Observations on *Lophomonas blattarum*, a flagellate inhabiting the colon of the cockroach, *Blatta orientalis*. [1])

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(With 4 Textfigures and Plates 7—8.)

Contents.

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Introduction.

In contrast with the intestinal flagellates of termites which have recently been quite popular objects of studies by several protozoologists, the flagellate inhabitants of the alimentary canal of the common cockroach have been given a much lesser degree of attention by modern workers. Among many flagellated Protozoa living in the intestine of cockroaches, *Lophomonas blattarum* and *L. striata* have been known to science for some time. As far as I am able to confirm, the last worker on *Lophomonas blattarum* is JANICKI who in 1910 published a detailed account of the organization, multiplication and encystment of this flagellate. He encountered however conditions which he could not observe or explain fully and which consequently await further investigations. During the last fifteen years, not a single paper dealing with either morphological or developmental phase of the flagellate has appeared, although frequent reference was made on it by numerous workers of parasitic flagellates.

Under these circumstances, it seemed highly desirable to undertake anew a study of this somewhat neglected flagellate of the cockroach. In the summer of 1921 I noticed that abundant material was obtainable here at Urbana, so begun its study and have continued it from time to time since that time. The data thus far obtained are summarized in the present paper and presented here in order to bring to light certain points of interest which were not fully understood by previous workers of the protozoon.

Material and methods.

The cockroaches, *Blatta orientalis*, were collected on the University grounds. During the warmer months — from March to November — they come out from the crevices at the base of various buildings in the evening and are to be found in a large number on the walks and lawn. The collected insects were kept in glass jars in which were placed water and various food stuffs and in this way were kept alive throughout the year. In all fourteen hundred cockroaches were examined and findings recorded. The majority of animals examined were adult females but a few males and immature individuals were studied also. The examination revealed that 32 per cent harbored *Lophomonas blattarum*, while 29 per cent contained *L. striata* in the similar habitat. Mixed occurrence of these two species was noticed in 15 per cent of the material. The

voracious females contained a far greater number of flagellates than the males. Since the material was only obtained out-of-doors from March till November and examinations during the winter months were carried on those insects that had been kept in the laboratory for a variable length of time or collected from artificially heated rooms, I have no definite data to determine the seasonal distribution of the protozoon in nature. But as a rule the largest incidence was recognized in July, August and September as was the case with *Endamoeba blattae* (KUDO, 1925). In Rome, JANICKI (1910) found that about 10 per cent of the female cockroaches were infected by *Lophomonas*, that the males were less frequently infected than the females and that ordinarily two species of *Lophomonas* occurred together. YAKIMOFF and MILLER (1922) examined 124 cockroaches at Petrograd, and found that the infection rates of *Lophomonas blattarum* [1]) and *L. striata* were 7. 2 and 9. 6 per cent respectively.

Lophomonas blattarum is found as is also the case with *L. striata*, *Endamoeba blattae* and *Nyctotherus ovalis*, only in the lumen of the host colon, particularly at the anterior portion of the latter. They are also found in its posterior portion as well as in the rectum, though in a small number. The encysted stages are found throughout the hindgut. The small intestine does not harbor it under ordinary circumstances.

For examination of the flagellate the contents of the colon were mixed with a small drop of sterile salt solution in order to dilute the usually thick fluid containing various solid matters found therein. *Lophomonas blattarum* are best observed in from 0.8 to 0.9 per cent salt solutions. This flagellate withstands artificial conditions better than the other Protozoa mentioned above which occur in the same habitat. To retard the flagellar movements, JANICKI employed a weak picric acid which I find also convenient to use. In its place, I have used vaseline successfully. A very thin coat of vaselin is smeared on the slide before the smear is made and in this way numerous active forms were studied satisfactorily with an oil immersion objective for several hours. *Lophomonas blattarum* lives longer in an ordinary slide covered with a vaselined coverglass than in a sealed hanging drop slide, which was also experienced by KOFOID and SWEZY (1915) in their study of trichomonad flagellates.

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[1]) YAKIMOFF and MILLER designated the flagellate probably by mistake as *Lophomonas blattae*.

For fixation Schaudinn's mixture, cold and warm; sublimate alcohol, cold and warm; Flemming's strong solution; the same modified by Gatenby; Bouin's fluid and Osmic acid were used. All the fixatives gave good results, showing the presence of glacial acetic acid does not hinder demonstration of the so-called parabasal apparatus of this flagellate of which mention will be made later. For staining, the usual cytological methods were employed, i. e., Heidenhain's iron haematoxylin, Dobell's alcoholic iron haematein, Giemsa's fluid and Delafield's haematoxylin. JANICKI stated that the last named stain did not bring out various organelles of the protozoon very distinctly. As will be stated later, my experience with this stain was totally different from JANICKI's. Intra vitum staining with janus green B, neutral red and methyl blue was also tried. For observations, compensating binocular lenses were used exclusively, by which various structures both in fresh and stained conditions were made out with certainty, ease and distinctness.

Tho trophic stage.

It seems unnecessary to give a historical review of the papers published by STEIN (1860, 1878), BÜTSCHLI (1878, 1887—1889) and GRASSI (1882) related to *Lophomonas blattarum*, since JANICKI (1910) gave an excellent account of them. I have made a brief remark upon the value of cockroach Protozoa as material for a class in parasitic Protozoology KUDO (1922). YAKIMOFF and MILLER (1922) made a survey of the intestinal fauna of the insect at Petrograd as stated elsewhere, but did not give special attention to any one of the organisms observed. Therefore our knowledge of *Lophomonas blattarum* has made little progress in the last fifteen years.

Form. — In its active trophic stage *Lophomonas blattarum* exhibits somewhat variable appearances. The free swimming individuals are as a rule spherical (Figs. 1, 4, 5) or oval (Figs. 2, 3, 9, 11). The anterior end is slightly flattened or drawn out, in which case the body assumes a pyriform shape (Fig. 3). The posterior extremity is usually broadly rounded, although when the axial structure extends beyond the surface of the body, the latter becomes slightly pointed at that end. When the flagellate stays in one place, its anterior region twists from left to right or up and down due to active movements of the flagella-tuft which is located at that extremity and consequently presents various appearances, while the posterior portion remains unchanged indifferent to this activity. When however

making its way through the masses of detritus present in the preparation, the organism shows remarkable change of form. When going straight through the detritus, it usually maintains a more or less pear shape, drawing out somewhat its anterior end, but when it turns round or encounters obstacles, it can assume almost endlessly manifold forms, interesting to watch. This latter state evidently demonstrates that the general protoplasm of this organism is extremely viscous and there is no specialized peripheral region to hinder the change in form.

The fact that the flagellate has unusual ability of undergoing changes in form, is manifested in Textfigure A which was drawn

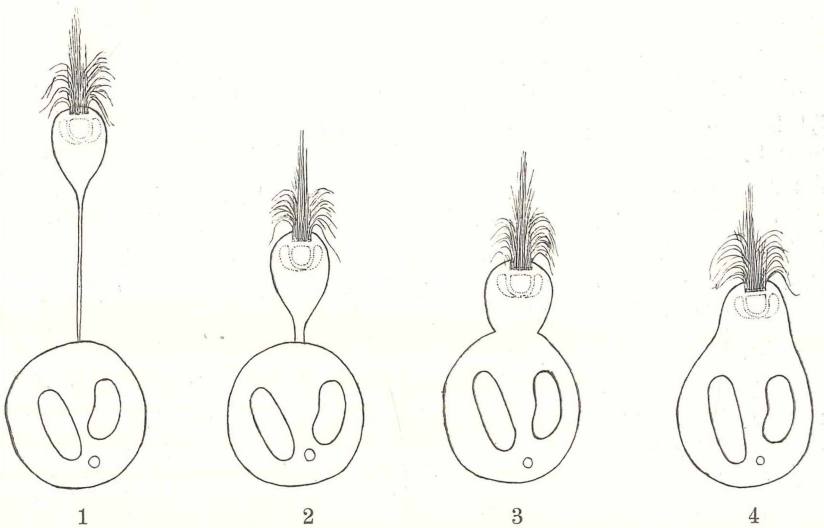

Fig. A. Four outline sketches of an active form of *Lophomonas blattarum*, showing the change of form due to the prolongation of the anterior region, as seen in a living individual.

from a single specimen. When found the larger portion of the body was rounded and from it extended a long „neck" at the end of which was found a small rounded cytoplasmic mass possessing a nucleus and an actively motile flagella-taft (1). The flagellar movements were so highly active that the „neck" bent right and left, up and down continuously. After about three minutes, the „neck" begun to shorten (2), in a minute it disappeared (3) and in another minute the outline of the body assumed a smoothly rounded ordinary pyriform shape (4). I have seen a number of individuals exhibiting such a change. Janicki (1910) noticed a similar change in his material

and supposed therefrom that „doch ist es nicht unwahrscheinlich, daß einmal die schwache, noch übrig bleibende Verbindung reißt, und dann schwimmt die reduzierte Form selbständig von dannen". I have been able to see frequently actual separation of the small nucleate and flagellate portion from the remaining general mass of cytoplasm in living individuals, and followed the former for several hours without noticing any unusual further behavior due to the reduction in the amount of cytoplasm. The remaining anucleate portion which is very often found and easily distinguished in stained preparations (Fig. 20), degenerates sooner or later. As to this pecular reduction of cytoplasm during the nuclear division, I shall describe it later. The significance of this phenomenon is not fully understood by me. It does not result in increase in number of individuals and hence cannot be considered as a multiplication process. It is highly probable that this is merely an accidental decrease in the volume of cytoplasm. As far as I can see, the flagellate does not seem to suffer from the sudden breaking up of the ratio between the volumes of the nucleus and cytoplasm. Under crowded conditions in the host gut the elongated „neck" may easily be cut off from the rest of the body due to the movements of the host body and consequent rapid disturbances of its contents or of nematodes, Nyctotherus, etc., which coexist in the same habitat.

Although larger specimens are as a rule broadly rounded at their posterior end, small individuals possess usually a more or less elongated cytoplasmic projection from the posterior end in which one can see faintly the axial structure. In some cases this projection is quite long and to it are attached many particles (Figs. 5, 7).

Food and method of feeding. — The cytoplasm is homogeneous and shows no special differentiation. Neutral red shows that there is a central, ill-defined area in the cytoplasm which becomes slightly stained reddish. When the flagellates are in active state, they contain numerous food particles of various kinds. The latter consist of, as were noted by former investigators, mainly starch grains, fungi and spores of *Coelosporidium periplanetae* which are ordinarily present in large numbers. Bacilli, spirilla or *Blastocystis* which abound the host's colon are rarely found in them. When smaller flagellates are present in abundance, they are also taken in by *Lophomonas blattarum* as food. JANICKI stated that bacilli were found in food vacuoles, but judging from the fact that in sixty-six figures of *Lophomonas blattarum*, he figured only two individuals in which bacilli were evidently observed, the majority of bacteria while

highly motile should be considered as seldom taken in by the flagellate. It is most probable that this flagellate can take only or at least in most cases immotile objects, although the reason why the *Blastocystis* escape the ingestion by the protozoon is not clear to me. The more active the animal, the greater the number of food particles therein. These food particles are, as were observed by Bütschli and Janicki, located ordinarily in the posterior half of the body. Immediately after the flagellates are removed from the host colon, one sees the majority of them being loaded with various food particles. After being on the slide for some time, the food particles found in the flagellate become however reduced in number, due apparently to their ejection by the organism. In many cases this process was noted as is shown in Figures 6 and 7. The food particles surrounded by a thin coat of cytoplasm leaves the body at the posterior end. As the animal moves forward, this small mass becomes entirely separated from the body and finally left behind.

There is no cytostome in *Lophomonas blattarum*. Stein (1860) thought that there was a small mouth opening at the anterior end where the basal ring of the flagella was broken. Bütschli and Grassi did not recognize such a cell-organ. Janicki holds that an erroneous observation was responsible for Stein's statement. As to the way in which the animal feeds upon solid food particles, Bütschli (1878) wrote that „auch sah ich zuweilen dem Hinterende unserer Tiere äußerlich zahlreiche Körner von ähnlicher Beschaffenheit wie die des Innern ankleben, so daß ich die Vermutung nicht ganz unterdrücken konnte, daß möglicherweise gerade das Hinterende eine Rolle bei der Nahrungsaufnahme spielt, eine Vermutung, die auch darin noch eine Stütze findet, daß das so eigentümlich gebaute Vorderende stets ganz frei von Nahrungseinschlüssen gefunden wird". Janicki (1910) observed more definitely the process of ingestion of food particles by *Lophomonas blattarum* and stated that „das Tier sucht mit seiner seitlichen Körperfläche sich dem Nahrungsobjekt anzuschmiegen, bildet unmittelbar unter diesen letzteren eine kleine Delle aus, und umfließt mit dem freien Körperrand die Beute, alles Vorgänge, die sich mit außerordentlicher Schnelligkeit abspielen". For illustration this author gave a figure of a fixed and stained individual.

With the material from several hundred hosts, I have watched living *Lophomonas blattarum* and succeeded only on a few occasions in convincing myself that I saw the process of feeding on solid particles. The difficulties involved in the present case, are mainly

comparative smallness and great activity of the organism. The active locomotion of the flagellate is remarkable, and when it ceases to move, it is in or round masses of detritus where the body changes in form continuously by the ever active flagellar movements and protoplasmic contraction. The entire body becomes in the mean time covered with onrushing particles due to the flagellar activities. When such an individual comes out into open space, it may carry with it a large number of solid matters (Fig. 5). Not infrequently were seen individuals dragging behind them potato starch grains which were twice as large as their body. I have so far not seen such free swimming forms taking food in, although observations were carried on numerous specimens for frome one to four hours at a time.

The actual taking in of the food particles has only been noticed in animals located in or round masses of detritus and without much change in location. In such a situation, if the detritus is thin and the circumstance favorable enough to allow observation, one can see that the food particle is first firmly attached to posterior surface of the protozoon as if it was glued (Textfig. B, 1), that the

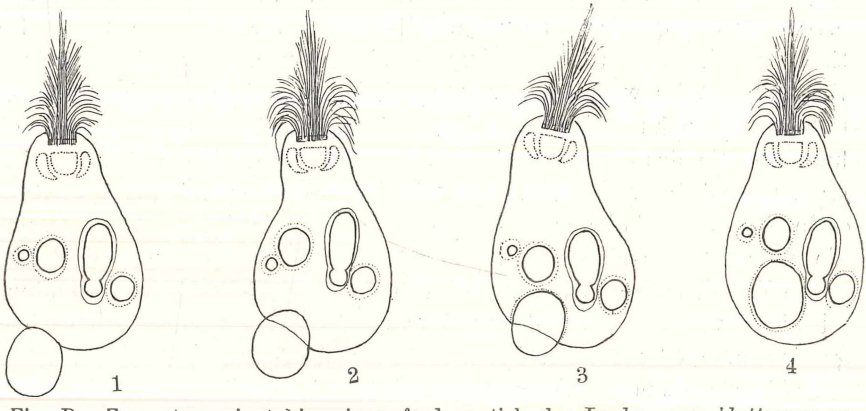

Fig. B. Four stages in taking in a food particle by *Lophomonas blattarum* as sketched from in a living individual.

cytoplasm is drawn in at the point of contact with the solid matter (2), which depression becomes deeper (3) and that finally it is completely included inside the body (4). The process here figured took approximately five minutes. How the solid matter is held so strong by the animal, is not clear. In stained smears, stages in the process of feeding are often met with (Figs. 11—13, 30, 48). In many cases the part of cytoplasm which partly surrounds the food

particle is marked with a deep coloration (Figs. 11, 30). I interprete this as due to either secretion of viscous substances by or to a temporary specialization of the cytoplasm in that region. Thus aside from the absence of myonemes and the range of body surface through which food matters are taken in in the present flagellate, the method of feeding is somewhat similar to that of *Trichonympha campanula* of termites as described recently by CLEVELAND (1925). The long protoplasmic projection such as shown in Figures 6 and 7 may suggest that a pseudopodial method occurs also; buth in both cases the structure was left behind the animal when the latter moved forward.

Structure. — A tuft of flagella is always located at the anterior extremity. These flagella are arranged so close to one another and so large in number that to determine their exact number is a matter of great difficulty. JANICKI (1910) suggested that they number over fifty. I believe there are more than sixty in normal active stage. These flagella are of unequal length with a definite arrangement as were noted by BÜTSCHLI and JANICKI and arise apparently from a ring of blepharoplasts which is regularly broken at one place (Figs. 14, 17, 18, 21), conditions recognized by STEIN, BÜTSCHLI and JANICKI. Careful examinations of slightly compressed living individuals with an oil immersion objective show however that each flagellum extends further down below the blepharoplast (Fig. 8) and becomes fine, although the posterior end is hard to make out. In stained specimens, one can frequently notice that this fine intracytoplasmic extension of the flagellum which I may call axial filament extends further down. The axial filaments unite into one bundle to form the so-called axostyle of JANICKI (Textfig. C, Figs. 10, 21, 24, 25, 34). This fibrillar bundle seems to be of same optical character as the cytoplasm and consequently cannot be observed in living state even with an oil immersion objective. When its posterior tip protrudes through the body surface, it may however be seen comparatively easily as noted by JANICKI. When seen in life it is straight, but it appears to be quite flexible viewed from the findings in the smears (Figs. 13, 16, 21, 25). JANICKI (1910) apparently observed this fibrillar nature of the structure, but could not interprete reasonably, stating that during the nuclear division when the nucleus is not located in its normal place, „beobachtet man vielfach, aber nicht immer, daß ein Bündel von zentral verlaufenden Fibrillen des Achsenstabes sich geradlinig an dem Kelchrand nach vorn fortsetzt und an der Stelle, wo die unteren Basal-

körner in ihrer kreisförmigen Anordnungslinie die Lücke frei lassen, in einer nicht näher analysierbaren Weise endet". This observation is faulty, but he seems to have seen the fibrillar character of the axial structure which he called axostyle, since he wrote that „dieses axiale Gebilde, an dem man bei geeigneten Präparaten längsfibrilläre Struktur erkennen kann, ist biegsam, aber, wie es auch GRASSI und A. FOÀ (1904) für *Joenia* hervorheben, wahrscheinlich nicht elastisch". This bundle of axial filaments stains deeply with various stains including Delafield's haematoxylin which according to JANICKI did not stain the structure, and is a structure quite different from the so-called axostyle found in trichomonad flagellates. JANICKI (1911, 1912, 1915) described a number of interesting flagellates of termites. In *Stephanonympha silvestrii*, he observed that one to three flagella grew from each of the numerous scattered blepharoplasts from which an „Achsenfaden" run through the center to the posterior margin of the body. Each animal possessed thus „ein stattliches Achsenfadenbündel". In *Calonympha grassii*, he (1915) further saw that „aus einem jeden Blepharoblasten entspringt nach Innen ein feiner, mit Eisen-Hämatoxylin gut darstellbarer Achsenfaden; in einem Bogen begibt er sich gegen die Körpermitte, wo die Gesamtheit der Fäden zu einem mächtigen, lockeren, geradlinig verlaufenden und über die hintere Körperbegrenzung nicht hinausragenden Achsenfadenbündel zusammentritt". Thus it appears that the structure here mentioned in these two species of termite flagellates are homologous — and most likely analogous — structure with the compact bundle of axial filaments found in *Lophomonas blattarum*.

JANICKI (1910) was of the opinion that each flagellum had two unequal basal granules, of which proximal granule was large, but hard to see in vivo, while the distal one was not „immer sichtbar zu machen" and was „feinsten punktförmigen Gebilden". My observations do not agree with JANICKI on this point. There is only one somewhat elongated blepharoplast for each flagellum, which can be made out distinctly in living material without any treatment (Fig. 8). The distal granule seen by JANICKI seems to be in all probability none other than the point where the flagellum leaves the anterior margin of the body. On the flagella that are attached to the surface of slide these points present a granuler appearance due to imperfect washing off of dyes at these particular points. That this view seems to be reasonably true is shown by focusing upon the upper half of the flagella taft which is not in direct contact with the slide where these granules have never been found.

Janicki states that Delafield's haematoxylin does not stain the blepharoplasts, but I find it stains the flagella as well as blepharoplasts fairly distinctly though not so clean-cut as those stained by Heidenhain's iron haematoxylin. But carefully controlled decolorization gave several good preparations with Delafield's haematoxylin.

The anterior end of the bundle of axial filaments forms a sort of a funnel to which Janicki gave the name Calyx („Kelch"). As was stated before, this calyx is formed by the axial filaments and shows typically a longitudinal gap which is in a line with the broken space of the blepharoplast-ring (Textfig. C, Figs. 21, 24, 25, 34). The clear zone roundthe calyx which Janicki thought always present, is not to be seen in living individuals (Figs. 1—8), but is observed in some fixed specimens, which state is without doubt due to the shrinkage of the general mass of cytoplasm caused by fixation (Figs. 9—12). When a slightly compressed active form is examined with an oil immersion objective, one frequently sees the rotatory movements of the calyx with its nucleus, due to flagellar movements, while the rest of the body shows only contraction.

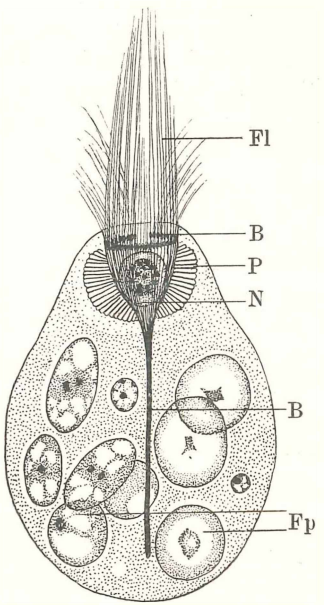

Fig. C. A schematic drawing of a stained *Lophomonas blattarum*. *A*, the bundle of axial filaments; *B*, ring of blepharoplasts; *Fl*, flagellata tuft; *Fp*, food particles; *N*, nucleus; *P*, parabasal apparatus.

A nucleus is located within the calyx (Textfig. C). In living specimen it appears as a homogeneous structure found just below the ring of blepharoplasts. In most cases it is semispherical in form, the flattened surface facing the anterior end (Figs. 1—3, 5, 6, 8), but it may be of different forms such as triangular (Fig. 4), oblong (Fig. 7), etc. Under an oil immersion objective it is of somewhat different optical texture compared with the general cytoplasm. When stained the nucleus shows a distinct membrane. In the resting nucleus, there is a narrow clear peripheral zone of nuclear sap and a central more or less compact mass composed of both chromatin and achromatin elements, the former appearing in granular form. In thinly made smears one can see numerous radiating achromatic threads running between

the membrane and the central mass (Fig. 9). As a rule, there are no chromatin granules attached to the nuclear membrane, but occasionally one or two chromatin granules may be seen located directly under the nuclear membrane (Fig. 19). As JANICKI noted a portion of the chromatic substances becomes condensed into usually one or sometimes two large bodies (Figs. 49—52). These latter may be spherical, oval or irregularly rod-like and are separated from the central mass by an achromatic zone. At present I do not understand their significance, although JANICKI supposed them to be „Stoffwechselumsatz des Kernes".

Around the calyx there occurs a specialized protoplasm which is distinctly visible in living specimens (Figs. 1—8). A high power objective reveals that this structure is composed of rod-like minute elements which are arranged radially round the calyx (Fig. 8). BÜTSCHLI (1887—1889) recognized it. JANICKI first called it a "collare" (1910) as composed of „einer großen Menge von feinen, dicht aneinander gedrängten, radial angeordneten Stäbchen", but later called it parabasal apparatus (1911). Of the so-called parabasal apparatus occurring in different genera of flagellates, which JANICKI (1911, 1912, 1915) attempted to homologize, I believe the present flagellate possesses one which is greatly different from others with a probable exception of that of *Joenia annectens*. In coining the term, JANICKI (1911) wrote that „als Parabasalapparat bezeichne ich ein zuerst von GRASSI bei *Joenia annectens* unter dem Namen Collare beschriebenes Organell, welches nach Untersuchungen von GRASSI, A. FOÁ sowie den meinigen bei parasitischen Flagellaten weite Verbreitung und recht mannigfache Ausbildung findet". More recent investigators report findings of a structure in different groups of flagellates which they termed parabasal body and which apparently seems to be entirely different from that of *Joenia annectens*. The parabasal apparatus of *Joenia* is composed of „Stäbchen- bzw. blättchenförmigen Parabasalia" and is somewhat similar to the structure found in *Lophomonas blattarum*. But its relations to the nucleus and to the axial structure are entirely different. In *Lophomonas blattarum* these rod-like structures are in direct contact with and centered around the calyx. Contrary to JANICKI's observation, I find the structure is lacking along the gap of the calyx (Fig. 15) of which mention was made before. That the parabasal apparatus is different chemically from other organelles is easily demonstrated by staining with Giemsa's mixture. With this stain this structure is bluish, while the nucleus, blepharoplasts, flagella and bundle of

axial filaments take various reddish colorations. The presence of glacial acetic acid in the fixatives such as Schaudinn's, Bouin's or Flemming's fluid, does not obscure this structure in smears. This is not the case, according to some authors, for instances, CUTLER (1919) on *Ditrichomonas termitis* and WENRICH (1921) on *Trichomonas muris*, with the so-called parabasal body in other flagellates which seems to be different from the parabasal apparatus of *Lophomonas blattarum* in form, structure, location and probably in function. In discord with JANICKI, I find that Delafield's haematoxylin stains the structure in my material.

Concerning the nature of the so-called parabasal apparatus found in various genera of flagellates, many suggestions have been published, none of which seems to be based upon evidences. With reference to the parabasal body of *Devescovina striata*, a termite flagellate, JANICKI (1911) writes that „die Anordnung des spiralförmigen Parabasale um den Achsenstab von Devescovina erinnert unwillkürlich an den aus Mitochondrien entstehenden Spiralfaden am Mittelstück vieler Spermatozoen". This suggestion does not apply to the parabasal apparatus of *Lophomonas blattarum* since it gives negative results with intra vitam stains or with Mann-Kopsch method. On the other hand, I agree with JANICKI (1915) in holding the parabasal apparatus of the present flagellate as protective organelle for the nucleus. It is interesting to note here that another species of the genus, *Lophomonas striata* which occurs in the same habitat, is covered with tough filamentous ectoplasm and is of definite form, does not possess any structure which can be compared with the parabasal apparatus of *L. blattarum* here described. In the latter form which is highly capable of undergoing very active changes in form and location amid the contents of the host colon, the nucleus seems to be fairly well protected by the parabasal apparatus. Semewhat similar structure was figured in the „männlicher Geschlechtskern" of *Didinium nasutum* by PRANDTL (1906). There seems to be a closer resemblance between the structure under consideration and the "radiation cytoplasmique" around the flagellum-bearing nucleus of *Mastigina hylae* observed by COLLIN (1913).

Size. Active forms of *Lophomonas blattarum* vary considerably in size. In fresh state they vary from 16 to 30 μ in largest dimeter which runs usually in antero-posterior direction. Mingled with normal active forms with a variable number of food particles, one always finds extremely small individuals highly active with a long projecting protoplasmic thread. These are most probably formed

by the separation of the nucleate and flagellate portion from the rest of the body as described elsewhere in this paper. They do not exceed 8 or 10 μ in diameter, excluding the posterior projection.

Multiplication. Although I succeeded in following the nuclear division in living *Endamoeba blattae* (Kudo, 1925), it has been impossible to do so in the present flagellate which is comparatively small and highly active in trophic stage. The changes during the nuclear division and of other organelles, were therefore constructed from observations in smears fixed and stained with various stains.

The first indication of nuclear division is a slight enlargement of the nucleus and the peculiar bending down of one end of the ring of blepharoplasts. The achromatic elements of the nucleus become transformed into filiform structure which winds round inside the nuclear membrane. The chromatin granules become enlarged and conspicuous and lodged on the threads in spherical form (Fig. 23). One end of the blepharoplast-ring bends down and becomes attached to the anterior region of the nuclear membrane (Figs. 22—24). This structure becomes separated completely from the remaining Part of the ring and firmly attached to the nuclear membrane. The nucleus now leaves its normal location and emerges into the cytoplasm through the gap in the calyx where no axial filament nor parabasal apparatus is present (Fig. 24). JANICKI (1910) however thought that the nucleus breaks through the calyx wall, since he saw „nicht selten eine Art Narbe in der Kelchmembran", which in my opinion is due to his overlooking the natural gap of the calyx. After the nucleus left the calyx, one can see numerous hexagonal or round markings on the wall of calyx which are apparently the end views of part of the parabasal apparatus located on the lower side of the calyx as was suggested by Janicki. JANICKI apparently observed the detached piece of blepharoplast-ring without seeing how it was formed and called it "Central spindle", but stated that „Beziehungen zwischen Spindelanlage und Basalkörpern wurden nicht beobachtet". The term central spindle is not applicable here, because the structure is a part of extranuclear blepharoplasts. According to my observations, this extranuclear deeply staining rod-like structure originates in the blepharoplast ring as stated above. In a few individuals, I have noticed that each flagellum appeared to possess two equally large blapharoplasts located very close to the nucleus, thus exhibiting two rings of blepharoplasts parallel to each other (Fig. 21). Such an individual gave me im-

pression that in preparation of nuclear division, each blepharoplast divided into two and one group moved down upon the nucleus to form the structure under consideration; but because of the rarity of this stage compared with the one in which one end of the blepharoplast-ring comes in contact with the nucleus, I am unable to determine whether this division of blepharoplasts is normal process or not.

The nucleus which migrated into the cytoplasm from the calyx shows usually a slight elongation in the direction of the elongating mass of blepharoplasts, which seems to be identical with the paradesmose coined by KOFOID and SWEZY (1915) for the structure connecting the divided daughter blepharoplasts in trichomonad flagellates, and proposed in place of blepharoplastdesmose of ALEXEIEFF (1914). The paradesmose and consequently the entire nucleus become elogated. The chromatin granules aggregate into rounded chromosomes which are usually six or rarely eight in number. JANICKI stated that the number of chromosomes ranged from ten to sixteen and also remarked that he found centrioles at the ends of the paradesmose, not connected with the latter, although he did not mention them in or on the nucleus at the resting stage. In the nuclear division in a trophic stage, I have very seldom seen any granule located at the ends of the paradesmose and when present, it was almost always noticeable at one of the poles only (Fig. 27).

When the elongation of the nucleus reaches a stage such as shown in Figure 27, several achromatic spindle fibers become prominent parallel to the paradesmose, over which the chromosomes become attached and each divides into two — a typical metaphase — is realized. These two groups of chromosomes move toward the opposite poles (Figs. 28, 29). Up to this stage, the paradesmose is a straight uniformly thick rod, but when the nuclear division is at anaphase, the ends of the paradesmose thickens conspicuously, resulting in forming a short rod-like structure, whose axis is usually at right angles to the paradesmose (Figs. 29, 31), and in many cases a new flagella tuft grows out from each of them (Figs. 31, 34). This however is not always true as will be seen in Fig. 33 where the paradesmose is still a uniform rod, while the nuclear division is in an advanced stage of anaphase. As the chromosomes approach the opposite poles, they may break up into numererous smaller granules (Figs. 32, 33, 34, 38), a condition noticed by JANICKI also, although they may retain their individuality until later stages (Fig. 40). JANICKI stated that late stages in nuclear division were found in

the posterior end of the body and the central spindle (paradesmose) was at right angles to the old axostyle, which is not always the case (Figs. 34, 37, 38, 42). The nuclear membrane persists throughout these changes and at telophase appears as a fine connecting thread between the daughter nuclei (Figs. 35—37).

The paradesmose is now less deeply stainable and at either end becomes broadened to form a calyx; the blepharoplasts circle round the extreme end of the calyx with a broken space (Figs. 35, 36). During the course of further construction of the nucleus, the calyx encases the daughter nucleus (Figs. 36, 39, 40). In Figure 40 one sees two constructed daughter nuclei with a still faintly visible connecting thread between them and the paradesmose whose ends become widened and surrounded the nuclei. In the mean time a parabasal apparatus becomes differentiated anew around each of the nuclei which now become widely separated from each other as the flagella grow longer and more active (Figs. 41, 42).

Finally the paradesmose divides through the middle portion and each of these develops into the bundle of axial filaments (Figs. 45—48). There have been diverse observations as to the fate of the old and origin of the new so-called axostyles in trichomonad flagellates a full discussion of which is beyond the scope of the present paper. As I have already stated elsewhere, the bundle of axial filaments in *Lophomonas blattarum* is not homologous with the axostyle of trichomonad flagellates. In *Lophomonas blattarum* as was described by JANICKI (1910) the new bundles of axial filaments develop from the paradesmose. The old flagella-tuft, blepharoplast-ring, calyx and the bundle of axial filaments persist until such a stage as shown in Figure 43 is reached, when they start to degenerate (Fig. 45) and finally disappear completely (Figs. 46—48). But in many cases during the various stages of nuclear division, the old organelles here mentioned appear to become separated from the general mass of cytoplasm in which the dividing nucleus or two daughter nuclei are situated. I have already stated that the anterior region of an actively motile individual of this flagellate breaks off with a little mass of cytoplasm from the rest of the body. A similar phenomenon was also noted during the nuclear division. One of the cases is shown in Textfigure D. This individual when first noted had three tufts of flagella (1), one located at the somewhat drawnout end of the body, apparently being the old organelle, and the other two were situated at the extremes of the opposite side. The somewhat solitary tuft bearing end was seen to extend to a considerable

extent (2) and it was seen busily thrusting itself into the mass of detritus, when suddenly it became disconnected with the main part of the body. Immediately the remaining portion with two apparently new tufts became stretched, the flagella-tufts occupying opposite ends (3) and the whole mass underwent manifold changes in form. After about ten minutes, the body showed a slight constriction midway between the two flagella tufts (4) and before I placed my

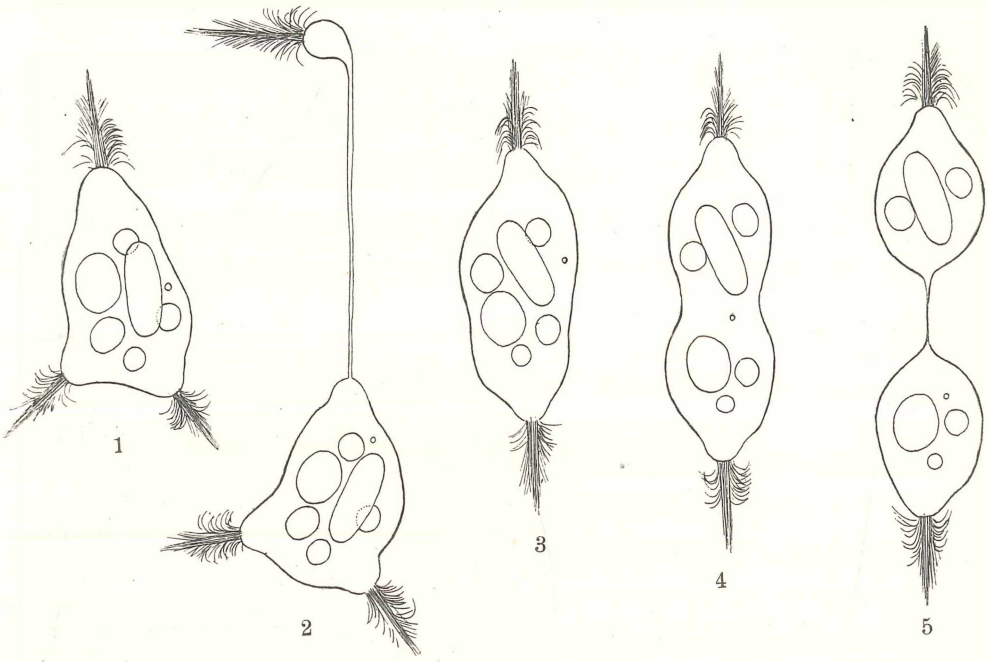

Fig. D. Five outline sketches of a dividing individual in life. 1. The two daughter nuclei are probably completely formed and the anucleated old calyx with its flagella tuft (above) is seen still actively motile. 2. The old calyx is elongating itself, before breaking off completely from the rest of the body. 3. The two daughter nuclei now occupy the opposite extremities. 4. The constriction of the body has started. 5. A stage just before complete separation of the two daughter individuals.

eyes upon the oculars, the body was almost completely separated in two masses, only a fine protoplasmic thread existing between them (5). In a minute, they were completely separated from each other. This breaking way of old organelles probably takes place almost at any time, and would bring out forms such as shown in Figures 33 and 44, where the old organelles are entirely lacking. In the earlier part of my study, I saw a comparatively large number

of the stages shown in Figures 20, 33 and 44, especially in smears made form the host colon filled with *Lophomonas blattarum* and was puzzled about their significance; but after observing the accidental separation between the active and passive parts of the flagellate, these forms were fully explained as formed by this peculiar phenomenon here noted.

As to the fate of old organelles which stay on the body and degenerate, I am inclined to think that they are most probably absorbed by the cytoplasm. JANICKI (1910) stated in this connection that „ob diese Teile (old axostyle and flagella-tuft) als Ganzes ausgestoßen oder im Plasma resorbiert werden, kann nicht bestimmt entschieden werden, die erstere Möglichkeit erscheint aber wahrscheinlich, indem in wenigen Fällen alte, vollständig über den Körperrand hinausragende Kelche an zweikernigen Formen beobachtet worden sind." The constriction of the body is completed in such a short length of time that such a competent observer as JANICKI failed to notice. JANICKI writes that „eine regelmäßige Durchschnürung des Plasmakörper in zwei gleiche Hälften, nachdem die Kernteilung vollendet, habe ich weder im Leben noch an konservierten Präparaten je beobachten können". JANICKI on the other hand, observed individuals with from four to eight nuclei. Compared with numerous other species of flagellates, one would naturally expect a multiple division in *Lophomonas blattarum*. The material I have studied, however, is in striking contrast with that of JANICKI in that I have failed to see a single active trophic stage with more than two nuclei. Therefore I hold that a binary fission is the main multiplication method of *Lophomonas blattarum* in the colon of a host.

The encysted stage.

As JANICK (1910) noted, the encysted forms of *Lophomonas blattarum* are found throughout the year. The feces of a cockroach which harbors a large number of this flagellate contain usually numerous cysts.

Precystic stage. — The precystic stage is characterized by a more spherical form, a small number of food particles, highly alveolated protoplasm and a comparatively large calyx. In life it is less active compared with a trophic stage. The parabasal apparatus degenerates first so that it is not to be recognized in smears (Figs. 53—55). The end of the blepharoplast-ring becomes bent down toward the nucleus (Figs. 53, 54, 57, 58) as in the case of the trophic stage and

finally this portion becomes separated form the rest. It is now very closely attached to the nuclear membrane (Figs. 55, 59). The remaining portion of the blepharoplast-ring as well as bundle of axial filament are absorbed by the cytoplasm. As to the fate of the flagella tuft, I am inclined to think that it is also absorbed by the body, comparing with the corresponding stages in *L. striata* in which the flagella-tuft is completely taken inside of the body at the time of encystation (KUDO, 1925 a).

Nuclear division. — The nucleus when stained is much more conspicuous than that in the trophic stage. The achromatic substances form at the beginning a coiled thread on which one sees that six to eight chromosomes are distributed (Figs. 53—55). Occasionally every two of them seem to be connected with an achromatic thread (Fig. 56). The chromosomes are frequently paired (Fig. 59) which I interprete as due to a premature division. JANICKI writes that he observed „acht große, rundliche bis ovale Chromosomen ohne besondere Anordnung verteilt." In the mean time, the piece of blepharoplast-rod grows conspicuously and together with the nucleus falls more deeply into the cytoplasm (Figs. 60—63). This paradesmose is so close to and apparently exercise so strong a pressure upon the nucleus that in an end view it appears as shown in Figure 63. The paradesmose become elongated and a few achromatic fibers appear inside the nuclear membrane parallel to it (Fig. 65). I have not seen a typical metaphase with an equatorial plate as I have described for the trophic stage; the chromosomes become broadly arranged in the intranuclear space (Figs. 64—67) and each sooner or later divides into two (Fig. 68). Contray to the circumstances noted in the trophic stage, here on finds often a distinct granule at the end of the paradesmose (Figs. 66, 71) or two compact granules (Fig. 68), which seem to correspond with the granules which JANICKI called centrioles. After passing through the anaphase (Figs. 70, 71), the daughter nuclei are formed (Figs. 72—75) in which at the beginning six or rarely eight peripheral chromosomes are to be distinguished. These chromosomes finally become condensed into a more or less compact mass staining deeply and assuming a central position. This chromatin mass appears to be connected with the extranuclear structure compared above with JANICKI's centriole, by an achromatic thread (Fig. 75).

Ordinarily in the host colon as well as in the fecal matter, these binucleated cysts appear to be the final stage in development. Occasionally, however, one finds each of the two nuclei undergoing

division at approximately right angles to the direction of the first division (Figs. 76—78). This division appears to be also mitotic in which three chromosomes can in most cases be distinguished (Fig. 77). The original paradesmose persists throughout the changes, although it is now less stainable (Fig. 78).

JANICKI suggested that the ultimate number of nuclei in the cyst was eight. JANICKI failed to determine how the flagellate gained entrance to a new host colon in experimental animals, but thought that probably cyst contents break up into eight uninucleated individuals before leaving the cyst membrane in the gut of a new host. Neither have I been successful in determining this point. There however does not seem to be any doubt in maintaining that the encysted stage serves as source of infection in a new host. Experiments and observations are now being carried on with the hope of bringing out the life cycle of *Lophomonas blattarum*.

Size. — The fulley formed cyst is covered by a homogenous membrane of uniform thickness and varies in size as does the trophic stage. In fresh condition the cysts range from 12 to 20 μ in diameter. The cyst membrane may show undulation as was also observed by JANICKI.

As to the relation between the cockroach and the flagellate, I do not see any harm done by the flagellate upon the host. The organism lives on indigestible starch grains and other objects which are present in the host colon and which are apparently of no more use to the host, and furthermore they have never been found in a host tissue, being always present in the lumen of the colon. CLEVELAND (1925a) recently removed „two kinds of flagellate Protozoa“ [1]) from the hindgut by confining the cockreaches in oxygen and found that the confinement did not injure the insects at all. On the other hand, the flagellates cannot live outside of the host colon where they find ample supply of food and are well protected from unfavorable external circumstances. Therefore, *Lophomonas blattarum* should be considered as a commensal of *Blatta orientalis*.

Summary.

1. *Lophomonas blattarum* was found in 32 per cent of 1400 *Blatta orientalis* examined, the largest incidence occurring in the summer months, which is associated with the food habit of the host.

[1]) CLEVELAND gives no specific names; but it is supposed that he observed the largest flagellates in the habitat, *Lophomonas blattarum* and *L. striata*.

2. The active individual possesses a remarkable power of locomotion and of change in form.

3. The food consists of solid particles especially starch grains. No cytostome is present. The food matter is taken in through the entive body surface except the anterior extremity. The manner with which the food is taken in inside of the body is described.

4. The axial structure is a bundle of axial filaments, each of which is continuous with the anterior flagellum. Its anterior end opens into a funnel-like calyx inside of which a nucleus and outside parabasal apparatus are located. The latter structure is a protective organelle of the nucleus. The calyx has a gap which corresponds with the broken space in the ring of blepharoplasts located anterior to the nucleus. Each flagellum passes through an elongated blepharoplast.

5. The nuclear division is mitotic in which six or rarely eight chromosomes and spindle fibers become prominent. A part of the blepharoplast-ring becomes attached to the nuclear membrane and then completely separated from the rest. It is extranuclear and very closely attached to the nucleus. It elongates itself as the nucleus elongates. No regular centrioles are present.

6. The paradesmose encase the newly divided nuclei and later when it divides into two, it develops into the hundles of axial filaments. During the nuclear division the calyx from which the nucleus had emerged, bundle of axial filaments, blepharoplasts, flagella-tuft and parabasal apparatus, persist and become absorbed by the general mass of cytoplasm or break off from the main part of the body and disintegrate.

7. Multiple division was not observed.

8. In cysts, a part of blepharoplast becomes detached from the blepharoplast-ring and attached to the nuclear membrane. The nucleus divides mitotically in which six or rarely eight chromosomes, achromatic spindle fibers and possibly centrioles are apparent. In the second division, there appear ordinarily three chromosomes. Cysts with more than four nuclei were not noted.

9. *Lophomonas blattarum* is a commensal and multiplies by a binary fission in trophic stage in the host colon after gaining entrance to the latter in encysted forms.

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Explanation of figures.

All the figures are related to *Lophomonas blattarum* found in the colon of *Blatta orientalis* and were made by means of Abbe's drawing apparatus attached to one of the oculars. Figs. 1—8 are from life, while the rest taken from stained smears or sections. The scale placed under Figs. 1 and 2 is to be applied for Figs. 1—7 which were originally magnified 1050 times; Figs. 9—78 were originally magnified 2300 times and should be measured by the scales placed under Figs. 36 and 43.

Plate 7.

Figs 1—3. Actively motile individuals with food particles.

Fig. 4. A somewhat smaller individual with the protruding bundle of axial filaments.

Fig. 5. An individual just emerged from the masses of detritus present in the host colon, with numerous solid matters attached to it.

Figs. 6, 7. Individuals with a long protoplasmic projection.

Fig. 8. A living individual, slightly compressed under the coverglass, showing the flagella tuft, blepharoplasts, the beginning of axial filaments which form the calyx, nucleus and parabasal apparatus.

Figs. 9, 10. Stained specimens showing various structures.

Figs. 11—13. Individuals fixed in the course of feeding.

Fig. 14. An end view of the anterior part of a trophic stage, showing the broken ring of blepharoplasts and the nucleus.

Figs. 15. A similar view to the last, showing besides the absence of parabasal body below the broken space of the blepharoplast-ring.

Figs. 16—19. Small individuals, probably formed by separation of the nucleate portion from the rest of the body.

Fig. 20. A rounded mass of cytoplasm with two food particles, in all probability, the part left behind when the nucleate portion broke of from the body and destined to disintegrate sooner or later.

Fig. 21. A highly compressed individual, showing the gap and fibrillar condition of the calyx.

Figs. 22—40. Stages in nuclear division.

Figs. 22, 23. Formation of the extranuclear body from the blepharoplast-ring.

Fig. 24. Emergence of the nucleus through the gap of the calyx.

Figs. 25, 26. Prophase.

Fig. 27. Metaphase.

Figs. 28—34. Anaphase.

Figs. 35, 36. Telophase, the ends of paradesmose enlarging to form calyx.

Figs. 37—39. Telophase.

Fig. 40. The two daughter nuclei are completely formed and included inside of the calyx formed by divided paradesmose.

Plate 8.

Fig. 41. Further development of organelles; parabasal apparatus is formed round each of the nuclei.

Fig. 42. A stage just before division of the paradesmose into two.

Fig. 43. Two daughter nuclei, each possessing independent organelles.

Fig. 44. Two daughter nuclei are completely formed. The old organelles broke off sometime before this stage was reached.

Fig. 45. The degeneration of the old organelles.

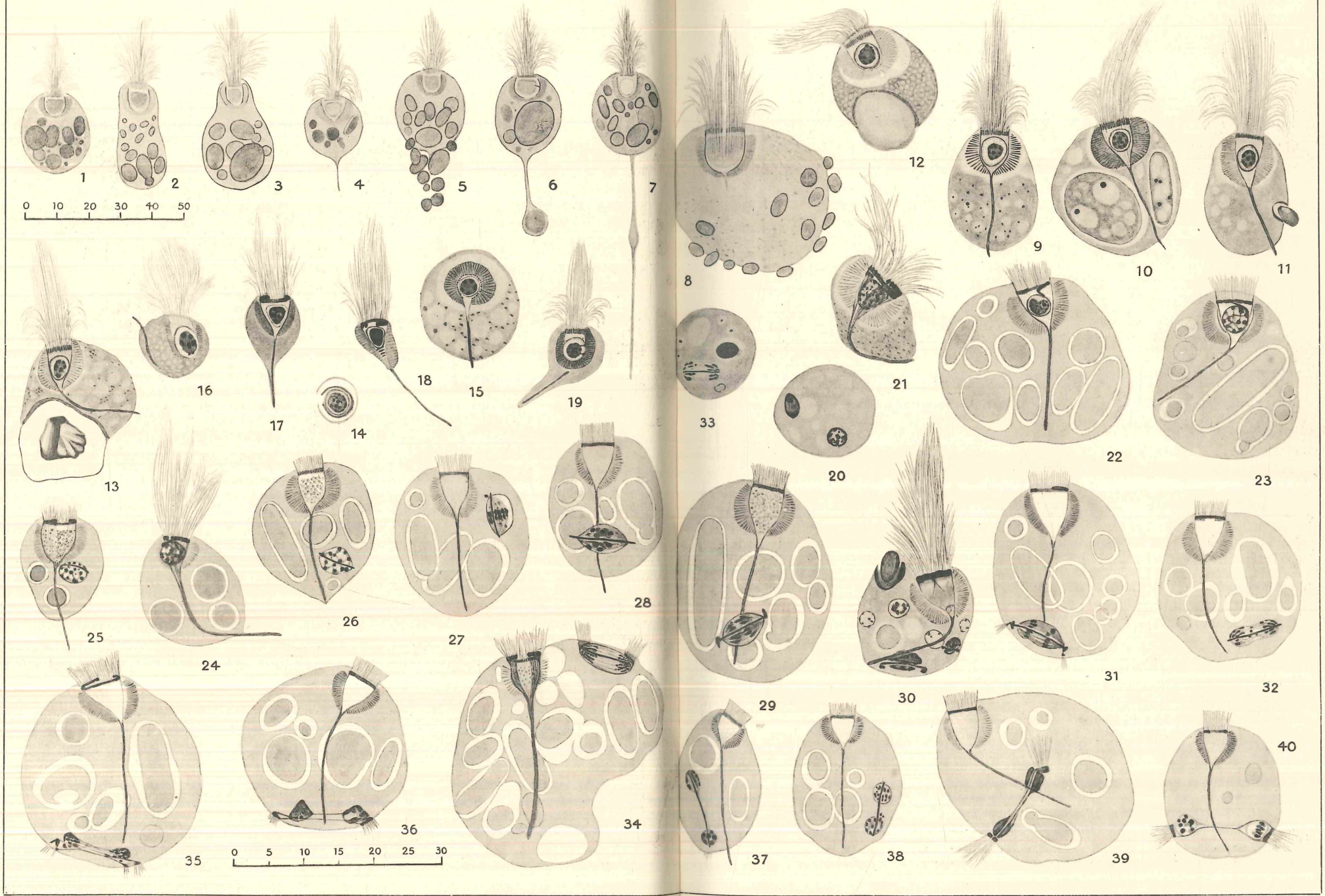
Figs. 46—48. Stages in binary fission.

Figs. 49—52. Different aspects of nuclei.

Figs. 53—59. Precystic stages.

Figs. 60—75. Stages of the first nuclear division in cysts.

Figs. 76—78. Stages in the second nuclear division in cysts.



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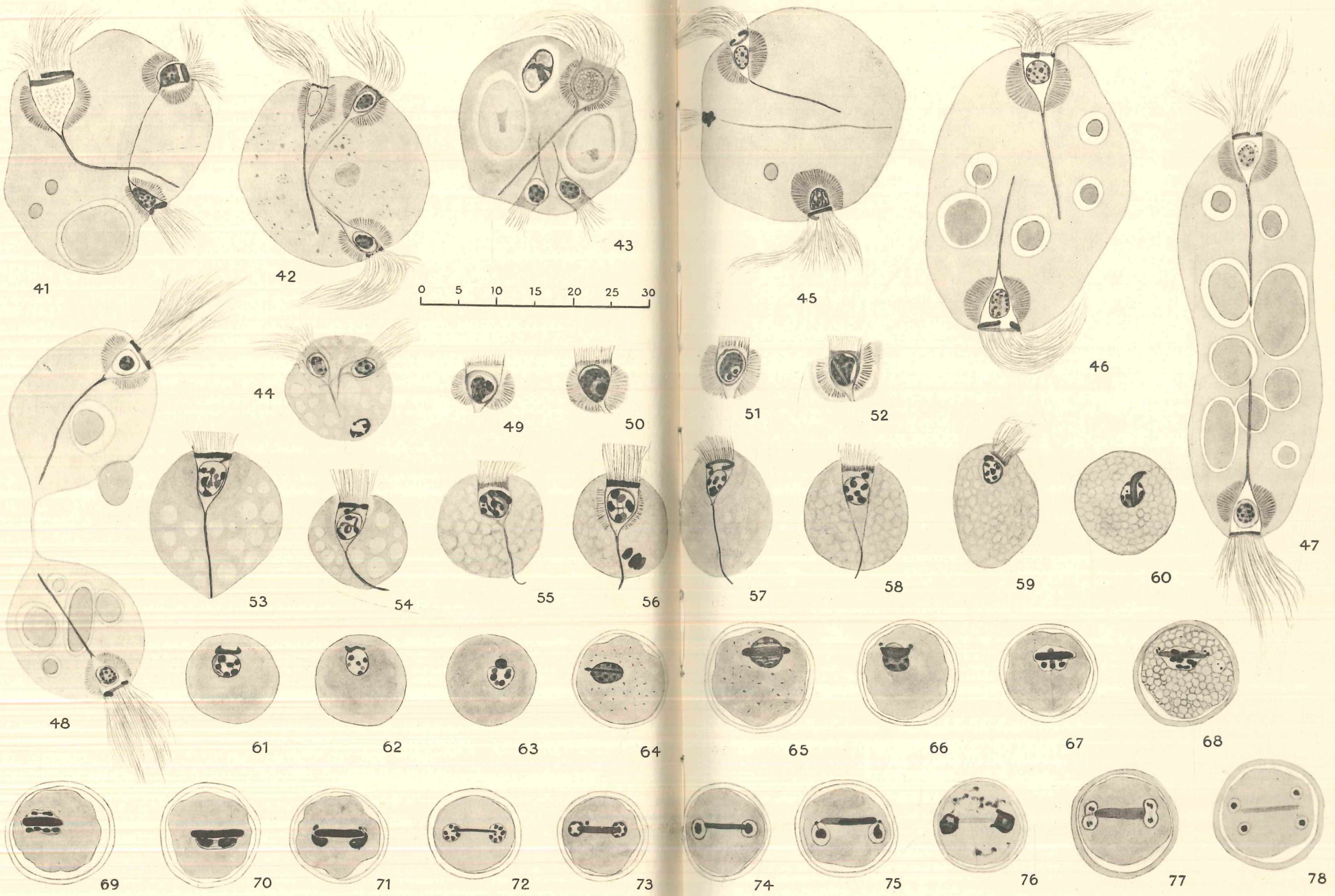

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