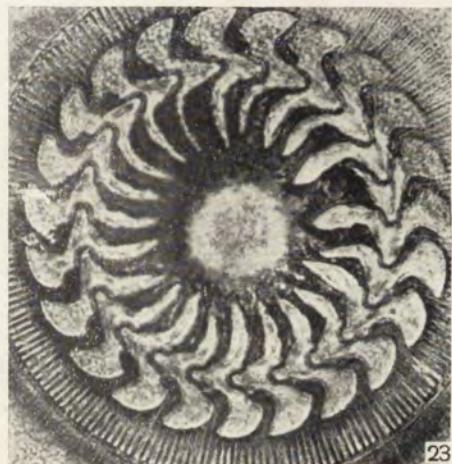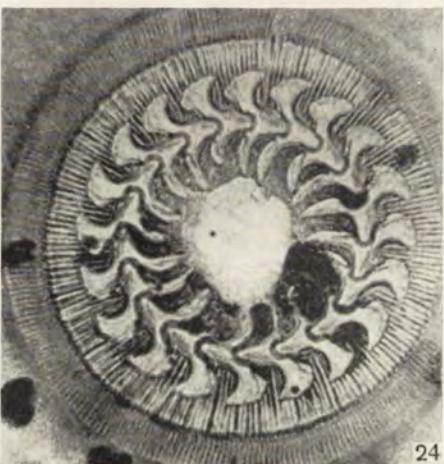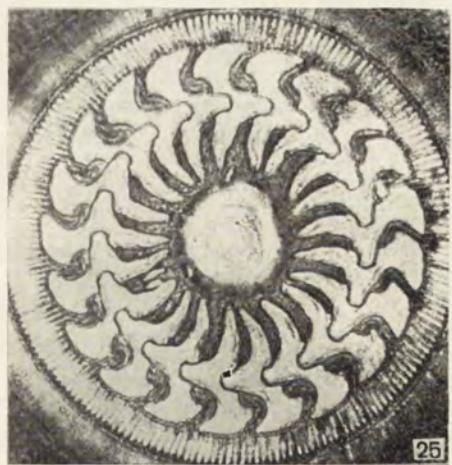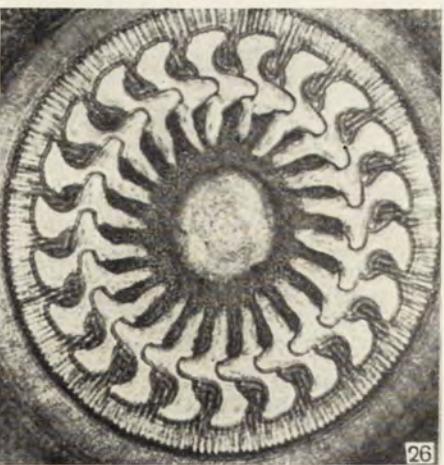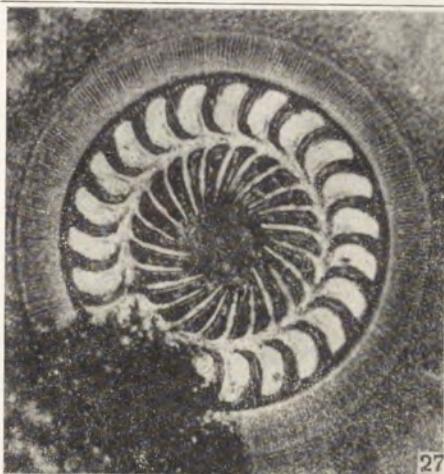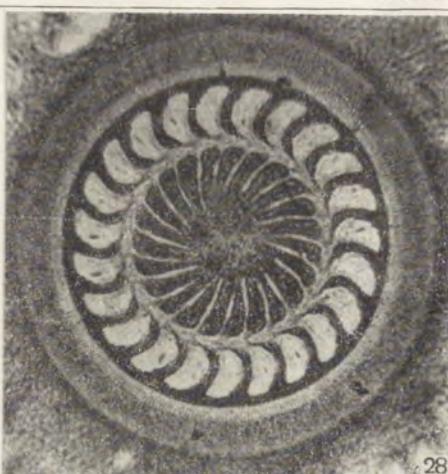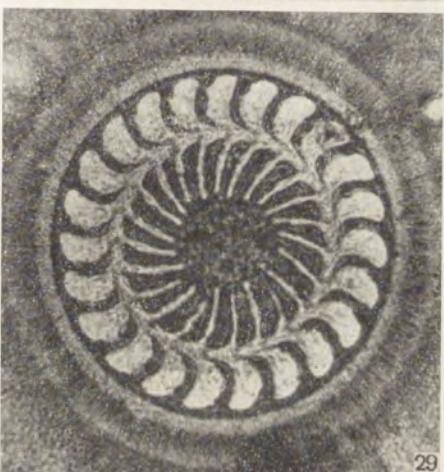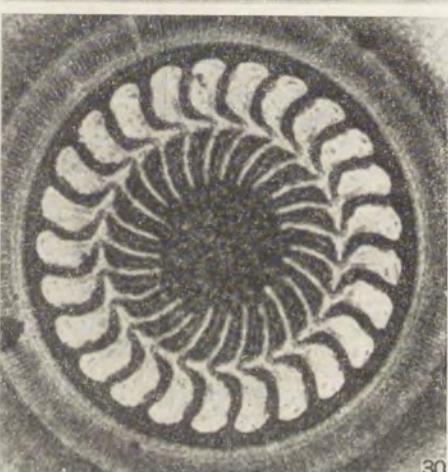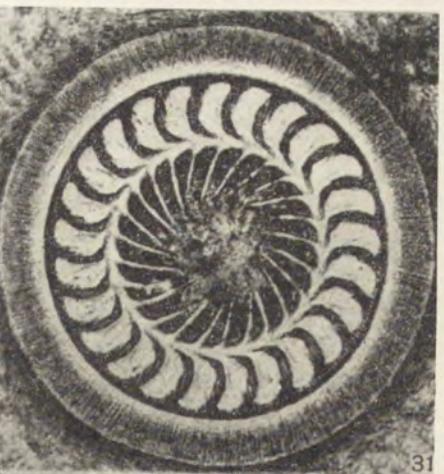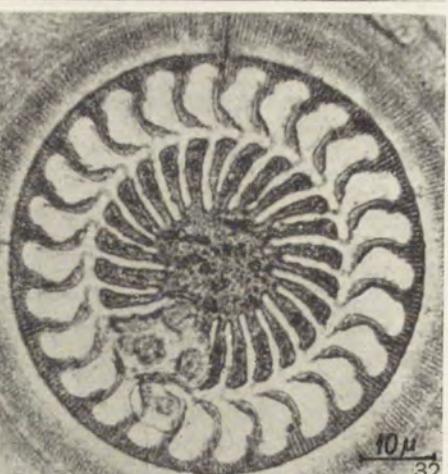
S. L. Kazubski et K. Migała

auctores phot.

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PLATE IV

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auctores phot.

PLATE V

S. L. Kazubski et K. Migała auctores phot.

PLATE VI

S. L. Kazubski et K. Migała

auctores phot.

S. L. Kazubski et K. Migała   auctores phot.

PLATE VIII
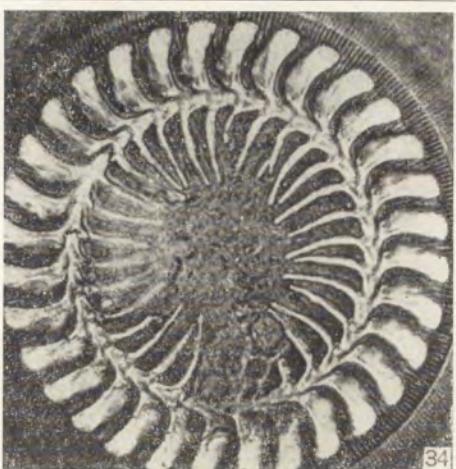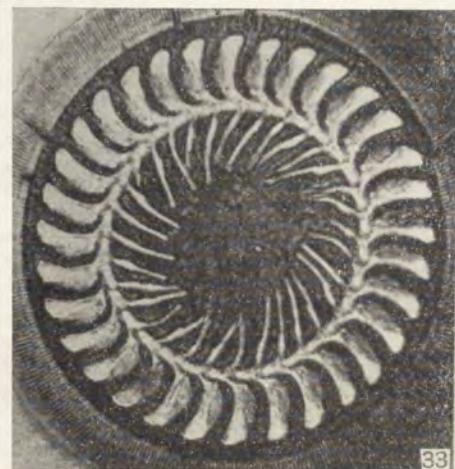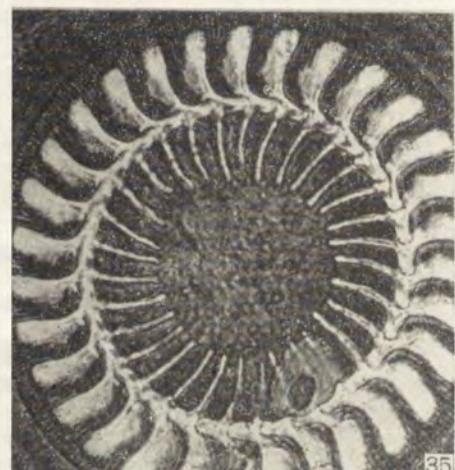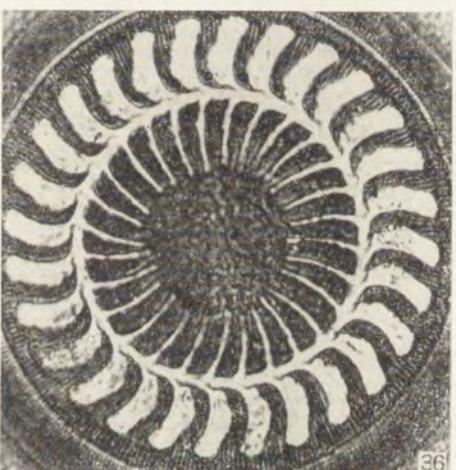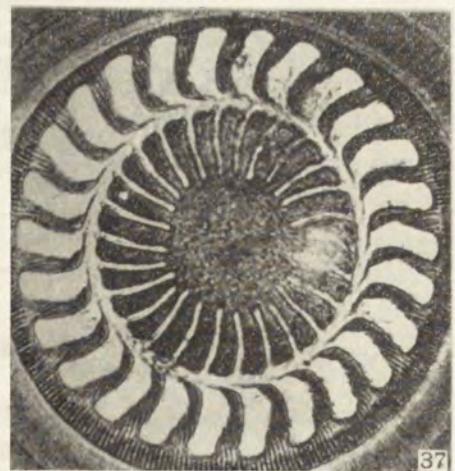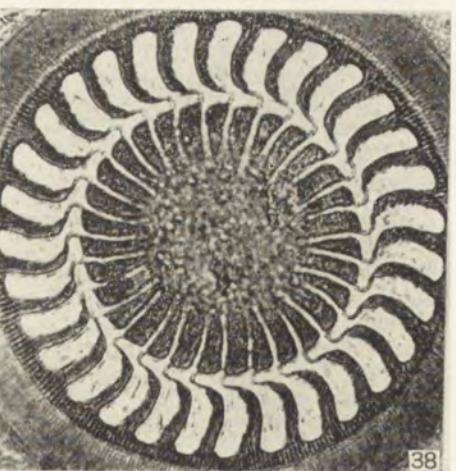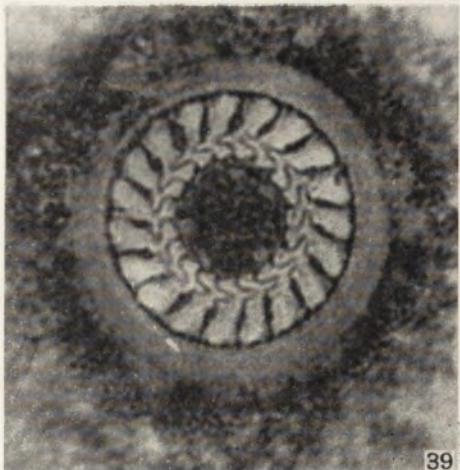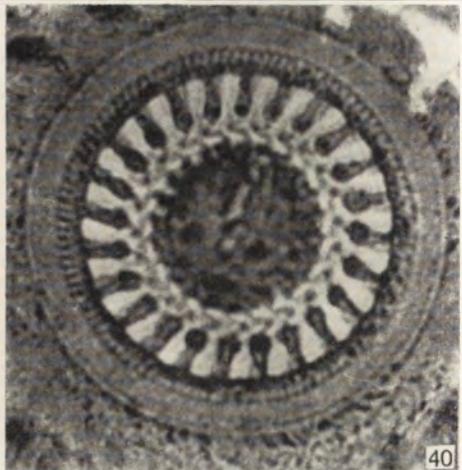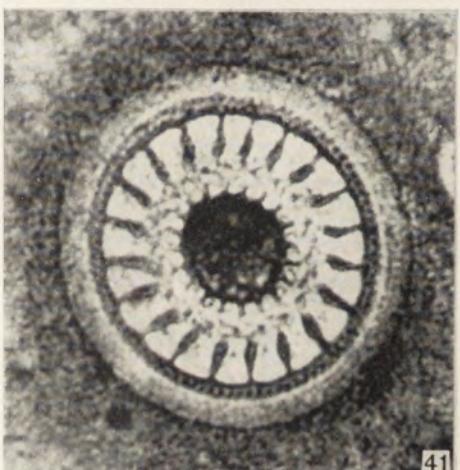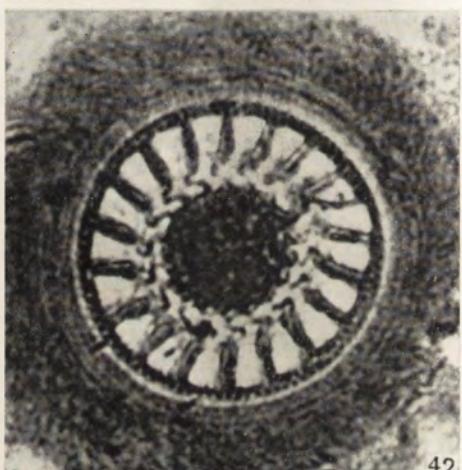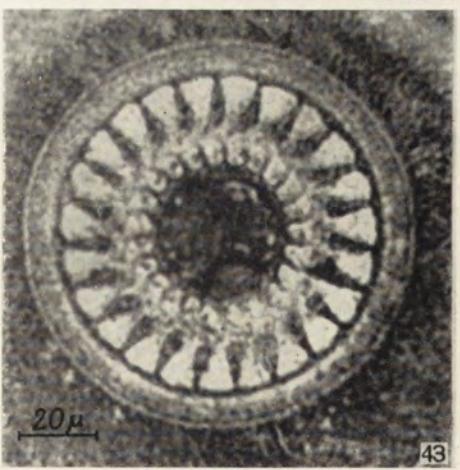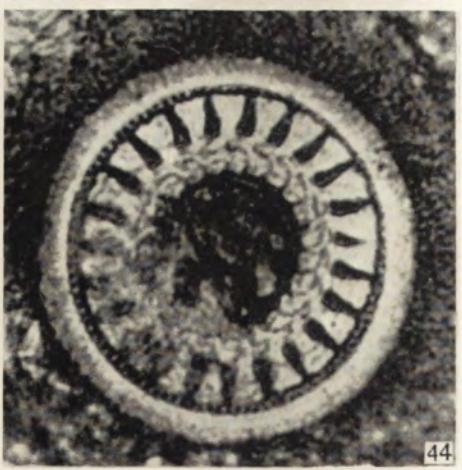
S. L. Kazubski et K. Migała

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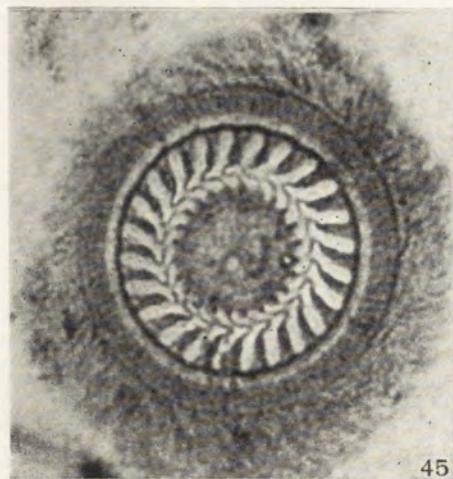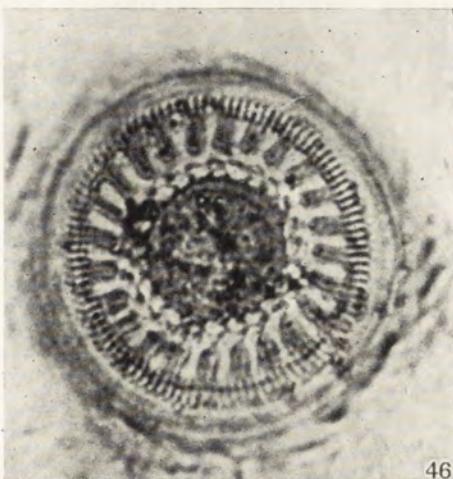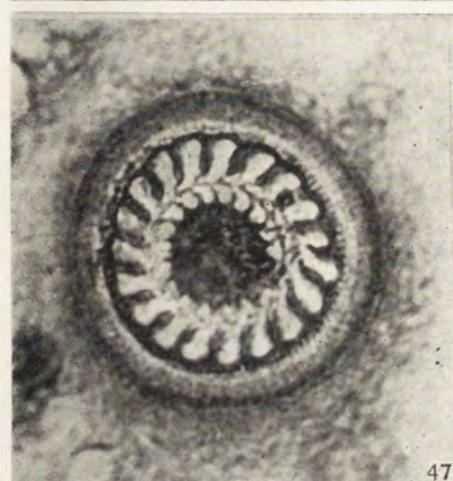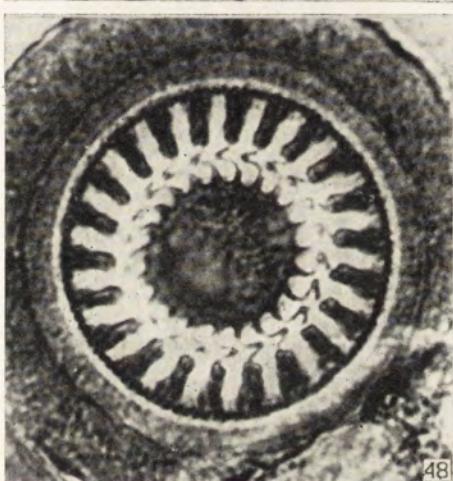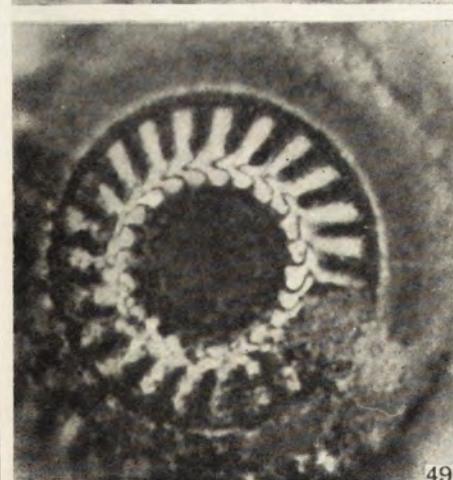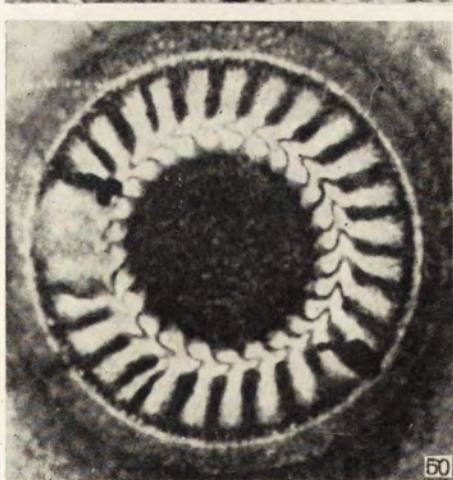
O. N. BORCHSENIUS and D. V. OSSIPOV

Polymorphism of micronuclei of *Paramecium caudatum*. II. Mitotical cycles of micronuclei of different morphological types

Полиморфизм микронуклеусов *Paramecium caudatum*.
II. Митотические циклы микронуклеусов разных морфологических типов

It has been stated in the previous paper (Borchsenius, Skoblo i Ossipov 1968) that various clones of *Paramecium caudatum* belonging to the same variety, possess types of micronuclei (Mi) essentially differing from one another. Definite features have been distinguished which are characteristic for any type of Mi: the shape of the nucleus, its size, the intensity of staining after the Feulgen reaction, presence of "tail", shape of the achromatic "cap" and structure of Mi. Those differences permitted to establish a morphological range of the interphase Mi for the given species of paramecia embracing the following clones: D-31a, DS-6a-1, D-66a, M-17, D-91a, ODSI-5a, D-199v-1k, MG-8a1, D-199v-5, DS-3a, DS-1a [1]. This morphological range of Mi is constituted from the barrel-shape, big, unusually compact and chromatin--rich nuclei of the type D-31a, to faintly stainable, comet-shaped with often twisted tail Mi of the DS-6a type, and the different transitory forms. Previously (Borchsenius, Skoblo i Ossipov 1968) a special study has been carried out concerning the stability of the morphological types of nuclei as revealed by us. As result, a capability of Mi was ascertained for a constant reproduction of the type of morphological organization in the agamic reproduction of the cells.

As known, mitosis is one of the forms of manifestation of the nucleic activity. The study of the mitotic division in various morphological types of Mi would possibly permit to throw some light upon the nature of differences between them as observed by us. Therefore the aim of the present study was to examine separate phase of the mitotic cycle (for Mi of different clones) which are accompanied by consecutive morphogenetic changes of Mi at every stage of division.

The authors of the present article express their thanks to I. I. Skoblo for her aid in their investigations.

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[1] Designations of the clones are transliterated from Russian, and are the same as in paper of Borchsenius, Skoblo i Ossipov 1968.

Material and methods

The same clones of *P. caudatum* were used as in the previous study. The methods of the ciliate cultivation, fixation and staining for revealing DNA in Mi whole preparations were the same as those described previously (B o r c h s e n i u s, S k o b l o i O s s i p o v 1968).

The attempt of inducing the synchronic mitotic divisions of Mi in the cultures were not successful. Consequently the prophase and anaphase stages were selected from a great number of whole preparations executed 9—10 hrs. after feeding of the culture. The frequency of Mi being at different phase of the prophase stage was rather high. For every morphological type of micronucleus occurring within our examination, 80—100 cells with nuclei at this stage were stated. The anaphase stage is evidently comparatively shortlasting. We succeeded to reveal 4—6 cells of every morphological type of nuclei being in the course of this division stage. For obtaining Mi at the telophase stage, individuals were examined on depression slide under the binocular microscope 8—10 hrs. after feeding. Single cells (25—50 individuals) being at different stages of cytokinesis were fished out and transfered into the Bouin fluid.

Microphotographs were executed by means of the MBI-3 microscope and the microphotographic set MFN-3 (lens 60×, eye piece 15×).

Results

The description of separate mitosis stages was executed for different morphological Mi types. We consciously did not attempt to study the picture of the mitotic division in all the 11 nuclei types. We found adequate to divide the studied Mi range into 4 groups, including the nuclei with a similar general structure into every group. The first group: D-31a, D-66a, M-17; the second group: D-91a; the third: D-199v-1k, MG-8a1, D-199v-5; the fourth: DS-3a, DS-6a, DS-1a. The Mi type ODS1-5a doubtlessly constitutes a separate group however this type is not to be considered in the present study. Each differs from the preceding group one by a gradual increase of the long axis of nucleus together with elongation of the tail. The morphological picture of mitotic division was studied in several representatives of each group.

The stages of the mitotic division of Mi in the first group was studied in the clone M-17 (Pl. I 1—13). This morphological type of nucleus has been described previously (B o r c h s e n i u s, S k o b l o i O s s i p o v 1968). It should be stressed that the interphase barrel-shaped nucleus is very compact, vividly and evenly stained (Pl. I 1). At the stage of the early prophase, formation of clods is observed, the nucleus becomes spherical and slightly swelled. At the stage of the late prophase the size of Mi increases 2—3 times, its consistency becomes loose and the clue of chromosome threads arises (Pl. I 2—5). Then Mi extends along the long axis and assumes an ovoid form with a highest concentration of chromatin on one of its poles (Pl. I 6). Prior to entering the anaphase stage, the nucleus becomes still more loose, enlarges 4—5 times when compared to its interphase conditions. After the metaphase stage which is marked very indistinctly (Pl. I 7), Mi enters the anaphase (Pl. I 8—9). At this stage the filaments of the achromatic apparatus and accumulations of chromatines are seen well. At the stage of a late anaphase, just prior to the onset of cytokinesis, the filaments of the spindle elongate, the anaphase asters — being diverged — diminish in size and become more

compact. In the period of an intense migration of micronuclei to the opposite cell poles, the chromatin material becomes gradually more concentrated (Pl. I 10—11). The thin achromatin filaments which connect the daughter nuclei disappear till the moment of cytokinesis. The telophase Mi are bigger and more elongated than usually are the interphase ones (Pl. I 12—13).

The character of mitotic division of Mi of the D-31a and D-66a types belong to the first group as well with all its conformity of this process in the Mi of the wild type (Pl. II 14—15, III 26—29). However in those biggest, most compact and intensely stainable, rounded nuclei an inclination is observed to a more rapid disappearing of achromatin filaments and to spherical telophase Mi (Pl. II 22—25, III 36—39). The process of transformation of Mi of both types into the usual interphase ones proceeds much quicker than in the Mi of the M-17 type.

The goblet-shaped, intensely stainable Mi of the type D-91a which are slightly elongated and symmetrical to the long axis, belonging to the second group — are much smaller and contain less chromatin (Pl. IV 40). The morphological pictures in nuclear changes at prophase resemble to those in Mi of the first group of clones (Pl. IV 41—45). However in the course of the whole period from the late telophase to interphase, a considerable narrowing and elongation of the dividing nucleus is observable (Pl. IV 52—54). In the phase of separation of the daughter cells, Mi has a different form from that of the interphase nucleus (Pl. IV 53—54). The tendency to elongation of the long axis is observed also even in the interphase nuclei (Pl. IV 40).

A characteristic feature of the mitotic cycle of the drop-shaped vividly stainable Mi of the type D-199v-1k (Pl. V 55) is the fact that the elongation of the nucleus long axis which terminates as a thin thread and resembles to the non-separated telophase Mi — keeps its form and its "tail" up to the stage of interphase. Prior to the onset of interphase, the thread, preserved till the last stages of cytokinesis, shortens and the elongated nucleus diminishes its size (Pl. V 65—67). Mi of the separated daughter cells have always their "tail" and differ from the interphase nuclei. A certain lapse of time is necessary for accomplishing some morphogenetic processes which transform the nucleus into the interphase conditions.

At last the mitotic division process of the faintly stainable comet-shaped, long-tailed Mi of the type DS-1a (Pl. VI 68) is characterized by "unrolling" of the anterior nucleus end and formation of clods (Pl. VI 69—70). Subsequently a further broadening of the anterior end (i.e. of the end with achromatic "cap") takes place (Pl. VI 71). Mi assumes a form of an obtuse cone (Pl. VI 72) and then the rounded stage of the late prophase follows (Pl. VI 73). The nucleus at this stage is unusually faintly stainable. The anaphase nucleus is of typical form, being however clear and of small size (Pl. VI 74). The most striking fact is that after telophase (Pl. VI 75—79), the form and size of the interkinetic cell Mi are practically preserved. Since the moment of late telophase up to the stage of early prophase, i.e. in the course of the whole interphase period, Mi of the type DS-1a have a long bright "tail" (Pl. VI 56—70, 76—79).

The differences between the morphological Mi types being at the stage of late prophase till the early telophase concern only the dimensions and the intensity of staining of the dividing nuclei but fail to reflect in the form of the nuclei (Pl. I 5—8, II 17—21, III 29—35, IV 41—49, V 58—64, VI 73—74). So, Mi of the type D-31a, D-66a and M-17, being at the stage of prophase and

anaphase, are much more intensely stained and show much bigger dimensions then the Mi type D-199v-1k and more so of DS-1a.

As follows from the description of morphological changes of different types of Mi in mitosis as presented above, the picture of transition of the telophase to the typical interphase nucleus, differs essentially. For the Mi of the first group (D-31a, D-66a and M-17) a rapid transformation of the telophase nucleus into the typical interphasal one is characteristic. The conclusion of this process occurs prior to accomplishment of the cytokinesis process. In the Mi of the fourth group (DS-1a), the telophase form persists really till the stage of phophase, i.e. in the course of the whole interphase period. Even at the stage of the prophase, the nuclei of the latter type have a comet-shaped form which is characteristic for the telophase stage. In this respect Mi of the second and third groups (D-99a and D-199v-1k) occupy a transitory position (between the first and fourth groups).

In this way, in the process of studying the stages of the mitotic Mi cycle of morphologically different types, a different duration of its single stages was revealed by us which is — in our opinion — of a great importance for understanding the nature of differences in the structure of interphase nuclei.

Discussion

In the study of the nuclear apparatus of the clones of *Paramecium caudatum* belonging to one non-identified variety, obtained as result of aberrant sexual process, we succeeded to distinguish 11 morphological types of nuclei which differ highly from one another as well as from Mi of the type M-17 usually occurring in paramecium species under study (B o r c h s e n i u s, S k o b l o i O s s i p o v 1968). Out of those types of Mi, a morphological range was constituted which may be looked upon as polyploidal. The morphological ranges of Mi described in ciliates (C h e n 1940, D i l l e r 1940, J a n k o w s k i 1965), include nuclei which do not differ essentially in form. A more important feature of the Mi range revealed in *P. caudatum* is the appearance of very different forms of nuclei: spherical, goblet-shaped, lancet-shaped, drop-shaped, comet-shaped and others, in contrast to the barrel-shaped Mi normally characteristic for this species.

Among the eleven types of interphase Mi those belonging to the series DS resemble by their morphology to the Mi of the wild type at the telophase stage as described by W i c h t e r m a n 1953. A suggestion arises that one of the division stages of nucleus (telophase) is the most prolonged and the morphological Mi type is simply one of the nucleus division stages.

The data gained by us after the examination of preparations executed at intervals and the results of subclonization, speak in favour of the stability of clones of series DS. Besides this, a doubtless prove of the existence of interphase Mi, type DS as a definite morphological nucleus type, is the picture of the mitotic division examined by us. After the description of the mitosis stages of Mi in the wild type M-17, a comparison may be done on the mitotic cycle of nuclear division characteristic for the given paramecium species and the mitosis stages in the Mi types described above and the essential peculiarities observed in division may be marked.

The data concerning the mitosis of Mi in *Paramecium*, have a very fragmentary character and the pictures presented are comparable only with Mi of the type M-17. W e n r i c h 1926 described most exactly the stages of the

mitotic Mi division in *Paramecium putrinum*. He sygnalized the absence of a clear metaphase stage with the formation of the equatorial plate and with the separation of chromatin filaments into two polar groups at the anaphase stage. The findings of W e n r i c h have been supported by the study of J a n k o w- s k i 1965. C h e n 1940 found in *P. caudatum* the presence of well marked individual chromosomes splitting longitudinally at the stage of anaphase. W i c h t e r m a n 1953 presented pictures of Mi division — without a detailed description — in this paramecium species at two different stages of telophase.

A comparative study of the mitotic Mi cycle stages of different morpholog- ical types permits to presume that the telophase stage — the step evidently most responsible for the form of the interphase nucleus — proceeds in various cases with an unequal rate, i.e. in different morphological nucleus types, the mitosis stages are reversibly blocked at the various stages of the mitotic division.

Consequently in the clones of the first group (D-31a, D-66a, M-17) the process of blocking the mitotic division occurs after the nuclei have assumed the form characteristic for the interphase Mi. In the clones D-91a (second group) and D-199v-1k (third group), the morphogenetic processes associated with the nucleus division, occur at the moment of the early interphase. Con- sequently the process of blocking the Mi division in the clones of the second group, and more when compared with the first morphological nucleus group, occurs much earlier, securing in this way more or less intensive elongation of the long axis of nuclei. In Mi of the type DS-1a, blockade occurs already at the late telophase, i.e. at the moment when the connection of daughter Mi by a thin achromatin filament has been broken but the process of the cell cyto- kinesis has not been concluded. In result the shape characteristic for the telophase Mi appears like "fixed" and is preserved in the interkinetic cell.

The facts stated above permit to state the following interesting regularity: evidently the duration of the conclusive stage of the mitotic division (the place of blocking) depends in some way of the DNA content in the nucleus. The less chromatin is contained in Mi, the more prolonged is the telophase stage and the transit to the typical interphase conditions. This interdependence is most clearly marked in the clone DS-1a in which the elongated nuclei contain a very small amount of chromatin. The interphase Mi of this type are practi- cally at the telophase stage (i.e. they are blocked) and are very little changed since the moment of the early telophase till the early prophase. The more chromatin Mi contains, the more clear is the reduction of the telophase dura- tion. So in the clones D-31a, D-66a, M-17, telophase and its stages transitory to interphase proceed exceptionally rapidly and are concluded prior to the moment of separation of the daughter cells.

It should be noted that in no one of the numerous clones examined, the big, spherical and faintly stainable nucleus has been revealed. The form and staining of Mi of different lines followed strictly the definite regularity: the less chromatin in the nucleus, the more elongated is the long axis of the interphase Mi.

Conclusions

Morphological pictures of the mitotic cycle were investigated in the prin- cipal morphological types of micronuclei (clones D-31a, D-66a, M-17, D-91a, D-199v-1k, DS-1a) of *P. caudatum*.

The pictures of the mitotic division of Mi of the wild type (M-17) as revealed by us, fully coincide with the data from the literature (C h e n 1940, W i c h t e r m a n 1953).

The separate stages of the mitotic process in the Mi of the types studied, are blocked reversaly at different stages of the paramecium division which may be accounted for by the presence of new morphological Mi types in species *P. caudatum*.

The inverse dependence between the quantity of chromatin in nucleus and the duration of the telophase stage has been observed which suggests the existence of the action of the genetic factors (namely the amount of DNA in Mi) upon the form of the interphase nucleus.

S u m m a r y

The results of the study of mitotic division of the principal morphological types of Mi in clones of *P. caudatum* (D-31a, D-66a, M-17, D-91a, D-199v-1k, DS-1a) of the same variety, have been reported in the study. A comparative study of the mitosis stages permit to postulate that the separate stages of this process (the telophase stage) proceed at various rate in the Mi of different morphological types. Blockade of the mitotic cycle of Mi, type M-17, occurs after the nucleus has assumed its form characteristic for the interphase Mi. The morphological processes associated with the division of nucleus in the clones D-199v-1k and D-91a are much delayed and conclude at the moment of the early interphase. In the Mi type DS-1a the process of blockade occurs already at the stage of the late telophase. Consequently, the earlier concludes the nucleus mitosis, which is accompanied by definite morphological changes, the more clearly appears the tendency to elongation of the long axis of the interphase Mi. In this way, the different rate of the morphogenetic processes brings about the formation of new morphological Mi types in species *P. caudatum.* An inverse dependence between the amount of chromatin in the nucleus and the rate of the course of telophase stage has been observed.

РЕЗЮМЕ

В работе представлены результаты изучения митотического деления основ-ных морфологических типов Ми клонов *P. caudatum* (Д-31а Д-66а, М-17, Д-91а, Д-199в-1к, ДС-1а) одного и того же сингена. Сравнительное изучение стадий митоза дает возможность предположить, что отдельные этапы этого процесса (стадия телофазы) протекают с различной скоростью у Ми разных морфоло-гических типов. Блокировка митотического цикла Ми типа М-17 наступает после того, как ядра приняли форму, характерную для интерфазных Ми. Мор-фологические процессы, связанные с делением ядра в клонах Д-199в-1к и Д-91а, значительно замедлены и завершаются к моменту ранней интерфазы. В Ми типа ДС-1а процесс блокировки наступает уже на стадии поздней тело-фазы. Следовательно, чем раньше прекращается митоз ядра, сопровождаю-щийся определенными морфологическими изменениями, тем ярче проявляет-ся тенденция к удлиннению продольной оси у интерфазных Ми. Таким образом, различная скорость морфогенетических процессов влечет за собой появление

новых морфологических типов Ми у одного вида *P. caudatum.* Отмечено суще-
ствование обратной зависимости между количеством хроматина в ядре и ско-
ростью протекания стадии телофазы.

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EXPLANATION OF PLATES I—VI

Mitotic cycle of micronucleus in different morphological types of *Paramecium cau-
datum.* Whole preparations. Feulgen reaction. Photographs were made with obj. 60×,
eye piece 15×

Clone M-17

1—2: Interphase nuclei
3—4: Early prophase. Chromatin clods are seen
5—7: Late prophase. The ball of chromosome filaments is well seen
8: Early anaphase. One of the anaphase asters lying on the Mi

9: Late anaphase
10—11: Telophase. Daughter nuclei are connected by thin filaments
12: Late telophase. The filament connecting the daughter nuclei has disappeared
Mi much elongated
13: Late telophase. Mi becomes spherical, and diminishes in size

Clone D-31a

14: Interphase nucleus
15—16: Early prophase. Nucleus is slightly increased in size. Achromatin cap is
seen well
17—19: Late prophase. Nucleus becomes loose. Ball of chromosome filaments is seen
20: Anaphase. One of the asters lying on Mi
21: Late anaphase
22: Telophase. Nucleus comet-shaped
23—25: Late telophase. Nuclei are distinctly spherical

Clone D-66a

26. Interphase Mi
27—29: Early prophase. Nuclei are slightly enlarged. Chromatin clods are seen well
30—33: Late prophase
34—35: Anaphase
36—37: Telophase nuclei
38—39: Late telophase. Nuclei became spherical and diminished in size

Clone D-91a

40—41: Interphase nuclei
42—43: Early prophase
44—47: Late prophase
48: Early anaphase
49—50: Late anaphase
51—52: Early telophase. Daughter nuclei are considerably diminished in size and
connected with thin filaments
53—54: Late telophase

Clone D-199v-1k

55: Interphase Mi
56—59: Early prophase
60—61: Late prophase
62: Early anaphase
63—64: Late anaphase
65: Telophase. Daughter nuclei are connected with thin filaments
66—67. Telophase comet-shaped nuclei

Clone DS-1a

68: Interphase nucleus
69—72: Early prophase. Broadening of the anterior end of nucleus. Clearly stained
chromatin granules are seen
73: Late prophase
74: Anaphase
75—76: Telophase. Clearly stained daughter nuclei are connected by thin spindle
filaments
77—79: Late telophase. The filament connecting the daughter nuclei has disappeared.
Mi of the form of interphase nucleus
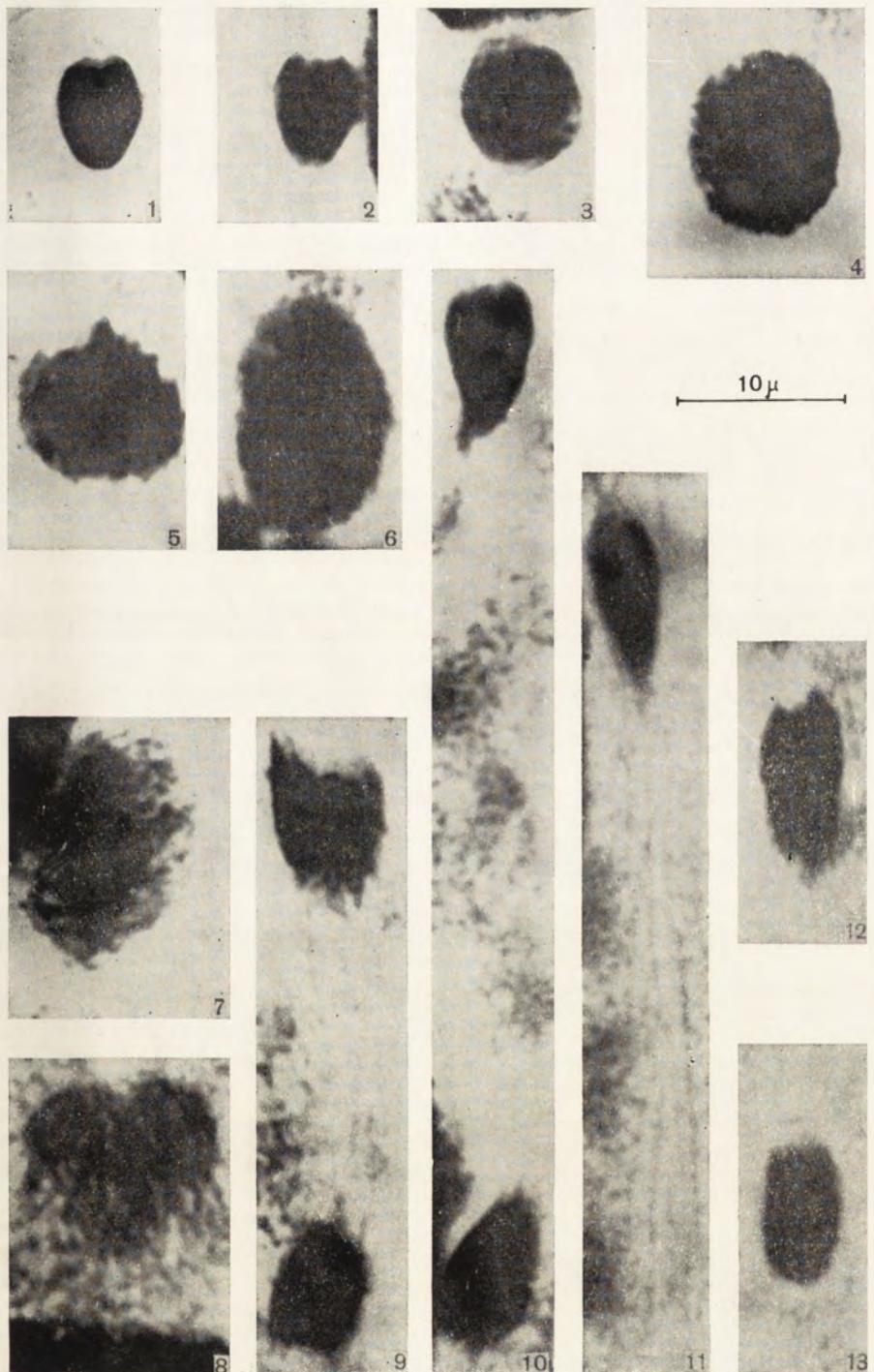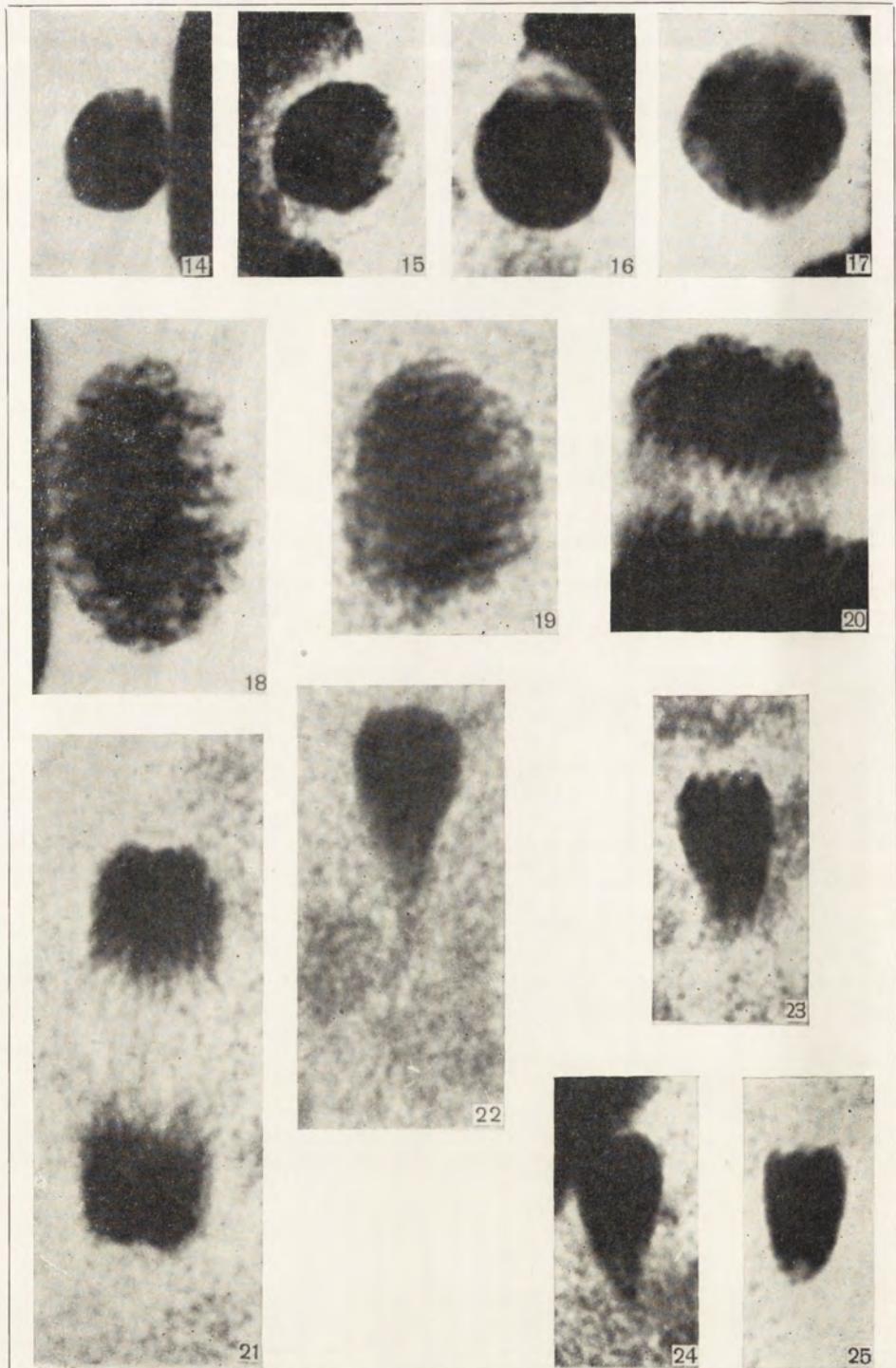
PLATE I

10 μ

O. N. Borchsenius et D. V. Ossipov auctores phot.

O. N. Borchsenius et D. V. Ossipov   auctores phot.

PLATE III

O. N. Borchsenius et D. V. Ossipov

auctores phot.

PLATE IV

O. N. Borchsenius et D. V. Ossipov

auctores phot.

PLATE V
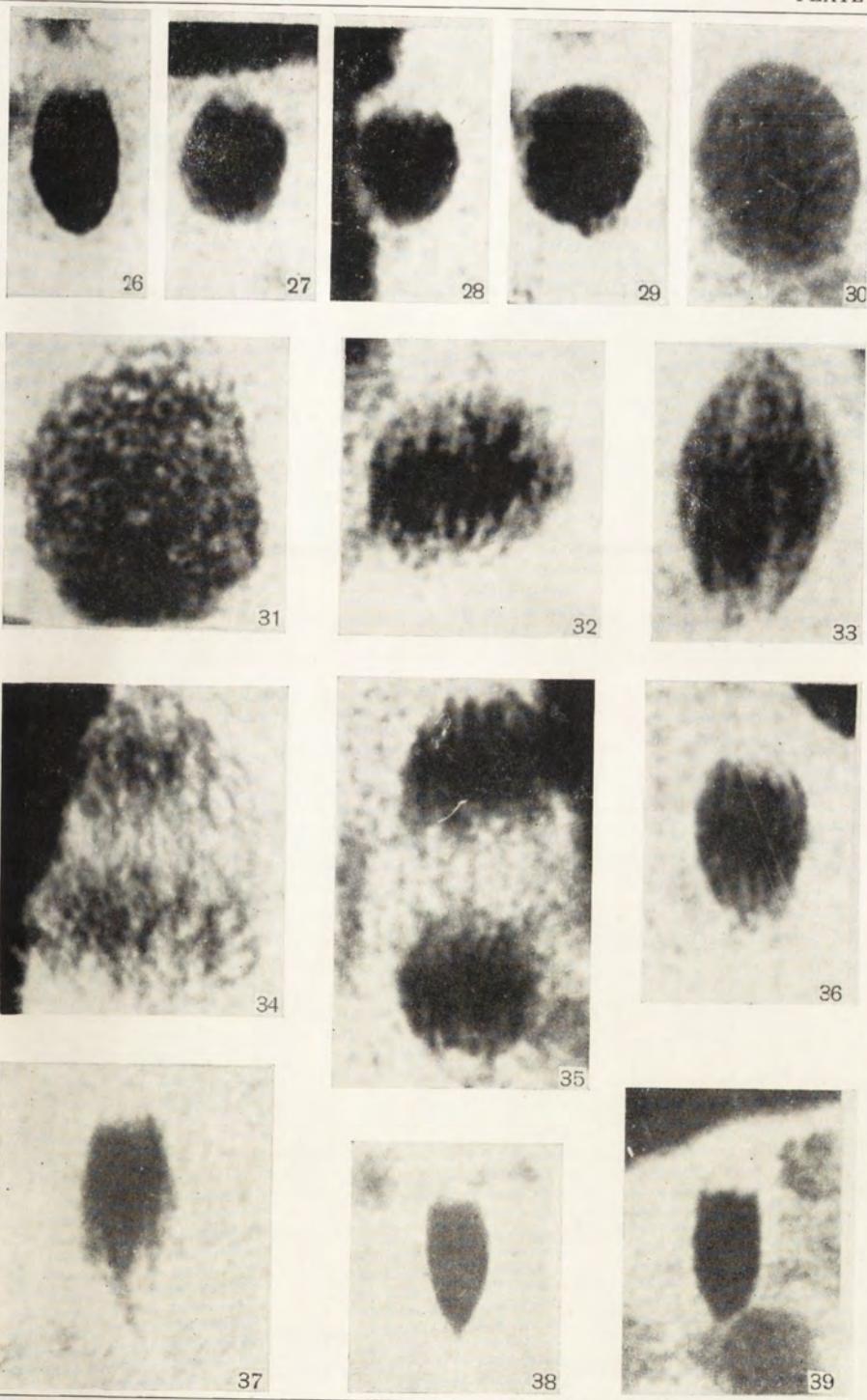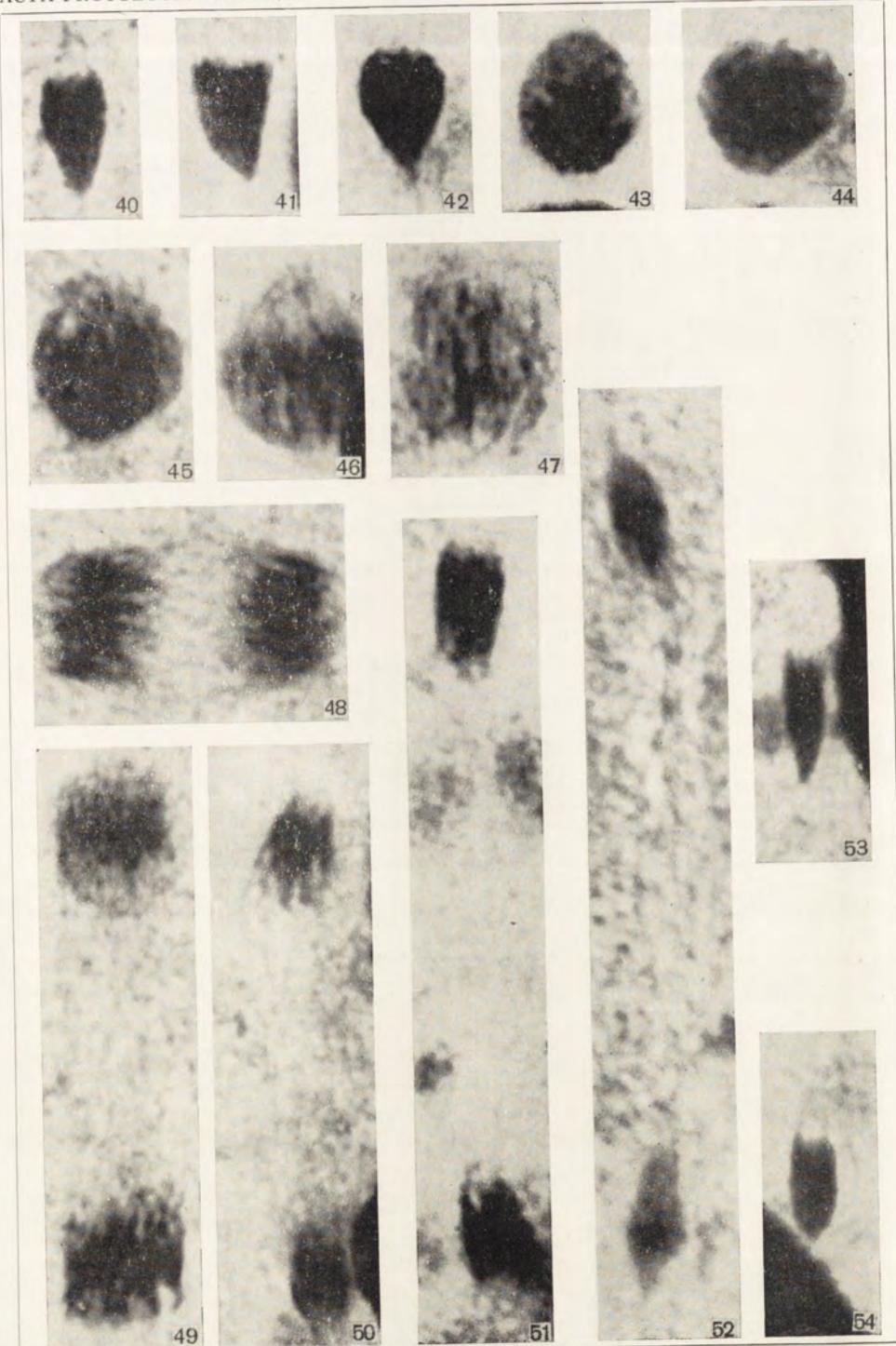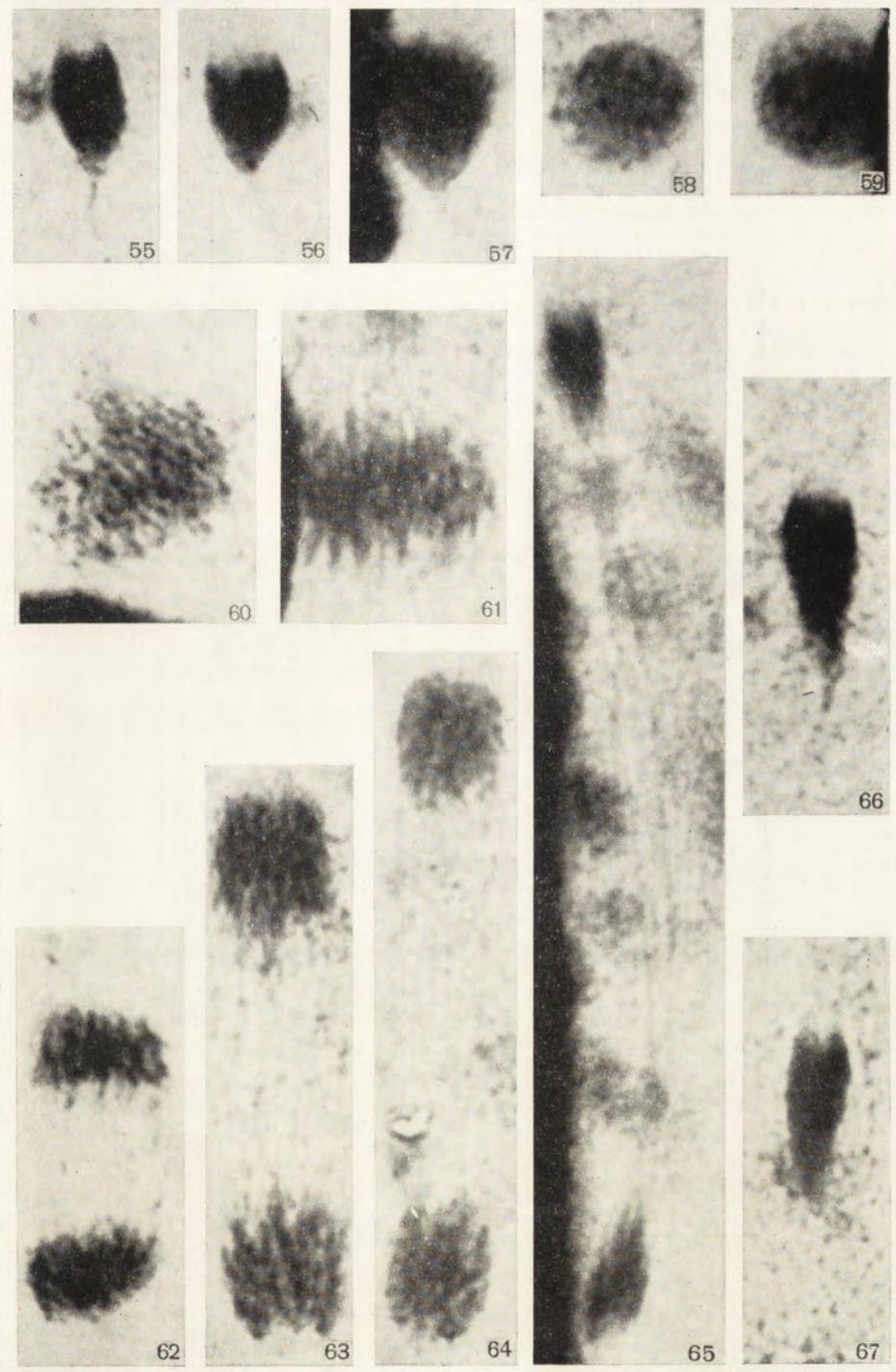
O. N. Borchsenius et D. V. Ossipov
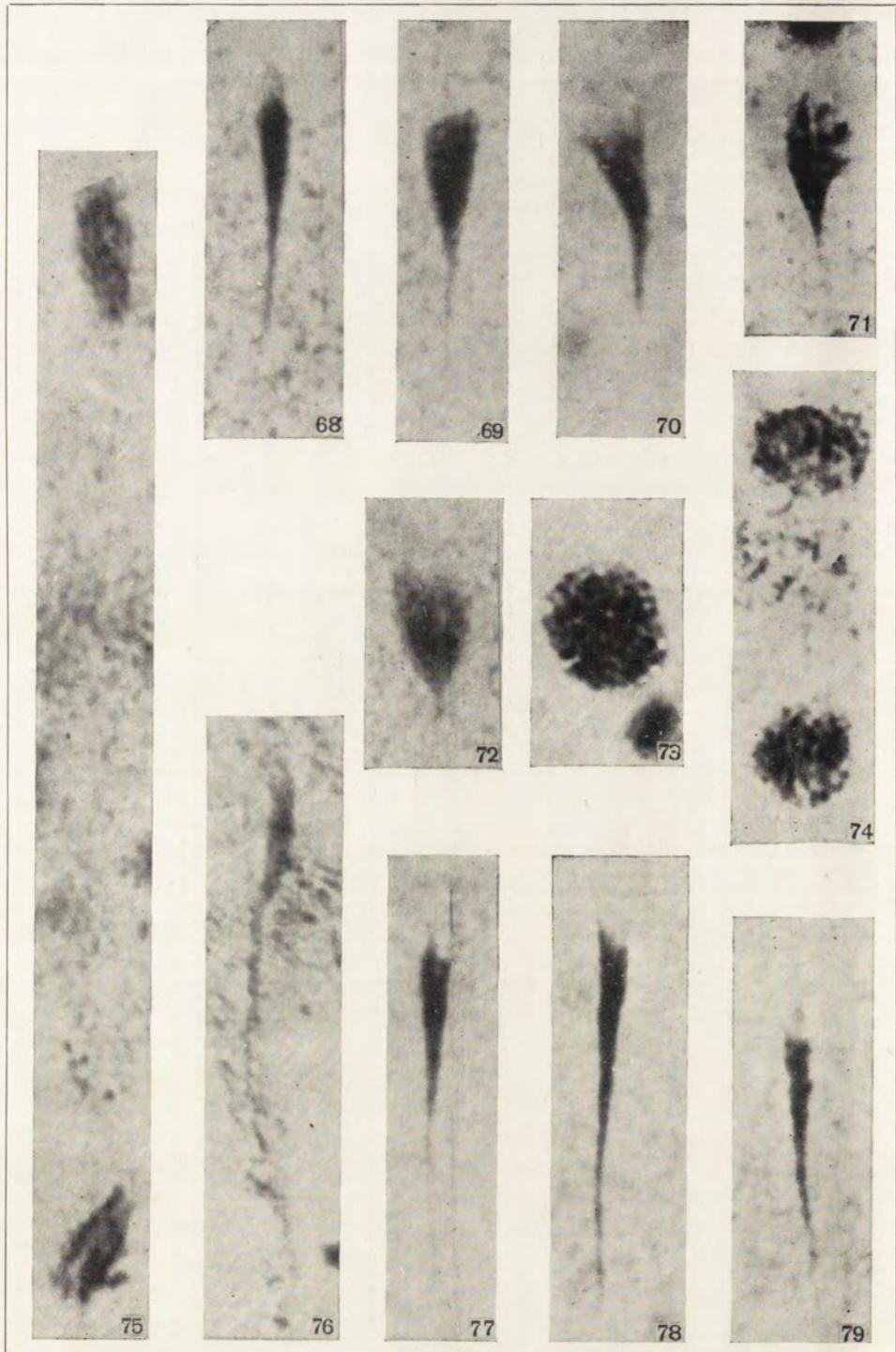
auctores phot.

O. N. Borchsenius et D. V. Ossipov   auctores phot.

Zdzisław RAABE

Two new species of *Thigmotricha* (*Ciliata, Holotricha*) from *Theodoxus fluviatilis*

O dwu nowych gatunkach *Thigmotricha* (*Ciliata, Holotricha*) z *Theodoxus fluviatilis*

The prosobranch *Theodoxus fluviatilis* (L.), being a species of an exceptionally broad geographical and ecological distribution, has a very limited parasitic ciliate fauna. According to my own investigations carried out in the south part of Baltic Sea, in brakish and fresh waters of Poland, in the Balaton Lake in Hungary, in the lakes and rivers of Yugoslavia — including the Ohrid Lake (R a a b e 1965) — only *Trichodina baltica* Quen. and *Scyphidia* sp. related to *Scyphidia physarum* Lachm., occur in this mollusc. These parasites, before all *Trichodina*, are transmitted by *Theodoxus* to the other *Gastropoda* coexisting with it (R a a b e and R a a b e 1959, R a a b e 1965) however *Theodoxus* itself fails to accept — as far as it is known — foreign parasites.

In the course of my investigations on *Thigmotricha,* I had the opportunity to work in September 7—12 1967 in the Department of Lake Fisheries (belonging to the Institute of Freshwater Fisheries) at Giżycko in the Mazury Lakeland. There I found convenient conditions for work which enabled me to carry out the studies on numerous molluscs and to collect an interesting material.

I express my most vivid tkanks to the director Prof. St. Bernatowicz and to his co-workers for enabling me to carry out my work and for their friendly attitude.

Among other molluscs, I could find the material of *Theodoxus fluviatilis* (L.) from several sites: from the stones in the Dargin Lake, from the scrubs of *Potamogeton* in the Kisajno Lake and from the canal connecting this lake with the Niegocin Lake. The population from stones, being the most numerous (30 specimens), was infected only by a few *Trichodina baltica* Quen. and *Scyphidia* sp. The population collected from *Potamogeton* (several specimens) and from the canal (58 specimens) proved to be infected with two unknown species of *Thigmotricha* in a rather high percentage (approx. 30%) besides the above mentioned parasites. In consequence I decided to report my findings in the present communication considering the unexpected occurrence of those ciliates and their peculiar position in the evolution of *Thigmotricha* and of the families to which they belong.

One of the species found belongs to the family *Hemispeiridae* (subfam. *Hemispeirinae*) and has been described here as a new genus and species

Protospira mazurica. The other one corresponds to the family *Ancistrocomidae* (subfam. *Hypocomellinae* Raabe, 1967) and has been described as *Hypocomella quatuor* sp. n.

Protospira mazurica gen. n., sp. n.

Body elongated, tapering forwards and rounded in its posterior part. Its shape approaches that of the representatives of the genus *Ancistrumina* Raabe, 1959. Body dimensions: length 40—45 μ, width approx. 15 μ. Body is slightly flattened laterally, bent to the left, sometimes twisted spirally (Fig. 1 A). Ma
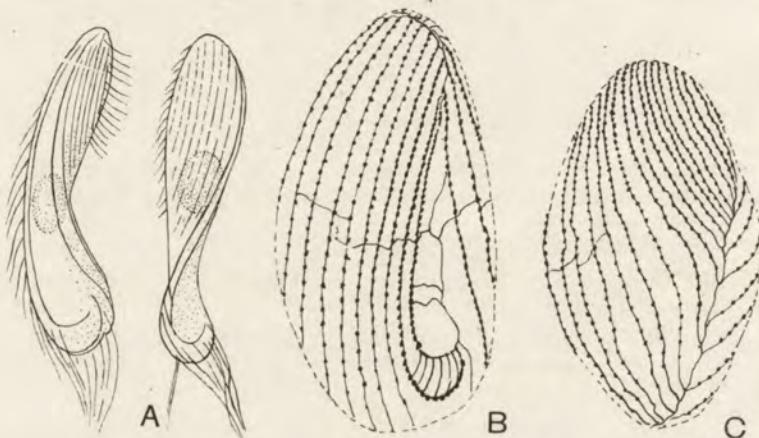
Fig. 1. *Protospira mazurica* g.n., sp. n. A — general aspect, B — the ciliary apparatus of the right-ventral side, C — of the left-dorsal side. B and C after AgNO₃ dry preparations

measures ca 8 μ, lies in the posterior body part. Mi lies on its side. C.V. in the posterior body region. Among the dense ciliary covering, long adoral cilia are distinguishable reaching up to 15 μ. Near the posterior body pole, a more rigid cilium (or perhaps a bundle of cilia stuck together) is differentiated which is usually seen in the living material.

The general ciliture is arranged in 26—29 kineties, out of which 8—9 run along the right and about 18—20 on the left body side. Adoral kineties initiate closely to the anterior body pole, run along the naked peristomal area and bend at the posterior pole producing a large loop (Fig. 1 B, C). Their course reminds that in *Ancistrumina* and in *Ancistrum.* The kineties of the general ciliature of the right body side run meridionally from the anterior suture to the posterior one. The marginal kineties of the left body side, the nearest the peristomal zone (*n, n—1, n—2* etc) behave in a similar way. Kineties running along the dorsal part of the left body side behave however in a different manner which is associated with a considerable forwards shift of the dorsal end of the posterior suture. These kineties (8—10 in number) initiate also their course at the anterior suture on the anterior body margin, however — beginning at both sides and running centripetally — they gradually become shorter, bend to form an arch and produce a system called by C h a t t o n et

L w o f f 1949 système sécant (parenthetical system). This feature deviates distinctly from the configuration present in *Ancistrumina, Ancistrum* and other *Ancistrinae* (= *Protophryinae* — R a a b e 1967), and approaches *Protospira mazurica* g.n., sp.n. to *Ancistrospira, Plagiospira* and other *Hemispeirinae* (C h a t t o n et L w o f f 1949).

Consequently I am inclined to include the new species to the subfamily *Hemispeirinae* and to place it at the base of the evolutionary range represented by this family. I assume it as a form the least advanced in this direction in which this subfamily was developing, namely in the direction of retrogradation and spiralization of the adoral kineties and of anterogradation of the thigmotactic zone.

Really *Protospira mazurica* g.n., sp. n. resembles distinctly to the most plesiomorphic forms of the subfam. *Ancistrinae* as *Ancistrumina* as well by its whole body form as by the situation of its adoral kineties. The presence of its fairly differentiated parenthetical system qualifies this species to *Hemispeirinae*, to a position of a highly plesiomorphic form. It differs from the genus *Plagiospira* Ch. Lw. — the next genus in this system — by the lack of retrogradation of adoral kineties and by the fact that the kineties n, $n-1$, $n-2$ etc. fail to interrupt at the periphery of the adoral arch but reach as far as the posterior suture. I did not succeed to ascertain and to localize the place of insertion of the differentiated cilium at the body end.

There was an opportunity to show in the case of *Protospira mazurica* gen. n., sp.n. — the first fresh water representative of the subfamily *Hemispeirinae* — what essentially is the système sécant and what is its genesis. Evidently it is not an alien and unexpected structure but rather a result of anterogradation of the posterior suture. Its kineties continue being bipolar, diverging from the anterior suture in their anterior ends and reaching as far as the posterior suture with their posterior ends. It seems to be an essential fact that the kineties which participate in formation of the thigmotactic system — enclosed in the parenthetical system — belong rather to the left part of the ciliature and consequently are not dorsal kineties as understood by C h a t t o n et L w o f f 1949, or became such ones only secondarily in connection with a certain symmetrization of their system on both sides of the posterior suture which has been deplaced forwards.

The lack of information concerning the nature and of genesis of système sécant in the studies of C h a t t o n et L w o f f among others, is the result of using the moist methods of silver impregnation which fail to preserve or to reveal the connective fibers of kinetosomes. The only reliable method is the dry silver impregnation after Klein which has been applied in the present study. It gives also satisfactory results on the salt water material.

Theodoxus fluviatilis (L.) is the host of *Protospira mazurica* g.n., sp. n. but this was stated only in the Mazury Lakes material as yet. The infection reaches 0—50⁰/₀ in various populations of the molluscs and its intensity amounts to 5—20 ciliates in one host specimen.

Protospira genus novum

Hemispeiridae, Hemispeirinae with slightly marked characters of this group. Body ovoid, elongated, the length about 40 µ. General ciliature rather dense, the number of kineties 25—30, out of which 8—10 kineties produce a thigmotactic system of a character of système sécant. Adoral kineties start nearly

4*

at the body apex, run backwards describing a broad loop at its posterior pole. Parasites of the mantle cavity of *Gastropoda*.

Typus generis: *Protospira mazurica* sp.n. from *Theodoxus fluviatilis* (L.).

Hypocomella quatuor sp. n.

Body pear-shaped, moderately elongated, bent and concave on one side on which the ciliature is present, the anterior part of the body is much elongated so as to form a proboscis (Fig. 2 A). Body length 11—18 µ, width ca 6 µ. Ma measuring ca 5 µ lies in the body middle, Mi lies near it.
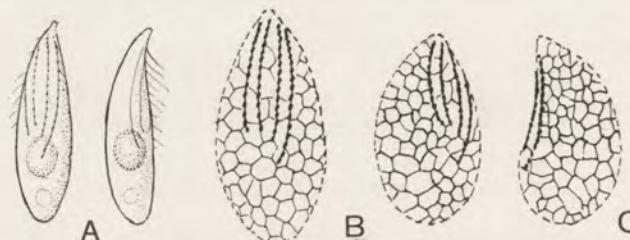
Fig. 2. *Hypocomella quatuor* sp.n. A — general aspect, B — the thigmotactic side, C — the naked side. B and C after AgNO₃ dry preparations

The ciliature comprises only 4 kineties which begin at the base of the proboscis and are symmetrically bent to the right — the right ones, and to the left — the left ones (Fig. 2B, C). The thigmotactic area reaches approx. as far as the half of the body length. After silver impregnation, on the body surface a network is revealed with meshes which are rather big as for the insignificant dimensions of the ciliate.

I included this species into the genus *Hypocomella* Chatton et Lwoff, 1922 and not — as it could be expected — to the genus *Hypocomatophora* Jarocki et Raabe, 1932, because in my opinion (following C h a t t o n e t L w o f f 1950) those two genera should be joined together into one common, and the name *Hypocomella* has the priority. Among the species of this genus — similarly as in other species known of *Ancistrocomidae* — *Hypocomella quatuor* sp. n. is distinguished by a far advanced reduction of the kineties number. In the other species of the genus *Hypocomella* (or *Hypocomatophora*) this number is at least 6.

The host: *Theodoxus fluviatilis* (L.). *H. quatuor* sp. n. is known only from the Mazury Lakes as yet.

The taxonomic position of both species described above will be analyzed in details in the subsequent parts of my monographic study: Ordo *Thigmotricha* (*Ciliata, Holotricha*) devoted to its single families. It should be stressed that both species described — so unexpectantly found in *Theodoxus fluviatilis* — represent some interesting points in the evolution of families to which they belong. *Protospira mazurica* g.n., sp. n. initiates an evolutionary range formed by the subfamily *Hemispeirinae* which continues further on, over *Ancistrospira* Ch.Lw., *Plagiospira* Ch. et Lw., *Cheissinia* Ch. et Lw. to *Hemi-*

speira König. *Hypocomella quatuor* sp.n. presents possibly the last link in the reduction of the kineties number among *Ancistrocomidae, Hypocomellinae* (see R a a b e 1967).

Summary

Occurrence of two new species of the order *Thigmotricha* has been ascertained in the mollusc *Theodoxus fluviatilis* (L.) from the Mazury Lakes. Till now, no representatives of this order were reported to occur in this host. *Protospira mazurica* g.n., sp.n. proved to be the most plesiomorphic species among *Hemispeiridae, Hemispeirinae* and may be looked upon as a model exit form for this group. *Hypocomella quatuor* sp.n. reaches the most advanced position among *Ancistrocomidae, Hypocomellinae* in regard to the reduction of its ciliature.

Streszczenie

W *Theodoxus fluviatilis* (L.), z którego nie opisano dotychczas żadnego przedstawiciela *Thigmotricha*, stwierdzono w jeziorach Mazurskich występowanie dwu nowych gatunków z tego rzędu. *Protospira mazurica* g.n., sp. n. okazał się najbardziej plezjomorficznym gatunkiem z pośród *Hemispeiridae, Hemispeirinae* i może być uważany za model formy wyjściowej dla tej grupy. *Hypocomella quatuor* sp. n. osiąga w redukcji swego urzęsienia najbardziej zaawansowane stanowisko wśród *Ancistrocomidae, Hypocomellinae*.

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Henry TAMAR

Observations on *Halteria bifurcata* sp. n. and *Halteria grandinella*

Beobachtungen an *Halteria bifurcata* sp. n. und *Halteria grandinella*

During February to June 1967, observations were made on what might best be considered a new species of *Halteria*. It has in common 7 groups of bristles and presumably 16 adoral membranelles with the following: *Halteria grandinella* O.F. Müller, 1786 (T a m a r 1965); two former varieties of this species later elevated to species level (K a h l 1935) — *Halteria cirrifera* Kahl, 1935 and *Halteria chlorelligera* Kahl, 1935; and *Halteria minima* Gelei, 1954 (G e l e i 1954).

Of these species the status of *H. chlorelligera* is in some doubt. The work of G e l e i and S z a b a d o s 1950 suggests that *H. chlorelligera* is only *H. grandinella* with ingested algae, and L ó p e z 1945 describes a *Halteria chlorelligera* var. *grandinelloides* which again appears to be *H. grandinella* or a similar type with ingested algae.

A description of the structure and behavior of the new species, *Halteria bifurcata*, and findings on comparable aspects of *H. grandinella*, follow.

Materials and methods

Four mixed cultures containing *H. bifurcata* sp.n. were obtained from the upper portion of the lake system in Deming Park, Terre Haute, Indiana. The collection site was characterized by a bed of *Cabomba*, over which floated a mat of algae and duckweed (*Lémnaceae*). Many snail egg masses were present. Only sparse material — some *Cabomba*, a piece of a dead deciduous leaf carrying a few snail egg masses, and some duckweed and algae — was collected with the lake water. Algae, diatoms, a *Strombidium* species containing numerous zoochlorellae, *Chlorohydra viridissima*, *Bodo*, etc. were present in the culture jars. The pH ranged from 8.0—8.5, and temperatures of 20—25°C were maintained. The addition of a small quantity of skimmed milk powder produced large numbers of *H. bifurcata*. The addition of skimmed milk powder to lake water samples is also effective for obtaining high numbers of *H. grandinella*.

H. grandinella were cultured in 2 balanced salt media developed mutually with Yee-wan Mark. The original medium consists of a solution of 117 mg. NaCl, 55.5 mg. $CaCl_2$, 37.2 mg. KCl, 24 mg. $MgSO_4$, 13.6 mg. KH_2PO_4, and 8 mg.

$FeCl_3 \cdot 6\,H_2O$ in a liter of distilled water, to 20 cc. portions of which in test tubes is added 2 mg. of skimmed milk powder. The pH is brought to 7 with 0.1 N NaOH or 0.1 N HCl. Heavily-inoculated test tubes kept at 28—29°C contain optimum numbers in 4—5 days. Various bacteria, including spirilla, were also present in the cultures.

In an effort to approximate the desired pH and provide buffering to better maintain this pH, a second, but somewhat less effective medium, was developed. This differs from the original medium in containing only 89.5 mg. NaCl and 33.5 mg. KCl, having the increased quantity of 20.5 mg. KH_2PO_4, and containing in addition 33.5 mg. Na_2HPO_4.

Live and deteriorating specimens were examined and photographed by phase contrast microscopy.

Results

Halteria bifurcata sp.n.

Bady form and size

As in the case of *H. minima*, the peristome is constricted. In *H. bifurcata* it makes up 1/3 to 1/4 of the body length (Pl. I 2, 4). The primarily globular body of 20 specimens ranged between approximately $20 \times 20\,\mu - 30 \times 30\,\mu$. Larger organisms were most frequent in the 1st culture, collected Feb. 11 1967, during the long period before skimmed milk powder was added. During this time the typical cell diameter in the 1st culture was approx. $27.5\,\mu$. Specimens which were initiating division could be recognized by their larger size, which approached the upper limits of the size range.

Adoral membranelles

H. bifurcata normally possesses 16 adoral membranelles, which are unusual in being compound (Pl. II 6). Each membranelle consists of 2 parts which are united proximally but then separate more distally. Their separated portions make up most of the length of these subdivisions. In many preparations the 2 subdivisions exhibit fraying at their distal tips, which is indicative of the ciliary rows of which they are composed.

The base of each adoral membranelle, where it enters the body surface, shows 3 short, dark lines under phase contrast (Pl. II 7 A.). The middle dark line was identified as the line of junction of the 2 subdivisions of the membranelle, and the lateral lines represent the lateral edges of these subdivisions, or the lateral limits of the membranelle.

H. bifurcata resembles *H. minima* in that the adoral membranelles arise from small indentations around the sides of the constricted peristome.

Forty measurements show the adoral wreath, with the adoral membranelles fully extended, to range between $39—50\,\mu$ in diameter.

Over 50 measurements indicate both that individual adoral membranelles, after straightening out, have a length of $17—21\,\mu$, and that adoral membranelles which approach their natural bent form to varying degrees cover a linear distance of $12—17\,\mu$.

During forward spiraling the adoral membranelles proximally extended out at almost 90 degrees to the anterior-posterior axis, and then more distally curved forward so that the tips pointed anteriorly.

In 4 observations the new adoral zone, during reproduction, was already active during mitosis of the micronucleus, when the latter was in anaphase.

Oral membranelles

Seven oral membranelles or their bases could be clearly counted. It is possible that sometimes 8 oral membranelles are present. As is the case for the adoral membranelles, the oral membranelles are each composed of 2 subdivisions, which results in the appearance under phase contrast of 3 dark lines at their bases (Pl. II 7 O). The area surrounding the oral membranelles, the wall of the buccal cavity, can be seen to have striations.

A reproducing specimen not yet showing a constriction had both active new adoral and oral membranelles.

Bristles

H. bifurcata almost always has 7 groups of bristles, which arise from equatorial furrows. However, 1 specimen undoubtedly possessed only 6 groups of bristles. The 4 bristles of each group were given consecutive numbers, from the most anterior to the most posterior (Pl. I 1, 3).

Bristles 2 and 4 are bifurcated distally, and have a straight stem (Pl. I 1, 2, 3, 5). The bifurcation of a bristle, especially of bristle 4, can often open to a greater length than is normally apparent. There seems to be a difference in length between the 2 branches. The anterior branch of bristle 4, which continues straight from the stem, seems to be longer than the posterior branch, which curves backward. Each of the branches of a bifurcated bristle is about 1/2 as thick as the stem of the bristle.

Nine measurements of the length of the longer branch of bristle 2 ranged from $4.7—6.9\,\mu$. The best values varied around $5.5\,\mu$. Thus the longer branch of bristle 2 is about 1/5 of the total length of the bristle $(24—27\,\mu)$.

Twenty measurements of the longer branch of bristle 4 ranged from $11.3—13.7\,\mu$. The best values varied around $12\,\mu$. Thus the longer branch of bristle 4 is about 1/2 of the total length of this bristle $(24—27\,\mu)$, and about 2 times the length of the longer branch of bristle 2.

Bristle 3 is straight and is not bifurcated (Pl. I 4).

Bristle 1 is bent forward in its distal 1/3, and then curves laterally again, in the shape of a Texas longhorn's horn (Pl. I 1—3).

Bristles 2,3, and 4 reach a length of $24—27\,\mu$. Thus the length of these bristles normally ranges around the body diameter of H. bifurcata (Pl. III 9). The bristles often exceed the body diameter, apparently especially in the smaller and younger specimens (Pl. III 10).

All the bristles of each specimen extend about the same distance from the body. Comparisons between bristles 2 and 4 (measurement of stem and longer branch) showed these to have approximately the same length in any specimen. However, the curves in bristle 1 give this bristle a length greater than that of the other bristles.

Bristle 1 usually extends anteriorly past the level of the peristome; and, during fast forward spiraling, bristles 3 and 4 can hang far back behind the body. In motionless specimens the angle of the bristles to the anterior-posterior axis of the body appears to be variable. Frequently bristles 1 and 2 are directed anteriorly, bristle 3 is directed slightly posteriorly, and bristle 4 has a strong posterior direction, or bristle 3 appears to be at a 90 degree angle to the

anterior-posterior axis. However, sometimes bristle 2 is at a 90 degree angle to the anterior-posterior axis, and 3 and 4 are directed posteriorly.

Macronucleus

The interphase macronucleus becomes visible under phase contrast, when a specimen swells, as an ovoid body ranging from about $6.5—9.5\,\mu \times 9—12.5\,\mu$ (15 measurements). It may expand considerably as the specimen swells and deteriorates. Most of the macronucleus is anterior to the middle of the anterior-posterior axis of the body. The macronucleus lies at the middle of the left-right axis.

A chromatic reticulum was seen in macronuclei before and after the division of organisms (Pl. III 11). This finding correlates well with the observation by K o r m o s und K o r m o s 1958 of the macronuclear reorganization band in *Halteria*.

Micronucleus

The spherical micronucleus has a diameter of between $3—5\,\mu$. Two sets of newly-divided micronuclei ranged from $2.5—3.5\,\mu$. There may be some expansion of the micronucleus before it becomes clearly visible under phase contrast microscopy.

During micronuclear mitosis the micronucleus elongates and, as is usual in ciliates, the micronuclear membrane remains intact. Micronuclear mitosis precedes the macronuclear process and constriction.

Contractile vacuole

The contractile vacuole is located to the left of the buccal cavity and just anterior to the equator. In this respect *H. bifurcata* resembles the other species of the genus *Halteria* (S z a b ó 1935), with the exception of *H. minima* (G e l e i 1954).

Chlorellae

In the first 2 cultures, kept under some fluorescent illumination, *H. bifurcata* containing relatively spherical, green algal cells were observed at 9—30 days, before skimmed milk powder was added. Most of the fraction of specimens carrying these spherical or subspherical algae contained only a few, but some had a larger number (Pl. IV 13). The diameter of 21 such algae ranged from $2.5—4\,\mu$. They did not appear to be inside food vacuoles, and none was seen in a partially digested state. Several were observed to possess concave chloroplasts and smaller convex spaces. Their structure thus seems to be that of *Chlorella*, and it is possible they were zoochlorellae.

Other algae

Large, elongated green algal cells present in many *H. bifurcata* were studied from the first 2 cultures, before the addition of skimmed milk powder. One specimen contained at least 5 (Pl. IV 12), 2 specimens had 3, and 3 possessed 2 such algae each. Two of the last 3 organisms also carried respectively 8 and over 20 chlorellae (Pl. IV 13). Many specimens contained 1 large, elongated green alga. The size of 9 of these algae ranged between $2.7—5\,\mu \times 7—12.5\,\mu$.

Whether the elongated algal cells were digested was not determined. No vacuolar membranes could be discerned around them. In each of 2 specimens

a clump of what appeared to be smaller, spherical green algae was seen in a food vacuole.

Four specimens each contained a diatom and 1 of these diatoms appeared to be in a food vacuole. Two of the diatoms measured $17 \times 5 \, \mu$ and $15 \times 6 \, \mu$ respectively.

Small globules

Smaller globules were seen in *H. bifurcata* from all cultures. In many specimens approximately 20 small globules could be counted, and 6 globules which were measured had diameters of approx. $1-2 \, \mu$. Frontal views of specimens showed these globules to be concentrated primarily near the periphery of the organisms (Pl. IV 14). The globules were apparently not in vacuoles. In degenerating specimens the globules showed various bulges, giving them highly irregular shapes (Pl. IV 15), and in some cases seemed to change shape. Because of chromatic aberration, it was not possible to determine the significance of a greenish tone, or even of a greenish area with a concave border, in some of the globules.

A great number of more minute granules were also present in the cytoplasm.

Response to nearby movement

When groups of *H. bifurcata* were observed under natural conditions, hovering around plant stalks etc. in dishes of lake water, it was seen that many specimens jumped away from rotifers, larvae, and annelid worms when approached, but before these larger animals came into actual contact with the ciliates. This early avoidance reaction was studied by moving the smaller end of a pin toward and after specimens by both continuous motions of fairly steady velocity and by jerks which stopped short of the specimens. In response to both types of pin motions (perhaps the jerks were more effective), a good number of *H. bifurcata* jumped away when the pin was still at least several body lengths from them. Some specimens, after thus jumping several times from a pin following behind them, escaped by a rapid reversal. A few responded to the first stimulation with reversal, which has a higher threshold (T a m a r 1967), perhaps because the uncontrolled pin movement was unusually powerful. A good early response to pin movement was obtained even in cultures containing viscous bacterial masses as a result of the addition of skimmed milk powder.

If a pin was lowered through the water surface, or pulled out through it again, numerous specimens within a considerable radius reacted to the water movement resulting from the breaking of the surface tension layer by bits of rapid reversal.

Response to nutrients

In unmodified lake water cultures the *H. bifurcata* gathered around snail egg masses and also around pieces of plant stalks.

Preliminary experiments indicate *H. bifurcata* can also be cultured in the original, weakly-buffered balanced salt medium for *H. grandinella*.

Halteria grandinella O. F. Müller, 1786

H. grandinella cultured in the original, weakly-buffered balanced salt medium gave the following results:

Body size

Specimens collected in November fell within their previously-reported size range of $23\times23\,\mu - 33\times36\,\mu$ (T a m a r 1965). In 1 culture tube they had a typical cell diameter of 30—35 µ [1].

Position of adoral membranelles

As in *H. bifurcata*, during forward spiraling the adoral membranelles proximally extended out at almost 90 degrees to the anterior-posterior axis, and then more distally curved forward. During reversal, induced by placing culture fluid on slides into a freezer for 1/2 minute intervals, the adoral membranelles were also bent.

Bristles

H. grandinella normally has 7 equatorial groups of bristles. However, 1 organism with 8 groups of bristles was observed.

The bristles reached a length of 17—19.5 µ. Thus their length ranged around, or somewhat exceeded 1/2 the body diameter. In any specimen all the bristles were of the same length.

Small globules

The specimens had a high number of small globules. When 1 organism dried up, its small globules gave rise to greenish cross-figures.

Response to nearby movement

There was only a relatively poor response to pin movement. Most of the organisms showed no response at all. Some jumped away, but many of these did so only after the pin had approached within a short distance. A few specimens responded to pin movement with reversal.

It was suspected that the poor response resulted from a considerable viscosity produced by bacterial masses in the medium. Therefore portions of medium containing numerous specimens were diluted with a minimum of 10 times as many parts of filtered lake water. The pH of these mixtures was near 8.5, and the temperatures during experiments were 20—25°C. Experiments were performed 1 hour and 18 hours after the mixtures were prepared, and there is little doubt, judging from the normal forward spiraling and jumping, that the specimens were fully adapted to the mixtures by the time of experimentation. The *H. grandinella* in these mixtures again showed a poorer response to the moving end of a pin than did the *H. bifurcata* in lake water. Fewer of the *H. grandinella* jumped away in response to the movement, and many of these jumped only when the pin was at a relatively shorter distance from them. Some of the organisms did rapid reversal after the needle came quite close to them.

Discussion

The new form can be distinguished by its double adoral and oral membranelles, and by the curving or bifurcation of three of its bristles. However, it also differs from the two species most similar to it, *H. grandinella* and *H. mi-*

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[1] The last figure was misprinted in Abst. 98, Suppl., J. Protozool. 14, 1967.

nima, in more basic characteristics. Thus *H. grandinella* does not possess the constricted peristome of the new form, although *H. grandinella* has a similar number of oral membranelles and its contractile vacuole is also found to the left of the buccal cavity. *H. minima* shares a constricted peristome with the new form, and the general bristle length of these two organisms is not radically different. However, *H. minima* has 9—10 oral membranelles, and its contractile vacuole is located to the right of the buccal cavity (G e l e i 1954).

If the new form were to be considered a new variety, it would at this time be almost impossible to decide whether it should be a variety of *H. grandinella* or of *H. minima.* Therefore at present it seems most satisfactory to give the new form the status of a new species.

One of the unique characteristics of *H. bifurcata* is the double nature of its adoral and oral membranelles. It is hardly surprising that the structure of the oral membranelles resembles that of the adoral membranelles, since S z a b ó 1934 already suggested that in *Halteria* the row of oral membranelles may have had its evolutionary origin in the adoral zone. S z a b ó 1935 later described the common origin of the adoral and oral membranelles from a single row of primordia during reproduction. F a u r é - F r e m i e t 1953 reported that in *H. grandinella* the oral membranelles and mouth differentiate at one end of the new adoral zone, opposite to that adjoining the old buccal ciliature. On the other hand, in *Halteria geleiana* each oral membranelle consists of 3 lamellae, whereas the adoral membranelles are not subdivided (S z a b ó 1935).

The observation of an occasional *Halteria* with an aberrant number of bristle groups indicates some intraspecific variation in this respect. Such variation is a common phenomenon among Protozoa.

The bifurcation of *H. bifurcata's* 2nd and 4th bristles may be of significance in providing sensitivity to water movement or vibration. Thus the curved posterior branches of the 4th bristles might be more easily displaced by a water current.

Branched bristles are unusual among ciliates. However, K u d o 1966 states that in some instances the distal portion of a cirrus may show 2 or more branches. *Cladotricha koltzowii* has 2 feathery frontal cirri, and *Euplotaspis* has cirri with fringed tips.

If the chlorellae present in some *H. bifurcata* are zoochlorellae, they would not be a unique example of symbiotic algae in *Halteria.* K a h l 1935 describes *H. chlorelligera* as being completely filled with large *Chlorella,* which have a stigma. K a h l 1935 also states that *Halteria* [*Strombidium*] *oblonga* frequently has a few zoochlorellae in its cytoplasm. A n d r é 1912 reports that a few *H. grandinella* contain zoochlorellae.

The capture of algae for nutrition has also been noted in *Halteria.* S z a b ó 1934 found that *H. grandinella* feeds on bacteria and small algae. He also observed *Halteria maxima* to swallow *Micractinia* (*Chlorococcales*), 3—4 of which could be seen in the body of one specimen. D i n g f e l d e r 1962 confirms the use of small algae by these 2 species. G e l e i és S z a b a d o s 1950 found *Halteria oviformis* to occasionally utilize green flagellates.

The determination of the nature of the described small globules, whether they are mitochondria, stored material, etc., presents a subject for further study. The presence of many such globules in the *H. grandinella* in the culture medium suggests that they bear no relationship to chlorellae.

The jumping response in *H. grandinella* is essentially equivalent to the avoidance reaction of other protozoa. It is a response to touching a particle, the substrate, or the surface, and to reaching an unfavorable chemical or osmotic environment (T a m a r 1967). In *H. bifurcata* the jumping response has enhanced significance because this species is sensitive to a low order of mechanical stimulation in the form of water movement or vibration. In *H. bifurcata* the jumping response also permits escape from predators before these have reached the organism.

H o r r i d g e 1966 reported that ctenophores, chaetognaths, crustaceans and the *Chaoborus* mosquito larva (*Culicidae, Diptera*) are also sensitive to small disturbances in the water, in this case by means of innervated non-motile cilia or modified bristles. Horridge considers these cilia and bristles a new class of receptors.

It is suggested that a small quantity of skimmed milk powder be added to collected water samples which are to be used as sources of *H. bifurcata* or *H. grandinella.*

The structure and function of *H. bifurcata,* and of other *Halteria,* are of evolutionary interest. Although the *Oligotrichida* offer a rare example of oligomerization in the Protozoa (D o g i e l 1965), since they have lost most of their somatic ciliation, within the genus *Halteria* we find complication as in polymerization, intensification of function, and the development of new function. *H. grandinella,* considered above the level of *H. oblonga* (K a h l 1935), probably represents the next simplest and second most primitive stage of the genus. *H. bifurcata* shows morphophysiological progress beyond *H. grandinella* in the following features: 1. The backward movement of the adoral zone to a position further on the sides of a constricted peristome. 2. The doubling of the adoral and oral membranelles, although probably only by a splitting process rather than by a doubling of membranellar area. 3. The increase in bristle length. 4. The complication of the bristles, by curves in the 1st and bifurcations in the 2nd and 4th. 5. The development of sensitivity to water movement or vibration, possibly through the bristles. Features 1, 2, and 3 represent an intensification of motor function. A posterior movement of adoral membranelles normally increases their locomotor effectiveness, and the presence in the middle of the adoral zone of a constricted peristome should also contribute to motor efficiency. Furthermore, the constriction of the peristome results in a more streamlined body shape. The polymerization of the adoral and oral membranelles increases both the efficiency of the locomotor apparatus and the efficiency of food capture. The greater bristle length should permit longer and more powerful saltatory movement (and earlier response to mechanical stimuli). Feature 5 represents the development of a new function.

Since the 2nd and 4th cirri of the new species are bifurcated, the name *H. bifurcata* is suggested for it.

S u m m a r y

A new oligotrich species, *Halteria bifurcata* sp. n., with double adoral and oral membranelles and bifurcated bristles as well as curved bristles, is described. It often contains chlorellae and other algae. The avoidance reaction,

jumping, is shown on movement in the water, permitting early escape from predators. The evolutionary advances of the new species are summarized.

New media consisting of balanced salts and skimmed milk powder are given for *Halteria grandinella*, and aspects of its structure and jumping response are reported.

ZUSAMMENFASSUNG

Eine neue Oligotrichen-Art, *Halteria bifurcata* sp. n., mit verdoppelten Adoral- und Oral- Membranellen, und mit in zwei gegabelten Borsten so wie gebogenen Borsten, wird beschrieben. Sie enthält öfters Chlorellen und andere Algen. Die Fluchtreaktion, das Springen, wird von Bewegungen im Wasser hervorgerufen, und das ermöglicht die frühe Flucht vor Raubtieren. Die evolutionären Fortschritte der neuen Art werden zusammengefasst.

Neue Nährmedien aus ausgeglichenen Salzen und getrockneter Magermilch werden für *Halteria grandinella* gegeben, und Eigenschaften des Baus und der Sprungreaktion dieser Art sind beschrieben.

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EXPLANATION OF PLATES I—IV

Halteria bifurcata sp. n.

1: One bristle group, numbered. Posterior branch of bristle 2 ends at dark center of bubble.

2: Side-view, showing constricted peristome and 2 bristle groups

3: A bristle group, numbered

4: Side-view. Adoral membranelles arise from constricted peristome. Bristle 3 is in focus

5: Side-view of swollen organism, showing bristle 4 of 2 bristle groups

6: Frontal view. A layer of bristles extends beyond double adoral membranelles

7: Bases of some adoral membranelles (A), and of oral membranelles (0). $1.000\times$

8: Track of saltatory movement. Jumping is the avoidance reaction of *Halteria*

9: Frontal view of specimen and the 4th layer of bristles, each equal in length to body diameter

10: Frontal view of another specimen and the 2nd layer of bristles, each here exceeding length of body diameter

11: Macronucleus (M) containing chromatic reticulum. New micronuclei (N), 2 groups of oral membranelles (0), and food vacuole (F) are visible. $1.000\times$

12: Specimen with at least 5 elongated algae

13: Specimen with over 20 chlorellae and 2 larger algae

14: Frontal view, showing small peripheral globules and indentations for adoral membranelles (upper right)

15: Small globules in degenerating specimen

(All photomicrographs by phase contrast. At $500\times$, except Figs. 7 and 11. Tri-X film, Diafine developer. Printed by Audio-Visual Center, Indiana State University)
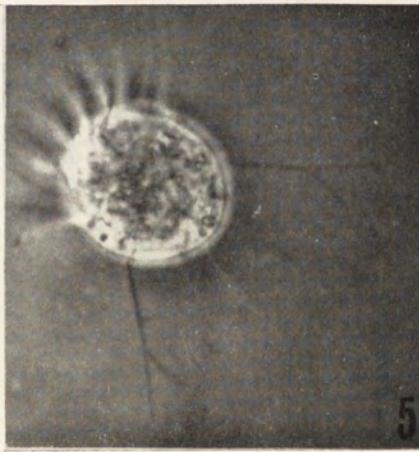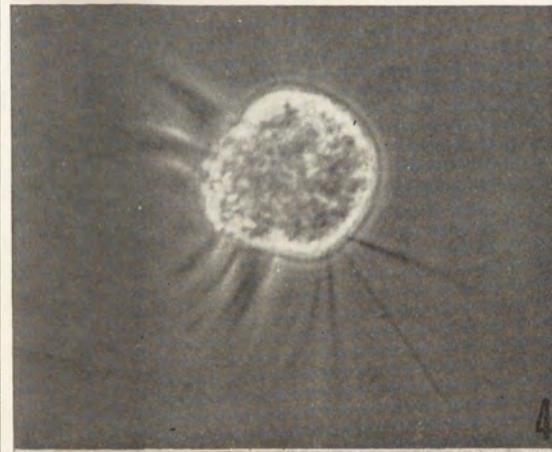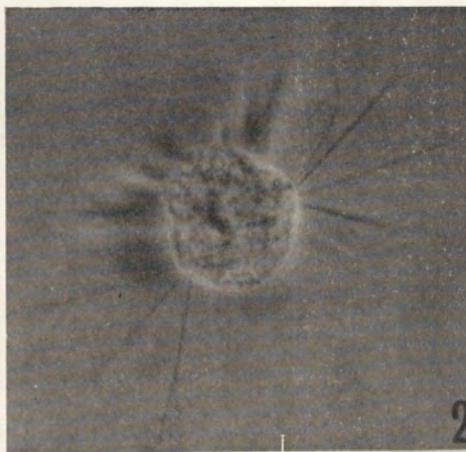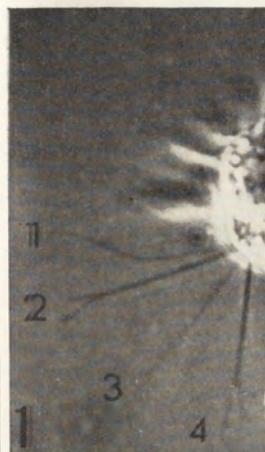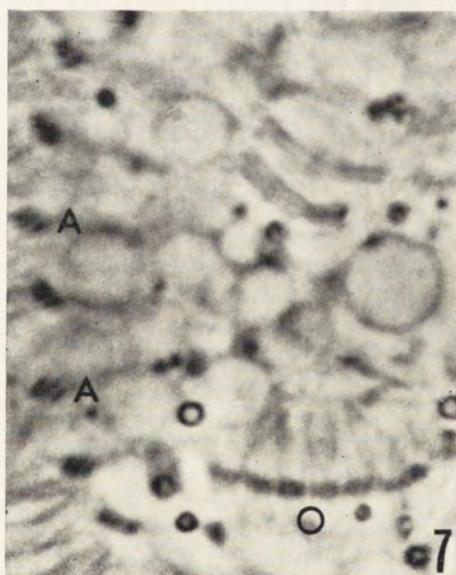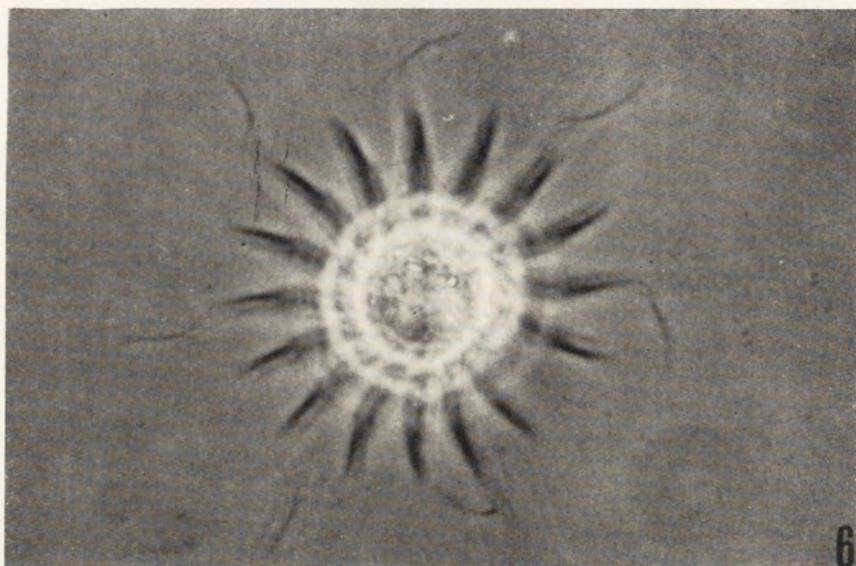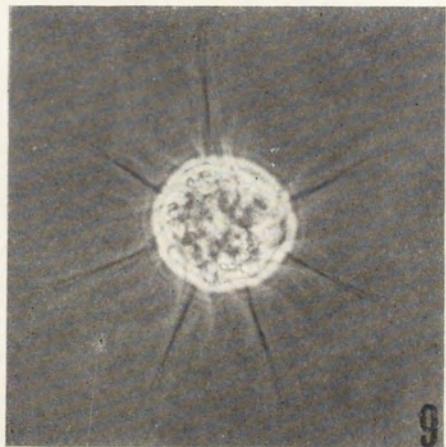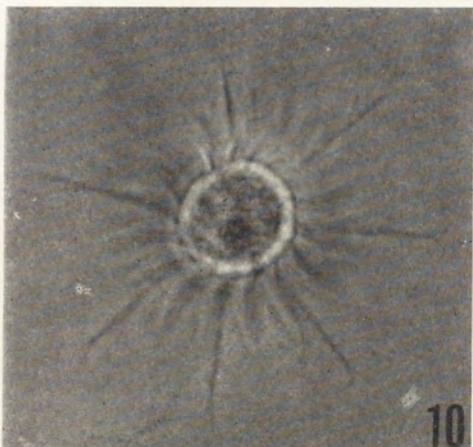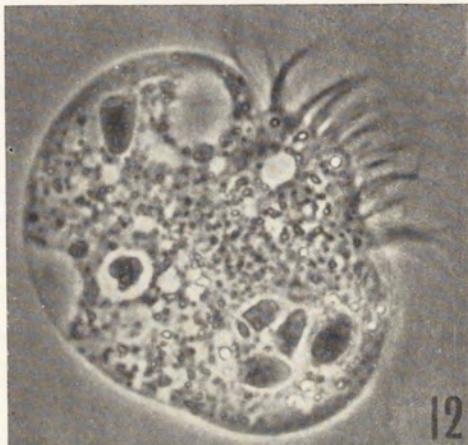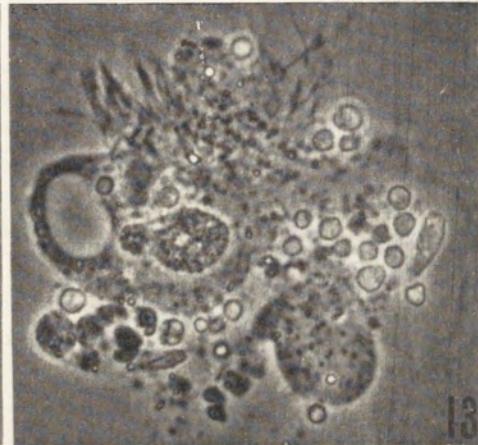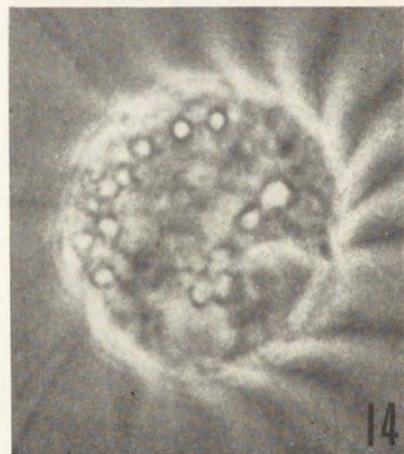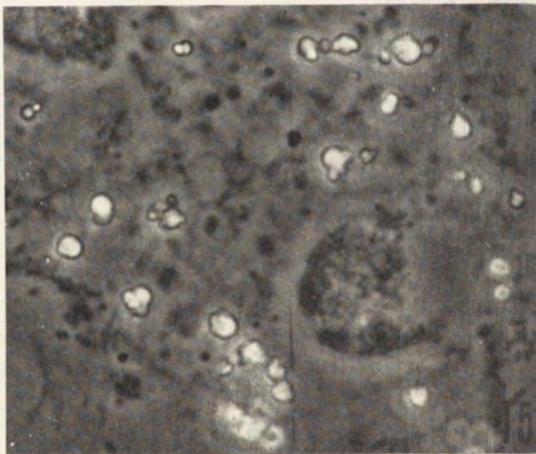
PLATE II

H. Tamar

auctor phot.

H. Tamar auctor phot.

PLATE IV

H. Tamar

auctor phot.

И. В. КУЛЕМИНА

I. V. KULEMINA

Паразитические инфузории (*Peritricha, Urceolariidae*) с молоди некоторых рыб оз. Селигер

Parasitic ciliates (*Peritricha, Urceolariidae*) from the fry and young fishes of lake Seliger

Материалом для настоящей статьи послужили урцеоларииды, обнаруженные среди прочих паразитов у личинок и мальков семи видов рыб озера Селигер в 1963—1965 г. (Poljansky et Koulemina 1965, Кулемина 1966, Кулемина, Полянский, Шульман 1967).

Для полного паразитологического обследования была взята молодь разных возрастов (от 5—7 дней до 2+ включительно) в количествах:

Rutilus rutilus	517	экз.	(0+, 1+, 2+)
Abramis brama	270	„	(0+, 1+)
Leuciscus idus	157	„	(0+, 2+)
Blicca bjoerkna	43	„	(0+)
Perca fluviatilis	111	„	(0+, 1+, 2+)
Lucioperca lucioperca	80	„	(0+)
Esox lucius	12	„	(0+)

В основном *Urceolariidae* собирались во второй половине июня с личинок 20—30 дневного возраста, когда молодь заражена триходинами на 44.0—100⁰/₀, при очень высокой интенсивности инвазии. Нередко нам попадались рыбки, вся поверхность тела которых и плавниковые складки были почти сплошь покрыты урцеолариидами. Инфузории активно перемещались с кожи личинок под жаберные крышки и обратно.

В литературе отмечено несколько случаев массового заражения триходинами рыб в естественных водоемах (Davis 1947; Schäperclaus 1954, Kozicka 1958, Grabda i Kozicka 1961, Любарская и Штейн 1967). Сведения о гибели от триходиниазиса молоди рыб разных возрастов приводятся Шеперклаусом 1954 и Козицкой 1958. Сообщения о триходинах прудовых хозяйств и рыбопитомников (Бауер и Стрелков 1959, Богданова 1963, 1967, Иванова 1966, Мусселиус 1966 и т.д.) посвящены, в основном, вопросу патогенного влияния паразитов на рыб ранних возрастов. Работы Любарской 1963, Кашковского 1966, Любарской и Штейн 1967 содержат данные по систематике, экстенсивности инвазии и сезонной динамике заражения урцеолариидами молоди рыб в природных условиях.

Обработка собранного нами материала выявила известное разнообразие видового состава *Urceolariidae* в оз. Селигер. Наряду с широко распространенными формами отмечены сравнительно редко встречающиеся. Некоторые виды оказались новыми для науки. Массовые популяции *Trichodina nigra* позволили сделать некоторые замечания относительно изменчивости внутри этого вида. Последнее обстоятельство имеет особое значение, так как существование высокой степени вариабильности у триходин требует детального морфологического анализа в систематике урцеоляриид (L o m and G. S t e i n 1966). Молодь для исследования отлавливалась в одном из плесов оз. Селигер — Городском. Сбор инфузорий проводился по общепринятой методике: сухие мазки обрабатывались на месте 2% раствором AgNO₃ по Клейну. Часть мазков фиксировалась жидкостью Шаудинна для последующей окраски по Гейденгайну. Определение *Urceolariidae* велось по системе, предложенной Г. А. Ш т е й н (1961, 1962). За постоянную помощь и ценные советы пользуемся случаем принести Г. А. Штейн искреннюю признательность.

Таблица 1
Table 1
Распределение урцеоляриид на молоди рыб в оз. Селигер
Distribution of urceolariids on fry and young fishes from the Lake Seliger

Хозяин Host	Вид паразита Species of parasite	Возраст хозяина Age of host
Плотва *Rutilus rutilus*	*Trichodina nigra* Lom, 1960	0+, 1+, 2+
	T. polycirra Lom, 1960	2+
	T. rostrata sp. nov.	0+
	Tripartiella copiosa (Lom, 1959)	0+
	T. incisa (Lom, 1959)	0+
	Trichodinella epizootica (Raabe, 1950)	1+
Лещ *Abramis brama*	*Trichodina nigra* Lom, 1960	0+, 1+
	T. rostrata sp. nov.	0+
	T. spathulata sp. nov.	0+
	Tripartiella copiosa (Lom, 1959)	0+
Язь *Leuciscus idus*	*Trichodina nigra* Lom, 1960	0+, 1+, 2+
	T. pediculus Ehrenberg, 1838	0+
	T. meridionalis (Dogiel, 1940)	0+
	Trichodina sp. 1	0+
	Tripartiella copiosa (Lom, 1959)	0+
	T. incisa (Lom, 1959)	0+
Густера *Blicca bjoerkna*	*Trichodina meridionalis* (Dogiel, 1940)	1+
	Trichodina sp. 2	0+
	Tripartiella copiosa (Lom, 1959)	1+
Окунь *Perca fluviatilis*	*Trichodina nigra* Lom, 1960	0+, 1+, 2+
	T. pediculus Ehrenberg, 1938	0+
	T. urinaria Dogiel, 1940	1+, 2+
	T. domerguei f. *acuta* Lom, 1961	0+, 1+, 2+
	Trichodinella epizootica (Raabe, 1950)	0+, 1+, 2+
Судак *Lucioperca lucioperca*	*Trichodina nigra* Lom, 1960	0+
	T. pediculus Ehrenberg, 1838	0+
Щука *Esox lucius*	*Trichodina demorguei* f. *esocis* Lom, 1960	0+
	Trichodinella epizootica (Raabe, 1950)	0+

Все приведенные микрофотографии сделаны с препаратов импрегнированных $AgNO_3$ при компенсационном окуляре 10× и объективе 90×. Рисунки выполнены с рисовальным аппаратом при окулярах 10×, 15× или 20× и объективе 90×.

Урцеолярииды распределялись по хозяевам в следующем порядке (Таблица 1).

Trichodina nigra Lom, 1960

Описана Ломом (L o m, 1960) с кожи различных рыб из водоемов Чехословакии. В СССР обнаружена: К а н д и л о в ы м 1964 у сазана в бассейне р. Куры, Л ю б а р с к о й 1965 на плотве в бассейне р. Волги, К а ш к о в с к и м 1965 — бассейн р. Урал — на сазане и соме, К о с т е н к о 1967 в течении Среднего Днепра, А л л а м у р а т о в ы м 1967 у маринки, сазана, ельца, пескаря, храмули, усача и гольца из водоемов Узбекистана, а также в рыбопитомниках — на Выгском заводе у годовиков семги и у радужной форели в Ропше (Ленинградской обл.) Б о г д а н о в о й 1967.

В оз. Селигер для *T. nigra* отмечен наиболее широкий круг хозяев — из семи исследованных видов молоди рыб личинки и мальки шести видов — *Rutilus rutilus, Abramis brama, Leuciscus idus, Blicca bjoerkna, Perca fluviatilis* и *Lucioperca lucioperca* были заражены этими инфузориями на 44—100% при очень высокой степени интенсивности инвазии (кроме *Blicca bjoerkna*).

Таким образом, в нашем распоряжении оказался массовый материал по *T. nigra*, собранный одновременно с различных одновозрастных хозяев (возраст молоди 25—40 дней, длина тела — 16—28 мм). Небольшое количество экземпляров того же вида цилиат получено с двухлеток язей (1+) (Табл. I 3, 4). На микрофотографиях (Табл. I, II) представлены инфузории с молоди пяти видов рыб. Структура зубцов, характер импрегнации и строения прикрепительного венчика, а также биометрические данные (Таблица 2) свидетельствуют об их несомненной принадлежности к *T. nigra*. Бросается в глаза одна особенность — каждый вид хозяев имеет две „габитуально" различные формы триходин — одна с тонкими наружными лопастями и сравнительно узкими внутренними шипами наиболее сходна с типичной *T. nigra* (L o m 1961). Другая „форма", имея более массивные наружные выросты и менее заостренные, иногда укороченные, широкие, внутренние отростки, напоминает *T. nigra* f. *gobii* (L o m 1961). Для простоты изложения мы условно первую из „форм" называем „узкой", вторую — „широкой" (Табл. I 3—6, II 7-12). Промеры обеих форм почти совпадают (Таблица 2). Просмотр гематоксилиновых препаратов выявил лишь общую картину ядерного аппарата. Детали строения зубцов в венчиках не удалось сравнить из-за недостаточной четкости окрашенных препаратов.

Как показывает Таблица 3, промеры ядерного аппарата *T. nigra* в материале Л о м а 1961 и нашем почти не разнятся.

Возможно, наличие на молоди таких двух „форм" связано с характерной для триходин широкой внутривидовой изменчивостью. В работе Лома и Штейн (L o m and S t e i n G. 1966) приводятся сведения о значительной вариабильности *Trichodina tenuidens* Fauré-Fremiet, 1944.

Внутри популяций *T. nigra* с молоди рыб наблюдаются заметные различия в строении зубцов. Как пример, на Рис. 1 изображены участки прикрепительного венчика *T. nigra* с окуня (А—Е) и плотвы (F—J). Следует отметить, что по

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Таблица 2—

Сравнение биометрических данных *Trichodina*
Comparison of biometric data od *Trichodina nigra*

Хозяин — Host	*Leucsicus idus* 0+		*Leucsicus idus* 1+	
Формы — Forms	,,широкие'' "wide"	,,узкие'' "narrow"	,,широкие'' "wide"	,,узкие'' "narrow"
Диаметр прикрепительного диска Diameter of adhesive disc	—	39.0—51.0 (44.0—46.0)	40.5—44.0 (40.5—41.5)	34.0—39.0 (36.0—37.0)
Диаметр венчика Diameter of denticulate ring		35.0—49.0 (37.0—41.5)	37.0—41.3 (38.0—39.0)	31.0—37.0 (34.0—36.0)
Длина наружного отростка зубца Length of outer blade of denticle		5.5—9.0 (7.0—7.6)	6.5—8.3 (7.0—8.0)	6.0—6.5
Длина внутренного отростка зубца Length of inner ray of denticle		5.5—8.2 (6.5—7.0)	5.5—6.5 (6.0—6.5)	5.0—6.5 (6.0)
Количество зубцов Number of denticles		20—27 (22—23—24)	21—24 (22—23)	20—24 (21—22)
Число полос диска Number of radial pins		8—9—10	9—10—11	9—10

Таблица 3
Table 3

Ядерный аппарат *Trichodina nigra* (измерения в μ)
Nuclear apparatus of *Trichodina nigra* (measurements in μ)

Автор Author	L o m 1961	Наши данные Present author
Диаметр макронуклеуса Diameter of macronucleus	32.0—52.0	31.2—48.1
Отрезок ,,x'' Distance "x"	—	9.09—21.2
Размеры микронуклеуса Dimensions of micronucleus	1.0—2.5×3.0—5.5	1.3—3.9×2.6—6.0
Отрезок ,,y'' Distance "y"	+5—22.0	0—27.3
Хозяин Host	различные карповые и окунь varoius *Cyprinidae*, *Perca fluviatilis*	молодь различных карповых, окунь и судак varoius young *Cyprinidae*, *Perca fluviatilis* and *Lucioperca lucioperca*
Локализация Localization	кожа, реже жабры skin, rarely gills	кожа, плавники, жабры, ротовая полость skin, fins, gills and buccal cavity

форме зубцы триходин с различных хозяев разнятся, в общем, не более, чем таковые внутри популяций с одного хозяина.

Наконец, на личинке плотвы нами обнаружен аберрантный экземпляр *T. nigra* (Табл. I 2). Диаметр розетки инфузории 51.0 μ, венчика 45.5 μ. Количество зубцов 19. Наружные и внутренние отростки приблизительно одинаковой длины (около 8.0 μ). От типичных *T. nigra* найденный экземпляр отличаетсъ формой зубцов, лопасти которых кнаружи более расширены и спереди заострены. Пальцевидные внутренние выросты направлены под углом к центру. Они одинаково расширены на всем протяжении и имеют закругленные концы.

Table 2

nigra различных хозяев (измерения в μ)
from different hiosts (measurements in μ)

Rutilus rutilus 0+		Abramis brama 0+		Perca fluviatilis 0+		Lucioperca lucioperca 0+	
"широкие" "wide"	"узкие" "narrow"	"широкие" "wide"	"узкие" "narrow"	"широкие" "wide"	"узкие" "narrow"	"шрокие" "wide"	"узкие" "narrow"
40.0—52.0	31.0—54.0	48.0—51.0	40.0—56.0	42.0—49.0	39.0—47.0	37.0—49.0	27.0—40.0
(49.0—52.0)	(49.0—52.0)	(48.0—49.0)	(48.0—51.0)	(46.0—47.0)	(42.0—43.0)	(44.0—45.0)	(36.0—37.0)
38.0—50.0	29.0—50.0	45.0—49.0	38.0—51.0	40.0—47.0	37.0—45.0	34.0—46.0	25.0—37.0
(48.0—50.0)	(39.0—46.0)	(48.0)	(46.0—50.0)	(44.0—46.0)	(40.0—42.0)	(38.0—41.0)	(34.0—35.0)
7.0—9.0	5.0—9.0	7.6—8.7	6.5—9.0	7.0—9.0	6.5—9.0	6.0—8.7	5.0—7.6
(8.0—8.6)	(7.0—7.6)	(8.7)	(7.0—7.6)	(8.3—9.0)	(7.0—7.8)	(7.0—8.0)	(6.0—6.5)
6.5—9.0	5.0—9.0	7.6—9.0	5.5—9.0	6.0—7.0	6.5—8.0	5.0—7.0	5.5—7.0
(7.6—8.0)	(7.0—7.6)			(6.5—7.0)	(7.0—7.2)	(6.0—7.0)	(6.0—6.5)
22—24	20—26	22—23	22—25	20—23	21—25	21—24	20—25
(22—23)	(24—25)	(22)	(23—24)	(22)	(22—23)	(21—22)	(22)
10—12	9—11	10—12	9—12	9—10—11	8—12	10—11	9—11
(11)	(10)	(11)	(11)		(10—11)		

Рис. 1. Вариации форм зубцов *Trichodina nigra* Lom, 1960. А—Е — с личинок и сеголеток *Perca fluviatilis*, F—J — с личинок и сеголеток *Rutilus rutilus*
Fig. 1. Variation of shape of the denticles of *Trichodina nigra* Lom, 1960. А—Е — from the fry and young specimens of *Perca fluviatilis*, F—J — from the fry and young specimens of *Rutilus rutilus*

Таким образом, *T. nigra* свойственна известная внутривидовая изменчивость в строении прикрепительного аппарата, обусловленная, по-видимому, паразитированием этих цилиат среди широкого круга хозяев. В настоящее

время *T. nigra* отмечена у 20 видов рыб. Нападая одной из первых на личинок, вскоре после их выклева, *T. nigra* в различной степени инвазирует молодь любых возрастов и половозрелые экземпляры.

Trichodina domerguti f. *acuta* Lom, 1961

Впервые описана Ломом (L o m 1961) с сазана, окуня, судака, верховки из водоемов Чехословакии, где распространена довольно широко.

Из пресноводных водоемов СССР известна для бассейная р. Куры (К а н д и л о в 1964) и р. Урал (К а ш к о в с к и й 1966), где встречена у судака. Ре-

Таблица 4
Table 4

Промеры *Trichodina domerguei* f. *acuta* (измерения в μ)
Biometric data of *Trichodina domergu.* f. *acuta* (measurements in μ)

Автор Author	L o m 1961	К а н д и л о в 1964	К а ш к о в- с к и й 1965	И в а н о в а 1966	Наши данные Present author
Диаметр тела Diameter of body	50.0—86.0	—	43.0—62.0	57.2—67.7	—
Диаметр прикрепи- тельного диска Diameter of adhesive disc	30.0—66.0	31.0—38.0	34.0—50.0	41.6—57.2	34.0—45.0
Диаметр венчика Diameter of denticu- late ring	18.0—40.0	30.0—35.0	31.0—42.0	28.6—36.4	29.0—40.0
Длина наружного отростка зубца Length of outer blade of denticle	4.5—6.0	6.0—7.0	5.0—6.2	5.2	5.5—8.0
Длина внутренного отростка зубца Length of inner ray of denticle	4.0—7.0	5.0—7.0	6.2—8.7	6.3	6.0—7.0
Количество зубцов Number of denticles	15—23	18—20	21—22	19—25	17—24
Число полос диска Number of radial pins	9—11	10	8—10	10—12	8—12
Хозяин Host	Сазан, окун, су, дак, верховка, гор-чак *Cyprinus carpio, Perca fluviatilis, Lu-cioperca lucioperca, Leucaspius delineat-us, Rhodeus sericeus*	судак *Lucioperca lucioperca*	судак *Lucioperca lucioperca*	сазан *Cyprinus carpio*	окунь 0+, 1+, 2+ *Perca fluviatilis*
Локализация Localization	поверхность тела (кожа и плав-ники, иногда жаб-ры) surface of the body (skin and fins, occa-sionally gills)	поверхность тела surface of the body	поверхность тела, плавни-ки, носовые ямки surface of the body, olfactory sacs	поверхность тела surface of the body	поверхность тела surface of the the body

ш е т н и к о в а 1965 обнаружила этих урцеоляриид у сазана разных возрастов Цимлянского нерестово-вырастного хозяйства. В рыбхозах Московской обл. отмечена И в а н о в о й 1966 на карпах.

В озере Селигер зарегестрирована на мальках окуня (поверхность тела, плавники и жабры) (Табл. III 13).

В Таблице 4 проводится сравнение биометрических данных известных нам популяций *T. domerguei* f. *acuta*.

Приведенная таблица свидетельствует об относительной однородности размеров указываемых инфузорий в различных водоемах Советского Союза.

Trichodina domerguei f. *esocis* Lom, 1960

Описана Ломом (L o m 1960) с поверхности тела щуки из пресноводных водоемов Чехословакии.

В пределах Советского Союза отмечена Л ю б а р с к о й 1963 на щуке в бассейне Волги, К а ш к о в с к и м 1965 у судака Ириклинского водохранилища (бассейн р. Урал), Б о г д а н о в о й 1967 на годовиках радужной форели в рыбхозе „Гостилицы" Ленинградской области. Нами *T. domerguei* f. *esocis* обнаружена в значительных количествах на коже, плавниках и жабрах сеголетка щуки (Табл. III 14).

Сравнение размеров элементов прикрепительного диска этого вида триходин из различных водоемов дано в Таблице 5.

Таблица 5
Table 5
Биометрические данные *Trichodina domerguei* f. *esocis* (измерения в μ)
Biometric data of *Trichodina domerguei* f. *esocis* (measurements in μ)

Автор Author	L o m 1960	Л ю б а р- с к а я 1963	К а ш к о в- с к и й 1965	Наши данные Present author
Диаметр тела Diameter of body	—	—	42.0—74.0	—
Диаметр прикрепительного диска Diameter of adhesive disc	38.0—62.0	64.4	33.0—56.0	47.0—53.0
Диаметр венчика Diameter of denticulate ring	22.0—39.0	41.6—44.8	29.0—42.0	42.0—49.0
Длина наружного отростка зубца Length of outer blade of denticle	6.2—8.1	6.2—6.4	5.0—6.2	6.5—8.0
Длина внутренного отростка зубца Length of inner ray of denticle	7.0—8.5	4.8—6.4	5.0—7.2	6.5—9.0
Количество зубцов Number of denticles	20—27	24—26	21—25	24—27
Число полос диска Number of radial pins		12—14	8—10	10—11
Хозяин Host	щука *Esox lucius*	щука *Esox lucius*	судак *Lucioperca lucioperca*	щука 0+ *Esox lucius*
Локализация Localization	поверхность тела, жабры surface of the body, gills	поверхность тела surface of the body	жабры gills	поверхность тела, плавники, жабры surface of the body, gills

Trichodina pediculus (O. F. Müller, 1786) Ehrenberg, 1838

Первоначально описана с гидр (Müller 1786, Ehrenberg 1836, James-Clark 1866); данные о *T. pediculus* Quennerstedt 1869 и Wallengren 1897 основаны на материале с рыб (Raabe 1959). В пресноводных водоемах Китая этот вид отмечен Chen Chih-leu 1956 у белого и черного амура, толстолобиков и *Aristichthis nobilis.*

В Советском Союзе зарегистрирована Османовым 1964 у годовиков белого амура и сеголеток толстолобика из водоемов Узбекистана, Ивановой 1966 у белого амура, белого толстолобика и карпов в рыбхозах Московской области.

Отмечена Wellborn 1967 на *Micropterus salmoides* в пресноводных водоемах США (Алабама).

Нами единичные экземпляры *T. pediculus* собраны с поверхности тела личинок язя, окуня и судака. Биометрические данные для инфузорий данного вида приведены в Таблице 6.

Из приведенной таблицы видно, что инфузории с молоди рыб оз. Селигер несколько мельче по размерам и числу зубцов, чем паразитирующие в рыбхозах Московской обл.

Trichodina meridionalis (Dogiel, 1940)

Описана Догелем 1940 с сома, морской иглы и одного из бычков Каспийского, Черного и Азовского морей.

Тимофеевым (цитиров. по Штейн 1962) этот вид отмечен для сома, судака и окуня Невской губы Балтийского моря, Османовым 1963 для судака и сома бассейна Сыр-Дарьи и Кандиловым 1964 у шемаи в бассейне р. Куры.

Довольно значительное количество экземпляров *T. meridionalis* обнаружено нами в обонятельных ямках густеры (1+) и единичные инфузории на коже личинок язя. Триходины имеют характерную форму наружных отростков в виде широких, закругленных, слегка изогнутых лопастей. Центральная часть зубца выражена относительно слабо. Внутренние отростки слабо изогнуты, их основания заметно расширены, а концы заострены (Табл. III 15). Биометрические данные обнаруженных в СССР *T. meridionalis* представлены в Таблице 7.

Сравнение *Trichodina meridionalis* из различных водоемов СССР показывает, что промеры инфузорий с шемаи и густеры почти совпадают. Возможно, это связано с одинаковой локализацией паразита.

Trichodina urinaria Dogiel, 1940

Этот обычный, широко распространенный паразит окуневых отмечен нами в мочевом пузыре окуней возраста 1+ и 2+. Зараженность молоди очень незначительна — 6.6—13.2% при низкой интенсивности инвазии (не более 12—15 особей в одной рыбе). Инфузории имели обычную для вида форму зубцов и следующие размеры: диаметр розетки 44.0—45.0 μ; диаметр венчика 42,0 μ; длина наружных лопастей 7.0—7.8 μ, внутренних — 7.0—7.8 μ. Число зубцов 33.

Таблица 6
Table 6

Биометрические данные *Trichodina pediculus* (измерения в μ)
Biometric data of *Trichodina pediculus* (measurements in μ)

Автор Author	Chen Chih-leu 1956	Иванова 1966	Wellborn 1967	Наши данные Present author		
Диаметр тела Diameter of body	53.9—75.5	62.4—75.4	69.0(61.0—86.0)	—	—	—
Диаметр прикрепительного диска Diameter of adhesive disc	41.7—68.2	49.4—65.0	48.0(45.0—57.0)	44.0—52.0	40.0	39.0
Диаметр венчика Diamater of denticulate ring	29.1—43.1	33.8—54.6	29.0(25.0—35.0)	39.0—44.0	30.0—35.0	34.0
Длина наружного отростка зубцов Length of outer blade of denticle	6.2	5.2	6.0(5.5—7.0)	6.0(5.5—6.5)	4.6—5.2	4.6
Длина внутреннего отростка зубца Length of inner ray of denticle	9.8	7.8	7.0—9.0	8.0—10.9	9.1	9.0
Количество зубцов Number of denticles	23—26	27—33	23(22—26)	24—27	26—27	25
Число полос диска Number of radial pins	—	8—10	—	8—9	8—9	9
Хозяин	белый и черный амуры, толстолобик	белый амур, белый толстолобик, карпы	язь		судак	окунь
Host	*Ctenopharyngodon idella, Mylopharyngodon piceus Hypophthalmichtys molitrix, Aristichthys nobilis*	*Ctenopharyngodon idella, Hypophthalmichtys molitis, Cyprinus carpio*	*Micropterus salmoides*	*Leuciscus idus*	*Lucioperca lucioperca*	*Perca fluviatilis*
Локализация	кожа, жабры	жабры	поверхность тела, плавники	поверхность тела, плавники, жабры		
Localization	skin, gills	gills	surface of the body, fins	surface of the body, fins, gills		

Таблица 7
Table 7

Биометрические данные *Trichodina meridionalis* (измерения в μ)
Biometric data of *Trichodina meridionalis* (measurements in μ)

Автор Author	Догель 1940	Тимофеев (по Штейн 1962)			Османов 1963	Кандилов 1964	Наши данные Present author
Диаметр тела Diameter of body	17.8—30.0	—	—	—	—	—	—
Диаметр прикрепительного диска Diameter of adhesive disc	—	19.8—27.5	18.7—29.7	17.6—27.0	37.7	23.0—30.0	24.0—27.0
Диаметр венчика Diameter of denticulate ring	—	22.0—27.0	17.0—23.0	17.0—19.0	31.0—34.8	20.0—25.0	22.0—26.0
Длина наружного отростка зубца Length of outer blade of denticle	—	4.4—5.5	4.4—6.6	4.4—5.5	5.7—6.5	5.0—7.0	5.0—6.5
Длина внутреннего отростка зубца Length of inner ray of denticle	—	—	—	—	3.9—5.2	3.6—4.0	5.0—5.5
Количество губцов Number of denticles	—	—	—	—	19—24	16—18	1 5—19
Число полос диска Number of radial pins	—	—	—	—	—	—	—
Хозяин Host	сом, морская игла, *Silurus glanis*, *Syngnathus* sp. *Mesogobius* sp.	сом *Silurus glanis*	судак *Lucioperca lucioperca*	окунь *Perca fluviatilis*	судак, сом *Lucioperca lucioperca* *Silurus glanis*	шемая *Chalcalburrus chalcoides*	густера 1+ *Blicca bjoerkna*
Локализация Localization	жабры gills	жабры gills			жабры gills	обонятельные ямки olfactory sacs	обонятельные ямки olfactory sacs
Характер водоемов Characteristic of waters	солоноводные salt	солоноватоводные brackish				пресноводные fresh	

Trichodina polycirra Lom, 1960

Впервые описана Л о м о м из плотвы в Чехословакии.

В СССР обнаружена у куринской воблы (К а н д и л о в 1964) в бассейне р. Куры и К а ш к о в с к и м 1965 у плотвы и густеры Ириклинского водохранилища (бассейн р. Урал). В обоих случаях локализация — мочевой пузырь.

Нами встречена один раз в мочеточниках плотвы 2+ (6 экз.). Из-за неудовлетворительного качества препарата, импрегнированного $AgNO_3$, мы не можем сравнить нашу находку с вышеописанными.

Trichodina spathulata sp. nov.

Паразитирует на жабрах личинок леща. Отмечена у 28.0% обследованной молоди. Интенсивность заражения довольно значительна. Единичные экземпляры собраны с плавников.

Сравнительно мелкие инфузории (Табл. III 16), диаметр тела 27.5—48.4 µ, диска — 23.0—29.0 µ (чаще 27.0 µ), венчика 22.0—27.0 µ (чаще 25.0 µ). Число зубцов, составляющих венчик, 18—20. Наружные лопасти 4.6—6.0 µ (чаще 5.2—6.0 µ), внутренние — 4.6—5.5 µ (чаще 5.0—5.2 µ). Число кутикулярных полосок на один зубец равно 8. Адоральная спираль образует дугу 360—370°. Макронуклеус подковообразный, его диаметр 16.9—30.0 µ, расстояние между концами 3.9—10.4 µ. Микронуклеус размером 2.5—5.0 × 1.0—2.6 µ, округлый или овальный, лежит снаружи от Ма, на некотором расстоянии от последнего. Величина отрезка „у" вариирует от + 6.5 до 19.8 µ (Рис. 2 А—Е).
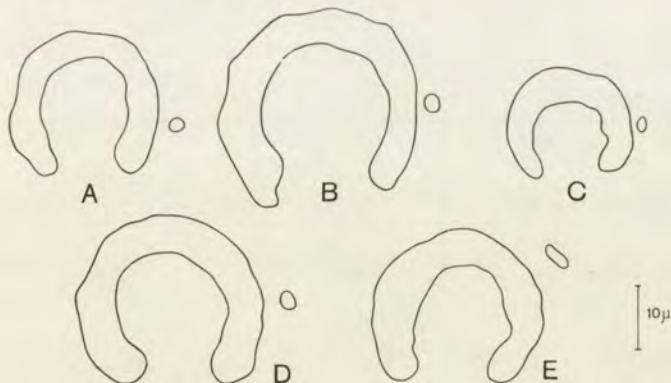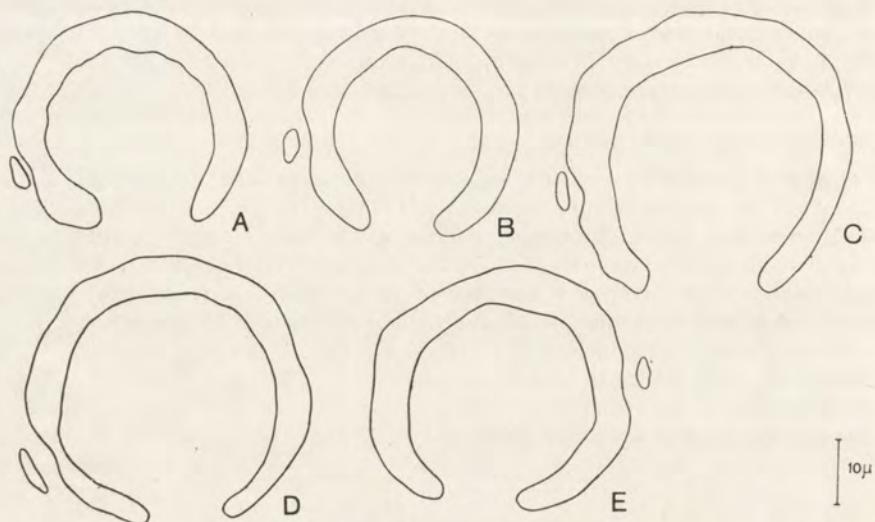

Рис. 2. *Trichodina spathulata* sp. nov. Вариации формы и положения микронуклеуса

Fig. 2. *Trichodina spathulata* sp. nov.; variation of shape and position of micronucleus

Характерной особенностью описываемых инфузорий является специфическая форма зубца. Его наружная ветвь, суженная около центральной части крючка, по направлению к перифории диска продолжается в широко закругленную, лопатообразную лопасть. Тело зубца имеет форму узкого и короткого тонкостенного конуса. Внутренние отростки слегка изогнутые, одинаково рас-

ширенные на всем протяжении. Их концы заострены в большей или меньшей степени.

Рис. 3. *Trichodina spathulata* sp. nov. Часть венчика зубцов (импрегнация AgNO₃)

Fig. 3. *Trichodina spathulata* sp. nov.; fragment of the denticulate ring (AgNO₃ impregnation)

Зубцы, составляющие венчик, сравнительно длинные (Рис. 3). Прикрепительная розетка тонкостенная, нежная. Центральная часть диска импрегнируется AgNO₃, так же, как у *T. nigra*. Однако от последней описываемый вид резко отличается формой зубцов и биометрическими данными (Таблица 8).

Более всего формой наружных лопастей зубцы *T. spathulata* напоминают *T. puytoraci* Lom, 1962 с *Mugil cephalus* (Lom 1962) отличаясь значительно

Таблица 8
Table 8

Сравнение некоторых биометрических данных *Trichodina spathulata*
sp. nov. *T. nigra* Lom, 1960 *T. microdenticulata* Wellborn, 1967
Comparison of biometric data of *Trichodina spathulata* sp. nov.,
T. nigra Lom, 1960 and *T. microdenticulata* Wellborn, 1967

Автор Author	*T. nigra* Lom, 1960	*T. microdenticulata* Wellborn, 1967	*T. spathulata* sp. nov.
Диаметр тела Diameter of body	—	26.0(22.0—37.0)	27.5—48.4
Диаметр прикрепительного диска Diameter of adhesive disc	40.0—56.0(48.0—51.0)	13.0(12.0—15.5)	23.0—29.0(27.0)
Диаметр венчика Diameter of denticulate ring	38.0—51.0(46.0—50.0)	6.9(6.0—8.0)	22.0—27.0(25.0)
Количество зубцов Number of denticles	22—25(23—24)	16(15—18)	18—20
Число полос диска Number of radial pins	9—12(11)	5	8
Адоральная спираль Adoral zone	более 360° over 360°	330°—350°	360°
Хозяин Host	*Cyprinidae,* *Percidae*	*Micropterus* *salmoides*	*Abramis* *brama*
Локализация Localization	кожа, плавники, ротов. и жаберн. пол. skin, fins, buccal cavity and gill chambers	кожа, плавники skin, fins	жабры, редко плавники gills, rarely fins
Местонахождение Locality	озеро Селингер Lake Selinger	США USA	озеро Селингер Lake Selinger

более короткими внутренними шипами, отсутствием неимпрегнируемых участков в центральной части диска и иной конфигурацией тела зубца.

От *T. microdenticulata* Wellborn, 1967, наш вид отличается формой зубцов (Рис. 7 А—В) и биометрическими данными (Таблица 8).

Дифференциальный диагноз

Trichodina spathulata sp. nov. — сравнительно мелкие инфузории. Наружные лопасти лопатообразно расширены к периферии диска и широко закруглены. Внутренние шипы длинные, слегка изогнутые. Центр прикрепительной розетки сильно импрегнируется AgNO₃. На каждый зубец приходится 8 кутикулярных полос. Число зубцов в венчике от 18 до 20. Адоральная спираль образует дугу не менее 360°. Макронуклеус подковообразный. Микронуклеус округлый или овальный, расположен с наружной стороны макронуклеуса.

Хозяин — Лещ *Abramis brama* (личинки).

Локализация — жабры.

Место нахождения — озеро Селигер.

Trichodina rostrata sp. nov.

В озере Селигер сравнительно редко встречающийся вид: обнаружена у пяти личинок плотвы и одного сеголетка леща. Паразитирует на коже, плавниках и жабрах. Интенсивность заражения незначительна.

Довольно крупные инфузории (Табл. III 17). Диаметр тела 49.5—84.7 μ, розетки — 43.0—53.0 μ, чаще 46.0—48.0 μ, венчика — 38.0—47.0 μ, чаще 46.0—47.0 μ. Число зубцов в розетке 23—25 (чаще 23). Длина наружной лопасти зубца 6.5—8.0 μ, внутренних отростков — 6.5—8.0 μ. На один зубец венчика приходится 9—11 кутикулярных полос. Центр диска ровно импреднируется AgNO₃, адоральная спираль образует дугу 360—390°. Диаметр подковообразного макронуклеуса 33.0—44.0 μ, расстояние между его концами 11.0—16.5 μ. С наружной стороны макронуклеуса, сбоку от одной из его ветвей лежит овальный микронуклеус. Его размер 3.8—6.5 × 2.0—3.8 μ. Значение „y" всегда положительно (11.0—27.5 μ) (Рис. 4 А—Е).

Основная особенность инфузорий данного вида — структура элементов прикрепительного венчика. На импрегнированных препаратах хорошо заметен выступ в основании наружной лопасти зубца, нависающий спереди над центральной частью в виде козырька (Рис. 5 А). От остальной части лопасти этот „рострум" отделен более или менее заметной выемкой. На очень слабо импрегнированных препаратах, где нижний край наружной лопасти виден наиболее отчетливо (Рис. 5 В), он оказывается лишенным и выроста, и выемки. Очевидно, в этом участке лопасти стенка очень тонка и легко обламывается при обработке препарата, создавая видимость вырезанного края наружной лопасти зубца. Таких инфузорий с „выломанной" лопастью Л о м 1963 отнес к подроду *Paratrichodina*. Однако представители этого подрода обладают укороченной адоральной спиралью. Последняя у описываемых нами экземпляров образует дугу более 360°. На основании этого признака мы относим описываемый вид к роду *Trichodina*.

Вторая характерная особенность *T. rostrata* — строение центральной части зубца, имеющей вид массивного, слегка изогнутого конуса с плотными стенками. Передняя часть тела зубца заметно расширена и несет хорошо выра-

Рис. 4. *Trichodina rostrata* sp. nov. Вариации формы и положения микро-
нуклеуса

Fig. 4. *Trichodina rostrata* sp. nov., variation of shape and position of micro-
nucleus
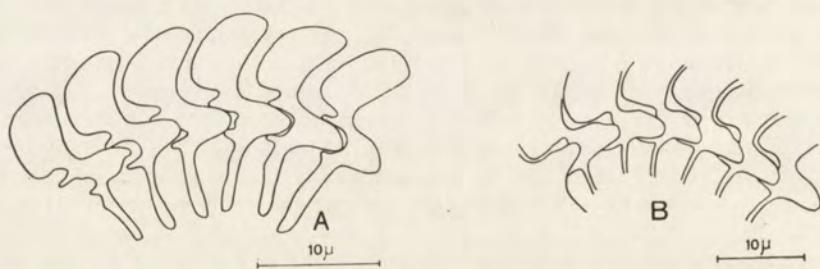
Рис. 5. *Trichodina rostrata* sp. nov. A — часть венчика зубцов, обычная им-
прегнация AgNO₃, B — Участок слабо импрегнированного прикрепительного
диска. Видно строение центральной части зубца и нижнего края наружной
лопасти

Fig. 5. *Trichodina rostrata* sp. nov. A — fragment of the denticulate ring (nor-
mal AgNO₃ impregnation), B — fragment of the denticulate ring weakly impregn-
ated. The shape of the lower part of blade and central part of denticle is visible

женные внутренние выросты, плотно прилегающие к внутренней стороне тела
предыдущего зубца (Рис. 5 A, B). Зубцы венчика прочно соединены и при по-
верхностной установке тубуса их центральные части имеют вид сплошного
широкого валика.

Внутренние отростки зубцов прямые или очень слабо изогнутые, более или
менее широкие, с закругленными концами. От описанной У Бао-хуа 1961
(цитир. по Штейн 1962) *T. rutili* Wu, 1961, наш вид отличается тонкостенными
зубцами и закругленными лопастями (Рис. 6). От *T. hypsilepis* Wellborn, 1967

и *T. funduli* Wellborn, 1967 *T. rostrata* sp. nov. отличается формой зубцов (Рис. 7 C—E).

Дифференциальный диагноз

Trichodina rostrata sp. nov. — довольно крупные инфузории. На импрегнированных препаратах расширенные и закругленные наружные лопасти имеют в основании заметный выступ („рострум"), отделенный от остальной части лопасти небольшой выемкой. Массивная конусообразная центральная часть
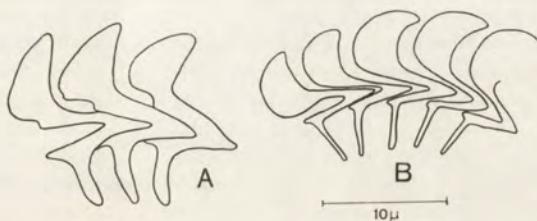
Рис. 6. Часть прикрепительного венчика *Trichodina rostrata* sp. nov. (A) и *T. rutili* Wu, 1961 (B) (гематоксилин)
Fig. 6. Fragments of the denticulate ring of *Trichodina rostrata* sp. nov. (A) and *T. rutili* Wu, 1961 (iron haematoxylin)

зубца имеет внутренний вырост. Внутренние отростки прямые или слегка изогнутые. Центр диска хорошо импрегнируется $AgNO_3$. Число зубцов в венчике 23—25. На каждый зубец приходится 9—11 кутикулярных полос. Адоральная спираль образует дугу более 360°. Макронуклеус подковообразный. Микронуклеус овальный или палочковидный лежит сбоку от наружной ветви макронуклеуса.
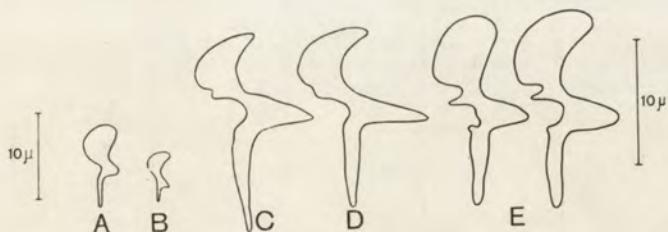
Рис. 7. Отдельные зубцы различных *Trichodina*: A — *T. spathulata* sp. nov.; B — *T. microdenticulata* Wellborn, 1967, C — *T. funduli* Wellborn, 1967, D — *T. hypsilepis* Wellborn, 1967, E — *T. rostrata* sp. nov.
Fig. 7. Denticles of various *Trichodina*: A — *T. spathulata* sp. nov., B — *T. microdenticulata* Wellborn, 1967, C — *T. funduli* Wellborn, 1967, D — *T. hypsilepis* Wellborn, 1967, E — *T. rostrata* sp. nov.

Хозяин — плотва *Rutilus rutilus* (личинки), редко — *Abramis brama* лещ (0+).

Локализация — кожа, плавники, жабры.

Место нахождения — озеро Селигер.

Trichodina sp. 1

Обнаружена на жабрах 6.3% обследованных сеголеток язей (интенсивность инвазии до 30 экз.) и один раз на поверхности тела малька 2+ (единственный экземпляр).

Инфузории средних размеров (Табл. III 18). Диаметр прикрепительного диска 34.0—46.0 μ, венчика зубцов — 30.5—44.0 μ, чаще — 33.0—37.0 μ. Длина наружной лопасти 6.5—8.5 μ, чаще 7.0—8.0 μ. Внутренние отростки 5.5—8.0 μ, чаще 6.5—7.2 μ. Число зубцов в венчике 23—27. На каждый зубец приходится 9—10 кутикулярных полос. Ядерный аппарат не исследован.

Характерная особенность данного вида — строение наружной лопасти зубца, края которой почти параллельны. Тело зубца имеет вид относительно короткого и узкого тонкостенного конуса. Внутренние отростки прямые или чуть заметно изогнутые. Центральная часть прикрепительного диска сильно импрегнируется AgNO₃. Зубцы по форме наиболее походят на *T. nigra* f. *cobitis* Lom, 1960. От последней наш вид отличается более широким расстоянием между лопастями зубцов. Хотя отмеченные особенности позволяют выделить этот вид инфузорий как самостоятельный, их недостаточно, чтобы дать ему видовое название.

Trichodina sp. 2 с *Blicca bjoerkna* собрать и определить не удалось.

Таблица 9
Table 9
Биометрические данные *Tripartiella incisa* (Lom, 1959) (измерения в μ)
Biometric data of *Tripartiella incisa* (Lom, 1959) (measurements in μ)

Автор Author	Lom 1959	Кашковский 1965		Наши данные Present author	
Диаметр тела Diameter of body	21.0—30.0	30.0—43.0	27.0—42.0	—	—
Диаметр прикрепительного диска Diameter of adhesive disc	16.0—21.0	27.0—32.0	26.0—34.0	22.0—23.0	21.0—27.0
Диаметр венчика Diameter of denticulate ring	10.0—13.0	21.0—25.0	18.0—25.0	17.0—20.0	16.0—22.0
Длина наружного отростка зубца Length of outer blade of denticle	—	3.7—4.0	3.7—4.3	3.5—4.0	3.0—4.0
Длин внутреннего отростка зубца Length of inner ray of denticles	—	3.0—3.7	2.0—3.7	2.0—2.6	2.6—3.3
Количество зубцов Number of denticles	20—26	23—26	23—25	19—20	17—22
Число полос диска Number of radial pins	—	—		4 (?)	5—6 (?)
Хозяин Host	голец *Nemachilus barbatus*	язь *Leuciscus idus*	голавль *Leuciscus cephalus*	плотва *Rutilus rutilus*	язь *Leuciscus idus*
Локализация Localization	жабры gills	жабры gills		поверхность, тела, плавники surface of the body, fins	жабры gills

Tripartiella (Paratrichodina) incisa (Lom, 1959)

Описана Ломом (L o m 1961) с жабр горчака (Чехословакия).

В Советском Союзе впервые зарегистрирована К а ш к о в с к и м 1965 в бассейне р. Урал у язя и голавля. К р а с и л ь н и к о в а 1966 отметила *T. incisa* среди паразитов рыб Верхнего Дона, К о с т е н к о 1967 — у рыб Среднего Днепра.

В нашем материале данный вид встречен у личинок плотвы и язя. Степень инвазии была очень незначительна.

Размеры инфузорий сведены в Таблицу 9.

Tripartiella copiosa (L o m, 1959)

Описана Ломом (L o m 1961) с жабр горчака из пресноводных водоемов Чехословакии.

В СССР впервые отмечена К а н д и л о в ы м 1964 в бассейне р. Куры. Затем Л ю б а р с к о й и Ш т е й н 1967 с мальков плотвы и окуня, (бассейн р. Волги), А л л а м у р а т о в ы м 1967 для ельца, пескаря, быстрянки, острслучки, гольца, щиповки (Узбекистан) и К о с т е н к о 1967 в бассейне Среднего Днепра.

Нами *T. copiosa* встречена у сеголеток плотвы, леща и язя.

Сравнение измерений инфузорий данного вида из различных водоемов представлено в Таблице 10.

Trichodinella epizootica (Raabe, 1950)

Этот чрезвычайно широко распространенный вид отмечен нами на поверхности тела, плавниках и жабрах окуней разного возраста, на жабрах сеголеток щуки и плотвы 1+.

Биометрические данные приводятся в Таблице 11.

Среди обнаруженных урцеоляриид два вида триходин известны как возбудители триходиниоза — *T. pediculus* явилась причиной гибели молоди белых амуров (C h e n Chih-leu 1956), *T. nigra* отмечена как один из возбудителей заболеваний у молоди лососевых (Б о г д а н о в а 1967).

В оз. Селигер мы не наблюдали ни признаков триходиниоза у обследованных личинок и мальков, ни их гибели. Однако трудно предположить, чтобы столь высокая степень интенсивности заражения не оказывала бы на молодь патогенного влияния, тем более, что на ранних этапах постэмбрионального онтогенеза плавниковые складки личинок играют существенную роль в газообмене.

Параллельно с исследованием паразитофауны молоди обследовались половозрелые *Cyprinidae*, *Percidae* и другие виды рыб, на которых обнаружены единичные экземпляры *Tripartiella copiosa* (плотва), *T. incisa* (лещ), несколько чаще — *Trichodina domerguei* f. *acuta* (20% у леща), *T. polycirra* (плотва), *Trichodinella epizootica* (ерш, налим, щука, густера). Взрослые караси на 60% оказались зараженными *Trichodina reticulata*. Таким образом, еще раз подчеркивается, что триходины, в основном „детские паразиты". Однако, они не являются „неизбежным злом" в развитии молоди. В нашем материале массовое заражение этими *Ciliata* наблюдалось лишь у рыб, проходящих личиночную стадию

http://rcin.org.pl

Таблица 10
Table 10
Биометрическое данные *Tripartiella copiosa* (Lom, 1956) (измерения в μ)
Biometric data od *Tripartiella copiosa* (Lom, 1956) (measurements in μ)

Автор / Author	Lom 1956	Кандилов 1964	Любарская и Штейн 1967		Наши данные / Present author	
Диаметр тела / Diameter of body	26.0—48.0	21.0—26.0	28.8—30.0	—	—	—
Диаметр прикрепительного диска / Diameter of adhesive disc	26.0—48.0	20.0—24.0	19.2—22.0	15.3—24.0	16.0—24.0	16.5—23.0
Диаметр венчика / Diameter of denticulate ring	9.0—16.0	18.0—22.0	17.6	15.0—23.0	15.0—18.5	15.3—21.0
Длина наружного отростка зубца / Length of outer blade of denticle	3.3	2.0—3.5	3.4—4.1	3.3—5.0	3.2—4.0	3.3—4.4
Длина внутреннего отростка зубца / Length of inner ray of denticle	2.2	1.5—2.5	3.4	—	—	2.5—4.0
Количество зубцов / Number of denticles	21—32	20—24	20	19—23	20—23	19—23
Число полос диска / Number of radial pins	4—5	—	—	4—5	4—5	5
Хозяин / Host	горчак / *Rhodeus sericeus*	горчак / *Rhodeus sericeus*	плотва и окунь, мальки / fry of *Rutilus rutilus* and *Perca fluviatilis*	плотва 0+ / *Perca fluviatilis* 0+	лещ 0+ / *Abramis brama* 0+	язь 0+ / *Leuciscus idus* 0+
Локализация / Localization	жабры / gills	жабры / gills	обонятельные ямки, голова, жабры / olfactory sacs, head and gills		жабры, реже плавники / gills, reraly fins	

развития в июне. Обследованные в июле личинки *Blicca bjoerkna* (отнерестившей-
ся в конце июля) были инвазированы единичными *Trichodina* sp. 2 (видовая
принадлежность не установлена), хотя процент заражения достиг 65%. Молодь
этого вида до 90% была поражена цистами *Myxobolus musculi*. Такое различие
в зараженности одновозрастной молоди объясняется, по-видимому, как сезонным
воздействием на паразитофауну, так и некоторыми различиями в биологии са-
мых личинок. И наоборот, пребывание личинок разных видов рыб в сходных
условиях хорошо прогреваемых мелководий и образование общих стай ведут
к интенсивной инвазии молоди одним и тем же видом *Trichodina nigra*.

Таблица 11
Table 11

Сравнение биометрических данных *Trichodinella epizootica* с молоди различных рыб оз. Селигер
(изменения в μ)

Comparison of biometric data *Trichodinella epizootica* from various young fishes from Lake Seliger
(measurements in μ)

Хозяин Host	Окунь *Perca fluviatilis*	Щука *Esox lucius*	Плотва *Rutilus rutilus*
Диаметр прикрепительного диска Diameter of adhesive disc	15.0—23.0(17.5—18.5)	21.0—24.0(23.0)	20.0—23.0
Диаметр венчика Diameter of denticulate ring	14.0—22.0(17.5—18.5)	19.0—22.0(21.0)	18.0—22.0(20.0)
Длина наружного отростка зубца Length of outer blade of denticle	3.0—4.0(3.0—3.0)	4.5—5.0(5.0)	4.5—5.0(4.0)
Количество зубцов Number of denticles	19—24(20—21)	20—23(21—22)	20—23(20—21)
Число полос диска Number of radial pins	5 (?)	4—5	5 (?)
Возраст хозяев Age of hosts	O+, 1+, 2+	O+	1+

Интенсивность заражения урцеоляриидами с возрастом, в общем, понижает-
ся. С одной стороны это вызвано изменением биологии хозяев, например, отхо-
дом мальков на большие глубины, с другой — сопутствующими сезонными изме-
нениями. По данным К а н д и л о в а 1964,, наибольшая зараженность рыб
эктопаразитическими простейшими в бассейне р. Куры обычно наблюдается
весной и летом. Согласно сообщению Л ю б а р с к о й и Ш т е й н 1967 экстен-
сивность инвазии урцеоляриид в осенние месяцы понижалась, либо падала
до нуля.

Trichodina nigra отмечена нами у молоди карповых всех исследованных
возрастов. Как упоминалось выше, наибольшая зараженность (до 100%) наблю-
далась у личинок. В июльских пробах экстенсивность инвазии снижалась до
50—70%, а у осенних сеголеток не превышала 33.0%, при очень низкой интен-
сивности. На молоди старших возрастных групп (1+ и 2+), обследованных
в августе-сентябре, обнаружены единичные *Т. nigra*.

На поздних личинках окуня *Т. nigra* с середины июня постепенно „вы-
теснялась" *Т. domerguei* f. *acuta*. Последняя инвазировала сеголеток, двух-
и трехлеток окуня на 60—80%. Интенсивность заражения в июле превышала
сентябрьскую.

Trichodinella epizootica у сеголеток окуня и щуки встречалась с июня по

октябрь. Процент заражения почти не менялся (соответственно 60.0—50.0%). Двухлетки окуня инвазированы *T. epizootica* на 26.6%, плотвы — на 6.6%

На сеголетках окуня наблюдалась несвойственная *T. epizootica* локализация — кожа и плавники. То же наблюдали Любарская и Штейн 1967 на мальках щуки.

Tripartiella copiosa и *T. incisa* на молоди карповых отмечены с конца мая по август. Заражение не превышало 53.0% первым видом; находки *T. incisa* немногочисленны. Кроме обычной локализации (жабры) встречена на плавниках и коже личинок плотвы и язя.

Trichodina urinaria впервые встречена у окуня 1+ (26.6%, единичные особи) и 2+ (20.0%, заметное повышение интенсивности). Единичные *T. polycirra* отмечены лишь однажды у плотвы 2+. По мнению Хайдера (Heider 1964) рыбы заражаются триходинами мочевых путей только при непосредственном контактировании (например, во время нереста). Возможно, эта находка свидетельствовала о возросшем контакте исследованной молоди со взрослыми.

Нахождение в оз. Селигер *T. meridionalis* заслуживает специального упоминания. Описанный из водоемов с высокой соленостью — Азовское, Черное и Каспийское моря (Догель 1940) солоноватоводного (Тимофеев, по Штейн 1962) и, наконец, из пресноводных — бассейн р. Куры (Кандилов 1964) и оз. Селигер (наш материал) — этот вид может быть охарактеризован как эвригалинный. О приспособленности к изменениям солевого режима *Trichodina domerguei* f. *latispina* (син. *T. domerguei* subsp. *domerguei*) сообщают Исаков и Шульман 1956.

Проведенный анализ выявил у молоди рыб оз. Селигер известное видовое разнообразие урцеоляриид по сравнению с другими водоемами Советского Союза. Так например, Кандилов 1964, изучавший эктопаразитических простейших бассейна р. Куры отметил 11 видов *Urceolariidae* у 30 видов хозяев. Кашковский 1966 для молоди леща и окуня выявил 3 вида триходинид. Костенко 1967 у 11 видов рыб Среднего Днепра обнаружила 10 видов *Urceolariidae*, Любарская и Штейн 1967 отметили 5 видов этих инфузорий у 5 видов молоди рыб Куйбышевского водохранилища.

Резюме

У молоди семи видов рыб в оз. Селигер обнаружено 13 видов урцеолярид. Наиболее широко распространена *Trichodina nigra*, заражающая молодь возраста 1 месяц до 100%. Этому виду у *Urceolariidae* свойственна внутривидовая изменчивость формы зубцов прикрепительного венчика. *T. polycirra* и *T. urinaria* обнаружены только у молоди рыб старших возрастов — 1+ и 2+. *T. spathulata* sp. n. и *T. rostrata* sp. n. описаны как новые виды.

SUMMARY

Thirteen species of Urceolaridae (*Peritricha*, *Mobilia*) have been found on 7 species of fry and young fishes from the Lake Seliger.

Trichodina nigra the most abundant species infects up to 100% of the fry at the age of 1 month. This urceolariid species is characterized by intraspecific variability of shape of denticles of the adhesive disc and two forms: "narrow" (Pl. II 3, 5, Pr. III 7, 9, 11) and "wide" (Pl. II 4, 6, Pl. III 8, 10, 12) are distinguished. Two species — *Tri-*

chodina polycirra and *T. urinaria* have been found on the young fishes (1+ and 2+) only. Two other species *Trichodina spathulata* sp. n. and *Trichodina rostrata* sp. n. are described as new ones.

Trichodina spathulata sp. n. (Figs 2 and 3, Pl. III 16) are small trichodinids. The biometric data are presented in Table 8. The blades of the denticles are widened in form of spade with rounded edges. Their length are 4.6—6.0 μ. The inner rays, long and slightly curved 4.6—5.5 μ of length. The central, conical part of denticle is short and narrow. The center of adhesive disc is dark after AgNO₃ impregnation. Adoral zone circumscribeds spiral over 360°. Diameter of the horseshoe macronucleus is 16.9—30.0 μ, the distance between the ends of macronucleus (the value "x") is 3.9—10.4 μ. Micronucleus round or oval, measuring 2.5—5.0×1.0—2.6 μ is situated at the outer side of macronucleus, distance "y" is 6.5—19.8 μ.

> Host: fry of *Abramis brama*.
> Locality: Lake Seliger.
> Localization: gills.

Trichodina rostrata sp. n. (Figs 4 and 5, Pl. III 17) are rather big trichodinids. Diameter of the body is 49.5—84.7 μ (most frequently 46.0—48.0 μ), of adhesive disc — 43.0—53.0 μ of denticulate ring 38.0—47.0 μ (46.0—47.0 μ), number of denticles 23—25 (23). Length of the blade is 6.5—8.0 μ, length of the ray 6.5—8.0 μ. The broad rounded blades have a "rostrum" on its base, separated from the other part of the blade with a small groove. The body of the denticle is solid and has a conical outgrowth. The inner rays are straight or little curved. Number of radial pins is 9—11 for one denticle. The center of the disc in AgNO₃ impregnated specimens are dark. Adoral zone circumscribes spiral of 360—390°. Diameter of the horseshoe macronucleus is 33.0—44.0 μ. Micronucleus oval or elongated measures 3.8—6.5×2.0—3.8 μ, the values "y" being 11.0—27.0 μ and varying even in the same population (Fig. 5). The distance between the ends of macronucleus (the values "x") is 11.0—16.5 μ.

> Host: young specimens of *Rutillus rutillus*, rarely — *Abramis brama* (0+).
> Locality: Lake Seliger.
> Localization: skin, gills and fins.

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ПОДПИСИ К ТАБЛИЦАМ I—III

1: *Trichodina nigra* Lom, 1960 с личинки *Leuciscus idus*
2: Аберрантная *T. nigra* с личинки *Rutilus rutilus*
3—4: *T. nigra* с *Leuciscus idus* 1+
5—6: *T. nigra* с личинки *Rutilus rutilus*
7—8: *T. nigra* с личинки *Abramis brama*
9—10: *T. nigra* с личинки *Perca fluviatilis*
11—12: *T. nigra* с личинки *Lucioperca lucioperca*
3, 5, 7, 9, 11 — „узкие" формы; 4, 6, 8, 10, 12 — „широкие" формы *T. nigra*
13: *Trichodina domerguei* f. acuta Lom, 1961 с *Perca fluviatilis* 0+
14: *Trichodina domerguei* f. esocis Lom, 1960 с *Esox lucius* 0+
15: *Trichodina meridianalis* (Dogiel, 1940)) с *Blicca bjoerkna* 1+
16: *Trichodina spathulata* sp. nov. с личинки *Abramis brama*
17: *Trichodina rostrata* sp. nov. с личинки *Rutilus rutilus*
18: *Trichodina* sp. с личинки *Leuciscus idus*

EXPLANATION OF PLATES I—III

1: *Trichodina nigra* Lom 1960 from the fry of *Leuciscus idus*
2: Aberrant form of *T. nigra* from the fry of *Rutilus rutilus*
3—4: *T. nigra* from *Leuciscus idus* 1+
5—6: *T. nigra* from the fry of *Rutilus rutilus*
7—8: *T. nigra* from the fry of *Abramis brama*
9—10: *T. nigra* from the fry of *Perca fluviatilis*
11—12: *T. nigra* from the fry of *Lucioperca lucioperca*
3, 5, 7, 9, 11 — „narrow" forms of *T. nigra*; 4, 6, 8, 10, 12 — „wide" forms of *T. nigra*
13: *Trichodina domerguei* f. acuta from *Perca fluviatilis* 0+
14: *T. domerguei* f. esocis Lom, 1960 from *Esox lucius* 0+
15: *T. meridionalis* (Dogiel, 1940) from *Blicca bjoerkna* 1+
16: *T. spathulata* sp. nov. from the fry of *Abramis brama*
17: *T. rostrata* sp. nov. from the fry of *Rutilus rutilus*
18: *Trichodina* sp. from the fry of *Leuciscus idus*
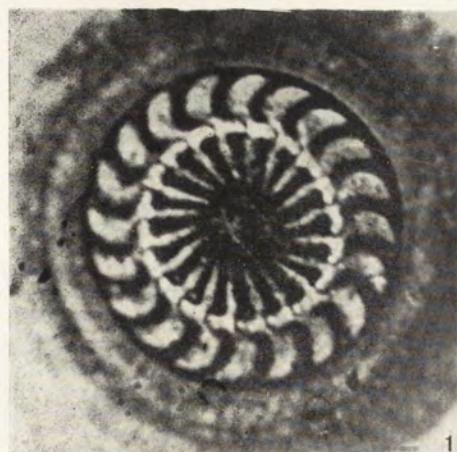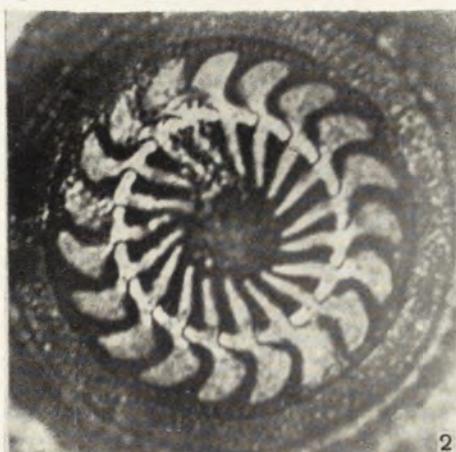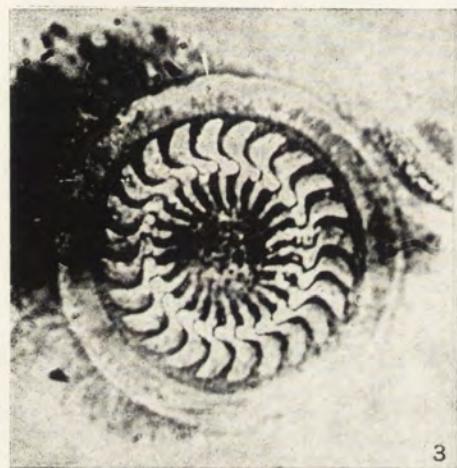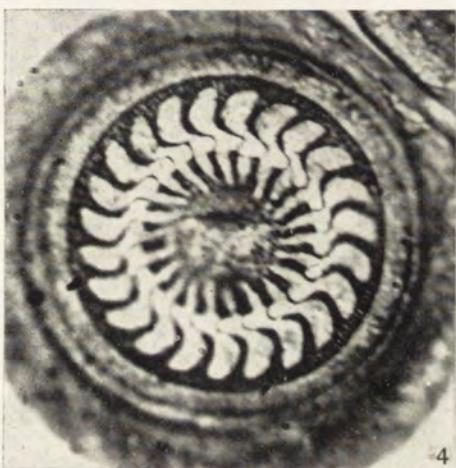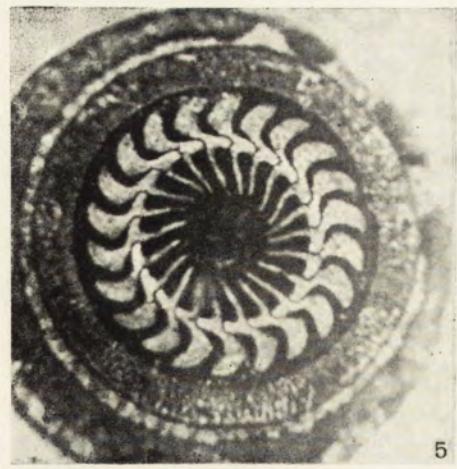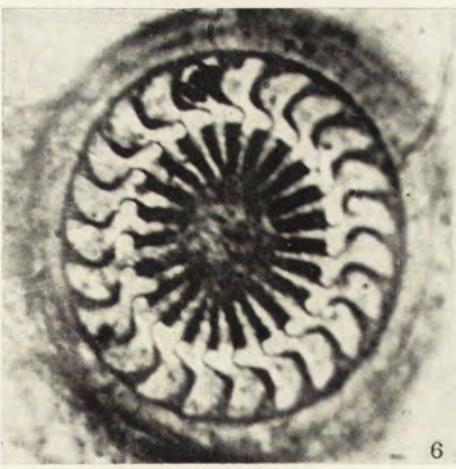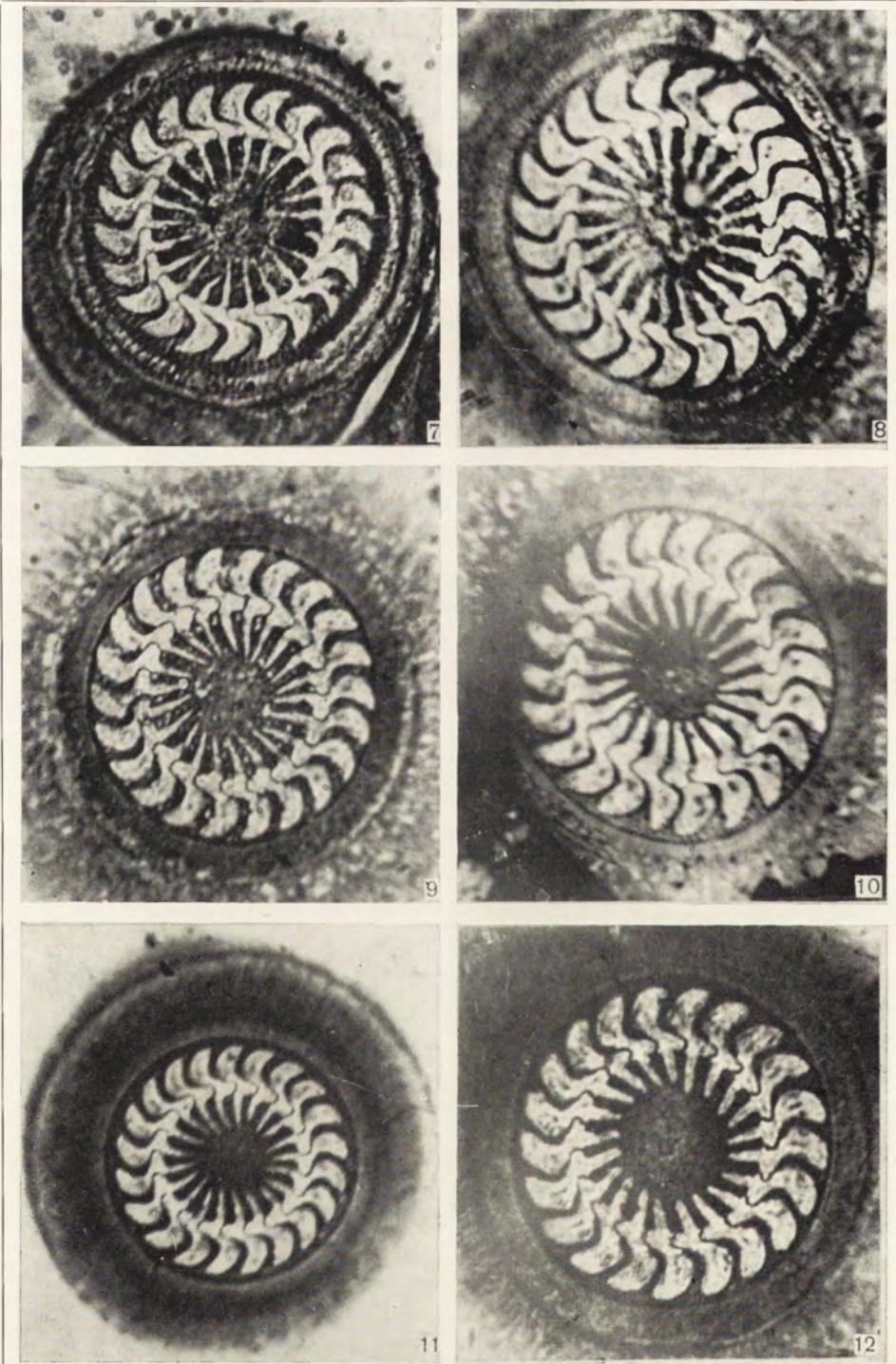
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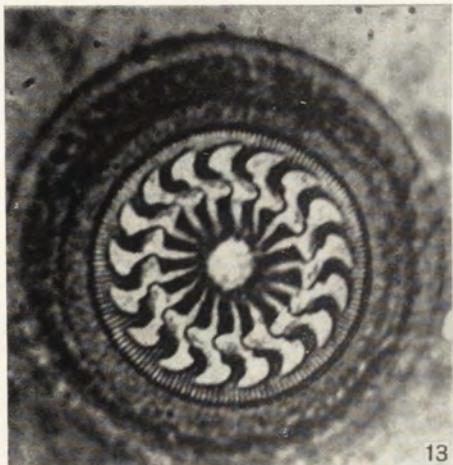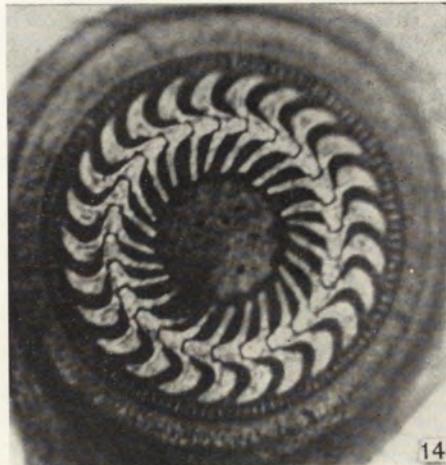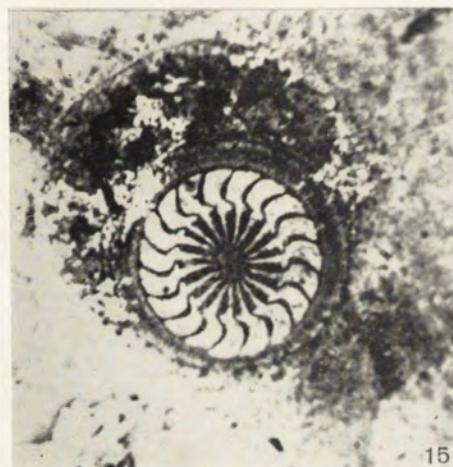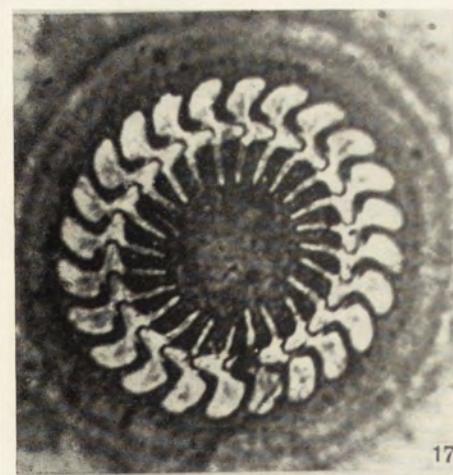
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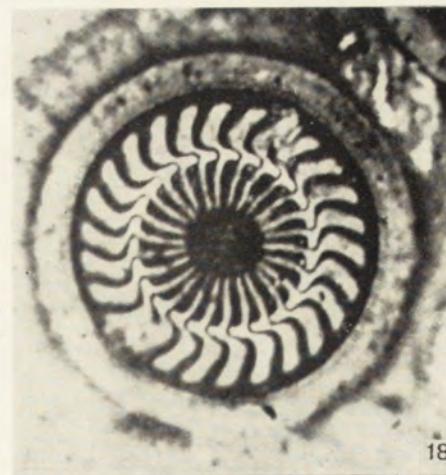
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Cytochemical investigations of *Isospora lacazei* and *I. chloridis* of the sparrow (*Passer domesticus*) and the greenfinch (*Chloris chloris*)*

Цитохимическое исследование *Isospora lacazei* и *I. chloridis* из воробья (*Passer domesticus*) и зеленого вьюрка (*Chloris chloris*)

Materials and methods

Infections of *Isospora* were obtained from the English sparrow (*Passer domesticus*) and the greenfinch (*Chloris chloris*) trapped at Imperial College Field Station, Ascot, Berkshire. All birds examined were parasitised by *I. lacazei* and two were doubly infected with *I. lacazei* and *I. chloridis* (A n w a r 1966 b).

The infected region of the intestine (the small region of duodenum just posterior to the gizzard) was fixed as follows: Carnoy's fluid was used for preservation of nucleic acids, protein and glycogen; B r a c h e t's 1953 modification of Serra's fixative for RNA; Helly's fluid for carbohydrate and nucleic acids and Bouin's fixative for acid mucopolysaccharide. After fixation, the tissues were dehydrated with alcohol, cleared and embedded in paraffin wax (M. P. 58°C). For the determination of acid and alkaline phosphatase the intestine was fixed by the method of G o m o r i 1952 embedded in low melting point paraffin wax (M.P. 49—52°). For the detection of lipids, Baker's formaldehyde calcium fixative was used (B a k e r 1949) followed by embedding of the tissue in gelatin.

Cytochemical studies were made on paraffin sections (7μ thick) of the parasitised tissue. The Feulgen reaction was used as a method for recognising sites of deoxyribonucleic acid (DNA) (G o m o r i 1952, D a n i e l l i 1953). Where the test samples were incubated in 1.0 N HCl at 60°C, to produce aldehyde groups reactive to Schiff's reagent, controls were either incubated in water at 60°C to prevent such groups forming on DNA or were incubated in deoxyribonuclease to remove the DNA. The DNase was used according to G i l b e r t, O v e r e n d and W e b b 1951 in a concentration of 1 mg/ml phosphate buffer pH 7.5 containing 0.003 M $MgSO_4$. Staining of ribonucleic acid (RNA) and DNA was carried out using the methyl green-pyronin Y staining method of K u r n i c k 1955. Pretreatment of slides in RNase (1 mg/ml

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* A part of this investigation was presented at the First International Congress of Parasitology, Rome, September 1964.

distilled water) at 37°C for one hour was carried out selectively to remove RNA components (B r a c h e t 1953).

For the detection of carbohydrate the PAS method of M c M a n u s 1946 utilising aqueous periodic acid as an oxidising agent followed by Schiff's reagent was employed. Glycogen was removed from control slides by incubation in 1% diastase in phosphate buffer pH 7.0 at 37°C for 2 hours.

For identification of acid mucopolysaccharide, the Alcian Blue method of S t e e d m a n 1950 was adopted. Control slides were incubated in hyaluronidase (phosphate buffer pH 6.8) at 37°C for one hour before staining.

Protein was identified by B o n h a g's 1955 modification of the mercuric bromphenol blue technique.

Acid and alkaline phosphatase activity was detected by Gomori's method (1952).

Frozen sections (8 μ thick) were employed for detecting the presence of lipid. The sections were treated by Sudan Black B in 70% alcohol (B a k e r 1949) and mounted in glycerine.

To facilitate identification of the parasite stages that were tested cytochemically, alternate strips of paraffin ribbons containing the sections were mounted on separate slides and stained with convenient cytological stains, e.g. Heidenhain's iron haematoxylin.

Acknowledgement

Results

Deoxyribonucleic acid (DNA)

DNA was restricted to an intra-nuclear ring in all asexual stages (Figs. 1, 2, 4, 10 and 11). The nuclei of the immature microgametocytes (Figs. 7 and 8) were similar in their DNA content to those of the schizonts and these stages could be distinguished from one another only by the number not the structure of their nuclei. Mature microgametocytes showed an increased DNA content, each entire nucleus being Feulgen positive and, as little cytoplasm could be detected around the individual nuclei, the microgametes appeared to be composed almost entirely of DNA. The two species were easily distinguished by the microgamete nuclei, those of *I. lacazei* being spindle-shaped (Fig. 9) and those of *I. chloridis* comma-shaped (Fig. 13).

No DNA was detected in the nucleus of macrogametes. This does not imply that DNA is absent but indicates rather that the Feulgen reaction is not sufficiently sensitive to detect the very small amount present.

Ribonucleic acid (RNA)

Sections stained with methyl green-pyronin Y revealed the presence of ribonucleic acid. RNA is chiefly responsible for basophilia in the cell and in addition to staining with basic dyes (e.g. haematoxylin), it reacts specifically with pyronin Y to produce a light red colour.

All stages showed RNA in the cytoplasm and most stages had an additional store of RNA in the nucleolus (Figs. 3, 5, 6 and 12). In general the cytoplasm of the mature form of any stage was more strongly basophilic than that of the young form. The schizonts of *I. chloridis* (Fig. 12) contained RNA in the residuum as well as in the merozoites. The macrogametes showed strongly basophilic cytoplasm due to accumulation of RNA (Fig. 5 and 6). The nuclei of the merozoites and macrogametes (Figs. 3, 5 and 6) possessed RNA in the nucleolus and in addition RNA was detected in the nucleoplasm of the macrogametes (Fig. 6). The nuclei of early microgametocytes (Fig. 7) showed no RNA within the DNA ring. The nuclei of microgametes were composed only of DNA.

Polysaccharides

Polysaccharides are laid down as reserves in the cytoplasm. They can be detected by the highly sensitive periodic acid Schiff technique, but the test is non-specific and shows most polysaccharides as a deep red colour. Further characterisation is necessary.

PAS positive material, confirmed as glycogen by digestion of control sections with diastase, was detected in the merozoites and schizonts of the first and late generations of both species (Figs 14, 15, 16 and 17). It occurred as fine granules in the cytoplasm but not in great quantity. In *I. chloridis* glycogen was present in the residuum as well as the merozoites. Glycogen was present throughout the development of the macrogametes (Figs 19—21). There was a considerable increase in the quantity in the mature form and on the whole the individual granules grew as the gamete increased in size. It was noted that the glycogen present in the newly encysted zygote (oocyst) was in the form of very fine granules, indicating that a sudden change took place in the glycogen content coinciding with fertilisation. The oocyst wall itself was strongly PAS positive with a glycogen content (Fig. 22). Similarly PAS positive material identified as glycogen by its complete removal with diastase was found as granules in the cytoplasm of microgametocytes (Fig. 18).

Sections of *I. lacazei* from which glycogen was removed by digestion with diastase showed a residual but a less intense PAS positive reaction in the cytoplasm of the mature macrogamete and in the wall of the oocyst (Fig. 23). In the wall, the PAS positive sites were in the form of small flat plates and in the cytoplasm as fine granules. This material was present in no other stages.

All sexual and asexual stages of *I. lacazei* contained acid mucopolysaccharide in the form of granules as indicated by a blue colouration with Alcian Blue (Figs 24—27). The granules were quite large, irregular in shape and obviously did not correspond to the fine granules remaining after diastase treatment. The nature of these granules was confirmed by pre-treatment of sections with hyaluronidase after which there was no blue colouration.

After removal of glycogen by diastase treatment the PAS technique, which does not stain acid mucopolysaccharide, reveals the presence of a third polysaccharide in mature macrogametes and oocyst only in the form of fine granules. By a further study of the development of the oocyst it is believed that this polysaccharide combines with protein to form a component of the cyst wall, possibly as a mucoprotein.

Thus at least three types of polysaccharides are present in *I. lacazei*: large granules of glycogen, large irregular granules of acid mucopolysaccharide
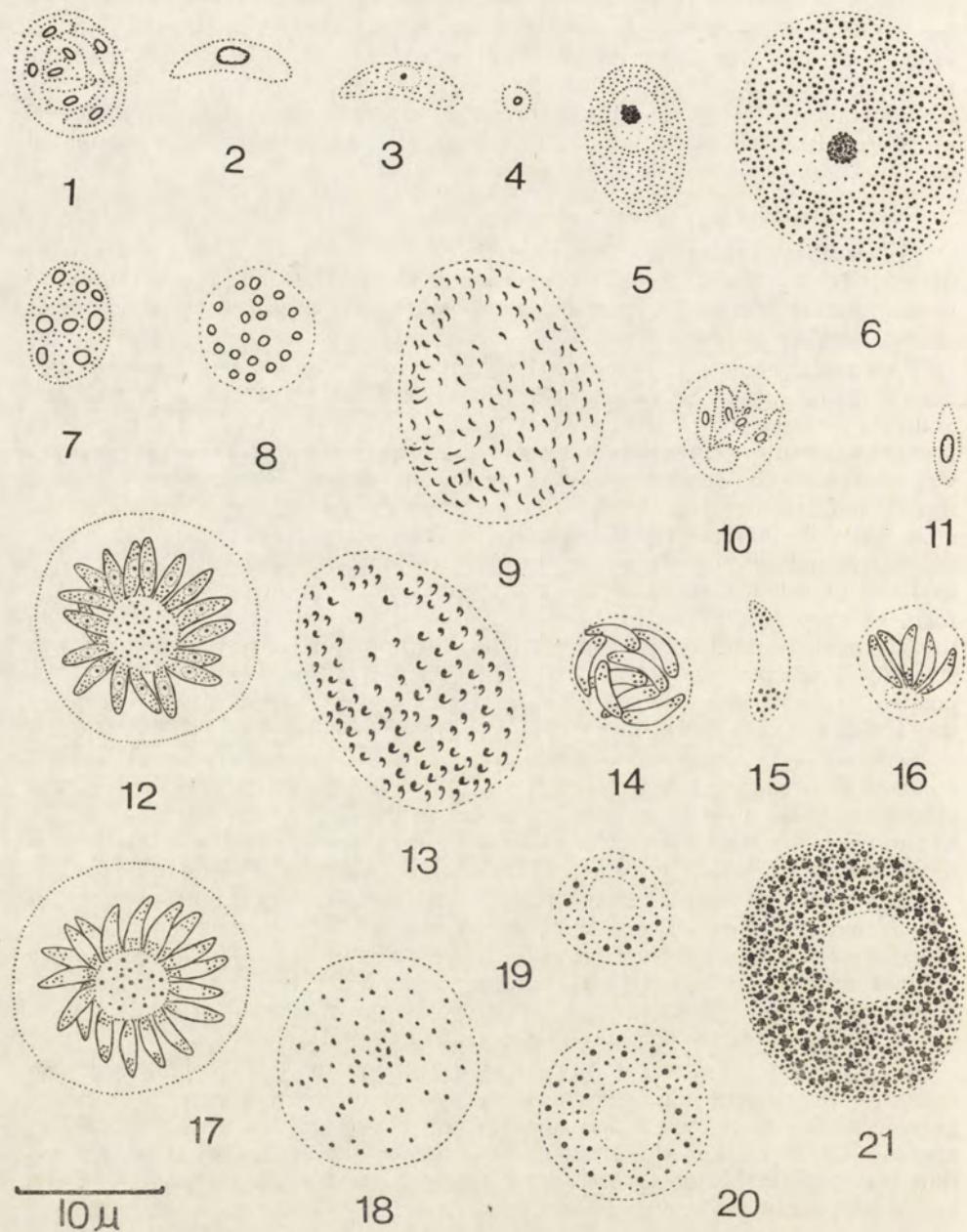
present in all stages and an unidentified polysaccharide in the form of fine granules in macrogametes and oocyst only.

Protein

The investigation of the protein content was carried out on the late generation merozoites, growing macrogametes and newly formed oocyst of *I. lacazei*.

Protein distribution is illustrated for the merozoites (Fig. 28), the immature macrogametes (Fig. 29), the mature macrogametes (Fig. 30) and the newly formed oocyst (Fig. 31).

In the formation of the oocyst wall of *I. lacazei* small plates are first laid down which can be detected by protein (Fig. 31) or polysaccharide stain (Fig. 23) and it is suggested that these substances combine as mucoprotein. Later the peripheral accumulations of protein diminish as protein is deposited in large thick plates in the cyst wall. Glycogen is also added (Fig. 22). These components merge with one another to produce a uniform cyst wall (Fig. 32).

Lipid

Only the merozoites and macrogametes of *I. lacazei* were examined for lipid content. Lipid was sparse in the merozoites (Fig. 33), occurring mainly in the cytoplasm at the two poles, with a little around the central nucleus. In the macrogametes lipid reserves were abundant with especially large globules in the vicinity of the nuclear membrane (Fig. 34).

Enzyme activity

Two enzymes, acid and alkaline phosphatase were tested for and located in certain stages of *I. lacazei*.

Using Gomori's methods, the presence of acid phosphatase is shown by a brownish-black precipitate and alkaline phosphatase by a definite black colour.

Acid phosphatase was detected in growing macrogametes. There were sites of enzyme activity in both the nucleus and the cytoplasm. In the cytoplasm

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Figs 1—21. *Isospora lacazei* and *I. chloridis*. 1—9, 14—15, 18—21 *I. lacazei*, 10—13, 16—17 *I. chloridis*, camera lucida drawings. 1. First generation schizont after the Feulgen reaction, showing DNA in nuclei. 2. Late generation merozoite after the Feulgen reaction. 3. Late generation merozoite after methyl green-pyronin Y, showing basophilia caused by RNA. 4. Rounded uninucleate body (developing gametocyte or schizont) in host cell after the Feulgen reaction. 5—6. Stages of developing macrogametes stained with methyl green-pyronin Y, showing basophilia removable by RNase. 7. Developing microgametocyte stained with methyl green-pyronin Y, showing DNA in nuclei as rings and RNA granules. 8. Developing microgametocyte after the Feulgen reaction. 9. Microgametocyte after the Feulgen reaction showing DNA in microgametes. 10. First generation schizont with residual body after the Feulgen reaction. 11. Late generation merozoite from a schizont with the residual body after the Feulgen reaction. 12. Late generation schizont with residual body after methyl green--pyronin Y, showing RNA. 13. Microgametocyte after the Feulgen reaction, showing DNA in microgametes. 14. First generation schizont after PAS, showing the presence of glycogen in the cytoplasm of merozoites. 15. Late generation merozoite after PAS, showing the location of glycogen in the cytoplasm. 16. First generation schizont after PAS, showing the presence of glycogen in the cytoplasm of the merozoites and the residual body. 17. Late generation schizont with the residual body after PAS, showing the presence of glycogen in the cytoplasm of the merozoites and the residual body. 18. Mature microgametocyte after PAS, indicating the site of glycogen. 19—21. Developing stages of macrogametes after PAS, showing polysaccharide mostly glycogen as accumulations in the cytoplasm

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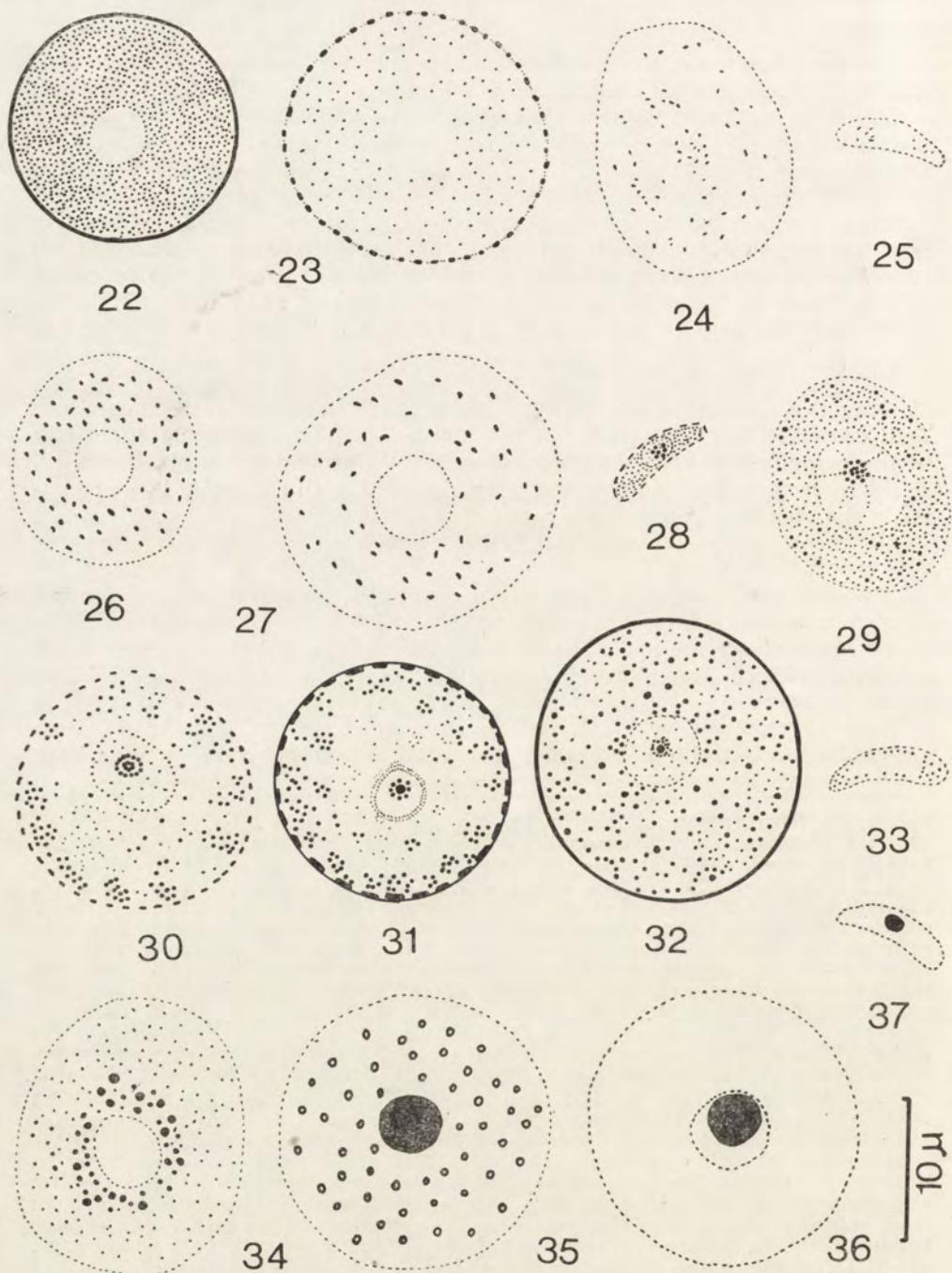
the precipitate occurred as small rings and in the nucleus there was a strong reaction in the membrane together with a diffuse intranuclear reaction (Fig. 35).

Alkaline phosphatase was detected in the macrogamete and late generation merozoites. In the macrogamete the only site of alkaline phosphatase activity was within the nuclear membrane which was shown as an eccentric dark globule (Fig. 36). This probably corresponded to the nucleolus. In the merozoites there was also a dark spot within the nucleus (Fig. 37) but as the nuclear membrane was not visible, it was impossible to say if the activity extended beyond the nucleolus.

Discussion

These cytochemical studies have given information about the distribution of nucleic acids, proteins, carbohydrates, lipids and two enzymes in the different stages of the life cycle of *Isospora*. The two species cannot be compared as insufficient material from *I. chloridis* was available. However inferences may be drawn regarding the function of the substances in the different stages of the parasite.

Both *I. lacazei* and *I. chloridis* were tested for DNA and similar results were obtained, namely that DNA was present in the nuclei of all stages except the macrogametes. Negative results for macrogamete DNA were obtained both with the Feulgen reaction and with the methyl green-pyronin Y test. This agrees with the results of P a t t i l l o and B e c k e r 1955 and C h e i s s i n 1958, 1959 but H o r t o n - S m i t h and L o n g 1963 and V e t - t e r l i n g 1966 obtained a weak reaction for DNA in the macrogametes of *Eimeria maxima* and *E. debliecki* respectively. It is unlikely that DNA is completely absent in the macrogamete of *Isospora* nucleus. It is usual to find only small quantities of DNA in interphase nuclei. DNA accumulates at the onset of nuclear division. The nucleus of coccidial macrogamete remains undivided throughout its growth and maturation and only after fertilisation does the nucleus undergo division. A similar situation obtains in the development of metazoan oocytes. S e r r a 1947 failed to demonstrate DNA in the oocyte

Figs 22—37. *Isospora lacazei*, camera lucida drawings. 22. Newly formed oocyst after PAS; showing polysaccharide in the cytoplasm and the oocyst wall. 23. Newly formed oocyst stained by PAS after diastase digestion, showing PAS+ve material, not removable by enzyme activity, in granules and plates. 24. Mature microgametocyte after Alcian Blue; showing the presence of granules of acid mucopolysaccharide. 25. Late generation merozoite after Alcian Blue; showing the presence of acid mucopolysaccharide in cytoplasm. 26—27. Stages of macrogametes stained with Alcian Blue showing the sites of acid mucopolysaccharide in the cytoplasm. 28. Late generation merozoite showing the site of protein, after staining with mercuric bromphenol blue. 29—30. Developing stages of macrogametes stained with mercuric bromphenol blue, showing the site of protein. 31. Newly formed oocyst showing the presence of protein in wall and also in contents, after staining with mercuric bromphenol blue. 32. Oocyst inside the host cell after mercuric bromphenol blue test, showing the sites of protein. 33. Late generation merozoite stained with Sudan Black B. showing lipid globules in the cytoplasm. 34. Macrogamete stained with Sudan Black B, showing lipid globules distributed in the cytoplasm and around the nucleus. 35. Macrogamete showing the presence of acid phosphatase in the cytoplasm and the nucleus. 36. Macrogamete showing the site of alkaline phosphatase inside the nucleus. 37. Late generation merozoite showing the site of alkaline phosphatase in the nucleus

of the snail and he concluded that the DNA was present in amount below the sensitivity of the Feulgen test. A l f e r t 1950 studying oogenesis and cleavage in the mouse obtained the same result. The macrogamete of *E. maxima* is of large size even for a coccidium and H o r t o n - S m i t h and L o n g 1963 suggested that its large nucleus contains sufficient DNA to be demonstrable with the Feulgen reaction. Nuclei of smaller coccidial species may contain DNA in a quantity just below the limit of sensitivity of this test.

All asexual stages of *I. lacazei* and *I. chloridis* contained DNA in the form of a ring at the periphery of the nuclei. The microgametes consisted almost entirely of DNA. Thus the amount of DNA in *Isospora* stages coincides with the nuclear activity. Dividing stages, schizonts and microgametocytes have active nuclei with an appreciable DNA content whereas the growing macrogametes have resting nuclei with little stainable DNA.

It is well known that strongly basophilic cells are active in protein synthesis (L o e w y and S i e k e v i t z 1963). Basophilia is conferred by the presence of RNA. RNA was found present in all stages of *I. lacazei,* where its distribution closely paralleled that of protein. This is to be expected as RNA plays a central role in protein synthesis. RNA was detected throughout the cytoplasm. The protein located in the nucleolus can be accounted for by the enzymes (acid and alkaline phosphatases) shown to be present.

Of the three carbohydrates detected, glycogen was the most widespread. It was found in varying amounts in all asexual and sexual stages of *I. lacazei* and *I. chloridis.* Glycogen has been determined as an important source of energy in the parasite (E d g a r et al. 1944; C h e i s s i n 1958, 1959). Therefore the quantity accumulated in the cytoplasm of the parasite can be related to its metabolic and physical activity. Glycogen present in the cytoplasm of microgametocyte is required to provide energy for the nuclear division and separation of the microgametes. Any reserve remaining after the separation of the gametes is left behind in the residuum. The absence of glycogen in the microgamete may be correlated with the fact that the gamete requires little energy for movement. It merely penetrates a nearby macrogamete. The absence of this reserve, suggests that the microgamete is incapable of extended "searching" for the macrogamete.

Glycogen was present in the growing schizonts, the separated merozoites, and the schizont residuum of *I. chloridis.* As in the microgametocytes, glycogen in the schizont provides a source of energy for nuclear and cytoplasmic division. The merozoites after release from the parent schizont leave the host cell and actively seek a new cell, for this migration a source of energy is required. The glycogen present in the schizont residuum of *I. chloridis* represents only that glycogen left after separation of the merozoites and does not imply active metabolism or movement on the part of the residuum.

The mature macrogamete was richest in its reserve of glycogen. This reserve was laid down throughout the development of the macrogamete and was seen in the mature gamete as closepacked coarse granules. After fertilisation, formation of the oocyst and expulsion from the host cell, the zygote receives no further nutriment from an external source. The energy for the entire process of sporulation comes from the reserves of the macrogamete. It is interesting to note that immediately after fertilisation the coarse granules of glycogen were immediately converted to fine granules. Thus the development of the zygote starts immediately, while still within the host cell. The oocyst wall itself remains strongly PAS positive with a glycogen component.

One point of particular interest concerns the "plastic granules" of the macrogamete. These granules, so characteristic of many coccidia, were not detectable in *I. lacazei* but were easily discernible in *I. chloridis*. Previous workers (P a t t i l l o and B e c k e r 1955, H o r t o n - S m i t h and L o n g 1963) have reported that the plastic granules are composed of a protein-carbohydrate complex and that they play a part in the formation of the oocyst wall. In *I. lacazei* fine granules of polysaccharides were distributed throughout the cytoplasm. As protein has an equally wide distribution in the cell it is possible that the protein-carbohydrate complex is evenly distributed in the cytoplasm and not accumulated in large globules.

After fertilisation of the macrogamete the oocyst wall is formed by the same process as in *Eimeria*. The results with *Isospora* also suggest that the oocyst wall is formed from a protein-carbohydrate complex present in the cytoplasm of the macrogamete together with a glycogen component. The oocyst wall is laid down first as flat plates at the periphery of the zygote and these are later joined up as more protein is added.

Acid mucopolysaccharide was found in the cytoplasm of the macrogamete and late generation merozoites of *I. lacazei*. It was also sparsely distributed in the cytoplasmic residuum of the microgametocyte. Previous results differed as to the presence or absence of this substance. C h e i s s i n 1958, 1959 and P a t t i l l o and B e c k e r 1955 did not find acid mucopolysaccharide in any stage of the rabbit coccidia or of *E. brunetti* and *E. acervulina*. G i l l and R a y 1954, however, working with *E. tenella* and P a t t i l l o 1957 with *E. tenella* and *E. necatrix* demonstrated its presence in these species. A n w a r 1966 a and 1966 c also reported the presence of acid mucopolysaccharide in *I. lacazei*. It seems possible, therefore, that there is variation between species, some producing acid mucopolysaccharide, others not. There is, as yet, no complete explanation of the function of this substance in tissues or cells, although it may be involved in water retention as was suggested by R o g e r s 1961. If this function is accepted as a possibility acid mucopolysaccharides in coccidians may be involved in osmotic control.

C l a u d e 1949 states that lipids are usually regarded as storage substances which can be drawn upon to produce a supply of energy. Thus those stages of the parasite which are most active and have a high metabolic rate will contain some stored lipids. Like the carbohydrate reserves, the lipids were found in merozoites and macrogametes of *I. lacazei,* the former being capable of locomotion and the latter when fertilised being involved in the development of the sporozoites.

Although a little work on enzyme activity in coccidial parasites has been done, the sites of activity have not been fully demonstrated and it is thus difficult to relate the occurrence of phosphatases to their known function.

According to D a n i e l l i 1946 the presence of alkaline phosphatase in the cell nucleus is probably related to the synthesis of nucleic acid. S u l l i - v a n 1950 working with *Colpidium campylum* reported alkaline phosphatase activity in the perinuclear area and suggested that it might be connected with the synthesis of ribonucleic acid. W i c k l u n d 1948 also suggested that the activity of the phosphatase was in some way connected with an increase in nucleic acid synthesis.

Alkaline phosphatase activity in this study was only demonstrated in the nucleus, probably in the nucleolus. The same result was reported by B e y e r 1960 in *E. magna*.

There is, as yet, no agreement on the functions of the phosphatases. In contrast to D a n i e l l i 1946 as above, M u g a r d 1951 held the opinion that they were concerned with cell growth. Thus acid phosphatase, which was demonstrated in both nucleus and cytoplasm in this investigation may be concerned with carbohydrate or protein metabolism.

S u m m a r y

Cytochemical investigations were carried out on two species of coccidia (*Isospora lacazei* and *I. chloridis*), parasites of the sparrow (*Passer domesticus*) and the greenfinch (*Chloris chloris*). Both species attack the epithelial cells of the villi of the small intestine.

DNA was detected in the nuclei of all stages except the macrogamete. RNA was found in the nucleolus and in the cytoplasm of all stages.

Glycogen was detected as fine granules in the cytoplasm, reaching its highest concentration in the mature macrogamete. Another polysaccharide was detected as granules in the cytoplasm of the macrogamete, and later in the oocyst wall. A similar distribution of protein in the macrogamete and oocyst suggested that the protein and polysaccharide granules combined to form the oocyst wall.

Acid mucopolysaccharide was found as coarse granules in the cytoplasm of different stages of the parasites.

Acid phosphatase activity was detected in the nucleus and cytoplasm, alkaline phosphatase activity was found only inside the nucleus.

Previous work on the cytochemistry of the coccidial parasites dealt mainly with the genus *Eimeria* which has been shown to contain glycogen, lipid, protein, DNA and RNA, and the site of enzyme activity including acid and alkaline phosphatase have been located. Little or no work has been performed on coccidial parasite of the genus *Isospora*. This paper reports the results of studies on the cytochemistry of the endogenous stages of *Isospora lacazei* and *I. chloridis* which are found in passerine birds. Preliminary reports have been published by A n w a r (1966 a and c).

РЕЗЮМЕ

Проведено цитохимиечское исследование двух видов кокцидий — *Isospora lacazei* из воробья и *I. chloridis* из зелёного вьюрка. Оба паразита локализуются в эпителиальных клетках ворсинок тонкого кишечника.

ДНК обнаружена в ядрах всех стадий жизненного цикла за исключением стадии макрогаметы. РНК найдена в ядрышке и цитоплазме на всех стадиях развития.

Гликоген выявляется в виде мелких гранул в цитоплазме, причём в зрелой макрогамете его количество достигает максимума. Другие полисахариды обнаружены в виде гранул в цитоплазме макрогаметы и позднее в оболочке ооцисты. Сходство в распределении белка в макрогамете и ооцисте позволяет предположить, что белковые и полисахаридные гранулы вместе формируют стенку ооцисты.

Кислые мукополисахариды обнаруживаются в виде крупных гранул в цитоплазме на разных стадиях развития паразита.

Активность кислой фосфатазы выявлена в ядре и цитоплазме, тогда как локализация щелочной фосфатазы ограничена только ядром.

Более ранние исследования по цитохимии кокцидий ограничены, в основном, родом *Eimeria*, для которого было показано распределение гликогена, липидов, белков, нуклеиновых кислот и мест локализации ферментов, включая фосфатазы, кислую и щелочную, а также сукцинатдегидрогеназу. В этом плане кокцидии рода *Isospora* оказались изученными очень мало. В настоящей работе приводятся результаты цитохимического исследования эндогенных стадий развития *Isospora lacazei* и *I. chloridis* из воробьиных птиц. Предварительные данные по этому вопросу были опубликованы автором (A n w a r, 1966 a и c).

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Amoeboaphelidium protococcarum sp. n. and *Amoeboaphelidium chlorellavorum* sp. n. — endoparasites of protococcous algae

Amoeboaphelidium protococcarum sp. n. и *Amoeboaphelidium chlorellavorum* sp. n. — эндопаразиты протококковых водорослей

In 1925 S c h e r f f e l described a peculiar parasite of diatom as a representative of the new genus *Amoeboaphelidium*. This genus included only one species, *A. achnanthides*. According to S c h e r f f e l the genus *Amoeboaphelidium* is related to the genus *Aphelidium* Zopf, 1885. The latter was precisely examined by S c h e r f f e l 1925 and recently by F o t t 1957. These parasites form characteristic amoeboid bodies in the cells of infected algae. *Aphelidium* species have flagellated zoospores. *Amoeboaphelidium* in free living stage appears as minute amoebas. T r e g o u b o f f 1953 placed *Amoeboaphelidium* in the *Pseudoheliozoa* group, but the real position of this organism in the system of *Protozoa* is not clear.

S c h e r f f e l found *A. achnanthides* only once in a single water sample. We have not found any other material concerning *Amoeboaphelidium* in the literature availiable.

In 1966 we obtained two strains of similar organisms in cultures free from contaminants. Our forms attack protococcous algae. Their life cycles and list of sensitive hosts were reported (G r o m o v i M a m k a e v a 1966). Two new strains were obtained later. Examination of these new forms and strains described before yielded some additional data for the consideration of structural characters and systematic position of these microorganisms.

Material and methods

Two strains of the parasites described before as X-1 and X-2 have been used, new strains have been obtained from the soil surface, X-3 from Putiatin island (Japanese Sea) and X-4 from Kamchatka.

Sensitive strains of *Scenedesmus obliquus* Kütz and *Chlorella vulgaris* Beijer. were used as hosts, being cultivated at 25°C on the following medium (per liter of distilled water): KNO_3 — 2 g, KH_2PO_4 — 300 mg, $MgSO_4$ — 300 mg, $ZnSO_4 \cdot 7H_2O$ — 0.022 mg, $MnSO_4$ — 1.81 mg, $CuSO_4 \cdot 5H_2O$ — 0.079 mg, $NaBO_3 \cdot$

$\cdot 4H_2O$ — 2.63 mg, $(NH_4)_6MO_7O_{24}$ — 1 mg, $FeSO_4 \cdot 7H_2O$ — 9.3 mg, $CaCl_2$ — 1.2 mg, $Co(NO_3)_2 \cdot H_2O$ — 0.02 mg, EDTA — 10 mg and agar 20 g.

Infected algae were continuosly illuminated by fluorescent lamps with light intensity about 2100 lux. Media with penicilline were used for the purification of new strains.

The life cycle of parasites has been examined in Peschkov's chamber (P e s c h k o v 1955). Material from the cultures of different age was stained according to Feulgen after fixation by Carnoy during 12, 24 or 48 hours. Lipids were stained with Sudan Black B. Infected algae in solutions of acridine-orange or tetracycline (I : 30 000 or I : 20 000) were examined under fluorescent microscope.

Results and discussion

Four examined strains of *Amoeboaphelidium* were similar in their morphology. A free living parasite is a motile amoeba, crawling on the solid surfaces. Amoeba forms numerous pseudopodia, thin trichopodia and thick lobopodia (Pl. I 1). Most amoebas have a single nucleus (Pl. I 2) and one or several fat globules stainable with Sudan Black B. The diameter of rounded amoeba is about $2\,\mu$, some animals however are bigger or even giant (Pl. I 3) and have several nuclei, 4 or more. In several cases it was possible to observe the liberation of such giant amoebas from cells of the host, therefore, their origin can not be a result of the fusion of usual organisms. The fate of these giants is unknown. In the microscopic preparations they always died after the short period of activity, but on the other hand the conditions in preparations are unfavorable for the development of *Amoeboaphelidium*. It seems possible that giant amoebas are formed in the case of uncomplete division of the parasite's body inside the algal cell.

When amoeba attacks algal cell it forms a round body attached to the cell wall by a small stalk. The protoplasm of parasite penetrates into the algal cell through the hole in the cell wall. This hole is clearly visible under the base of the stalk of parasite. Parasite forms a thin sometimes long risoid inside the infected cell. S c h e r f f e l 1925 observed such risoids in *Aphelidium*. He believed that walls of the risoid are secreted by algae as a defence reaction to the penetrating parasite. Vegetative protoplasmatic body of the parasite is formed at the end of the risoid. This body grows digesting the contents of algal cell. It is possible to observe a round stalked body outside algal cell and the risoid formed by the parasite until the digestion of alga is completed (Pl. I 4). These bodies are hardly vacuolated, contain little protoplasm and have rigid walls. Their shape is constant. Numerous round bodies separated from the infected cells are present in old cultures.

A small orange globule is visible inside of the vegetative body of the parasite growing in the algal cell (Pl. I 5). This globule is easily stainable by Sudan and soluble in Carnoy fixative. It remains in the destroyed algal cell after the release of new amoebas. It seems to be excrete of the parasite.

One alga cell can be sometimes infected by several amoebas. Several bodies of the parasite are growing separately, but later they fuse (Pl. I 5). Only one mature body of the parasite is formed in each cell of infected algae.

Nuclear structures are unstainable in the growing body of the parasite by Feulgen method or by acridine orange. During this period tetracycline staining gives a good picture of the membranous structure surrounding a growing body

in the place of contact with the algal protoplasm (Pl. I 6). Staining with tetracycline is the best method for the determination of a growing parasite inside the host cell.

When the content of the host cell is digested, the body of mature parasite (sporangium) occupies all space inside the cell wall. Numerous nuclei of sporangium are situated around the central excrete globule (staining by Feulgen method: Pl. I 7—9). The nuclei are round, oval, doubled, in the latter case it is obviously a division of nuclei. Nuclei in the sporangium are distinct in the acridine orange preparations as well. They may be round (Pl. I 10) or elongated and curved (Pl. I 11).

Tetracycline does not stain the membrane in mature sporangium, on the other hand, it reveals ring like structures surrounding nuclei (Pl. I 12). According to the recent results (B i r j u s o v a   i   M e i s s e l 1964, B i r j u s o v a i   N i k i t i n a 1966) tetracycline is selectively adsorbed by membranous structures of microorganismes, primarily by mitochondria. In growing *Amoeboaphelidium* the membrane is a place where active metabolic processes connected with the digestion of the host take place. In mature sporangium membraneous structures are distributed between the parts of protoplasm which correspond to future amoebas.

Later, the body of sporangium is divided into uninucleate amoebas which leave the destroyed cell.

In strain X-2 and X-4 mature sporangium can also give rise to a dormant spore. In this case the excrete globule is forced to the space between the cell wall of alga and the surface of the parasite (Pl. I 14). The parasite's body excretes a thick yellowish wall. In the parasite of *Chlorella* (strain X-2) dormant spores are round, 3—7 μ in diameter. In the parasite of *Scenedesmus* (strain X-4) dormant spores are oval, 4—6×5—7 μ (Pl. I 13). Nuclear material in the spore is aggregated into the single irregular mass (Pl. I 15). The thick spore wall looses its yellowish colour and becomes thin during germination. The content of the spore divides into round bodies, which apparently are the future amoebas, but we have never seen their liberation from the spore.

Amoeboaphelidium is much alike *Aphelidium* (F o t t 1957) when growing inside the host cell, but it differs from the latter in amoeba like free living stage. We have not observed flagellated cells in our strains although we examined them under different conditions during a long period of time. We can consider them only as members of the genus *Amoeboaphelidium*.

S c h e r f f e l 1925 described only one species of *Amoeboaphelidium A. achnanthides,* the parasite of diatom. Our strains are specific for the protococcous algae. 226 cultures of green and yellow green algae were examined as possible hosts for our parasites. As it was noted before (G r o m o v i M a m k a e v a 1966), strain X-2 can be cultivated only in the cells of some *Chlorella* strains. All these strains are resistant to other parasites. Strains X-1, X-3, and X-4 attack different *Scenedesmus* species and some other protococcous algae (*Muriella, Scotiella, Protococcus* and some *Chlorella* forms). In some hosts all three strains can grow, but many algae are sensitive only to one or two strains. All algae, which are sensitive to these strains, are resistant to the strain X-2.

According to the different host range of the parasites and character of dormant spores they can be regarded as two new species of *Amoeboaphelidium* described below.

Amoeboaphelidium protococcarum sp. n.

Parasite of *Scenedesmus, Protococcus* and some other genera of protococcus algae. Size of the vegetative body and sporangium depends on the size of the host cell. Dormant spore is oval $4—6\times5—7\,\mu$ freely lying in the host cell. Three strains are now available. Strains differ in the potential host range, but in some algae all three strains can grow. Till now dormant spores were observed only in strain X-4.

Amoeboaphelidium chlorellavorum sp. n.

Parasitizing only in *Chlorella* strains. The size of the growing body and sporangium depends on the size of the host cell, but since all sensitive forms of *Chlorella* have comparatively smaller cells sporangia in this species are smaller than they are in *A. protococcarum*. The nuclei in sporangia are smaller too (compare Pl. I 7 and 9). Dormant spores are round, $3—7\,\mu$ in diameter. The spore is occupying the whole space under the wall of the host cell. Sometimes spores are smaller than the host cell, but in these cases the rest part of the cell is undigested by the parasite.

One strain was obtained from the mass culture of *Chlorella vulgaris* near Leningrad.

Summary

Two new species of *Amoeboaphelidium* (*Pseudoheliozoa*), obtained and examined in cultures, are described. Both species are endoparasites in the cells of protococcous algae and are characterized by amoeba like free living stage. The general morphology of parasites is described.

Amoeboaphelidium protococcarum sp.n. attacks species of *Scenedesmus, Protococcus* and some other genera of protococcous algae. It forms oval dormant spores inside algal cell.

Amoeboaphelidium chlorellavorum sp.n. is specific for some *Chlorella* strains. It has round dormant spores.

РЕЗЮМЕ

Описано два новых вида рода *Amoeboaphelidium* (*Pseudoheliozoa*), представители которых получены в культурах. Оба вида паразитируют в клетках протококковых водорослей, в свободноживущей стадии они представлены мелкими амебами.

Amoeboaphelidium protococcarum sp. n. поражает *Scenedesmus, Protococcus* и другие протококковые водоросли. Для этого вида характерны овальные покоящие споры, свободно расположенные внутри разрушенной клетки хозяина.

Amoeboaphelidium chlorellavorum sp. n. специфичен для некоторых штаммов *Chlorella*. Покоящиеся споры этого вида круглые.

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EXPLANATION OF PLATE I

1—8: *Amoeboaphelidium protococcarum* sp.n. strain X-1
1: Motile amoebas. Phase contrast
2: Giant amoebas. Phase contrast
3: Nuclei in amoebas. Feulgen staining, phase contrast
4: Destroyed cell of *Scenedesmus obliquus*. Round bodies on the cell wall and risoids remaining after infection are visible. Phase contrast
5: Algal cells each infected by two amoebas. Phase contrast
6: Infected cell stained with tetracycline. Membranous structure of the parasite is visible. Fluorescent microscope
7—8: Nuclei in the sporangium. Feulgen staining
9: Nuclei in the sporangium of *Amoeboaphelidium chlorellavorum* sp. n. Feulgen staining
10—11: Sporangia of *A. protococcarum* stained with acridine orange. Numerous nuclei are visible. Fluorescent microscope
12: Sporangium of *A. protococcarum* stained with tetracycline. Membranous structures are visible. Fluorescent microscope
13: Dormant spore of *A. protococcarum*, strain X-4. Phase contrast
14: Dormant spore of *A. chlorellavorum*. Phase contrast
15: Nuclear material in the dormant spores of *A. chlorellavorum*. Feulgen staining

All microphotographe in the magnification 2000×

PLATE I

B. V. Gromov et K. A. Mamkaeva

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