Kleinere Mitteilungen.

Notes on some new or little known Protococcales.

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(With Plate 28.)

1. *Dichotomococcus capitatus*, n. gen. et sp.

(Plate 28, Figs. 1—2.)

This alga was found in the plancton of a small shallow pond ("Gigirev-pond") in the environs of the Hydrophysiological Station of the Moskow Institut of Experimental Biology on the river Moskow near Zvenigorod. It developed there in considerable abundance in August 1927, while in the same month of 1926 it was absent in this basin. The samples were taken with the plancton net and kept in the laboratory in a jar, where all immotile organisms concentrated at the bottom. In these conditions the alga in question felt fairly well for several days, but later on began to die off. In hanging drop preparations only very slight development took place.

The structure of the organism is very simple. The cells are elongated and rather variable in shape, of which the best notion is given by Figs. 1—2 Pl. 28. In general they are club-shaped, consisting of a thicker main part more or les obtusely rounded at one end and sharply narrowed at the other end, that looks as an appendix on the cell, usually somewhat capitate at the end. Often this appendix is bent aside and the whole cell may also be somewhat

curved. A slight constriction may often be seen at the thick end of the cell too. The whole length of the cell is 7—9 μ, width 2—2,5 μ.

The protoplast contains the chromatophor in form of a thick lateral plate extending through the whole length of the thicker part of the cell. Pyrenoid is absent in *Dichotomococcus*. The rest of the protoplast ist occupied by the colorless protoplasm with the nucleus, which latter however is indiscernible in living cells.

The cells multiply by division into two, which is preceded by division of the chromatophor (Fig. 2). The very process of the cell division and formation of the daughter-cells was not followed up, but there is no doubt, that here autospores are produced, transforming further into young daughter-cells when probably still in the mother-cell wall.

The cells of *Dichotomococcus* are joined together into loose colonies, which is due to the persisting old cell-envelops forming a regular system of dichotomically diverging branches. The origin of the colony and its growth take place in the following way:

An originally solitary cell divides into two. The daughter cells come out through a longitudinal split in the mother cell wall and remain to be loosely attached to the middle of the latter diverging at certain angles to one another. After these two cells have in their turn produced two pair of cells of the third generation, their empty walls remain adhering to that of the primary cell of the young colony each bearing in its middle two cells of the last generation, as shown in Fig. 1. All the cells of the colony divide apparently simultaneously and thus a regular and symmetrically built colony arises, all the constituting cells being of one and the same generation. Fig. 2 represents such a colony with the cells of the sixth generation. Sometimes four daughter-cells may be produced (Fig. 2 at *a*), but this is an utmost rare case.

Old cell-envelops undergo no subsequent gelatinisation, nor is there a general slimy mass surrounding the colony, as in *Dictyosphaerium*. The colonies can thus fall into separate parts giving rise to new colonies. This proceeds very easily, and it is very seldom that the more complex colonies are met with than that in Fig. 2.

Any other developmental stages were not observed.

As to the taxonomical position of *Dichotomococcus*, it may be included into the family Selenastreae Brunnth. (Coelastraceae Printz) among which *Dictyosphaerium* and *Dimorphococcus* will be the nearest types to it. It is however a noteworthy peculiarity of

Dichotomococcus that its cells are usually capable of bipartition only, which results in a somewhat deviating structure of the colony.

Taking the general structure of the colony and form of the cells for generic characters, one may give the following diagnoses of the new genus and species:

Dichotomococcus, n. gen.: Cells elongated, covered with thin adherent, not gelatinised walls, with a chromatophor deprived of pyrenoid, and with a nucleus. Assimilat starch. Multiplication only by autospores arising normally two in a mother cell. Daughter cells, liberated through the longitudinal split in the mother cell wall, remain attached to the latter, to form a loose colony consisting of a system of dichotomically disposed empty cell walls, the youngest of which bear the cells of the last generation. There is no general gelatinous envelope round the colony, which can thus fall into parts and so multiply. Resting cells, zoospores, or gametes are unknown.

D. capitatus mihi: Cells elongated, 7—9 μ long, 2—2,5 μ broad, obtusely rounded and often somewhat narrowed or slightly consticted at the attached end, and at the free end sharply narrowed into a short, usually capitate and bent aside appendix. The whole cell may also be slightly curved, or at least asymmetrical. Chromatophor plate-shaped, lateral, extending throughout almost the whole length of the thick part of the cell. Living cells, or empty walls, are attached to the middle of their mother cell wall on its split side.

Found in the plancton of a shallow pond, in Moskow district.

2. *Bernardinella bipyramidata* Chodat.

(Plate 28, Figs. 3—10.)

This curious alga has as yet been found only in Switzerland by Chodat, who in 1920 gave the first brief account of it [1]). The structure and development of *Bernadinella* having not been studied, its taxonomical position has remained the subject of conjectures. Chodat was of the opinion that *Bernardinella* belongs to the Heterocontae, which view was followed by Pascher [2]) and Printz [3]).

In his more recent notice on *Bernadinella* Chodat [4]) writes as follows:

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[1]) Chodat, R.: Algues de la Region du Grand St.-Bernard. Bull. de la Soc. Bot. de Genève 1921 p. 300.

[2]) Pascher, A.: Süßwasserflora, Heft 11, Heterocontae p. 55.

[3]) Printz, H.: Chlorophyceae in „Nat. Pflanzenfam." 1927 p. 393.

[4]) Chodat, R.: Observations faites à la Linnea 1923—25. Bull. de la Soc. Bot. de Genève 1925 p. 206.

„J'ai de nouveaux observé cette algue rare cette année, provenant cette fois-ci des eaux tourbeuses de Champex d'en-Bas. Il s'agit d'eaux qu'on exprime du Sphagnum dense et demitourbifié. Cette fois-ci, on pouvait bien observer le plastide vert, dépourvu de pyrénoide et le gouttelettes huileuses. On voit souvent, entre la membrane distante et singulièrement sculptée et le contenu arrondi, une espèce de la gelée brunâtre. Dimensions 9—11 μ. Le genre *Bernardinella,* comme *Aurantiella* et les Chrysostomatacées, représentent sans doute des états quiscents de Flagellées colorées."

The nature of *Bernardinella,* and consequently its taxonomical position, remain thus quite obscure. I think therefore that my own observations on this enigmatic organism will be of interest, in spite of their incompleteness in some points.

I happened to find *Bernardinella* in the nearest environs of the Zvenigorod Hydrophysiological Station, namely in the marginal zone of a Sphagnum-bog (so-called "Lutzino-Moor"), in August 1927. Its development was a very slight one, which rendered it rather difficult to find it out of the mass of other algae and detritus. Therefore, in spite of my efforts to study this organism throughout its life-history, this task remained unaccomplished. I succeeded however in obtaining data as to the most important points: cell structure and method of multiplication, which permits to make out the taxonomical position of the organism.

The characters of the alga from my samples are in good accordance with those given by Chodat. It may only be mentioned, that the number of longitudinal ribs on the envelope was somewhat greater than that indicated by Chodat, namely 8—10 instead of 5—6. As regards the shape of the organism, it is rather a variable one, especially in point of the ratio between length and width, and the form of the middle part of the cell, variations being largely due to differences in age. For details I refer to my Figs. 3—6. Owing to the ribs the cell is star-shaped in the pole view (Fig. 4). In adult individuals envelope is to 30 μ long and to 18 μ broad, cells 9—12 μ in diameter.

The outer envelope of the cell consists of two equal bell-shaped halves, joined by their margins so strongly as not to be separated by pression or in any other way. The cause of the reddish brown tint of this envelope is not understood. One could suspect the presence of iron-hydroxyd there as was the case with many other algae in that basin. But the walls of these latter were not of such a reddish tint. Microchemical analysis could not be made due to

scantiness of material. As to the ground substance of the envelope, it appears to be of pectic matter, judging from the characteristic orange coloration when treated with safranin solution.

The line of junction of both halves of the envelope is very distinct and makes an impression of an equatorial diaphragm inside the envelope, in the middle of which the very cell is placed. This latter however lies freely within its investment, probably surrounded by an aqueous mucilage, responsible for its always central position. A thin and colourless wall is to be seen round this cell closely adhering to the protoplast. There are thus two envelopes in *Bernardinella*, only the inner one being an actual cell wall according to its position and function.

The protoplast contains a peripheral, wall-sided, green chromatophor, with a pyrenoid in a large thickening protruding inwards. Assimilat is starch in form of small granules in the stroma of the chromatophor and round the pyrenoid. Judging from an optical section of the chromatophor, the latter is not entire, but has narrow slits or, which is more probable, is partially subdivided into lobes (four in number?), in which case the chromatophor will be cup-shaped, with an opening at the pole opposite to the pyrenoid. I could not establish any constant position of the pyrenoid relative to the long axis of the outer envelope.

There is evidently only one nucleus in *Bernardinelli*, but in living cell it was not to be discerned through the thick walls of the chromatophor. Contractile vacuoles are absent.

Such is my knowlege of the structure of *Bernardinella*, obtained by studying living objects. As to the staining methods, they were unapplicable to my utmost poor material. This latter circumstance hindered me from following up all stages of development. At the beginning I hoped to gain something by examining a large number of preparations, but all the individuals I found were in a more or less mature condition, and only once I met with a group of four young cell lying freely admidst other algae and obviously being descendants of a single mother cell (Fig. 5). After long searching I made an attempt to trace up the multiplication on one and the same individual, in which I failed, however. For this purpose I chose a particularly large cell (Fig. 6) in a preparation closed with paraffin. This cell was 12 μ in diameter and the chromatophor was clearly divided into four parts: in the equatorial plane and longitudinal one. Pyrenoids were there invisible, probably due to abundance of starch in the chromatophor. The conditions in such

a preparation were not very oppressive judging from the fact that many other organisms there were capable of multiplication. *Bernardinella* was however hindered in its further development. So, on the third day the only change observed was that in the dimensions of the cell, that had grown so as to fill up the whole outer investment (Fig. 7). The four chromatophores were quite close to one another and now a pyrenoid was clearly seen in each of them. The outer investment was slightly distended by the cell within it and deprived of its former characteristic colour. Several days afterwards it disappeared quite, and in the cell, that became still larger and rounded, many small rounded polygonal chromatophors were seen each bearing a pyrenoid placed close at the inner surface of the chromatophor. Fig. 8 shows the superficial view of the cell, and Fig. 9 its optical section. What became of the nucleus I could not establish due to the untransparency of the cell contents.

During the following next days no noteworthy changes occurred, and since the conditions of existance in the preparation became obviously worse further observations were discontinued. I was thus induced to return to the former method of collecting daily fresh material and searching for any critical stage of development. Finally I succeeded in finding such a stage, which is represented in Fig. 10. There are seen eight quite young individuals surrounded by a general almost indiscernible colourless mucilagineous vesicle deriving obviously from the strongly distended and gelatinised outer envelope of the mother cell. Each of the daughter cells was already surrounded by its own vast envelope having assumed its characteristic bipyramidate form but yet colorless. The inner walls of the cells were still not secreted and the protoplasts lay naked inside their outer investments. It is clear that these latter were formed at the earliest stage of the development of the reproductive cells, but the very method of their formation was too late to be observed.

As regards the protoplasts, they were shortly ellipsoidal in form, the long axis lying mostly in the equatorial plane of the envelope. In some cells the protoplast was also slightly compressed in the direction of the axis of the outer investment, appearing elliptical from the side and round in the polar view. In each of them was seen a thick cup-shaped chromatophor disposed mostly on the broad side of the protoplast and furnished with a small elliptical pyrenoid. In mature cells the structure of the pyrenoid was difficult to see, but in those young cells the starch envelope round the pyrenoids consisted of two valves, the plane of their contact being parallel

to the long axis of the cells. As to the nucleus, it was indiscernible in living cells. In each protoplast were seen one or two pulsating vacuoles situated at the margin of the chromatophor. After some time they disappeared and simultaneously an utmost thin membrane was secreted by the protoplast, to become later an actual cell wall.

The further fate of these cells was not of interest to be followed up. Of course, it would have been exceedingly interesting to find some earlier stages of multiplication in order to observe the structure of reproductive cells and the formation of outer envelopes round them. But this proved practically impossible.

My observations on *Bernardinella*, though not complete, give a sufficient base for the judging of taxonomical position of this organism, which doubtless is identical with *Bernardinella bipyramidata* as it was described by Chodat. We see that Chodat's description though quite insufficient, is correct in that there was no contraction of the protoplast in the cells from his material, as Pascher supposes; the condition in which the organism was described was not a resting one however. Here we are dealing with an utmost original type and must find an adequate place for it in the system of other organisms.

It is evident that now there is no ground to rank *Bernardinella* among the Heterocontae, as it was done by Chodat, Pascher and Printz, because assimilat in *Bernardinella* is not oil, but starch stored directly in the chromatophor and round the pyrenoid. That the outer envelope consists of two parts, as in some Heterocontae, is a feature of little importance in this question. We have here only a convergency, as in the case of double walls in the Desmids and Diatoms.

The proper place of *Bernardinella* is among the Protococcales. In possessing a characteristic bipyramidate and ridged envelope it resembles much *Calyptrobactron indutum* recently described by Geitler [1]). These two organisms are in general so similar in structure, that they might be considered as representatives of the same genus, in which case the name of *Calyptrobactron indutum* ought to be changed into *Bernardinella induta*. But there is a feature in *Bernardinella* that prevents us to do so, namely the method of multiplication. Though I did not see its reproductive cells, but only

[1]) Geitler, L.: Über *Acanthosphaera Zachariasi* und *Calyptrobactron indutum* nov. gen et sp., zwei planktonische Protococcaceen. Österr. bot. Zeitschr. 1924.

those on the way of their transforming into young cells, I think that they are not zoospores, as is the case with *Calyptrobactron*. This is seen from the following facts. Firstly, young cells are from the very beginning surrounded with a strongly expanded envelope, which is probably nothing but an outer envelope of their mother cell, the inner wall of the latter being converted into aqueous mucilage. I did not observe the very process and a priori it is not impossible that the case is a reverse one: the outer envelope disappearing and the inner one transforming into the mucilagineous vesicle with daughter cells within it. But it is of no importance for our considerations: in either case the fact is that reproductive cells are not set free here, while this ought to have occurred, if the reproductive cells were motile. Secondly, the daughter cells I observed were deprived of stigmata notwithstanding that they were so young that contractile vacuoles were still preserved in them, whereas I newer saw the stigma to disappear, by germination of zoospores, earlier than the contractile vacuoles. On the contrary, the stigma is preserved sometimes for a very long time, as for instance in *Characium ocellatum* Korsch.[1]), or in Oedogonium. The stigma is accordingly absent in the reproductive cells of *Bernardinella*. If it is so, these latter may hardly be expected to be zoospores, since cases of the absence of the stigma in zoospores are quite exceptional.

There is thus a very great probability that *Bernardinella* multiplies by means of immotile reproductive cells, which hinders us from uniting *Calyptrobactron* with *Bernardinella*, similar as they may be in other points.

The structure of reproductive cells in *Bernardinella* is of much theoretical interest. The Protococcales are known to multiply by two methods: by zoospores and by autospores. This led Brunnthaler[2]) to divide the Protococcales into two groups: Zoosporinae and Autosporinae. This is not very convenient from the practical point of view, because there is a series of forms obscure with respect to their method of multiplication. But from the theoretical stand-point the above division is very adequate, because the two above groups correspond to two subsequent stages of phylogenetical development of the Protococcales, autospores being treated as reduced zoospores.

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[1]) Korschikow, A. A.: Protistologische Beobachtungen. Arch. Russes de Protistol. III 1924 p. 67.

[2]) Brunnthaler, Jos.: Pascher's Süßwasserflora, Heft 5 Chlorophyceae 2, 1915.

There may however be reproductive cells not to be qualified as either zoospores or autospores, and representing a something intermediary between these two cathegories. So, in 1915 I found [1]) that *Glaucocystis Nostochinearum* Itz. multiplies by forming, inside the mother cell, four or two naked daughter cells provided with contractile vacuoles but without any cilia and transforming there into daughter individuals. With respect to the type of reproductive cells, *Glaucocystis* may be considered as intermediary between zoosporic *Gloeochaete* [2]) and autosporic *Asterocystis*, for instance. The life-history of *Gloeochaete* strikingly resembles that of *Apiocystis*: in both genera the cells are capable of vegetative divisions leading to the growth of the colony, but under certain conditions they come out in the form of biciliate zoospores, in which way propagation takes place.

Thus, in the group of the Glaucophyceae we have a subsequent passing over from zoosporine to autosporine forms through the structural reduction of zoospores.

The existance of zoospores with reduced cilia was with full certainty shown, for *Marthea tetras* Pasch. (Protococcales) by Pascher [3]), who gave an utmost coherent picture of the elaboration of the autosporine forms from the zoosporine ones. Pascher's detection was the more interesting, that it showed a remarkable parallelism between the phylogenetical development of the Glaucophyceae and that of the Isocontae with respect to the method of multiplication, while until then only morphological convergencies of the vegetative stages were known (*Apiocystis* and *Gloeochaete*, *Glaucocystis* and *Oocystis*, *Chroocystis* and *Hormotila*, etc.).

In view of such analogies as *Marthea* and *Glaucocystis*, there may be little doubt that typical motile zoospores in *Bernardinella*

[1]) Korshikov, A.: Contribution à l'études des algues de la Russie. Travaux de la Stat. biol. "Borodinskaja" Vol. 4 1917. In this paper, apparently neglected by subsequent authors dealing with the Glaucophyceae (Chodat, Geitler, West), I have supposed *Glaucocystis* to produce zoospores (see French résumé), considering it not inprobable, "that in some cases zoospores may form no cilia, but contractile vacuoles only, in which case they do not come out of the mother cell wall" (l. c. p. 253). Now I have no doubt that just this second assumption is correct; as to the formation of true zoospores, it is rather not to be expected.

[2]) Geitler (in Arch. f. Protistenk. 1923) considers that zoospores in *Gloeochaete* are reduced. According to Dangeard (Le Botaniste, ser. I 1889), whose observations were confirmed by mine (l. c. 1917), this alga is capable of producing zoospores, Geitler's view being thus incorrect.

[3]) Pascher, A.: Amoeboide Stadien bei einer Protococcale, etc. Ber. d. d. bot. Ges. 1918 p. 259.

are actually absent and replaced by somewhat reduced homologous structures. It is of interest, that reduction goes here still farther that in *Marthea*. According to PASCHER, reproductive cells of the latter still have both stigma and contractile vacuoles. Those of *Bernardinella* are deprived of stigmata, and need only lose contractile vacuoles to transform into true autospores.

We have also to note the interesting similarity between the phylogenetical history of the reproductive stage and that of the vegetative one. Taking into account PASCHER's considerations, the succession in the elaboration of the reproductive stage is such: *Chlamydomonas*-like zoospore (*Macrochloris* KORSCH.) — naked zoospore (*Chlorococcum*) — arrested zoospores (*Pediastrum*) — not-ciliated zoospores (*Marthea*) — zoospores with reduced cilia and stigmata (*Bernardinella*) — autospores (*Chlorella*). A similar line in the evolution of the vegetative part of the life cycle is represented, with some deviations, by *Chlamydomonas* — *Chlorangium* or *Gloeodendron* (both stigma and vacuoles presenting) — *Hypnomonas* (only vacuoles presenting) — *Chlorococcum*.

As to the position of both *Calyptrobactron* and *Bernardinella* in the system of the Proctococcales, the question seems to be not so obscure. I do not consider *Calyptrobactron* to belong to "ungenügend bekannte Gattungen von unsicherer systematischer Stellung", as PRINTZ (l. c.) writes. It is a true Protococcacea to be ranked among the Chlorococcaceae, according to its method of multiplication by zoospores. As regards *Bernardinella*, it occupies, like *Marthea* but in the series of unicellular forms, an intermediary position between the Zoosporinae and Autosporinae, with the nearest relations to *Calyptrobactron*, and consequently may also be placed into the same family, Chlorococcaceae, the same as *Marthea* found its place among the Coelastraceae in the system of PRINTZ. It is true that both genera will be in a rather isolated position there, and it might thus be reasonable to separate them into a new subfamily: Bernardinelleae, to solve the question of the taxonomy of both forms.

As to the relationship between *Bernardinella* and *Desmatractum* W. & G. S. WEST [1]), there is nothing to say until the latter is studied more precisely.

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[1]) WEST & G. S. WEST: Contribution to freshwater Alga of Ceylon. Transact. Linn. Soc. 2. ser. Bot. Vol. 6 1902 p. 198.

Explanation of Plate.

Plate 28.

All the figures are made by use of Abbe's drawing-apparatus from living objects.

Figs. 1—2. *Dichotomococcus capitatus* n. gen. et sp.

Fig. 1. Young colony of four cells of the second generation, of which two with chromatophor divided into two and one (right hand below) with chromatophor dividing into four. 1500 ×.

Fig. 2. Large colony; at *a* a group of four daughter-cells. 1500 ×.

Figs. 3—10. *Bernardinella bipyramidata* Chod.

Fig. 3. Mature cell, side view. 1000 ×.

Fig. 4. The same, nearly apical view. 1000 ×.

Fig. 5. Group of four young cells. 1000 ×.

Fig. 6. Very large cell with chromatophor divided into four. 1000 ×.

Fig. 7. The same cell after $1^1/_2$ days. 1000 ×.

Fig. 8. The same cell on the fourth day. 1000 ×.

Fig. 9. The same, optical section. 1000 ×.

Fig. 10. Eight very young daughter cells still surrounded by mother envelope and with contractile vacuoles. 1500 ×.

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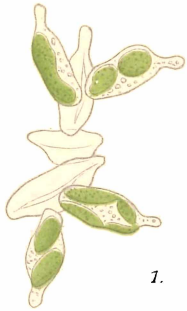

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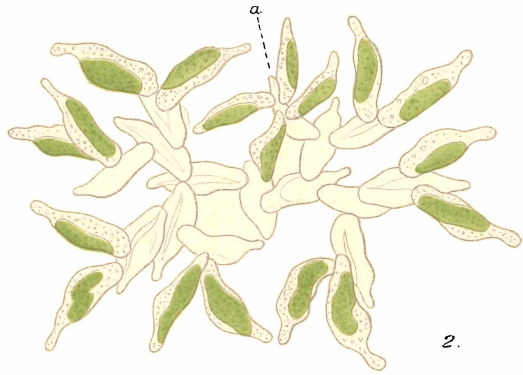

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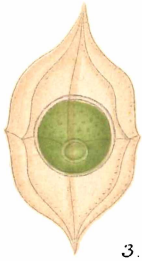

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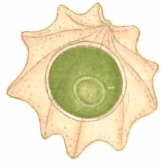

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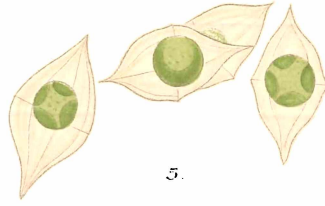

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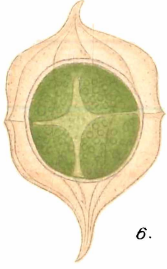

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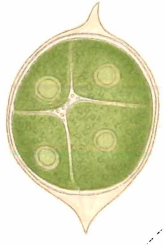

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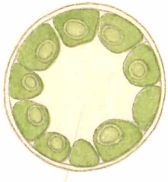

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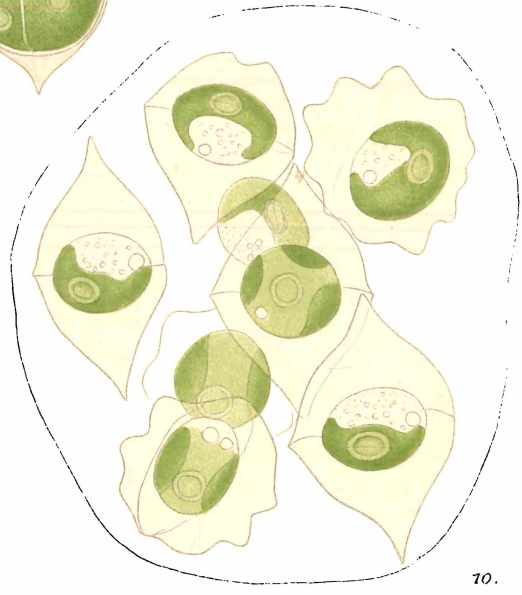

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