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POLSKIEJ AKADEMII NAUK

ACTA PROTOZOOLOGICA

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

Janina KACZANOWSKA

Topography of cortical organelles in early dividers of *Chilodonella cucullulus* (O. F. M.)

Topografia organellów kortykalnych *Chilodonella cucullulus* (O. F. M.) we wczesnych stadiach podziału

In the previous study the description was given of the formation of new contractile vacuole pores (CVPs) in divisional morphogenesis in *Chilodonella cucullulus* (Kaczanowska and Kowalska 1969). New CVPs are distributed differently in the proter and opisthe (intraclonal dimorphism) (Fig. 2). Total resorption of all old CVPs takes place simultaneously in divisional morphogenesis.

Subsequent studies (Kowalska and Kaczanowska 1969) showed that in individuals with a kinetome of 18–20 kineties the five-kinetal interval of places of occurrence predominates between the upper vacuoles (termed CVP-1 and CVP-2). This five-kinetal interval is maintained in proters, but turns to six in the group of opisthes. Increase in the number of kineties between CVP-1 and CVP-2 in opisthes takes place through intercalar ingrowth of one somatic kinety in later phases of divisional morphogenesis (i.e. immediately after the period of forming new CVPs for the future opisthe) (Fig. 4).

This increase in the number of kineties between CVP-1 and CVP-2 in the opisthe simultaneously compensates for the loss of one kinety. This loss is due to the non-participation in division of the extreme left kinety, which in its entirety falls to the proter (l'évolution par decalage — Chatton et al. 1931).

At the same time even superficial observations show that individuals with the same pattern (e.g. proters) with the same total number of kineties, the same interval between CVP-1 and CVP-2, and the same length, and finally even in the same ontogenetic stage and from the same stock, may differ fundamentally in respect of the reciprocal topography of these CVPs. For instance CVP-1 may occur above or below the level of CVP-2 (Figs. 5, 6). This would mean that there are factors (or a factor) which cause the given vacuole to occur in different places in a definite interkinetal space.

In order to examine the original topographical relations and places of occurrence of new CVPs attention was focussed on individuals in the same ontogenetic stage, and the location of CVPs was analyzed in relation to the mouth and body length.

Protozoa in the early stage of division were used for the investigations, at the time when the localization of the new CVPs is established, but before the stage of morphogenetic movement (Figs. 1, 3).

Examination of the topography of mouth and CVPs was made in respect of three groups of questions. The first of these groups is concerned with the reciprocal topographical relations of mouth, old CVPs and body length. The second group is concerned with the topographical relations of old and new CVPs, and the third with the spatial relations of new centres and CVPs.

The detailed plan for investigations is as follows:

1. Localization of the mouth and old CVPs in relation to body length. These investigations are based on an analysis of:

a. Whether there is a definite correlation between the length of the preoral and the postoral parts of the body.

b. Whether there is a correlation between length of the postoral part and the distance between upper and lower vacuoles, e.g. the distance between CVP-2 and CVP-3. Answers to these questions will permit of drawing out the conclusions concerning the region of the cortex increase during cell growth. It will be valid if no autonomic movements exist of CVPs.

c. Whether there is a definite distance of CVP-3 from the posterior pole of the body, and if this character is not a constant one, whether the length of this part is in proportion to total body length, or to its postoral part.

2. The next series of investigations was concerned with the question of the spatial relations of old CVPs to new ones. Observations of intraclonal dimorphism (Kaczanowska and Kowalska 1969) would appear to show that there is no such relation. Interesting information on possible growth of the cortex in the posterior part of the protozoon may, however, be supplied by data from comparison of the localization of the old CVP-3 and new CVP-3 for the opisthe so called CVP-3''. Comparison was therefore made of sections of lengths measured from these vacuoles to the end of the body.

3. Finally examination was made of topographical relations within different newly-forming organelles. Many of the former studies, both descriptive and experimental, justify assuming that there is a definite topographical relation between length of the dividing protozoon, localization of its oral rudiments and the localization of other cortical structures. Suzuki 1957 draws attention to the topographical relation of the localization of the cytoproct and mouth in *Blepharisma*. The relation between localization of the mouth and excretory canal in *Spirostomum* was investigated by Eberhardt 1962. Tartar 1962 showed that in *Stentor* with reversed asymmetry there was reversed formation both of the mouth primordium and also of the contractile vacuole. Nanney 1966 examined the induction angle between the stomatogenic kinety and CVPs in *Tetrahymena*. Similar investigations in respect of different *Tetrahymena* were also carried out by Loefer et al. 1966, and for *Glaucoma* by Klug 1968. Investigations of this type of relation in *Chilo-*

donella may supply information on the cortical pattern of the topography of new structures, and the results obtained could be compared with cytogeometric models described for Ciliates (Uhlig 1959, 1960, Tartar 1961, 1962, Hanson 1967, Nanney 1966, 1967, Schwartz 1969).

In connection with this, the following questions were raised:

a. Previous studies (Kowalska and Kaczanowska 1969) show that only certain interkinetal space are activated in early dividers and are capable of forming new CVPs. In view of this, can the localization of the mouth mark the place of differentiation of the activated kinety?

b. Do similar, or identical, spatial relations occur in the localization of CVPs in relation to the mouth in the forming proter and opisthe? In other words within what range does homothetigenity of the two offsprings occur?

c. Does a relation occur, and what kind of relation, or reciprocal localization of the mouth of proter and opisthe occur, and what is their relation in respect of length of the parent individual?

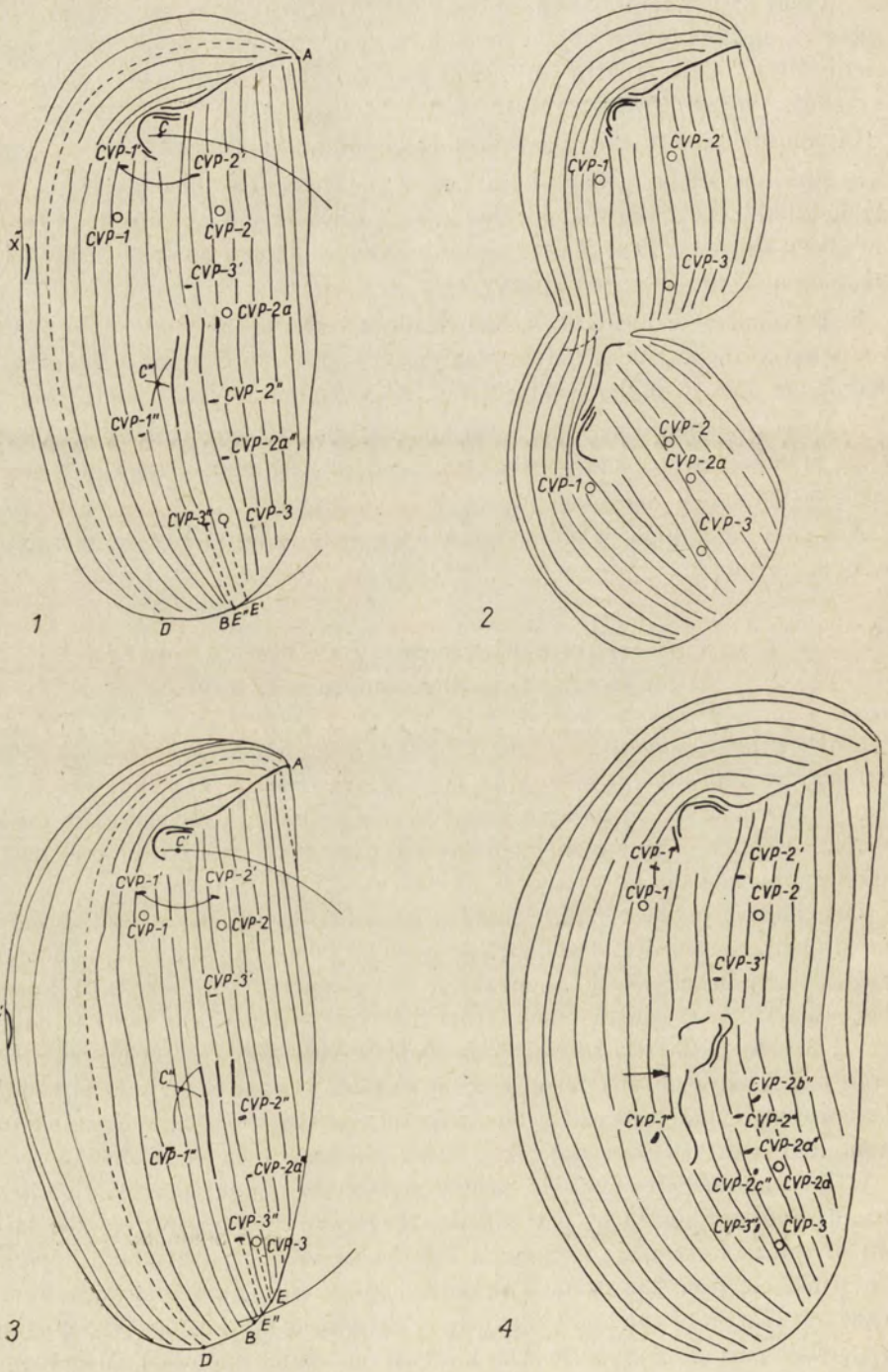
d. Can a description be given of the topography of the site of the presumptive divisional fissure?

Topography of cortical organelles of *Chilodonella cucullulus* in the morphostatic stage and in early dividers

Chilodonella cucullulus is a ciliate of the *Holotricha–Gymnostomatida* group. Within *Gymnostomatida* it belongs to the *Cyrtophorina* Corliss 1961 group. This ciliate is characterized by an apex markedly and asymmetrically shifted to the left, subapical location of mouth and complicated structure of what is known as the oral basket supporting the cytostome.

The ciliature of the ciliate is situated on a flat, asymmetrical ventral surface. The strongly convex dorsal surface is devoid of cilia, with the exception of one oblique kinety in the apical region, the so-called kinety X — after Radzikowski 1966. This kinety originally comes from the ventral side of the body (Chatton et al. 1931). As in the related species, in *Chilodonella* (Dragesco et Deroux 1968) in cases of distension of the dorsal side, the extreme kineties of the ventral side may occasionally occur, at least partly, on the dorsal side. These are cases deviating from the normal ones and were not taken into consideration in these studies.

In the anterior part of the flat ventral surface the mouth apparatus is situated centrally and surrounded by oral kineties. There are three or four of the latter. Two or three of them are circumoral kineties surrounding the mouth apparatus in a semicircle from the front. The preoral kinety runs from the apex situated on the left margin of the body obliquely to the circumoral kineties, and continues for a certain part of their course. This kinety is undulating and its apical end approximately indicates the apex of the protozoon.



Figs. 1–4

The remaining part of the ventral surface is evenly covered with meridional kineties. These kineties are all parallel to each other in the equatorial region, but the assymetry of the ventral surface causes that the right margin of the ciliate is longer than the left. The right meridional kineties are longer than the left ones, and run in an arch from the apical region and preoral kinety as far as to the posterior margin of the body. The postoral kineties begin behind the mouth and run longitudinally, breaking off at the back slightly earlier than the right kineties. The next kineties to the left run parallel to the postoral kineties and their anterior ends extend to the preoral kinety. Three or four extreme left kineties are consecutively increasingly shorter and shorter. The extreme kinety of the left system may consist of a few kinetosomes not reaching the equatorial region, and not participating in division falling in their entirety to the proter.

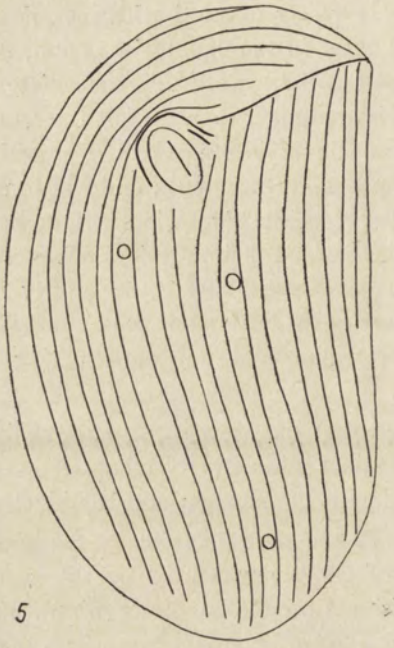
The above description of the ciliature shows that the apex is strongly shifted towards the left margin of the body, that a distinct allometry of ciliature occurs and that the preoral kinety separates the apical ends of the right and left kineties.

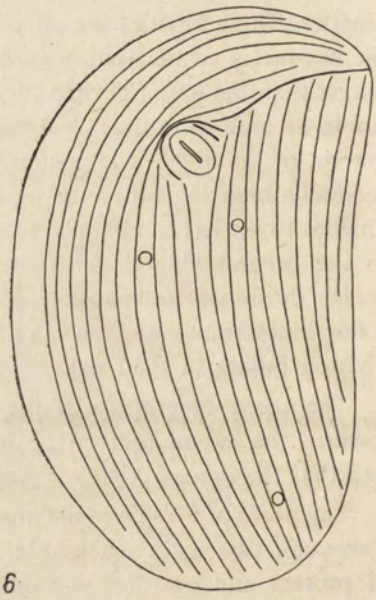
The pores of CVs lie in the interkinetal space on the ventral surface of the body. There are two main patterns of distribution of CVPs represented by the group of proters and opisthes during the last division (intraclonal dimorphism). For individuals with a total number of 18–20 kineties from the stocks given here these patterns were as follows:

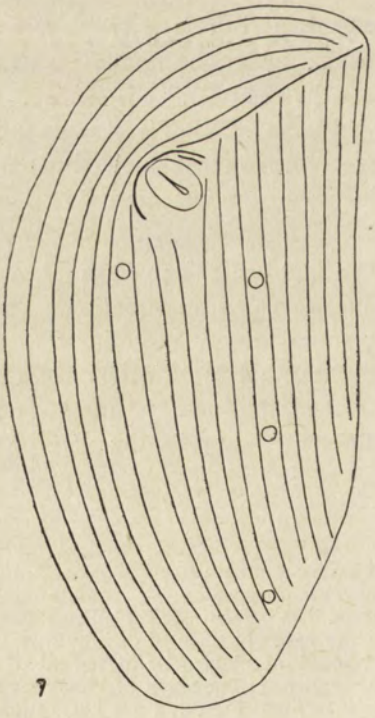
For the proter (Figs. 2, 5, 6) three CVPs are characteristic. The two upper vacuoles, represented by CVP-1 and CVP-2, are located under the mouth in the interval of five kineties. In the vast majority of cases the right CVP-1 is situated between the right postoral kinety and in the inner right kinety. CVP-2 is situated to the left in relation to CVP-1′. CVP-3 is the opening of the lower vacuole situated in the interkinetal space in the interval of four kineties to the left from CVP-1 and respectively one kinety from CVP-2.

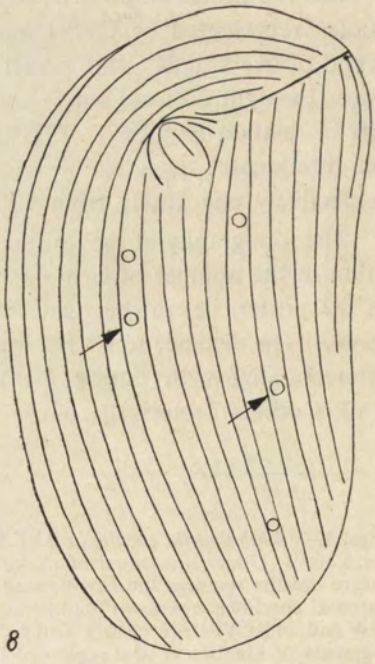
The topography of the opisthe is more modified and exhibits considerable variation in the number of vacuoles. CVP-1 occurs in an analogical situation to that in the proter, i.e. to the right from the right postoral kinety posteriorly to the mouth. The distance to CVP-2 was increased from the initial five kineties to six, by intercalar ingrowth during the last division of the additional postoral kinety. CVP-3 occurs posteriorly, but is formed by a different kinety during division in

Figs. 1–4. *Chilodonella cucullulus* (O.F.M.) 1. Opisthe during early stage of cell division. 2. Early cytokinesis. Upper individual of proter's characteristic of CVPs disposition. Lower individual — future opisthe requires the new pattern of CVPs disposition. The pattern of CVPs disposition of parental specimen unknown (dedifferentiation). 3. Proter during early stage of cell division. The new and old CVPs are clearly visible. 4. Stage more advanced-morphogenetic movement of the segments of kineties in oral region of future opisthe. The ingrowth of segment of kinety supplementing the total number of the kineties of future opisthe (arrow). Figs. 1, 3 are illustration of markings and abbreviations used in text. Figs. 1–4 are drawings of specimens after Camera-lucida









Figs. 5–8

comparison with CVP-3 of proters. In effect the interval between CVP-2 and CVP-3 is two kineties too.

An additional CVP-2a characteristic for the developed opisthe in located in the same interkinetal space as CVP-2′. In some more elongated individuals yet a third vacuole, represented by the opening of CV-2c, may occur in the same interkinetal space. CVP-2a usually occurs in the equatorial region, and CVP-2c at approximately two-thirds of the body length.

Like CVP-2c, CVP-2b may occasionally occur in some opisthes. This vacuole occurs in the next interkinetal space to the left in relation to CVP-2 and slightly above it.

However, the number of vacuoles in the opisthe does not exceed five CVPs (Figs. 2, 7). Some vacuoles may be represented by two pores, but such cases have not been taken into consideration in these studies.

The cytoproct is situated in the posterior part of the dorsal side of the body in both proters and opisthes. It occurs in preparations in the form of an undulating line parallel to the right margin of the body. In the examinations it served merely as proof that no injury to the body had taken place from the rear.

During the period of early divisional morphogenesis all the old cortical structures are retained, but the rudiments of new structures occur simultaneously. For the purpose of these investigations individuals were used in which the rudiments of all new CVPs occurred. New CVPs are indicated (identified analogically to those in interdivisional individuals) as follows in CVPs for the future proter being indicated by the appropriate numeration with the addition of ′, and for opisthe with the addition of ″. The pattern of disposition of new CVPs does not depend on the pattern for CVPs represented by the parent individual (Fig. 1, in comparison with Fig. 3). During the period of early divisional morphogenesis segment X″ also forms in the supraquatorial region, on the right margin of the ventral side. Segment X″ is the rudiment of the future dorsal kinety of opisthe. If this segment is already formed i.e. consists of more than ten kinetosomes, its upper end demarcates the right margin of the presumptive divisional fissure, not as yet visible at this stage.

In some individuals slightly more advanced in development the segments of the mouth kineties are completely formed and bent in a bow-shape, which indicates

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Figs. 5–8. *Chilodonella cucullulus* (O.F.M.) 5 and 6. Drawings of two proters fixed six hours after last division from one tested sample. Note the difference in topography CVP-1 in relations to CVP-2 between these specimens. 7. Young opisthe. Note nearly terminal position of CVP-3. 8. The case of individual six hours old. Probably it retained two CVPs from the former generation (arrows). If so, it is the proter resulting from division of the parental cell of opisthe's pattern of CVPs disposition. Figs. 5–8 are drawings made after photomicrographs

All drawings (Figs. 1–8) made after the specimens silvered after Chatton Lwoff in Corliss 1953 modification

that they have entered into the stage of morphogenetic movement (mouvement girotoire — Deroux 1968). Individuals were used for the investigations in which the segments of kineties had not shifted, and all the rudiments were in the most initial topographical situation as possible.

The topography of these organelles during the period of early divisional morphogenesis is illustrated by Fig. 1 and 3.

Only topographical data are given in the description. The remainder of the data on morphology and morphogenesis of this ciliate are to be found in studies by Fauré-Fremiet 1950, Radzikowski 1965, Kaczanowska and Kowalska 1969, Kowalska and Kaczanowska 1969.

Material and methods

Material originating from different stocks were used for the investigations. This lack of homogeneity in the material was intended to eliminate regularities which would apply only to homogeneous material. It was a question of grasping topographical regularities applying to the divisional morphogenesis itself, irrespective of stock, state of the culture etc.

All individuals in the preparations in the early stages of division were used for the investigations, bearing in mind the following conditions:

1. In order to ensure uniformity of measurements, only individuals with a similar total number of kineties (18–20) and completely normal course of formation of CVPs were taken into consideration.

2. Only those individuals with five-kinety interval of occurrence sites of new single CVP-1′ and CVP-2′ were analyzed. The question of the localization of new CVPs was thus narrowed down to the class of individuals of the same width of median sector (Kowalska and Kaczanowska 1969). The question of choice of activated kineties and problems of induction angle (Nanney 1966), or circular gradient (Uhlig 1960) were not therefore considered here.

3. Individuals were taken into consideration in which the degree to which division had advanced did not extend to morphogenetic movement of mouth kineties.

4. Examination was made of individuals embedded in gelatine in the preparation, sufficiently parallel to the cover-slip to enable the outline of the ventral surface of the protozoon to be made using one microscopic focus, and to mark all the topographical points discussed here.

The protozoa originated from B_1, B_2, S and D stocks. The method of culturing the protozoa has been described previously (Kaczanowska and Kowalska 1969). The preparations were silvered after Chatton and Lwoff, in accordance with Corliss' modification 1953. The protozoa were examined using an immersion objective × 60 and a Zeiss drawing apparatus with × 4 eyepiece. Outlines were made on millimetre paper at a distance of 37.2 cm from the eyepiece.

All the measurements were made on outlines only. The material analysed included the full drawing of ciliature and localization of the organelles of 114 individuals in the early phases of division. The examinations were supplemented by drawings of 50 individuals fixed at times varying from 0–5 minutes from the moment of separation of offspring, and 100 individuals from the later periods of the interdivisional stage (3- and 6-hour old protozoa).

All the results given here are expressed as converted to microns.

Measurements and analysis of errors

Errors in the measurements obtained may be due to three causes. They may be errors in the drawing itself, in making the measurement itself of the distance of points on the drawing, or may be errors in estimation of points between which the measurement was made.

The first and second category of errors can be evaluated by experiment. For this purpose 10 individuals were drawn twice independently, and measurements made twice independently from each drawing. In this way four sets of data were obtained for each individual. Comparison of the results showed that the maximum absolute error in the mean value of these four measurements was not more than $\pm$ 0.7 μ in the case of measurements of distance of CVPs from each other. This was therefore an error which for the majority of the measurements should not exceed 5%. It was however decided not to take this error into consideration in calculations on account of the arbitrary way in which other topographical points were determined, in relation to which the localization of CVPs was analyzed.

It is primarily a question here of the way in which the centre of the mouth of the future proter is determined. This point, indicated as C′, was determined only by geometrical assessment on the drawing as the centre of the oral field, marked by the semicircular course of circumoral kineties.

The second arbitrarily determined point was the apical point, indicated as A. This point was determined as the intermediate point between a point determined on the basis of the outline of the ventral surface, the end of the preoral kinety and finally as the extreme point of localization of the somatic ciliature of the right and left system.

Both these options make it impossible to determine the degree and significance of errors and reduce the accuracy of conclusions. A partial improvement of accuracy was obtained referring the majority of measurements to the postoral length of the protozoon.

Denominations of measurements (Figs. 1, 3)

Interval — this is the number of kineties counted between different structures. Distance — this is the distance between two points on the drawing made. Long axis of the body — the length of the section from the apex (point A) to the middle of the oral field (point C′) and then parallel to the course of postoral kineties to the posterior margin of the body was taken as the long axis of the body. Measurements of the section A–C′ showed that it varies little, and independently of the length of the postoral part of the body (see test 1). In many cases therefore it was decided not to asses section A–C′, which is burdened with considerable error, and to use only the index of length I.

I — length index — covers the postoral part of the long axis of the body and occupies the section from point C′ to the posterior margin of the body B. The distance between upper vacuoles and the lower vacuole was measured using the distance between CVP-2 and CVP-3. The fact was overlooked here that there is an interval between these vacuoles. In large individuals however the section CVP-2 and CVP-3 is almost parallel to the postoral part of the long axis.

The distance of CVP-3 to the posterior margin of the body was measured along the course of the somatic kineties, and this section was given the symbol E.

The distance of CVP-3″ to the posterior margin of the body was also measured along the course of somatic kineties and was given the symbol E″. The distance between C′ and CVP-1′ was termed R_1, and distance between C′ and CVP-2′ as R_2. R indicates the arithmetical mean of R_1 and R_2.

The length of the right margin measured from point A to the posterior end of the body D was taken as the right margin of the body. Point D on the posterior margin of the body was the point reached by the rear of the kinety of the right system which attained the apex in the anterior part. The right margin of the body A–D fell into two sections: one was the section from the apex to the upper end of segment X″ and was termed section A–X″, and the second from point X″ to

point D — as section X''–D. Sections A–X'' and X''–D corresponded to the right margins of the future proter and opisthe.

Measurements were made by means of compasses and bow compasses. The results obtained were analysed statistically in accordance with the explanations given. Column H_0 indicates the qualification of the numerical results obtained. Statistical calculations were made on a computer in the Numerical Computer Centre of Warsaw University. I should like to express my gratitude to Dr. E. Pleszczyńska and K. Styś, M. Sc. for their help in selecting and interpreting the results obtained.

Results

1. Localization of the mouth and old CVPs in relation to body length

a. Examination of correlation of the length of the preoral and postoral section of the body axis (test 1)

Statistical examination of the ratio of length A–C′ to I (test 1) does not reveal correlation of these two characters. There is a slight standard deviation for section A–C′ suggesting a relatively small range of variations in the length of this section. This deviation is not, however, so slight as to be able to speak of the constancy of this character. In view of this ambiguous result observations were extended to include 50 postdivisional individuals and 100 morphostatic individuals, separately, in the classes of proters and opisthes.

The results showed that in all the groups of protozoa examined, the range of variations in length of section A–C′ is the same. Comparative examinations also showed an approximately similar range of variations in the angle contained between section A–C′ and the postoral section I. This angle was slightly greater in the group of proters immediately after division, but in all the groups exhibited variation irrespective to the length of the individual.

These results would point to the considerable variation in the allometry of individuals, regardless of the stage of ontogenesis and length of I. If we compare these observations with formation in situ of the proter's mouth and absence of perceptible changes during division in the region of kinety X of the parent individual, we must reach the conclusion that the preoral part of the cortex is successively, almost without growth, transmitted during division to the proter.

b. Correlation of length of postoral section I to distance between CVP-2 and CVP-3 (test 2)

The previous investigations suggest that the preoral part does not grow. If this is in fact the case, then the chief increase in body length is connected with postoral section I. If we assume that CVPs do not carry out autonomic migrations in the interkinetal space during the morphostatic period, then by comparing the distance between CVP-2 and CVP-3 to I, it will be possible to deduce whether the main growth takes place in the equatorial region of the cortex. Test 2 demonstrates the

Table 1

Statistical calculations

No. of test	Character I			Character J						(6)		
	designation	(1)	(2)	designation	(1)	(2)	(3)	(4)	(5)	a	b	m
1 H_0	AC′	30.3	3.6	I	92.9	10.2	—	0.52 —	6.15 —	—	—	102
2 H_0	I	92.9	10.2	CVP-2-CVP-3	60.8	7.6	—	0.77 +	12.26 —	0.58	6.38	,,
3 H_0	AC′B	123.3	12.4	E	12.5	3.7	—	0.42 —	4.66 —	—	—	,,
4 H_0	I	92.9	10.2	E	12.5	3.7	—	0.42 —	4.68 —	—	—	,,
5 H_0	I	92.9	10.2	E	16.6	4.0	—	0.67 +	9.77 —	—	—	,,
6 H_0	R_1	11.0	1.2	R_2	11.1	1.2	1.52 +	0.92 +	26.14 —	0.95	0.56	114
7 H_0	R_1	11.0	1.2	I	92.9	10.2	—	0.51 —	6.35 —	—	—	,,
8 H_0	C_3′-CVP3′	27.5	3.5	C′′-CVP-3′′	28.6	3.8	5.28 —	0.81 +	15.16 —	0.9	3.8	,,
9 H_0	C′′-CVP3′	20.4	2.9	C′′-CVP2a′′	18.8	2.4	4.61 —	0.13 —	1.40 +	—	—	,,
10 H_0	C′-CVP3′	27.5	3.5	C′′-CVP2a′′	18.8	2.4	24.2 —	0.22 —	2.40 +	—	—	,,
11 H_0	C′-C′′	46.5	5.1	C′′-B	46.8	5.6	0.77 +	0.80 +	14.24 —	—	—	,,
12 H_0	C′-C′′	46.5	5.1	C′-CVP3′	27.5	3.5	—	0.69 +	10.34 —	0.47	5.38	,,
13 H_0	C′′-B	46.8	5.6	C′′-CVP-3′′	28.6	3.8	—	0.71 +	10.92 —	0.59	5.62	,,
14 H_0	A-X′′	87.8	8.5	X′′-D	87.6	9.1	0.40 +	0.81 +	14.6 —	0.86	0.11	,,

Explanations

(1) Mean value.

(2) Standard deviation defines the extent to which the individuals of a given community differ in respect of the character examined from the mean value of this character.

(3) T_S statistics $= T(S\,\mathrm{I} = S\,\mathrm{J})$ serve as a criterion for accepting or rejecting, on the level of significance of $\alpha = 0.01$, of hypothesis H_0 as to uniform mean values of character X I and X J. From tables of the t-Student distribution we determine, with m-1 degrees of freedom such a T_0 that $P(|T| \geqslant T_0) = \alpha$. If T_S is greater than T_0 we reject hypothesis H_0. If not there are no grounds for rejecting it.

(4) Correlation coefficient of character I and J.

(5) T_S statistics $= T(R\,\mathrm{I}\,\mathrm{J} = 0)$ serve as a criterion for accepting or rejecting hypothesis H_0 as to lack of correlation of characters I and J on the level of significance of $\alpha = 0.01$. From the tables of the t-Student distribution, with m-2 degrees of freedom we determine such a T_0 that $P(|T| \geqslant T_0) = \alpha$. If T_S is greater than T_0 we reject hypothesis H_0. If not there are no grounds for rejecting it.

(6) b and a are coefficients of simple regression of equation $y = a + bx$.

(m) Degree of freedom.

positive and significant correlation of these two characters and suggests that the growth of section I is connected with a greater distance between CVP-2 and CVP-3.

c. Examination of the correlation of body length with localization of CVP-3

Investigations of the variation in length of section E were made in relation to section A C′ B and in relation to I. The results agree (test 4 and 3). No correlation was shown between localization of CVP-3 in relation to the long axis of the body, although certain faint relations between these characters were not ruled out. The negative result of statistical investigations also agrees with observations of post-divisional and morphostatic individuals, where considerable variation in localization of CVP-3 is observed even in the same class of size of individuals. In two large individuals which were 6-hour uninjured proters almost terminal localization of CVP-3 was observed (see also Fig. 7).

All four tests 1, 2, 3, 4 jointly show that the chief increase in length probably takes place in the equatorial region and is almost, or totally, absent in the preoral part. The localization of CVP-3 however is not at a definite distance from the posterior end of the body nor is it in definite proportion in relation to body length, even in individuals in the same stage of ontogenesis and belonging to the same group (proters or opisthes).

2. Analysis of topographical relations between old and new CVPs

Investigation was made of the correlation of characters I to E″, and the faint positive correlation of these characters demonstrated. In view of this CVP-3″, in comparison with the situation of CVP-3, in more closely connected topographically with body length (test 5). The faint correlation coefficient, however, may suggest a less direct relation between these characters.

On the other hand direct observations of dividers gave unexpected results. It proved that distance E″ may be equal to, greater, and only in two cases smaller, than the homological section E. These results applied to all stocks. It was also found that differences between the situation of CVP-3 and CVP-3″ may vary greatly, even in individuals belonging to the same class of size, in both proters and opisthes.

The investigations on the one hand did not show that there was a definite localization of CVP-3 in relation to new-forming CVP-3″, and in addition revealed a phenomenon difficult to interpret, that CVP-3″ is in general formed nearer the equatorial region than the parent CVP-3. This astonishing result (Figs. 3, 4) suggests that during the course of divisional morphogenesis far more complicated shift of structures in relation to each takes place than is suggested by the static pictures of the kinetom in the preparations.

This observation points to the possibility that autonomic movement of CVP-3″ exists in a certain period of morphogenesis like that shown by King 1954 for *Paramecium*.

3. Topographical relations of C′, C″ and new CVPs

a. Topographical relations of C′ and CVP-1′ and CVP-2′

Comparison of length of sections C′–CVP-1′ and C–CVP-2′ showed that there is a small standard deviation of these two characters. There is a distinct and high correlation coefficient of these characters, this correlation is significant and there are no grounds for rejecting the hypothesis as to equality of the mean value of these sections (test 6). In other words it appears that CVP-1′ and CVP-2′ are formed at a uniform distance from C′. Test 7 reveals a faint correlation, or absence of correlation of this distance in relation to section I. The small standard deviation for R_1 and R_2 permits of speaking of a more or less constant radius R for all the individuals examined. Faint correlation and rejection of the lack of correlation between R and I suggests that in certain strains of large individuals this radius R may be slightly greater. This result also coincided with the observations made.

b. Determination of point C″ and comparison of topography of CVPs of the future proter and opisthe

C″ was determined geometrically, assuming that CVP-1″ and CVP-2″ in the forming opisthe will be situated at distance R characteristic of the homological proter.

The point determined in this way falls subequatorially in the region of the second postoral kinety. C″ is thus shifted during this period of morphogenesis to the right in relation to C′. This would also form evidence of the temporal distortion of the postoral axis of the body. It would appear that this phenomenon is very important to an analysis of intraclonal dimorphism.

C″ is determined geometrically, but the significance of this way of determining C″ is borne out by the occurrence of CVP-2b″ exactly at distance R in all 17 cases of the occurrence of this vacuole.

Comparison of distance from C′ and C″ to the localization of new lower vacuoles (CVP-3′ and CVP-3″ respectively) revealed significant and marked correlation (test 8). Contrary to expectations, however, uniform mean values of these two characters were not shown. As a rule the distance falling to the opisthe (C″–CVP-3″) was longer than in the proter (C′–CVP-3′). We do not know the underlying cause of this phenomenon, but it undoubtedly influences the appearance of intraclonal dimorphism.

Occurrence of distortion of the postoral axis and the longer distance from C″ to the lower vacuole of opisthe in relation to the proter C′–CVP-3′ are the two causes resulting, the distance between CVP-2″ and CVP-3″ being greater in the future opisthe than in the proter. The appearance of CVP-2a″ and possible CVP-2c′ may be connected with the possibility of development of other places of differentiation in the activated kinety. Support for this interpretation is provided by cases

of the absence of these vacuoles in small dividing individuals, or individuals with amputated posterior part (Kaczanowska 1969).

Statistical calculations did not reveal a correlation of lengths of sections C''–CVP-3' and C''–CVP-2a'' (test 9), and C'–CVP-3' and C''–CVP-2a'' (test 10), despite the fact that CVP-3' and CVP-2a'' form slightly later in divisional morphogenesis and may disappear in some starved individuals (Kowalska and Kaczanowska 1969).

c. Localization of C'' in relation to the postoral section of the body

Comparison of length of sections C'–C'' and C''–B revealed a marked and significant correlation of these characters and equality of the mean values of these characters (test 11). Thus C'' determined geometrically is situated midway along the oral-caudal section, and slightly to the right from C'. Consequently a positive correlation was also shown between sections C'–C'' and C'–CVP-3' (test 12) and C''–B and C''–CVP-3'' (test 13).

d. Topography of the right margin of the presumptive divisional furrow

Initially segment X'' forms in the supraequatorial region of the parent individual. In the early phases of division it was found that the length of sections A–X'' and X''–D exhibit marked correlation and that there are no grounds for rejecting the hypothesis that the presumptive right margin of the divisional furrow forms midway along the apical-caudal margin of the body (test 14). It must be added that the accuracy of measurements is not very great here on account of the difficulty in determining point A.

Discussion

Growth of the cortex and topography of cortical organelles

During these studies the considerable variation in the localization of CVPs in relation to the ciliature was demonstrated. This variation makes it impossible to treat CVPs as landmarks for drawing up a growth map.

The mouth of a proter, formed in situ, justifies the assumption that the preoral part of the cortex of a proter does not grow and is successively transmitted to consecutive generations of proters. It can be deduced from this that the various parts of this cortex composing the whole of an individual vary in age. This conclusion agrees with the data given by Ehret et al. 1964, Tartar 1941, Dippell 1965, Shan 1969. The preoral part of the opisthe is formed during the course of division. During the period of its formation both allometric growth (Fauré-Fremiet 1950) and intensive morphogenetic movements (Deroux pers. commun.) is suggested. Observations of the localization of CVP-3'' and CVP-3 also lead to assuming that

during this period CVP-3'' also shifts in relation to the rest of the ciliature of the opisthe. This problem requires separate investigation. If, however, the morphogenetic movements of CVPs were limited only to the division period, and did not take place during the postdivisional period, it may be assumed that the main growth of the cortex takes place in the equatorial region of the ciliate. This conclusion agrees with the observations made on *Paramecium* (Sonneborn 1963, Gillies and Hanson 1968).

Relation of old and new structures during the period of divisional morphogenesis, causes of appearance of intraclonal dimorphism

Division of the ciliate is preceded by characteristic changes in the nuclear apparatus. These changes are connected with the emergence of the nucleole outside the macronucleus (Radzikowski 1965). This suggests genetic conditions of the morphogenetic process (de Haller 1964, Hanson 1967). The problem remains to be solved, however of what cytoplasmatic factors determine the place in which the morphogenetic processes begin. Offspring take the polarization of the cortex from the parent individual (anisotropy of cortex Fauré-Fremiet 1950). The organization of the proter progeny is connected both with the formation in situ of the mouth and with the same system of the ciliature. Thus many elements of the organization are taken by the proter from the organization of the parent individual. The position with the forming opisthe is, however, different. The right meridional ciliature is subject here to continuation, while l'évolution par decalage takes place in the left (Chatton et al. 1931). In principle with the same kineties in proter and opisthe the upper vacuoles form in opisthe and proter, but not necessarily in conformity with the pattern of the parent individual (Kowalska and Kaczanowska 1969). The mouth of opisthe forms completely autonomically. The site of its original localization is not known. In any case the earliest rudiments of the rosette are observed between the second and third postoral kinety, and thus in the same axis in relation to the mouth of the future proter. The geometrically determined point C'' is, however, shifted to the right and very posteriorly in relation to the first position of the rosette. The reason for this discrepancy is not known. The shift to the right of point C'' results in the activated kineties capable of differentiation being situated at a different distance in relation to C' and C''. This is a phenomenon influencing the appearance of intraclonal dimorphism. Other causes of which are not known are: activation of a different kinety in the forming proter to that in the opisthe for the creation of the lower vacuole, the greater distance of CVP-3 from C'' in the opisthe than is the case in the forming proter and the capacity for forming CVP-2b'' occurring only in the opisthe.

The consequence of the mechanism of intercalar ingrowth of the postoral kinety during the period of morphogenetic movement is a change in the interval between CVP-1 and CVP-2 of the opisthe. This is both a factor affecting intraclonal di-

morphism and a mechanism for bringing about a return shift of C'' to their proper position in relation to the meridional kineties. This might be spoken of here as the mechanism of retrogression of the slippage phenomenon (Nanney 1967) of C'' during each divisional morphogenesis. The occurrence of additional vacuoles CVP-2a'' and possibly CVP-2c'' is connected with activation of other places of differentiation. Taken jointly all the phenomena given influence the occurrence of intraclonal dimorphism, but still do not permit of its full interpretation.

The studies made do not show that there is any relation between the localization of old and new CVPs. Intraclonal dimorphism, the different course of morphogenesis in the forming proter and opisthe and absence of constant topographical relations between old and new CVPs form evidence of the lack of continuation of the organization plan of the parent individuals in creating new topographical relations in the offspring. Therefore in searching for a different type of cortical conditionings of the course of divisional morphogenesis attention was paid to the possibility of determining the sites of differentiation by cortical gradients.

Cortical gradients

Experimental studies of ciliates showed that cortical gradients may influence the determination of site and course of stomatogenesis. These data taken jointly show that the circular gradient should be expected to determine the meridional sector capable of stomatogenesis (Uhlig 1960, Tartar 1962). The inhibiting action of both the preexisting mouth structure and of the posterior pole of the body should also be expected (Tartar 1958, Uhlig 1960). May be that these gradients are not separated but form only one morphogenetic gradient of cortex (König 1968, Schwart 1969). As in many cases of motor cortical gradients, so in morphogenesis it would seem that the localization of the mouth and posterior pole are the centres of these gradients, e.g. the mouth as the centre from which metachronic waves originate (Seravin 1962, Doroszewski 1963), oral-caudal gradient of excitability in *Paramecium* (Grebecki 1965), differentiation of reactions of the anterior and posterior parts of *Dileptus* reproduced during divisional and regenerative morphogenesis (Doroszewski 1965). These analogies should not however be over-estimated, and the possibility that motor cortical gradients and morphogenetical ones are basically different cannot be excluded. All that can be said about the differences between the oral and caudal regions in *Chilodonella* is that a larger number of fibrous structures and membranes and greater enzymatic activity is centered in the oral region in comparison with the caudal one. It is not impossible that in the case of morphogenetic gradients revelation of their action is not possible just after a certain loosening of the structure of the parent individual. In the light of what we know about inhibition of morphogenesis by the old mouth (Weisz 1956, Tartar 1958) and of the activation of the eggs of *Metoza* connected with proteolysis (Mano 1966) this possibility cannot be ruled out.

The morphological expression of this proteolysis in *Chilodonella* would be the dissolution of the parent oral basket. In a large protozoon in this state of activation, two sites of stomatogenesis are formed, one in situ and the other at a certain distance from it and behind it, in accordance with the polarization of the cortex. Although it is not known what the geometrical point C'' is in essence, it does not however constantly form at the same distance from C' as might be expected. Point C'' is correlated with the length of the oral-caudal axis. In this case C'' was situated midway along the axis, which was of course a convenient but certainly not decisive argument for its validity and not random determination of its position.

Data, as yet far from complete, permit of distinguishing the oral-caudal axis with determination of point C'' from the apical-caudal axis for determination of the presumptive divisional fissure. It is not however known what the relation is of localization of stomatogenesis and divisional furrow.

Organization centre and induction

Uhlig 1960, Tartar 1962, Eberhardt 1962, Sonneborn 1963 draw attention to the formation of structures at the place of contact of two cortical zones by means of induction of one zone on the competent second one. There are no corresponding data on this in the case of *Chilodonella*.

Studies of the topography of cortical organelles in *Chilodonella* show that there is non-random localization of newly-forming CVPs in relation to determined C' and C''. The results presented here suggest that the localization of C' and C'' influence the site of differentiation of activated kineties (competent sensu Jerka-Dziadosz and Frankel 1969). Thus points C' and C'' would play the part of organization centres which would be one of the factors determining the topography of CVPs. Such a statement does not of course exclude other factors also which might affect this process. For instance, the more intensive processes of differentiation in future opisthe in relation to the proter permitting of revealing additional CVPs. This view would agree both with the discussions on the subject of the organization centre and differentiation of the morphogenetic field (Nieuwkoop 1962, 1967, 1967 and also Uhlig 1960). It is thus not impossible that from C' and C'' a factor would be radiated with more or less equal intensivity and velocity in all individuals, which would stimulate the appropriate kineties to differentiation of CVPs.

This factor must not necessarily accompany each stomatogenesis. The studies by Janus (inedit.) on conjugational stomatogenesis in *Chilodonella cucullulus* showed that there is then absence both of resorption of old and formation of new CVPs.

The same was proved for autogamy and postautogamous reorganization for *Paramecium aurelia* (Diller 1969).

Similarly it would in fact appear that formation of new CVPs in not unequivocally connected with the resorption of old CVPs, since cases are observed which have been interpreted by me as inhibition of resorption of old CVPs from the pre-

ceding generation (Fig. 8). It has not however been shown that after the period of morphogenesis there was control of the localization of cortical organelles in relation to the whole kinetome.

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Summary

The study was based on various strains of *Chilodonella cucullulus* but on the cells in the same ontogenetic stage of early differentiation of CVPs (Contractile Vacuole Pores).

It was suggested, but does not prove that the growth of cell surface takes place in the equatorial zone of ciliate and does not involve the preoral zone.

The disposition of CVPs is stabilized in early division in defined interkinetal space. The exact places of differentiation of CVP for future proter and opisthe is highly correlated with position of oral field (C′) and hypothetical center for opisthe (C″). In this and only in this period the geometrical relations between ciliated area, oral field and C″ on the one part and CVPs disposition on the other part can be detected.

In the next stages this conformity is highly disturbed by such events as morphogenetic movement, cytokinesis and various ratio of cell growth in different specimens.

Then only for early dividers of *Chilodonella cucullulus* the cytogeometric model of topography of cortical organelles is proposed.

STRESZCZENIE

Studia dotyczyły osobników *Chilodonella cucullulus* pochodzących z różnych szczepów, ale badanych w tym samym stadium ontogenezy. Było to stadium przed podziałem komórki, w momencie pojawiania się otworków wodniczek tętniących CVPs.

Sugerowana, ale nie udowodniona, jest strefa równikowa jako miejsce najintensywniejszego przyrostu powierzchni. Przyrost powierzchni nie obejmuje okolicy przedgębowej pierwotniaków. Rozmieszczenie CVPs ustala się w okresie wczesnego podziału i odbywa się w określonych pasmach międzykinetalnych.

Miejsca, w których tworzą się CVPs są wyraźnie skorelowane z położeniem gęby (C′) i hipotetycznym centrum (C″) dla tworzącego się opistora. W tym i tylko w tym okresie istnieje zależność między orzęsioną powierzchnią, gębą i C″ z jednej, a miejscem pojawiania się CVPs z drugiej strony. W późniejszych stadiach te zależności są zakłócone przez ruch morfogenetyczny, cytokinezę i różne tempo wzrostu poszczególnych pierwotniaków.

Tak więc jedynie dla wczesnych form przedpodziałowych *Chilodonella cucullulus* może być podany cytogeometryczny model topografii organellów kortykalnych.

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Laboratory of General Parasitology, Department of Parasitology, Polish Academy of Science, Warszawa 22, Pasteura 3, Poland

Stanisław L. KAZUBSKI

Morphological variability of *Semitrichodina sphaeronuclea* (Lom, 1956)

Morfologiczna zmienność *Semitrichodina sphaeronuclea* (Lom, 1956)

Lom 1956 described under the name *Trichodinella sphaeronuclea* an interesting species of ciliates of a spherical or somewhat oval macronucleus, occurring in the mantle cavity of the land snails *Limax maximus* L. and *Bielzia coerulans* (Bielz), family *Limacidae*, originating from Bohemia and Slovakia.

Two years later Kazubski 1958 described a very similar trichodine occurring in *Oxychilus (Cellarius) orientalis* (Cless), (syn. *Schistophallus orientalis* Cless.) of the *Zonitidae*, gathered in the environs of Sanok in southeastern Poland. This trichodine was classified to the same species. At the same time a separate genus *Semitrichodina* Kazubski, 1958 for this species was erected. Certain differences, which were observable between specimens from *Oxychilus* and the ciliates described by Lom taken from the *Limacidae* were qualified as probably resulting from the parasitization in different hosts. However, it was not possible at the time to clarify the matter definitively, until Lom 1956 gave the dimensions from both hosts originating from various parts of Czechoslovakia inclusively.

Kazubski 1959 makes note of *S. sphaeronuclea* also in *Bielzia coerulans* and indicates the occurrence of the ciliate as well in the environs of Krosno (southeastern Poland). In another report Kazubski 1960 informs of the occurrence of numerous specimens of *S. sphaeronuclea* in *Oxychilus orientalis*, *Bielzia coerulans* and singular specimens in *Semilimax semilimax* (Fér.) of the *Vitrinidae* and in *Aegopinella epipedostoma* (Fagot) of the *Zonitidae*.

The earliest account of *S. sphaeronuclea* from the *Limacidae* from Poland was given by J. Raabe and Z. Raabe 1961 describing this species from *Bielzia coerulans* from the Gorce mountains in the Carpathians. The ciliates found by the authors were described as having greater size and number of denticles than specimens described by Lom in 1956.

In the same year Kazubski 1961 describing a new species of trichodine, *Semitrichodina convexa* Kazubski, 1961, and comparing it with *S. sphaeronuclea* calls attention to serious differences in the number of denticles, occurring with the latter

species, between specimens originating from *Limacidae* and *Zonitidae*. It was underscored that both of these "forms" inhabit the same area, and are not therefore geographical forms. The matter of their distinctiveness has not been however discussed.

Lom 1964 mentions *S. sphaeronuclea* additionaly in *Limax cinereoniger* Wolf. (*Limacidae*). In the same year Haider 1964 gave an account of a find near Schwabach, Mfr., Bavaria, of a single *Limax maximus* infected with *S. sphaeronuclea* and presented a detailed description and dimensions of these ciliates approximating the dimensions given by Lom 1956.

The presented work has as its aim the demonstration of more extensive material concerning both discussed forms, the consideration of their distinctiveness, and as far as possible, an explanation of seasonal changes in the searched ciliates.

Material and method

The ciliates serving as material for examination were gathered in the Carpathian mountains in the years 1957–1959 at which time I conducted extensive faunistical research on parasitic ciliates occurring in land snails. A list of the infected snails and the localities where they originated will be discussed in the following part of this work. Preparations of the protozoans were made by the method of dry smears according to Klein. Material for the arrangement (Tables 1 and 2) was employed irrespective of place or year of collection, after previous examination as to whether a given group of ciliates did not in fact differ from the remaining ciliates included in the same sub-division. For research on the morphology of the examined ciliates extensive use was made of analyzing photographs made to identical scale.

The technical assistance of Mrs. Grażyna Mierzejewska is gratefully acknowledged.

Results

Numerous specimens of *Semitrichodina sphaeronuclea* (Lom) were examined originating equally from *Bielzia coerulans (Limacidae)* as from *Oxychilus orientalis* (*Zonitidae*). The results of the measurements of these ciliates are presented in Tables 1 and 2. No new structural details were found. The trichodines found in *Bielzia coerulans* correspond basically to the primary description of Lom 1956, while specimens from *Oxychilus orientalis* are in agreement with the description of Kazubski 1958. Only a description of the shape of the denticles requires more precision. Namely the centrifugal blades of the denticles only very rarely have as parallel lateral margins, especially in ciliates from the *Zonitidae*, as has been presented in the drawings of Lom 1956 and Kazubski 1958. Most oftenly the blade narrows gradually and ends relatively sharply (Figs. 1 A, B, and 2 B, C, E).

The conducted research further on confirmed the existence of differences between forms from *Limacidae* and *Zonitidae*. These differences however appear to be prin-

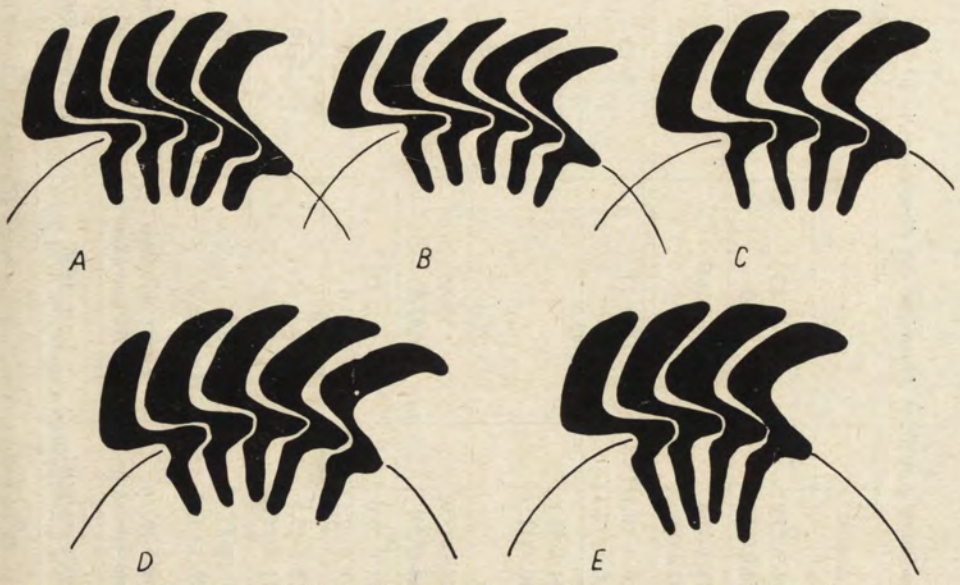

Fig. 1. *Semitrichodina sphaeronuclea* (Lom, 1956) forma *macrodentata* f. n. from *Bielzia coerulans* (Bielz), shape of the denticles. Specimens from 20 June (C and E), from 28 July (A and B) and from 21 Aug. (D)

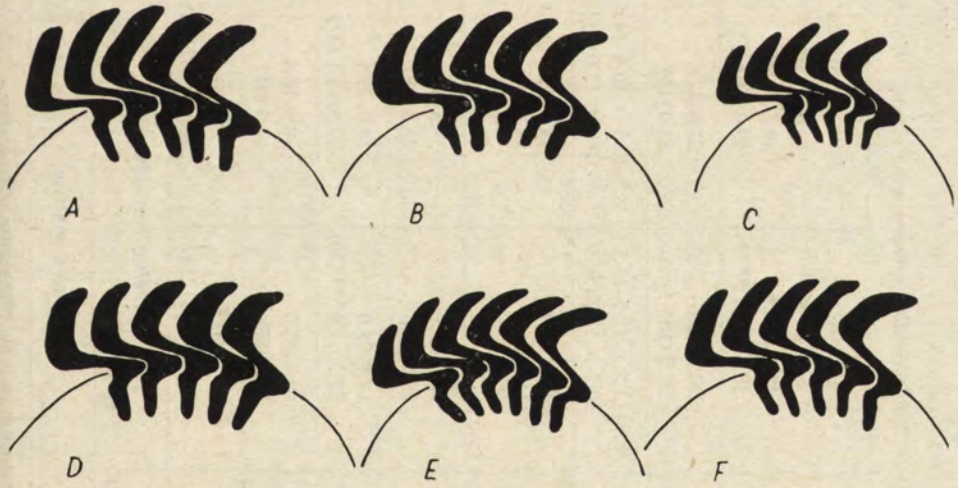

Fig. 2. *Semitrichodina sphaeronuclea* (Lom, 1956) forma *microdentata* f. n. from *Oxychilus orientalis* (Cless.) shape of the denticles. Specimens from 21 May (A and B), from 24 June (C and D), from 4 Sept. (E) and from 3 Oct. (E)

cipally numerical in nature (Tables 1 and 2). Thus, the diameter of the denticulate ring is practically the same in both forms, while the adhesive disc is larger in specimens from *Limacidae* than from *Zonitidae*, whereas the number of denticles is lesser in specimens from *Limacidae* than from *Zonitidae*. It depends on the arran-

Table 1

Measurements of *Semitrichodina sphaeronuclea* (Lom, 1956) forma *macrodentata* f. n. from *Limacidae* (all dimensions in μ)

Dates of collection	13 May	21 June–21 August	August	—	—
Diameter of the body	47.8–66.6* 58.0** (29)****	40.6–79.0 60.0 (50)	34.3–75.9 58.0 (176)	44–83 62.0	41–65 50.0
Dimension of the adoral spiral	32.2–42.6 36.2 (10)	27.0–42.6 34.6 (16)	23.9–37.4 33.6 (9)	—	—
Dimensions of the macronucleus	8.3–12.5 × 12.5–13.5 10.2 × 12.7 (5)	8.3–13.5 × 10.4–14.6 10.7 × 13.0 (6)	9.4–14.6 × 10.4–19.8 11.0 × 12.3 (26)	9–15	8–15 10
Diameter of the adhesive disc with the border membrane	41.6–49.9 62.3 (21)	39.5–55.1 46.6 (29)	34.3–55.1 44.9 (113)	—	—
Diameter of the adhesive disc	33.3–43.7 39.0 (20)	34.3–50.0 41.3 (26)	28.1–48.9 38.6 (84)	35–49 42.0	35–49 43.0
Diameter of the denticulate ring	18.7–25.0 22.2±1.9*** (20)	14.6–29.1 22.6±3.1 (37)	15.1–27.0 21.8±2.1 (158)	18–32 23.0	18–32 25.0
Length of the denticle	9.4–13.0 10.9 (22)	8.3–15.6 11.3 (31)	8.3–13.5 11.2 (161)	—	8–10
Number of the denticles	26–31 27.8±1.24 (21)	26–32 28.8±1.42 (30)	26–33 28.3±1.32 (154)	26–31 28.0	26–35 29.0
Hosts	*Bielzia coerulans*		*Limax* sp. sp. *Bielzia coerulans*	*Limax maximus* *Bielzia coerulans*	*Limax maximus*
References	own material		Raabe's material	Lom 1956	Haider 1964

* Range, ** mean value, *** standard deviation, **** number of dimensions.

Table 2

Measurements of *Semitrichodina sphaeronuclea* (Lom, 1956) forma *microdentata* f. n. from *Oxychilus orientalis* (Cless.) (all dimensions in μ, own material)

Dates of collection	19–21 May	24 June–12 Aug.	2–18 Sept.	2 Oct.–16 Nov.
Diameter of the body	49.9–78.0* 61.8 ** (51) ****	45.8–64.5 54.8 (20)	48.9–69.7 57.8 (30)	45.8–74.9 58.5 (116)
Dimension of the adoral spiral	25.0–43.7 38.9 (32)	28.1–40.6 33.5 (7)	32.2–41.6 36.4 (10)	22.9–43.7 36.0 (51)
Dimensions of the macronucleus	7.3–16.6 × 8.3–18.7 11.5 × 13.6 (38)	8.3–13.5 × 9.4–15.6 10.4 × 11.4 (5)	9.4–11.4 × 10.4–14.6 10.6 × 12.3 (5)	7.3–14.6 × 8.3–16.6 10.7 × 11.9 (53)
Diameter of the adhesive disc with the border membrane	37.4–51.0 44.0 (19)	36.4–51.0 42.6 (14)	22.9–51.0 41.7 (14)	32.2–60.3 43.4 (47)
Diameter of the adhesive disc	33.3–46.8 40.7 (17)	30.2–42.6 37.0 (14)	34.3–43.7 37.1 (12)	27.0–54.1 38.5 (53)
Diameter of the denticulate ring	19.8–27.0 22.7±2.1 *** (26)	15.6–26.0 21.7±2.7 (17)	18.7–25.0 22.7±1.8 (15)	17.7–27.0 22.8±2.2 (48)
Length of the denticle	8.3–11.4 9.5 (20)	8.3–9.7 9.7 (9)	8.3–12.5 9.7 (13)	7.3–11.4 9.6 (39)
Number of the denticles	32–36 33.5±1.09 (21)	29–35 31.9±1.54 (14)	31–34 32.9±0.94 (10)	31–38 33.1±1.62 (29)

* Range, ** mean value, *** standard deviation, **** number of dimensions.

gement of denticles in both forms. The denticles of the specimens from *Limacidae* are larger, whereby especially clear is the difference in height, and they are more rarely arranged (Plate I, Figs. 1 and 2). Both forms in spite of marked similarity thus differ considerably.

As I have already indicated in the introduction, the dimensions of specimens from *Limacidae* given by J. Raabe and Z. Raabe 1961 differ significantly from the dimensions given by Lom in 1956. Thanks to the kindness of Prof. Dr. Z. Raabe I was able to re-examine the same material coming from the Gorce mountains and gathered in August of 1960 on the slopes of the Mt. Turbacz facing Nowy Targ. The examination and repeated measurements of all of the material permitted the confirmation that differences in dimensions of ciliates gathered by Raabe and ciliates described by Lom 1956 and by other authors, as well as the ciliates collected by me from *Bielzia coerulans*, are not great and can be acknowledged as insignificant (Table 1).

Simultaneously with the comparison of trichodines from various hosts, an analysis of the dimensions and number of denticles within the separate groups of *Semitrichodina sphaeronuclea* was made. Interesting results were gained in the case of ciliates from *Oxychilus orientalis*, collected in different seasons of the year. Ciliates in this group gathered in the summer were somewhat smaller and had a lesser number of denticles than specimens collected in the spring and autumn. As results from Table 2, these seasonal changes are relatively small but sufficiently marked (Fig. 3).

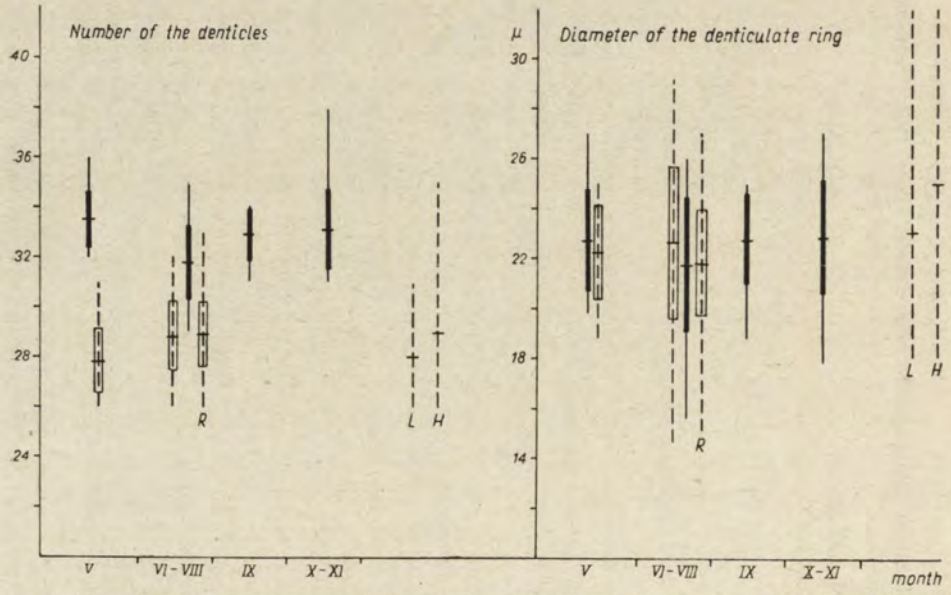

Fig. 3. *Semitrichodina sphaeronuclea* (Lom, 1956), variability of the number of the denticles and diameter of the denticulate ring; full line *S. s.* forma *microdentata* from *Oxychilus orientalis*, dotted line *S. s.* forma *macrodentata* from *Limacidae*, *R* — Raabe's material, *L* — after Lom 1956, *H* — after Haider 1964; range, mean and standard deviation are denoted

Disagreement with this regle shown by the ciliates from *Limacidae* (Table 1) is probably due to fact that all the ciliates examined in May were derived from only one population originating from one snail.

Distribution of *Semitrichodina sphaeronuclea*

Semitrichodina sphaeronuclea in *Bielza coerulans* (Bielz) was found by me in 6 individuals of snails of this species among 12 collected in the Carpathian mountains. The infected individuals were found in the Bieszczady Mts. in the area of the village Beniowa-Sianki and in the area of Komańcza in the Osławica river basin; in the Beskidy mountains along the sides of Mt. Luboń Wielki, in the valley Wielka Puszcza (Andrychów district), and near the town Wisła. The extensiveness of infection was great. As many as several hundred specimens and more were found in each snail.

Semitrichodina sphaeronuclea from *Oxychilus orientalis* (Cless.) was found in the Bieszczady in 17 of 65 examined snails (26.2%) in several places near the village Beniowa-Sianki, Stuposiany, in the area of Komańcza in the valley of the rivers Osława and Osławica. In the Carpathians in the area of Sanok 97 of 392 snails examined (24.7%) were infected. Near Krosno 6 of 15 examined (40%) were infected. An intensity of infection of several dozens of specimens was found.

In *Aegopinella epipedostoma* (Fagot, 1879) (*Zonitidae*) *S. sphaeronuclea* was found twice in the environs of Sanok on 104 snails examined in the Carpathians, number 79 of which were taken from Sanok. In *Semilimax semilimax* (Ferussac, 1802), *Vitrinidae*, only single snails were infected by *S. sphaeronuclea* near Sanok and Krosno among 35 snails of this species examined in the Carpathians. In both these latter mentioned species the degree of infection was low and barely a few specimens occurred. It is worthy of note that in all of these cases, the infected snails were collected from the places inhabited by *Oxychilus orientalis*, which was found to be heavily infected with *S. sphaeronuclea*. In other species of *Zonitidae* the list of which was given by Kazubski 1963 (Table 5) *Semitrichodina sphaeronuclea* was not noted.

Discussion

The above presented data indicate that under the name of *Semitrichodina sphaeronuclea* there has been described two separate forms of trichodines similar to one another but also posing many differences. These differences concern mainly the structure of the denticular ring. The denticulate ring of specimens derived from the *Limacidae* are characterized by a lesser number of the larger denticles in comparison with the form from the *Zonitidae*. The separate forms are specific in relation to their respective hosts, the one being noted exclusively in the snails from *Lima-*

cidae, the other mainly in the *Zonitidae*. With regard to this feature both live in various biological and ecological conditions: the form from *Limacidae* in places drayer and warmer than the form occurring in *Zonitidae*, which inhabit moist and shaded banks of mountain streams. It appears equally that certain differences in the distribution of both forms also exist. The form from *Zonitidae* has up to this time been noted in the Carpathians in south-eastern Poland and probably it occurs in the whole area of *Oxychilus orientalis* to the eastern borders of the Tatry mountains. This form has been also found in several species of *Zonitidae* in Bulgaria (Kazubski unpublished). On the other hand, the form from *Limacidae* has been noted along the entire area of the Carpathians in Poland, Slovakia and Bohemia (Lom 1956) and in Bavaria in G. F. R. (Haider 1964).

Taking into account all the forementioned considerations I should like to distinguish within the species *Semitrichodina sphaeronuclea* two separate forms: *macrodentata* described from *Bielzia coerulans*, *Limax maximus*, and *L. cinereoniger* by Lom 1956, 1964, J. Raabe and Z. Raabe 1961, Haider 1964 and Kazubski (present paper) and *microdentata* described by Kazubski 1958, 1959, 1960 from *Oxychillus orientalis* and other *Zonitidae*. Underscoring in this fashion the differences between these two groups of trichodines, I should like to call attention to them, thereby encouraging further collection of new material and the undertaking of further research concerning this species. It appears to me that especially interesting would be a re-examination of the distribution of the two forms, recognition of their hosts, as well as research on the environmental conditions in which these hosts occur and which indirectly influence on the parasitic ciliates in them. Perhaps this is a case in which two closely related forms are in the process of differentiation into separate species. In this regard it is especially interesting, that the form occurring under warm conditions is characterized by a lesser number of denticles than the form from *Zonitidae*, which occurs in cooler areas.

In *Semitrichodina sphaeronuclea* f. *microdentata* there has been noted seasonal changes associated with decreasing of the dimensions of the body, adhesive disc, diameter of the denticulate ring and the number of denticles in summer specimens in relation to spring and autumn specimens (Table 1). This places this species next to many other species Ciliata, in which the same regularity has been observed (Kazubski 1969).

Summary

An analysis of the species *Semitrichodina sphaeronuclea* (Lom, 1956) has been made on the basis of author's materials as well as data from literature. Two forms have been distinguished within the species: f. *macrodentata*, occurring in snails of the family *Limacidae* and f. *microdentata* parasitizing mainly *Zonitidae*. In *S. sphaeronuclea* f. *microdentata* seasonal changes have been ascertained.

STRESZCZENIE

Na podstawie materiałów własnych oraz danych z piśmiennictwa przeprowadzono analizę gatunku *Semitrichodina sphaeronuclea* (Lom, 1956). W gatunku tym wyróżniono dwie formy: f. *macrodentata*, występująca u mięczaków z rodziny *Limacidae* i f. *microdentata* pasożytująca głównie u *Zonitidae*. U *S. sphaeronuclea* f. *macrodentata* stwierdzono zmienność sezonową.

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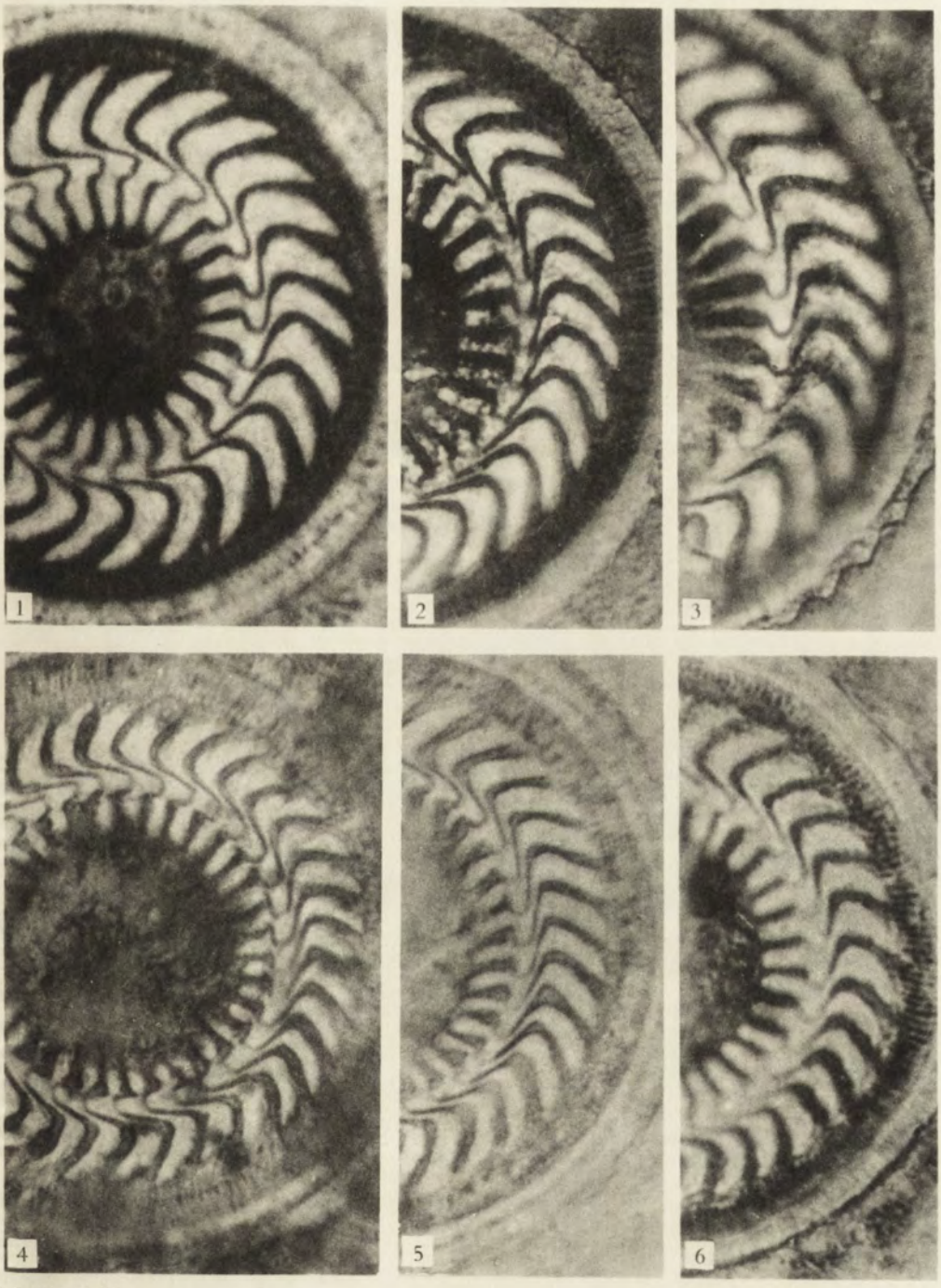
EXPLANATION OF PLATE I

1–3: *Semitrichodina sphaeronuclea* (Lom, 1956) forma *macrodentata* f. n. from *Bielzia coerulans* (Bielz), adhesive disc; specimens from 13 May (1), from 28 July (2) and from 20 June (3)
4–6: *Semitrichodina sphaeronuclea* (Lom, 1956) forma *microdentata* f. n. from *Oxychilus orientalis* (Cless.), adhesive disc; specimens from 21 May (4 and 5) and from 24 June (6)



S. L. Kazubski auctor phot.

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Научно-исследовательская станция пресноводного промысла Динь-Банг, ДРВ,
и Лаборатория паразитологии Зоологического института АН СССР, Ленинград В-164, СССР

Research Station of freshwater fisheries Dinh-Bang, DRV and
Laboratory of Parasitology, Zoological Institute of the Academy of Sciences of the U.S.S.R., Leningrad V-164, U.S.S.R.

Ха КИ

На КУ

Новые инфузории (*Ciliata*) из кишечника пресноводных рыб Северного Вьетнама

New *Ciliata* from the intestine of freshwater fishes of Northern Vietnam

При исследовании паразитофауны некоторых пресноводных рыб Северного Вьетнама в их кишечнике были обнаружены 6 интересных видов инфузорий, оказавшихся новыми для науки; один из них отнесен к новому роду *Inferostoma* gen. n.

Нами просмотрено фиксированное содержимое кишечника от следующих рыб: *Spinibarbichthys denticulatus* (Oshima, 1926). *Squaliobarbus curriculus* (Pell. et Chev., 1934), и *Cirrhina molitorella* (Cuv. et Val., 1844), добытых в оз. Ба-Бэ (провинция Бак-кан) и в речке Бо (провинция Лао-кай).

Материал, фиксированный 70° спиртом, окрашивался гематоксилином Гейденгайна с последующим заключением в канадский бальзам. В соответствии с правилами зоологической номенклатуры и с практикой, принятой в протозоологии (Corliss 1962 a, b), мы обозначали не типовых особей, а типовые препараты новых видов, все остальные препараты каждого вида обозначены как синтипы. Типовые препараты всех 6 новых видов паразитов хранятся в коллекции Зоологического института АН СССР, Ленинград.

В статье применяется следующая терминология. Кинетом — совокупность ресничных рядов, мембран и мембранелл особи; инфрацилиатура — совокупность кинетосом особи; кинета — ряд ресничек или кинетосом; префундибулюм — наружный отдел адоральной зоны мембранелл, лежащий на поверхности тела; инфундибулюм — внутренний отдел, погруженный в цитоплазму; инфундибулярное отверстие — место погружения зоны мембранелл вглубь тела. Термин рот (ротовое отверстие) мы понимаем в том же смысле, что и Корлисс (Corliss 1959) — это отверстие, за которым уже нет каких-либо мембранелл или ресничек; от рта отходит лишь трубчатая глотка.

Система высших таксонов инфузорий (отряды, подклассы и классы) дается по курсу „Общей протозоологии" Догеля, Полянского и Хейсина 1962, с модификацией Полянского и Хейсина 1964.

За помощь при обработке материалов, написании статьи и подготовке ее к печати приношу глубокую благодарность А. В. Янковскому (Зоологический институт АН СССР).

Подкласс *Holotricha* Stein, 1859
Отряд *Trichostomatida* Bütschli, 1889
Семейство *Balantidiidae* Reichenow, 1929
Род *Balantidium* Clap. et Lachm., 1858

Balantidium strelkovi sp. n.

Хозяин: *Cirrhina molitorella*.
Локализация: кишечник.
Место нахождения: озеро Ба-Бэ (провинция Бак-кан) речка Бо (провинция Лао-кай).

Крупная инфузория; тело удлиненно-яйцевидной формы, величиной 133–161×68–77 μ. Передний и задний концы тела плавно закруглены (Рис. 1).

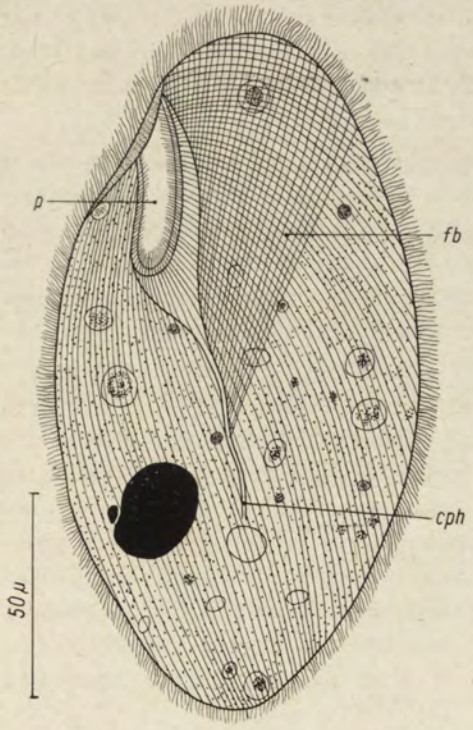


Рис. 1. *Balantidium strelkovi* sp. n., p — перистом, fb — пучок фибрилл, cph — глотка
Fig. 1. *Balantidium strelkovi* sp. n., p — peristome, fb — fibrillar bundle, cph — cytopharynx

Ротовой аппарат включает перистом (несущий предротовые реснички) и глотку; перистом имеет вид почти продольного желобка в передней части тела; глотка широкая, удлиненная, доходит до задней четверти тела. В передней половине тела хорошо видные крупные фибриллы, отходящие пучком от переднего конца тела и идущие более или менее параллельно друг другу. Пучок расширен в верхней[1] части тела, сужен в экваториальной. Он напоминает таковой у примитивных инфузорий типа *Spathidium, Amphileptus, Legendrea* из близкого к трихостоматидам отряда *Gymnostomatida,* а также пучок фибрилл у *Stentoropsis barbi* — ресничноротой инфузории из кишечника аральского усача (Гаврилова личное сообщение, Шульман 1962). Цилиатура перистома на препаратах плохо видна, разобрать ее строение не удалось. Соматические кинеты тесно сближены, расположены строго меридионально, идут от переднего до заднего конца тела. Макронуклеус овальной формы относительно некрупный (22.8–32.3×13.3–19.0 μ); микронуклеус один, величиной 3.8×1.9 μ, лежит в выемке макронуклеуса. В цитоплазме (исключая зону, занятую пучком фибрилл) видны многочисленные мелкие пищеварительные вакуоли; одна сократительная вакуоль лежит у заднего конца тела.

Вид назван в честь Ю. А. Стрелкова.

От *B. ctenopharyngodonis,* описанного (Chen 1955) из кишечника белого амура *Ctenopharyngodon idellus,* данный вид отличается большими размерами тела, от *B. polyvacuolum* Lee, 1963, из кишечника *Xenocypris argentea, X. davidi, Plagiognathops microlepis* (Lee 1963) от *B. piscicola* Daday, 1905, найденного в кишечнике *Piarectus brachypomus* (Daday 1905, Entz 1913) и *Pimelodus clarias* (Cunha et Penido 1926, Pinto 1928) отличается также формой и размерами макронуклеуса.

Из кишечника рыб ранее описан еще один вид *Balantidium grevolosum* (Ganthier, цит. по Lee 1963); вид обнаружен в северо-американской рыбе *Salvelinus fontinalis.*

Balantidium spinibarbichthys sp. n.

Хозяин: *Spinibarbichthys denticulatus.*
Локализация: кишечник.
Место нахождения: Озеро Ба-Бэ (провинция Бак-кан); речка Бо (провинция Лао-кай).
Интенсивность и процент заражения: обнаружен у 16.5% исследованных рыб в количестве от 4 по 25 экземпляров.

Сравнительно некрупная инфузория; тело широкое, овальное или почти округлое, величиной 95–117×70–100 μ. Передний и задний концы тела закруг-

[1] По отношению к субстрату правая сторона тела — нижняя, левая — верхняя. Если ориентировать особи так, как и других инфузорий, безотносительно к субстрату, верхняя — передняя часть тела, нижняя — задняя.

ленные (Рис. 2). Перистом широкий и глубокий, расположен не вдоль тела, как у *B. strelkovi*, а на переднем конце тела, почти поперек. Глотка воронковидная, доходит до экваториальной части тела. Пучок фибрилл такого типа, как у *B. strelkovi*, не найден. Макронуклеус овальной формы, величиной 20.9–22.8×11.4–13.13 μ; микронуклеус крупный, веретеновидный (что характерно для многих видов *Balantidium*), величиной около 4.7×1.9 μ, тесно прилегает к макронуклеусу. В цитоплазме видны немногочисленные пищеварительные вакуоли; одна сократительная вакуоль лежит у заднего конца тела.

Рис. 2. Fig. 2. *Balantidium spinibarbichthys* sp. n.

B. spinibarbichthys sp. n. отличается от *B. strelkovi* округлой формой тела, поперечным расположением перистома; глотка короче, чем у *B. strelkovi*, размеры макронуклеуса меньше. От *B. polyvacuolum* и *B. ctenopharyngodonis* данный вид отличается удлиненно-овальной формой макронуклеуса, от *B. piscicola* — большими размерами макронуклеуса.

Balantidium steinae sp. n.

Хозяин: *Spinibarbichthys denticulatus*.
Локализация: кишечник.
Место нахождения: озеро Ба-Бэ (провинция Бак-кан), речка Бо (провинция Лао-кай).
Интенсивность и процент заражения: был встречен у 18% исследованных рыб в количестве от 5 до 20 экземпляров.

Очень мелкая инфузория; размеры тела 51–56×32–43 μ. Тело яйцевидное, передний конец шире заднего, закруглен (Рис. 3). Перистом такого же типа, как у *B. spinibarbichthys*, расположен почти поперек тела. Глотка веретеновидная, трубчатая, не достигает средней части тела. Пучка фибрилл в передней

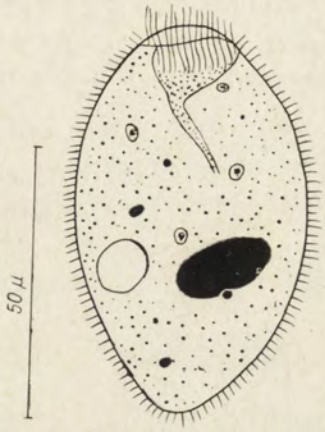


Рис. 3. Fig. 3. *Balantidium steinae* sp. n.

части тела не найдено. Макронуклеус овальный, величиной 13.3–17.1 × 4.7–6.6 μ; микронуклеус круглый, расположен в выемке макронуклеуса, его диаметр 1–1.5 μ. В цитоплазме видны мелкие пищеварительные вакуоли и одна сократительная вакуоль.

Вид назван в честь Г. А. Штейн.

От остальных видов рода из кишечника рыб, найденных в нашем материале (*B. strelkovi, B. spinibarbichthys*), и от *B. polyvacuolum* (Lee 1963) данный вид отличается (Табл. 1) прежде всего мелкими размерами тела: он мельче этих

Таблица 1

Table 1

Размеры тела и ядер у видов *Balantidium* из кишечника рыб

Measurement of the body and nuclei of *Balantidium* species from intestine of fishes

Виды Species	Размеры (μ) Measurement (μ)			Авторы References
	тело body	макронуклеус macronucleus	микронуклеус micronucleus	
B. polyvacuolum	100–159 × 72–108	30–48 × 12–15	—	Lee 1963
B. ctenopharyngodonis	48–75 × 27–66	15–24 (длина–lenght)	—	Chen 1955
B. piscicola	36 × 28	12 × 6–7	—	Entz 1913
B. strelkovi sp. n.	133–161 × 68–77	22.8–32.3 × 13.3–19	3.8 × 1.9	По нашим данным Present data
B. spinibarbichthys sp. n.	95–117 × 70–100	20.9–22.8 × 11.4–13.3	4.7 × 1.9	
B. steinae sp. n.	51–56 × 32–43	13.3–17.1 × 4.7–6.6	1–1.5 (диаметр) (diameter)	

форм примерно в 2–2.5 раза; от *B. ctenopharyngodonis* и *B. piscicola* отличается формой тела и макронуклеуса. От близкого рода *Stentoropsis* Dogiel et Bychowsky, 1932, *B. steinae* отличается также поперечным расположением перистома.

Подкласс *Spirotricha* Bütschli, 1889
Отряд *Heterotrichida* Stein, 1859
Семейство *Plagiotomidae* Bütschli, 1887
Род *Nyctotherus* Leidy, 1849

Nyctotherus schulmani sp. n.

Хозяин: *Squaliobarbus curriculus.*
Локализация: кишечник.
Место нахождения: озеро Ба-Бэ (провинция Бак-кан).
Интенсивность и процент заражения: был обнаружен у 28.24% исследованных рыб при высокой интенсивности заражения.

Крупная инфузория веретеновидной формы, резко расширенная в средней части тела и сужающаяся к переднему и заднему концам (Рис. 4). Размеры

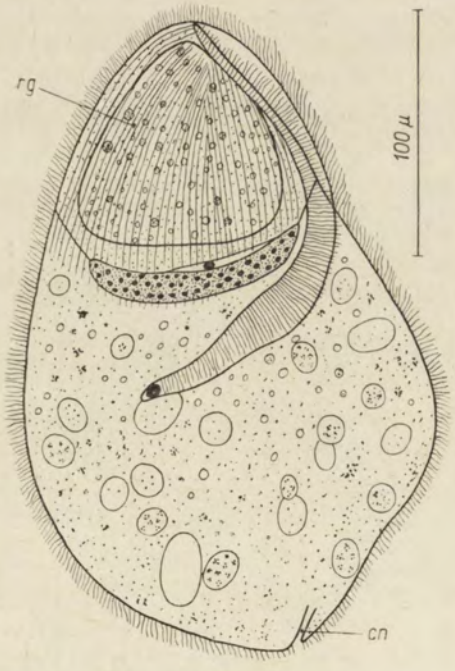


Рис. 4. *Nyctotherus schulmani* sp. n., rg — светопреломляющие тельца, cn — канал сократительной вакуоли
Fig. 4. *Nyctotherus schulmani* sp. n., rg — refractile granules, cn — contractile vacuole channel

тела 180–249×116–154 μ. Тело сплющено латерально, так, что адоральная зона мембранелл проходит по краю тела. К субстрату прилегает левая сторона тела. Отношение длины передней части тела (от переднего конца тела до рта)

к длине задней части тела (от рта до заднего конца тела) составляет 1.6–1.9 : 1. На препаратах, окрашенных гематоксилином, хорошо видно строение кинетома *N. schulmani*. Топография кинет усложнена: кинеты идут не строго параллельно друг другу по всему телу и они не симметричны; видны безресничные швы, разделяющие специализированные отделы кинетома. На левой стороне тела видно 2 таких шва, которые можно обозначить как апикальный (Рис. 5 A as) и каудальный (cs). Кинеты, расположенные правее апикального шва, достигают переднего конца тела. Еще сложнее топография кинет на правой стороне тела. Здесь видны 3 отчетливых безресничных шва: апикальный (Рис. 5 A as),

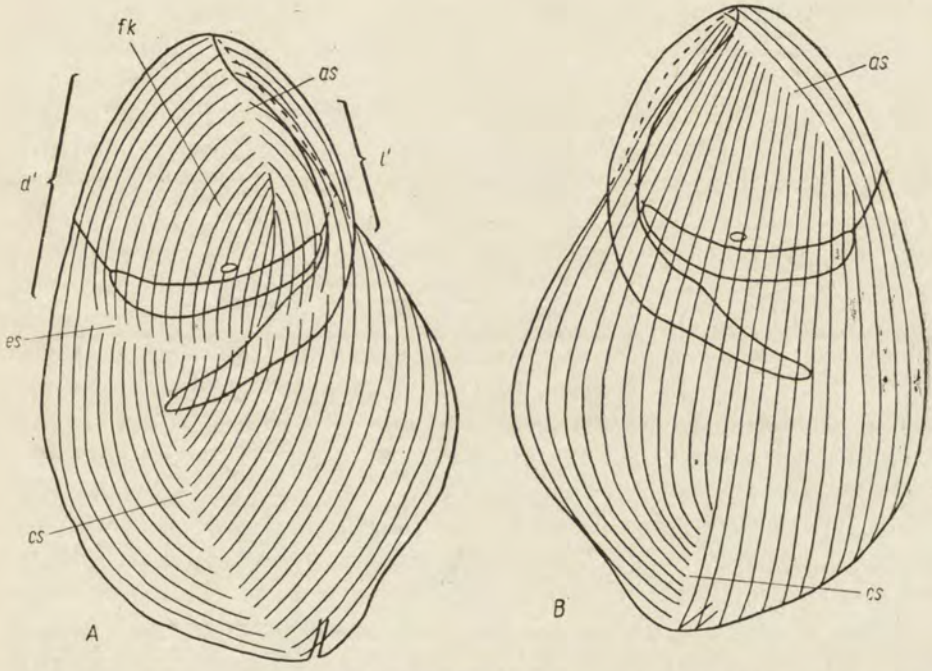


Рис. 5. *Nyctotherus schulmani* sp. n. Организация кинетома, *A* — правая, *B* — левая сторона тела. fk — фрагменты кинет (d′ — правосторонние, l′ — левосторонние): as, es, cs — апикальный, экваториальный и каудальный безресничные швы

Fig. 5. *Nyctotherus schulmani* sp. n. Kinetome organization, *A* — right, *B* — left body side. fk — fragments of the kineties (d′ — dextral, l′ — sinistral), as, es, cs — apical, equatorial and caudal cilia-free sutures

экваториальный (es) и каудальный (cs). Экваториальный шов отсекает верхние участки почти всех соматических кинет на правой стороне тела; обособившаяся группа фрагментов кинет (fk) образует специализированное тигмотактическое поле. В этой зоне поверхность тела *Nyctotherus* несколько вдавлена. У описываемого ниже нового рода *Inferostoma* мы найдем в этом месте сложную присоску.

Ротовой аппарат *N. schulmani* включает адоральную зону мембранелл, рот и глотку. Наружная (префундибулярная) и внутренняя (инфундибулярная) части адоральной зоны мембранелл примерно равны по всей длине (отклонение около 0.80–0.93 : 1, Рис. 4). Мембранеллы в обеих зонах расположены с одинаковой густотой. Как и у других видов *Nyctotherus*, правая стенка префундибулюма (имеющего вид неглубокого продольного желобка) значительно выше левой. По-видимому, такое возвышение задерживает пищеварительные частицы, отбрасываемые мембранеллами, и не позволяет им проноситься мимо тела инфузории. Ротовое отверстие лежит глубоко, на дне инфундибулярного канала. Глотка очень короткая, едва заметна. Передняя треть тела занята мелкозернистой, плотной эндоплазмой; эта зона имеет почти треугольные очертания (Рис. 4 rg). Здесь нет пищеварительных вакуолей или каких-либо других включений; возможно, что это зона запасных питательных веществ. Ниже ее находится лентовидный макронуклеус с многочисленными нуклеолами; размеры ядра 57–85.5×9.5–13.3 μ. Ядро „подвешено" к вентральному и дорзальному краям тела с помощью хорошо видимых кариофоров. Хроматин макронуклеуса грубозернистый. Микронуклеус один, прилегает к верхней стороне макронуклеуса; его размер 3.8×2.8 μ. Вся цитоплазма от макронуклеуса до заднего конца тела занята пищеварительными вакуолями. Сократительная вакуоль одна, лежит у нижнего конца тела. На препаратах четко виден канал длиной 13–19 μ, близь заднего конца тела (Рис. 4 cn). Судя по описаниям видов *Nyctotherus* (Grassé 1928, Rosenberg 1937, Entz 1913) это выводной канал сократительной вакуоли.

Вид назван в честь С. С. Шульмана.

Род *Nyctotherus* — один из крупнейших, наиболее богатых видами родов подтипа *Ciliophora*. Это объясняется тем, что виды *Nyctotherus* заселяют большой круг хозяев (амфибий, рептилий, насекомых, многоножек и т. д.), будучи при этом видоспецифичными для хозяев. К настоящему времени известно около 100 видов рода: Амаро и Сена (Amaro et Sena 1967 a, b) приводят список 66 видов в составе „рода" *Nyctotheroides* и 33 вида в составе рода *Nyctotherus* s. str. Грассе (Grassé 1928) предложил различать 2 подрода — *Nyctotherus* s. str. (с кариофорами, типовой вид *N. cordiformis* Ehrenberg, 1838) и *Nyctotheroides* без кариофоров. Корлисс (Corliss 1961) считает их возможными самостоятельными родами, а Амаро и Сена (Amaro et Sena 1967 a, b) — родами, не высказывая уже сомнений в их самостоятельности. *N. piscicola* в этом случае попадает в состав рода *Nyctotherus* s. str., а *N. pangasia* в „род" *Nyctotheroides*. Различия этих „родов" (наличие или отсутствие кариофоров) при полной идентичности плана строения по всем остальным признакам представляются нам столь ничтожными, что их едва ли можно использовать для различения даже подродов. Хотя род *Nyctotherus* выводится от свободноживущих инфузорий рода *Metopus* (Villeneuve-Brachon 1940, Янковский

1968) находки видов *Nyctotherus* в водных хозяевах очень редки, род получил расцвет в земноводных и в наземных хозяевах. Насколько нам известно, до сих пор в литературе было описано только 2 вида рода из рыб — *Nyctotherus piscicola* из *Piarectus* (*Colossoma*) *brachypoma* в Южной Америке, Парагвай (Daday 1905), *P. brachypoma* в Южной Америке (Entz 1908, 1913) и *Pimelodus clarias* в Бразилии (Cunha et Penido 1926, Pinto 1928) и *N. pangasia* из кишечника *Pangasius pangasius* в Индии (Tripathi 1954). Возможно при этом, что в *Piarectus* и в *Pimelodus* обитают разные виды рода.

Описываемый нами вид *N. schulmani* отличается от *N. piscicola* более крупными размерами тела, формой макронуклеуса, от *N. piscicola* и *N. pangasia* также более высоким расположением адоральной зоны мембранелл. К сожалению, цитированные авторы не изучали кинетома *N. piscicola* и *N. pangasia* — поэтому мы не можем пока провести более подробного сравнения организации видов *Nyctotherus* из рыб. Если учесть специфичность *Nyctotherus* к определенным видам хозяев, а также приведенные выше различия, мы можем считать *N. schulmani* бесспорно новым видом. От *N. baueri* данный вид отличается формой и крупными размерами тела, овальной формой и меньшей величиной микронуклеуса.

Nyctotherus baueri sp. n.

Хозяин: *Spinibarbichthys denticulatus*.
Локализация: кишечник.
Место нахождения: озеро Ба-Бэ (провинция Бак-кан).
Интенсивность и процент заражения: найден у 21.00% исследованных рыб при небольшой интенсивности заражения.

Небольшая инфузория веретеновидной или ромбической формы (Рис. 6 А), не столь резко расширенная в средней части тела как *N. schulmani*. Размеры тела 138–184×95–97 μ. Отношение длины передней и задней части тела 2.3–2.8 : 1. Ротовой аппарат такого же типа, как у *N. schulmani*, но инфундибулюм несколько крупнее, изогнутый. Префундибулюм несколько длиннее, чем инфундибулюм (отношение — около 1.2 : 1, у *N. schulmani* — 0.80–0.93 : 1). На препаратах, окрашенных гематоксилином, в нижней части инфундибулюма (у рта) обоих видов *Nyctotherus* видно темное пятно или полоска (Рис. 4, 6 А). Это не артефакт: она встречается у всех осмотренных особей, имеет постоянную, строго определенную локализацию. Скорее всего это образование, которое ранее в литературе по *Plagiotomidae* и *Clevelandellidae* описывали как „нейромоториум") (Rosenberg 1937, Kidder 1937). Можно отметить в этой связи, что электронно-микроскопические данные показывают наличие в нижней части инфундибулюма *Nyctotherus* сгустка фибрилл, отходящих от кинетосом; в основном это опорные фибриллы, укрепляющие стенки полости (Oktem Nimet 1966, Paulin 1967, King et al. 1961). Макронуклеус *N. baueri*

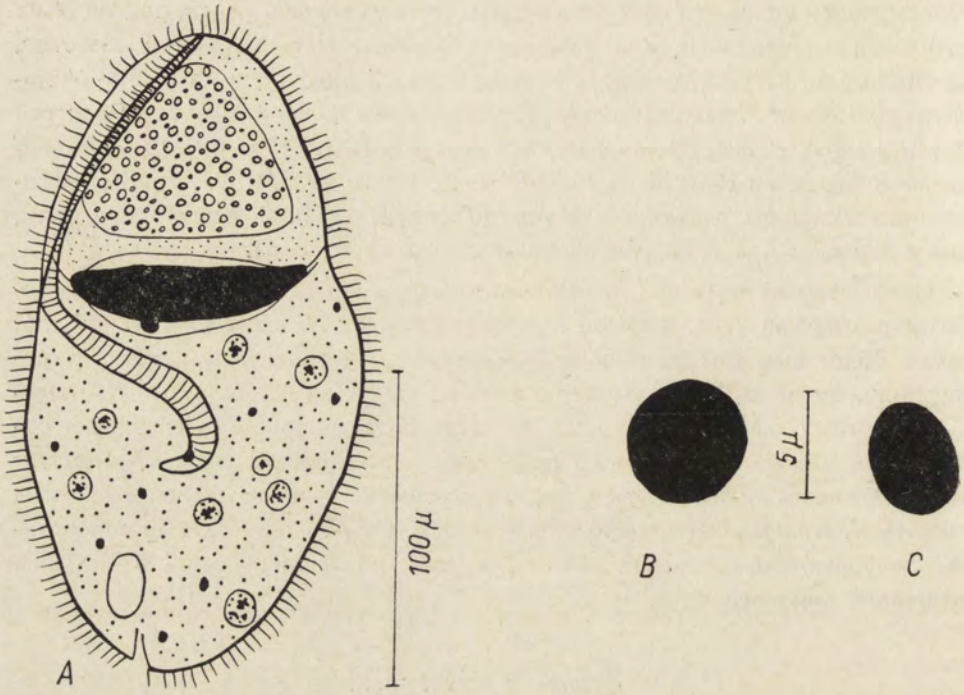


Рис. 6. *Nyctotherus baueri* sp. n. *A* — общий вид. Микронуклеусы *Nyctotherus baueri* (*B*) и *N. schulmani* (*C*) при одном увеличении

Fig. 6. *Nyctotherus baueri* sp. n. *A* — general view. Micronuclei of *Nyctotherus baueri (B)* and *N. schulmani (C)* at the same magnification

Таблица 2

Table 2

Размеры тела и ядер у видов *Nyctotherus* из кишечника рыб

Measurement of the body and nuclei of *Nyctotherus* species from intestine of fishes

Виды Species	Размеры (μ) Measurement (μ)			Авторы References
	тело body	макронуклеус macronucleus	микронуклеус micronucleus	
Nyctotherus piscicola	152 × 112	30–90 × 12–30	5–10	Pinto 1928
N. pangasia	150.8–262.2 × 114–197.6	—	—	Tripathi 1954
N. baueri sp. n.	138–184 × 79–95	51.5–68.4 × 15.2–19.0	3.8–4.7	По нашим данным Present data
N. schulmani sp. n.	180–294 × 116–154	57–85.5 × 9.5–13.3	3.28 × 2.8	

удлиненный, сужающийся у обоих концов; величина ядра 55.1–68.4×15.2–19.0 μ. Хорошо выражены кариофоры. Микронуклеус один, круглой формы, обычно лежит у нижней поверхности макронуклеуса; его диаметр 3.8–4.7 μ. Сократительная вакуоль одна, расположена у заднего конца тела; длина выводного канала около 11.4 μ.

Вид назван в честь О. Н. Бауера.

N. baueri отличается от *N. piscicola* формой макронуклеуса, от *N. schulmani* целым рядом особенностей: тело меньше по размерам, не столь расширено в средней части (Табл. 2); глотка длиннее, изогнута; микронуклеус *N. baueri* значительно крупнее такового у *N. schulmani* (Рис. 6 B, C).

Род *Inferostoma* gen. n.

Крупные латерально-сплющенные инфузории с хорошо развитым, сложно дифференцированным кинетомом. Правая (обращенная к субстрату) сторона тела несет крупную, четко выраженную присоску со специализированным кинетомом и аргиромом. Кинетом левой (верхней) стороны тела примерно такой же, как у *Nyctotherus*. Ротовой аппарат исключительно своеобразен: префундибулюм резко удлинен, растянут от переднего до заднего конца тела; он сдвигает инфундибулярное отверстие к нижнему концу тела (отсюда родовое название *Inferostoma* — нижнеротая). Инфундибулярный канал отходит вверх от нижнего конца тела; строение его по сравнению с *Nyctotherus* не изменено. Ядерный аппарат такого же типа, как у *Nyctotherus* — имеется лентовидный макронуклеус с кариофорами и с одним прилегающим к нему микронуклеусом. Сократительная вакуоль с выводным каналом, как у *Nyctotherus*.

Единственный, описанный ниже (типовой) вид *I. jankowskii* gen. n., sp. n., обитает в кишечнике пресноводных рыб Северного Вьетнама.

Inferostoma — несомненно самостоятельный, четко очерченный род, отличающийся от других родов семейства *Plagiotomidae* (*Plagiotoma* и *Nyctotherus*) резким удлинением адоральной зоны мембранелл, сдвигом инфундибулярного отверстия к заднему концу тела и наличием присоски.

Inferostoma jankowskii sp. n.

Хозяин: *Spinibarbichthys denticulatus*.
Локализация: кишечник.
Место нахождения: озеро Ба-Бэ (провинция Бак-кан), речка Бо (провинция Лао-кай).
Интенсивность и процент заражения: был обнаружен у 73.5% исследованных рыб, в задней части кишченика, при большой интенсивности заражения, в то время как в передней и средней части кишечника найден только у 6–9% исследованных рыб в количестве от 3 до 15 экземпляров.

Крупная инфузория угловатых, несколько неправильных очертаний, с расширенным и закругленным верхним и менее широким, как бы срезанным по горизонтали нижним концом (Рис. 7–9). Размеры тела 90–129×62–86 μ. На

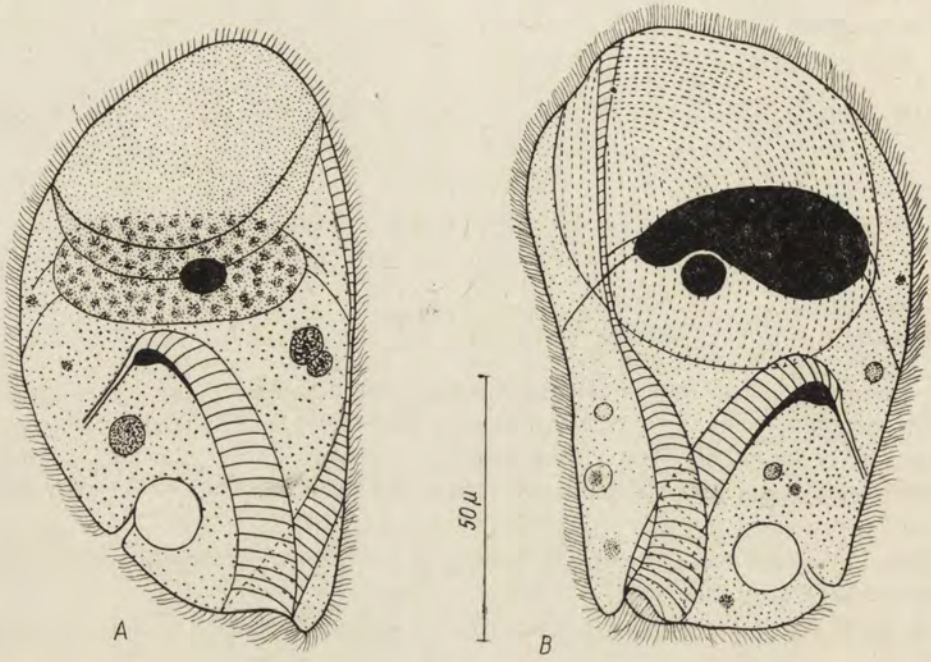


Рис. 7. *Inferostoma jankowskii* gen. n., sp. n., общая морфология
Fig. 7. *Inferostoma jankowskii* gen. n., sp. n., general morphology

Рис. 8 схематически изображены контуры тела, присоски, ядер и инфундибулюма нескольких особей *I. jankowskii* видны некоторые различия в размерах присоски, высоте инфундибулюма, но в целом план строения разных особей в популяции однотипен.

Тело инфузории сплющено латерально, но так, что префундибулярный отдел адоральной зоны мембранелл проходит не строго по краю тела, а несколько левее (как у *Odontostomatida* и у некоторых других спиротрих). Кинетом левой стороны тела значительно проще, чем на правой стороне: мы видим здесь многочисленные продольные кинеты, значительная часть которых идет от переднего до заднего конца тела, часть подходит к четкому каудальному шву cs. Кинетом правой стороны тела усложнен: видны, фактически, те же 3 безресничных шва, что и у *Nyctotherus* (as, es, cs), но зона выше экваториального шва es превращена в присоску (Рис. 9 A). Ниже шва es кинеты идут V-образно, сходясь друг с другом попарно под углом вдоль шва cs. Фактически кинеты нижней половины тела, справа, разделены на 2 группы, обозначенные

на рисунке, как d (от „dexios", правая) и l („laevos", левая). Интересно, что 2 такие же группы кинет, d′ и l′, можно различить и в присоске; границей их является шов as. Кинеты d′ и l′ — это фрагменты обособившиеся в резуль-

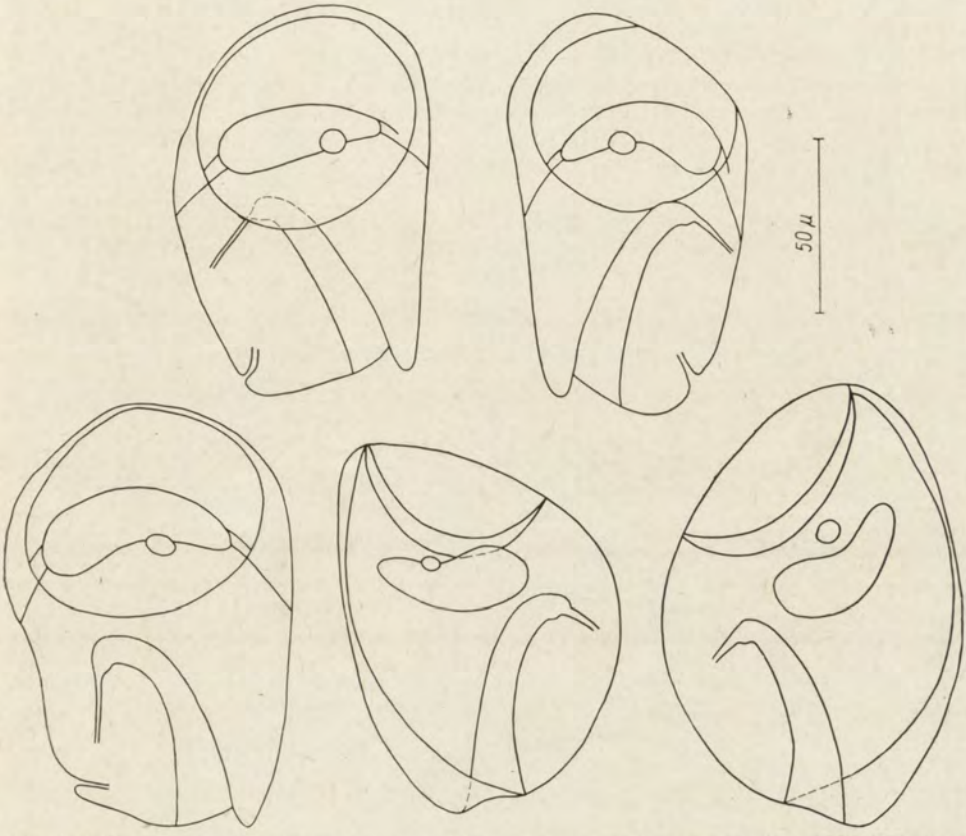


Рис. 8. *Inferostoma jankowskii* gen. n., sp. n. Изменчивость относительного положения присоски, ядер и инфундибулюма

Fig. 8. *Inferostoma jankowskii* gen. n., sp. n. Variation of the relative position of the sucker, nuclei and infundibulum

тате образования экваториального безресничного шва es. Только в зоне присоски на гематоксилиновых препаратах видна система прямоугольников пелликулы, т. е. аргиром; в остальных участках тела ее нельзя видеть даже на наиболее удачно окрашенных препаратах (Рис. 10, A, B).

Строение ротового комплекса *Inferostoma*. Как и у *Nyctotherus*, ротовой аппарат *Inferostoma* включает префундибулюм, инфундибулюм, рот и глотку. Префундибулюм, редко достигающий средней части тела у видов *Nyctotherus*, резко удлинен у *Inferostoma* — он вытянут продольно от переднего до заднего конца тела. Ширина адоральной зоны мембранелл в разных местах различна:

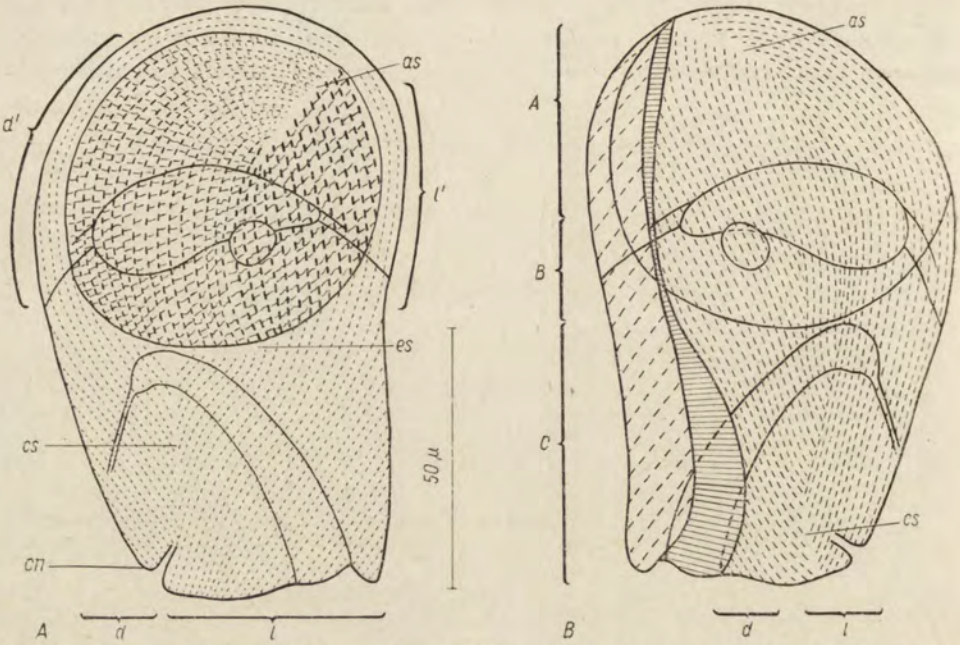


Рис. 9. *Inferostoma jankowskii* gen. n., sp. n. *A* — Кинетом и аргиром правой и *B* — левой стороны тела. as, es, cs — апикальный, экваториальный и каудальный швы, cn — канал сократительной вакуоли, d, l — правые и левые соматические кинеты, d′, l′ — фрагменты тех же кинет в присоске. *A*, *B*, *C* — зоны префундибулюма

Fig. 9. *Inferostoma jankowskii* gen. n., sp. n., *A* — kinetome and argyrome of the right and *B* — left body side. as, es, cs — apical, equatorial and caudal sutures, cn — contractile vacuole channel, d, l — dextral and sinistral somatic kineties, d′, l′ — fragments of the same kineties in sucker area. *A*, *B*, *C* — 3 zones of prefundibulum

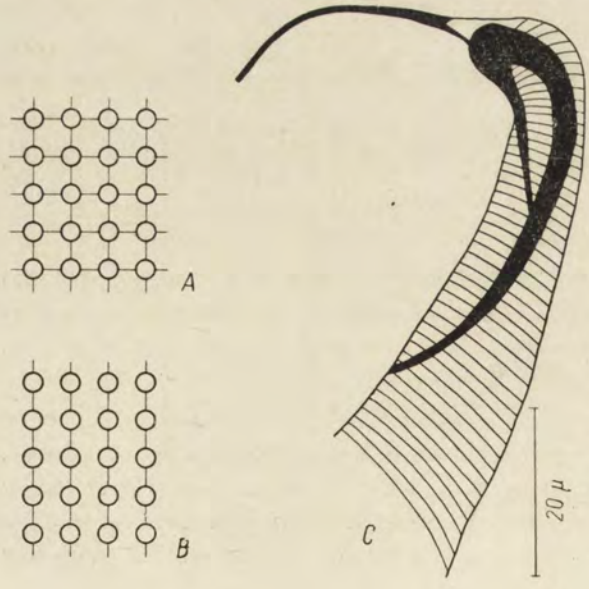


Рис. 10. *Inferostoma jankowskii* gen. n., sp. n. Аргиром в зоне присоски (*A*) и в зоне соматических кинет (*B*). *C* — инфундибулюм, глотка и „нейромоториум"

Fig. 10. *Inferostoma jankowskii* gen. n., sp. n. Argyrome in sucker area (*A*) and in somatic kineties zone (*B*). *C* — infundibulum, cytopharynx and "neuromotorium"

в верхней части префундибулюма это неширокая полоска (около 2.8–3.8 μ) (Рис. 9 В *A*); она плавно сужается к средней части тела (шириной около 1 μ (*B*)) и, примерно на уровне нижнего края присоски, резко расширяется вновь (*C*). Максимальной ширины (7.6–9.5 μ) адоральная зона мембранелл достигает у входа в инфундибулярное отверстие. Мембранеллы по всей длине адоральной зоны расположены неравномерно: густо у верхнего и нижнего концов префундибулярной полосы и редко в ее средней части. Подобное удлинение префундибулярной части адоральной зоны мембранелл и неравномерная ее толщина нетипичны для семейства *Plagiotomidae* и для отряда *Heterotrichida* в целом.

Поскольку инфундибулярное отверстие сдвинуто к нижнему концу тела, инфундибулярный канал обращен ротовым отверстием не вниз или вбок, как у *Nyctotherus*, а вверх (Рис. 7 В, сравни Рис. 4). В строении же инфундибулюма *Inferostoma* нет каких-либо особенностей по сравнению с *Nyctotherus* — это канал, плавно сужающийся по направлению ко рту; мембранеллы расположены довольно густо. Рот ведет в удлиненную (около 18 μ) трубчатую глотку. На стенках инфундибулюма близ ротового отверстия гематоксилином хорошо окрашиваются несколько полосок; конфигурация их у разных особей в целом сходна (Рис. 10 С). По аналогии с *Nyctotherus*, такое образование можно было бы назвать „нейромоториум", если бы многие современные авторы не отрицали правильность понятия о „нейромоториуме" у инфузорий. Скорее это опорный фибриллярный комплекс, служащий, в частности, для укрепления стенок инфундибулюма и глотки.

Макронуклеус *I. jankowskii* банановидной формы, величиной 30.4–47.5 × × 10.4–17.1 μ; один из концов макронуклеуса, обращенный к дорзальной стороне тела, расширен, другой (обращенный к адоральной зоне мембранелл) сужен, его ширина 5.7–8.5 μ. Кариофоры отчетливо выражены; они прикрепляются к пелликуле не выше ядра (как у *Nyctotherus*, макронуклеус которого как бы подвешен к стенкам тела), а ниже ядра (Рис. 7 В). Неясно, как кариофоры удерживают массивное ядро в столь необычном положении. Микронуклеус один, крупный, овальный, реже округлой формы, расположен у нижней стороны макронуклеуса; его размеры 4.7–6.6 × 3.8–4.7 μ. Сократительная вакуоль одна, с выводным каналом, расположена у заднего конца тела, близ дорзального края. Цитоплазма содержит мелкие пищеварительные вакуоли (только в нижней половине тела, ниже присоски).

Вид назван в честь А. В. Янковского.

Родственные связи *Inferostoma* и положение в системе

При всем своеобразии внешнего облика, *Inferostoma* обнаруживает ряд признаков, общих с *Nyctotherus*. Тело этих форм сплющено латерально, пре-

фундибулюм проходит по краю тела; кинетом сложен, на левой стороне тела имеются безресничные швы as и cs, на правой швы as, es и cs; макронуклеус прикреплен к пелликуле с помощью кариофоров. Присоска *Inferostoma* — сложный, четко обособленный отдел кинетома — соответствует тигмотактической блюдцевидной ямке, свойственной ряду видов *Nyctotherus*. Уже отмечалось, что у *N. schulmani* экваториальный шов es отсекает верхние фрагменты всех правосторонних соматических кинет; они и образуют тигмотактическое ресничное поле (можно отметить, что такое обособление не было найдено у *N. cordiformis*, *N. tipulae*, *N. ovalis* и др. (Grasse 1928)). Более того, в зоне присоски или тигмотактического ресничного поля у *I. jankowskii* и *N. schulmani* видны две группы кинет, d′ и l′ (Рис. 5 A, 9 A), различимые по длине и направлению. *Inferostoma* отличается от *Nyctotherus* главным образом двумя признаками — резким удлинением префундибулюма и превращением тигмотактического поля кинет в типичную присоску.

Близкий к *Nyctotherus* род *Plagiotoma*, единственный достоверный вид которого *P. lumbrici*, обитает в кишечнике дождевых червей Европы (Pertzewa 1929, Heidenreich 1935, Dworakowska 1966), имеет в отличие от рода *Inferostoma* префундибулюм обычного типа и резко укороченный инфундибулюм; макронуклеус фрагментирован. Инфундибулярное отверстие лежит в верхней половине тела.

Род *Paranyctotherus* с единственным видом *P. kirbyi* (= *Balantidium xenopi*) (Puytorac et Grain 1965, Sandon 1941, Rodriguez 1939) не родствен не только *Inferostoma*, но и роду *Nyctotherus*; это один из родов семейства *Balantidiidae* (Янковский 1968).

В кишечнике тропических тараканов *Panesthia javanica*, *P. spadica*, *P. angulipennis* в Японии, Китае и на Филиппинах обитают виды семейства *Clevelandellidae* Kidder, 1938, выводимого Киддером от *Nyctotherus* (Kidder 1937, 1938, Yamasaki 1939). По Киддеру, это семейство включает 2 рода — *Clevelandella* (син. *Clevelandia*, *Emmaninius*) и *Paraclevelandia*. В кишечнике тараканов эти формы живут совместно с *Nyctotherus*. Киддер (Kidder 1937) и Ямасаки (Yamasaki 1939) изучали, независимо друг от друга, один и тот же вид *Nyctotherus*, описав его под разными названиями — *N. uichancoi* Kidder, *N. panesthiae* Yamasaki. Амаро и Сена (Amaro et Sena 1967 a, b) по наличию или отсутствию (?) кариофора отнесли эти виды к разным „родам": *N. uichancoi* Kidder, 1937 к *Nyctotherus* s. str., а *N. panesthiae* Yamasaki, 1939 к *Nyctotheroides* (см. выше).

Clevelandellidae резко отличаются от *Nyctotherus* и не столь существенно от *Inferostoma*. Как и у *Inferostoma*, инфундибулярное отверстие у всех *Clevelandellidae* находится у нижнего конца тела, инфиндибулюм направлен не вниз, а вверх, несет хорошо красящийся „нейромоториум". Нижний конец тела клевланделлид обычно вытянут в виде короткого или удлиненного хо-

ботка. Присоска у *Clevelandellidae* отсутствует; верхняя половина тела не расширена, как у *Inferostoma* и ряда видов *Nyctotherus*. Нет и следа префундибулюма; на месте его сохранился лишь продольный краевой безресничный шов, указывающий путь сдвига инфундибулярного отверстия. В целом *Clevelandellidae* отличаются от *Nyctotherus* отсутствием тигмотактического ресничного поля, префундибулюма, сдвигом инфундибулярного отверстия к нижнему концу тела, от *Inferostoma* — отсутствием префундибулюма и присоски.

Основным отличительным признаком семейства *Clevelandellidae* Киддер (Kidder 1937) считал „заднее положение рта". Формально по этому признаку *Inferostoma* удовлетворяет диагнозу семейства и могла бы быть включена в его состав. Однако делать это нецелесообразно по следующим соображениям. У *Clevelandellidae* произошел не только сдвиг префундибулюма (что нередко встречается у инфузорий), но и полное исчезновение этой зоны, что заметно отличает данное семейство от всех остальных известных *Heterotrichida*. Пока известны гетеротрихи с уменьшенной в размерах префундибулярной зоной (например, различные *Odontostomatida*), но совершенно неизвестны формы с полной редукцией этого отдела адоральной зоны мембранелл. Такое видоизменение ротового комплекса должно считаться, несомненно, важнейшим отличительным признаком семейства *Clevelandellidae*. Поэтому в состав семейства следует включать лишь формы, не имеющие префундибулюм. Между тем *Inferostoma* не принадлежит к числу таких форм; префундибулюм не только не редуцирован, но, напротив, резко удлинен, протянут лентой от переднего до заднего конца тела. Это растяжение и вызвало сдвиг инфундибулярного отверстия к нижнему концу тела.

Важно отметить также, что у *Clevelandellidae* и у *Inferostoma* наблюдаются разные тенденции в развитии ротовой цилиатуры — ее редукция у первых и усложнение у вторых. Едва ли формы с разными эволюционными тенденциями могут быть объединены в одном семействе.

Таким образом род *Inferostoma* оказывается наиболее близким к представителям семейства *Plagiotomidae* — родам *Plagiotoma* и *Nyctotherus*.

Несмотря на наличие таких отличительных признаков, как резкое удлинение префундибулюма и сдвиг инфундибулярного отверстия к нижнему концу тела, мы не считаем возможным выделить *Inferostoma* в отдельное семейство. Аналогичным образом растянут перистом, к примеру, у гетеротрихи *Brachonella* из семейства *Metopidae* (Schulze 1958, Villeneuve-Brachon 1940, Tuffrau 1967). *Brachonella* дает начало целой группе своеобразных, узко специализированных форм с редукцией соматической цилиатуры (сем. *Caenomorphidae*), но сама недостаточно отличается от рода *Metopus*, чтобы быть выделенной в отдельное семейство. Аналогично, *Boveria*, рот у которой сдвинут к нижнему концу тела, не выделяется из состава семейства *Ancistridae*.

Inferostoma отличается от *Nyctotherus* не более, чем *Brachonella* от *Metopus* и *Boveria* от *Ancistrum*. Поэтому *Inferostoma* включается нами в состав семейства *Plagiotomidae*, это третий (кроме *Plagiotoma* и *Nyctotherus*) достоверный род семейства. У *Inferostoma* наивысшего выражения достигает тенденция к тигмотактизму: специализированная тигмотактическая зона, постепенно обособляющаяся у видов *Nyctotherus*, превращается в присоску. У эндопаразитических форм тело обычно сплющивается; с развитием присоски рот смещается к противоположному концу тела (*Hemispeiridae*, *Hysterocinetidae*, *Licnophoridae* и др. — Raabe 1947). *Inferostoma* не представляет исключения в этом отношении.

В составе семейства *Plagiotomidae* целесообразно различать 2 группы родов — *Plagiotoma* и *Nyctotherus*, имеющие ротовой аппарат обычного типа, и *Inferostoma*, у которых инфундибулюм смещен в нижнюю часть тела вследствие чрезмерного удлинения префундибулюма. Можно различить, соответственно, 2 подсемейства, получающие следующие характеристики.

Семейство *Plagiotomidae* Bütschli, 1887

1. Подсемейство *Plagiotominae* s. str. Bütschli, 1887

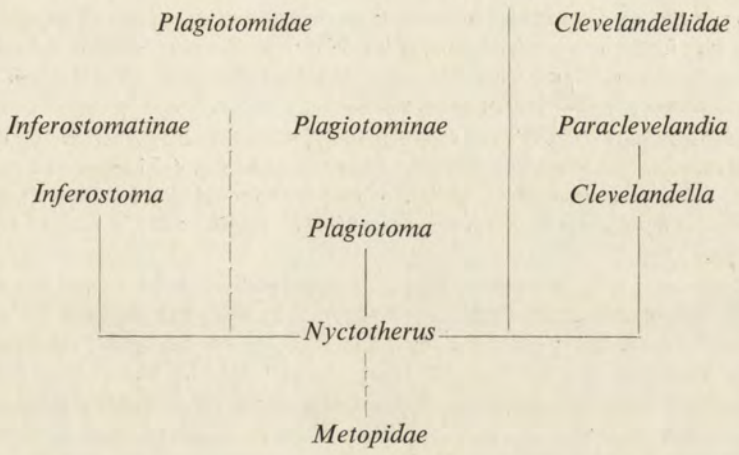
Префундибулюм относительно короткий, не длиннее средней части тела. Инфундибулярное отверстие лежит в середине тела или в верхней части тела; инфундибулюм направлен вниз. Присоски нет, но может быть различима группа специализированных тигмотактических кинет по правую сторону. Эндопаразиты насекомых, многоножек, амфибий, рептилий и рыб. Два рода, *Plagiotoma* Dujardin, 1841, типовой род, и *Nyctotherus* Leidy, 1849.

2. Подсемейство *Inferostomatinae* subfam. n.

Префундибулюм резко удлинен, растянут лентой от переднего до заднего конца тела. Инфундибулярное отверстие сдвинуто к нижнему концу тела; инфундибулюм направлен вверх. Имеется четкая присоска на правой стороне тела. Эндопаразиты рыб. Один (типовой) род *Inferostoma* gen. n.

Исходя из приведенных выше материалов автора и литературных данных, родственные связи различных родов семейств *Plagiotomidae* и *Clevelandellidae* можно выразить следующей схемой, в которой роды *Plagiotoma*, *Inferostoma* и представители семейства *Clevelandellidae* происходят независимо друг от друга, непосредственно от рода *Nyctotherus*.

Структура и родственные связи семейств *Plagiotomidae* и *Clevelandellidae*:



Резюме

При паразитологическом изучении 3 пресноводных рыб обнаружено 6 новых видов инфузорий; один из них выделяется в новых род, отнесенный к новому подсемейству. Приведены диагнозы и описания всех новых форм. Роды *Balantidium* и *Nyctotherus*, очень редко отмечавшиеся из рыб, представлены целой группой видов: *Balantidium strelkovi* sp. n. (в кишечнике *Cirrhina molitorella*), *B. spinibarbichthys* sp. n. (из *Spinibarbichthys denticulatus*), *B. steinae* sp. n. (из *S. denticulatus*), *Nyctotherus schulmani* sp. n. (из *Squaliobarbus curriculus*), и *N. baueri* sp. n. (из *Spinibarbichthys denticulatus*). Новый род *Inferostoma* отличается от *Nyctotherus* резким удлинением и дифференцировкой префундибулюма, достигающего заднего конца тела, развитием гигантской правосторонней присоски выше экваториального безресничного шва; инфундибулюм направлен вверх. Единственный вид, *I. jankowskii* sp. n. найден в кишечнике *S. denticulatus*. Сравнивается кинетом *I. jankowskii* и *N. schulmani*. Сходство *Inferostoma* с *Clevelandellidae* считается конвергенцей, результатом параллелизма в эволюции. Для рода *Inferostoma* в семействе *Plagiotomidae* предлагается новое подсемейство *Inferostomatinae* subfam. n.

SUMMARY

Parasitological examination of 3 species of freshwater fishes (*Spinibarbichthys denticulatus*, *Squaliobarbus curriculus* and *Cirrhina molitorella*) revealed 6 new intestinal ciliates of the genera *Balantidium*, *Nyctotherus* and a new genus *Inferostoma* in a proposed new heterotrich subfamily *Inferostomatinae* (*Plagiotomidae*).

1. *Balantidium strelkovi* sp. n. (Fig. 1) (*Trichostomatida: Balantidiidae*) from *Cirrhina molitorella*, Lake Ba-Be (province Bac-can) and river Bo (province Lao-kay).

Large ovoid ciliates with rounded body ends; peristome long, ovoid, lateral, nearly longitudinal in position, with long cytopharynx. An extensive fibrillar bundle runs between the anterior body end and pharyngeal tube. Kineties numerous, closely spaced. Body size 133–161 × 68–77 μ; macronucleus (Ma) 22.8–32.3 × 13.3–19.0 μ; micronucleus (Mi) 3.8 × 1.9 μ.

2. *B. spinibarbichthys* sp. n. (Fig. 2) found in 16.5% of examined *Spinibarbichthys denticulatus*, Lake Ba-Be (province Bac-can) and river Bo (province Lao-kay). 4–25 specimens per host.

Large rounded, nearly spherical ciliates, peristome wide and deep, subapical; cytopharynx small. No fibrillar bundle was detected. Body size 95–117 × 70–100 μ; Ma 20.9–22.8 × 11.4–13.13 μ; Mi 4.7 × 1.9 μ.

3. *B. steinae* sp. n. (Fig. 3) found in 18% of studied *Spinibarbichthys denticulatus*, Lake Ba-Be (province Bac-can) and river Bo (province Lao-kay). 5–20 specimens per host.

Very small ovoid ciliates; peristome small and wide, transversal, apical; cytopharynx short, anterior, fibrillar bundle is absent. Body size 51–56 × 32–43 μ, Ma 13.3–17.1 × 4.7–6.6 μ, Mi 1–1.5 μ.

4. *Nyctotherus schulmani* sp. n. (Figs. 4, 5) (*Heterotrichida, Plagiotomidae*). Found in 28.24% of examined hosts *Squaliobarbus curriculus*, Lake Ba-Be (province Bac-can) in large numbers.

Large laterally flattened ciliates, widest equatorially, sharply narrowing to both anterior and posterior ends. Kinetome complicated: left side with apical and caudal cilia-free sutures, right side with much longer apical, caudal and transversal equatorial sutures, Ma elongated, with caryophores directed forwards. "Neuromotorium" looks like a small spot near the cytostome. Body size 180–249 × 116–154 μ, Ma 57–85.5 × 9.5–13 μ, Mi 3.8 × 2.8 μ. Relation anterior/posterior body part 1.6–1.9: 1.

5. *N. baueri* sp. n. (Fig. 6 A, B). Found in small number in 21% of examined *Spinibarbichthys denticulatus*, Lake Ba-Be (province Bac-can).

Small narrow ciliates with weak equatorial extension. Infudibulum long, hook-like. Body size 138–184 × 95–97 μ, Ma 55.1–68.4 × 15.2–19.0 μ, Mi 3.8–4.7 μ. Relation anterior/posterior body part 2.3–2.8 : 1. Ma with caryophores, Mi of remarkably large size, in contrast with small body size.

Inferostoma gen. n. (*Plagiotomidae*). Large laterally flattened ciliates with highly complicated kinetome. Right body side with a giant prominent sucker, that may extend on more than 1/2 body surface, left one with two polar sutures, like in *Nyctotherus*. Peristome is of peculiar configuration: the external (prefundibular) zone extends from the anterior to the posterior (not equatorial) body part where the infundibular opening is dislocated and subdivided into 3 zones of different length, width and membranellar spacing. Infundibulum long directed forwards (unlike *Nyctotherus*) with large "neuromotorium". Ma with caryophores directed backwards (again unlike *Nyctotherus*). With sole species dwelling in the intestine of fishes.

6. *I. jankowskii* sp. n. (Figs. 7–10) Found in 73.50% of examined *Spinibarbichthys denticulatus*, Lake Ba-Be (province Bac-can) and river Bo (province Lao-kay) in large numbers in the posterior intestine, and in 6–9% in anterior and middle intestine (3–15 specimens per host). Body large, pyriform, widest anteriorly on sucker level, with pointed posterior part. Body size 90–129 × 62–86 μ Ma 30.4–47.5 × 10.4–17.1 μ (in widest part), 5.7–8.5 μ (in narrowest part), Mi 4.7–6.6 × 3.8–4.7 μ. Perfundibular width: zone *A*, 2.8–3.8 μ, zone *B*, 1 μ, zone *C*, 7.6–9.5 μ. Membranelles are rarefied in zone *B*, closely shifted in zones *A* and *C*.

The structure of *Plagiotomidae*. A new genus *Inferostoma* has some resemblance to *Clevelandellidae* from the intestine of *Panesthia*, wood-eating roach, but differ in possesing a long, well developed prefundibulum, entirely lacking in clevelandellids. This suggests the existence of 2 separate lines (to *Inferostoma* and to *Clevelandellidae*) rising from a single ancestral family, *Plagio-*

tomidae. Prefundibular reduction in clevelandellids, a unique phenomenon in *Spirotricha* (except *Odontostomatida*) confirms the desirability of conservation of a separate family for these ciliates, but its conservation in *Inferostoma* suggests their retention in *Plagiotomidae* Bütschli, 1877, subdivided into 2 well-defined subfamilies: *Plagiotominae* s. str., Bütschli, 1877 (type-genus *Plagiotoma*, with no sucker, infundibular opening in the middle body part, infundibulum directed backwards, and *Inferostomatinae* subfam. n. (type-genus *Inferostoma*, with sucker; prefundibular area extensive, differentiated into three zones, infundibular opening shifted on wide posterior body end, infundibulum directed forwards. *Inferostoma* is derived directly from *Nyctotherus*, parallel to *Nyctotherus-Clevelandella-Paraclevelandia* and *Nyctotherus-Plagiotoma* lines.

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ACTA PROTOZOOLOGICA

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Научно-исследовательская станция пресноводного промысла Динь-Банг, ДРВ
и Лаборатория паразитологии Зоологического института АН СССР, Ленинград В-164, СССР

Research Station of freshwater fisheries Dinh-Bang, DRV and
Laboratory of Parasitology, Zoological Institute of the Academy of Sciences of the U.S.S.R., Leningrad V-164, U.S.S.R.

Ха КИ

На КУ

Некоторые миксоспоридии пресноводных рыб Северного Вьетнама

Some myxosporidians of freshwater fishes of the North Vietnam

Во время исследования паразитофауны пресноводных рыб Северного Вьетнама в 1961–65 гг. у 5 видов рыб (*Cyprinus carpio viridiolaceus*, *Hypophthalmichthys harmandi*, *Aristichthys nobilis*, *Cirrhina molitorella*, *Spinibarbichthys denticulatus*) было обнаружено 17 видов миксоспоридий. Кроме того один вид был найден на жабрах *Anabas testudineus* при дополнительных неполных вскрытиях.

Большинство обнаруженных видов оказалось новыми. Поскольку в литературе нет никаких данных о миксоспоридиях Вьетнама, наши сведения о них представляют интерес. Ниже приведены описания найденных видов. Учитывая большую вариабельность размеров спор, мы приводим результат наших измерений также для уже известных видов.

За помощь при сборе материала приношу благодарность своим товарищам по работе на станции Динь-Банг. Выражаю также признательность С. С. Шульману за консультации и советы при обработке и определении собранных паразитов. Подготовил рукопись к печати А. В. Гусев.

Семейство *Myxidiidae* Thélohan, 1892

Zschokkella donecae sp. n.

Хозяин: *Hypophthalmichthys harmandi*.
Локализация: желчный пузырь.
Место нахождения: водоемы Ха-бак (окрестности Ханоя).
Интенсивность и процент заражения: найден у 2.69% исследованных рыб. Интенсивность заражения невысокая.

Вегетативные формы неизвестны. Споры удлиненно-овальные, без перетяжки посредние, с закругленными концами и слабо выраженной исчерчен-

ностью (Рис. 1 А). Шовная линия слегка изогнутая. Полярные капсулы почти сферические. Длина спор 14.4–17.1, ширина 5.4–6.3 (изредка 7.2), толщина 5–6, длина полярных капсул 3.6–4.5, их диаметр 3.2–3.6 μ.

Этот вид отличается от *Z. nova* Klokacewa, 1914, большей длиной споры (5–6 μ) и полярных капсул, от *Z. parasiluri* Fujita, 1927, большей длиной и шириной споры и меньшей величиной полярных капсул, что дает основание считать его новым. Вид назван в честь З. С. Донец.

Семейство *Myxobolidae* Thélohan, 1892

Myxobolus toyamai Kudo, 1915

Син. *Thelohanellus toyamai* (Kudo) Kudo, 1933.
Хозяин: *Cyprinus carpio.*
Локализация: соединительная ткань жаберных лепестков.
Место нахождения: озеро Ба-Бэ (провинция Бак-кан).
Интенсивность и процент заражения: обнаружен у 0.83% исследованных рыб. Интенсивность заражения обычно невысока.

Длина спор 15–18, ширина 5.4, толщина 4.5, длина большей полярной капсулы 9–10.3, диаметр 3.6, длина меньшей полярной капсулы 3.6–4.5, диаметр около 1 μ.

Myxobolus koi Kudo, 1919

Хозяин: *Cyprinus carpio.*
Локализация: соединительная ткань жаберных лепестков.
Место нахождения: водоемы Хай-фон и Ханой.
Интенсивность и процент заражения: встречен у 0.17% исследованных рыб при небольшой интенсивности заражения.

Длина спор 17–18.5, ширина 9–10, толщина 5–7, длина полярных капсул 10–11.2, их диаметр 2.7 μ.

Myxobolus pavlovskii (Achmerov, 1954)

Син.: *Disperospora pavlovskii* Achmerov, 1954.
Хозяин: *Hypophthalmichthys harmandi.*
Локализация: почки, жаберные лепестки.
Место нахождения: водоемы Ханоя и Ха-бак.
Интенсивность и процент заражения: был обнаружен на жаберных лепестках у 4.17% толстолобиков при высокой интенсивности заражения в почках, 0.83% при небольшой интенсивности.

Длина спор 10–11, ширина 9–10, толщина 7.2, длина полярных капсул — большей 6.3–7.2, меньшей 3.6–5.2, их диаметр — большей 2.7–3.6, меньшей 1.5–2 μ.

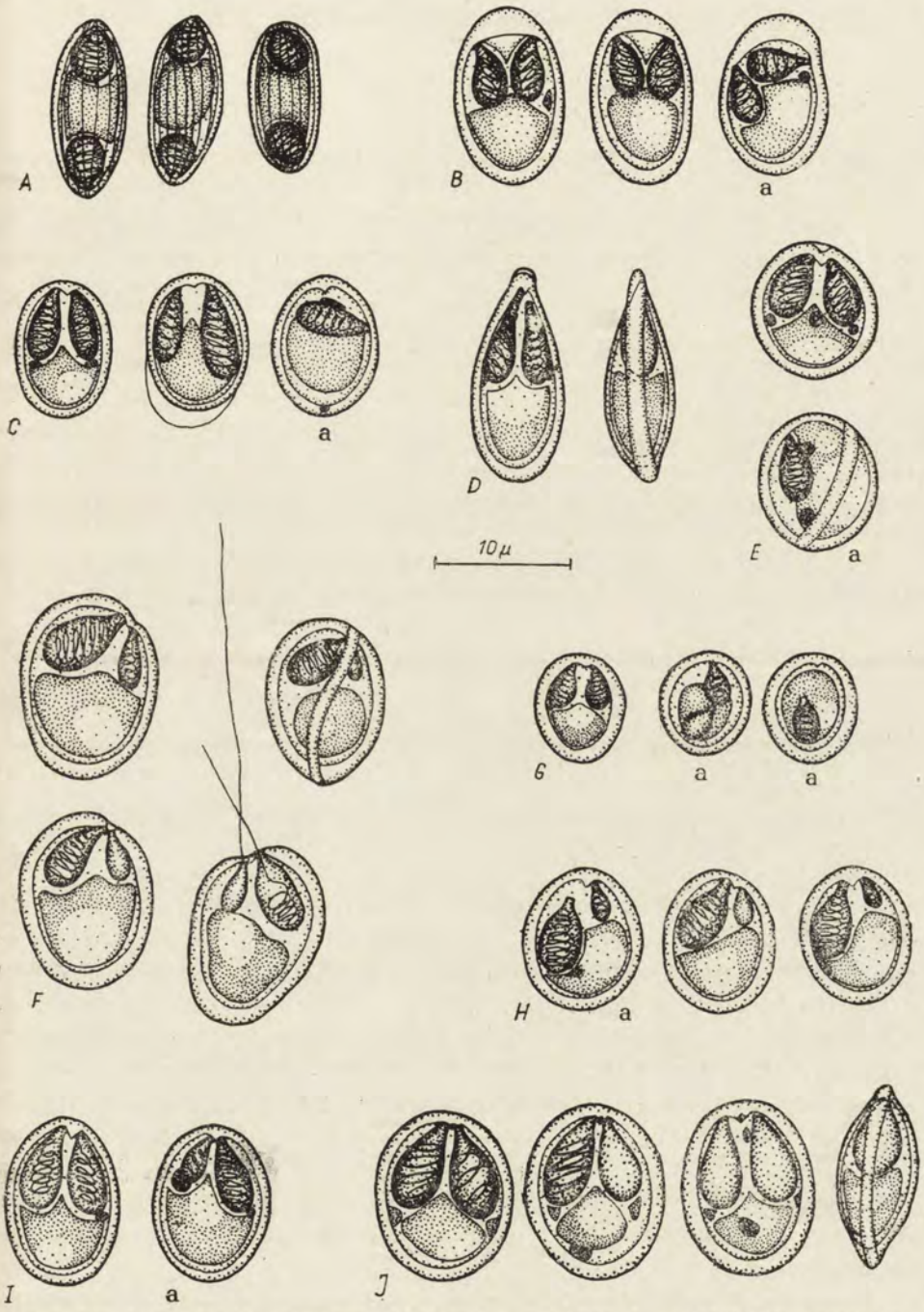

Рис. 1. Fig. 1. Споры (a — атипичные) Spores (a — atypical): A — *Zschokkella donecae*, B — *Myxobolus divergens*, C — *M. uyeni*, D — *M. semeniformis*, E — *M. lanfyongi*, F — *M. assymmetricus*, G — *M. humilis*, H — *M. discapsularis*, I — *M. ellipticus*, J — *Myxobolus* sp.

Myxobolus anisocapsularis Schulman, 1962

Хозяин: *Cyprinus carpio.*
Локализация: жаберные лепестки.
Место нахождения: водоемы Ханоя.
Интенсивность и процент заражения: найден у 1.62% карпа. Интенсивность заражения невелика.

Длина спор 12.6–14.5, ширина 7–7.5, толщина 5.5, длина полярных капсул — большей 7.2–8.1, меньшей 2.7–4, их диаметр — большей 2.7–3.6, меньшей около 1 μ.

Myxobolus achmerovi Schulman, 1966

Хозяин: *Cyprinus carpio.*
Локализация: кожа, жаберные лепестки, стенка кишечника.
Место нахождения: озеро Ба-Бэ (провинция Бак-кан) и водоемы Ханоя.
Интенсивность и процент заражения: обнаружен у 0.83% рыб на коже, у 1.62% на жаберных лепестках и на стенке кишечника при небольшой интенсивности.

Длина спор 10.8–13.5, ширина 9–11.7; длина полярных капсул 4.5–5.4, их диаметр 2.7–3.6; длина полярной нити 57.6–68.4 μ.

Myxobolus divergens sp. n.

Хозяин: *Aristichthys nobilis.*
Локализация: жабры, кожа, печень, почки, селезенка.
Место нахождения: водоемы Ханоя и Ха-бак.
Интенсивность и процент заражения: встречен у 9.63% рыб на жабах при большой интенсивности, у 1.20% на коже и в печени, у 7.20% в почках, у 2.4% в селезенке при небольшой интенсивности.

Вегетативные формы: цисты мелкие, темные, овальной формы. В цистах образуется сравнительно небольшое количество спор. Споры удлиненно-овальные с закругленными концами (Рис. 1 В). Створки неравной величины с довольно толстыми передними концами и слабо расширенными задними. Грушевидные полярные капсулы почти равные, широко расставлены, так что каждая капсула сильно сдвинута на одну сторону. Внутри полярных капсул видна спирально свернутая нить, образующая 4 витка. Интеркапсулярный отросток большой и широкий, хорошо заметен. Иодофильная вакуоль в амебоидном зародыше слабо заметна. Длина спор 14.4–16.2, ширина 9–10, длина полярных капсул 5.4, их диаметр 3.6 μ. Среди спор встречается небольшое количество атипичных форм с неправильно расположенными полярными капсулами.

Данный вид отличается от всех известных видов миксоспоридий необычайно широко расставленными полярными капсулами и очень крупными широкими интеркапсулярными отростками.

Myxobolus uyeni sp. n.

Хозяин: *Cirrhina molitorella.*
Локализация: стенка кишечника.
Место нахождения: речка Бо (провинция Лао-Кай).
Интенсивность и процент заражения: обнаружен у 5.69% исследованных рыб. Интенсивность заражения высокая.

Вегетативные формы: мелкие белые цисты округлой формы. В цистах образуется огромное количество спор. Споры правильной эллипсоидной формы (Рис. 1 C). Полярные капсулы грушевидные с резко суженными передними концами, длинные, занимают больше половины полости спор. В полярных капсулах хорошо заметна длинная спирально свернутая стрекательная нить, дающая 7 витков. Между полярными капсулами заметен маленький интеркапсулярный отросток. Иодофильная вакуоль в амебоидном зародыше плохо видна. Длина спор 9.9–10.8, ширина 8.0–8.5, длина полярных капсул 5.4 (в виде исключения — 6.3), их диаметр 2.7 μ. Изредка встречаются атипичные споры или только с одной полярной капсулой, которая сильно сдвинута на одну сторону, или с неравными капсулами: одна из них удлинена и в 1.7 раза больше другой и занимает почти всю полость споры.

Этот вид сближается по форме спор с *Myxobolus cheni* Schulman, 1962, с лобана — *Mugil cephalus* из бассейна р. Ляохэ (КНР). Но у найденного нами вида размеры спор в 2–2.5 μ крупнее, чем те же у *M. cheni.* Кроме того у последнего полярные капсулы теснее сближены своими передними концами, чем у *M. uyeni.* От другого близкого вида *Myxobolus uniporus* Fujita, 1927, наш вид отличается относительно меньшей длиной и большей шириной споры.

Вид назван в честь товарища Тхан Ван Уэн, оказавшего большую помощь при сборе материала.

Myxobolus semeniformis sp. n.

Хозяин: *Cirrhina molitorella.*
Локализация: кожа.
Место нахождения: водоемы Ханоя.
Интенсивность и процент заражения: этот вид был найден у 6.03% исследованных рыб при очень высокой интенсивности заражения.

Вегетативные формы: крупные белые цисты неправильно округлой формы диаметром до 4 мм. Цисты заполнены многочисленными спорами. Споры грушевидной формы, суждающиеся по направлению к заостренному концу (Рис. 1 D). Шовный валик толстый, достигает 1.6 μ. Грушевидные полярные капсулы одинаковой величины, имеют длину меньше чем половина длины споры. Концы грушевидных полярных капсул сближены, вследствие чего интеркапсулярный отросток плохо виден. Амебоидный зародыш сравнительно больших размеров. Длина спор 13.2–14.4, ширина 4.8–6, толщина 3.6–4.2, длина полярных капсул 5.4–6, их диаметр 1.4–1.8 μ.

Myxobolus semeniformis сходен с *Myxobolus poecilichthidis* Fantham et al., 1939, по размерам, но отличается от последнего значительно большей величиной шовного валика.

Массовое заражение цистами *H. semeniformis* на поверхности тела рыб особенно хвостовой части ее вызывает нарушение нормальной функции кожи, деформацию тела, выражающуюся искривлением хвостовой части в одну сторону. Заболевание приводит к гибели мальков рыб.

Myxobolus lanfyongi sp. n.

Хозяин: *Spinibarbichthys denticulatus.*
Локализация: стенка кишечника.
Место нахождения: речка Бо (провинция Лао-кай).
Интенсивность и процент заражения: невелика, встречен у 3.84% исследованных рыб.

Вегетативные формы: шаровидные цисты желтовато-белого цвета. В цистах образуется огромное количество спор. Споры округлые с воронковидным углублением на переднем полюсе (Рис. 1 E). Полярные капсулы грушевидной формы, длина их примерно равна половине длины споры. В полярных капсулах имеются 7 витков стрекательной нити. Интеркапсулярный отросток очень хорошо заметен. Иодофильная вакуоль крупная и четко видна. Диаметр спор 10.8–11.7, длина полярных капсул 4.5–5.4 (изредка 6.3), диаметр 2.7–3.6 μ. Среди спор встречаются единичные атипичные формы с одной полярной капсулой.

Этот вид отличается от *Myxobolus albovi* Krassilnikova, 1964, и *M. amurensis* Achmerov, 1960, круглыми спорами, от последнего сравнительно меньшими размерами полярных капсул и отсутствием воронки на переднем полюсе спор

Вид назван в честь товарища Нгуен Тхи Лан Фыонг.

Myxobolus asymmetricus sp. n.

Хозяин: *Hypophthalmichthys harmandi.*
Локализация: почки.
Место нахождения: водоемы Ха-бак (окрестности Ханоя).
Интенсивность и процент заражения: обнаружен у 3.84% исследованных рыб. Интенсивность заражения невысокая.

Вегетативные формы неизвестны. Споры овальной формы, с суженным задним полюсом и несколько изогнутым на одну сторону передним концом (Рис. 1 F). На переднем полюсе имеется воронкообразное углубление. Створки сравнительно толстые. Грушевидные полярные капсулы различной величины, сближаются своими концами к переднему полюсу. В больших полярных капсулах заметна спирально свернутая нить, образующая 7 витков, а в маленьких она плохо видна. Интеркапсулярный отросток, несмотря на небольшую величину, хорошо заметен. Иодофильная вакуоль большая. Атипичные формы

не обнаружены. Длина спор 14.4–16.2, ширина 11.7–12.6, толщина около 9, длина полярных капсул — большей 6.8–7.2, меньшей 4.5 (изредка 5.4), их диаметр — большей 3.6, меньшей 1.8 (изредка 2.7), длина полярных нитей — большей 44–53, меньшей 28–30.6 μ.

Описанный вид наиболее близок к *Myxobolus drjagini* Achmerov, 1954, и *M. pseudodispar* Gorbunova, 1936, но отличается от них большими размерами спор и наличием воронки на их переднем полюсе. Кроме того от *M. drjagini* он отличается тем, что обе полярные капсулы несколько сдвинуты на одну сторону, а от *M. pseudodispar* — большей разницей в размерах полярных капсул.

Myxobolus humilis sp. n.

Хозяин: *Hypophthalmichthys harmandi.*
Локализация: селезенка.
Место нахождения: водоемы Ха-бак (окрестности Ханоя).
Интенсивность и процент заражения: невелики, встречен у 1.25% толстолобика.

Вегетативные формы неизвестны. Споры найдены в селезенке, овальные, с суженным задним концом и закругленным передним, состоят из толстых створок (Рис. 1 G). Грушевидные полярные капсулы почти одинаковых размеров, сравнительно широко расставлены, занимают примерно половину полости споры. Иодофильная вакуоль в амебоидном зародыше, хорошо заметна. Длина спор 8.1–9, ширина 6.3–7.2, длина полярных капсул 3.6–3.8, их диаметр 1.8–2.7 μ. Среди спор встречается небольшое количество атипичных форм: у одних одна полярная капсула полностью редуцирована, другая сильно сдвинута назад; у других значительно менялась ориентировка капсул.

Данный вид больше всего похож на *Myxobolus spherium* Tripathi, 1953, но отличается от него формой полярных капсул (у *M. spherium* они более округлые). Отношение длины полярных капсул к длине спор у *M. humilis* 2.2–2.3, а у *M. spherium*: 2.6–3.1. Кроме того, этот вид отличается от *Myxobolus squamosus* Kudo, 1934 большей шириной полярных капсул, от *M. circulus* (Achmerov, 1960) меньшими размерами спор и полярных капсул.

Myxobolus discapsularis sp. n.

Хозяин: *Hypophthalmichthys harmandi.*
Локализация: желчный пузырь.
Место нахождения: водоемы Ха-бак (окрестности Ханоя).
Интенсивность и процент заражения: невелики, обнаружен у 0.83% исследованных рыб.

Вегетативные формы неизвестны. Споры овальные с двумя неравными грушевидными полярными капсулами (Рис. 1 H). Внутри больших полярных капсул имеются 8 витков стрекательной нити. Створки сравнительно толстые. Маленький интеркапсулярный отросток хорошо выражен. Иодофильная ва-

куоль в амебоидном зародыше может достигать 3.5 μ в диаметре. Длина спор 12.6–13.5, ширина 9–10.8, длина полярных капсул — большей 7.2, меньшей 2.7–3.6, их диаметр — большей 3.6, меньшей 1.8 μ. Нередко встречаются атипичные споры, у которых большая полярная капсула сдвинута к середине полости споры.

Этот вид наиболее близок к *Myxobolus diversicapsularis* Sluchai, 1966, но отличается от него менее округлыми спорами и большей длиной больших полярных капсул.

Myxobolus ellipticus sp. n.

Хозяин: *Hypophthalmichthys harmandi.*
Локализация: жабры.
Место нахождения: водоемы Ханоя.
Интенсивность и процент заражения: встречен у 2.16% толстолобиков при невысокой интенсивности.

Вегетативные формы: цисты овальной формы, заполненные многочисленными спорами. Споры удлиненно-овальные, состоящие из относительно тонких нежных створок (Рис. 1 I). Грушевидные полярные капсулы одинаковой величины. В одной цисте встречались два типа спор, различающиеся по длине полярных капсул: у спор первого типа (80–83%) полярные капсулы имеют длину меньше половины длины споры, а у спор второго типа (15–20%) длина полярных капсул равна половине длины споры. Внутри полярных капсул хорошо заметна спирально свернутая стрекательная нить, дающая 5 витков. Интеркапсулярный отросток маленький. Иодофильная вакуоль сравнительно круглая. Длина спор 12.6–14.4, ширина 9–10.8, длина полярных капсул 5.4–7.2, их диаметр 3.6 μ. Среди спор встречаются единичные атипичные формы с сильно уменьшенной одной полярной капсулой.

Наиболее близок к *Myxobolus ellipsoides* Thelohan, 1892, и *M. barbi* Tripathi, 1953, но отличается от первого относительно меньшей длиной споры, большей длиной полярных капсул, от *M. barbi* — длиной полярных капсул.

Myxobolus sp.

Хозяин: *Cirrhina molitorella.*
Локализация: кожа.
Место нахождения: водоемы Ханоя.
Интенсивность и процент заражения: был найден у 7.87% исследованных рыб, интенсивность заражения очень высокая.

Вегетативные формы: эллипсоидные или шаровидные цисты желтовато-белого цвета, достигающие размеров 0.5–1.8 мм. Споры овальные, с закругленными концами и толстыми створками (Рис. 1 J). Грушевидные полярные капсулы неодинаковой величины, однако сравнительно мало отличаются друг от друга, занимают больше половины полости споры, сближаются своими

узкими концами к переднему полюсу. В полярных капсулах видна короткая спирально свернутая стрекательная нить, образующая 3 витка. Интеркапсулярный отросток, несмотря на небольшую величину, очень хорошо заметен. Иодофильная вакуоль в амебоидном зародыше сравнительно большая. Длина спор 13.5–14.5 (в виде исключения 16.2), ширина 10.8–12.6, толщина 7.2–8.1, длина полярных капсул — большей 7.2–9, меньшей 6.3–7.2, их диаметр — большей 3.2–3.6, меньшей — 3.2 μ. В некоторых цистах наблюдается 1–5% атипичных спор с широко расставленными полярными капуслами.

Описанный вид по форме и размерам спор сходен с *Myxobolus lintoni* Kudo, 1919. К сожалению, мы не можем полностью сравнить данный вид с *M. lintoni* из-за того, что автор не приводит размеров полярных капсул.

Henneguya schulmani sp. n.

Хозяин: *Anabas testudineus*.
Локализация: жабры.
Место нахождения: водоемы Ханоя.
Интенсивность и процент заражения: был найден на 7 из 12 рыб при большой интенсивности заражения.

Вегетативные формы: мелкие цисты, темные, шаровидной или овальной формы, их размеры от 0.25 до 0.30 мм. Споры веретеновидные с суженными передним и задним полюсами (Рис. 2 А). Грушевидные полярные капсулы занимают половину полости споры. Длина спор 16.8–20.4, ширина 4.8–6, толщина 3.6–4.2, длина полярных капсул 8.0–10.2, их диаметр 1.44–2.16 μ.

Найденный вид больше всего похож на *Henneguya alexeevi* Schulman, 1962, но отличается от него закругленным передним и широким задним концами, сравнительно меньшими размерами спор и большей длиной хвостовых отростков.

Вид назван в честь С. С. Шульмана, оказывавшего постоянную помощь при обработке материала.

Thelohanellus acuminatus sp. n.

Хозяин: *Cyprinus carpio*.
Локализация: жабры.
Место нахождения: водоемы Хай-фон.
Интенсивность и процент заражения: встречен у 1.11% исследованных рыб при высокой интенсивности заражения.

Вегетативные формы: цисты округлые, заполнены многочисленными спорами. Споры грушевидные, с суженным, несколько изогнутым на одну сторону и заостренным передним концом и закругленным задним (Рис. 2 В). Грушевидные полярные капсулы крупные, длинные, занимающие больше

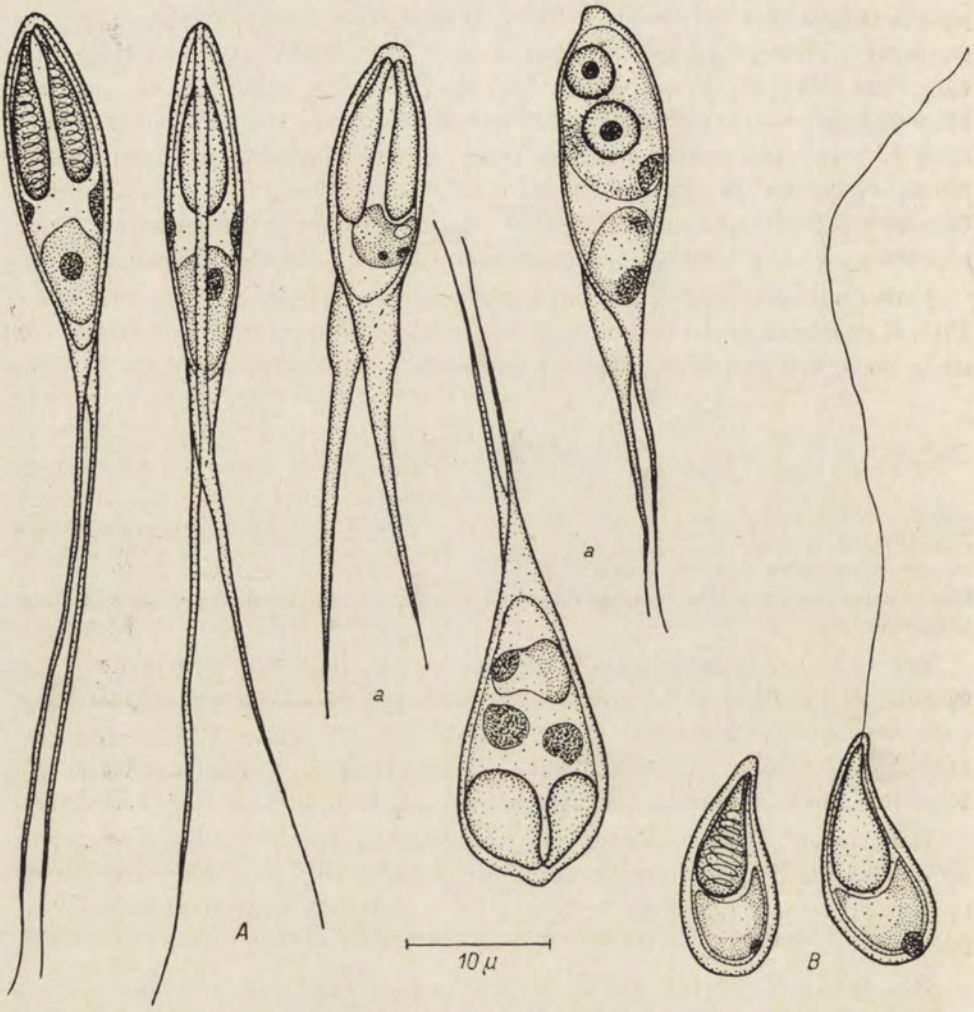

Рис. 2. Fig. 2. Споры (а — атипичные). Spores (a — atypical): A — *Henneguya schulmani*, B — *Thelohanellus acuminatus*

половины длины спор. В полярных капсулах имеются 7 витков свернутой стрекательной нити, которая хорошо заметна. Иодофильная вакуоль сравнительно большая. Длина спор 19.8–21.6, ширина 7.2–8.1, длина полярных капсул 10.8–14.4, их диаметр 4.5–5.4, длина стрекательной нити 50–59.4 μ. Среди спор встречается небольшой процент атипичных форм с двумя полярными капсулами, у которых одна очень маленькая плохо заметна.

Данный вид наиболее близок к *Th. oculi-leucisci* Trojan, 1909, *Th. misgurni* Kudo, 1919; *Th. gantetius* Tripathi, 1953, но отличается большими размерами спор и полярных капсул.

Thelohanellus callisporis sp. n.

Хозяин: *Cyprinus carpio*.
Локализация: кожа, жабры.
Место нахождения: водоемы Ханоя.
Интенсивность и процент заражения: Обнаружен у 2.22% рыб на коже и у 4.17% на жабрах, при высокой интенсивности.

Вегетативные формы: овальные цисты. В цистах образуется огромное количество спор. Споры удлиненно-овальные со слабым углублением на переднем полюсе (Рис. 3). Створки массивные, с мощным, хорошо заметным шовным валиком, толщиной до 3.6 μ. Толщина спор несколько меньше ширины. Полярные капсулы крупные, широкие, колбовидной формы с хорошо развитой четко заметной крышечкой. В полярных капсулах хорошо видна длинная, спирально свернутая стрекательная нить, образующая много витков, причем у зрелых спор имеет место чередование витков с большим и меньшим диаметром. Иодофильная вакуоль в амебоидном зародыше крупная, может достигать 7 μ в диаметре. Длина спор 23.4–25.2, ширина 12.6–16.2, толщина 12.2 длина полярных капсул 10.8, их диаметр 7.2–8.1, длина стрекательной нити 108–113.4 μ. Встречаются единичные атипичные споры меньших размеров или округлой формы.

Этот вид близок к *Thelohanellus catlae* Chakravarty et Basu, 1958 и *Th. dogieli* Achmerov, 1955, но отличается от последнего большими размерами спор и капсул, а от наиболее близкого вида *Th. catlae* — несколько большей шириной спор. Кроме того он отличается от обоих видов правильной овальной формой спор без сужения на переднем полюсе.

В результате исследования 15 видов рыб мы обнаружили 18 видов миксоспоридий, относящихся к 4 родам, из них только представитель рода *Zschokkella* относится к подотряду *Bipolaria*. Остальные 17 видов — представители подотряда *Platysperea*, весьма характерного для пресных вод. Наиболее богато представлен род *Myxobolus* — 11 видов плюс один *Myxobolus* sp.

Обращает на себя внимание большое своеобразие фауны миксоспоридий Вьетнама — из 18 видов, обнаруженных нами, 12 видов, т. е. 2/3 оказались новыми. Еще один вид *Myxobolus* sp. по-видимому, тоже новый, однако мы были лишены возможности описать его из-за отсутствия материала для сравнения. 5 видов *Myxobolus pavlovskii*, *M. achmerovi*, *M. koi*, *M. toyamai* и *M. anisocapsularis* известны для рыб бассейна Амура, а также для водоемов Китая или Японии. Хозяин одного из них (*M. pavlovskii*) — толстолобик, типичный представитель китайского равнинного комплекса. Остальные 4 вида, хотя и паразитируют на сазане, т. е. на рыбе, имеющей широки ареал, однако встречаются только на его восточноазиатском (*Cyprinus carpio haematopterus*) и южноазиатском (*Cyprinus carpio viridiolaceus*) подвидах. По этому можно считать, что эти виды имеют восточное происхождение.

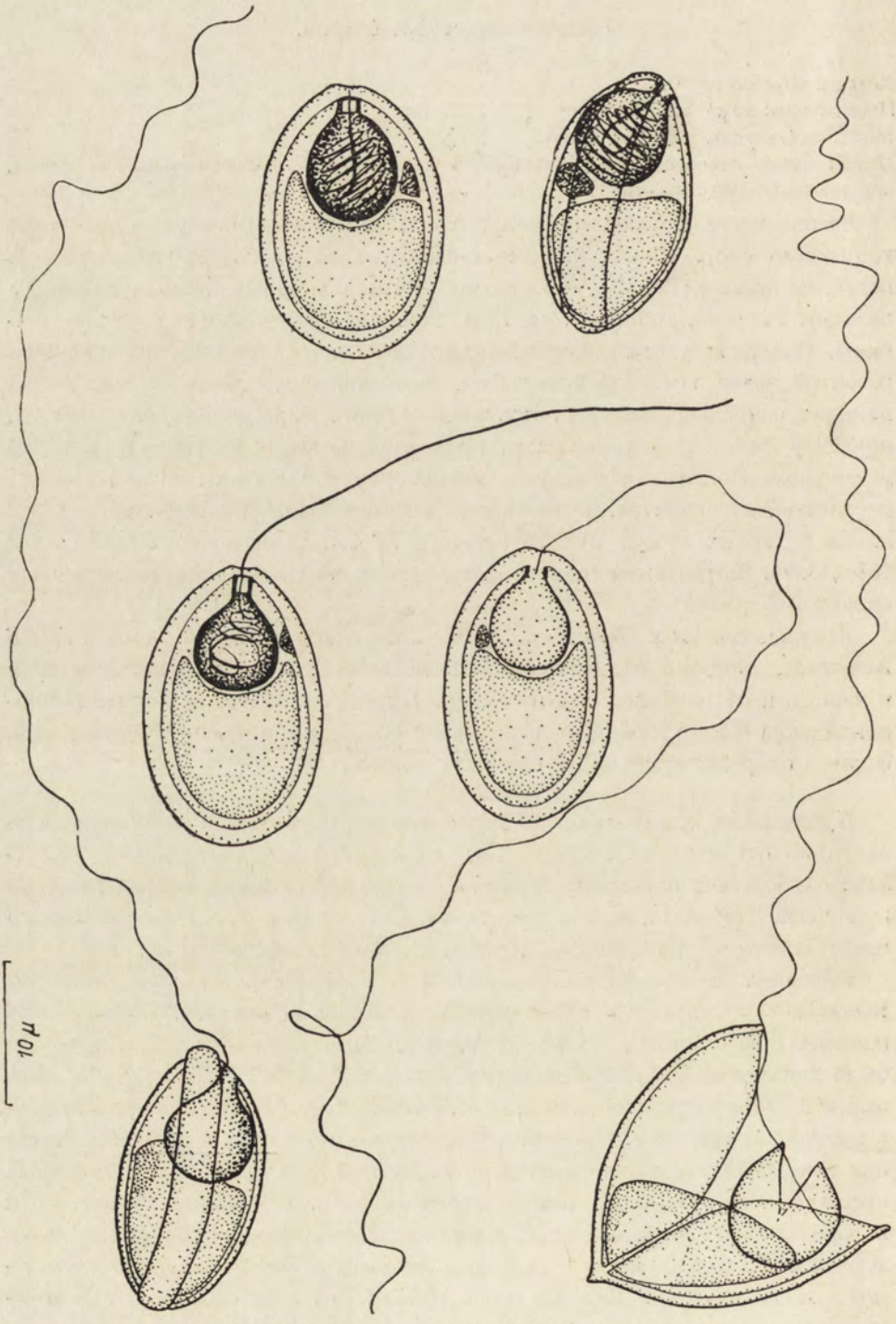

Рис. 3. Fig. 3. Споры — Spores. *Thelohanellus callisporis*

Для того, чтобы проверить зависимость заражения рыб миксоспоридиями от питания и способа приема пищи, мы разделили всех зараженных рыб на 3 группы: I группа — рыбы, берущие пищу из толщи воды — планктофаги толстолобик и пестрый толстолобик, а также анабас, который во время пребывания в воде питается преимущественно плавающими насекомыми. II группа — рыбы, берущие пищу как со дна, так и из толщи воды; сюда входит только один вид *Cirrhina molitorella*, питающийся илом и диатомовыми водорослями. III группа — рыбы, берущие пищу преимущественно со дна —сазан (типичный бентофаг) и *Spinibarbichthys denticulatus* (растительноядная рыба).

Как видно из Таблицы 1, у рыб I группы встречаются только миксоспоридии с медленно опускающимися спорами или со спорами, занимающими по скорости погружения промежуточное положение (Шульман 1966). Виды с быстро опускающимися спорами у них отсутствуют. С другой стороны у рыб II, III группы не обнаружены виды с медленно опускающимися спорами,

Таблица 1

Table 1

Экологические группы миксоспоридий

Ecological groups of *Myxosporidia*

Группы рыб * Groups of fishes	Виды с медленно опускающимися спорами Species with slowly immersing spores	Виды со спорами, занимающими промежуточное положение Species with moderate speed of immersing	Виды с быстро опускающимися спорами Species with quickly immersing spores
I			
Hypophthalmichthys harmandi	*Zschokkella donecae*	*Myxobolus ellipticus*	
	Myxobolus pavlovskii	*M. humilis*	
	M. asymmetricus	*M. discapsularis*	
Aristichthys nobilis		*M. divergens*	
Anabes testudineus	*Henneguya schulmani*		
II			
Cirrhina molitorella		*Myxobolus seminiformis*	*Myxobolus uyeni*
			Myxobolus sp.
III			
Cyprinus carpio		*Myxobolus achmerovi*	*Myxobolus anisocapsularis*
		M. koi	*Thelohanellus callisporis*
		M. toyamai	*Th. acuminatus*
Spinibarbichthys denticulatus			*Myxobolus lanfyongi*

* Обяснения в тексте.

* I — Plancton-eating fishes, II — plancton- and benthos-eating fishes, and III — benthos-eating fishes.

а встречаются только миксоспоридии, споры которых или быстро опускаются на дно (5 видов) или занимают по скорости погружения промежуточное положение (4 вида). Таким образом характер питания рыб, в первую очередь способ приема пищи, заметно отражается на зараженности их миксоспоридиями.

Поскольку большинство рыб бралось из прудов или небольших рек мы в праве предполагать, что имели дело с обедненной паразитофауной, поэтому в будущем при исследовании других видов рыб или тех же видов, но взятых из более крупных водоемов, можно ожидать значительного увеличения списка видов миксоспоридий, паразитирующих на пресноводных рыбах Вьетнама. Особенно богатой фауны миксоспоридий можно ожидать в Красной реке, для которой известно более 100 видов пресноводных рыб.

Резюме

У 15 видов рыб, исследованных в разных водоемах Северного Вьетнама, найдено 18 видов миксоспоридий, из них 11 — из рода *Myxobolus*. Пять видов известны для водоемое Китая или Японии, 12 — новые для науки. Около 50% из них имеют споры с умеренной скоростью погружения и встречаются они и у планктоноядных и у бентосоядных рыб. Виды с медленно опускающимися спорами встречаются только у рыб, берущих пищу из толщи воды. Виды с быстро опускающимиса спорами — только у бентосоядных рыб.

SUMMARY

Eighteen species of myxosporidians have been found in fifteen species of fishes in different waterbodies of North Vietnam. Eleven of them belong to the genus *Myxobolus*. Five species had been found also in waterbodies of China and Japan, twelve were new species. About 50 percent of them have spores with a moderate speed of immersion. Both plancton-eating and benthos-eating fishes harbour the *Myxosporidia* with such spores. Species with quickly immersing spores are specific only for benthos-eating fishes. Species with slowly immersing spores are specific only for plancton-eating fishes (Table 1).

Zschokkella donecae sp. n. (Fig. 1 A). Vegetative forms unknown. Length of spores 14.4–17.1 μ, width 5.4–6.3 μ, thickness 5–6 μ length of polar capsules 3.6–4.5 μ, diameter 3.2–3.6 μ.
Host: *Hypophthalmichthys harmandi*.
Locality: waterbodies Ha-bac (environs of Hanoi).
Localization: gall-bladder

Myxobolus toyamai Kudo, 1915
Host: *Cyprinus carpio*.
Locality: lake Ba-be (province Bac-can)

Myxobolus koi Kudo, 1919
Host: *Cyprinus carpio*
Locality: waterbodies of Hai-phong and Hanoi.

Myxobolus pavlovskii (Achmerov, 1954)
Host: *Hypophthalmichthys harmandi*
Locality: waterbodies of Hanoi and Ha-bac

Myxobolus anisocapsularis Schulman, 1962
Host: *Cyprinus carpio.*
Locality: waterbodies of Hanoi

Myxobolus achmerovi Schulman, 1966
Host: *Cyprinus carpio.*
Locality: lake Ba-be (province Bac-can) and waterbodies of Hanoi.

Myxobolus divergens sp. n. (Fig. 1 B). Vegetative forms: cysts small, dark, oval with few spores. Intercapsular appendix big and width. Length of spores 14.4–16.2 μ, width 9–10 μ, length of polar capsules 5–4 μ, diameter 3.6 μ. Polar capsules widely apart.
Host: *Aristichthys nobilis.*
Locality: waterbodies of Hanoi and Ha-bac.
Localization: gills, skin, liver, kidney, spleen.

Myxobolus uyeni sp. n. (Fig. 1 C). Vegetative forms: small white round cysts with numerous ellipsoidal spores. Intercapsular appendix small. Length of spores 9.9–10.8 μ, width 8.0–8.5 μ, length of polar capsule 5.4 μ, diameter 2.7 μ.
Host: *Cirrhina molitorella.*
Locality: river Bo (province Lao-kay).
Localization: intestine.

Myxobolus semeniformis sp. n. (Fig. 1 D). Vegetative forms: big, incorrectly-round, white cysts, diameter to 4 mm. Many spores pear-shaped. Their length 13.2–14.4 μ, width 4.8–6.0 μ, thickness 3.6–4.2, length of polar capsule 4.5–6 μ, diameter 1.4–1.8 μ.
Host: *Cirrhina molitorella.*
Locality: waterbodies of Hanoi.
Localization: skin.

Myxobolus lanfyongi sp. n. (Fig. 1 E). Vegetative forms; spherical yellow-white cysts with numerous spores. Diameter of spores 10.8–11.7 μ, length of polar capsule 4.5–5.4 μ, diameter 2.7–3.6 μ.
Host: *Spinibarbichthys denticulatus.*
Locality: river Bo (province Lao-kay).
Localization: wall of intestine.

Myxobolus asymmetricus sp. n. (Fig. I F). Vegetative forms unknown. Spores oval, with bend anterior end. Their length 14.4–16.2 μ, width 11.7–12.6 μ, thickness about 9 μ. Polar capsules of unequal size. Length of one 6.8–7.2 μ, diameter 3.6 μ, length of second capsule 4.5 μ, diameter 1.8 μ.
Host: *Hypophthalmichthys harmandi.*
Locality: ponds Ha-bac (environs of Hanoi).
Localization: kidney.

Myxobolus humilis sp. n. (Fig. I G). Vegetative forms unknown. Spores oval with thick shell. Their length 8.1–9 μ, width 6.3–7.2 μ, length of polar capsules 3.6–3.8 μ, diameter 1.8–2.7 μ.
Host: *Hypophthalmichthys harmandi.*
Locality: ponds Ha-bac (environs of Hanoi).
Localization: spleen.

Myxobolus discapsularis sp. n. (Fig. 1 H). Vegetative forms unknown. Spores oval with unequal pear-like polar capsules. Length of spores 12.6–13.5 μ, width 9–10.8 μ, length of one capsule

7.2 μ, diameter 3.6 μ, length of second capsule 2.7–3.6 μ, diameter 1.8 μ.
Host: *Hypophthalmichthys harmandi.*
Locality: ponds Ha-bac (environs of Hanoi).
Localization: gall-bladder.

Myxobolus ellipticus sp. n. (Fig. 1 I). Oval cysts with numerous spores. The latter have thin shell. Their length 12.6–14.4 μ, width 9–10.8 μ, length of polar capsules 5.4–7.2 μ, diameter 3.6 μ.
Host: *Hypophthalmichthys harmandi.*
Locality: waterbodies of Hanoi.
Localization: gills.

Myxobolus sp.
Host: *Cirrhina molitorella*
Locality: waterbodies of Hanoi.
Localization: skin.

Henneguya schulmani sp. n. (Fig. 2 A). Small dark, spherical or oval cysts, 0.25–0.30 mm diameter. Length of spores 16.8–20.4 μ, width 4.8–6.0 μ, thickness 3.6–4.2 μ, length of polar capsules 8.0–10.2 μ, diameter 1.24–2.16 μ.
Host: *Cyprinus carpio.*
Locality: waterbodies of Hanoi.
Localization: gills.

Thelohanellus acuminatus sp. n. (Fig. 2 B). Round cysts with numerous spores. Length of spores 19.8–21.6 μ, width 7.2–8.1 μ, length of polar capsules 10.8–14.4 μ, diameter 4.5–5.4 μ.
Host: *Cyprinus carpio.*
Locality: waterbodies of Hai-phong.
Localization: gills.

Thelohanellus callisporis sp. n. (Fig. 3). Oval cysts with numerous spores. Length of spores 23.4–25.2 μ, width 12.6–16.2 μ, thickness 12.2 μ, length of polar capsule 10.8 μ diameter 7.2–8.1 μ.
Host: *Cyprinus carpio.*
Locality: waterbodies of Hanoi.
Localization: skin, gills.

ЛИТЕРАТУРА

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Laboratory of Protozoology, Department of Zoology, University College of Science, Osmania University, Hyderabad-7, A.P. India

E. SUSAN NAVARATHNAM

Studies on the morphology of some intestinal flagellates of the guinea-pig *Cavia cutleri*

Etudes sur la morphologie des certains Flagellés de l'intestin du cobaye *Cavia cutleri*

Davaine 1875 was the first to study the caecal protozoa of guinea-pig. Since that time there have been many reports of observations on the intestinal protozoa of guinea-pig. Lynch 1922 reported a flagellate from the guinea-pig. Ray and Mitra 1937 found a new flagellate *Monocercomonas caviae*. Ray and Singh 1949 described a new species of *Trichomonas* from the Indian guinea-pig *Cavia cutleri*. Nie 1950 gave a detailed account on the morphology of the intestinal protozoa and Ray 1950 described two new flagellates from the guinea-pig *Cavia porcella*. The present paper gives an account of the morphology of three new species belonging to the genera *Monocercomonas*, *Hexamastix* and *Trichomitus* from the Indian guinea-pig *Cavia cutleri*.

Material and methods

During the course of investigation of intestinal flagellates of mammals, flagellate infection was encountered in a laboratory bred guinea-pig which died of some unknown cause. On examination of the intestinal contents and caecum they were found to harbour *Monocercomonas*, *Hexamastix* and *Trichomitus*. Subsequently six guinea-pigs were examined but only two were infected with flagellates. The infection of *Monocercomonas* and *Hexamastix* was moderate while of the *Trichomitus* was very scanty.

The parasites were examined in the living condition with the help of vital stains such as Methylene blue and Toluidine blue. Permanent preparations were stained with Giemsa's stain following fixation in methanol or with Heidenhains iron haematoxylin after fixation in Schaudinn's fluid. The drawings were made with camera lucida at a magnification of about 2000 ×.

Monocercomonas cutleri sp. n.

Morphology

In stained preparations the shape of the body is variable. It is oval (Fig. 1 G–J), elongated (A–C) or spherical (E). The nucleus is situated at the anterior third of

the body below the blepharoplast. It is spherical (Fig. 1 G, J) or oval (B, D–F) in shape. It contains fine chromatin granules. A single blepharoplast is situated at the anterior end of the body. In some individuals it is situated beneath the pelta. Four flagella arise from the blepharoplast out of which three are unequal and are directed

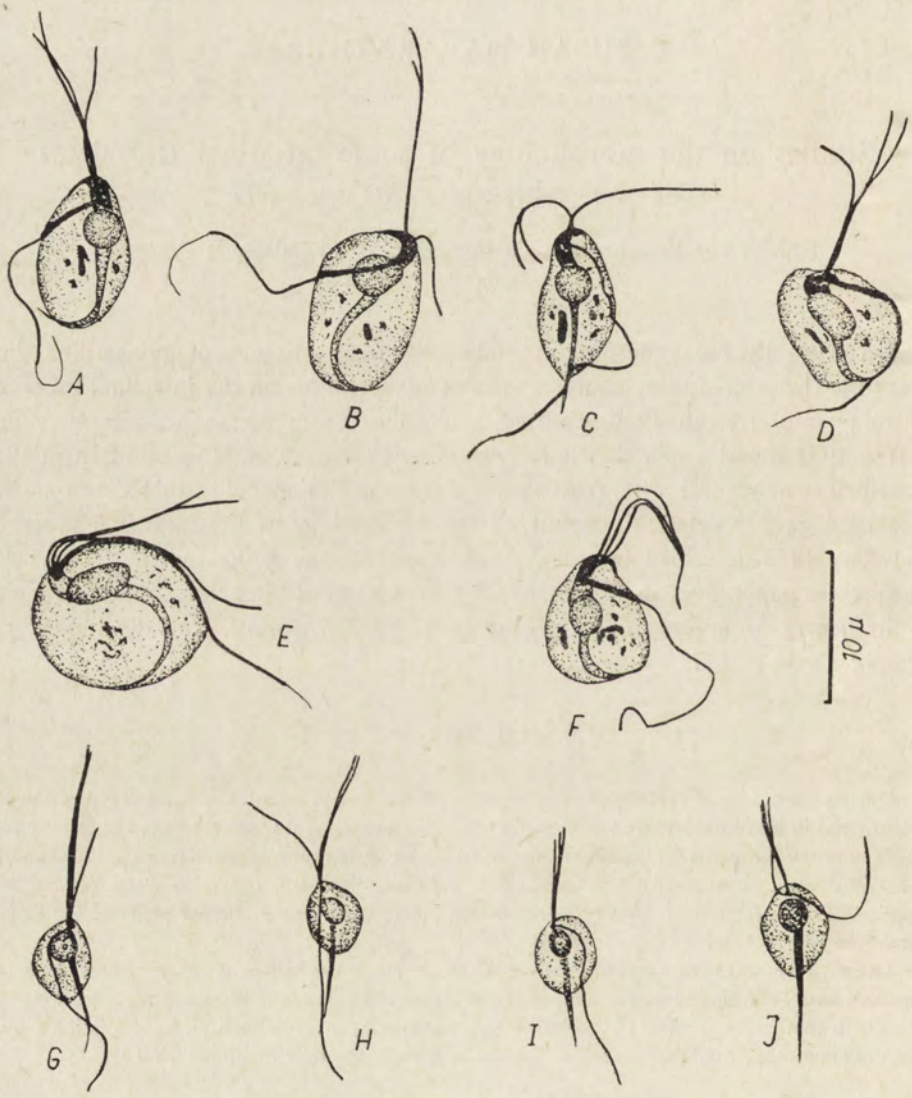

Fig. 1. *Monocercomonas cutleri* sp. n. A–C — showing acronemetic trailing flagellum, accessory filament running along with the trailing flagellum and the pelta, D, E — showing elongated nucleus, blepharoplast, accessory filament and an acronemetic trailing flagellum, F — showing pelta and the bulbous capitulum of the axostyle, G–J — showing fine chromatin granules in the nucleus and a long projecting axostyle, A–F — from smears exposed to 4% Osmic acid vepours, fixed in methyl alcohol and stained with Giemsa's stain, G–J — from smears fixed in Schaudinn's fluid and stained with Heidenhain's iron haematoxylin

anteriorly. In a few forms the two anterior flagella are very close to each other for some distance and then becomes separated (Fig. 1 B, D, E). The third anterior flagellum remains isolated throughout its length. They are uniform in thickness throughout and never terminate in knobs or in acronemes. The trailing flagellum is very long when compared with the three anterior flagella. It always passes backwards along the surface of the body in association with an accessory filament for some distance and then becomes free at the posterior region (Fig. 1 A, B, D–F). The trailing flagellum terminates in a long acroneme (Fig. 1 A–E). The axostyle originates from the blepharoplast, extends posteriorly and projects outside the body for a considerable length (Fig. 1 G–J) or curves inside the cytoplasm (B, D–F). It is broad at the anterior end and forms a capitulum. The axostyle gradually becomes narrow and ends in a fine point. A well developed pelta is present. It is inverted "comma" shaped and appears as an extension of the axostyle. It is directed anteriorly along with the anterior flagella (Fig. 1 A–D).

Measurements: Length of the body 5.0–11.0 μ average 8.16 μ; Breadth of the body (3.5–11.0 μ) 7.0 μ; Length of the nucleus (2.0–4.5 μ) 2.9 μ; Breadth of the nucleus (2.0–4.0 μ) 2.64 μ; Length of the anterior flagellum I (6.0–13.5 μ) 10.4 μ; Length of the anterior flagellum 11 (9.5–17.5 μ) 12.87 μ; Length of the anterior flagellum III (9.5–17.5 μ) 12.87 μ; Length of the recurrent flagellum (11.0–24.0 μ) 17.64 μ; Length of the axostyle (6.0–13.5 μ) 9.96 μ.

Discussion

In the range of dimensions the present species is the largest one sofar reported from mammals. A comparative statement of the dimensions of the different species along with the present species is given in the Table 1. Apart from the dimensions

Table 1

The dimensions of the species of *Monocercomonas* (measurements in microns)

Name of the species	Length of the body	Breadth of the body
M. caviae (Davaine, 1875) Nie, 1950 from *Cavia porcella*	(4.4 – 8.5) 6	(2.2 – 4.3) 3.1
M. cuniculi Tanabe, 1926 from Rabbit	5.0 –14.0	—
M. verrens Honigberg, 1947 from *Tapirus malayanus*	5.3 – 8.6	3.0 – 7.9
M. pistillum Nie, 1950 from *Cavia porcella*	(4.0 – 6.5) 5.4	(3.0 – 3.6) 3.3
M. minuta Nie, 1950 from *Cavia porcella*	(2.7 – 6.0) 4.2	(2.0 – 2.7) 2.3
M. lori Abraham, 1962 from *Loris tardigradus*	(6.2 –10) 8.4	(5.2 – 9.0) 6.8
M. gerbilli Todd, 1963 from *Gerbillus indicus*	(4.23–10.36) 7.38	(2.82– 7.05) 4.46
M. cutleri sp. n. from *Cavia cutleri*	(5.5 –11.0) 8.16	(3.5 –11.0) 7.0

the present parasite shows some marked differences when compared with the previously described species. The new species resembles *M. caviae* and *M. pistillum* in the structure of the nucleus but differs from them in not having an axostylar ring, siderophilic bodies in the cytoplasm and in the presence of only one blepharoplast, an accessory filament and the flagella without terminal knobs. The presence of an accessory filament along with the trailing flagellum distinguishes it from all other species sofar described from mammals. Further it differs from *M. cuniculi* in the absence of an endosome and cytostome. It is marked off from *M. verrens* in the absence of an endosome, terminal knobs for the flagella and in the number of blepharoplasts. The presence of chromatin granules in the nucleus and a conspicuous long axostyle projecting outside the body separate the new species from *M. minuta*. The species under discussion also differs from *M. lori* in the absence of four plaques in the nucleus and in having a single blepharoplast and pelta. The new organism can be distinguished from *M. gerbilli* in having a tubular axostyle, a pelta and an acronematic trailing flagellum. In view of the characters discussed above it is proposed to designate this parasite from the guinea-pig *Cavia cutleri* as *Monocercomonas cutleri* sp. n. after the specific name of the host.

Hexamastix hyderabadensis sp. n.

Morphology

In stained preparations the parasite is oval (Fig. 2 J) or pyriform (C, H). The nucleus is situated at the anterior part or at the middle of the body. It is spherical (Fig. 2 A, H, I, K) or elongated (D, G, J) and contains large and fine chromatin granules (G–K). Blepharoplast is situated at the anterior end of the body. From it all the flagella originate in two units — the anterior and the trailing. The anterior flagella are five in number and they are separate throughout their length (Fig. 2 A, H). They are unequal in length and are longer than the body. The trailing flagellum is longer than the anterior flagella and is twice or thrice the body length. It is always accompanied by an accessory filament (Fig. 2 A, B, F) for a short distance and terminates in a long acroneme. The axostyle is conspicuous and projects outside the body for a considerable length (Fig. 2 A, D, E, G–K). It has a distinct bulbous capitulum and a narrow trunk which terminates into a fine point (Fig. 2. A, D, E, G, K). A well developed pelta is present at the anterior part of the body as an extension of the axostyle (Fig. 2 A, C, E, F). It is sickle-shaped with the pointed end directed anteriorly. There is a clear cytostomal slit at the anterior part of the body either on the lateral side (Fig. 2 H) or in the centre (I). The cytoplasm is granular with a few food vacuoles containing bacteria.

Measurements: Length of the body (6.5–13.5 μ) average 9.35 μ; Breadth of the body (4.5–12.5 μ) 7.56 μ; Length of the nucleus (2.0–4.5 μ) 3.36 μ; Breadth of the

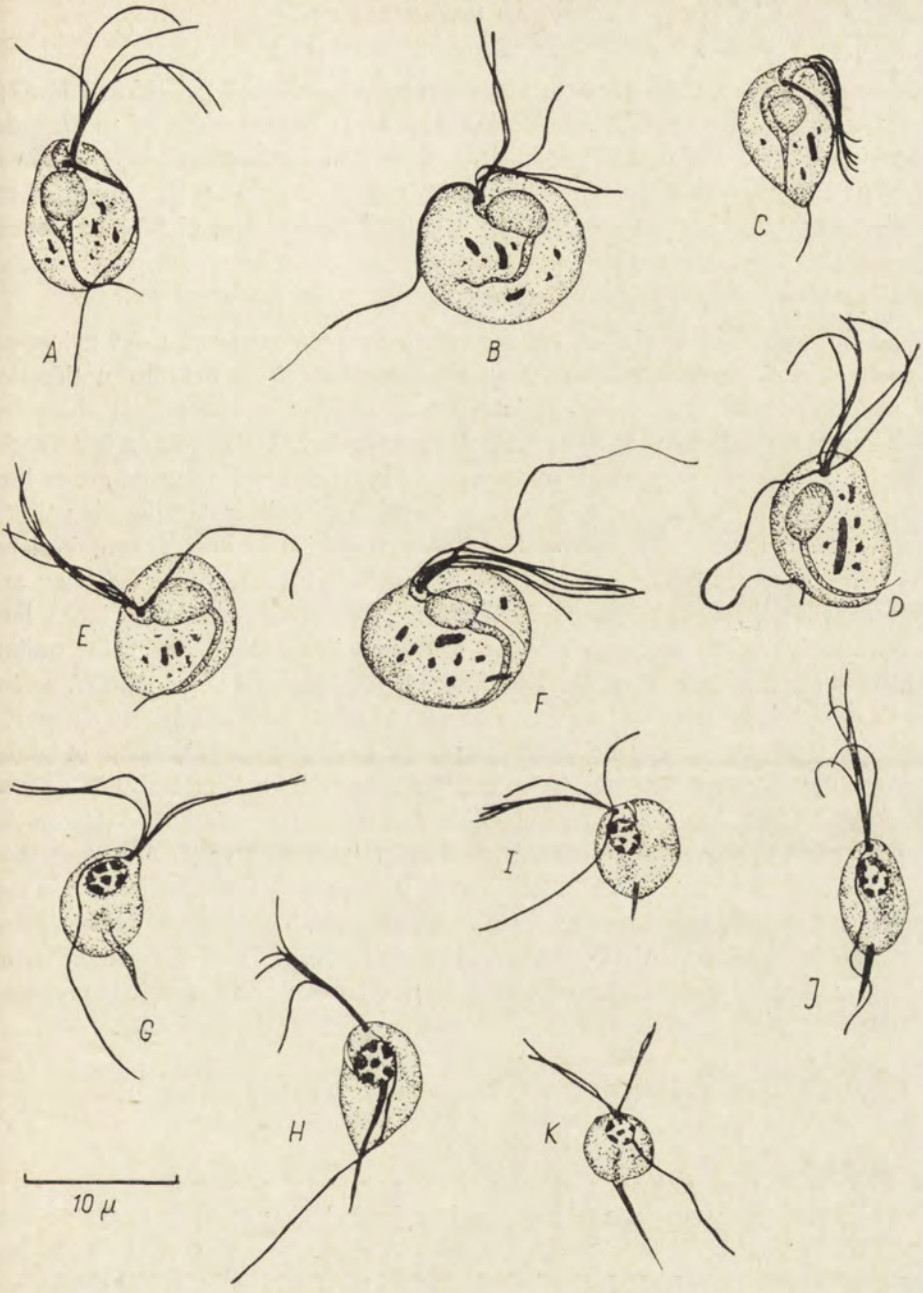

Fig. 2. *Hexamastix hyderabadensis* sp. n. A — showing spherical nucleus, blepharoplast, funis and pelta, B — spherical form showing elongated nucleus, trailing flagellum with an acroneme and a curved axostyle, C — showing blepharoplast, bulbous capitulum of the axostyle and the pelta, D — showing the elongated nucleus, large blepharoplast and a curved axostyle, E — showing the general structure, F — showing acronemetic trailing flagellum, accessory filament and a curved axostyle, G, J, K — showing large compact chromatin granules in the nucleus, long trailing flagellum and a long projecting axostyle, H, I — showing the cytostome, A–F — from smears exposed to 4% Osmic acid vapours, fixed in methyl alcohol and stained with Giemsa's stain, G–K — from smears fixed in Schaudinn's fluid and stained with Heidenhain's iron haematoxylin

nucleus (2.0–4.0 μ) 2.84 μ; Length of the anterior flagellum I (8.0–16.5 μ) 10.53 μ; Length of the anterior flagellum II (6.0–17.0 μ) 11.18 μ; Length of the anterior flagellum III (8.0–17.0 μ) 11.73 μ; Length of the anterior flagellum IV (7.0–16.0 μ) 10.94 μ; Length of the anterior flagellum V (7.0–16.5 μ) 10.94 μ; Length of the trailing flagellum (10.0–19.5 μ) 18.93 μ; length of the axostyle (5.5–14.5 μ) 9.61 μ.

Discussion

So far only two species of *Hexamastix* have been reported from guinea-pig namely *H. caviae* and *H. robustus*. Thus the present species is the third notification of infection in the guinea-pig. It has certain striking morphological characters which are not similar with the above mentioned species. *H. caviae* has a small endosome in the nucleus with fine granules around it. The anterior flagella are as long as the body and they always terminate in knobs. The trailing flagellum is uniform in its diameter throughout, whereas in the present species the nucleus contains large and fine chromatin granules. The anterior flagella are longer than the body and they never terminate in knobs. The trailing flagellum always terminates into a long acroneme. A distinct accessory filament is present in association with the trailing flagellum. In addition to these characters the present species is larger than *H. caviae*. In *H. robustus* the nucleus contains a small endosome with clumps of chromatin around it. The anterior flagella are as long as the body and they terminate in knobs. The trailing flagellum has no acroneme. The axostyle forms a lancet-like tip at the posterior end. Around the nucleus, there is a chromatic area full of siderophilic granules. But in the species under discussion the anterior flagella are longer than the body and never terminates in knobs. The trailing flagellum terminates in a fine acroneme and is associated with an accessory filament. The axostyle ends in a fine point. Siderophilic granules are absent in the cytoplasm. These differences justify the recognition of the present parasite as a new species and hence it is proposed to name it as *Hexamastix hyderabadensis* sp. n. after Hyderabad City.

Trichomitus honigbergi sp. n.

Morphology

In stained preparations the parasite is spherical (Fig. 3 A, D, F, G) or oval (B, C, H) in shape. The nucleus is situated at the anterior part of the body just below the blepharoplast. It is usually present on the lateral side of the capitulum. It has a very large endosome (Fig. 3 F–H). In some individuals in addition to the large endosome fine chromatin granules are seen on one side (Fig. 3 G). There is a large blepharoplast at the extreme anterior end of the body (Fig. 3 A–D, G, H). It gives rise to all the mastigont elements. The three anterior flagella originate from the blepharoplast and they are unequal in length. One of the flagella is long, the other is intermediate and the third is short. All of them are uniform in their thickness.

The undulating membrane extends on one side of the body upto half or three fourths of the body length. It shows three to four unequal folds. The undulating membrane is bordered by a marginal filament which extends posteriorly as a free flagellum. This free posterior flagellum is shorter than the anterior flagella. The costa arises from the blepharoplast and extends on the basal portion of the undulating membrane upto the middle or three fourths of the body length and very rarely upto the posterior

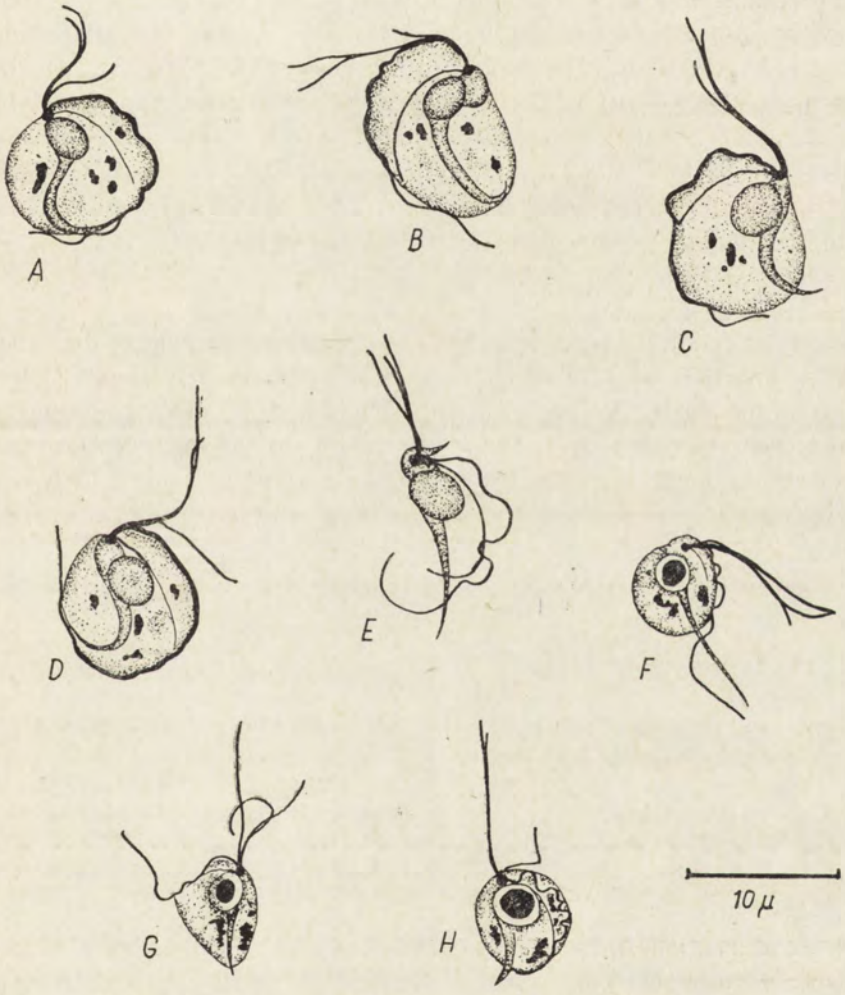

Fig. 3. *Trichomitus honigbergi* sp. n. A–E — showing a large blepharoplast at the extreme anterior end three subequal flagella, filament-like costa and a curved axostyle, F, H — showing a large endosome in the nucleus, G — showing chromatin granules in one side of the nucleus in addition to the endosome, A–E — from smears fixed in 4% Osmic acid vapours, fixed in methyl alcohol and stained with Giemsa's stain, F–H — from smears fixed in Schaudinn's fluid and stained with Heidenhain's iron haematoxylin

end. The axostyle is well developed. The anterior portion of the axostyle is expanded into a bulbous capitulum (Fig. 3 B–E), while the remaining portion gradually tapers into a point. The axostyle either projects outside the body (Fig. 3 C, G, H) or curves inside the cytoplasm (A, B, D). The costa is filamentous and no paracostal granules are observed. The pelta is seen extending at the anterior part of the body (Fig. 3 E). It is broad at the base and pointed at the free end. Cytostome is not observed in any individual. The cytoplasm is granulated and contains a few darkly stained cytoplasmic inclusions.

Measurements: Length of the body (7.5–15.0 μ) average 11.18 μ; Breadth of the body (7.5–15.0 μ) 10.32 μ; Length of the nucleus (3.0–5.0 μ) 3.86 μ; Breadth of the nucleus (2.5–4.0 μ) 3.13 μ; Length of the anterior flagellum I (7.5–16.0 μ) 11.3 μ; Length of the anterior flagellum II (5.6–15.5 μ) 10.9 μ; Length of the anterior flagellum III (5.0–15.0 μ) 10.3 μ; Length of the undulating membrane (10.0–29.5 μ) 18.2 μ; Length of the free posterior flagellum (2.5–8.5 μ) 6.0 μ; Length of the costa (7.5–14.0 μ) 10.67 μ; Length of the axostyle (6.0–14.0 μ) 10.67 μ.

Discussion

According to the Honigberg's (1963) comprehensive account of the structure, synonymy and host list of *Trichomitus*, only four species were placed under this genus from mammals. The present species differs from all of them in dimensions and in certain other characters. The dimensions of various species so far reported from mammals along with the present species are given in the Table 2. In *T. wenyoni*

Table 2

A comparison of the body dimensions of the species of *Trichomitus* (measurements in microns)

Name of the species	Length of the body	Breadth of the body
T. wenyoni (Wenrich et Nie, 1949)	(4.0 – 8.80) 5.80	(3.0 – 5.50) 3.64
T. marmotae (Crouch, 1933) Gabel, 1954	(5.2 –10.50) 7.53	(3.30– 7.10) 5.11
T. ulmeri (Gabel, 1954)	(4.00– 9.00) 5.78	(1.00– 4.00) 3.18
T. rotunda (Hibler et al., 1960)	(6.83–11.4)	(4.56– 7.41)
T. honigbergi sp. n.	(7.50–15.0) 11.18	(7.5 –15.0) 10.32

Wenrich and Nie, 1947 from rat, the nucleus contains a small eccentric endosome, flagella adhere together at the base and have knobs at the tips, the undulating membrane is sharply spiral in position, while the new species is larger than *T. wenyoni*. The nucleus possesses a large central endosome either with or without chromatin granules. The undulating membrane does not possess a spiral course and is feebly developed. *T. marmotae* from *Marmota monax* is pyriform in shape, the nucleus contains a small endosome, the costa is rod-like and the cytostome is present, whereas

the new species is spherical in shape, the nucleus contains a large endosome and chromatin granules on one side, the costa is filamentous and the cytostome is absent. The new organism resembles *T. ulmeri* (Gabel, 1954) in the absence of a cytostome but differs from it in the presence of pelta and in not having a long posterior flagellum. The present species differs from *T. rotunda* Hibler et al., 1960 in the presence of unequal anterior flagella and the pelta. Further the anterior flagella never terminate in knobs and the posterior flagellum never terminates in acroneme in the new species. The differences discussed above are considerable and merit the assignment of this parasite to a new species. *Trichomitus honigbergi* sp. n. is therefore the name proposed after Dr. B. M. Honigberg.

Acknowledgements

The work was carried out under the guidance of Dr S. S. Qadri, Osmania University, to whom the author is grateful for his help and encouragement. The author is thankful to Dr S. N. Singh, Head of the Zoology Department, Osmania University for providing laboratory facilities. Thanks are due to the Council of Scientific and Industrial Research for the award of a fellowship.

Summary

During the course of investigation, flagellate infection was encountered in a laboratory bred guinea-pig which died of some unknown cause. On examination of the intestinal content three new species, *Monocercomonas cutleri* sp. n., *Hexamastix hyderabadensis* sp. n., and *Trichomitus honigbergi* sp. n. were found. The infection of *Monocercomonas* and *Hexamastix* was moderate while that of *Trichomitus* was scanty.

RÉSUME

Au cours de l'étude on a trouvé une infection causée par des Flagellés chez un cobaye élève dans le laboratoires qui était mort pour des raisons inconnues. Après l'examen du contenu de l'intestin on a trouvé trois éspèces nouvelles: *Monocercomonas cutleri* sp. n., *Hexamastix hyderabadensia* sp. n., *Trichomitus hongibergi* sp. n. L'infection par *Monocercomonas* et *Hexamastix* était moderée pendant que celle de *Trichomitus* était parcimonieuse.

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ACTA PROTOZOOLOGICA

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Institute of Inland Fishery, Department of Fish Culture, Żabieniec near Warsaw, Poland

Kazimierz MIGAŁA

Studies on natural populations of parasitic protozoa on *Cyprinus carpio* L. in pond culture. Carps in the first year of life

Badania naturalnych populacji pasożytniczych pierwotniaków u karpi (*Cyprinus carpio* L.) w stawach. Karpie w pierwszym roku życia

Only a few papers (among others Lajman 1951, Lucky 1965) can be found in the literature concerning the occurrence of parasitic protozoa on carps in ponds all over the year. They generally deal with the incidence and intensity of infection. These papers are based on uneven materials originating from several or many ponds situated in different ecological conditions, and where the qualitative composition of certain groups, of parasites even those very widespread on fishes, e.g. *Ciliata*, is inadequately elaborated. Admittedly, more comprehensive descriptions of *Urceolariidae* on pond fishes have recently appeared (Ivanova 1966, 1967, 1970). In sum, however, there is a lack of accurate quantitative studies and of a complex concept of many species or groups of parasites which compose parasitocenoses. According to Noble 1960 and Raabe 1964 the matter of relationships within, such a complex are not yet sufficiently understood. On the other hand, Bauer (1959, 1961) considers that a knowledge of these problems, particularly concerning carp fingerlings provides the key to work out the methods of suppressing epizooties. Thus the necessity occurs of broadening the knowledge of the biology of the parasites, and especially of their ecology. This is a condition for the correct elaboration of methods of prevention of parasitic fish diseases.

The aim of this work is to expand the knowlegde of the qualitative and quantitative changes in parasitic protozoa during the full-year period and, if possible, to determine the general rules in the process of parasitic infection under the conditions of a single fish-farm.

This paper comprises a part of doctor's dissertation. The author wish to thank Prof. Dr Eugeniusz Grabda, for his care and scientific guidance; and Prof. Dr Janina Janiszewska and Prof. Dr Zdzisław Raabe for their estimation and remarks. Thanks are also due to Dr Stanisław L. Kazubski for his assistance in presenting the material and for hints during the preparation of the paper for printing.

The area and conditions of research

Material for this paper was obtained from the ponds of the Inland Fishery Institute Department of fish culture in Żabieniec. These reservoirs, built on sandy soils with a clay-sand substrate, situated in a compact complex, are fed by one common supplying water ditch from the river Jeziorka.

On the segment up the river from Żabieniec to the springs the river is free of industrial sewage. In the upper course of the river there are several small carp fish farms. From these farms carps, going into the composition of the ichtiofauna of Jeziorka (Rembiszewski 1964) may originate. Carps in Jeziorka have probably been occurring for many years; already Wałecki mentions them in 1864, although with a question mark.

The ponds in Żabieniec were used in the previous years to produce stocking måterial of carp. These reservoirs possess an individual inflow and outflow. The average depth of the water at full flood amounts to 90–100 cm: it is possible to maintain it at a uniform level. Experimental ponds, however, have an area of 0.2 hectare and identical shape: a rectangle of 40 × 50 m. They have been used since 1957. Commercial ponds, especially fingerling ponds are considerably larger. Their area can reach up to 5 hectares. The utilization of commercial ponds has taken place for many decades. Water for experimental ponds is delivered by means of pumps. Commercial ponds are filled up partly by gravitation, and partly by pumps. The time of utilization of the particular ponds depends on their purpose, and thus: spawning ponds are utilized for about a week, fry ponds for about one month, fingerling ponds for about four months, and winter ponds for over five months. Some fingerling ponds were left without fishing until the spring period. This was an intended wintering of the carp fingerling in these ponds. All the ponds are dry during a part of the year.

During the period of conducting the studies, the bottom and banks of the ponds were overgrown with a small amount of rotted plants; algae occurred also.

In the ponds in which fertilizing and feeding of fishes were performed, the overgrowth by the mentioned plants was generally less extensive when compared with the ponds without fertilization and feeding. These ponds were in turn more abundant in small algae occurring in the water.

Together with water from the water supplying ditch certain amounts of coarse fish enter into all the ponds through the grids of the pumps and bellows. These are mostly the youngest generations of certain species of fish found in the river Jeziorka: *Gasterosteus aculeatus* L., *Rutilus rutilus* (L.), *Perca fluviatilis* L., *Leucaspius delineatus* (Heck.), *Alburnus alburnus* (L.), *Gobio gobio* (L.), *Nemachilus barbatulus* (L.), *Leuciscus cephalus* (L.), *Esox lucius* L., *Carassius carassius* (L.), *Tinca tinca* (L.), *Rhodeus sericeus* (Pall.) and *Scardinius erythrophthalmus* (L.). They are mentioned in succession beginning from the most numerous ones in the ponds. In autumn the quantity of fished coarse fish in experimental ponds reached up to 16 kg per hectare and up to 25 kg per hectares in commercial ponds.

In fry ponds during the period of fishery utilization, great quantities of tadpoles *Rana esculenta* L. and *Bombina bombina* L. were usually found. The amount of tadpoles during the fishing time in extreme cases reached several hundred kg per hectare.

The investigations were carried out in two one-year periods of time (1963–1964 and 1964–1965). These periods differed among others in the temperature pattern, in rainfall and in the number of sunny days (Table 1).

The ponds in which material was collected were stocked in the particular years with carps from one spawn.

In both periods of studies, in the particular pond categories with the exception of winter ponds, equal stock densities were used, recognized as optimum on the basis of experiments from the previous years (Wolny 1962). And thus the stock density in fry ponds at the time of stocking was 125 000 of just hatched fishes per hectare and 15 000 carp fry per hectare in carp fingerling ponds. In winter ponds sometimes a differentiated stock density was used, from 9500 to 122 000 carp fingerling per hectare.

Table 1

Average monthly temperatures of water in ponds (°C) and falls (mm) and the number of sunny days in the periods of investigations

Months	Temperature		Falls		Sunny days	
	1963–1964	1964–1965	1963–1964	1964–1965	1963–1964	1964–1965
May	19.6	16.3	88	51	9	12
June	21.3	23.5	22	57	17	11
July	23.8	22.7	47	26	25	19
August	21.1	19.9	58	28	14	16
September	18.8	16.4	59	23	9	4
October	10.8	10.0	22	10	5	13
November	7.3	3.6	55	70	2	3
December	1.0	1.8	10	13	13	2
January	0.6	1.6	12	19	12	6
February	0.6	0.9	55	18	11	11
March	0.7	2.0	63	20	10	9
April	6.8	7.8	9	55	12	7
	11.0 *	10.5 *	500 **	390 **	139 ***	113 ***

* Average annual temperature.
** Annual fall.
*** Total number of sunny days in the year.

Fry ponds and carp fingerling ponds were fertilized with nitro-phosphate fertilizers. The administration of fertilizers into ponds took place in a manner which enabled the longest possible preservation of the phosphorus and nitrogen compounds in water (Wolny 1969, 1970).

The feeding of fishes was performed in carp fingerling ponds. Forage was administered three times a week, in quantities sufficient for the fishes till the next feeding.

For the duration of the studies, in accordance with the assumptions of the paper, the fishes in ponds were not treated for disease.

Material and methods

The parasites were collected from the skin and gills of the fishes, within weekly or fortnightly intervals of time, with the exception of winter months. Fishes for studies were taken in the number of a least 80 individuals from eight ponds, beginning from just hatched fishes from spawning ponds until the end of the first year of life (fishing time in winter ponds). Altogether in both years, quantitative parasitologic studies have been conducted on 2970 carps from 81 ponds.

Since during the course of investigation it turned out that spawners, coarse fishes and frog tedpoles may play a certain role in the infection as a reservoir and vector of parasites, for comparative purposes studies were also carried out on 10 spawners, 100 specimens of fishes found in the water supplying ditch, and 50 tadpoles.

Smears were prepared from mucus collected quantitatively from the skin and gills, which were covered by glass slides and stored in 4% formalin after drying. With the view to a more detailed knowledge of the species composition of parasitofauna, $AgNO_3$ silvering by Klein's method was performed, fixing by means of Schaudinn's solution and dying by hematoxyline according to Meyer, or Giemsa dying. Biometric measurements were carried out for each species of parasites on 20

individuals selected at random from several fishes. Only in the case of *Trichodina pediculus* and *Chilodonella uncinata* all the occurring specimens were investigated. For measurements the preparations were used impregnated with silver and stained as well. A more detail description of the method is included in the paper of Migała 1970.

In the text of the present paper the term "population of parasites" is used to denote all the individuals of parasites of a given species occurring in a determined population of the host species. Multi-species aggregations, e.g. *Trichodina* on skin or *Sessilia*, are designated by the general term of "group parasites".

All the tables and charts have been constructed on the basis of the real data obtained from microscopic observations without computations. The data from spawning ponds and fry ponds indicate therefore the number of parasites found over the entire host. Data from carp fingerling ponds and winter ponds, on the other hand, concern the number of parasites found on 1/4 of the fish's surface.

Results

Species composition, biology and ecology of particular species

As a result of the conducted investigations 9 identified and 2 unidentified species of protozoa were found. The dimensions of these protozoa were generally in agreement with previous descriptions in the literature.

Ichthyobodo necatrix (Henneguy, 1884)

Syn.: *Costia necatrix* (Henneguy, 1884).

I. necatrix appeared in both years on the skin and gills of just hatched carps in spawning ponds and on carp fry during the initial period after the stocking of fry ponds. After about a fortnight's stay of the fishes in the fry ponds the parasite disappeared. Later it has not been observed.

The highest intensity and incidence of infection was found in 1964 in a spawning pond. The maximum number of parasites on the particular carp larvae, which were during the period of studies in the stage of the beginning of active feeding, approached 800 (averages 81.8, Table 2). In 1963 the infection was less intensive. A phenomenon deserving particular attention was the appearance and disappearance of *I. necatrix* during both years in the same period of culture (Fig. 1).

The occurrence of *I. necatrix* in spawning ponds and fry ponds was frequently observed (Bespaly 1939, Lajman 1946). During this period of culture of carp, *I. necatrix* caused a very high mortality. With the growth of fish the intensity and incidence of infection decreased. According to Bespaly 1939, the percentage of infected carps with the age up to 20 days was 87, of autumn carp fingerlings 37 of one-year carps 15.8, and of spawners 4.2.

Sources of the parasitic infection.

In view on the possibility of the formation of cystes by the parasite, and also of the development on fish eggs in the spawning ponds (Hłond 1963), most probably

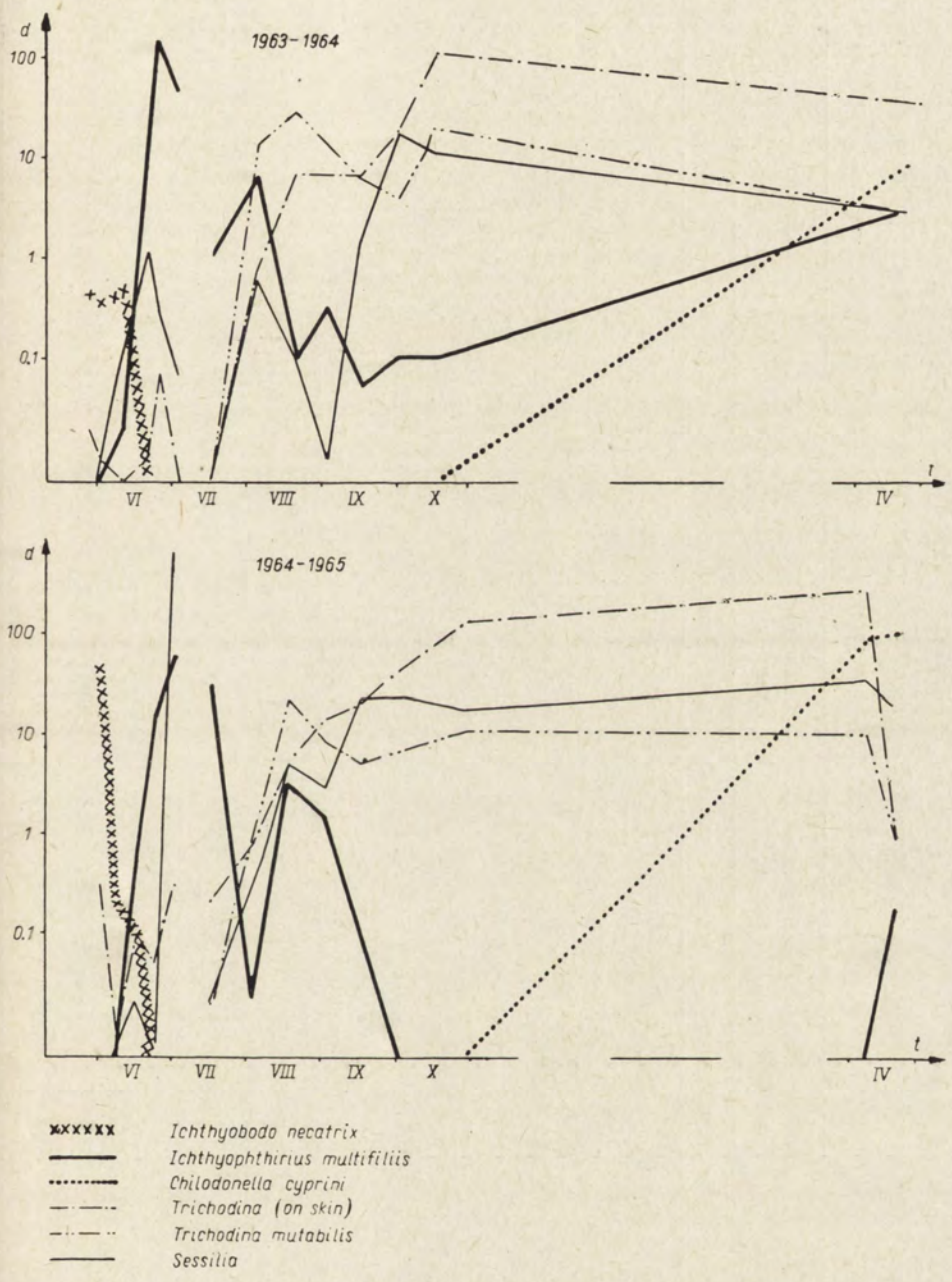

Fig. 1. Variations in the number of particular species of parasitic *Protozoa* on carps in annual periods. d — number of parasites computed per one examined fish (logarithmic scale used), t — time (period of wintering of fishes for which studies were not performed are denoted by dashed axis).

Table 2

Comparison of intensity and incidence of the infection with *Protozoa* on the example of carps in most frequently studied variants of experiments

1963–1964

Dates of observations	*Ichthyophthirius multifiliis*		*Trichodina* on skin*		*Trichodina mutabilis*		*Sessilia* **		*Chilodonella cyprini*		*Ichthyobodo necatrix*	
	int.***	ext.	int.	ext.	int.	ext.	int.	ext.	int.	ext.	int.	ext.
27 May 63			1.0	2.5							3.6	12.5
1 June 63			1.0	1.2							4.3	7.5
11 June 63	1.0	2.5					3.5	2.5			5.2	10.0
21 June 63	24.7	70.0	1.0	1.2			14.0	8.7				
25 June 63	188.8	100.0	2.0	3.3			6.0	5.0				
3 July 63****	56.4	81.2					4.0	1.2				
16 July 63	4.0	22.5										
5 Aug. 63	8.7	72.5	2.5	30.3	28.4	45.0	8.2	7.5				
20 Aug. 63	1.0	7.5	10.4	65.0	34.9	75.0	1.5	2.5				
2 Sept. 63	2.9	11.2	8.9	71.2	18.4	71.2	1.0	1.2				
16 Sept. 63	1.0	5.0	8.2	76.2	10.7	58.7	20.4	6.2				
1 Oct. 63	1.5	7.5	20.0	90.0	5.6	68.7	108.7	15.0				
16 Oct. 63	1.0	6.2	110.1	100.0	23.0	81.2	22.8	52.5				
23 April 64	6.9	44.0	37.9	96.0	3.7	76.0	45.3	64.0	13.5	64.0		

Dates of observations	*Ichthyophthirius multifiliis*		*Trichodina* on skin *		*Trichodina mutabilis*		*Sessilia* **		*Chilodonella cyprini*		*Ichthyobodo necatrix*	
	int.***	ext.	int.	ext.	int.	ext.	int.	ext.	int.	ext.	int.	ext.
2 June 64			6.5	5.0							81.8	60.0
9 June 64			1.0	1.2							2.8	7.5
16 June 64	5.5	11.2	4.0	2.5			2.0	1.2			6.0	1.2
23 June 64	51.6	26.2	2.0	2.5								
1 July 64	106.8	57.5	2.9	11.2			1108.1	65.0				
16 July 64 ****	31.2	93.7	2.0	8.7	1.0	2.5	2.0	1.2				
3 Aug. 64	1.0	2.5	2.7	28.7	3.6	30.0	3.7	8.7				
17 Aug. 64	4.7	67.5	8.5	65.0	29.4	71.2	46.2	10.0				
1 Sept. 64	4.0	37.5	15.3	86.2	10.5	78.7	27.2	11.2				
15 Sept. 64	1.4	10.0	20.5	91.2	8.1	63.7	74.2	30.0				
1 Oct. 64			45.1	96.2	8.4	78.7	50.5	47.5				
28 Oct. 64			134.4	100.0	13.7	81.2	21.7	81.2				
8 April 65			312.8	100.0	11.0	95.7	41.7	94.3	88.6	100.0		
27 April 65	2.3	22.5	224.2	92.5	8.7	92.5	24.7	77.5	135.8	77.5		

* The group *Trichodina* on skin comprises *Trichodina domerguei* f. *acuta* and *Trichodina nigra*.
** The group *Sessilia* comprises *Apiosoma* sp. and *Sessilia* indet.
*** Numbers concerning the intensity of infection denote the average number of parasites per one of the infected fishes in the sample. A sample usually consists of 80 carps.
**** Parasites were d onlycollecte from 1/4 of the body surface of the fish. See material and methods.

the spawners of carp were the source of infection. Before spawning, individual *I. necatrix* were found on spawners. Also coarse fishes could be the source of infection (Bespaly 1939, Ivasik 1960).

The action of environmental factors: concentration of hydrogen ions

In the occurrence of *I. necatrix* on carps, the behaviour of these parasites in water environment with different pH merits discussion. In the literature there can be found divergent views on this problem. In 1929, Schäperclaus observed that parasites appeared in winter ponds with a low pH in the water (from 4.5 to 5.8), whereas at a basic pH they did not occur. Bauer (1959), analyzing the data of Schäperclaus (1929) and other authors which indicated the appearance of *I. necatrix* in winter ponds with pH 5.0 to 5.5, suggests that *I. necatrix* develop better in low pH. According to Bauer 1959, *I. necatrix* is distinguished precisely by this feature from the other fresh-water fish parasites. In the meantime Benish 1936, on the basis of aquarium experiments with two-year old carps, arrived at the conclusion that both low pH and high pH affects the parasite negatively. Further data were provided by Hłond 1963, who observed *I. necatrix* occurring numerously on just hatched carps, causing their death in the spawning ponds, at a water temperature 20–22°C and pH from 7.2 to 7.5. After transferring the just hatched fish to fry ponds the infection decreased after 2–3 weeks. The observations of Hłond 1963 under pond conditions confirm the possibility of the appearance of *I. necatrix* also in an environment with an alkalescent reaction, and thus are in agreement with the data of Benish 1936.

In the studied material attention has been paid to the pH of the water. It was found that in a pond where spawners had wintered the pH was 7.2, whereas in the remaining ponds, and particularly in fry ponds, in carp fingerling ponds and in winter ponds the pH was higher. Furthermore, with the passage of time, in majority of fry ponds the pH indicated a tendency to increase. In the extreme case it approached 10.2 (Table 3). Since simultaneously, as time passed, the number of *I. necatrix* in fry ponds showed a decrease in all the ponds (Fig. 1, Table 2), it is possible to consider the limiting influence of a basic reaction of water upon the investigated parasite, but only above pH 7.5.

Ichthyophthirius multifiliis Fouquet, 1876

I. multifiliis appeared on the skin and gills, sometimes abundantly.

The course of the infection was as follows: *I. multifiliis* appeared in the beginning of June on most of the fishes. In 1963, early stages occurred already on carp fingerlings in spawning ponds. With the passage of time and with an increase of the water temperature the number of parasites slowly increased, and simultaneously they attacked an ever increasing number of carps. At the end of July, just before fishing in the fry ponds, there occurred a strong, abrupt increase in the number of *I. multifiliis*. During this period in three ponds (No. 5g, 11 and 4—see Migała 1970) the

infection attained its maximum. It was followed by the death of single fishes harbouring above 1000 adult parasites. The weight of the fishes during that period was 1.7–2.9 grammes on the average. In two other ponds the number of parasites on a fish reached several hundred. In the remaining ponds the number of parasites was on the average a dozen or more on one fish. It is worth to note the fact of the absence of *I. multifiliis*, even during periods of the greatest intensification of the infection, in ponds fertilized with urea (No. 2 and 10), and furthermore, in 1963, in two commercial ponds fishing slightly earlier, and in 1964 in pond No. 14. Explanation of these phenomena will be discussed in detail in the further part of the paper.

Table 3

Water pH in fry ponds

Date of examining the water samples	Kind of fertilizer									
	Without fertilizer		Super-phosphate		Ammonium carbonate super-phosphate		Ammonium carbonate + super-phosphate		Urea + super-phosphate	
	Number of ponds									
	4	12	3	11	5	13	6	14	2	10
30 May 1963	7.7	7.8	7.5	8.0	7.8	8.0	7.8	8.0	8.1	8.2
7 June 1963	7.6	7.7	7.8	7.5	8.4	8.0	8.3	8.6	9.3	8.5
11 June 1963	7.5	7.6	7.6	7.6	8.8	8.4	9.6	8.3	9.5	**10.0**
18 June 1963	7.8	7.5	7.6	7.8	9.0	7.3	8.9	8.1	7.7	9.5
1 June 1964	7.5	7.7	7.9	7.9	7.8	7.9	8.0	7.9	7.9	7.6
15 June 1964	7.8	8.0	8.0	8.0	7.8	8.2	8.0	7.8	8.1	8.0
30 June 1964	8.0	8.0	8.5	8.3	8.4	8.0	8.4	8.1	**10.2**	8.7

After transferring the fishes from fry ponds to carp fingerling ponds the number of *I. multifiliis* decreased considerably. As the time passed, till autumn, in 1964, they even disappeared.

In winter ponds, during the spring examination of fishes in 1964, a weak reinfection was found. Similarly the spring examination in 1965 also indicated a reinfection, but at a later date — the end of April.

It follows from the performed observations that the occurrence of *I. multifiliis* during the particular years was cyclic to a certain degree. Causing the death of carps it has turned out to be the most dangerous fish parasite in the given set of conditions of pond culture.

Sources of infection

The appearance of *I. multifiliis* in the successive years has most probably been influenced by the infected ichtiofauna of the river Jeziorka. Examinations of coarse

fish from the water supplying ditch indicated the occurrence of this parasite on three species of fishes: *Gasterosteus aculeatus*, *Rutilus rutilus*, and *Alburnus alburnus*. Infected carp spawners could also have been a source of infection in the ponds. It was established before spawning in 1963.

The effect of environmental factors: temperature

The peak of abundance of *I. multifiliis* at the end of June can partly be accounted for by the favourable thermal conditions. The temperature of water during that time was close to the optimum temperature for the parasite (above 20°C — Stchupakow 1954, Bauer 1955).

The concentration of hydrogen ions

As has been mentioned, despite the occurrence of *I. multifiliis* in the majority of ponds — in several reservoirs these protozoa have not been observed. A singularly striking example is provided by ponds fertilized with urea (No. 2 and 10).

Initially it was suspected that the direct reason for not finding *I. multifiliis* in ponds No. 2 and 10 was the presence of urea in the water, since urea is known to possess certain toxic properties. However, aquarium studies conducted separately have not confirmed this assumption, since *I. multifiliis* survived and even multiplied in unfertilized aquaria as well as in aquaria with an addition of urea. During the aquarium experiment it was found, however, that the pH of the water in the aquaria with fertilized was 7.4 to 8.0, whilst in the aquaria without fertilizer it amounted from 7.5 to 7.6, whereas in the fry ponds, in ponds No. 2 and 10, it approached 10.2. In the remaining fry ponds the pH amounted to only 9.6 (Table 3). According to Wagner 1960 *I. multifiliis* may occur only in the range of pH between 6.0 and 10. Perhaps therefore a relatively high pH in the ponds No. 2 and 10 was the cause of the absence of the parasite there.

Biotic factors

Besides the pH factor, some explanation of the lack of *I. multifiliis* on fishes in certain ponds can be sought for in the phenomenon, described by Ahmerov 1960 and Bauer 1955, of the devouring of parasites, being separated from the fish, by predatory forms of zooplankton from the genus *Cyclops*. Ahmerov 1940 noticed that *Cyclops (Macrocyclops) albidus* and *Cyclops strenuus* eat free forms of *I. multifiliis*. Bauer 1955 confirmed this under laboratory conditions for *Cyclops viridis*. On the margin of the problem under consideration, it may be added that protozoa and probably young *I. multifiliis*, constitute food also for *Macrocyclops fuscus* and *M. distinctus* (Monakov 1963). Anyway, certain *Cyclopidae* are so predacious that in aquarium conditions they can even devour just hatched fishes (Lillelund 1967). In ponds fertilized with urea (No. 2 and 10) and in pond No. 14 in 1964 (fry pond) in which *I. multifiliis* did not occur, the highest number of *Mesocyclops leuckarti* Claus was found (Grygierek, unpublished). A certain

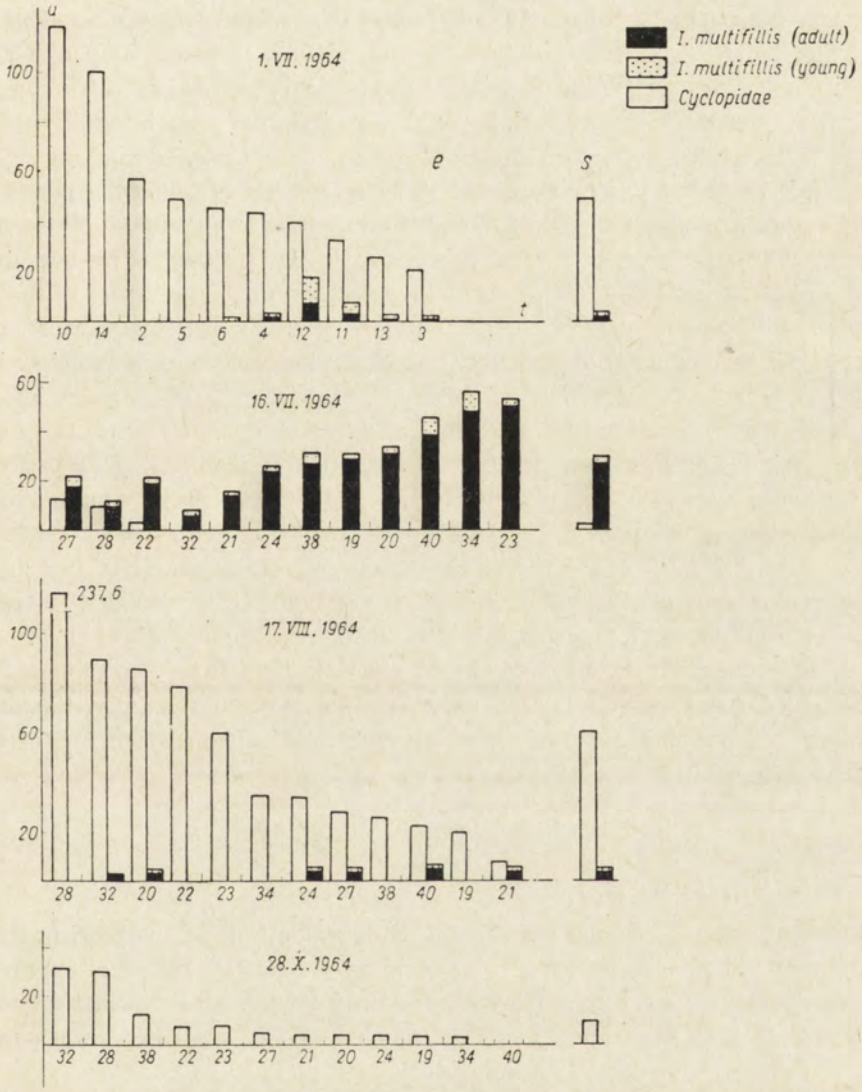

Fig. 2. Occurrence of *Ichthyophthirius multifiliis* in relation to the number of predatory forms of plankton from the group *Cyclopidae*. d — number of *Cyclopidae* per 1 l of water in the pond and the number of *I. multifiliis* per studied host, s — averages for pond groups investigated at the same date, e — ponds aligned according to decreasing numbers of *Cyclopidae*. Numbers denote the numeration of ponds

trend towards the appearance of a lower number of *I. multifiliis* in the presence of a large number of *Cyclopidae* can also be found in other ponds during various periods. This is presented on the example of the year 1964 (Fig. 2). The discussed tendency is emphasized by the mean values. This hypothesis therefore seems quite probable.

From the paper of Bauer 1955 it results that *I. multifiliis* appeared in carp fingerling ponds most numerously at the end of July, and according to Čečina 1960 — in Byelorussia (White Russia) at the beginning of August, at a comparatively highest water temperature. Following this period a fall of the infection occurred. In the U.S.S.R., however, one transfer of fish to another pond during the summer is applied. Most likely, in view of the different periods of filling the ponds with water and of fishing — the ecological spectrum changes to a certain extent and this causes a shift of the peak occurrence of the parasite. On the basis of his own studies on *I. multifiliis*, Čečina 1960 comes to the conclusion that the causes of the drop in the number of this parasite in the autumn lie in the low temperature of the water and in the immunity to secondary infection, thus confirming the previous views of Bauer 1953. In the conditions of Żabieniec, despite a stronger primary infection of fishes in the fry ponds in 1963, the number of *I. multifiliis* in fry ponds was higher, and the reinfection in the spring 1964 stronger than during analogous periods in the following year, with a weaker primary infection. This is rather contradictory to the conclusions of Bauer.

By making a preliminary recapitulation of the considerations of the changes within populations of *I. multifiliis* in time, it is possible to ascertain the existence of factors limiting the number of this parasite which are not a direct effect of the action of temperature and of immunity to superinfection. It may be supposed that in described cases various factors were involved: temperature, pH, liquidation by predators, but only separate investigations could explain which one of them was dominant.

Chilodonella cyprini (Moroff, 1902)

Syn.: *Chilodon cyprini* Moroff, 1902.

In both years *Ch. cyprini* were found at the same time of the year, in spring, on the skin and gills of carps from all the winter ponds. This period of appearance is typical of the studied species (Kraschennikov 1939, Golovkov and Abrosov 1952, Bauer 1959). On some fishes the number of *Ch. cyprini* on the examined part of the body exceeded 1000 individuals.

The action of environmental factors: temperature and light

Admittedly the ecology and biology of *Ch. cyprini* are not well known — nevertheless a number of authors have reached the conclusion that this parasite develops better at low temperatures. Among others, Bauer and Nikolskaya 1957 express the view that at the temperatures of wintering of the fishes, form +1 to +2°C, the process of multiplication of *Ch. cyprini* is still sufficiently intensive to bring about a mass infection. This has been observed in many carp fish farms in the northern climatic zone. According to the mentioned authors the spring increase of temperature has a stimulating effect on the course of the epizooty. In later works, Bauer 1959

indicates an optimum temperature for *Ch. cyprini* from +5 to +10°C. According to Golovkov and Abrosov 1952, at a temperature of the order of +1°C the increase of the number of *Ch. cyprini* takes place at a slow rate, whilst an increase of the temperature up to +3°C and up to +6°C accelerates the process of reproduction.

High temperature, above 20°C, has a limiting effect on *Ch. cyprini*. At this temperature, in laboratory tests, the majority of *Ch. cyprini* died after one hour. It has been shown in the same paper that besides a high temperature, also sunlight is a factor which limits the numerosity of *Ch. cyprini* on fishes in aquaria (Bauer and Nikolskaya 1957).

The presented observations and views have to some extent found confirmation in the studied material. The higher temperature of the winter 1964–1965 and spring 1965, and the smaller number of sunny days during these periods in comparison with the same periods of 1963–1964 (Table 1, Fig. 1) were associated with a higher number of *Ch. cyprini*.

Chilodonella uncinata (Ehrenberg, 1838)

Protozoa belonging to this species were found only once, in 1964, in the number of 6 individuals on the skin of two larvae of carp from the spawning pond.

Ch. uncinata, known as saprobionts, having occurred on the studied fishes sporadically and in an ustable manner, did not constitute a hazard to them. Regarding this, *Ch. uncinata* were treated as an incidental case, hitherto unencountered on carps.

Trichodina pediculus Ehrenberg, 1838

Single specimens of *T. pediculus* were found only once in spring, in winter ponds, on the skin and gills of four carps. In this case these parasites did not constitute a hazard to the fish in the given ecological conditions. In different conditions, e.g. on carps in ponds in the vicinity of Moscow, a gradual year by year increase of the number of *T. pediculus* has been noted, succeeding even the numerously occurring *Trichodina domerguei* f. *acuta* (Ivanova 1966, 1967, 1970).

The occurrence of *T. pediculus* on carps may be attributed to the wide specificity of this protozoan in relation to fishes (Kazubski 1965) and to a broad tolerance to various intensities of environmental factors or their combinations. This was confirmed by the studies of coarse fishes, mentioned in the introduction of the present paper. They have indicated, among others, the presence of *T. pediculus* on the skin and gills of four species of fishes caught in the water supplying ditch (*Rutilus rutilus Leucaspius delineatus*, *Tinca tinca*, *Perca fluviatilis*). It is noteworthy that most frequently (four times) *T. pediculus* were observed on *Rutilus rutilus*.

Trichodina domergueri f. *acuta* Lom, 1961

They occurred on the skin, very rarely on gills.

Fluctuations in the numerosity of *T. domerguei* f. *acuta* to a great extent were in accordance with the logistic curve (Fig. 3). This species began to appear in fry ponds and the number of parasites increased very slowly during the summer period.

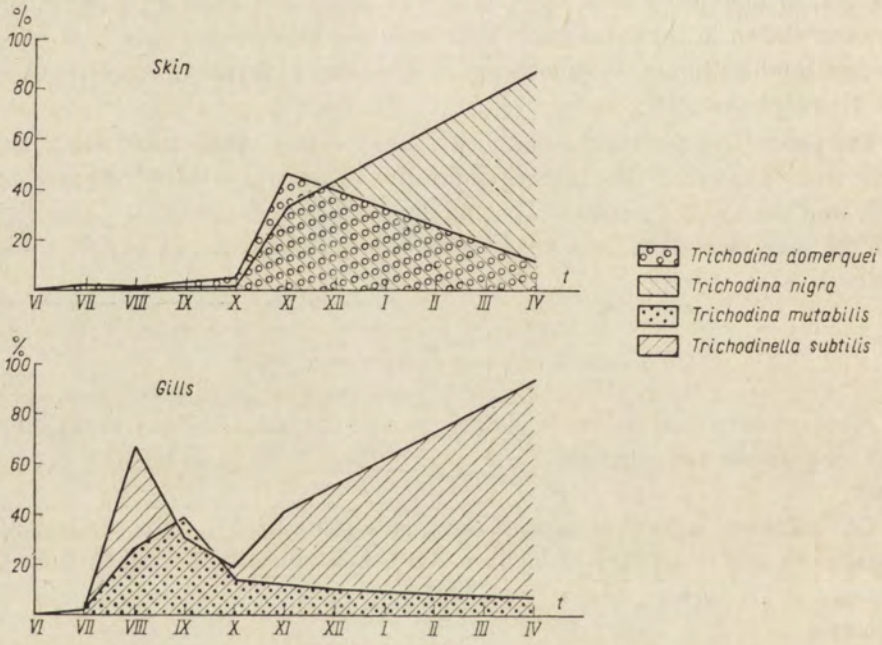

Fig. 3. Comparison of the relative numerosity of *Urceolariidae* in the annual period, computed per one examined fish on the basis of silvered preparations according to Klein. t — time, % — per cent, (as 100% was assumed the highest average number of protozoa from the group *Urceolariidae* on one fish (skin and gills are presented separately)

In early autumn an abrupt increase of their number took place. The peak of abundance was found in October. *T. domerguei* f. *acuta* were found then on all the examined carps, sometimes abundantly. In springtime a rather strong decrease of their number was noted.

Under different conditions, according to Ivanova 1970, the greatest infection of one-year carps by *T. domerguei* f. *acuta* may also take place in spring at water temperature of 1–5°C.

Sources of infection

The initial appearance of *T. domerguei* f. *acuta* on carps in fry ponds was most probably related to roach (*Rutilus rutilus*) infected by these ciliates in the water

supplying ditch. Infection of roaches by *T. domerguei* f. *acuta* have been observed during studies of coarse fish.

T. domerguei f. *acuta* has also been found on the tadpoles of *Rana esculenta* and *Bombina bombina* in fry ponds. The tadpoles here played the role of the medium on which *Trichodina* multiplied. Studies have shown the extensiveness of infection of tadpoles to be 22%. At the same time 11% of the carps were infected. This constitutes a link in the mechanism of penetration of the parasite from the river environment.

In the literature from the period before the introduction of the silvering method for the study of *Urceolariidae* there exist some references by various authors on the occurrence of "*Trichodina domerguei*" on carps. These data probably could be referred to different species which occur on carp. This has made it impossible to utilize these data for interpretation.

Trichodina nigra Lom, 1960

T. nigra occurred on the skin of the examined carps and very rarely on gills, similarly as *T. domerguei* f. *acuta*.

The appearance of *T. nigra* was observed in carp fingerling ponds at the beginning of August. During the months of August and September, the number of parasites increased very slowly. In October there followed a period of rapid growth. Occurrence on all the carps and the peak of abundance was found in the spring (Fig. 3). In two successive springtime periods the number of *T. nigra* was always greater in relation to the number of *T. domerguei* f. *acuta*. The ratio was 2.4 to 1 (1964) and 7.4 to 1 (1965).

Ivanova 1967 also found *Trichodina nigra* on carps in ponds. However, the drawing of *T. nigra* presented by that author showed close resemblance to *Trichodina mutabilis*, and the occurrence of the parasite in the material of Ivanova mainly on gills, suggests that most of them were *Trichodina mutabilis*.

Sources of infection

Since *T. nigra* has been found in five cases on the skin of *Rutilus rutilus* from the water supplying ditch, it may be supposed that this species of fish constituted the source of the infection.

For methodological reasons (Migała 1970), in the further considerations of *Urceolariidae* in this paper, *T. nigra* is considered together with *T. domerguei* f. *acuta*. Both these species are designated by the name "*Trichodina* on skin".

Trichodina mutabilis Kazubski et Migała, 1968

T. mutabilis occurred primarily on gills, very rarely on the skin.

T. mutabilis appeared in fry ponds at the end of June. Shortly afterwards, since from the middle of July, a phase of rapid increase in number has started. The ma-

ximum abundance was found during the second half of August. Following this period, until October, a certain drop in their number took place; in October, at the time of fishing, a renewed slight increase was observed. In the spring the number of *T. mutabilis* decreased; this was particularly visible after the colder winter of 1963–1964. A characteristic feature was the recurrence of the described fluctuations, with small deviations, in both the annual periods (Fig. 1, Table 2).

Ivanova 1970 presents the occurrence of *T. mutabilis* during the warm season of the year from May till October.

Sources of infection

In one case, in the spring, several specimens of *T. mutabilis* has been found on the gills of *Rhodeus sericeus*. Single *T. mutabilis* have been also encountered on carp spawners.

Trichodinella (Foliella) subtilis Lom, 1959

T. subtilis occurred on gills, sometimes very abundantly. Exceptionally it was found on the skin.

T. subtilis appeared in July in carp fingerling ponds, and the numerosity of this species increased rapidly, reaching a certain peak at the beginning of August. At the end of August and in September, a drop occurred in the number of *T. subtilis*. A renewed slight increase was observed during the autumn fishing, and a maximum in the spring (Fig. 3).

It should be mentioned here that Ivanova (1966, 1967, 1970) did not report *Trichodinella subtilis* in her material from carps, instead, she mentioned *T. epizootica* Raabe 1950, without any broader discussion. In view of the comparatively slight morphological differences between both species, this problem merits discussion as well as further studies, which has been already mentioned in the paper of Kazubski and Migała 1968.

Sources of infection

T. subtilis has been found abundantly on carp spawners. Single specimens have been also found on *Carassius carassius* and *Tinca tinca* from the water supplying ditch. Since *T. subtilis* have not been found on just hatched fish in spawning ponds and on the material from fry ponds, despite the abundance of this ciliate on spawners, this reveals a different source of infection in Żabieniec. Most probably coarse fishes constituted such source.

The uselessness of formalin materials in the quantitative studies of *T. subtilis* limited the possibility of wider discussion of this species. Nevertheless, on the basis of silver materials one can find that *T. subtilis* occur abundantly during the warm and cold seasons of the year as well.

Recapitulation of the observations on *Urceolariidae* and discussion

Amongst *Urceolariidae* occurring on carps, two groups inhabiting various habitats have been distinguised: a. species living on the skin, b. species from the gills (Fig. 3). These groups differed in the periods of occurrence. The maximum number of adult and young specimens of *Trichodina* on skin (= *T. domerguei* f. *acuta*+*T. nigra*) was found in the autumn and spring. In the particular months (July, August, September, October, April), the number of postdividers and young individuals of *Trichodina* on skin was as 0 : 2 : 2 : 33 : 29. On the other hand, the maximum quantity of *Urceolariidae* from gills was found in the summer (*T. mutabilis*), or in the summer and spring (*Trichodinella subtilis*). In the respective months mentioned before, the number of young and dividing *T. mutabilis* was as 2 : 6 : 2 : 5 : 1 (the highest in August). According to Sukhanova 1968 the high number of young specimens and adults of the given species of protozoa usually occurs at an optimum temperature for the development of these protozoa.

Since in the development of protozoa the most essential role among the climatic factors is played by the temperature, the performed observations may indicate that *T. domerguei* f. *acuta* and *T. nigra* are rather cool-water species. One may arrive at a similar conclusion on the grounds of the data of Ivanova 1967, 1970, concerning the maximum appearance of dividing and adult forms of *T. domerguei* f. *acuta* in springtime, especially in the vicinity of Moscow. This image in the material of Ivanova 1970 becomes blurred due to unnecessary, joint presentation of the data from fish farms located in the South (Krasnoyarski Land) and in the vicinity of Moscow, and because of disregarding the existence of geographical variation in *Trichodina* (Kazubski 1969, Stein 1969). Junchis 1969 draws attention to the sppearance of *T. nigra* rather during the cool periods, among four month old roach in the Lake Vrevo, indicating that the peaks of intensive multiplication of this species are related to water temperature of 15–16°C. With an increase of the water temperature up to 20°C Junchis found *T. nigra* extremely seldom. In turn, Kulemina 1968, 1969 encountered very great quantities of *T. nigra* on young fishes in the Lake Seliger admittedly in June. However, as indicated by Shulman and Grozdilova, the mean daily temperature of the water in that lake in June does not exceed 15°C. A certain exception in the estimation of *T. nigra* as a cool water species are the views of Kostenko 1969. This author points out that generally in the middle course of the Dnieper River, the representatives of the family *Urceolariidae* are characterized by two seasonal maxima: one in spring, and a weaker one in autumn. For *T. nigra*, however, she indicates maximum occurrence at a temperature 18–22°C. Since Kostenko 1969 does not present the taxonomical features, of the species of protozoa studied by her, the discussion is rendered impossible.

A rather opposite tendency of behaviour than *T. nigra* and *T. domerguei* under given thermal conditions is shown by *T. mutabilis*. It is most probably a warm-

water species, although this feature may undergo certain changes. Also Ivanova 1970 indicates the appearance of *T. mutabilis* during the warm period of the year. In the case of *Trichodinella subtilis* the influence of the temparature in the studied range was imperceptible.

Besides the modal values of the occurrence of *Trichodina* in certain periods, it is necessary to note the finding of a small number of representatives of the investigated species of *Urceolariidae* on carps also during the remaining seasons of the year, both cold as well as warm. This may be explained by the adaptation of ciliates to survive unfavouring periods. These observations also reveal a large thermal tolerance of the studied species of *Urceolariidae* on carps.

Considering the possibility of the existence of other factors, besides the temperature, which affect the abundance of *Trichodina* on skin during the cold period of the year, one can mention also the lower intensity of insolation connected with shorter day in autumn and spring, contributing for example to the growth of *Chilodonella cyprini*. Against such an interpretation, however, is the appearance of *Trichodina* on skin during the summer, despite the long day and intensive sunlight, during which *Chilodonella cyprini* do not occur.

Besides the mentioned factors, probably many others as the level of oxygen and carbon dioxide in the water exert an influence on the development of *Trichodina*. This may be expected on the basis of extensive studies by Noland 1925 conducted on 60 species of free living ciliates. This author demonstrates that the minimum level of oxygen for various species of ciliates varies from 0.0 to 6.8 cm^3 O_2/litre. According to Noland 1925 the oxygen requirements of ciliates are related to the kind of food they take. Oxygen, as an indispensible factor for the breathing of free living ciliates, is reported also by Sukhanova 1968. Hitherto, however, there was a lack of studies on the behaviour of *Trichodina* under different oxygen and CO_2 concentration.

According to Danielewski 1970 the oxygen level in the studied experimental ponds did not fall even during the critical period in the day (measured at 2–5 a.m.) less than 4.8 mg O_2/litre of water in fry ponds and 3.8 mg O_2/litre of water in carp fingerling ponds on the average. During the investigations, however, it was impossible to observe the fact which would indicate a negative effect of the oxygen level upon *Trichodina*.

In the successive periods of fish growth the directions of the variations in the number of particular species of *Urceolariidae* were subjected to very small deviations. Larger deviations took place after the winter season.

Under the studied environmental conditions the infection by urceolarids has not mass character. The characteristic white spots on the skin, described by Haider 1964, have not been observed too, nor the other symptoms of negative influence of these parasites on the health of investigated fishes, even among those most severly attacked.

Apiosoma sp.

Syn.: *Glossatella* sp.

The body is pear-shaped, elongated with distinctly marked massive stalk. Dimensions of the body: length 40–80 μ (average 65.2 μ), width 16–31 μ (average 25.7 μ). The avoid macronucleus is well visible in preparations stained in Giemsa. It is 20–30 μ (average 13.5 μ) long. Oval or bacilliform micronucleus is short and rounded; it is situated beside the Ma. It measures 3.5–9.0 μ (average 6.7 μ) in length, and 1.5–4.0 μ (average 2.5 μ) in width.

The encountered individuals of *Apiosoma* sp. resemble to a great extent *Apiosoma magna* Banina, 1968, in their shape and dimensions of the body, macronucleus and micronucleus.

Individuals of *Apiosoma* sp. occurred on the skin and gills during the whole year. At the same time, however, they showed irregular fluctuations. They were particularly abundant during the fishing period in fry ponds in 1964.

Apart from carps, *Apiosoma* sp. was found also on *Rhodeus sericeus* from the water supplying ditch.

Sessilia indet.

The body of *Sessilia* indet. is calyx-shaped, with a thin stalk. The body length of stained specimens measures 36–60 μ (average 51.6 μ), and width 23–40 μ (average 31.8 μ). The macronucleus has an irregular, ribbon-like shape. Its dimensions are 12–35 μ (average 25 μ) in length, and 5–12 μ (average 7 μ) in width. The micronucleus was invisible in the available preparations.

Sessilia indet. appeared irregularly during the whole year, on the skin and gills of the examined carps. They were abundant in fry ponds.

They show a number of common ecological features with *Apiosoma* sp.: coexistence, both on the skin as well as on gills, and identical periods of appearance and disappearance. Both species show a similar tendency to aggregate.

Sessilia indet. was found also on *Rhodeus sericeus*.

In the further discussion *Sessilia* indet. and *Apiosoma* sp. are necessarily considered as one group *Sessilia*, as mentioned previously by the present author (Migała 1970).

The studies concerning *Sessilia* on fishes are very few in the literature. With a certain insufficiency of taxonomic criteria in this group (Lyubarskaya and Stein 1967) there arose contradictory estimations of its harmfulness to fish farming. For example, some cases of death have been reported among two-months old carps due to mass infection with *Glossatella (= Apiosoma)* (Fijan 1962). Lyubarskaya and Stein stress the view that indeed there are no data dealing with the pathogeness of ciliates of the genus *Glossatella*, but it may be supposed that by settling in huge numbers on the fins, skin and gills of fishes, particularly young ones, they disturb

the normal gas exchange between the fish organism and the external environment. Banina 1969 comes to the conclusion that regardless the lack of a visible relation with the host as concerning feeding. *Apiosoma* are considerably more approximate to parasitic *Infusoria* than to commensals. According to Schäperclaus 1954, *Apiosoma* are not harmful to fishes. Raabe 1964 mentions that *Petritricha–Sessilia* are related to their hosts by feeding links — they benefit from food remnants. On the other hand, Shulman 1962 describes the food of a few *Apiosoma* — it consists of various microorganisms, *Flagellata* and small ciliates.

During my own investigations, no macroscopic pathological changes were found on the skin or gills of carps in the studied material, in spite of mass infection by *Sessilia*, even in 1964.

Some aspects of the occurrence of parasites

Dispersion

During the analysis of the collected material concerning the course of infection by the particular species of parasites, attention was paid to the dispersion of the studied protozoa in space and time.

This problem has been investigated on the basis of the quantitative data from eight selected ponds most frequently investigated (q.v. the scheme of collecting carp samples for quantitative studies, Migała 1970). The possibility of a combined analysis of the results from these ponds was based on the similarity of the occurrence of each species or group of parasites, generally rather independent of the factors intensifying fish production. This shows a possibility of studying the character of the infection, among others, its spread and frequency, on great number of fishes.

A method of construction of tables concerning dispersion (Table 4) is presented on the example of *Ichtyophthirius multifiliis*.

In the process of infection by *I. multifiliis* shown in Table 4 several stages can be distinguished; the initial stage, intensification, achievement of peak, and extinction. Furthermore, during the years 1963–1964, certain special cases occurred which brought about that the infection did not disappeared during autumn. In springtime the infection began again. A similar character of infection, though with certain modifications, was exhibited by the majority of the examined species (Fig. 4). Thus the dispersion of the studied fauna may be presented with some simplification as follows:

a. Initially single parasites appear on single fish (e.g. *I. multifiliis*, Fig. 4 D — 11 June 1963, *Trichodina* on skin, Fig. 4 E — 1 July 1964, *Sessilia*, Fig. 4 F — 16 June 1964).

b. Subsequently the number of infected fishes rises strongly, and the number of protozoa per unit of space they occupy (unit of space = a host specimen) is generally still small (examples: *I. multifiliis*, Fig. 4 D — 21 June 1963, *Trichodina* on skin, Fig. 4 E — 28 Oct. 1964).

Table 4

Numerical distribution of adult *Ichthyophthirius multifiliis* on skin and gills of carps in the successive periods of the year (numbers in the Table denote the frequency of fishes infected by the number of parasites according to class)

Numbers of parasites on the host / Date	0	1–100	101–200	201–300	301–400	401–500	501–600	601–700	701–800	801–900	901–1000	1001–1100	1101–1200	1401–1500
1963–1964														
27 May 63	40													
1 June 63	80													
11 June 63	78	2												
21 June 63	24	52	4											
25 June 63	0	34	9	4	3	2	2	3	1	1			1	
3 July 63	15	52	13											
16 July 63	62	18												
5 Aug. 63	22	58												
20 Aug. 63	74	6												
2 Sept. 63	71	9												
16 Sept. 63	76	4												
1 Oct. 63	74	6												
16 Oct. 63	75	5												
23 June 64	28	22												
1964–1965														
2 June 64	40													
9 June 64	80													
16 June 64	71	9												
23 June 64	59	18	1	2										
1 July 64	34	39	2	2								1	1	1
16 July 64	5	69	5	1										
3 Aug. 64	78	2												
17 Aug. 64	26	54												
1 Sept. 64	50	30												
15 Sept. 64	72	8												
1 Oct. 64	80													
28 Oct. 64	80													
8 June 65	70													
27 June 65	19	21												

c. Under favourable conditions the infection achieves its maximum. Almost all the fishes are infected. The average number of protozoa on a fish increases. The frequency distribution classes fall into a pattern approximate to the Poisson distribution. In the described cases many carps slightly infected have been found, considerably less medium infected ones, and single specimens very strongly infected.

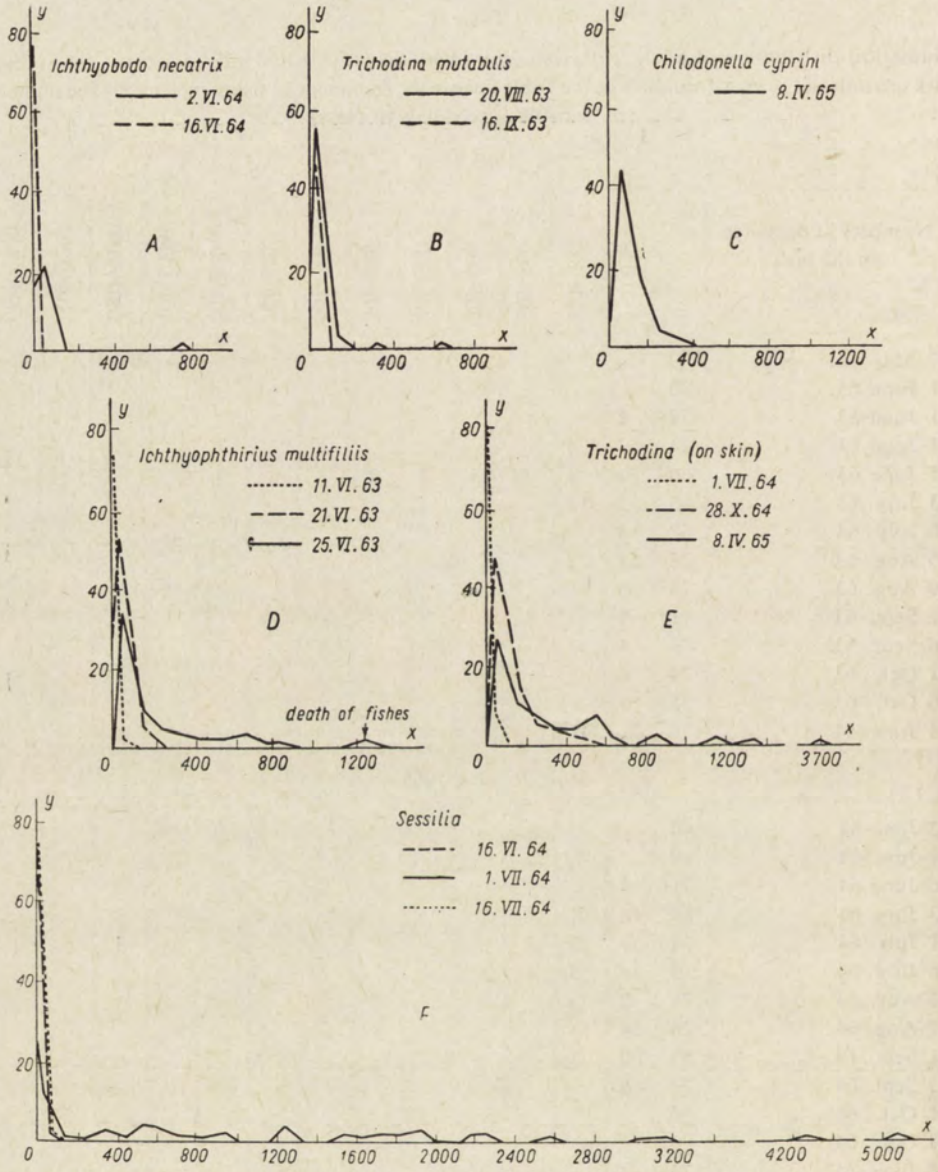

Fig. 4. Occurrence of protozoa on carps in various periods of studies. x — number of parasites per one fish, y — number of fishes (frequency)

During this period the death of fishes may occur due to infection by *I. multifiliis* (Fig. 4 D — 25 June 1963), and the most severely attacked fishes are in the stage of agony.

d. In the following period the strength of infection diminishes. The parasite gradually disappears from the environment (examples: *Ichthyobodo necatrix*,

Fig. 4 A — 16 June 1964, *T. mutabilis*, Fig. 4 B — 16 Sept. 1963, *Sessilia*, Fig. 4 F — 16 July 1964).

In some cases all the phases follow very rapidly one after the other. Rapid multiplication is then observed during a short time (two weeks), and shortly afterwards a strong drop in the number of protozoa, also within a short interval of time (see *Sessilia*, Fig. 4 F). All these observations seem to indicate that infection by the studied parasites has a similar character, regardless of the species.

Insofar as the presented form of the dispersion of parasites in relation to time does not require any special discussion, since generally speaking all populations adhere to it during the entire history of their life (Allee et al. 1958) — however, the causes of the described formation of dispersion in space may be sought for in the tendency of all the studied protozoa to aggregate. This is revealed in the so-called "contagious distribution" with reference to the unit of space which a fish constitutes. This is shown in Fig. 5, and is particularly visible in Fig. 6.

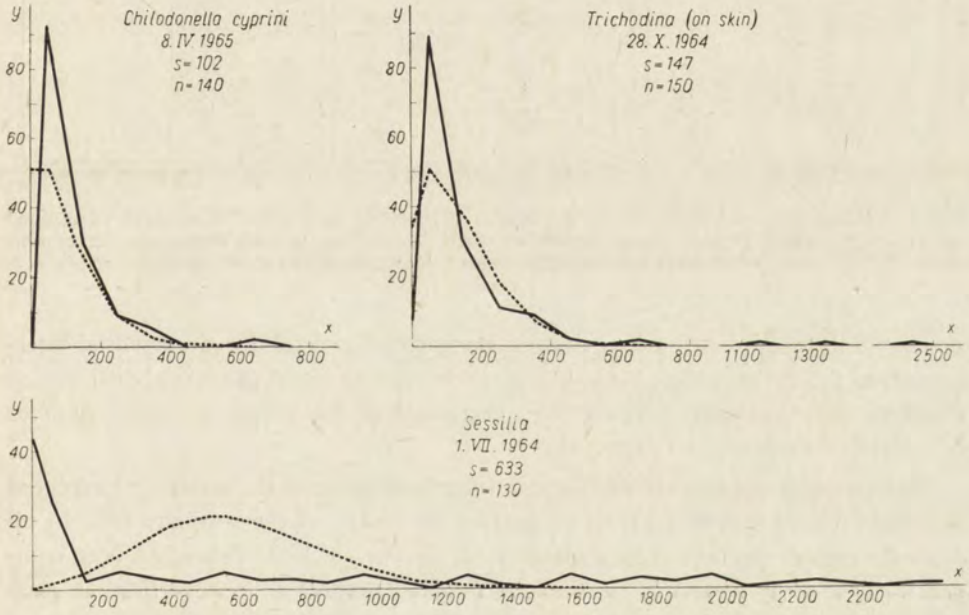

Fig. 5. Comparison of the empirical distribution of individuals in some populations of protozoa with theoretical Poisson distribution indicating their unaccidental (clumped) distribution. x — number of parasites per one fish, y — number of fishes (frequency), n — number of fishes examined at the same date, s — average number of parasites per one fish examined in the given date. ——— actual frequency, ····· expected frequency according to Poisson

As has been mentioned during the description of the model of dispersion in time (the third peak stage), the frequency distribution classes are found in a manner approximate to the Poisson distribution, which according to Bliss 1941 is in agreement with the individuals equally exposed to invasion. However, these classes do

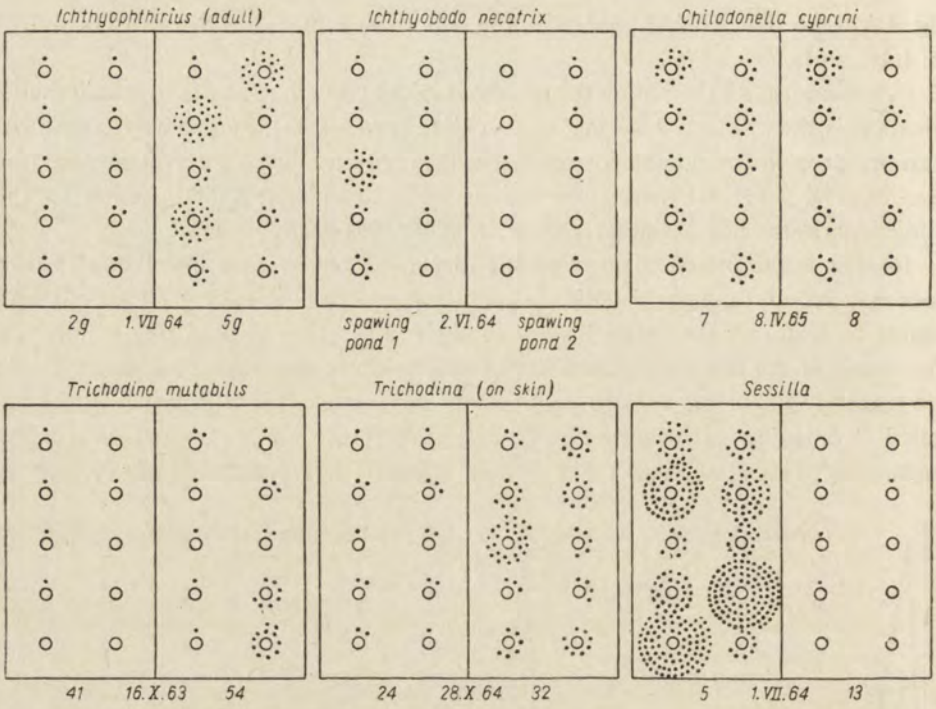

Fig. 6. Selected examples of clumped distribution of protozoa within the variants. o — examined fish, • — multiple of 50 individual parasites on the particular fish. In each variant the numeration is given for both ponds and date of examination

not fit the Poisson distribution, since in every case there follows an essential intersection of the curves according to Poisson with the curves of the actual distribution (Fig. 5), and, particularly in the final fragments of the graph, a greater number of parasites appears than expected.

The unaccidental occurrence of these differences between the actual and expected distribution was indicated by an evaluation by means of the χ-square test. In all cases the period was taken into consideration during which the fishes had been comparatively severely attacked. The highest possible number of observations in each series was taken into account. A likelihood of hypothesis of the compatibility of the theoretical curve and the empirical curve was obtained less than 0.0005. The distribution of parasites is therefore not a Poisson distribution. It may be added that the obtained distributions are not normal or binomial distributions, which makes it impossible to apply the commonly used statistical tests.

The outlined characteristic of the groups of protozoa may be treated as a contribution to the problems of infection. Irrespective of this it appears that the performed observations might have practical significance in the diagnosis of fish diseases caused by *Protozoa*. The first period of appearance (a) for example could be de-

signated at the "carrying period" or "incubation period". As a beginning of the parasitic infection one may assume the moment of transition of the first stage (a) into the second stage (b). The symptom of acute infection would be the third stage (maximum occurrence) in which dead fishes appear. Such a formulation would constitute some progress in the hitherto rather arbitrary determination of the degree of morbidity.

In view of the establishment of a very high dispersion frequency of the number of parasites on the particular fishes within ponds treated equally as a determined variance of carp production (Fig. 6), and in view of the disagreement with known theoretical distributions, appears the problem of the selection of criteria to estimate sources of the quantitative variation of the parasites.

Cyclicity and domination

In the previous sections attention has been drawn to the appearance and disappearance of certain populations of parasites similar during both annual periods of studies. This became a stimulus to seek for this kind of phenomena among the remaining aggregations. These researches are revealed in a compilation of the variations of the numerosity of the particular species (Table 2, Fig. 1). From the present data it follows that a dominating majority of protozoa occur cyclically during the year. This cyclicity consists of the occurrence of a high intensity and incidence of the infection and the achievement of a maximum of development during a given season of the year or in a definite phase of fish culture. It is evident that certain differences may occur between the particular years which, as has been mentioned, may be partly explained by climatic factors.

Besides cyclicity, attention is also merited by the observed phenomenon of domination of the particular species of parasites during certain periods (Fig. 7 a). And thus initially, for a short time, *Flagellata (Ichthyobodo necatrix)* were dominant, and next *Ciliata* (early summer — *Ichthyophthirius multifiliis*, late summer — *Trichodina mutabilis* and *Trichodinella subtilis*, subdominant — *Trichodina* on skin, early autumn — *Trichodina* on skin, subdominant — remaining *Urceolariidae*, spring — *Trichodina* on skin, subdominant — *Chilodonella cyprini*). Amongst the species composing the group *Trichodina* on skin, in the autumn *Trichodina domerguei* f. *acuta* were dominant, whereas in the spring — *Trichodina nigra* (Fig. 3).

A separate discussion is merited by *Sessilia*. This group occurred irregularly in both annual periods of studies, passing from subdominant into dominant or conversely, or also occurring in a relatively small number (Fig. 7 b). A particularly interesting moment in the occurrence of *Sessilia* was found in 1964 during the fishing period in experimental fry ponds. In this period *Sessilia* were abundant. The subdominant turned out to be *I. multifiliis*, whilst *Sessilia* occurred in a few numbers. This phenomenon suggest the existence of a relation of competition or antagonism between these protozoa in the sense of Cross 1938 and Noble 1941; according to them the presence of a large number of parasites of one species on a fish usually

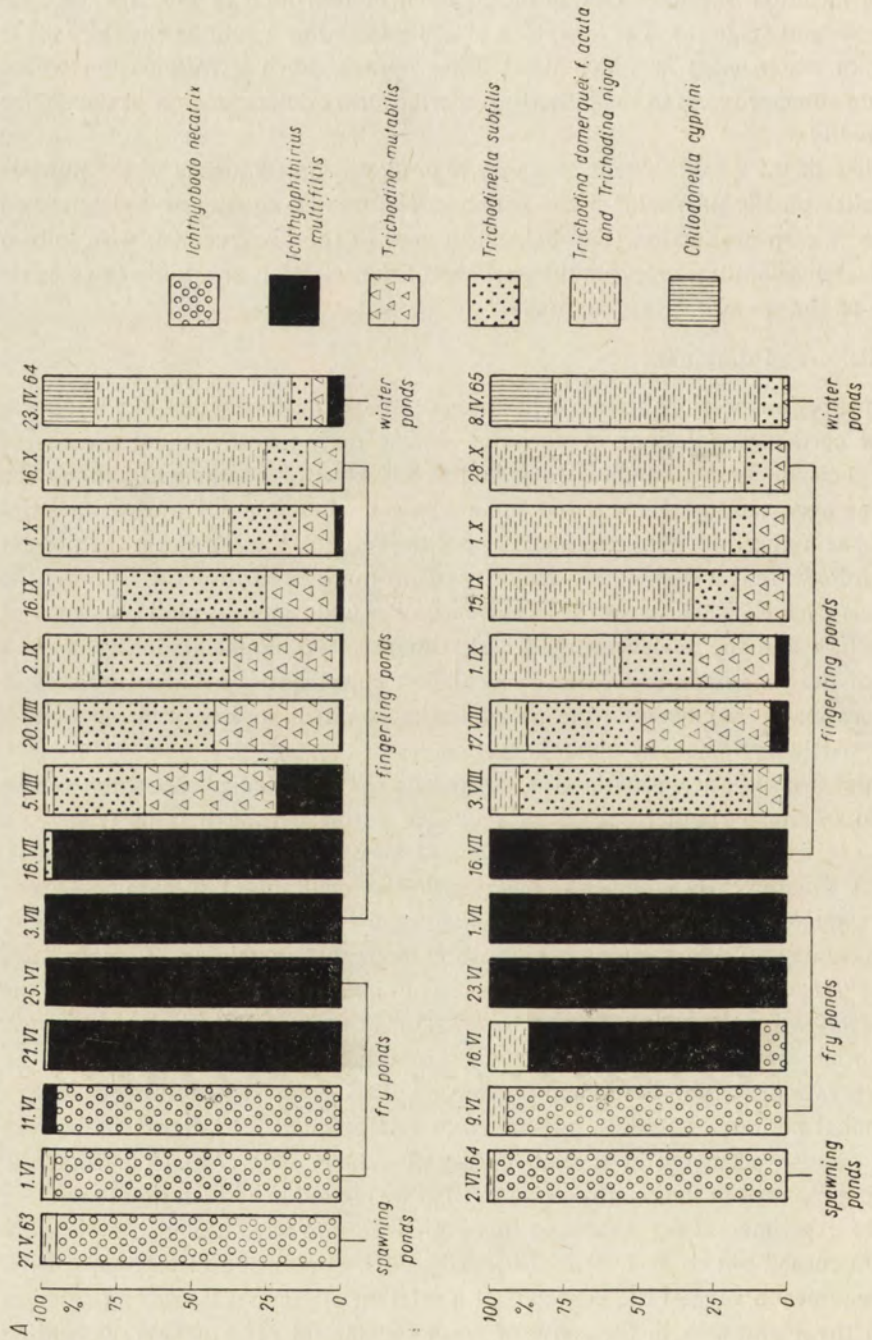

Fig. 7A

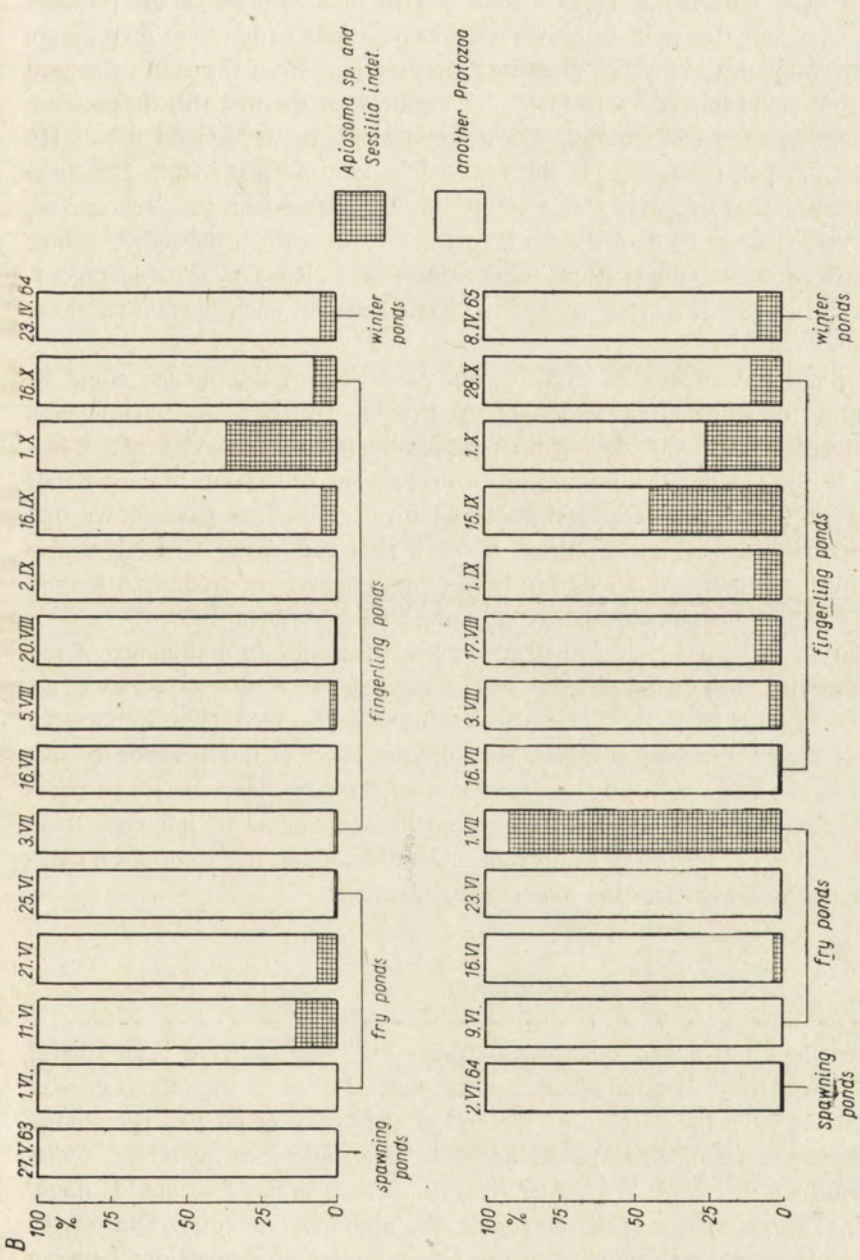

Fig. 7B

Fig. 7. The succession in dominance of parasitic *Protozoa* on carp during one-year periods. A — all *Protozoa* exception *Sessilia* group. B — *Sessilia* versus another *Protozoa*. t — time, % — frequency of specimens of given species in comparison to total number of ones

excludes the presence of a large number of parasites of another species. In the case under discussion this relationship may be, however, very complicated, since *Sessilia* show also a weak tendency towards a reduction of their number in the presence of *Urceolariidae*, and also as if certain competitive relations within their own group. In the latter case detailed studies of stained preparations from the skin of several carps from one pond have demonstrated, for example, in the first fish the presence of 542 specimens of *Sessilia* indet., 273 *Apiosoma* sp., in the second fish — 112 *Sessilia* indet., 327 *Apiosoma* sp., in the next fish — 1920 *Sessilia* indet., 155 *Apiosoma* sp. It seems that one may count among similar phenomena the "succession" of *T. domerguei* f. *acuta* by *T. pediculus* (Ivanova 1970), and furthermore, in lake conditions on perches — the gradual "succession" of *T. nigra* by *T. domerguei* f. *acuta* (Kulemina 1968). Among other investigated species such phenomena have not been observed.

The observed phenomena of cyclicity and variation of dominance could be a manifestation of seasonal succession of the examined species — a phenomenon characteristic of small pools, drying up periodically (Chodorowska and Chodorowski 1958). This is all the more probable that the utilization of carp ponds provides certain features of periodical pools. Closer examinations have shown that succession actually exists (Fig. 7 a). Proof of this is provided by the distinctly visible succession in dominance, similar during both annual periods of studies. Attention was drawn to this phenomenon earlier in a report by Migała 1969.

According to Poljanskij and Shulman 1969, succession in dominance of fish parasites may also exist in lakes.

The observed phenomena of seasonal succession may also be of great importance in combating the parasitic fish diseases. Having knowledge of the direction of succession and of the periodicity of the occurrence of the particular species of parasites may facilitate the anticipation of phenomena and enable to influence these phenomena for the benefit of the fish-breeders. The succession of *Protozoa* on cultivated carps has not been learned hitherto in literature.

Summary

Two-year quantitative and qualitative studies on *Protozoa* have been carried out on 2970 carps from 81 ponds from one fish-farm. Fishes for examination were collected starting from the hatch until the end of the first year of life. The studies indicated the presence of *Ichthyobodo necatrix*, *Ichthyophthirius multifiliis*, *Chilodonella cyprini*, *Ch. uncinata*, *Trichodina pediculus*, *T. domerguei* f. *acuta*, *T. nigra*, *T. mutabilis*, *Trichodinella subtilis*, *Apiosoma* sp. and *Sessilia* indet. During the studies particular attention was paid to the correlations and connections between parasites and the habitat, and to the dispersion of parasites in "space and time". As a result of the investigatison the following final conclusion haves been formu-

lated. Responsible for the system of relations formed between the external parasites from the group *Protozoa* and the hosts are foremost the ecological factors in a broad sense, and thus both abiotic factors (e.g. the temperature of water related to the season of the year, increase of water pH in the ponds) as well as biotic factors (e.g. the presence of infected coarse fishes in the water supplying ditch, of carps in the river, the appearance of large amounts of predatory *Cyclopidae*, tadpoles in ponds). 2. Parasitic protozoa on carp show clumped distributions. This phenomenon can be considered as the cause of a number of "irregularites" and "deviations" in the studies. 3. There exists a possibility of limiting the aboundance of *Ichthyophthirius multifiliis* by means of a biological method, by bringing about an intensive development of predatory forms of zooplankton in the water. 4. The individual species of *Urceolariidae* from carps most probably has a different optimum temperature. 5. Under determined conditions in the fish-farm there exists a seasonal succession of parasitic *Protozoa*, despite the drying of ponds every year.

STRESZCZENIE

Przeprowadzono dwuletnie ilościowe i jakościowe badania pasożytniczych *Protozoa* z 2970 karpi w 81 stawach jednego gospodarstwa. Ryby do badań pobierano począwszy od wylęgu do ukończenia pierwszego roku życia. Badania wykazały obecność *Ichthyobodo necatrix*, *Ichthyophthirius multifiliis*, *Chilodonella cyprini*, *Ch. uncinata*, *Trichodina pediculus*, *T. domerguei* f. *acuta*, *T. nigra*, *T. mutabilis*, *Trichodinella subtilis*, *Apiosoma* sp. i *Sessilia* indet. W badaniach zwrócono szczególną uwagę na współzależności i powiązania między pasożytami i siedliskiem oraz na rozproszenie pasożytów w "przestrzeni" i w czasie. W rezultacie badań sformułowano następujące wnioski końcowe: 1. Za układ stosunków powstałych między pasożytami zewnętrznymi z grupy *Protozoa* i żywicielami (młodymi karpiami) przede wszystkim odpowiedzialne są czynniki ekologiczne w szerokim rozumieniu, a więc zarówno abiotyczne (np. temperatura wody związana z porą roku, wzrost pH wody w stawach) jak i biotyczne (np. obecność w donośniku zarażonego chwastu rybnego, karpi w rzece, pojawianie się w stawach dużej liczby drapieżnych *Cyclopidae*, kijanek żab). 2. Pasożytnicze pierwotniaki na karpiach występują skupiskowo. Zjawisko to uważać można za przyczynę szeregu „nieprawidłowości" i „odchyleń" w badaniach. 3. Istnieje możliwość ograniczenia liczebności *Ichthyophthiris multifiliis* metodą biologiczną, poprzez doprowadzenie do intensywnego rozwoju drapieżnych form zooplanktonu w wodzie. 4. Poszczególne gatunki *Urceolariidae* z karpi posiadają najprawdopodobniej różną temperaturę optymalną. 5. W określonych warunkach obiektu stawowego istnieje sukcesja sezonowa pasożytniczych *Protozoa*, mimo corocznego osuszania stawów.

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Laboratory of Toxoplasmosis of the Gamaleya Institute of Epidemiology and Microbiology of the Academy of Medical Sciences of the U.S.S.R., Moscow, Gamaleya 2, U.S.S.R.

G. T. AKINCHINA and D. N. ZASUKHIN

Intranuclear localization of *Toxoplasma gondii* and *Besnoitia jellisoni* in conditions of tissue culture and some remarks on the intranuclear parasitism

Внутриядерная локализация *Toxoplasma gondii* и *Besnoitia jellisoni* в условиях культур тканей и некоторые замечания по внутриядерному паразитизму

In study on biological and cytological properties of *Toxoplasma gondii* and their interaction with the host cell, carried out on the tissue culture model, the occurrence of the parasite inside the host cell nuclei was observed by one of us years ago (Akinchina 1959). However, this fact has not been discussed because of the scarcity of information. Presently some new results of the electron microscope study support the possibility of intranuclear localization of toxoplasme. Similar results have been gained in the study of tissue culture of *Besnoitia jellisoni*, an obligatory intracellular parasite of the same morphology as *Toxoplasma gondii*.

We failed to find in the literature any defined information on the intranuclear localization of Protozoa in the cell infected by them. However, the literature concerning the occurrence of bacteria and fungi in the nucleus of Protozoa is extensive. A detailed revue of the research on the parasites of Protozoa has been published by Kirby 1941. Averburg 1954 signalized the intranuclear localization of tuberculosis bacillus in the human blood leucocytes, Vulchanov 1955 described the process of phagocytosis of the staphylococci by the nucleus in human blood leukocytes. A number of authors reported the possibility of intranuclear localization of *Rickettsia* (Wolbach 1919, Pinkerton and Hass 1932). The capability of rickettsia of intranuclear reproduction was stated by Hass and Pinkerton 1936 in *Dermacentroxenus conori* and was applied by him for classification of the antigenic strains of rickettsia. According to Zdrodovsky and Golinevič 1953 the intranuclear localization of rickettsia occurs in the scrotal fever forms after an experimental infection in guinea pig. Kokorin and Rybkina 1966 showed by the microphotographic method that rickettsia (*Dermacentroxenus conori*, and *D. sibiricus*) reproduce in the nuclei of tissue culture cells and move actively within them. An extensive

literature exists on the localization of viruses in the nuclei of vertebrate and invertebrate cells.

We found no data in the literature about the presence of *Protozoa* in the cell nuclei. However, a number of publications prove a definite tropoism of some pathogenic Protozoa towards the nucleus (*Karyolysus lacertarium* and other species of this genus: *Leucocytozoon*, *Lankesterella*). In these cases, the parasite is gradually "surrounded" by the nucleus, however not completely.

In the present study the problem is considered of the intranuclear localization of two obligatory intracellular parasites *Toxoplasma gondii* and *Besnoitia jellisoni* — pathogenic parasites — in the tissue culture cells. These parasites possess the full assembly of structures characteristic of the cells of a multicellular organism. We present here the information gained by the light and electron microscopy and discuss it in the light of the above data provided by other microorganisms beside *Protozoa*.

Material and methods are described in our former publications (Akinchina 1963, Goltzen and Akinchina 1967, Akinchina et Doby 1969).

Results

Toxoplasma gondii

Usually toxoplasma are localized in the cytoplasm vacuoles after having penetrated the cell. However their trend to localize near or around the host cell nucleus is characteristic. Single parasites or their accumulations often deform considerably the nucleus, producing invaginations in the place of contact (Pl. I 1). The nucleus is often shifted towards the cell periphery. Despite the distinct changes of the nucleus, the cases of intranuclear localization of toxoplasma occurred rarely and it was not certain if it was not a result of overlapping the nucleus by the parasite. The submicroscopic studies carried out simultaneously, which could resolve this problem, were unsuccessful for a long time. Only in the last years we succeded in finding a support of this fact. It is interesting that the possibility of revealing toxoplasma inside the nucleus of the degenerating host cell increases in the case of a simultaneous infection with toxoplasma and some viruses, especially with the vesicular stomatitis and pox vaccine viruses as well as the virus ECHO-II (Miller and Akinchina 1967). Toxoplasma, in contrast to besnoitia, appear directly in the karyoplasm (Pl. IV 12, 13) without forming a characteristic vacuole in the cytoplasm. The membrane of the infected nucleus has an usual double structure and contains many holes. Sometimes the space between the two nuclear membranes is enlarged in some places or on the whole periphery of the nucleus. The chromatinous structures shift sometimes to the periphery of the nucleus which is generally filled with the electron-transparent material. The structure of the intranuclear toxoplasma does not differ essentially from that of the cytoplasmic ones

(Pl. III 10, 11). However, in the case of a mixed infection with viruses, the membrane of parasite assumes a slightly wavy outline, mitochondria and the nucleolus become hypertrophied. The increase of the electronoptic density is observed in the entire cell of toxoplasma on account of the presence of a great number of free ribosomes.

Besnoitia jellisoni

The study on reproduction of *B. jellisoni* in the cultures of cells of different origin (Akinchina et Doby 1969) demonstrated again the possibility of intranuclear localization of parasite in the infected cell. After having penetrated the cell, besnoitia similarly as toxoplasma, continue development in the host cell cytoplasm in the great majority of cases. They become localized near the nucleus which undergoes significant changes. It often increases in size (giant nuclei), however, the integrity of its envelope is not affected in this case (Pl. I 2). Despite such a typical response of the host nucleus, besnoitia were found in the dividing cells (Pl. II 3). This may be naturally explained by the penetration of besnoitia in the moment of division of the cell nucleus. However, the presence of dividing parasites in the mitotic cell as well as the division of the besnoitia clone into two, does not exclude the possibility of division of the already infected cell. Inside the nucleus of the infected cell (Pl. II 4–9) besnoitia were found much more frequently (sometimes several cells in one preparation) than toxoplasma. If this was a rare and uncertain fact with toxoplasma in the light microscope examination so the localization of besnoitia inside the nucleus, in a distinctly limited vacuole, was an indisputable fact. More complicated is its interpretation. Besnoitia are in a vacuole, their number may vary (1, 2, 4) which indicates an equal possibility of their division inside the nucleus as of penetration of several individuals. The rather rare occurrence of such pictures complicates their study in vitro. However, the extensive study of stained preparations in the course of the invasion development inclines us to accept the hypothesis of karyophagy rather than that of an active penetration of parasites. Possibly those two postulations do not exclude but supplement each other.

Discussion

The data presented above demonstrate for the first time the possibility of intranuclear localization of toxoplasma and besnoitia which usually are the parasites of host cell cytoplasm. The possibility of a simple superposition of the parasite upon the nucleus was excluded after having proved this phenomenon on the submicroscopic level. In the case of intranuclear localization, toxoplasma are revealed directly in the karyoplasm, whereas besnoitia lie in distinctly limited vacuoles.

Toxoplasma and viruses are in some way similar as obligatory intracellular parasites. However, the factor involving the necessity of intracellular life of toxoplasma remains till the present time unknown, in contrast to the data concerning viruses.

In the case of parasitism of microorganisms in the cells, several variations are possible. Sometimes it is accounted for by a mutual advantage, sometimes the parasites produce enzymes which interfere with the host cell metabolism. Many parasites destroy the host cell causing its death. This concerns especially the microorganisms which penetrate the nucleus of the host cell.

This problem has been elucidated in an extensive and interesting way in the review of Kirby 1941 which remains till now the only publication existing in this aspect. Kirby presents his own results and the data of the above authors concerning different questions of this problem, namely the parasitism of microorganisms in the Protozoa cell, especially in its nucleus. So, a group of bacteria exists, parasites of flagellates, which live in the intestine of termites and in amoebae of the *Pelomyxa* genus. Bacteria become localized near the nucleus, surrounding it sometimes. In this case the hypertrophy and gradual decay of the nucleus is observed. Bacteria apparently of the Schizomycetes group were found in the nuclei and in the cytoplasm of free-living *Ciliates* and in *Sporozoa* (Bütschli 1889). The nuclear parasites of ciliates have been studied by many investigators (Engelman 1876, Bütschli 1889, Hafkine 1890, Metschnikoff 1892, Balbiani 1893, Calkins 1904, Bozler 1924 and others). Some authors indicate the possible action of bacterial toxins upon the nucleus (Fiveiskaya 1929). Decomposition of chromatin involves subsequent impairment of the normal metabolism of *Paramecium* controlled by the macronucleus, as well as the mechanical influence of a great number of reproducing bacteria.

Fungi included to the genera *Nucleophaga* and *Sphaerita* (as accepted) were found in the protozoan nuclei, in particular in the intestinal amoebae and in *Trichonympha* (Dangeard 1895, Dogiel 1916, 1917, Epstein 1922, 1940, Zasukhin 1928, 1931, 1934, Brumpt et Lavier 1935 and others).

The protozoa infected with *Sphaerita* often perish especially in the sporulation period of the parasite. In *Euglena* the colour disappears and chromatophores degenerate. Sometimes a slowing down of their flagella movement is observed (Zasukhin 1928). Degenerative changes of the nuclei were observed in *Jodamoeba buetschlii* (Wenrich 1937), in *Entamoeba citelli* (Becker 1926) and in the macronucleus of *Nyctotherus* (Zasukhin 1928).

Nucleophaga were nearly always observed in the nuclei of trophozoites of the human intestinal amoebae. It was postulated that they may impede encystation or cause a quick degeneration of cysts. In the process of vital activity of *Nucleophaga* in the amoeba nuclei (Dangeard 1895) and in *Trichonympha* (Kirby 1932), nuclear chromatin seems to be expended and disappears gradually and the parasite occupies the whole nucleus. The nucleus increases in size, sometimes even by several times. Evidently the parasite gains subsequently its food from the cytoplasm through the nuclear membrane. In some cases the nuclear chromatin is pushed towards periphery or concentrated in the center. Till the moment when parasites have attained their maximal dimensions, the host nucleus may be enlarged the 10–30 times

(Epstein 1922). In spite of the considerable hypertrophy of the nucleus in *E. blattae* (Mercier 1910), the parasite spores enlarge insignificantly. In this case, the parasite growth seems to be limited by a usually thick nuclear membrane. Lavier 1935 observed an increase of size and of activity in the infected *Entamoeba ranarum* and postulated that the hyperactivity stimulated by the reactivity for the penetration of *Nucleophaga* into the nucleus may be a defence reaction of amoeba. *Nucleophaga* occurs evidently in nuclei only, however, in *Amoeba verrucosa* it may penetrate the nucleolus (Dangeard 1895). In all the cases, the protozoan cell with infected nucleus lives till the complete filling with the parasite, and then desintegrates and perishes. No one of the authors cited above has attempted to determine the mechanism of the parasite penetration into the cell nucleus of the protozoan, namely whether it is associated with the active penetration of the parasite or with the phagocytosis of the nucleus. In what conditions is this process possible? Is it indispensable for the vital activity of the parasite or is it associated with some structural reconstructions of the nucleus in the conditions of a pathological process ? In any case, the possible variety in the structure of nuclear membrane should be taken into account as well as its connection with the cytoplasmic structures.

The microcinematographic observations of reproduction in rickettsia of the tick group (Kokorin and Rybkina 1966) in the tissue culture proved for the first time on the living material the possibility of their intranuclear localization. The authors assume that it is connected with the active penetration of the parasite into the nucleus and revealed their active motility and their reproduction within the nucleus. The possibility of phagocytosis by the nucleus has been postulated in the work of Vulchanov 1955, carried out on the study of phagocytosis of leukocytes in the case of infection with staphylococcal infection. The author analysed over thousand preparations observing cocci (most frequently 1–2, rarely 4–5, sometimes 13–14), directly included into the nucleus and surrounded with a light zone resembling food vacuoles with bacteria in the cytoplasm. The phagocyting nucleus is a less frequent phenomenon than phagocytosis usually observed in the cytoplasm and therefore a more precise technique and analysis were necessary. However — in the opinion of Vulchanov — the share of nucleus in the phagocytic process is a manifestation of hyperactivity of phagocyting leukocyte, as it often occurs as a single case of the rise of general reactivity of the organism.

Similar data have been gained independently in this country by Averburg 1954 who observed the tuberculosis bacillus inside the leukocyte nuclei. In this case, bacteria were surrounded with a light zone. It is known that under some pathological conditions, the perinuclear cell space is able to enlarge in some places and to form semilunar vacuoles in this way with a colourless content. This is observed as well in the phase contrast as in electron microscope. Other cellular structures may penetrate into this perinuclear space. It was shown by the phase contrast cinematography that the cytoplasmic vacuoles of phagocyte outside the given cell shift between the cytoplasm and nucleus.

All the above facts induce a postulation that the nuclear membrane produces a quite special zone. Its behaviour cannot be considered as the functioning of a simple limiting membrane. This was supported by the investigations of the permeability of the nuclear membrane. It is well known that toxoplasma and besnoitia reproduce only inside the living host cell. It has been postulated (Norrby and Lycke 1967) that the actively moving toxoplasma may penetrate into the cell disrupting its envelope. After being deprived of their active motility, toxoplasma evidently attach to the cell by means of a secretion of their paired organelles, and penetrate the cell after a certain time. Norrby and Lycke 1967 have associated the activity of penetration with the action of lysozyme or hyaluronidase and perhaps with both factors. The more frequent occurrence of toxoplasma in the nuclei in the cases of a mixed infection with viruses, may be explained in two ways: 1. by the active penetration of the parasite into the nucleus. This is promoted by the lower resistance to injury of the nuclear membrane which is associated with the disturbance of the cell metabolism by 3 agents: 2 viruses and toxoplasm. It should be assumed that the penetration nucleus is also faciliated — to a certain degree — by the secretory function of the paired organelles of the parasite. 2. The intranuclear localization of parasites may be the consequence of the phagocytic action of the host cell nucleus. Presumably for the effective phagocytosis of the cell, its cytoplasm should be weakly gelatinized. This accounts for the absence of such a capability in young cells as well as a comparatively high activeness in old forms being on their way to degeneration. This fact may evidently explain the more frequent occurrence of both toxoplasma and besnoitia in the nuclei of degenerating cells. At the present level of investigations, considering the lack of the vital observations, we are more inclined to accept the hypothesis of karyophagy which is indirectly supported by some microphotograms reflecting the dynamics of this hypothetic process in besnoitia (Pl. II 4–9). However, before we are able to draw decisive conclusions, all those facts should be the subject of further investigations, especially of the observations "in vitro" with the application of microcinematography.

Summary

Light and electron microscope studies have supported our previous hypothesis about the possibility of intranuclear localization of *Toxoplasma* gondii and *Besnoitia* jellisoni, multiplying in conditions of cell culture.

The difference in manner of localization within the nucleus has been revealed: *Toxoplasma* is found directly in the karyoplasm, and *Besnoitia* — in the nuclear vacuole. A possible mechanism of penetration of parasites into the nucleus of the host cell (active penetration or karyophagy) is discussed in the light of data about intranuclear localization and multiplication of some bacteria, fungi, rickettsia and viruses.

РЕЗЮМЕ

Представлены новые данные, полученные в световом и электронном микроскопе и подтверждающие выдвинутое нами ранее предположение о возможности внутриядерной локализации облигатного внутриклеточного паразита *Toxoplasma gondii* в условиях культуры тканей. Аналогичные результаты получены в отношении *Besnoitia jellisoni* — патогенных простейших, морфологически очень сходных с токсоплазмами. Отмечается различие в характере внутриядерной локализации: токсоплазмы обнаружены непосредственно в кариоплазме, бесноитии — в четко ограниченной вакуоли. Подробно обуждается возможный механизм проникновения паразитов в ядро клетки-хозяина (активное проникновение и кариофагия) в свете имеющихся данных о внутриядерной локализации бактерий, грибов, вирусов, риккеттсий.

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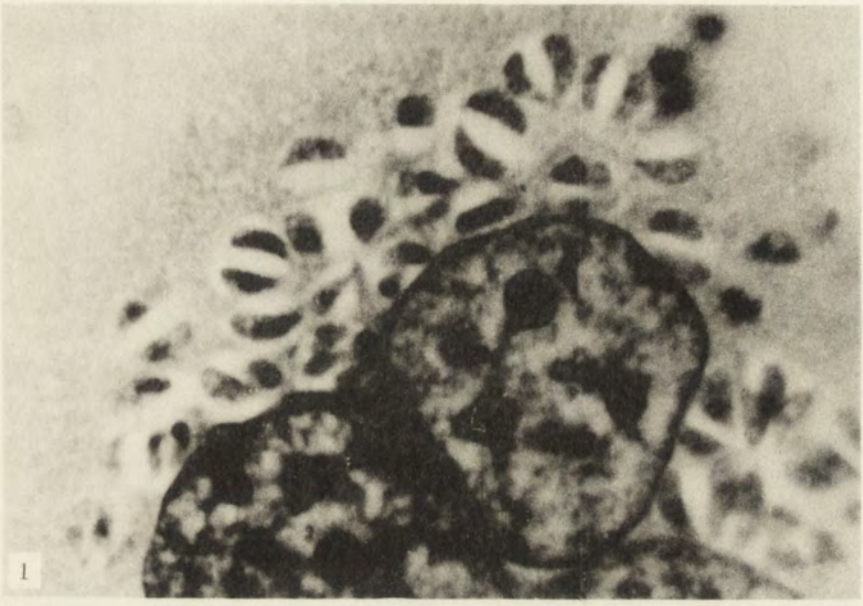
EXPLANATION OF PLATES I–IV

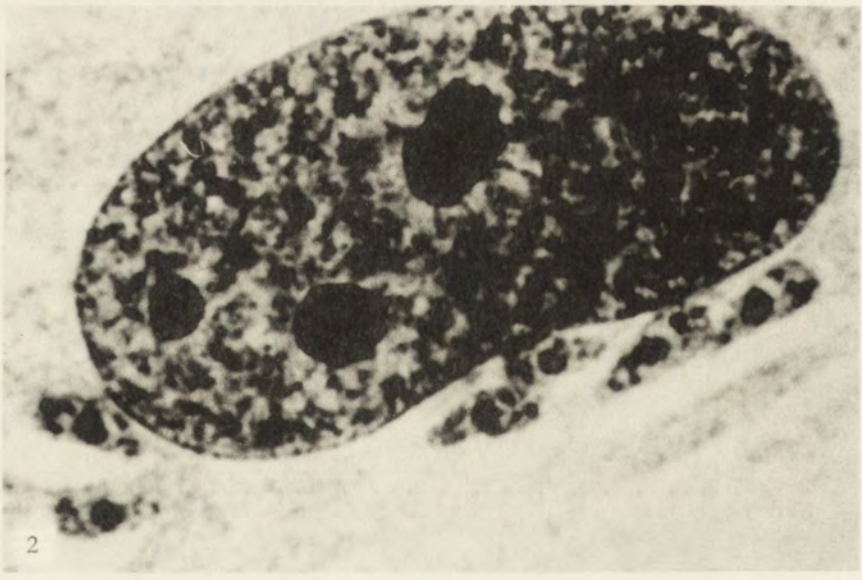
Toxoplasma and *Besnoitia* parasites in chicken embryonic cells. (Romanovsky-Giemsa; ob. ×100, oc. ×10)

1: In the cytoplasm of the cell, close to the nuclear membrane *Toxoplasma* are seen. Note deformed shape of the cell nucleus
2: *Besnoitia* parasitizing in a juxtanuclear position are seen. Note the giantic nucleus of the infected cell
3: Three pairs of *Besnoitia* parasites in mitotic cell
4–9: Some stages of hypothetical process of karyophagy of *Besnoitia* by the nucleus of the infected cell

Ultrathin sections of cells culture (chicken fibroblasts and HeLa cells), infected by *Toxoplasma* and by *Toxoplasma* with some viruses ×18000)

10: Intracellular division of *Toxoplasma* (endodyogenia) in cytoplasmic vacuole in conditions of monoinfection
11: 3 extracellular *Toxoplasma* in the vacuole of the cytoplasm in mixed infection with virus ECHO-II
12–13: In the karyoplasm of nuclei of HeLa cells (mixed infection with viruses) *Toxoplasma* are seen. Note the extreme position of chromatin of the host cell nucleus





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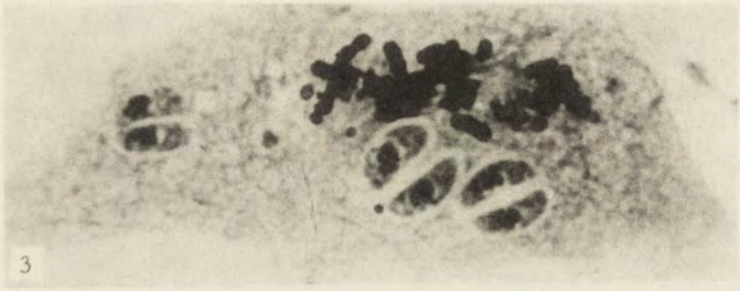

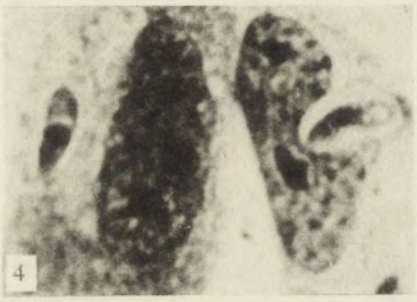

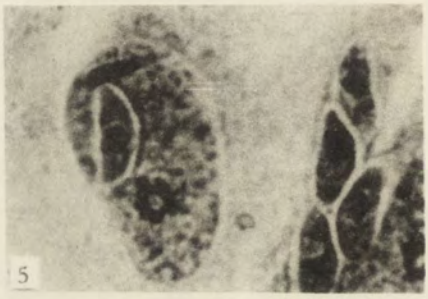

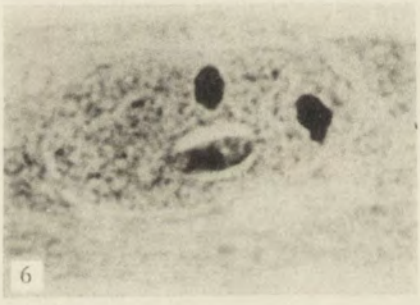

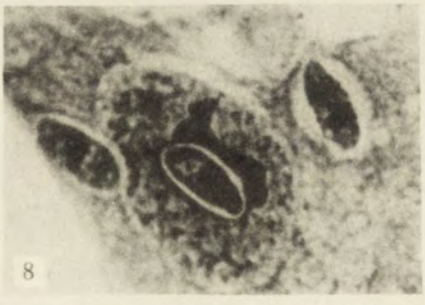

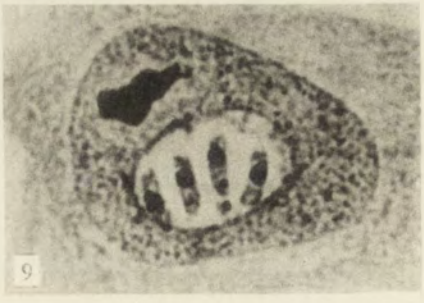

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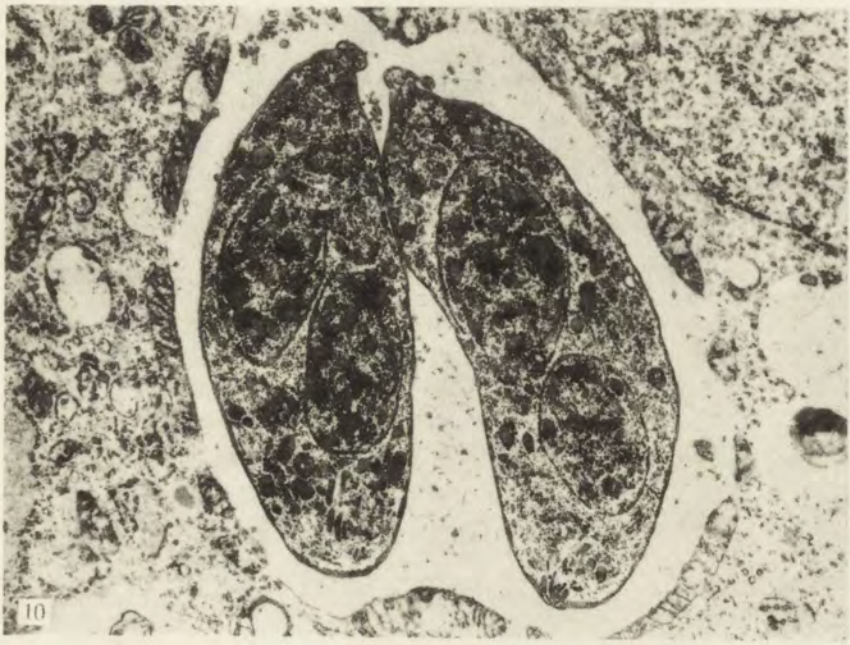

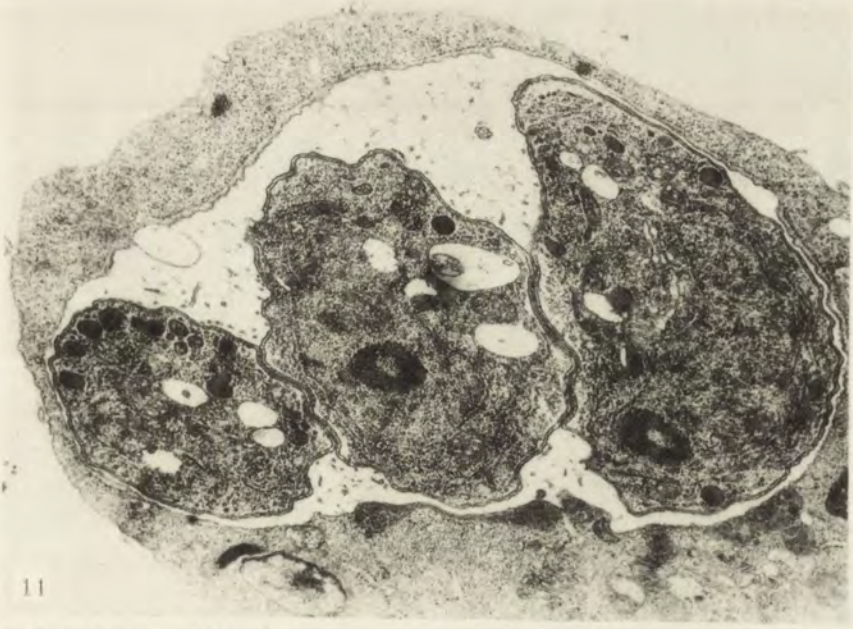

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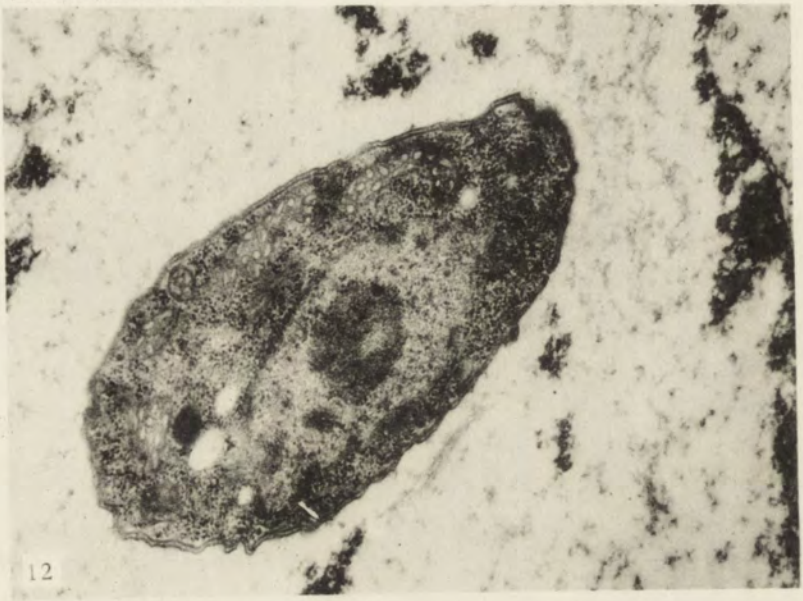

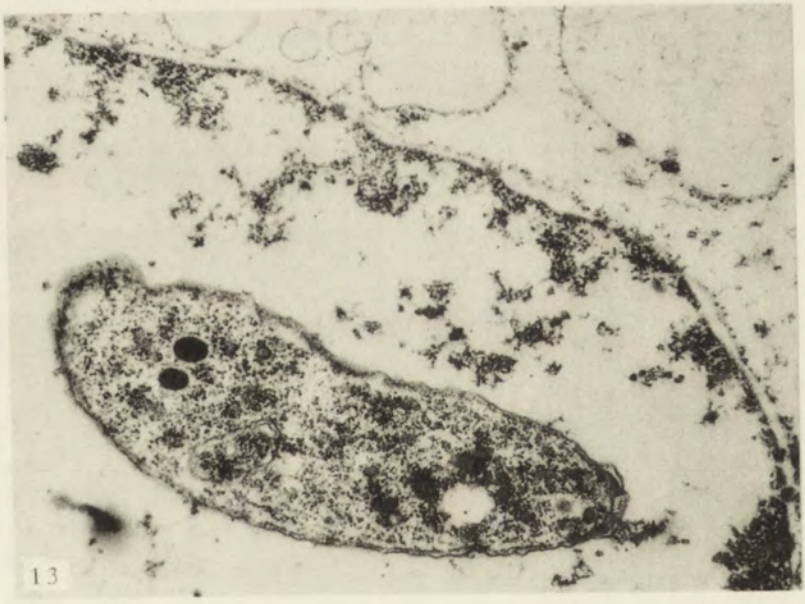

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T. A. SHIBALOVA and A. M. KOROLEV

Cultivation of chicken coccidia in chick embryos. I. The development of *Eimeria tenella*

Культирование кокцидий кур на куриных эмбрионах. I. Развитие *Eimeria tenella*

The *Eimeria* parasites are known to possess not only a narrow host specificity, but also a strict preference to some particular tissues and cells of the host. Of more than 300 species of this genus recorded from birds and animals, where the life histories have been traced, only two species appear to develop in sites other than the intestinal tract. These two, *Eimeria stiedae* and *E. truncata*, occur in the liver of the rabbit and the kidney of the goose, respectively, and are not known to develop at other sites. A strict preference to some particular site of the host body, on the part of the *Eimeria parasites*, has been largely reported.

In view of this all, of great interest is evidence provided recently by experiments, both in vivo and in vitro, showing the growth and development of some *Eimeria* in conditions that strictly differ from their normal environment.

Horton-Smith and Long 1965 showed that the sporozoites of *E. brunetti* and *E. necatrix* injected directly into the caeca of the sensitive fowl were capable of invading the caecal wall and of developing through schizogony and gametogony to the production of viable oocysts. These authors (1966) extended their studies to *E. acervulina*, *E. maxima* and *E. mivati* with positive results obtained for the latter only.

The apparent differences between the above parasites traced at caecal inoculation appeared to be consistent with results of embryo inoculation of the same species (Long 1965, 1966). When injected into the allantois of chick embryos, the sporozoites of *E. brunetti*, *E. mivati* and *E. tenella* (which is known to be essentially a caecal species at all intracellular stages) invade the chorioallantoic membranes and continue to develop there through their life-cycles to the production of oocysts. The sporozoites of *E. necatrix* also invade the chorioallantoic membrane, where they continue the life-cycle up to the second generation schizogony but no gametogony is initiated. No development of *E. acervulina* and *E. maxima* was observed following embryo inoculation. Of negative results appeared to be attempts (Long 1966)

to infect chick and turkey embryos with *E. stiedae* and quail and turkey embryos with *E. tenella*.

In view of great theoretical and practical value of studies like these and due to some puzzling results so far obtained, we decided to carry out experiments with poultry coccidia species, available in our conditions, involving both in vitro cultivation and embryo inoculation. The results of the former have been recently reported (Shibalova 1968, 1969b), whereas the present paper gives the results of attempts to infect embryos with *E. tenella*.

Materials and methods

Pure culture of *E. tenella* oocysts was obtained from a single oocyst inoculation of chickens free of coccidian infection. The oocysts thus obtained were maintained through passages into 14–30 day old chickens (Russkaya Belaya and Cross-288) hatched in the laboratory incubator at the Institute of Poultry Diseases or received from poultry farms of Leningrad district in an age of 1 day. The chickens were kept under sterile conditions precluding coccidial or any other infection.

Oocysts for further experiments were removed from the caeca and immediately treated with sodium hypochlorite (Jackson 1964) to preclude any contamination with intestinal microflora. For sporulation, the oocysts were placed into flasks and provided with continuous aeration system. All the manipulations involving oocyst, sporocyst and sporozoite collection were conducted under sterile conditions.

Excystation of sporulated oocysts

Sporozoites were obtained by excystation of sporulated oocysts using both in vivo (several experiments) and in vitro methods. In the latter case, sporozoites were released by mechanical fracture of the oocysts using technique, similar to that described by Doran and Farr 1961 in our modification (Shibalova 1968). In addition, sporozoites were hatched from intact sporulated oocysts. The oocysts used for this aim were treated first with cysteine, then with carbon dioxide and afterwards were kept at 41°C. Oocysts thus treated were suspended at 41° in the phosphate buffered saline containing 1% trypsin and 10% chick bile (Shibalova 1969a).

The same solution was employed for suspending sporocysts released by mechanical fracture of the oocysts. Excysted sporozoites were concentrated by centrifugation, twice washed in warm (37–39°) phosphate buffered saline before inoculation into embryos.

Embryo inoculation

Russkaya Belaya and Cross-288 chick embryo aged 7–12 days were employed. Embryos were incubated at 37° before inoculation and at 41° after inoculation.

Before inoculation, each embryo underwent ovoscope examination, with strongly viable embryos being chosen for further experiments. Each embryo was given between 10 000 and 250 000 sporozoites into the allantoic cavity. The number of sporozoites was counted in the calculating chamber. The total volume of the fluid injected never exceeded 0.2 ml.

In addition, attempts were made of inoculation of sporulated oocysts or sporocysts into the allantoic cavity of embryos, in doses between 10 000 and 25 000; each inoculum being accompanied with 2000 i.u. of penicillin and streptomycin injection.

The infected embryos were subjected to every day ovoscope examination, chorioallantoic and aminotic membranes and various tissues of these underwent microscopic examination (MBI-3 and MBI-13) every 24 hr. Histological examination involved fixation of the material with the Stiva fluid and 10% formol; paraffin sections were stained with haemotoxylin and eosin.

Microfilming involved phase contrast under MBI-13. In all, 60 embryos served for the *E. tenella* inoculation experiments, with the same number is as controls.

Results

Attempts to induce the development by the introduction of sporulated oocysts or sporocysts of *E. tenella* into the allantoic cavity of embryos were unsuccessful. The in vitro hatched sporozoites appeared to be the only suitable material for this purpose. Doses of sporozoites between 100 000–150 000 and 200 000 gave the best infection. Doses of 10 000–50 000 sporozoites resulted in light infection only, whereas doses over 250 000 sporozoites most readily caused early deaths of embryos with haemorrhage into chorioallantoic membranes. Besides, early embryonic deaths were seen when more than 0.2 ml volume inoculum was injected.

Following embryo inoculation of sporozoites, the development of *E. tenella* was confined to chorioallantoic membrane and allantoic fluid. No parasites were found in other sites into the embryo despite a thorough examination suggesting their absence in fact.

Non-mature schizonts (trophozoites), products of sporozoite development were first seen in the allantoic fluid at 40–44 and 48 hr (Pl. I 1). Mature schizonts, morphologically similar to first-generation schizonts of *E. tenella*, were found from 68–72 to 96 hr, sometimes at 108 hr (Pl. I 2). At 96 and 108 hr, merozoites, similar to first-generation merozoites, were registered in the allantoic fluid (Pl. I 3). Rarely at 120 and more frequently at 144 hr, schizonts, morphologically similar to second-generation schizonts of *E. tenella*, were seen at 144 hr, mature segmented schizonts were observed, sometimes merozoites were seen on their way into the allantoic fluid. Pl. I 3, 4, II 5.

Gamonts, morphologically similar to those of *E. tenella* appeared in the chorioallantoic membranes at 168, 192 and 216 hr (Pl. II 6). At the same time chorioallantoic and allantoic fluid were populated by segmented schizonts very likely corresponding to those of a third generation. However, due to the asynchroneous development of sporozoites and later stages of the life cycle as well as to the infection of embryos with heavy doses, a question of the origin of the first gametocytes that appear remains still open to question. The data observed do not allow to say with certainty if these may be products of a 2nd or a 3rd generation.

Neither did we follow the process of fertilization of macrogametes with microgametes. Nevertheless, the first oocysts appeared already at 168 hr after sporozoite inoculation, their number being increased within 192 and 216 hr (Pl. II 7, 8).

The oocysts obtained following embryo inoculation sporulated normally at 27° or room temperature and when fed to 10–15 day old chickens caused typical patterns of coccidial infection.

Discussion

The results just presented are in good agreement with previous discoveries relevant to the subject (Long 1965, 1966). In our material, neither sporulated oocysts nor sporocysts of *E. tenella* caused the infection, and in all the experiments sporozoites obtained by in vitro technique were employed. *E. tenella* was shown to complete its life-cycle in the chick embryo following the introduction of sporozoites by the allantoic route. The parasites were confined to the chorioallantoic membrane and allantoic fluid and were never seen at other sites. The same is true to other two species, *E. brunetti* and *E. mivati*, known to complete their life-cycles in the chick embryo (Long 1966).

Despite apparent similarity between our findings and previously reported data on *E. tenella*, some curious differences must be stressed. In our material the life cycle of *E. tenella* was not so markedly delayed if compared with P. L. Long's findings. Schizonts, morphologically similar to second-generation schizonts of *E. tenella*, appeared from 5 to 6 days (120–144 hr) after infection in our material, and from 7 to 12 days in P. L. Long's experiments. However, the appearance of oocysts in both cases coincided on the 7th day after infection which is similar to that of the appearance of oocysts in the faeces of infected chickens. It is not unlikely that some strain differences may be involved in this case. Nevertheless, the ability of the parasite (in the Long material) to make up for the lost time within a prepatent period seems rather astonishing.

Experiments involving the parasite's development beyond the host body seem of great scientific value and potentialities. In fact, infection of chick embryos affords the opportunity to study the development of the parasites at a time when host resistance is minimal. In other words, in this respect a chick embryo provides an equal environment for all the *Eimeria* species employed. And therefore the first and most curious thing one is faced with is the difference between these species rather than their likeness.

Morphological studies, involving cytochemistry and electron microscopy, tend to bring different species together which is not stricking in view of an apparent similarity in the pattern of life cycles not only within *Coccidia*, but also within the *Sporozoa* as a whole.

Differences revealed between seven species of chicken coccidia — *Eimeria tenella*, *E. brunetti*, *E. mivati*, *E. necatrix*, *E. acervulina*, *E. maxima*, *E. praecox* (Long 1966, 1967) may primarily involve some biochemical aspects of their life-history. Horton-Smith and Long 1965, 1966 and Long 1966 observed a relation between caecal localization of even a part of the parasite and its embryo development. How-

ever, this must be only formal coincidence. One may suppose that nutritional requirements of the parasites involving a caecal phase in their life-cycle may be easily met in the chick embryo, whereas those of eimerians lacking this phase may not. Needless to say, this is a mere speculation. Of course, to answer this and many other questions some new studies and facts are urgently needed.

Summary

Sporozoites of *Eimeria tenella* inoculated into the chorioallantois of chick embryo aged 7–12 days give rise to the whole endogenous cycle with production of viable oocysts. The rate of development following embryo inoculation differed only slightly from that in natural infection. Schizonts, morphologically similar to 1st generation schizonts of *E. tenella*, were found from 68–72 to 96–108 hr. Second-generation schizonts appeared at 120–144 hr, gamonts and oocysts at 168, 192 and 216 hr. The parasites were confined to chorioallantoic membranes and allantoic fluid only.

No development was obtained following oocyst or sporocyst inoculation of chick embryo.

РЕЗЮМЕ

Спорозоиты *Eimeria tenella*, введенные в хорион-аллантоис 7–12-дневных куриных эмбрионов приводили к развитию полного жизненного цикла вплоть до образования жизнеспособных ооцист. Скорость развития в эмбрионе лишь немного отличалась от таковой при естественном заражении. Шизонты, морфологически сходные с шизонтами 1-ой генерации *E. tenella*, обнаруживались через 68–72–96–108 часов. Шизонты 2-ой генерации появлялись на 120–144 час., гамонты и ооцисты на 168, 192 и 216 час. Развивающиеся паразиты были локализованы на оболочках хорион-аллантоиса и в аллантоисной жидкости.

При заражении куриного эмбриона спорулированными ооцистами или спороцистами развитие паразита не имело места.

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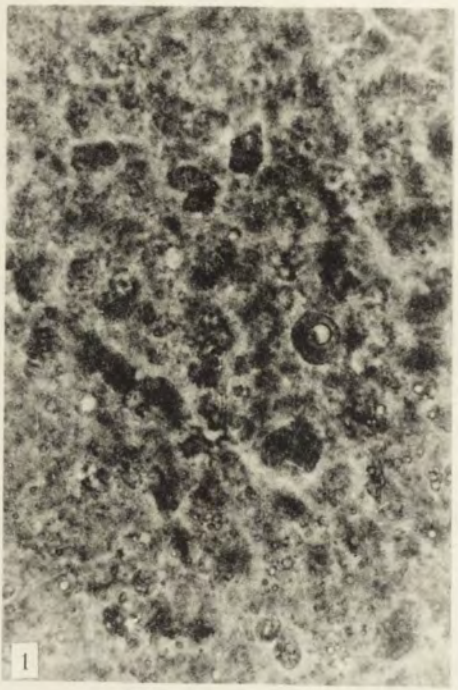
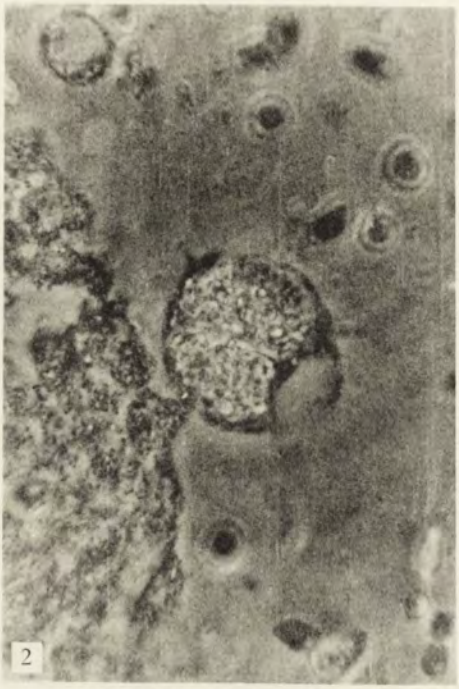
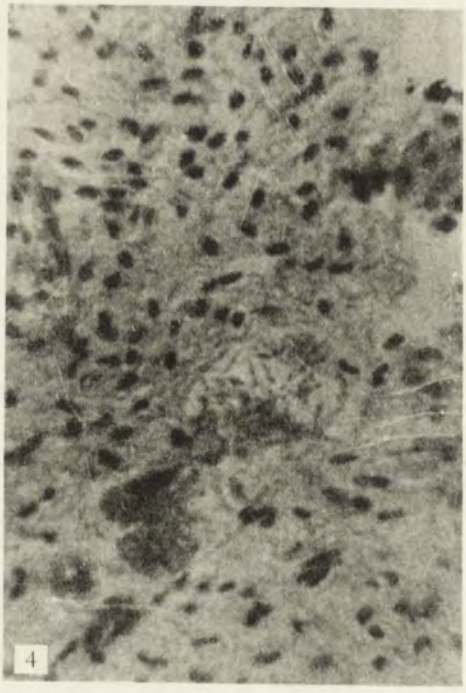
EXPLANATIONS OF PLATES I–II

1: Trophozoites of *Eimeria tenella* in chorioallantois at 44 hr after sporozoite inoculation 1500×
2: First-generation schizont of *E. tenella* in chorioallantois at 96 hr, 1512×
3: Second-generation schizont of *E. tenella* in chorioallantois at 144 hr, 1500×
4: Second-generation schizont of *E. tenella* at 144 hr. Stained by the Bemer haematoxylin and eosin, 1510×
5: Disrupting mature second-generation schizont of *E. tenella* at 144 hr, 1512×
6: Gamatocytes of *E. tenella* in chorioallantois at 192 hr, 1500×
7: Oocyst of *E. tenella* in chorioallantois at 216 hr, 1500×
8: Oocysts of *E. tenella* at 216 hr. Stained with the Bemer haematoxylin and eosin, 1500×







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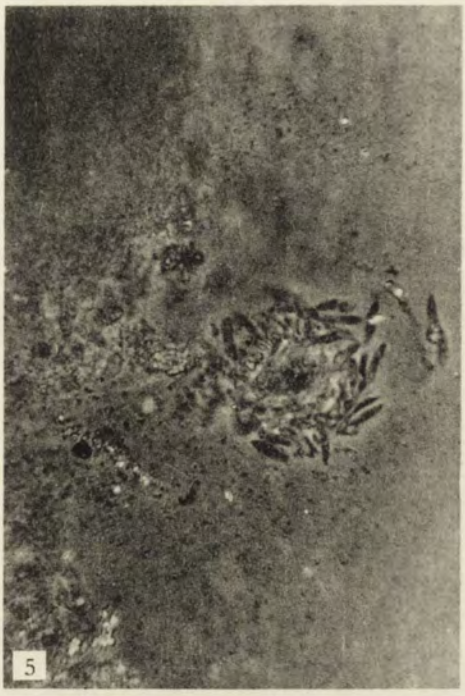

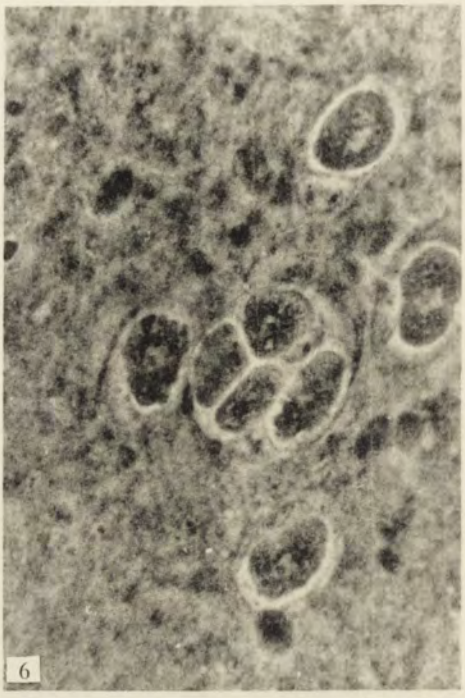

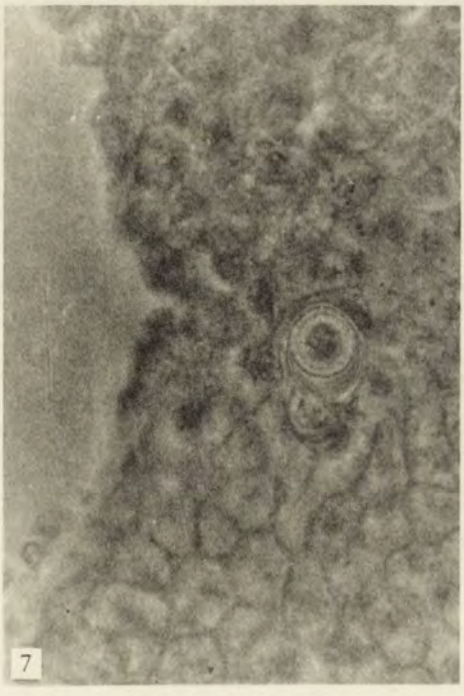

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