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ROSKIN, Gr. (1927)

To the knowledge of the genus Pseudospora
CIENKOWSKI.

Arch. Protistenk. 59, 350-368.

Translated by: J.E. Wightman

CONTENTS

1. INTRODUCTION

The work is devoted to the observation of the two types of Pseudospora.

The observations were made "in vivo" as well as on various dyed preparations. The "in vivo" observations were made with the help of a humidity chamber. Preparations of Pseudospora volvocis were prepared in the following way:- A very small drop of chicken egg white diluted half with water was introduced onto a microscope slide. Into the drop some infected Volvox was introduced. The drop was spread somewhat, then the slide was put onto a recessed object glass filled with a fixative fluid in such a way that the drop was on the fixative wetted side. The fixative consisted of a saturated sublimate solution with the addition of one drop of 1% osmicacid per 1 c.c. solution. Fixing lasted 15 minutes. The smears were then dyed with Hoemalaun + Eosin, Ferrohoematoxylin but also by the method of BENDA and that of MALLORY.

2. PSEUDOSPORA EUDORINI n. sp.

In the search for Pseudospora living in Volvox, I came across an organism which lived in Eudorina elegans and from all appearances belonged to the genus Pseudospora. For a description of this parasite of the Eudorina elegans, it is advisable to start with a description of the so called Heliozoa-like form.

The culture in which the Eudorina with Pseudospora existed, was taken out of a small pond in the grounds of the Zwenigorode Hydrophysiological Station. One could see immediately in the culture a considerable number of relatively small Heliozoa-like forms whose general appearance is given in fig. 1.

From a small rounded body (about 10 - 12 μ dia), a small number of fine straight plasma-rays branch out, which are two to three times the body diameter in length. The protoplasm of the body usually includes a small number of yellow or brownish globules, - products of the chlorophyll digestion. The pulsating vacuole is relatively large and easy to see. From observations "in vivo", I could by no means detect a characteristic structure, in the composition of the plasma rays, because they were too fine and hardly available for study. It is therefore difficult to decide the question:- whether we have to deal here with genuine Axopodia, or the plasma rays are just so built like the raylike Pseudopodia of the Amoeba radiosa i.e. special axial skeletal threads are lacking. There are more grounds to accept that the plasma strands of the sunlike form are constructed similarly to the ray formed Pseudopodia of the Amoeba radiosa. The resistance force of the ray formed Pseudopodium and the property to maintain its form, probably depends in our organism, on the ability of the central part of the pseudopodium plasma to change to the "stable" condition, analogous to what DOFLEIN for Rhisopodia, and I for the ray formed Pseudopodia of the Vampirella had observed.

The swimming heliozoa-like form displays a calm appearance. Its pseudopodia are usually unmoving or exhibit rare and slow movement. The picture becomes quite different if a Eudorina elegans swims by into the immediate vicinity. The ray-like Pseudopodia direct themselves quickly towards the Eudorina and make contact, thereby bringing the Pseudospora to the body of the Eudorina to stick to it. Afterwards one can make the following observations quickly one after the other: the pseudopodia of the Pseudospora is lined up. The pseudopodia approach one another and come to lie parallel to one another. The pseudopodia slowly attract the body of the Pseudospora to that of the Eudorina. Complete pseudopodia flow together to a single plasma tulip which begins to spread over the complete body of the Eudorina until the whole Pseudospora has completely covered the Eudorina. The stages of this process are shown in fig. 2.

The transition of the Pseudospora from a free swimming condition, onto the Eudorina is accompanied by a change in its outward form. The heliozoa-like Pseudospora becomes amoeba like.

As soon as the Pseudospora has penetrated into the Eudorina, it begins at once to devour the cells of the Eudorina. To do this it surrounds first the Eudorina cells in question with its wide pseudopodia, then it flows all around the captured cells. The process of the digestion of the cells of the Eudorina lasts about an hour. After it has devoured one cell it creeps forthwith onto the next and surrounds it with its complete body.

Fig 3 f-l gives a presentation of the process of the Pseudospora devouring the cells. The Pseudospora eats up not only the vegetative cells of the Eudorina but also the reproductive cells. Specific stages of the digestion of the Eudorina cells within the Pseudospora body are identified by the alteration in the pigmentation of the cells being digested, from green to yellow and brown colour. After some time, the body of the Pseudospora is blocked in such a way by the remains of the Eudorina cells that its structure is nearly completely indistinguishable any longer. The satiated Pseudospora is hardly moving in the Eudorina body, mostly it lies there quite motionless.

Before going over now to the description of the further stages of the Pseudospora-cyclus, it is necessary to refer to the very polymorphic appearance which the Pseudospora can assume during the course of its life in the interior of the Eudorina; one finds individuals with a single thick and long pseudopodium, next to those with multiple pseudopodia whose type, form, length and arrangement are themselves strongly varying. Some of these amoeboid stages are given in Fig. 3. A pulsating vacuole always exists in Pseudospora.

During the amoeboid stage a division of the Pseudospora takes place and indeed in the same manner one observes with amoeba, but since the observations were made "in vivo" and the body of the Pseudospora was fully blocked with digested Eudorina cells, I was not in a position to be able to ascertain any details of the process.

After a period of comparative calm, during which the Pseudospora finishes the digestion of the substantial parts of the absorbed Eudorina cells, the Pseudospora begins anew to move energetically and falls on the remaining Eudorina cells or comes to lie on the surface of them in order to separate itself from them. At this time, one can often observe a transition from the "amoeboid form" to the "flagellate form". This transition occurs fairly quickly. Fig. 5 shows the successive stages of transition from "amoeboid form" to "flagellate form" over a period of three minutes. The flagellate example so developed, now swims away very quickly. At the start the flagella are to be seen as stationary fine plasma threads, then after a few weak movements they begin to work quickly and the Pseudospora swims away.

The "flagellata form" is capable of changing back to the "amoeboid form". The following observations prove this:- After the Eudorina is digested, the amoeboid Pseudospora normally swims away under transformation to "flagellata form". I was once able to observe an amoeboid form on the devoured Eudorina, which had developed flagella, but the pseudopodia were in motion while the flagella lay completely still, (fig. 6a, b, c). Then the flagella began to function to work (fig. 6d, e), the Pseudospora remained lain in the body of the host; the flagella worked for another 2 - 3 minutes then they stayed still (fig. 6g). After another three minutes the Pseudospora became round and had lost its flagella again (were retracted or lost?) and after a six minute period of inactivity, changed again into a typical amoeboid form creeping around the inside of the destroyed Eudorina body (6 m, n). As soon as the Eudorina examples in the culture I observed had begun to disappear, annihilated by numerous Pseudospora, one could verify the formation of cysts by the Pseudospora. The cyst development starts with a rounding of the Pseudospora. In such specimens coming to cyst development, one finds normally, the not quite digested Eudorina cells in large numbers which fill considerable parts of the Pseudospora body. Besides the yellow, green and brown contents (remains of the digested Eudorina cells,) one finds white glistening pieces (fig. 7), which from outward appearance, as far as one can judge from the nature of these contents based on simple observation and without using appropriate reactions, reminds one of starch (glycogen?). The illustration of the starting stage of the development of the cysts is given in figures 7a, b. The further process consists in compression of the Pseudospora and the demarkation by an integument (membrane), indeed the first cyst integument.

Afterwards small or larger lumps of yellow or brown pigment are scattered out of the body of the Pseudospora which evidently are to be looked upon as excrement. Immediately afterwards, the second cyst integuments develop which lie directly against the plasma body of the Pseudospora. The mentioned yellow and brown lumps come to lie between the first and second cyst integuments and remain there during the whole of the observation period. Out of the row of cysts, by transferring them into a fresh culture, amoeboid Pseudospora were apparent which were in a position again to attack the Eudorina at once, or after they had just taken on a heliozoa-like form. Finally one must refer to fig. 8 which shows the three stages of transformation of the free heliozoa-like form into the flagellate-form. This transformation happens very quickly, in 3 - 5 minutes during which, the ray-like pseudopodia retract, at the same time the fine spiralled plasma branches carry out, at first, weak unco-ordinated movements which then take on a typically flagellum form. The body of the Pseudospora takes on an elongated form instead of its spherical form, and so proceeds from a heliozoa-like form to a flagellata-form.

Does the form of Pseudospora living in an Eudorina now represent a new type, or is it identical with that already described? In all there are indeed three species of known Pseudospora, (Pseudospora volvocis CIENK, Pseudospora parasitica CIENK, and Pseudospora aculeata ZOPF). They are mainly differentiated by the structure of their cysts. The cysts of the Pseudospora living in Eudorina however, possess two integuments: the outer with somewhat irregular contours of the sphere, and the regular sphere. One of this kind of cyst form is known in one of the previously described Pseudospora.

In the district where the observations were gathered, the Pseudospora volvocis was also observed by me. I have not been able to find any other species of Pseudospora here. By comparing the cysts of the Eudorina-dwelling Pseudospora with the cysts of the Pseudospora volvocis, one can record that these cysts are different from one another, not only because of the structure and number of integuments, but also because of the dimensions themselves. The cysts of the Eudorina-dwelling Pseudospora are 15 μ, those of the Pseudospora volvocis about 25 μ. Finally I must add that all my attempts to infect the Volvox with the Eudorina-dwelling Pseudospora came to nothing. The Eudorina-dwelling Pseudospora never attacked the Volvox even after several days hunger. These observations allow one to suppose that the Eudorina-dwelling Pseudospora represents an independent species. I propose to call it the Pseudospora-eudorini.

3. PSEUDOSPORA VOLVOCIS CIENKOWSKI

Pseudospora volvocis was first described by CIENKOWSKI (1865) whereby he observed the heliozoa-like form, the amoeboid form and described the cysts. The Pseudospora volvocis has been mentioned by BÜTSCHLI ('83), ZOPF ('85) and KLEBS ('92). It was studied in most detail by ROBERTSON ('06). Their particulars are listed in the surveys by DOFLEIN ('16) and NÖLLER ('22).

ROBERTSON has made the following resume of their observations in the following manner:-

1. A Pseudospora may adopt three forms, an amoeboid form, a flagellata form and a radial form. The last at least appears to be a direction to external conditions.

2. A single nucleus is present. It is bounded by a membrane which contains the karyosome surrounded by a clear space; fine rays pass from the karyosome to the membrane.

3. The nucleus divides by mitosis. The chromatin forms chromosomes, which are apparently rod-shaped. The spindle appears to be formed from the achromatic cutra-nuclear substance.

4. Pseudospora reproduces by fission. After fourteen to twentyone days gametes are formed.

5. Gametogenesis. The nucleus of the Pseudospora becomes converted into a sphere, the nuclear substance of which appears to be derived from the rays of the original cell nucleus. The karyosome is extruded from the sphere.

6. The sphere segments to form a large number of gametes.

7. The gametes conjugate in pairs, forming zygotes, which develop into the adult Pseudospora.

My observations however do not completely confirm the fundamental conclusions just stated.

It is most suitable to begin the description of our observations of Pseudospora volvocis with a description of the heliozoa-like form itself which was to be seen in the large numbers in my culture. In general, these are similar to the Pseudospora eudorina already described above, there are however, besides perfectly identical ones, those which from their outward appearance are somewhat different, as can be seen from fig. 9. The body dimensions of the heliozoa-like forms are exactly 12 μ, the majority of the ray-like Pseudopodia are twice as long as the body diameter. As regards their structure, the ray like pseudopodia are not typical axopodia. There is only one vacuole and I was not able, - like ROBERTSON, to observe 4 or 5 pulsating vacuoles in this form. The heliozoa-like form swims around in a similar manner to the Actinosphaerium. Under the influence of external conditions, the heliozoa-like form changes its outward appearance, in that it transforms into a creeping amoeba (fig. 8 f), or it takes on a transitional stage between the typical heliozoa-like form and the amoeboid form as illustrated in fig. 9 d. These last forms are not in themselves free swimming in the same way as most heliozoa, but creep slowly around on their substrate with help from their ray like pseudopodia. At times these pseudopodia sprout secondary branches (fig. 9 a) but they never anastromose with one another.

The free swimming amoeboid form as well as the transitional form both have pulsating vacuoles. Very often the endoplasm appears crammed full with green, yellow and brown granules, which are none other than Volvox cell elements in various digested stages. The heliozoa-like form and the amoeboid form are both capable of reproduction and indeed divide by fission into two daughter individuals. Both the helizoa-like form and the amoeboid form freely creeping around, are capable of attacking the Volvox. The method of attack on the Volvox by the Pseudospora volvocis is identical to that described above for the Pseudospora eudorina.

As soon as the Pseudospora reaches the Volvox, it takes on an amoeboid-like form and immediately begins eating up the Volvox cells figs 10a & 10 b show the amoeboid stage of the Pseudospora inside the body of the Volvox. The appearance of the Pseudopodia is fairly variable, mostly they are ray like, tapered at the end; from time to time they are merely developed on a piece of the body; a single pulsating vacuole exists. I could not find two or three contractile vacuoles in any of the amoeboid Pseudospora such as ROBERTSON describes. The body of the amoeboid Pseudospora is crammed full of the remains of the digested Volvox cells, the granules of reserve material; from time to time lipid bodies are to be found as well.

In the plasma body of the amoeboid Pseudospora, the ectoplasm and endoplasm can be discerned fairly clearly. These cell layers can best be seen on preparations stained with hoemalaun + eosin, whereby the ectoplasm is dyed a soft rose and the endoplasm a light violet in contrast. By this staining one can also see that in the majority of the amoeboid forms, many round grains are stored which can be intensely stained with eosin. On staining with ferrohaematoxylin, one can often see small rods or dumb-bell shaped small bodies in the ectoplasm which look intensely black (fig. 10 c - d). Perhaps these are the mitochondria.

The nucleus is oval or round and contains a clearly visible covering (membrane). ROBERTSON says the following about the construction of the nucleus:- "it is a single well defined body lying in the centre of the creature. It is bounded by a fine membrane staining with chromatin stains. Inside the membrane lies a deeply-staining spherical body or karyosome, surrounded by a very definite clear space.

The chromatin lies diffused through the karyosome which presents in the resting stage, an almost homogeneous appearance. The karyosome is produced into fine rays which pass to the nuclear membrane, these stain somewhat less intensely than the Karyosome".

In the description of the various stages of the life of the Pseudospora, ROBERTSON gives the same description everywhere of the nucleus structure:- this is a nucleus with a clearly discernible nucleus membrane with a coarse homogeneous karyosome which is connected by fine straight threads to the periphery of the nucleus.

In reality however, this is not quite correct. The nucleas construction appears more complicated and not so constant as has been described by ROBERTSON.

Fig. 12 shows various forms of nucleus of the Pseudospora volvocis. Under the clearly visible membrane one sees a fine layer of peripheral chromatin, sometimes in the form of single grains, sometimes as compact groups of grains in which the sizes of the grains seemed fairly variable. The periphery of the nucleus is sometimes connected with the karyosome by fine rectilinear threads, in other cases one sees a complete network of threads. Very often the chromatin grains lie not only in the periphery but also within the threads aimed at the karyosome. Finally one can observe that the chromatin grains can stick to the karyosome to some extent, sometimes camouflaged (as one can see in fig. 12a) and the picture of a relatively normal structure of the Pseudospora nucleus presented. A homogeneous karyosome is an exception; normally one sees there various structure pictures, most frequently rings which by ferrohaematoxylin appear black coloured against the dark green background of the karyosome, then one finds vacuoles also. The basic stages of development of the nucleus division were correctly nominated and described by ROBERTSON. It remained completely unclear from his description and illustrations however, whence the rodlike chromosomes of the nucleus originate. One can believe that ROBERTSON was of the opinion that chromosomes originate in the karyosome. This notion would be false however, for the karyosome does not consist of chromatin, although it can appear to have chromatin grains adhering around it on occasion.

The chromosomes originate in the peripheral chromatin during the division of the Pseudospora. The spindle is indistinctly marked. Fig. 11a shows the telophase of the nucleus division of the amoeboid form of the Pseudospora. In fig 11 b are two daughter nuclii with spirema structure. The nucleus division does not always result in the division of the plasma substance. This explains the often met double nucleus form (fig. 10 b) whose existence was not mentioned by ROBERTSON.

As soon as the majority of the Volvox cells have been devoured by the Pseudospora, one can observe the appearance of the flagellate-forms correctly described by ROBERTSON. To the author's description, the following must be added:- two flagella do not come directly from the edge of the plasma substance, but originate in the basal granules, which for their part were connected by a fine thread to the karyosome of the nucleus (fig. 13). Very often the flagellata-form shows itself ready, on receipt of the flagella, once again to develop pseudopodia. It is important to record that the flagellata stages also cannot at all achieve the development.

Very often I could observe the development of cysts. This process went on in the same way as is described for Pseudospora eudorina. The structure of the cysts is the same as was described by CIENKOWSKI (fig. 14). The cyst diameter at 25 μ is the same.

Because of this ROBERTSON's words seem to me to be extraordinary, that he should on no single occasion have seen cysts, but only the spherical form with thickened membrane.

ROBERTSON described the development of gametes of Pseudospora and the copulation. By and large, the development of gametes according to ROBERTSON proceeded in the following manner:-

The nucleus grows and develops a spherical mass within itself which gradually completely fills the nucleus and pushes the karyosome out to the periphery. Then the sphere divides repeatedly until up to 116 segments are formed, out of which the flagella gametes arise, which come to the outside and are in the position to conjugate in pairs. It can be not just one, but also two or three are formed. ROBERTSON says that sometimes all the Pseudospora in the culture start to form the spheres within the nucleus (resp. gametes) at the same time. However, when we consider more exactly the picture presented by ROBERTSON of the process of gamete formation and remember in addition what is now known to us from the literature, it is clear that there can be no discussion here of a reproductive process. Rather, ROBERTSON has observed here the development of a nucleus dwelling parasite, which had infected the complete culture of the Pseudospora at the same time. What ROBERTSON has pictured as haematogonie, coincides completely with what Epstein described for the Chitridia in the nucleus of the amoeba. I have on various occasions had the opportunity to observe the infection of the Pseudosporia by the Chitridia with the nucleus and the pictures I observed are identical with those which were used by ROBERTSON to illustrate the reproductive process.

4. THE SYSTEMATIC CLASSIFICATION OF THE GENUS PSEUDOSPORA

At the conclusion of my work, I must very shortly dwell on the question of the systematic classification of the genus Pseudospora.

Pseudospora volvocis was described by CIENKOWSKI in 1865 as "zoospore forming monads" which belong to the group monadinae zoosporae.

BÜTSCHLI, in BRONN'S "Klassen und Ordnungen" (1883) classed the Pseudospora amongst the group of flagellata, classification Isomastigoda.

KENT had likewise added the Pseudospora to flagellata protozoa and indeed to the group Pantostomata.

DOFLEIN (1916) classed the Pseudospora amongst the primitive Heliozoa which form the group Protomyxidea (RAY LANKESTER). In this group of Protomyxidea, as DOFLEIN pointed out, belong the organisms in which "one saw most primitive relatives of the Mycetozoae". But as DOFLEIN opines, "above all, closest relations to heliozoa are unmistakeable; indeed it seems to me that the majority of them have such close relations with genuine lower heliozoa that we may classify them immediately with the heliozoa." DOFLEIN appoints the Pseudospora to the family Zoosporidae (ZOPF-DELAGE) "The forms of this family are also heliozoa-like, have axopodia, but lack the flagella always in the heliozoa condition. They encyst themselves before multiplying. Out of the cysts creep flagella-bearing zoospores". NÖLLER (1922) and CALKINS (1926) agree the system given by DOFLEIN.

Of all the opinions found in the literature up till now on the systematic classification of Pseudospora, that of DOFLEIN about the relationship of this family to primitive heliozoa is the most important. The acceptance by BÜTSCHLI and KENT of the relationship of the Pseudospora to the flagellata group has too little foundation in comparison with it. In any case the flagellata stages can be completely absent in a number of Pseudospora as I have observed occasionally. Also there are few grounds to accept that the Pseudospora of the Mycetozoa should belong, particularly because it seems necessary to reject completely the method of reproduction described earlier by ROBERTSON, as one standing in no relation to the Pseudospora itself.

But also the interpretation of DOFLEIN seems incorrect to me and not in accordance with the reality. Firstly, during its helizoa-like stage, the Pseudospora develops, not axopodia, but pseudopodia, which seem quite similar in structure to the pseudopodia of the so-called "radiosa-form" of some amoeba. Then, the heliozoa-like stage can also not be met for a whole series of Pseudospora generations, as I also have been able to observe.

I believe it would be most correct to include the Pseudospora in the family Bistadiidae (DOFLEIN) and indeed the Pseudospora seems to stand nearest to the genus Naegleria. The general appearance of the amoeboid form and the structure of the nucleus as well as the general structure of the flagella form all indicate this situation. This similarity is particularly noticeable when one compares the flagella form of the Pseudospora with the same form of the Naegleria gruberi as portrayed by C.W. WILSON.

But however with one reservation:- the type of nucleus division of the Pseudospora is different from the type of nucleus division of the Naegleria-gruberi (ref. ALEXEJEFF 1912). When one takes into consideration what has been said above about the morphology of the Pseudospora and does not forget that the existence of the heliozoa-like stage is not obligatory in Pseudospora and the axopodia existence is not very likely, it seems to me that the classification of the Pseudospora in the family Bastadiidae appears to be completely rational.

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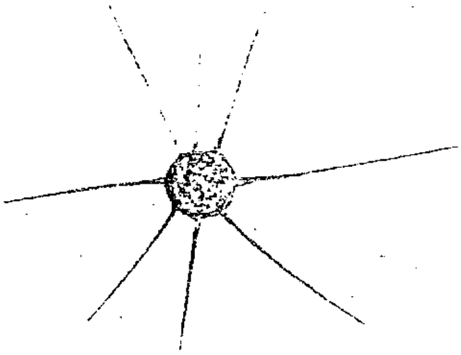

Fig. 1.

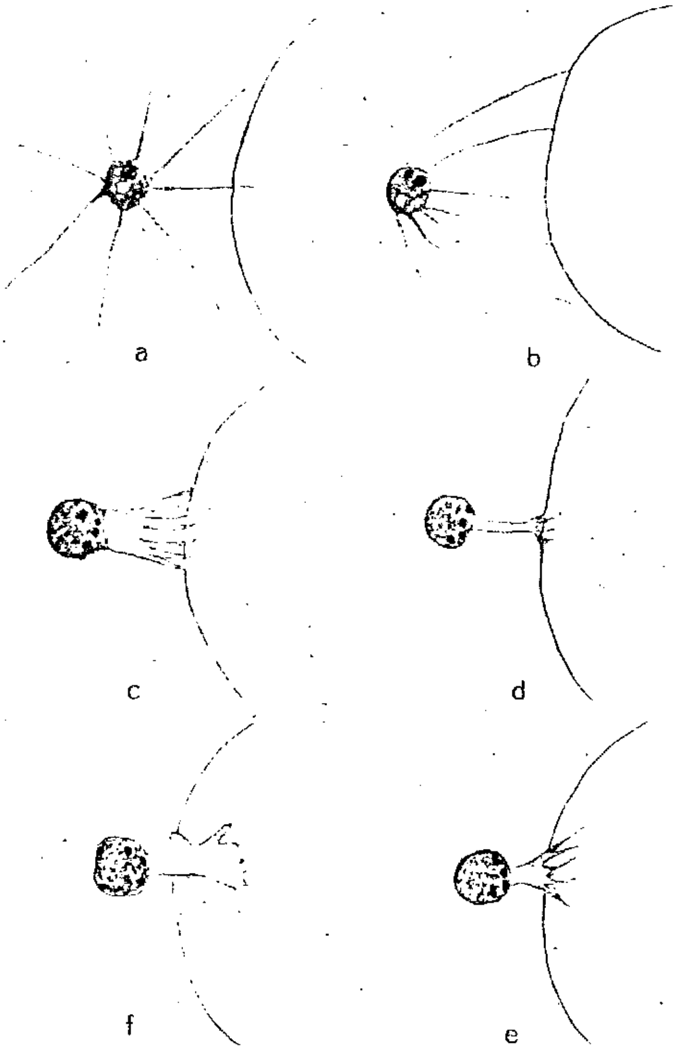

Fig. 2.

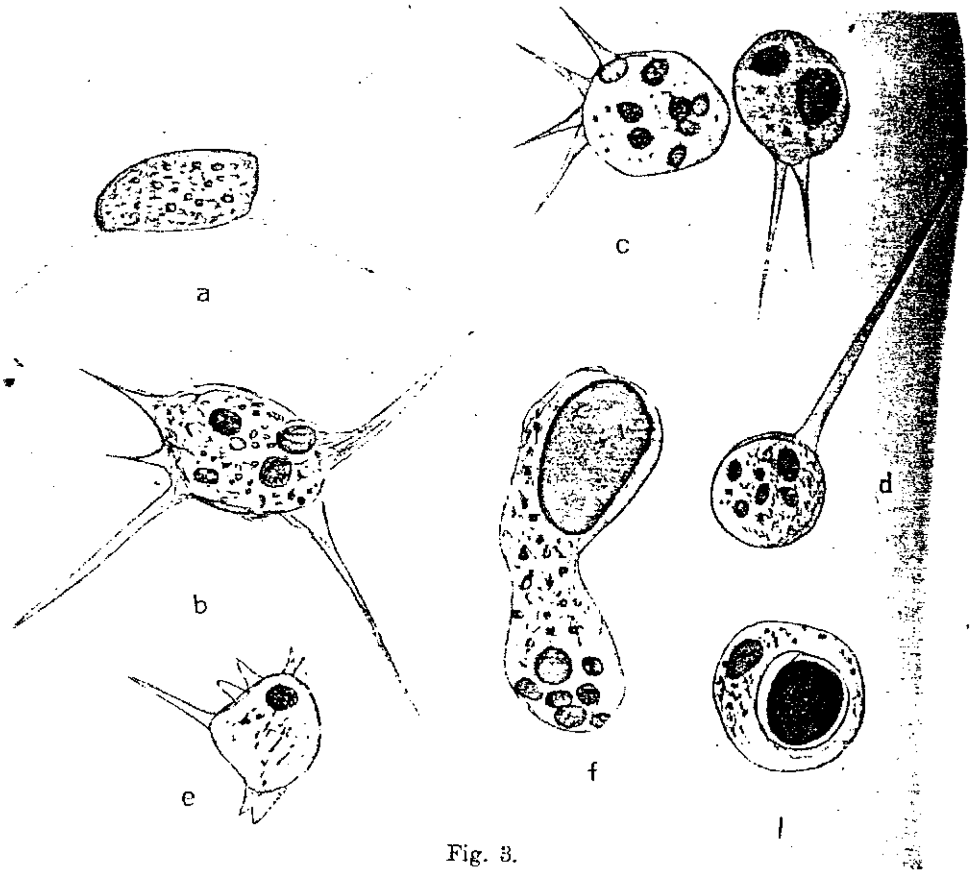

Fig. 3.

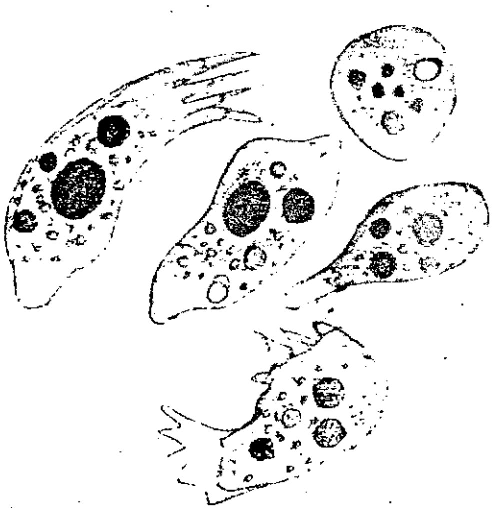

Fig. 4.

Fig. 5.

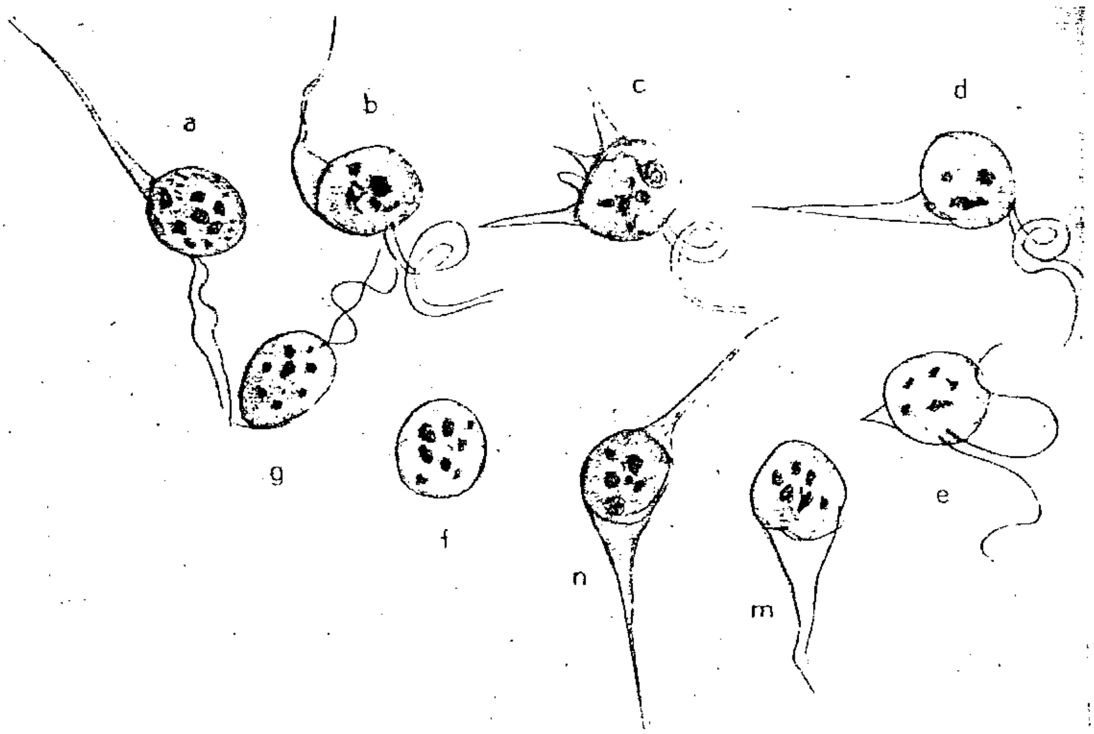

Fig. 6.

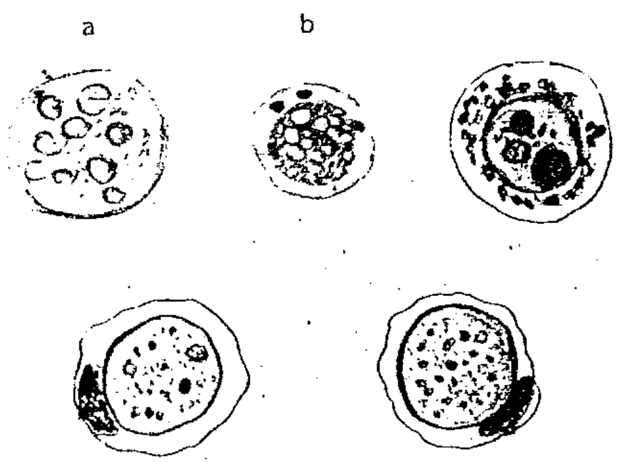

Fig. 7.

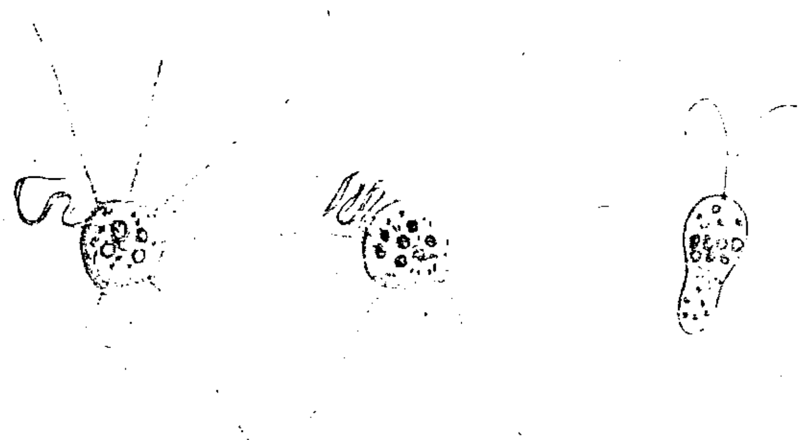

Fig. 8.

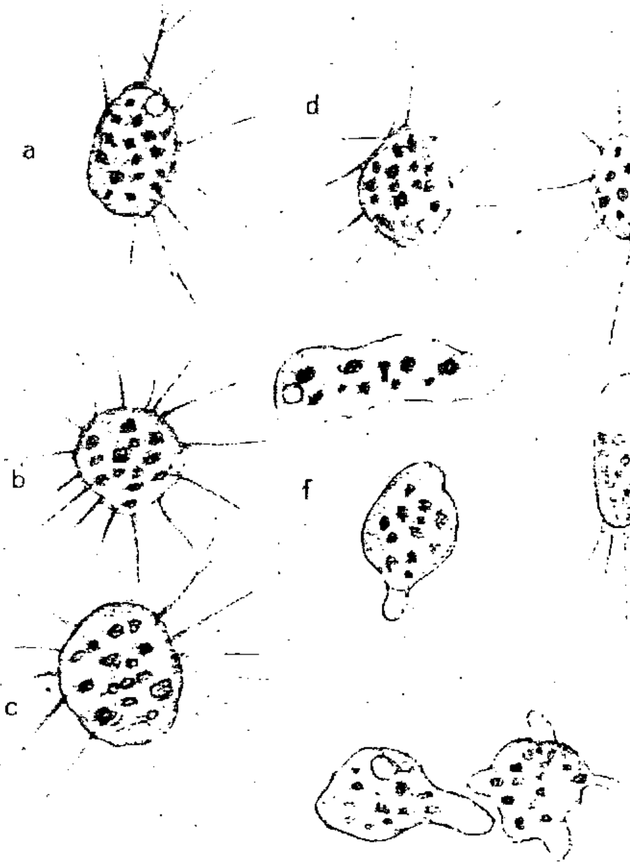

Fig. 9.

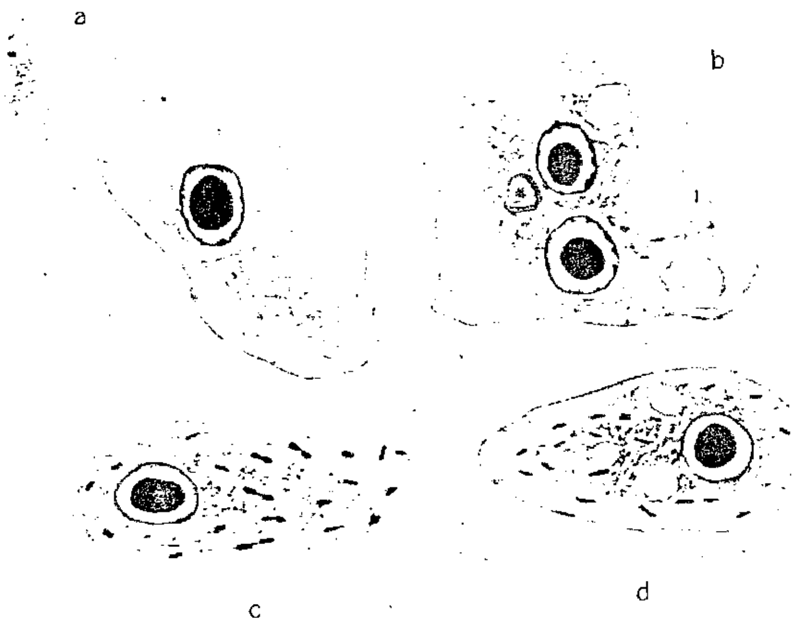

Fig. 10.

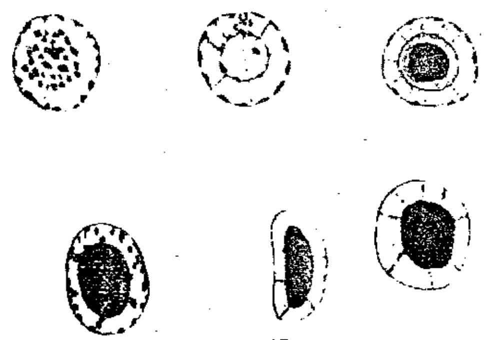

Fig. 12.

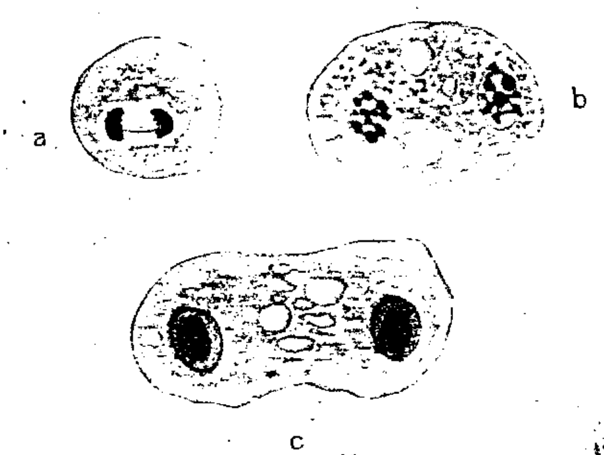

Fig. 11.

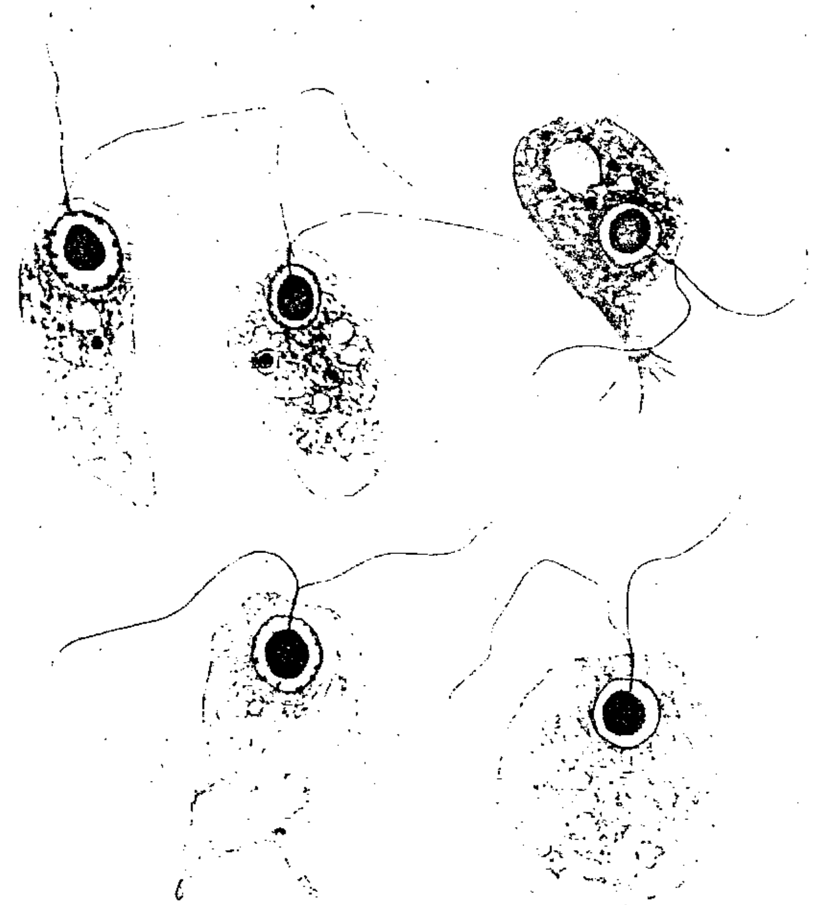

Fig. 13.

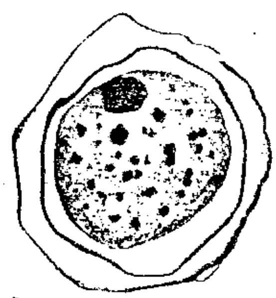

Fig. 14.

Erklärung der Textfiguren.

Die Fig. 1, 3—9 sind mit Hilfe Zeiss' Zeichenapparat und Obj. hom. Imm. 1,8 mm nebst Com. Oc. 4 von den lebenden Objekten gezeichnet.

Die Fig. 2 ist mit Hilfe Zeiss' Zeichenapparat und Obj. 3 mm nebst Comp. Oc. 4 von dem lebenden Objekt gezeichnet.

Die Fig. 10—13 sind mit Hilfe Zeiss' Zeichenapparat und Obj. hom. Imm. 2 mm nebst Comp. Oc. 12 nach den fixierten und mit Eisenhämatoxylin gefärbten Präparaten gezeichnet.

Die Fig. 14 ist von dem lebenden Objekt gezeichnet (Obj. 2 mm Comp. Oc. 12).

Die Fig. 11 a ist mit Hilfe Obj. hom. Imm. 1,8 mm und Comp. Oc. 6 gezeichnet.

Auf den Fig. 1—8 ist *Pseudosphora eudorini* und auf den Fig. 9—14 ist *Ps. volvocis* gezeichnet.

Notice