THE LIFE-HISTORY OF SPHACELARIA FURCIGERA KÜTZ. (PHAEOPHYCEAE)

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SUMMARY

Cultural and caryological investigations on *Sphacelaria furcigera* from Hoek van Holland give evidence of a slightly heteromorphic diplohaplontic life-history in this species. A relatively slender (13.5—31 μ) haploid gametophytic phase alternates with a more robust (19—41 μ) diploid sporophytic phase. Female gametophytes form plurilocular macrogametangia at 12° C and 4° C, male gametophytes form plurilocular microgametangia at 12° C and 4° C. Zygotes of female macrogametes and male microgametes grow into diploid sporophytes which form unilocular meiotosporangia at 4° C. Meiosis takes place in the initials of these sporangia.

About 50 % of the spores produced by them grow into male gametophytes, about 50 % into female gametophytes. Propagules are formed at higher temperatures, i.e. at 12° C and 20° C.

In nature gametangia are formed during the winter half of the year, and propagules during the summer half of the year.

Female gametes can develop parthenogenetically into new female gametophytes, but also into haploid plants forming unilocular zoidangia instead of plurilocular macrozoidangia. Such plants are repeatedly formed and represent dead ends in the life-history of *S. furcigera*. The result of our investigations are summarized in fig. 4.

Sphacelaria britannica Sauv. and *S. saxatilis* (Kuck.) Kuck. *ex* Sauv. are synonyms of *S. furcigera* Kütz.

1. INTRODUCTION

On the basis of incomplete observations the life-history of the *Sphacelariales* and of *Sphacelaria* in particular is generally inferred to be *isomorphic diplohaplontic* (cf. Fritsch, 1945, p. 291; Papenfuss, 1951, p. 125; Chapman, 1962, p. 137; Chadefaud, 1960, p. 292; Boney, 1966, p. 67).

The evidence regarding the life-history of *Sphacelaria* is given by Clint (1927) and Papenfuss (1934). Both authors investigated *Sphacelaria bipinnata*.

Clint investigated plants bearing plurilocular and unilocular zoidangia. She considered the zoids produced by the plurilocular zoidangia diploid. Their development could not be followed. She gave caryological evidence of meiosis in the initials of the unilocular zoidangia, whose zoids were observed to fuse among each other. The further development, however, of these fused zoids was not observed.

Papenfuss was not able to confirm the fusion of zoids produced by unilocular zoidangia. Such zoids in his material grew into germlings that died at the unicellular stage.

According to Papenfuss, some plants with unilocular zoidangia also bore plurilocular zoidangia whose zoids did not fuse. He interpreted plants bearing only plurilocular zoidangia as haploid gametophytes although he did not investigate their chromosome-number. Zoids produced by these presumptive gametophytes were observed to fuse, and such fusions were interpreted as evidence for a sexual, homothallic, isogametic process.

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It is possible to combine Clint's and Papenfuss's observations into the image of the isomorphic diplohaplontic life-history which is generally accepted as the 'fundamental life-history' of the *Phaeophyceae*. However, important details are lacking to make this picture fully acceptable.

In figure 1 facts and hypothetical connections are diagrammatically represented. The vegetative multiplication by propagules is not considered in the figure.

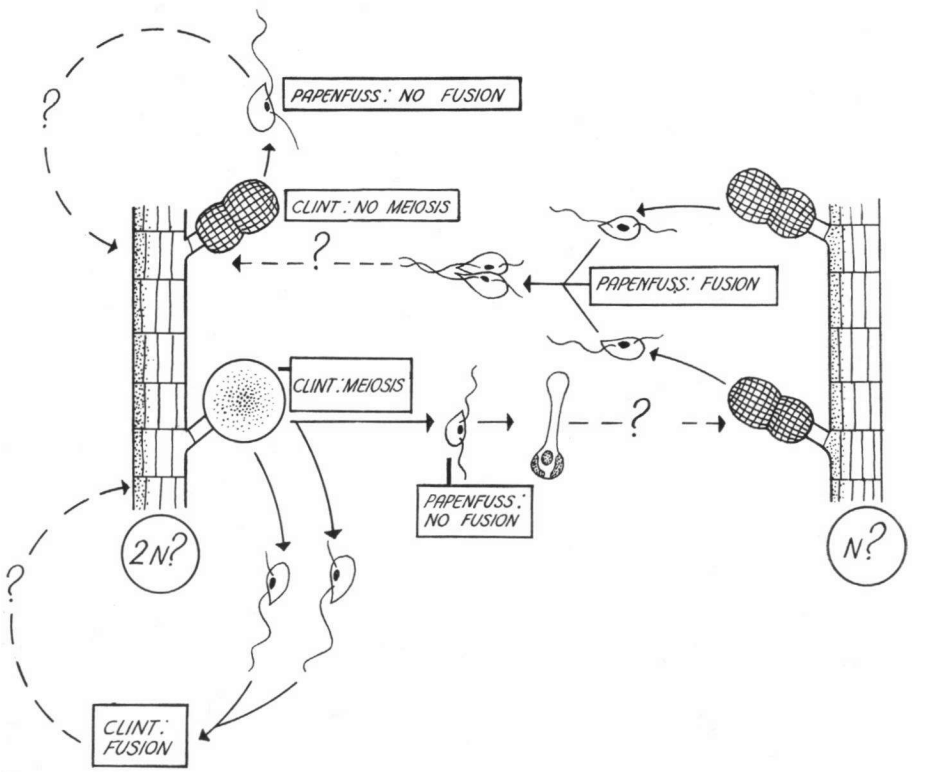

Fig. 1. Life-history of *Sphacelaria bipinnata* as this is generally conceived, after data of Clint (1927) and Papenfuss (1934). Query-marks indicate missing evidence.

New cultural and caryological investigations into life-histories of *Sphacelariales* were evidently needed. We succeeded in obtaining a reasonably complete picture of the life-history of *Sphacelaria furcigera*. For this success it was necessary to culture our material at three different temperatures. Müller (1962) found that *Ectocarpus siliculosus* plants (Mediterranean material) cultured at 20° C formed plurilocular zoidangia, plants cultured at 10° C formed unilocular zoidangia, and plants cultured at intermediate temperatures formed both types of zoidangia. He also found that lower light intensities clearly stimulated the formation of unilocular zoidangia and relatively inhibited the formation of plurilocular zoidangia. Müller's data suggested experiments to test the existence of comparable correlations in the life-history of *Sphacelaria furcigera*.

2. MATERIAL AND METHODS

2.1. Material.

A unialgal culture of *S. furcigera* was isolated in the spring of 1960 from material collected at Hoek van Holland. In March 1965 a stock-culture of this isolate kept at 12° C and under reduced light-intensities appeared to contain many very young germlings growing attached to the glass of the culture vessel (a conical flask) at the water-air interface. Since this stock culture bore unilocular zoidangia (figs. 37a, b) it could be reasonably assumed that the germlings had developed from zoids produced by the unilocular zoidangia.

25.III.1965 a number of germlings were isolated and cultured in culture tubes containing Erdschreiber medium (prepared as in van den Hoek, 1963, p. 12). Ten germlings were grown in each of three constant temperature cabinets at 20° C, 12° C, and 4° C resp. The cultures were illuminated by white fluorescent tubes (Philips TL de luxe 25 W/34) at a distance of 20 cm, and were exposed, during daily photoperiods of 12 hours, to light intensities of 1000—3000 lux at 20° C, 900—2400 lux at 12° C, and 600—1700 lux at 4° C.

Only two of the thirty germlings grew into mature plants, all other germlings died. One germling kept at 12° C appeared, by September 1965, to have grown into a plant bearing plurilocular macrozoidangia (loculi 6—10 μ in diameter) (figs. 21, 45) and another germling kept at 4° C appeared, by June 1966, to have grown into a plant bearing plurilocular microzoidangia (loculi 3.5—7.5 μ) (fig. 46). Both plants with plurilocular zoidangia were vegetatively propagated in order to have sufficient material available for further investigations. Also, zoids released from the plant bearing plurilocular macrozoidangia were grown at 12° C, initially on the watch-glass in which the zoidangia had sporulated. Subsequently these germlings were reared to maturity in culture tubes also kept at 12° C; in a period from 15.XII.1965—7.III.1966 many of them had grown into plants with plurilocular macrozoidangia, while others had grown into plants without plurilocular zoidangia but with propagules.

Thus five types of plants were available for experiments:

Type 1: plants from stock-material with ability to reproduce by unilocular zoidangia.

Type 2: plants obtained by vegetative propagation of a germling grown from a zoid released by a unilocular zoidangium of type 1. Type 2 plants are able to produce plurilocular macrozoidangia.

Type 3: the same as type 2, but bearing plurilocular microzoidangia.

Type 4: plants grown from zoids produced by type 2 and bearing also plurilocular macrozoidangia.

Type 5: plants grown from zoids produced by type 2 and without plurilocular macrozoidangia, but with propagules.

2.2. Experiments to test the influence of temperature and light-intensity on the formation of the reproductive bodies.

Fragments of plants belonging to the types 1, 2, 4, and 5 mentioned under 2.1 were cultured at 4° C, 12° C, and 20° C under two different ranges of light-intensities (600—1700 lux and 200—600 lux at 4° C; 900—2400 lux and 250—700 lux at 12° C; 1000—3000 lux and 500—900 lux at 20° C). Under each of these six combinations of temperature and light conditions 10 fragments of each type (1 fragment per culture tube) were grown so that in all these were 60 fragments of each type making a total of 240 fragments. In addition, fragments of stock material and of macrozoidangial

material (types 1 and 2) were cultured at 20° C and 12° C under very reduced light intensities: 3—10 lux.

The light intensity was simply regulated by covering the culture tubes with one or more layers of white paper.

2.3. Testing the influence of emersion on the formation of reproductive bodies and on the morphology of the plants.

Fragments of plants belonging to types 1 and 2 were cultured on discs of filter-paper covering the bottom of slightly sloping petri dishes. These petri dishes were filled with medium so that one half of the bottom was immersed and one half emersed. On both the immersed and emersed half a fragment was deposited. Sets of two petri dishes, one for type 1 and another for type 2 material, were cultured at 4° C, 12° C, and 20° C.

2.4. Copulation experiments.

Massive release of zoids from mature zoidangia can easily be obtained at the start of the light period. It is useful, therefore, to make the light period start at 9 a.m. Release of zoids is further stimulated by a sudden increase in temperature (e.g. when cultures kept at 12° C and 4° C are brought, for investigation, into a room of 20° C and/or by the light-source of the microscope).

Plants with mature zoidangia were observed in watch-glasses under the binocular dissecting microscope (magnification up to 64 ×). Dense swarms of zoids gathered at the light margin of the sterile sea-water contained in the watch-glass; these swarms were searched for copulations and 'clumping' ('*Gruppenbildung*'). In addition plants with mature zoidangia were observed under the high-power binocular microscope.

To prevent evaporation of the sea-water in which the plants were immersed the coverglasses were sealed off with vaseline. Observations under higher magnifications give a better insight into the fusion of zoids, whereas observations under the binocular dissecting microscope are more suitable for the detection of 'clumping'.

Observations under higher magnifications were made on plants releasing their zoids and on groups of zoids picked out from clumping swarms by micropipetting. Mostly these plants or zoids were embedded in a drop of Sodium-alginate solution (2 % in sea-water) to restrict the mobility of the zoids.

The following combinations were tried:

a) macrozoids from different plants (several plants bearing plurilocular macrozoidangia together in one watch-glass);
b) microzoids from different plants (several plants bearing plurilocular microzoidangia together in one watch-glass);
c) macrozoids and microzoids (several plants of both types together in one watch-glass);
d) zoids from unilocular zoidangia from different plants (several plants together in one watch-glass).

These combinations were repeated 5 times.

2.5. Rearing of new generations from unfused and fused zoids.

To be absolutely sure about the fate of zoids and copulants it would be necessary to pick them out singly by micropipetting and then culture them separately. This is very difficult because the zoids, particularly the microzoids, are so small and move very fast. Moreover 'fusion' cannot be unequivocally interpreted as 'copulation'. Only a

fusion resulting in a zygote that gives rise to a diploid generation, or that divides meiotically, should be considered a copulation. Therefore an indirect method had to be employed to detect the fate of zoids and 'copulants'. This method consisted of putting the watchglasses mentioned under 2.4. a—d into culture glasses with erdschreiber after swarming had occurred. But first, the parent plants were removed and the culture rinsed with sterile medium so that only the zoids and copulants that had attached themselves remained. Combined observations on morphology and caryology of the offspring permitted inferences to be made about the possible sexual nature of observed fusions and clumpings.

On the assumption that plants with plurilocular macrozoidangia were female and those with plurilocular microzoidangia male the percentages of both types in the offspring of plants bearing unilocular zoidangia were determined.

Quite a few unilocular zoidangia released their contents as one spherical 'monospore'. These 'monospores' could be easily picked up with a micropipette and cultured separately in culture tubes. Also aggregates of zoids from unilocular zoidangia were separately cultured. The cultures mentioned under 2.5 were all kept at 12° C.

2.6. Microscopic observations on living zoids.

Living zoids could be almost immobilized and hence easily kept under observation in a 2 % solution of Na-alginate in sea-water. Before use this solution must be filtered through filter-paper in a Büchner-funnel, to remove impurities from the commercially available alginate.

2.7. Caryological methods.

Whole mounts were stained with acetocarmine and embedded in phenol-balsam, according to the method of von Stosch (1952) with only slight alterations. This procedure comprises the following steps:

a) Fix in methanol-acetic acid 3 : 1 (½—1 hour);
b) Preserve in 96 % ethanol for at least 24 hours, to remove lipids and pigments;
c) Transfer, through progressively diluted ethanol, into 10 % iron alum for mordanting (½—¾ hour);
d) Stain (after rinsing in aquadest) in acetocarmine (2 hours in cold acetocarmine or ¼—½ hour in a water bath near 100° C);
e) Transfer into 50 % acetic acid; if necessary, differentiate under slight and careful heating;
f) Transfer over dilutions, into phenol (15 % water in phenol); steps a—f can be accomplished in one small glass tube (e.g. 1 cc), by removing and adding fluids with a Pasteur-pipette;
g) Mount in a small droplet of phenol (15 % water in phenol) on slide; apply a cover slip;
h) Surround cover slip on three sides with a narrow band of phenol-balsam; the phenol-balsam will gradually replace the phenol.

For the preparation of the phenol-balsam and for other details see von Stosch (1952). The chromosomes are stained deep red against a pale background.

Mitoses in the apical cells are not very suitable for counting chromosomes as the metaphase is always seen from its side. Good counts can be made in the longitudinally dividing upper and lower secondary segments.

Plants bearing initials of unilocular zoidangia were fixed and stained for the caryological demonstration of meiosis. Such whole mounts are also very useful to make observations on the internal structure of the plurilocular zoidangia and their primordia.

The cell-walls are stained faintly red by acetocarmine, the cell contents are made transparent by the removal of lipids and pigments, and the relatively high refractive index of phenol-balsam permits precise observation in one optical plane, if the object is not too thick.

3. RESULTS

3.1. Influence of the temperature and the light-intensity on the formation of the reproductive bodies.

The temperature appeared to have a very marked influence on the type of reproductive bodies formed. No difference, however, was observed in the results of the cultures kept under different light-intensities other than the very low ones from 3—10 lux. Therefore, the results of the cultures grown under both ranges of light-intensities first mentioned under 2.2 are combined and diagramatically given in figures 2 and 3.

Cultures kept at 12° C and 20° C under very reduced light-intensities (3—10 lux) grew extremely slowly and after 9 months showed only vegetative growth. These cultures will not be further considered.

When small fragments are put into fresh culture fluid, resting initials (generally in the upper secondary segments[1]) start to grow into new shoots. These shoots consist at first of an axis which later gives off primary laterals in a rather indistinct acropetal sequence (figs. 39—42). The laterals almost invariably arise from upper secondary segments, though sometimes this generally strict organisation may skip one segment (fig. 41, arrow). When these laterals are still young, they are much shorter than the axis. They are, however, of indeterminate growth and later have a development comparable to that of the axis (fig. 5). The relatively thick and long apical cells are expression of the considerable vegetative growth at this stage (figs. 39, 40).

The system as a whole is determinate and attains a maximum height which is, however, rather variable (*ca.* 3 cm; cf. de Haas-Niekerk, 1965, as *S. fusca*). The younger stages of a regenerative shoot and of plants grown from zoids develop initially into a characteristically robust axis bearing robust primary laterals (figs. 39, 40). The system, after having 'inflated' itself to a maximum width, subsequently exhausts itself in the formation of the more slender mature plant (compare, for haploid plants, figs. 39, 41, 43, and for diploid plants figs. 40, 42, 44). The diameter of diploid plants, for example, grown at 4° C reaches 30—45 μ in the young stage (fig. 40) but diminishes later to *ca.* 20—40 μ (fig. 36). For haploid plants these measurements are *ca.* 22—37 μ (fig. 39) and 13,5—32 μ (figs. 10, 11) respectively. Similar results are obtained at 12° C.

Young plants differ also from mature plants by a denser pigmentation.

Axis and laterals of the mature plants initiate, again in a rough acropetal sequence, large numbers of determinate fertile laterals.

Summarizing, four phases can be distinguished in the growth of a *S. furcigera* plant: 1) development of a robust axis, 2) first wave of lateral formation giving vegetative indeterminate laterals, 3) vegetative maturation of the system into a relatively slender axis with relatively slender indeterminate vegetative laterals, 4) second wave of lateral formation giving determinate fertile laterals. However, secondary indeterminate vegetative laterals are often formed which bear fertile determinate laterals. It depends on the temperature whether the determinate fertile laterals grow into zoidangia or propagules.

Shorter and narrower apical cells, and longitudinal septation of the subapical segments are an expression of the diminished growth of the mature system (compare for the

[1]) For terminology, see Fritsch, 1945.

diploid phase fig. 40 with figs. 6 and 7, for the haploid phase fig. 39 with figs. 10 and 11).

After having formed and emptied zoidangia, and/or produced and shed propagules, the plants degenerate at 20° C much more rapidly than at 12° C and 4° C. The cells fill with laminarin (highly refractive vacuoles) and fucosan vesicles, they grow paler, the cell-walls thicken, and the filaments start unorganized proliferation.

At about this stage a second type of vegetative reproductive bodies, termed nodules, are abundantly formed. Nodules (figs. 17—20) originate from swelling intercalary cells in both upper and lower secondary segments. The larger nuclei of these cells bear witness to their renewed division-activity (fig. 18). A nodule initial divides into a cell which remains incorporated in the filament and into a nodule-cell which subsequently septates in a rather irregular way (figs. 17, 18). In fact, a nodule shows a development comparable to that of an embryonic nodule growing from a zoid (compare figs. 17 and 18 with figs. 98 and 106—111). In both cases, one cell after swelling divides into a stout cylindrical or spherical mass of cells from which one or more axes may arise.

In contact with a solid substrate nodules may grow into irregular attachment discs (figs. 20, 12—14).

At the start of maturity the plants bear very few or no phaeophycean hairs, but by the beginning of the degenerative phase of the culture the plants bear great numbers of them (fig. 38). In our experience the rich production of phaeophycean hairs is indicative of a depleted culture-medium as it is also in a number of other *Phaeophyceae*. Furthermore, germlings growing in crowded cultures easily form phaeophycean hairs (figs. 99, 102, 105, 106). The hairs are always terminal; growth of a filament ending in a hair is continued by the cell underlying the hair-meristem and the system becomes sympodial (figs. 38, 112, 113).

3.1.1. Plants grown from fragments of stock-material: type 1 (diploid plants, cf. 3.6) (diagram of results: fig. 2).

At 20° C and 12° C the fertile determinate laterals grow into propagules (figs. 38, 42, 44), at 4° C into unilocular zoidangia (diameter 45—85 μ; zoids 9—13 μ long) (figs. 5, 36).

Vegetative apical cells of mature plants bearing unilocular zoidangia quite often show zoidangial characters: they may form apical pores through which the undivided contents are discharged (fig. 54).

Growth at 20° C is much more rapid than at lower temperatures, so that at 20° C maturity is reached within one month, at 12° C after *ca.* 3 months, and at 4° C after *ca.* 5 months (fig. 2).

Nodules are abundantly formed in the degenerative phase (see above, 3.1).

Old cultures finally consist of tubes filled with the white, dead remains of the plants and dense masses of proliferating propagules and nodules.

To obtain normal plants from these it is necessary to inoculate tubes containing fresh culture-fluid with a few of them.

3.1.2. Plants bearing plurilocular macrozoidangia: types 2 and 4 (haploid plants, see under 3.6) (diagram of results: fig. 3).

At 20° C the fertile determinate laterals grow into propagules (fig. 43), at 12° C and 4° C into plurilocular macrozoidangia (figs. 8, 9, 41). At 12° C at first only plurilocular macrozoidangia are formed but later a few propagules also appear. When plants kept at 12° C and bearing plurilocular zoidangia are transferred to 20° C the mature zoidangia release their contents while the immature ones die off. Their stipes mostly give off propagules but some produce vegetative laterals (figs. 24—27).

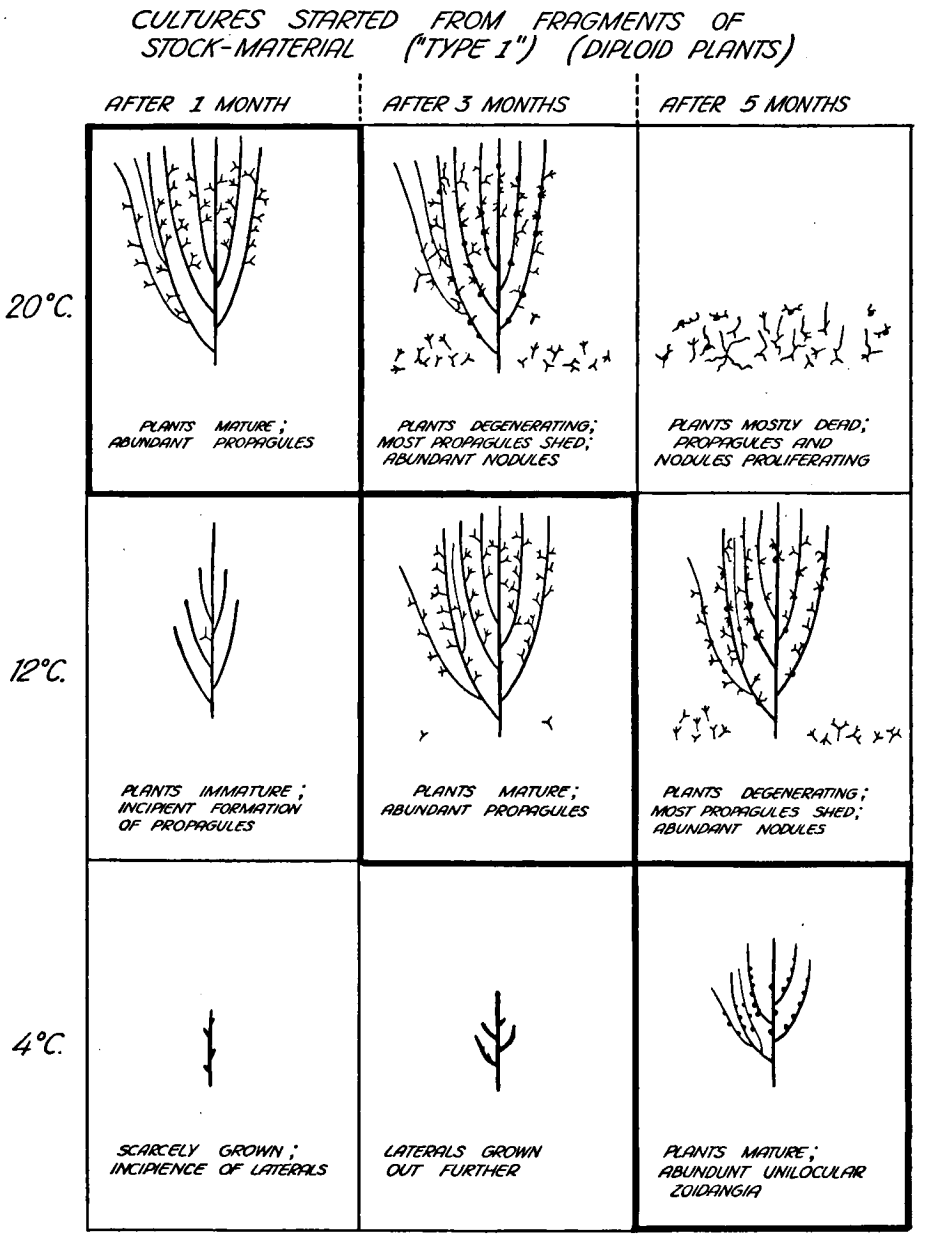

Fig. 2. Diagram of cultures grown from fragments of stock-material ('type 1') (diploid plants). Sizes of propagules and zoidangia are slightly exaggerated as compared with vegetative parts.

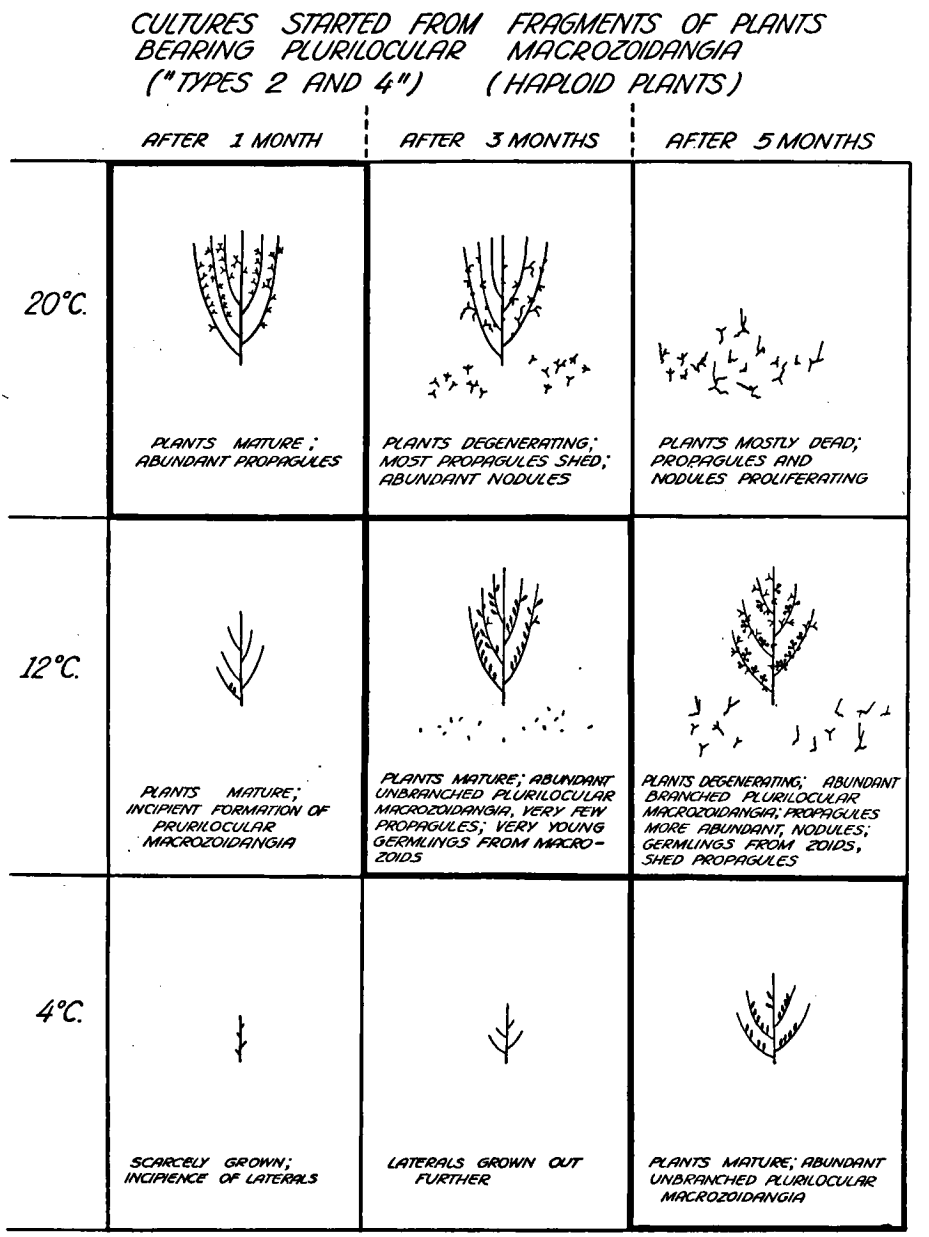

Fig. 3. Diagram of cultures grown from fragments of plants with plurilocular macrozoidangia ('types 2 and 4') (haploid plants). Sizes of propagules and zoidangia are slightly exaggerated as compared with vegetative parts.

At 4° C no propagules are formed at all.

At 20° C the plants reach maturity after about one month, at 12° C after *ca.* 3 months, at 4° C after about 5 months (fig. 3).

In the degenerative phase the cultures form nodules after having shed the propagules and/or having released the zoids.

Fertile determinate laterals of young mature plants bear one plurilocular zoidangium each (figs. 21, 41) whereas those of older, mature plants bear branched stands of plurilocular zoidangia. These stands mostly have a dichasium-like construction; below the terminal, originally single zoidangium two opposite stemcells grow into new zoidangia (figs. 143—147). Often the new lateral zoidangia grow into the emptied wall of the old terminal zoidangium. The stems of the lateral zoidangia in their turn may branch in the same way (fig. 144). Monochasium-like ramifications of the zoidangial stems, in combination with dichasium-like ones, are also possible.

The aspect of a plant bearing mainly branched zoidangial stands differs markedly from a plant bearing unbranched zoidangial stands.

Depending on their mode of origin four types of plurilocular macrozoidangia can be distinguished:

a) 'Stout cylindrical type' (figs. 21, 45, 49, 51, 150).
The zoidangium initial divides transversely into an apical and a subapical cell, these subsequently divide by a longitudinal wall. The resulting quadrants partition, by consecutive divisions, into the loculi. This is the most common type on young plants which are just starting the formation of plurilocular zoidangia;

b) 'Spherical type' (figs. 21, 47, 48, 50).
The initial divides longitudinally into two cells which each then divide by transverse walls. The resulting quadrants partition, by consecutive divisions, into the loculi. This type is very common on older plants with branched zoidangial stands;

c) 'Spherical type with oblique divisions' (fig. 52).
This differs from the previous type by its oblique primary divisions. Rather rare, in older cultures only.

d) 'Slender cylindrical type' (fig. 22).
The initial at first gives off a row of cells which later partition by consecutive divisions into loculi. This type was very rare.

Once only an emptied unilocular zoidangium was found on a plant bearing plurilocular macrozoidangia (fig. 53).

In the first three types the primary walls in the mature zoidangia can often be recognized by the schizogenous cavities formed in them (figs. 47, 51, 52, 150).

Young mature plants are practically devoid of phaeophycean hairs, older mature plants, in more or less depleted culture fluid, can be densily covered by them.

3.1.3. Plants grown from zoids produced by plurilocular macrozoidangia, but bearing no plurilocular macrozoidangia (type 5 of plants mentioned under 2.1).

At 20° C and 12° C the fertile determinate laterals grow into propagules (figs. 32a, b, 33), at 4° C into unilocular zoidangia (figs. 31, 142). This is curious as these plants appeared to be haploid after chromosome-counts (see 3.6). The diameter of the filaments and the size of the plants are also of the order of plants bearing plurilocular zoidangia (diameter 13.5—31 μ).

3.2. Difference in size between plants producing unilocular zoidangia (diploid plants) and plants producing plurilocular zoidangia (haploid plants).

Diploid plants are very obviously thicker and larger than haploid plants, although there is a considerable overlap. There are no differences in filament-diameter between mature haploid plants grown at 4° C, 12° C, and 20° C. These diameters vary from 13.5 μ to 31 μ. Mature diploid plants cultured at 4° C, 12° C, and 20° C also have the same filament-diameters which vary from 19 μ to 41 μ.

3.3. Influence of emersion and substrate on the formation of reproductive bodies and morphology.

All plants grown on partly immersed discs of filter-paper in petri dishes showed a very obvious reduction in the tendency to form reproductive bodies although the vegetative growth was as vigorous as in culture tubes. These plants, however, particularly the completely emersed ones, are built up mainly from creeping rhizoidal axes and laterals from which arise only short upright axes.

The growth pattern of these cultures demonstrates how easily the erect and creeping axes can interchange. Apical cells from shoots that were originally erect grow into rhizoids when they are in contact with the filter-paper. These rhizoids penetrate between the fibres of the filter-paper thus firmly anchoring the plant to the substrate. The originally cylindrical upright axes show the irregular twisting and swellings of rhizoidal creeping axes (figs. 28—30).

As well as obvious differences in the diameter of the filaments, haploid and diploid plants show very marked differences in the size of the plants. At 4° C for instance, the haploid and diploid fragments on emersed filter-paper had grown after 3 months into circular creeping plants, the former 0,8 cm in diameter, the latter 1,5 cm in diameter. For cultures kept at 12° C these measurements after 3 months were 0,6 cm and 2,5 cm, respectively. The 20° C plants were dead after three months.

It seems probable that only when the upright system is optimally developed is the optimum production of reproductive bodies possible. This probably accounts for the fact that diploid stock cultures kept at 4° C only form unilocular zoidangia very rarely because in the dense masses of plants the systems never grow to completion (fig. 14).

Type 1 plants (diploid ones, mentioned under 2.1) in filter-paper cultures produce a few propagules at 20° C and 12° C, and no unilocular zoidangia at 4° C (cf. 3.1.1.). Type 2 plants (haploid ones) produce a few propagules at 20° C, and few plurilocular macrozoidangia at 12° C and 4° C (cf. 3.1.2).

3.4. Copulation experiments.

The following results of our observations were rather unexpected.

a) Macrozoids (figs. 75—77) could clump and fuse in pairs (figs. 78—83) or groups of more than two zoids;

b) Microzoids (figs. 61—64) could clump and fuse in pairs or groups of more than two zoids (figs. 65—74);

c) Micro- and macrozoids could clump and fuse in pairs. No fusions of more than one macrozoid with more than one microzoid were observed (figs. 57—60);

d) Zoids produced by unilocular zoidangia (figs. 55, 56) did not clump. They often fused in pairs and even often in groups and into aggregates (fig. 84). Aggregates could also be the result of incomplete divisions. The most extreme forms of aggregates were the released, undivided contents of unilocular zoidangia (as 'monospores').

A condition necessary for the clumping of either microzoids, or macrozoids, or both together, was a sufficiently dense suspension of them. Only in dense swarms of active zoids at the light margin of the watch-glass did clumping occur. This repeatedly confirmed observation suggested that clumping was a random tactile response and not chemotactic.

Clumps were formed by agglutination of the tip of the anterior flagellum of one zoid with the body of another zoid. In this way large clumps could be formed (figs. 67—69 for smaller clumps). Several zoids could be attached by their flagella to the body of one zoid that had its own anterior flagellum still free. This latter zoid could drag forward the group of immobilized zoids which were attached to its body. A clump could consist of anything from three to more than a hundred zoids.

Clumping usually occurred at a definite time after the release of the zoids had started. If the release started at 10.00 hours for example, clumping would start suddenly between 11.30 and 12.00 hours and gradually disappear towards 13.00 hours as the activity of the zoids diminished.

Clumps of zoids were several times observed to loosen into long trails of interconnected zoids. The distances between the zoids of such trails were much greater than the length of the flagella so that probably strands of invisible mucilaginous material kept the zoids linked with each other.

During fusion the anterior flagellum of one zoid appeared to be gradually 'sucked in' by the body of the other zoid, certainly this flagellum shortened as the distance between the zoids diminished. Eventually the bodies of the zoids came to lie against each other and gradually fused (figs. 78, 79, 80).

When plurilocular micro- and macrozoidangia and unilocular zoidangia were observed separately when embedded in Na-alginate solution in seawater, many zoids fused in pairs or in groups immediately after leaving the zoidangium. They were unable to swim away as their mobility was impaired by the alginate micelles.

In mixtures of macrozoids and microzoids the microzoids often showed 'creeping' movements on the surface of the macrozoids (figs. 58, 59).

A plurilocular macrozoidangium releases its zoids through many pores in its surface (figs. 148, 149). Each pore corresponds to an underlying row of 2—4 loculi (figs. 47, 50—52) the lower zoids of which have to pass through emptied overlying loculi to reach freedom. A plurilocular microzoidangium releases its zoids partly as does a macrozoidangium, partly by disintegration of its extremely thin walls so that it falls into pieces from which the microzoids escape.

Apart from the size of the loculi, the plurilocular macrozoidangia differ from the microzoidangia by their dark brown colour a scompared with the yellowish white colour of the larter. This is because the macrozoids contain several large brown chromatophores on one of which lies the eyespot, and microzoids only one diminutive pale chromatophore and one very obvious eyespot (compare fig. 45 with fig. 46, and figs. 75—77 with figs. 61—64).

3.5. Rearing of new generations from unfused zoids and from fused zoids.

It took about three months for zoids and presumed zygotes to grow into mature plants in the 12° C culture cabinet. This period roughly agrees with the one necessary for resting initials to grow into mature shoots (see fig. 3).

3.5.1. Cultures from mixtures of macrozoids from different plants.

Such cultures contained two types of plant:

a) Plants bearing macrozoidangia. Chromosome-counts demonstrated that these plants were haploid (see 3.6). They are therefore to be considered the parthenogenetic offspring of the macrozoids. This type of offspring dominated (type 4 plants mentioned under 2.1);

b) Plants bearing propagules (and when cultured at 4° C, unilocular zoidangia). These plants very much suggested a secondary diploid generation, i.e. a product of copulation between pairs of macrozoids. However, they were thinner than diploid plants should be and chromosome-counts proved them to be haploid (type 5 of plants mentioned under 2.1). In principle these plants reacted to differences in temperature in the same way as type 1 (diploid plants). No true diploid plants were observed in the offspring of macrozoids.

Observations were made on the mode of germination of the macrozoids (figs. 85—88, 91—111). An attached macrozoid starts to germinate by the formation of a germination tube which, at its end, swells up into an embryospore (figs. 91—96). The protoplast can move through the germination tube without (fig. 96) or with (figs. 95, 97) cell-divisions. In fig. 97 diminutive nuclei can be seen which have been left behind in the partitions of the germination tube. After having reached its maximum size the embryospore divides into a short cylindrical or spherical parenchymatous group of cells, an embryonic nodule. The number of cells in an embryonic nodule and the sequence of their origin are variable. In figs. 107—111 a diagram of one of the possibilities is given.

One or more cells of an embryonic nodule can function as an axis initial (fig. 106), consequently one or more axes can grow from one embryonic nodule (figs. 98—102, 104, 112, 113).

Quite often the attached zoid swells up and divides into an embryonic nodule directly, without embryospore formation (figs. 103—106).

In the crowded cultures the axis of a germling often ends in a phaeophycean hair, in which case the growth is continued by a new apical cell originating from the cell underlying the hair-meristem (figs. 99, 101, 102, 105, 106, 112, 113) (cf. 3.1).

Quite often macrozoids did not escape from the plurilocular zoidangium but germinated in situ into plants which often bore plurilocular zoidangia at a very young stage (fig. 23). This 'neoteny' was not shown by plants grown from released zoids.

3.5.2. Cultures from mixtures of microzoids.

Such cultures did not give any plants at all, although microzoids and fused microzoids were observed to attach after swarming (figs. 89, 90).

3.5.3. Cultures from mixtures of microzoids and macrozoids.

As would be expected from the results given under 3.5.1. and 3.5.2. these cultures contained *a*) plants bearing plurilocular macrozoidangia and *b*) plants bearing propagules. It was now necessary to differentiate, in this group of plants bearing propagules at 12° C, between a haploid and a possible diploid generation. For this purpose relatively thick propagule-bearing plants were isolated vegetatively, propagated, and caryologically investigated. The caryological test confirmed the presence of a secondary, diploid generation.

3.5.4. Cultures from mixtures of zoids produced by unilocular zoidangia.

These cultures consisted of plants with plurilocular macrozoidangia and plants with plurilocular microzoidangia. No plants bearing propagules at 12° C and without pluri-

locular zoidangia were observed in this offspring. Counts of four cultures gave totals of 180 microzoidangial and 166 macrozoidangial plants, this suggests segregation of sexes at meiosis. Meiosis was caryologically demonstrated in the initials of the unilocular zoidangia (see 3.6).

Aggregates of zoids from unilocular zoidangia (in total 9 isolated) died. Released undivided contents of unilocular zoidangia died (10 such 'monospores' were isolated).

3.6. Results of caryological investigations.

3.6.1. Mitosis.

The chromosomes showed, in the metaphase and anaphase, the extreme contraction characteristic of the *Phaeophyceae* (figs. 115—123) (cf. Evans, 1966; Roberts, 1960). At the moment of maximum contraction most chromosomes are characteristically dumb-bell shaped. This is the stage (metaphase) that is most suitable for making counts (figs. 115, 119, 120, 122).

The haploid number of chromosomes is 25—30, the diploid 50—60. Relatively exact counts could be made in only few metaphase-plates; usually only approximate counts were possible but these mostly gave sufficient information to differentiate between haploid and diploid numbers. Sometimes clear spindles could be observed (fig. 123). The nucleolus gradually disappears through vacuolization, in the course of the prophase (fig. 114). Smaller nuclei contain one nucleolus, larger nuclei one or several nucleoli.

Vigorously growing material was fixed at different times during the light and dark periods but as there was no indication of synchronous cell-divisions, much material had to be fixed and stained in order to have sufficient metaphases available.

Nuclei of actively dividing cells are much larger than those of non-dividing cells. Compare, for instance, the nucleus of an apical cell with the nuclei from the mature part of a filament (figs. 112, 117).

It is possible, by their larger nuclei, to recognise in nodules the cells which are initiating new shoots.

3.6.2. Meisosis (figs. 124—140).

Characteristic stages of meiosis were observed in the initials of unilocular zoidangia. The initial of a unilocular zoidangium is the apical cell of a determinate fertile lateral (fig. 124; interphase preceeding meiosis). Diplotene (figs. 125, 126), pachytene (or diakinesis?) (fig. 127), and first metaphase (figs. 128—132) were observed several times. In the course of diplotene the nucleus swells considerably and the chromosomes congregate at one side of the nucleus (also observed in other *Phaeophyceae*, e.g. Clint, 1927; Higgins, 1931; Knight, 1931; Schussnig and Kothbauer, 1934; Mathias, 1935; Evans, 1966, p. 136; Roberts, 1966, p. 157). The nucleolus gradually disappears through vacuolization. At diplotene the chromosomes appear as knobbly filaments which suggests spiralling. This suggestion was confirmed by the presence of small loops which could sometimes be observed (fig. 126, arrow). At pachytene (or early diakinesis? cf. fig. 127) the nucleolus had entirely disappeared and the nucleus had reached its greatest inflation. During inflation, the nuclear membrane gradually disappeared. As in mitosis, metaphase I is characterised by extreme contraction. Nevertheless, the metaphase I of meiosis remains easily distinguishable from that of mitosis. The bivalents are spherical, circular, or rhomboidal, and more rarely dumb-bell shaped, they are thicker than the chromosomes at mitosis metaphase (compare figs. 115, 116, 119 with figs. 128—132). In a few cases the number of bivalents could be counted, and it amounted to 25—30. In other

cases the bivalents seemed to be interconnected in a reticulum and it was difficult to delimit each bivalent exactly (fig. 131).

Metaphase II is characterised by the most extreme degree of contraction observed (figs. 134—138). Here the haploid number was determined several times.

Meiosis in the initials of the unilocular zoidangia corresponds with characteristic structures seen in living material. The mass of the protoplast containing the chromatophores contracts around the centrally suspended nucleus. At meiosis interphase the two hyaline nuclei protrude from the central clump of protoplasm containing the chromatophores (fig. 139). After completion of meiosis II the four nuclei form a tetrad protruding from the central clump of protoplasm and chromatophores (fig. 140). After that, most chromatophores migrate to the periphery of the zoidangium (fig. 141) where nuclear divisions and cleavage of the protoplast lead to the formation of zoids.

The initials of unilocular zoidangia on type 5 plants (mentioned under 2.1) (haploid plants with unilocular zoidangia) showed meiotic configurations both in vivo (fig. 142) and in stained preparations: in vivo the characteristic contraction of the protoplast with chromatophores was seen and in stained preparations diplotene with inflation of the nucleus. No other phases were observed. As these unilocular zoidangia were never observed to have reached maturity and to have produced viable zoids, their divisions are interpreted as apomeiotic.

4. CONCLUSIONS AND DISCUSSION

4.1. Life-history.

The life-history of the *Sphacelaria furcigera* culture isolated from a plant collected in the spring of 1960 at Hoek van Holland in all probability follows the course shown in fig. 4.

S. furcigera is a feebly heteromorphic, anisogamous diplohaplont. Sexual reproductive organs are only formed at lower temperatures while propagules (organs for vegetative multiplication) are formed at higer temperatures.

The diplophase, whose filaments are thicker and whose plants are larger than those of the haplophase, forms unilocular meiotosporangia at 4° C (figs. 36, 55, 56). Sex determination takes place at meiosis and about one half of the zoids produced by unilocular zoidangia grow into male haploid plants, and the other half into female haploid plants. Male haploid gametophytes form plurilocular microgametangia (fig. 46) at 12° C and 4° C (loculi 3,5—7,5 μ), and female haploid gametophytes plurilocular macrogametangia (fig. 45) at 12° C and 4° C (loculi 6—10 μ). Female macrogametangia are dark brown, male microgametangia yellowish-white. Female gametes are 4—13 μ long and bear several brown chromatophores each on one of which the red eyespot. The two flagella are inserted near the eyespot. The anterior flagellum looks thicker than the posterior one probably because it is pleuronematic (figs. 75—77). Male gametes are 3—7 μ long and each one contains a rudimentary pale yellowish chromatophore and a distinct eyespot (figs. 61—64). Male gametes show a greater mobility than female ones.

Fusions of male with female gametes have been observed (figs. 57—60) and secondary diploid plants have been grown from mixtures of both types of gametes. No secondary diploid plants were retrieved from swarms of either microzoids or macrozoids, although homosexual clumping and fusion were regularly observed. Therefore diploid zygotes are very probably the result of fusions between male and female gametes.

Female gametes develop parthenogenetically into two types of plant, both haploid: firstly plants with plurilocular macrozoidangia (female gametophytes), secondly haploid

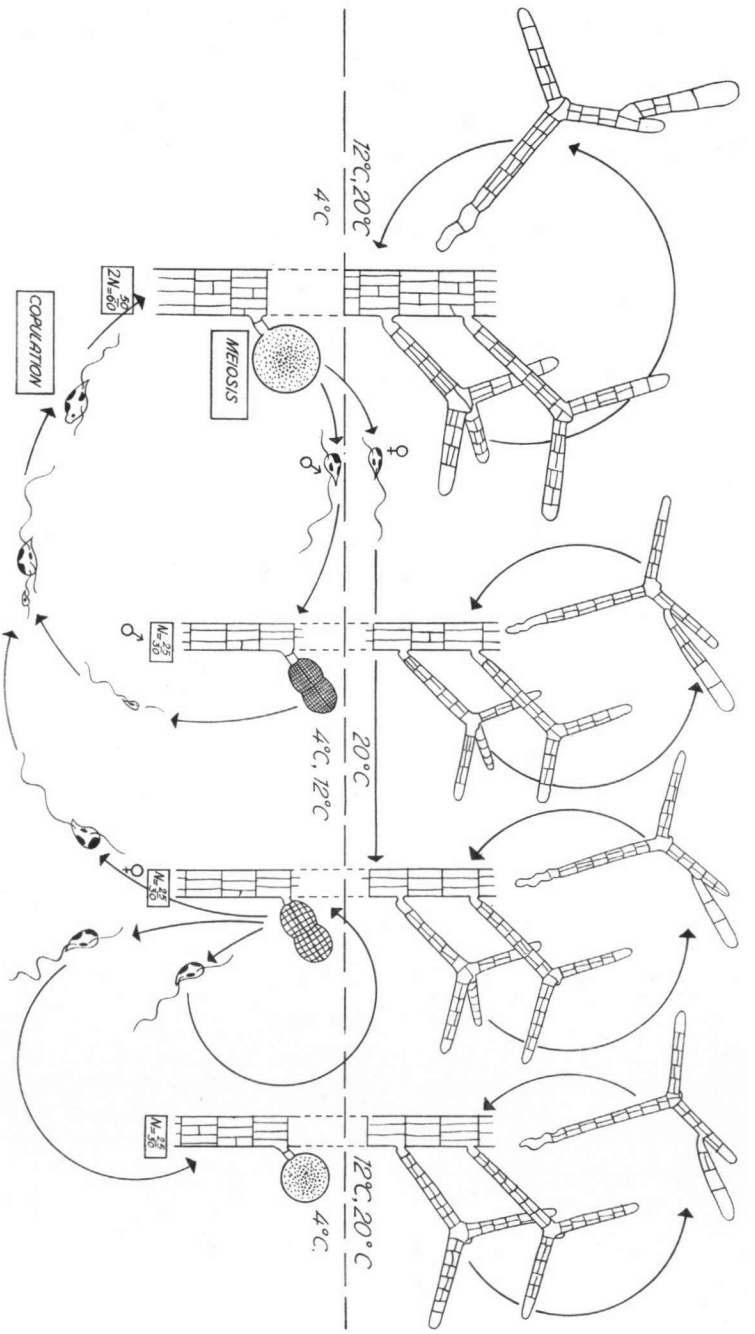

Fig. 4. Diagram of the life-history of *Sphaeclaria furcigera* according to the results of our investigations.

plants bearing unilocular zoidangia (fig. 31) that are not able to produce viable zoids (possibly as a consequence of apomeiotic divisions).

This haploid unilocular zoidangium-bearing phase reproduces only vegetatively by propagules and nodules and does not form part of the main life-history of *S. furcigera.*

The following generalisations seem permissable, particularly because they are corroborated by data from the literature on *S. furcigera* and the 'species' that are presumed to be synonymous with it (table I).

At our latitudes in summer *S. furcigera* multiplies only vegetatively by propagules, nodules, and stolons. Because of the rapid maturation of the plants at higher temperatures the species is able to invade and cover large areas. Most probably the diploid phase predominates as it grows more vigorously than the haploid phases. It is indeed the first author's experience that *S. furcigera* is an extremely common alga in the moderately exposed to very sheltered eulittoral zones of Brittany, NW. Spain, and the Côte Basque, where in shady places it is able to penetrate as high as the *Fucus spiralis* belt. Zoidangia were never encountered on plants collected in the summer, but propagules were nearly always abundant.

It is, of course, difficult to distinguish, in vegetative mixed populations, between haploid and diploid phases as their sizes overlap considerably. However, the smallest diameter of haploid plants (13,5 μ), which always occurred in cultures of haploid plants, are not known from these summer populations and it seems probable therefore that the summer populations of *S. furcigera* at our latitudes predominantly consist of diploid plants.

The records of unilocular and plurilocular zoidangia at our latitudes are from material collected in the winter half of the year (table I).

Sphacelaria furcigera seems to be a cosmopolitan species (table I) occurring from tropical to arctic regions. Our results would suggest that in warmer regions *S. furcigera* only multiplies vegetatively by propagules and nodules, whereas the sexual cycle can be accomplished only in temperate to cold regions. Therefore, at the northern and southern limits of its distribution-area it could be expected to be more often encountered as zoidangia-bearing plants than in warmer regions. Data from the literature support this hypothesis with regard to the northern temperate to cold regions (table I). The most northerly record, from Greenland, is of material bearing unilocular sporangia.

However, more evidence is of course necessary. It is conceivable that there are geographic races differing in the temperatures at which the various reproductive bodies are formed (Müller, 1962, observed such differences between *Ectocarpus siliculosus* populations from Helgoland and Naples). *S. furcigera* plants bearing zoidangia are reported from New Caledonia and Australia, Dirk Hartog Island (Sauvageau, 1900—24, pp. 145—156). The latter material contained plurilocular micro- and macrozoidangia.

Our conclusions differ from those reached by Clint (1927) and Papenfuss (1934) for *S. bipinnata.*

According to Clint *S. bipinnata* is a diplont reproducing by haploid gametes which are formed by meiosis in the unilocular zoidangia, and by asexual diploid spores formed in plurilocular zoidangia. She convincingly demonstrated meiosis in the initials of the unilocular zoidangia so that the zoids produced by them can reasonably, though not undisputably, be considered haploid. The fusion of zoids directly after release from the unilocular zoidangium was taken as evidence of the gametic nature of these zoids. Our results clearly demonstrate that clumping and fusion of zoids cannot be considered indisputable evidence for the gametic nature of zoids. Clint's conclusions therefore are founded on insufficient evidence especially as she did not succeed in culturing any material.

Papenfuss considered *S. bipinnata* as having an isomorphic diplohaplontic life-history.

Table 1. Reproductive bodies of *S. furcigera* and its presumptive synonyms, according to data in literature. I—XII: months in which reproductive bodies were observed (between brackets: emptied zoidangia). W: reproductive bodies observed in winter. Su: reproductive bodies observed in summer. +: present (time of the year not mentioned).

author	region	as	propagules	pluriloc. zoidangia	uniloc. zoidangia
Sauvageau, 1900—24	Adriatic	*S. furcigera*	X		
Sauvageau, 1900—24	SW. France	*S. furcigera*	VIII—IX		
De Haas-Niekerk, 1965	Holland	*S. fusca*	VI—X		
Sauvageau, 1900—24	Faroes	*S. furcigera*	+		
Jorde & Klavestad, 1960	Hardanger Fjord, Norway	*S. furcigera*	Su		
Jorde, 1951	Bergen, Norway	*S. furcigera*	Su		
Lund, 1948	Denmark	*S. furcigera*	I, VI, VIII		
Vickers & Shaw, 1908	Barbados	*S. furcigera*	I—III		
Sauvageau, 1900—24	Canaries	*S. furcigera*	+		
Sauvageau, 1900—24	Red Sea	*S. furcigera*	+		
Sauvageau, 1900—24	Malabar	*S. furcigera*	+		
Sauvageau, 1900—24	Madagascar	*S. furcigera*	+		
Sauvageau, 1900—24	Martinique	*S. furcigera*	IV		
Sauvageau, 1900—24	Mauritius	*S. furcigera*	+		
Sauvageau, 1900—24	Réunion	*S. furcigera*	+		
Sauvageau, 1900—24	Port Denison, Australia	*S. furcigera*	+		
Sauvageau, 1900—24	Port Denison, Australia	*S. divaricata*	+		
Sauvageau, 1900—24	Rockingham, Australia	*S. divaricata*	+		
Sauvageau, 1900—24	Geographe Bay, Australia	*S. furcigera*	+		
Sauvageau, 1900—24	Straits of Torres	*S. divaricata*	+		
Sauvageau, 1900—24	New Caledonia	*S. divaricata*	+		
Sauvageau, 1900—24	New Caledonia	*S. furcigera*	+		
Sauvageau, 1900—24	New Caledonia, Port Bouquet	*S. furcigera*	XI		XI
Sauvageau, 1900—24	New Caledonia, Nouméa	*S. furcigera*	IV	IV	
Sauvageau, 1900—24	Dirk Hartog Island, Australia	*S. furcigera*	IV	IV	IV
Sauvageau, 1900—24	Ceylon	*S. ceylanica*		+	
Kuckuck, 1897	Helgoland	*S. furcigera* var. *saxatilis*	VI—IX	XII—III	XII—III
Waern, 1945	W. Coast, Sweden	var. *saxatilis*	VII		(VII)
Lund, 1948	Kattegat	*S. saxatilis*		III	
Sundene, 1953	Oslofjord	*S. saxatilis*		(IV)	
Blackler & Jackson, 1966	St. Andrews, Scotland	*S. britannica*		I	W
Sauvageau, 1900—24	Berwick-on-Tweed, England	*S. britannica*			I
Sauvageau, 1900—24	Greenland	*S. britannica*			+
Van den Hoek, 1958	Roscoff, Brittany	*S. britannica*			IV
Lund, 1948	Copenhagen	*S. britannica*			I, (IV)
Irvine, 1956	St. Andrews, Scotland	*S. britannica*			X—IV
Irvine, 1956	'British Isles'	*S. britannica*			IX—IV
Waern, 1945	W. Coast, Sweden	*S. britannica*			V
Sundene, 1953	Oslofjord	*S. britannica*			I—IV
Jorde & Klavestad, 1960	Hardanger fjord, and vicinity	*S. britannica*			(VII)
Waern, 1952	Baltic, Öregrund	*S. britannica*			(VI)
Traill, 1890	Orkneys	*S. radicans* var. *olivacea*			W
Kylin, 1947	W. Coast, Sweden	*S. olivacea*			W

His evidence: zoids from unilocular zoidangia did not fuse, zoids from plurilocular zoidangia on plants bearing exclusively this type of reproductive organ fused. No caryological and cultural investigations were carried out. Since fusions are no evidence for the sexual nature of zoids Papenfuss's investigations do not prove anything at all, whereas those of Clint prove at least that meiosis takes place in the initials of the unilocular zoidangia.

All investigations on other *Sphacelariales* are equally incomplete.

Sauvageau (1900—24, pp. 174 and 191) described plurilocular microzoidangia and macrozoidangia interpreted as male and female gametangia for *S. hystrix* and *S. harveyana.*

The life-history of *Halopteris filicina* at Naples probably resembles that of *S. furcigera.* Mathias (1935) found diploid plants (2n = *ca.* 32) bearing only unilocular zoidangia in the initials of which meiosis took place, and haploid plants (n = *ca.* 16) bearing plurilocular macro- and microzoidangia. Haploid plants were more slender than diploid plants. The plurilocular macrozoidangia were interpreted as female, the microzoidangia as male, although Mathias did not succeed in observing copulations. He confirmed the earlier observations of Sauvageau (1898) on *Halopteris filicina* from Villefranche; the latter author, however, did not investigate his material caryologically.

Ernst Schwarzenbach (1957) repeated Mathias's incomplete observations. Higgins (1931) demonstrated meiosis in the initials of the unilocular zoidangia of *Halopteris scoparia* from Naples.

According to Moore (1951) five species of *Halopteris* from New Zealand have an isomorphic diplohaplontic life-history the gametophytes of which are oogamous. Plurilocular zoidangia, interpreted as antheridia, and unilocular 'oogonia', occurred in mixed sori in the axils of the branchlets. The undivided contents of the unilocular organs were released and fusions were observed to take place between zoids produced by the 'antheridia' and these supposed ova. Presumptive zygotes grew into germlings that died at an early stage. Relatively few plants bore only unilocular organs the contents of which were released as zoids. Moore interpreted such plants as diploid sporophytes. She did not carry out caryological investigations and she failed to culture her species of *Halopteris.* Although her observations certainly suggest her interpretations, other possibilities should not be excluded. In our cultures, for instance, quite a few unilocular zoidangia of the diplophase of *Sphacelaria furcigera* released their undivided contents as naked, spherical 'monospore'-like protoplasts.

Moore's conclusions are in accordance with Sauvageau's (1907) interpretation of herbarium specimens of *Halopteris* species from southern regions and one herbarium specimen of *Halopteris scoparia* from Biarritz.

The same objections can be made with regard to the classical investigations, by Knight (1931) and Papenfuss (1935), on the life-history of *Ectocarpus siliculosus.*

According to Knight, *Ectocarpus arctus* in the Irish Sea is a diplont (2n = 16) reproducing asexually by zoids from plurilocular zoidangia and sexually by zoids meiotically produced in unilocular zoidangia. Her evidence was the caryological demonstration of meiosis in the initials of the unilocular zoidangia and fusion of the zoids produced by them. She interpreted material from Naples as haplontic (n = 8) with gametes mitotically produced by plurilocular zoidangia because the zoids thus produced were seen to fuse.

Papenfuss regarded the life-history of *Ectocarpus siliculosus* as isomorphic diplohaplontic. He based his evidence on the frequent observation, in nature, of plants bearing plurilocular and unilocular zoidangia the zoids from neither of which fused, and on the infrequent observation, in nature, of plants bearing only plurilocular zoidangia the zoids from which were observed to fuse. Some plants of the first group gave chromosome-

counts of 16, and of the second group 8. He used fusion as the principle criterion for deciding whether a zoid was a gamete and the plant producing it a gametophyte. His attempts to culture his material throughout its complete life-history were unsuccessful.

Schussnig and Kothbauer (1934) made the same observations on material from Rovinj as Knight on material from the Irish Sea. Accordingly they interpreted the life-history of *E. siliculosus* in that region as diplontic. In contrast to the opinion of Knight, they interpreted the life-history of *E. siliculosus* at Naples as diplohaplontic because they found diploid plants (2n = 16) with meiotically dividing unilocular zoidangia and haploid plants (n = 8) with plurilocular zoidangia.

Kornmann (1956) interpreted the life-history of *E. siliculosus* from List (Sylt, NW. Germany) as slightly heteromorphic diplohaplontic on the basis of cultures. Unfortunately he did not investigate these cultures caryologically.

Müller's (1962) observations on the temperature-dependent formation of unilocular and plurilocular zoidangia complicated the information on the life-history of *E. siliculosus* still further. In two recent publications (Müller, 1966, 1967) this author demonstrated that the fundamental life-history of *E. siliculosus* is a slightly heteromorphic diplohaplontic one and thus of principally the same type as the life-history of *Sphacelaria furcigera*. Sex is also genotypically determined. Male and female gametes were physiologically differentiated but morphologically alike. The haploid chromosome number was 20—28, the diploid number 48—61. These numbers make it unlikely that earlier much smaller counts (Knight, 1931; Papenfuss, 1935; Schussnig and Kothbauer, 1934) were correct. The life-history of *E. siliculosus*, however, showed a number of deviations from the fundamental type although these were much less frequent than the normal pathway of the life-history. For instance, double male gametes (probably the results of incomplete division in the gametangium) could develop into diploid sporophyte with the male factor duplicated. Unfused gametes never developed into new haploid gametophytes, but into haploid sporophytes. (In our experiments unfused female gametes of *Sphacelaria furcigera* developed into new female gametophytes as well as into haploid sporophytes). Clumping in *Ectocarpus siliculosus* was observed to take place only between male and female gametes, whereas in our experiments with *Sphacelaria furcigera* gametes showed typical clumping in dense swarms of either male or female ones, or in mixtures of both.

4.2. Taxonomy.

Recently de Haas-Niekerk (1965) synonimized *S. furcigera* Kütz. with *S. fusca* (Huds.) C. A. Ag., and according to her opinion our material should belong to *S. fusca*.

Undoubtedly the collections she investigated and our material are conspecific.

It is our opinion, however, that it is preferable for the time being to use the specific. epithet *furcigera* Kützing (1855) instead of *fusca* because nomenclatural and taxonomic confusions can better be avoided in this way. No nomenclatural type-specimen of Hudson's (1762) *Conferva fusca* is available, so that its nature remains obscure, whereas the type of *S. furcigera* Kützing (1855) is present in the collections of the Rijksherbarium, Leiden. It is conspecific with our material (cf. de Haas-Niekerk, 1965). Moreover, Sauvageau (1900—24, pp. 206—211) interpreted *S. fusca* as a species having the indefinite organisation which is also typical for *S. furcigera*, but with larger diameters than the latter species (60—80 μ; *S. furcigera* is 13—45 μ in diameter). Both *S. furcigera* and *S. fusca* have bifurcate and trifurcate propagules with cylindrical arms, but in *S. fusca*, according to Sauvageau, bifurcate ones are rare. Sauvageau based his taxonomic opinion of *S. fusca*

on five collections from European coasts. The possibility should not be excluded, therefore, that two morphologically similar *S. furcigera*-like species, differing only in their diameters, inhabit the European coasts.

This taxonomic and nomenclatural tangle will be more fully considered in the forthcoming revision of the European *Sphacelariales* by W. F. Prud'homme van Reine (Rijksherbarium, Leiden).

However, on the basis of our results we can be reasonably certain that *S. furcigera* Kützing (1855), *S. saxatilis* (Kuck.) Kuck. *ex* Sauvageau (1901—14, pp. 5, 69, 152) (= *S. furcigera* var. *saxatilis* Kuckuck, 1897, pp. 373—376), and *S. britannica* Sauvageau (1901, pp. 50—54) are synonymous. The name *S. furcigera* has generally been used for plants bearing only propagules, the name *S. britannica* for plants bearing unilocular zoidangia, and the name *S. saxatilis* for plants bearing either plurilocular zoidangia and bifurcate propagules, or only plurilocular zoidangia, or unilocular zoidangia and propagules (see table 1).

The degree of differentiation between prostrate and erect filaments was sometimes used as a criterion to distinguish between *S. britannica* and *S. saxatilis* (Irvine, 1956; Lund, 1950). From our cultures it appears that in a plant growing on wet filter-paper the prostrate system dominates, whereas in completely submerged plants the upright system dominates. This suggests that the dominance of the prostrate system (stolons and attachment discs) in high-littoral populations is an expression of environmental modification.

The same conclusion is valid regarding the presence or absence of phaeophycean hairs: these are roughly an expression of rapidly growing cultures becoming depleted of nutritive substances.

On the basis of comparative cultures de Haas-Niekerk reached the conclusion that *S. britannica* is distinct from *S. furcigera* (as *S. fusca*) although its vegetative organisation showed great resemblance to that of *S. furcigera*. Cultures of *S. 'britannica'* (isolated from a population growing at Roscoff) remained consistently more slender than those of *S. 'furcigera'*. Very probably this *S. 'britannica'* from Roscoff represents the haploid unilocular sporangium-bearing phase of *S. furcigera* (see fig. 31).

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EXPLANATION OF FIGURES

Fig. 5. Mature diploid plant grown for *ca.* 5 months at 4° C, from fragment of stock-material. Unilocular zoidangia (cf. fig. 36).

Figs. 6, 7. Apical cells of the mature diploid plant given in fig. 5. These cells demonstrate declining vegetative growth. In fig. 6 the apical cell shows some properties of a unilocular zoidangium: it releases its (undivided) protoplast through an apical pore (cf. fig. 54). The subapical segments are already vertically divided. In fig. 7 the shortness and relatively narrow diameter of the apical cell are indicative of diminished activity (compare with the apical cells of the actively growing plant of fig. 40).

Fig. 8. Mature haploid female plant grown at 4° C for *ca.* 5 months, from a fragment. Plurilocular macrozoidangia.

Fig. 9. Mature haploid female plant grown at 4° C for *ca.* 5 months, from an unfertilized female gamete. Note attachment disc from which two plants originate.

Figs. 10, 11. Apical cells of the mature haploid female plant illustrated in fig. 8. These cells demonstrate declining vegetative growth: they are shorter and narrower than apical cells of plants with active vegetative growth (compare with the apical cells of the actively growing plant of fig. 39).

Figs. 12—14. Plants of crowded diploid stock-culture kept at 4° C. Abundant formation of nodules that develop into attachment discs. Unilocular zoidangia are very rarely formed in such crowded cultures, probably because the shoots arising from the nodules seldom have a chance to grow into mature systems.

Figs. 15, 16. Apical cells of a crowded diploid stock-culture (cf. fig. 14). These cells are indicative of limited activity. Compare with the apical cells of the actively growing diploid plant illustrated in fig. 40.

Figs. 17—20. Nodules of diploid stock-material. 17, 18. Young nodules. 19. Freshly proliferating nodules. 20. Old nodule grown into adhesive disc and bearing three upright axes.

Fig. 21. Plurilocular macrozoidangia. The larger one ('stout cylindrical type') has grown by partitioning of an apical and a subapical cell, the shorter one ('spherical type') by partitioning of only an apical cell.

Fig. 22. Plurilocular macrozoidangium of the rare slender cylindrical type, which has grown by partitioning of a row of vegetative cells.

Fig. 23. Neoteny of parthenogenetically grown macrogametophyte. This occurs only in germlings grown from macrozoids which germinated in the plurilocular macrozoidangium.

Figs. 24—27. Propagules and vegetative laterals grown from zoidangial stipes in female gametophytes transferred from 12° C to 20° C.

Figs. 28—30. Inversion of polarity in macrogametophytes transferred from fluid medium to moist filter-paper. Originally upright axes changed into rhizoidal axes. In fig. 29 a rhizoidal axis penetrating into a cellulose-fibre.

Fig. 31. Haploid plant parthenogenetically grown from a female gamete, and bearing unilocular zoidangia at 4° C ('type 5' of plant). These zoidangia do not produce viable zoids.

Figs. 32, 33. Propagules of the haploid type of plant illustrated in fig. 31 ('type 5' of plant). These propagules were produced in a culture kept a 12° C, at which temperature no plurilocular zoidangia are formed.

Figs. 34, 35. Nodules of the haploid type of plant illustrated in fig. 31 ('type 5' of plant). These nodules were formed at 4° C. They are characteristically sickle-shaped which character is not shown by the nodules of other types of plants.

Fig. 36. Detail of mature diploid plant grown for *ca.* 5 months at 4° C, from fragment of stock-material. Unilocular zoidangia (cf. fig. 5).

Fig. 37. Diploid stock-material, emptied unilocular zoidangia found after a thorough search.

Fig. 38. Detail of mature diploid plant grown from fragment of stockmaterial for *ca.* 3 months. Propagules, one of which germinating. Phaeophycean hairs.

Fig. 39. Detail of haploid plant (macrogametophyte) grown at 4° C for *ca.* 3 months (cf. fig. 3). Immature plant, active vegetative growth. Note large size of apical cells.

Fig. 40. Detail of diploid plant grown at 4° C for *ca.* 3 months (cf. fig. 2). Immature plant, active vegetative growth. Note large size of apical cells.

Fig. 41. Detail of haploid plant (macrogametophyte) grown at 12° C for *ca.* 1 month (cf. fig. 3). Plant reaching maturity, incipient formation of plurilocular macrozoidangia.

Fig. 42. Detail of diploid plant grown at 12° C for *ca.* 1 month (cf. fig. 2). Plant reaching maturity, incipient formation of propagules.

Fig. 43. Detail of haploid plant (macrogametophyte) grown at 20° C for *ca.* 1 month (cf. fig. 3). Mature plant, abundant formation of propagules.

Fig. 44. Detail of diploid plant grown at 20° C for *ca.* 1 month (cf. fig. 2). Mature plant, abundant formation of propagules.

Fig. 45. Mature plurilocular macrozoidangium (= female gametangium), from living material ('stout cylindrical type').

Fig. 46. Mature plurilocular microzoidangium (= male gametangium), from living material.

Fig. 47. Mature plurilocular macrozoidangium, median optical plane ('spherical type'). Acetocarmine stained, phenol-balsam embedded whole mount. Only the nuclei of the zoids are visible. Each pore corresponds to a row of underlying loculi. Note the central schizogenous cavity.

Fig. 48. Initial of plurilocular macrozoidangium after one division which is here vertical. This leads to the formation of the 'spherical type' of figs. 47 and 50. Acetocarmine stained, phenol-balsam embedded.

Fig. 49. Initial of plurilocular macrozoidangium after two divisions. The first division was here transverse. This leads to the formation of the 'stout cylindrical type' of figs. 21, 45, and 51. Acetocarmine stained, phenol-balsam embedded.

Fig. 50. Mature plurilocular macrozoidangium, spherical type; median optical plane. Most zoids have escaped. Acetocarmine stained, phenol-balsam embedded.

Fig. 51. Mature plurilocular macrozoidangium bearing an immature one on its stipe. Median optical plane. Both belong to the 'stout cylindrical type'. Note the schizogenous cavity in the centre of the mature zoidangium. Acetocarmine stained, phenol-balsam embedded.

Fig. 52. Mature plurilocular macrozoidangium, median optical plane. The planes of the first two divisions are oblique. This type of zoidangium ('spherical type with oblique divisions') occurs rather rarely on older female plants. Acetocarmine stained, phenol-balsam embedded.

Fig. 53. The sole unilocular zoidangium found on a gametophyte (female) during our investigations.

Fig. 54. Apical cell of diploid plant bearing unilocular zoidangia. This apical cell has an oblique apical pore, as have the unilocular zoidangia.

Figs. 55, 56. Zoids produced by unilocular zoidangia, lateral view and ventral view, respectively.

Figs. 57—60. Copulation-configurations between haploid macro- and microzoids. In 58 and 59 the microzoids show creeping movements on the immobilized macrozoid. In 60 the microzoid is 'pricking' its anterior flagellum into the macrozoid.

Figs. 61—64. Microzoids (male gametes). 61, 63, 64. Lateral views. 62. Ventral view. Note the single diminutive, pale chromatophore.

Figs. 65—74. Copulation-configurations among microzoids. Figs. 65, 66. Completely fused microzoids.

Figs. 67—74. 'Clumping' among microzoids.

Figs. 75—77. Macrozoids (female gametes). Note the thicker anterior most probably pleuronematic flagellum.

Figs. 78—83. Copulation-configurations among macrozoids. Fig. 78. Fusion completed in *ca.* 2 minutes. Fig. 79. Fusion completed in *ca.* 8 minutes. Figs. 80, 81, 83. Pseudoanisogamy. Small zoids produced by plurilocular macrozoidangia fuse with larger ones.

Fig. 84. Aggregation and fusion of zoids produced by unilocular zoidangium.

Figs. 85—88. Germinating attached macrozoids. Incipient formation of bulge into which moves the protoplast and which grows into a new macrogametophyte (embryospore formation).

Fig. 89. Germinating microzoid. Such a germling is not viable.

Fig. 90. Germinating fusion-product of two microzoids (note the two stigmata). Such a germling is not viable.

Figs. 91—111. Germlings of parthenogenetically developing macrozoids. Acetocarmine stained, phenol-balsam embedded. 91, 92. Recently attached zoids. 93, 94. Formation of bulge, inception of embryospore-formation. 95—97. Embryospores completed and swelling. Note, in 96, the long tube through which the protoplast migrated, and, in 95, the two walls which the migrating protoplast left behind; in 97 the three diminutive nuclei are left behind in the partitions of the tube. 98—102. Primary nodules grown from embryospores and each bearing a first upright axis. In 99, 101, 102 this axis bears a terminal phaeophycean hair. 103—106. Directly germinating zoids, without embryospore-formation, 104 with the first axis ending in an apical cell, 105 and 106 with the first axis ending in a phaeophycean hair. 107, 111. Diagram of formation of primary nodule from swollen embryospore. This is only one of the many possibilities of the septation of an embryospore into a primary nodule.

Figs. 112, 113. Young female gametophytes parthenogenetically grown from macrozoids. Sympodial construction of first upright axes. After formation of a terminal phaeophycean hair the first cell underlying the hair meristem takes over the function of apical cell. This is particularly clear in fig. 112. The nucleus of such a new apical cell swells considerably. Abundant formation of phaeophycean hairs is characteristic of crowded cultures.

Figs. 114—116. Mitoses in the secondary segments of the diploid phase, original material. 114. Late prophase with the vacuolized nucleolus still partly showing, and prometaphase with the chromosomes not yet fully contracted. 115, 116. Metaphase, 115 permitting a reasonably exact count of 56 or 57 chromosomes.

Figs. 117—119. Mitoses in the secondary segments of the haploid phase, in this case the haploid unilocular zoidangium bearing phase. 117. Prometaphase in the upper secondary segment and a metaphase in the lower secondary segment, a prometaphase in the underlying cell. 118. Metaphase. 119. Metaphase permitting a reasonably exact count of 28 chromosomes.

Figs. 120—123. Mitoses in the secondary segments of the diploid phase obtained by copulation of micro- and macrozoids (male and female gametes). 120—122. Metaphases permitting counts varying from 52—60. 123. Anaphase permitting count from 45—55.

Figs. 124—140. Meiosis in the initial of the unilocular zoidangium. 124. Interphase nucleus in the apical cell of determinate lateral. This apical cell is about to grow into a unilocular zoidangium. 125, 126. Diplotene. Nucleus distended, chromosomes congregating at one side of nucleus, near nucleolus. Note loop (arrow) in 126. 127. Pachytene (diakinesis?). 128—132. Meiosis metaphase I. 25—30 diads can be counted (particularly fig. 132). 133. Meiosis interphase. Chromatine filaments drawn in only one of the two nuclei. 134—137. Second meiotic metaphase. Chromosomes show an extreme degree of contraction. 25—30 chromosomes. 138. Anaphase of second meiotic division. 139. Meiotic interphase in vivo. Contraction of protoplast with chromatophores in centre of cell. 140. Initial of unilocular zoidangium after completion of meiosis, in vivo. Tetrad of nuclei grouped around central mass of protoplasm and chromatophores behind which the fourth nucleus is hidden.

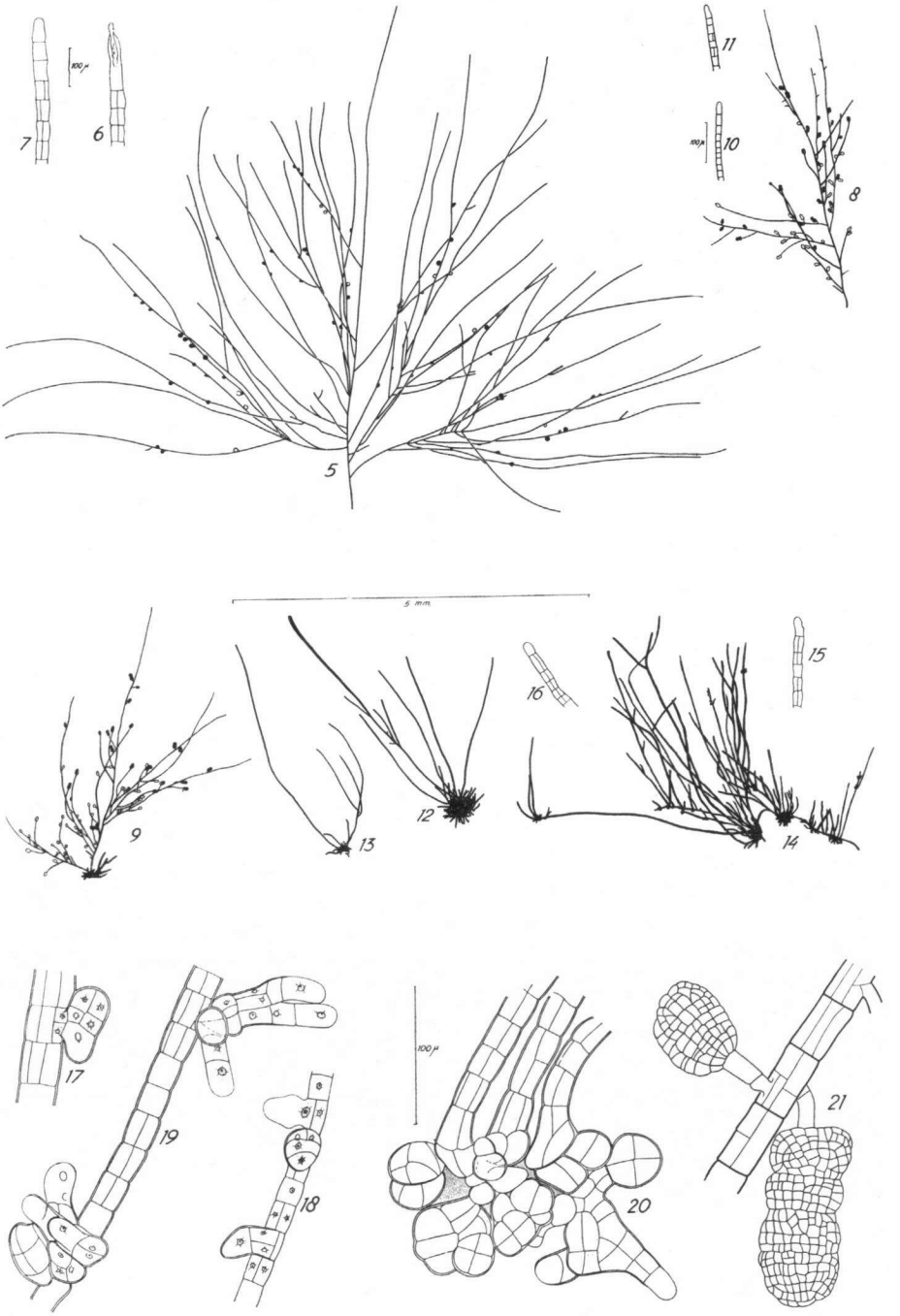
Fig. 141. Young unilocular zoidangium in vivo. Protoplasm and chromatophores redispersed. Nuclei not visible.

Fig. 142. Initial of unilocular zoidangium of haploid unilocular zoidangium bearing phase. Contraction of protoplasm and chromatophores in centre of cell.

Figs. 143—147. Branched stands of plurilocular macrozoidangia on older macrogametophytes. 143—146. Acetocarmine stained phenol-balsam embedded material. 147. Living material.

Figs. 148, 149. Surface views of plurilocular macrozoidangia showing pores through which zoids are released. Acetocarmine stained, phenol-balsam embedded.

Fig. 150. Median optical section of plurilocular macrozoidangium, showing schizogenous cavity.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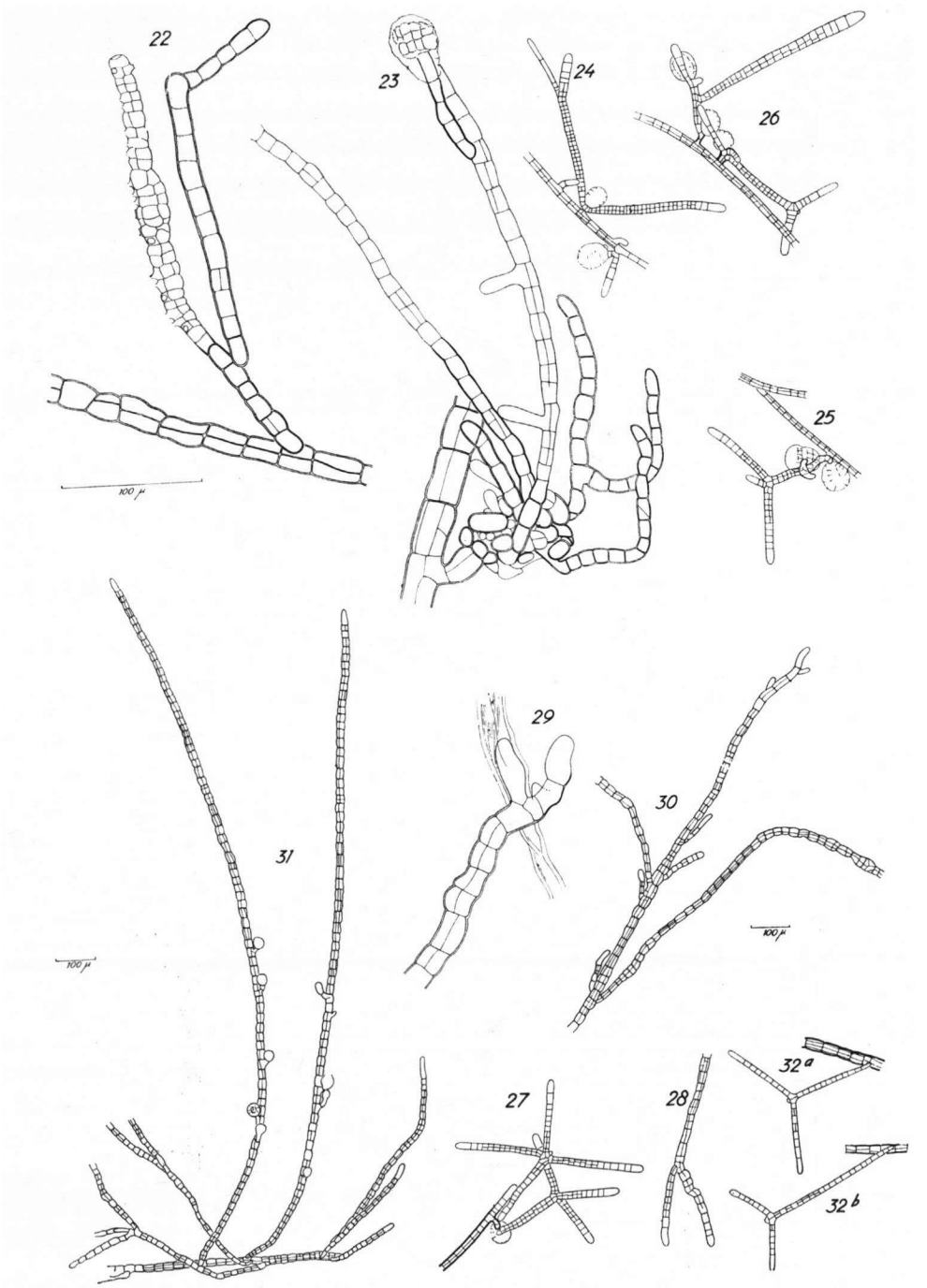
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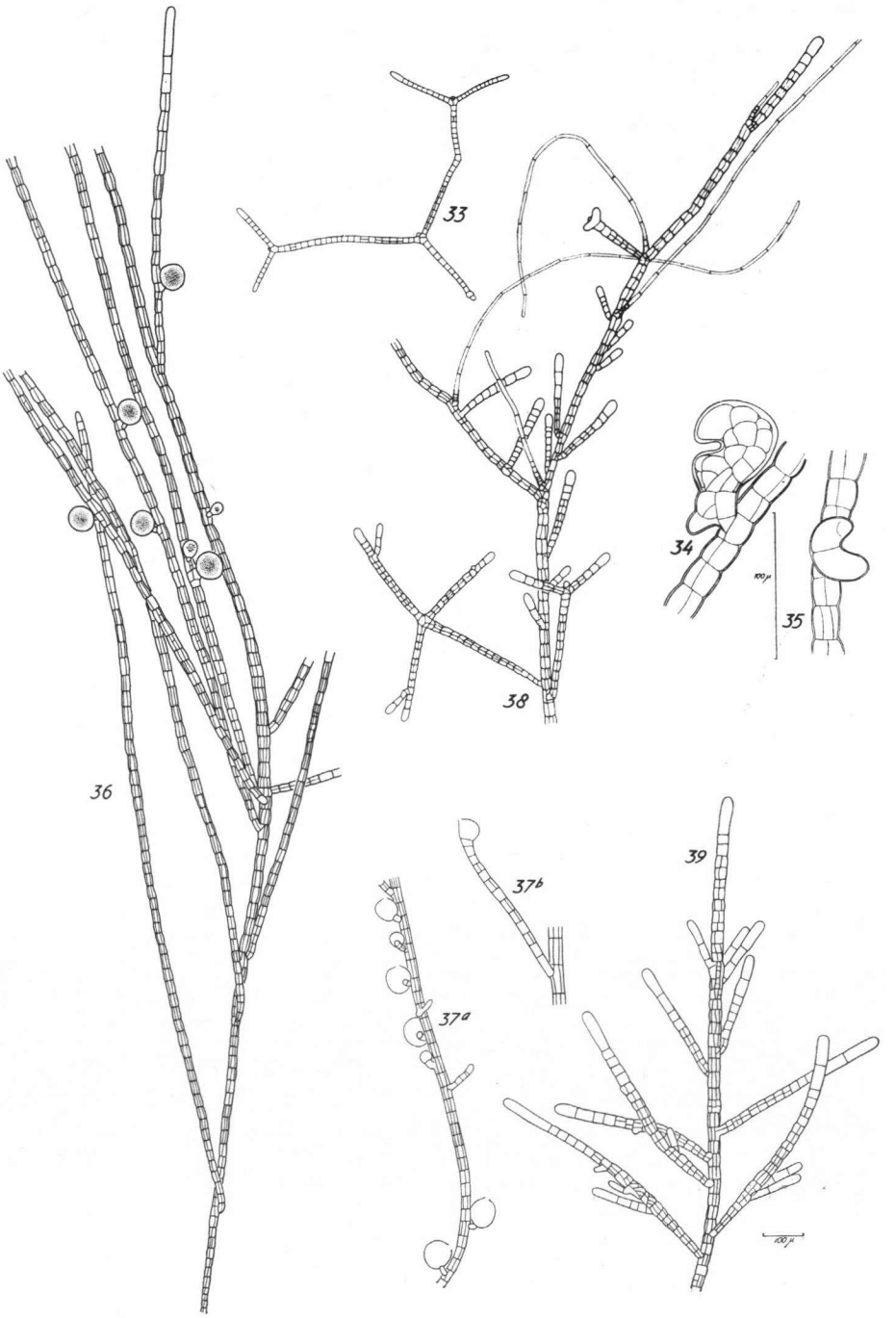
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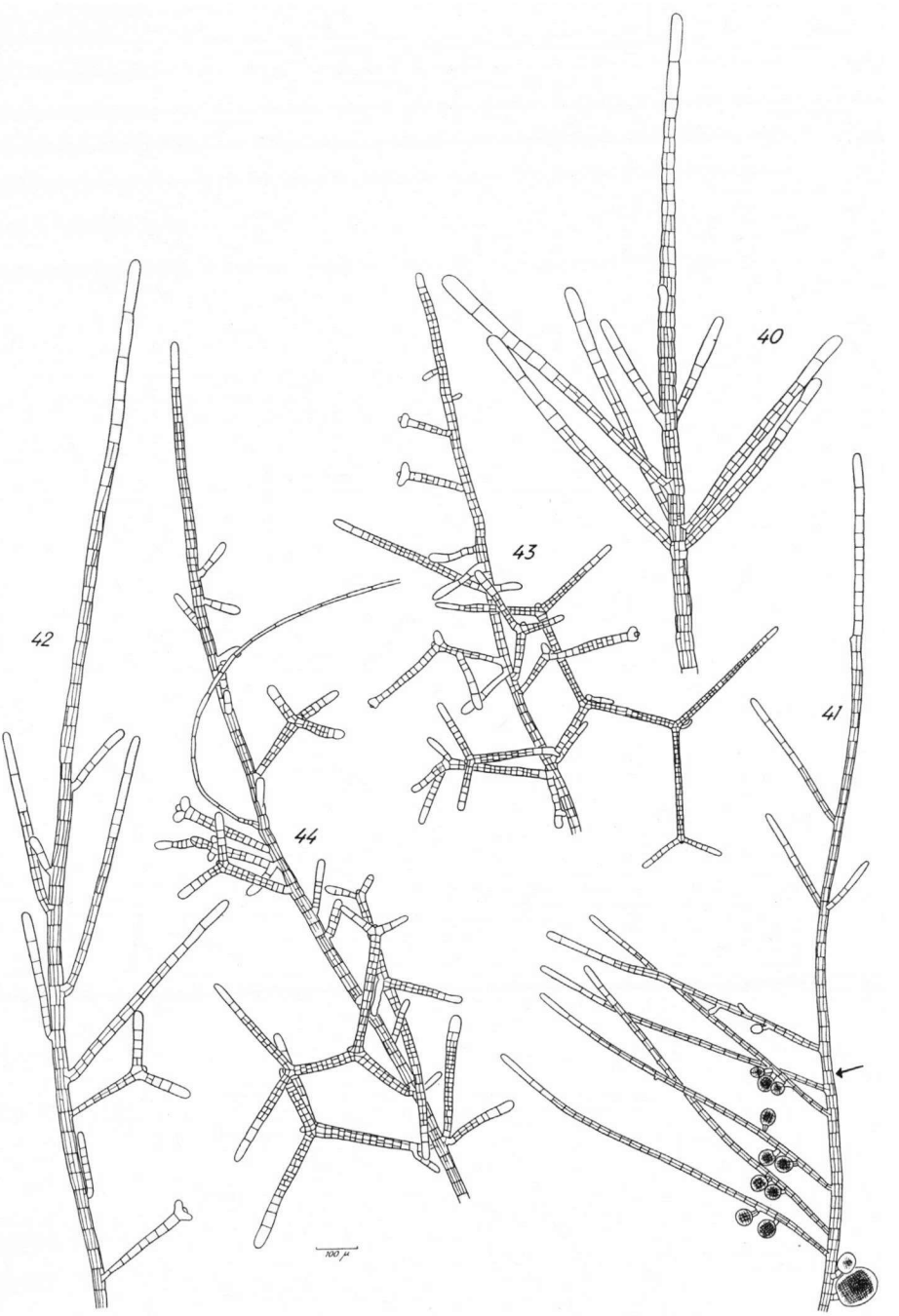
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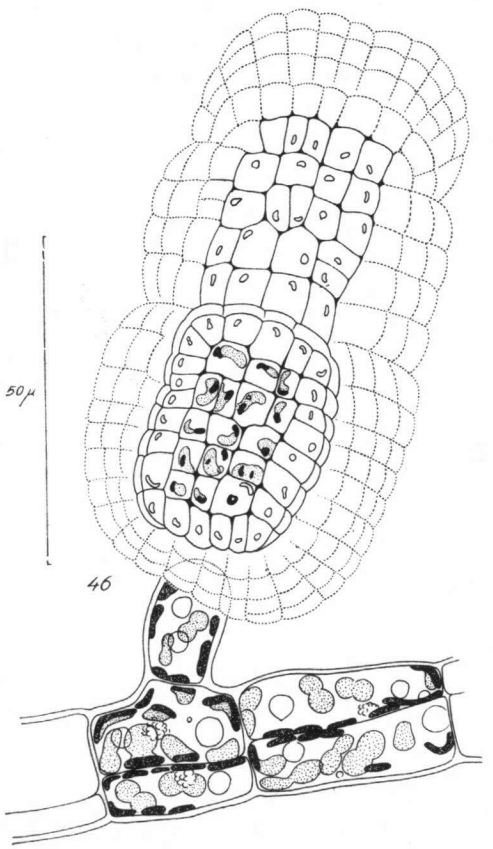
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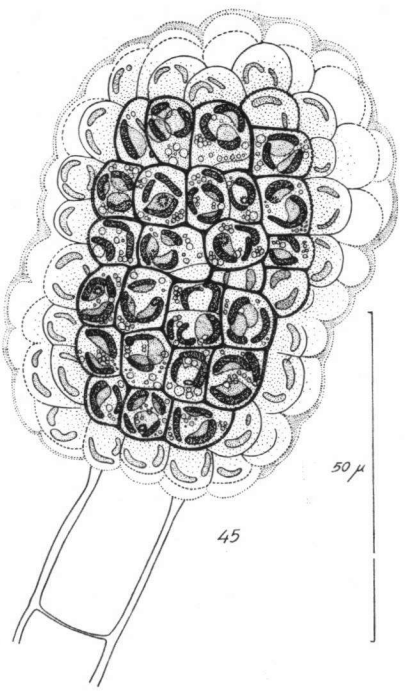
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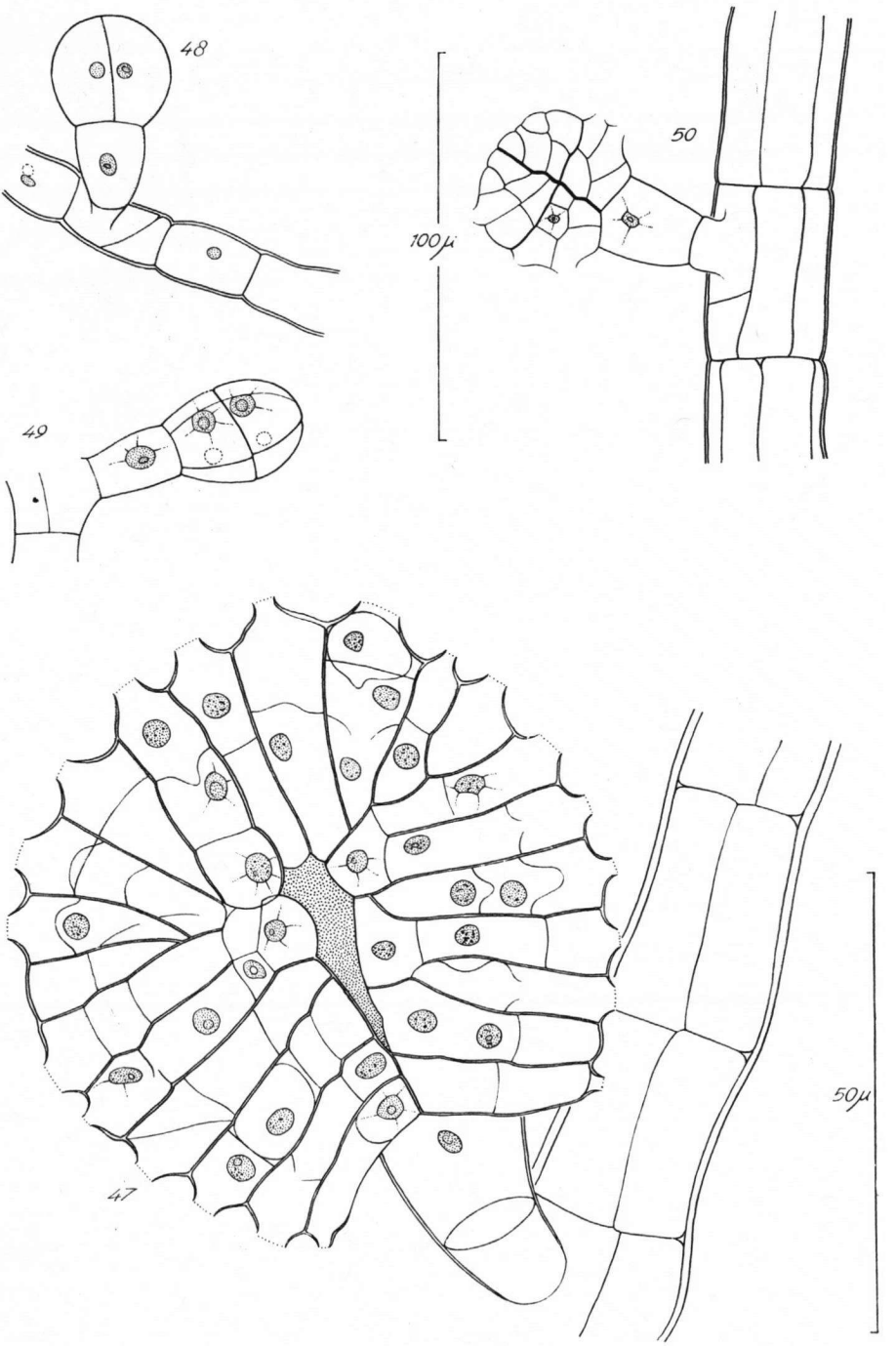
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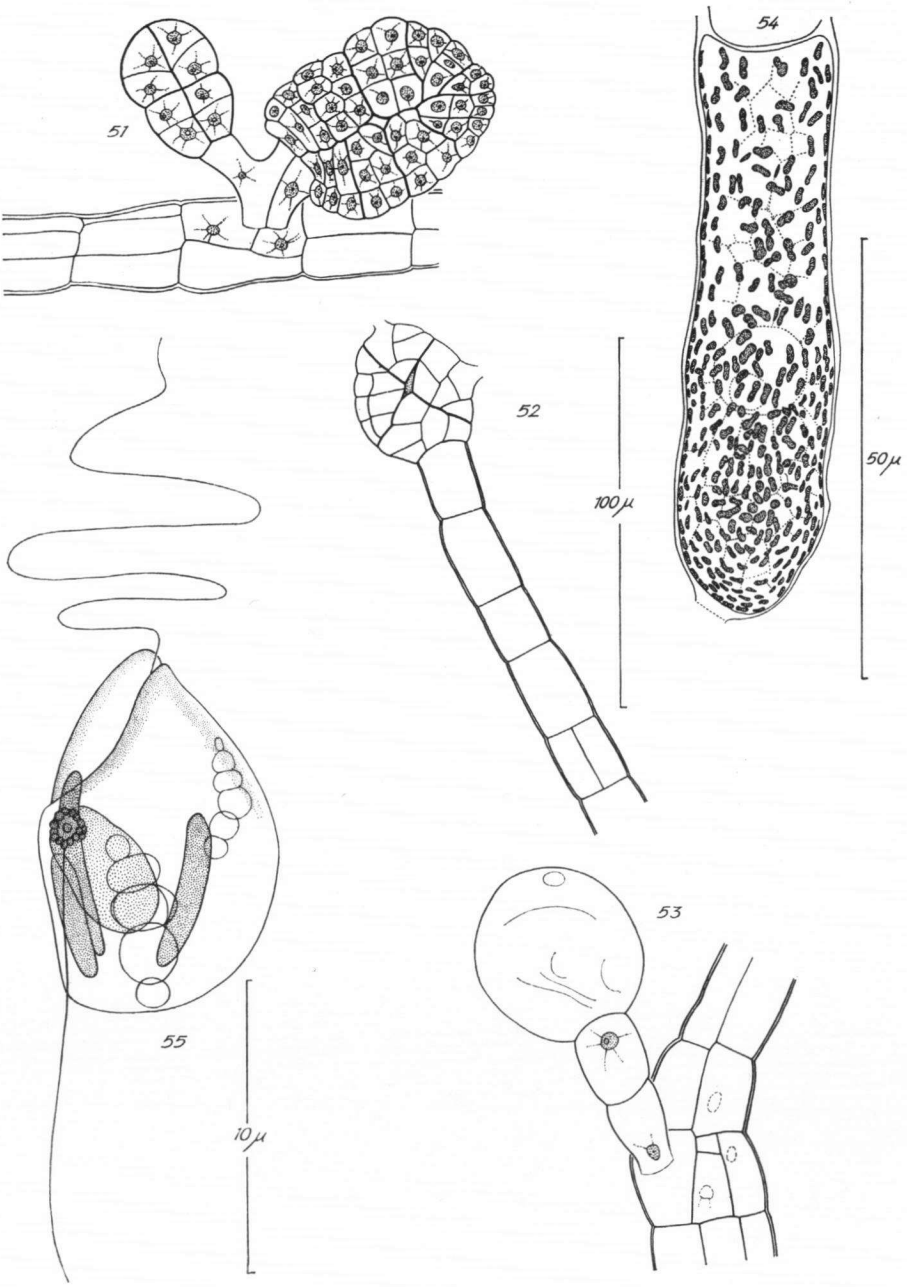
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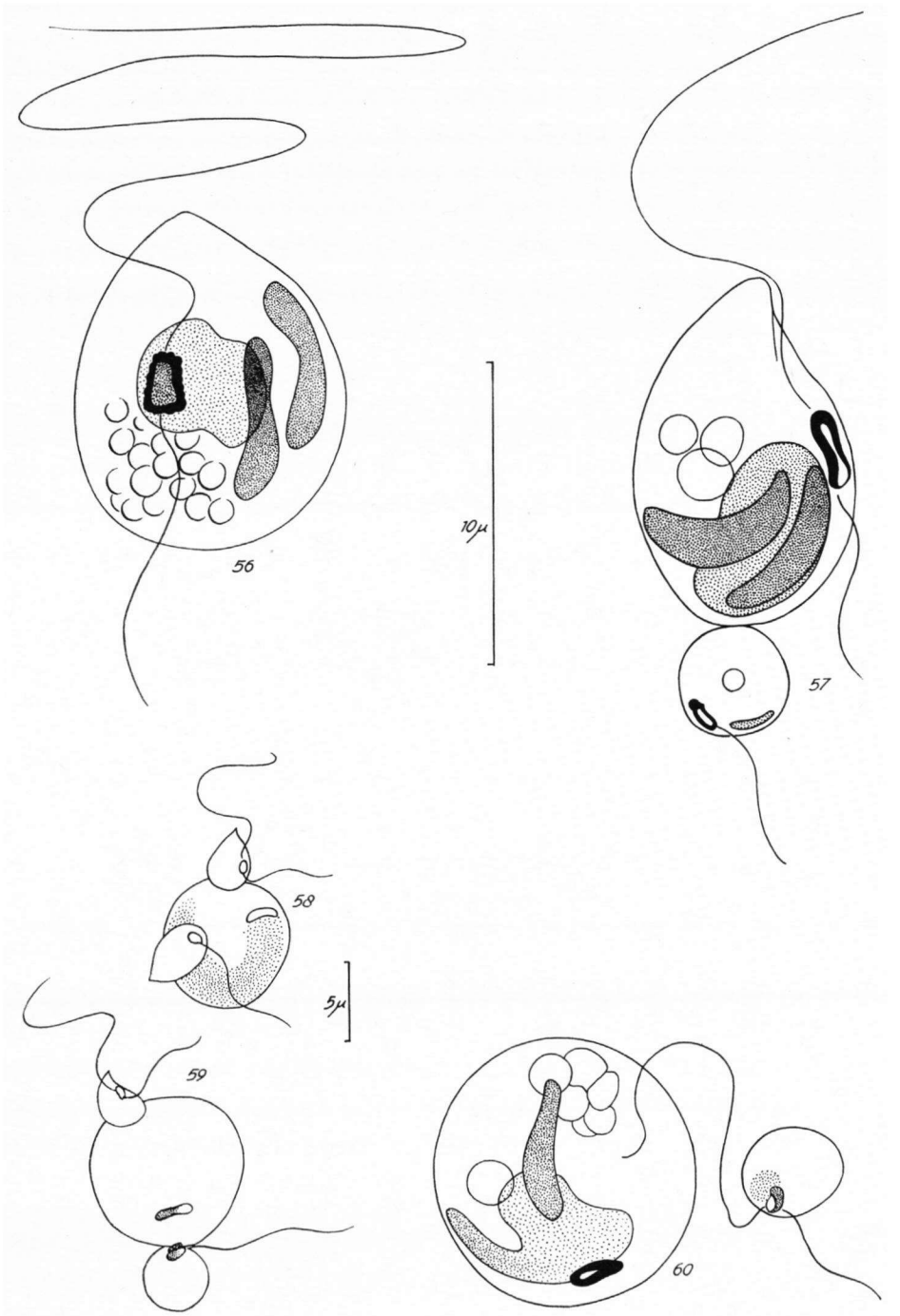
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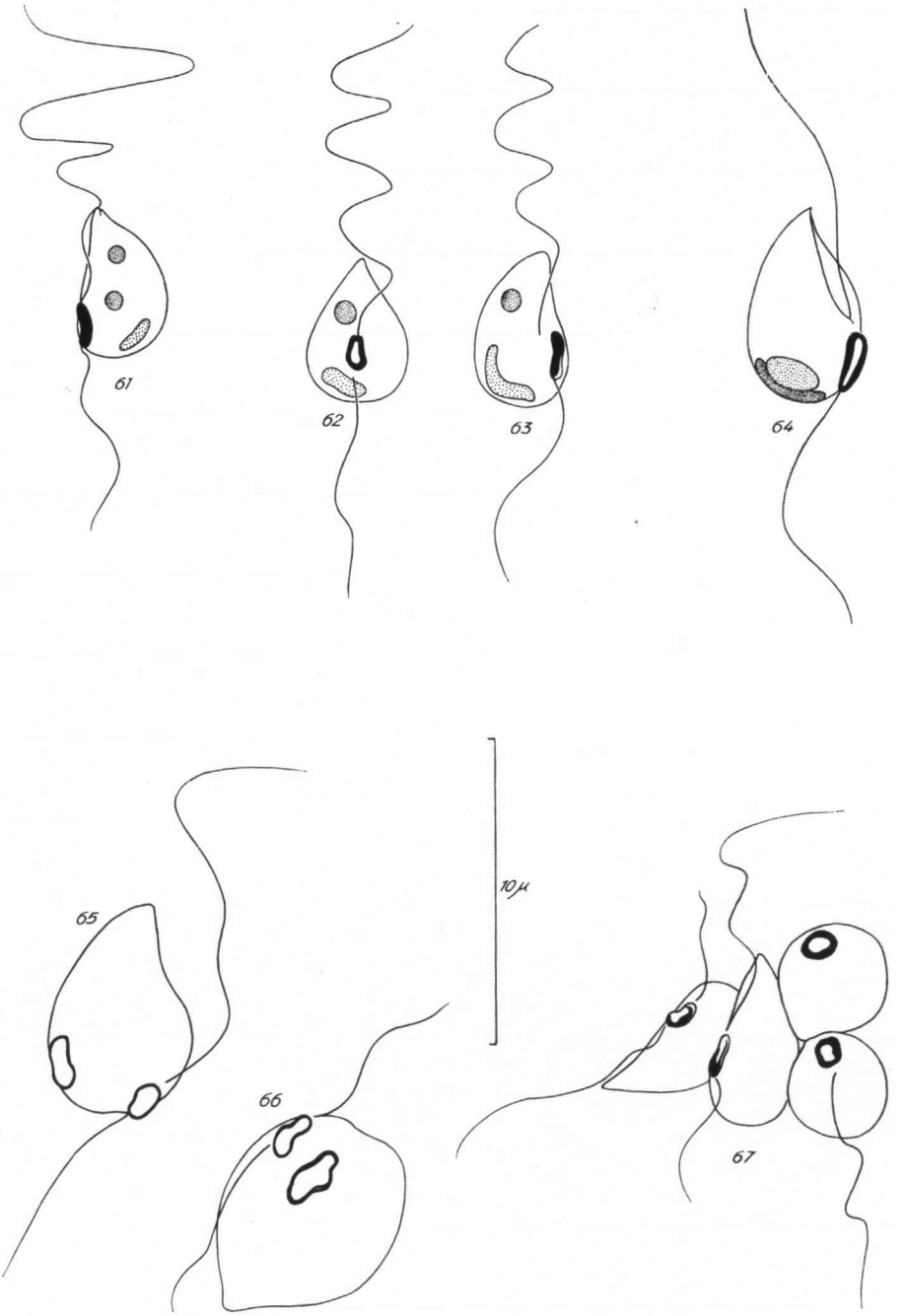
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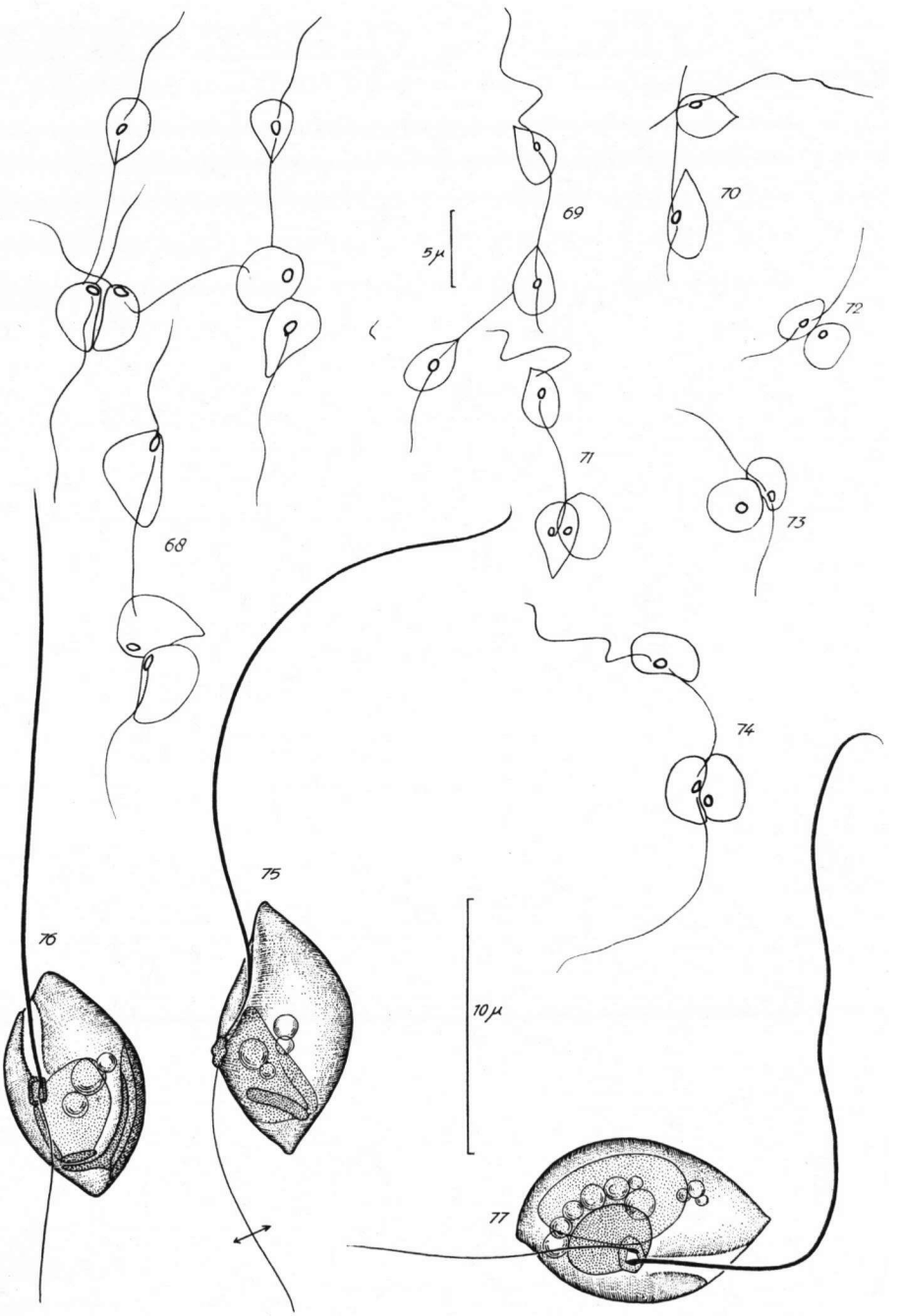
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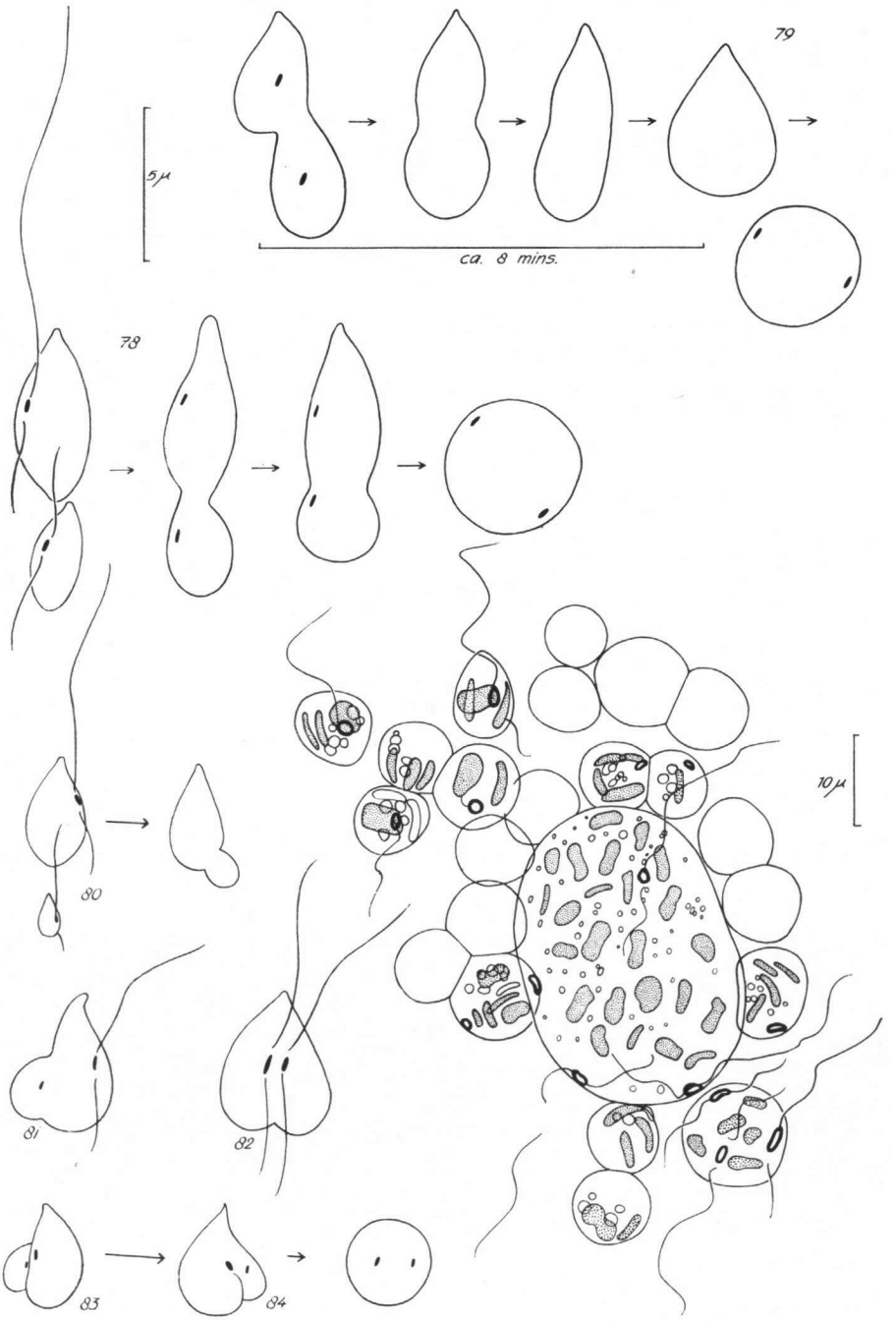
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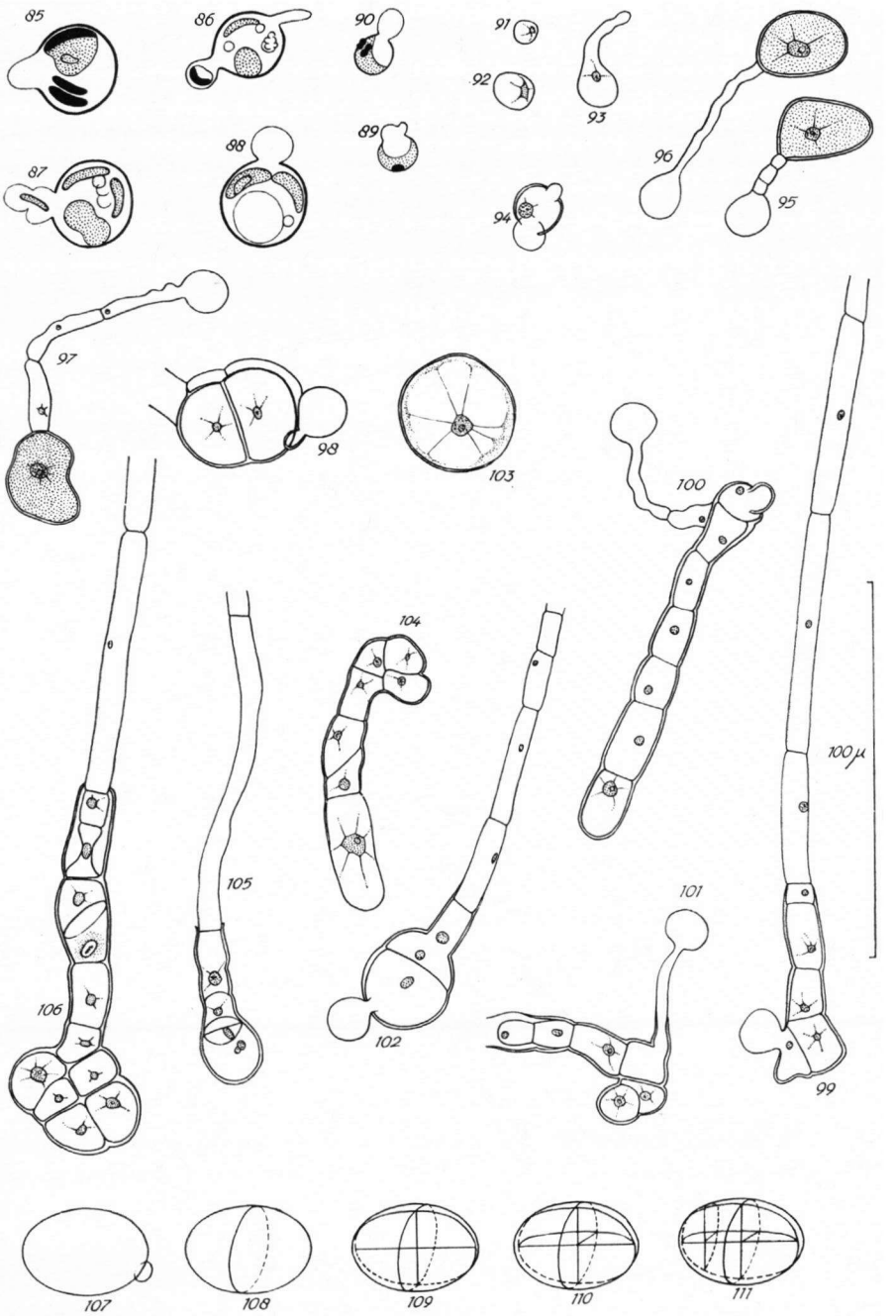
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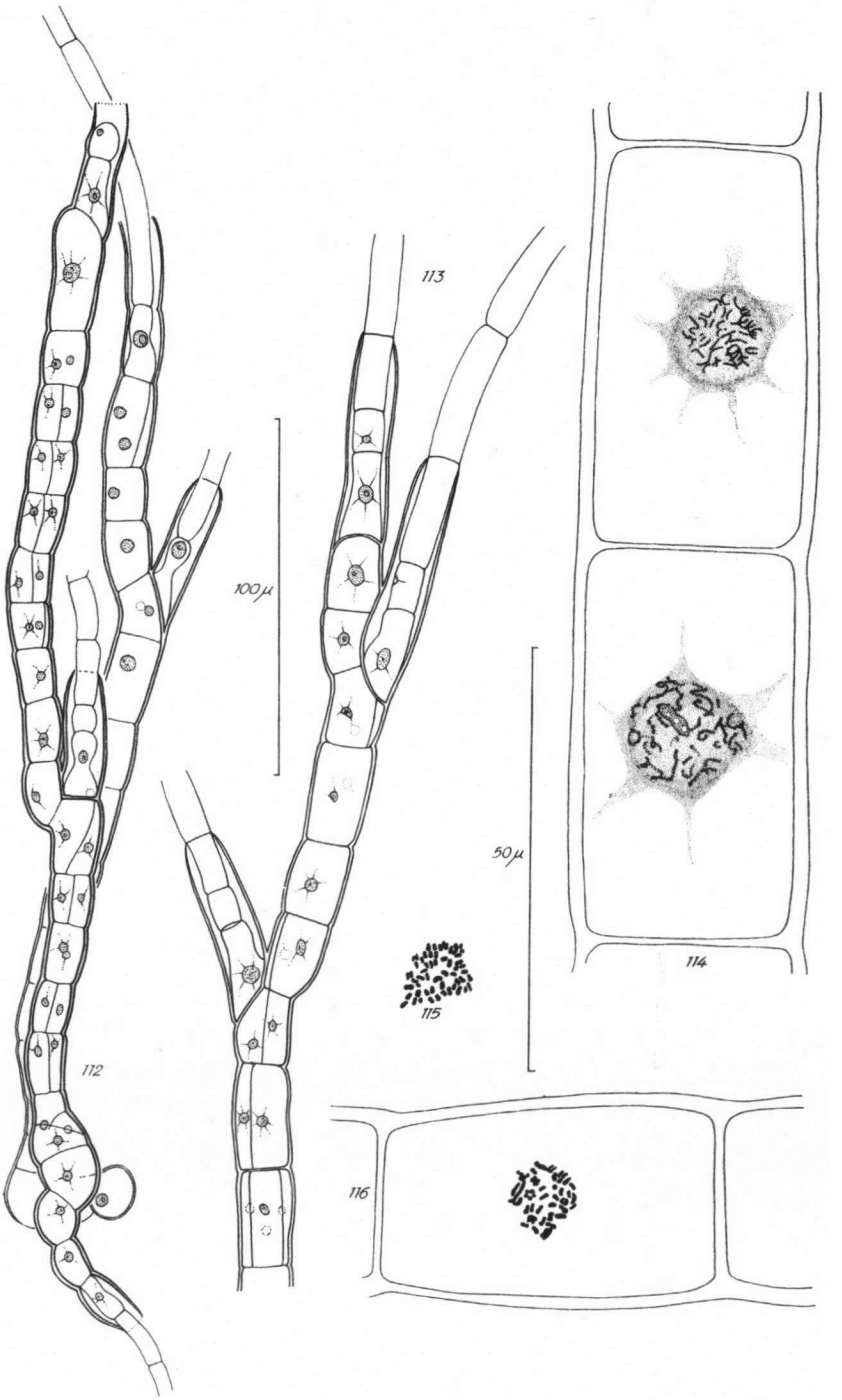
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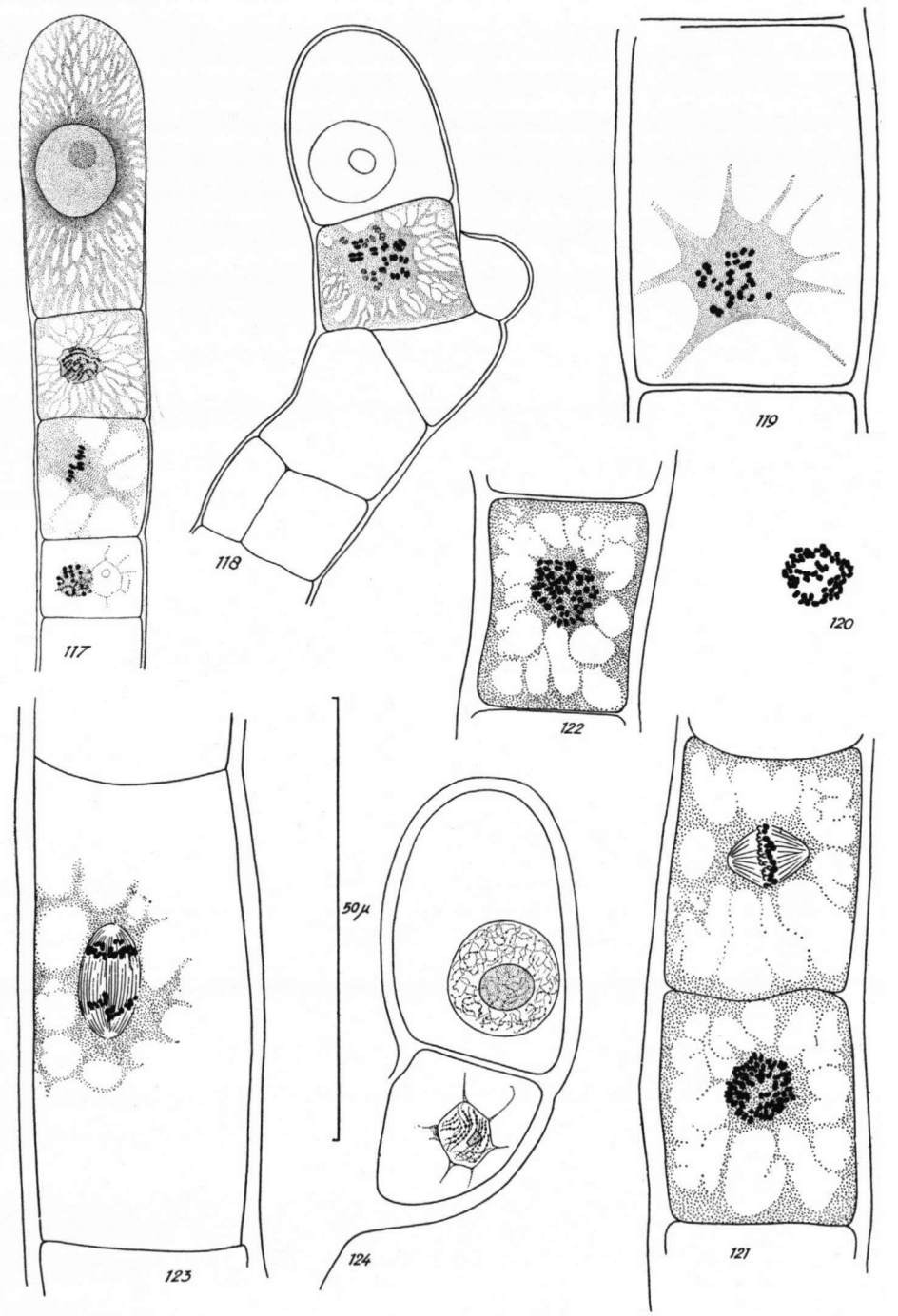
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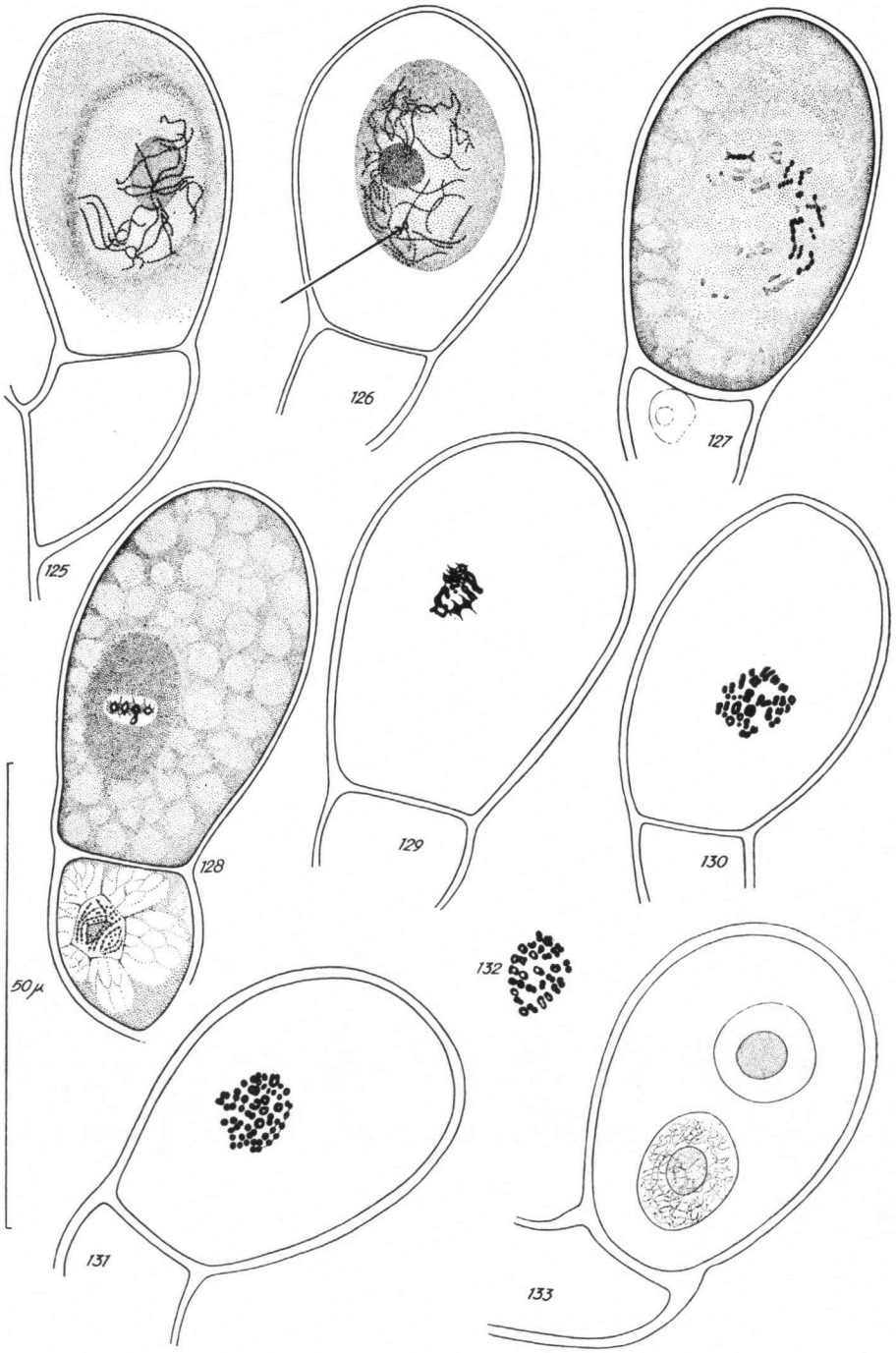
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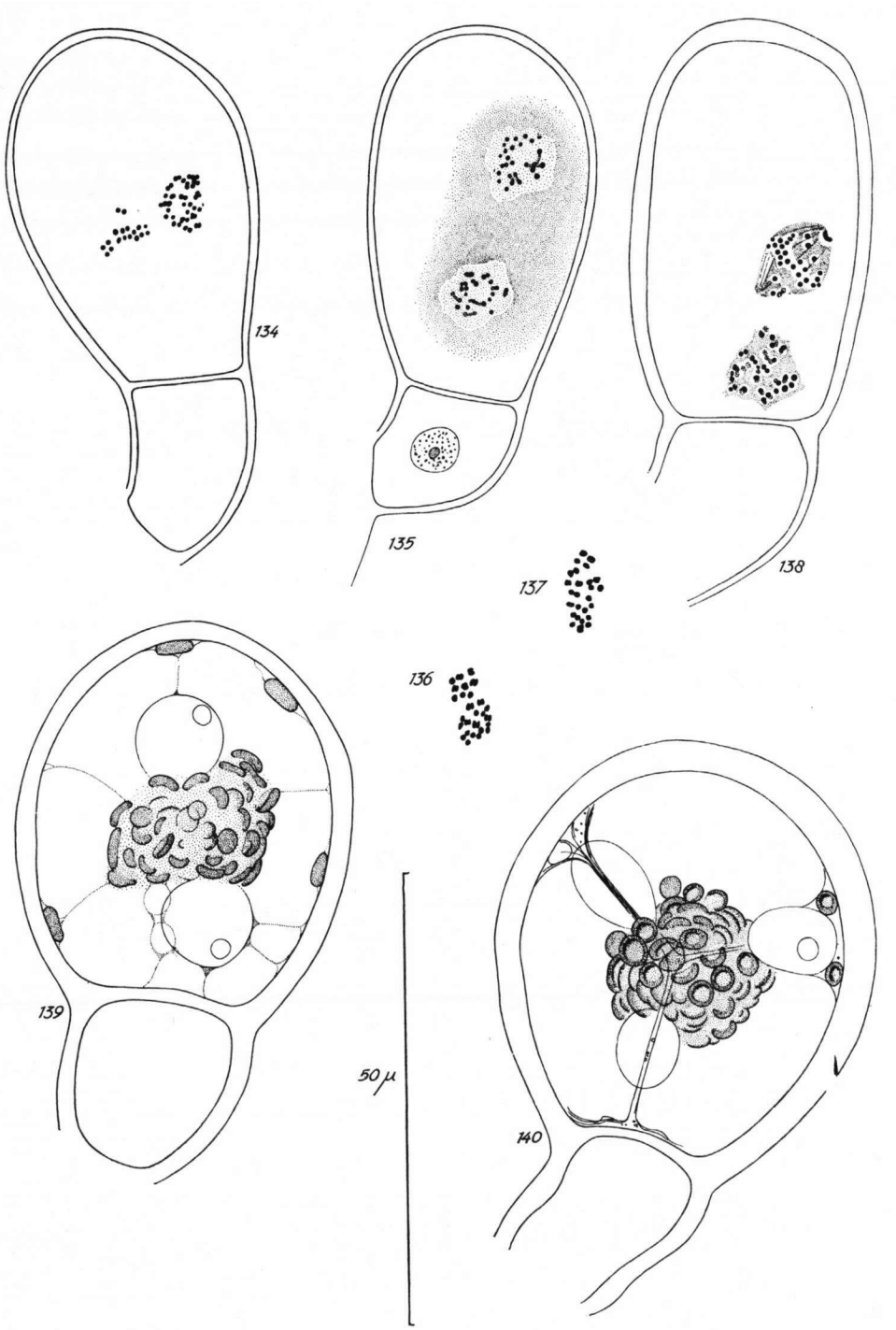
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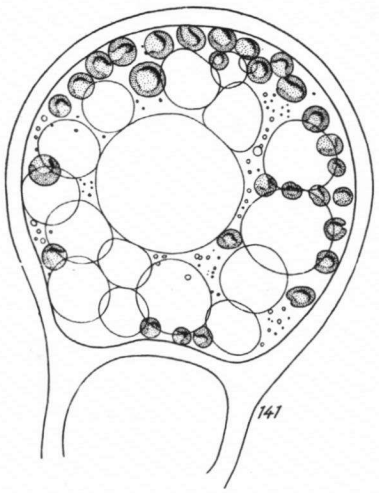
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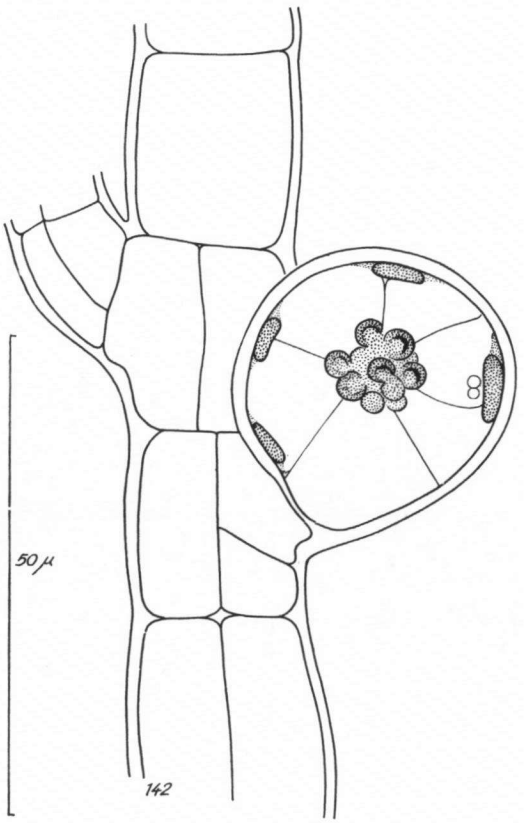
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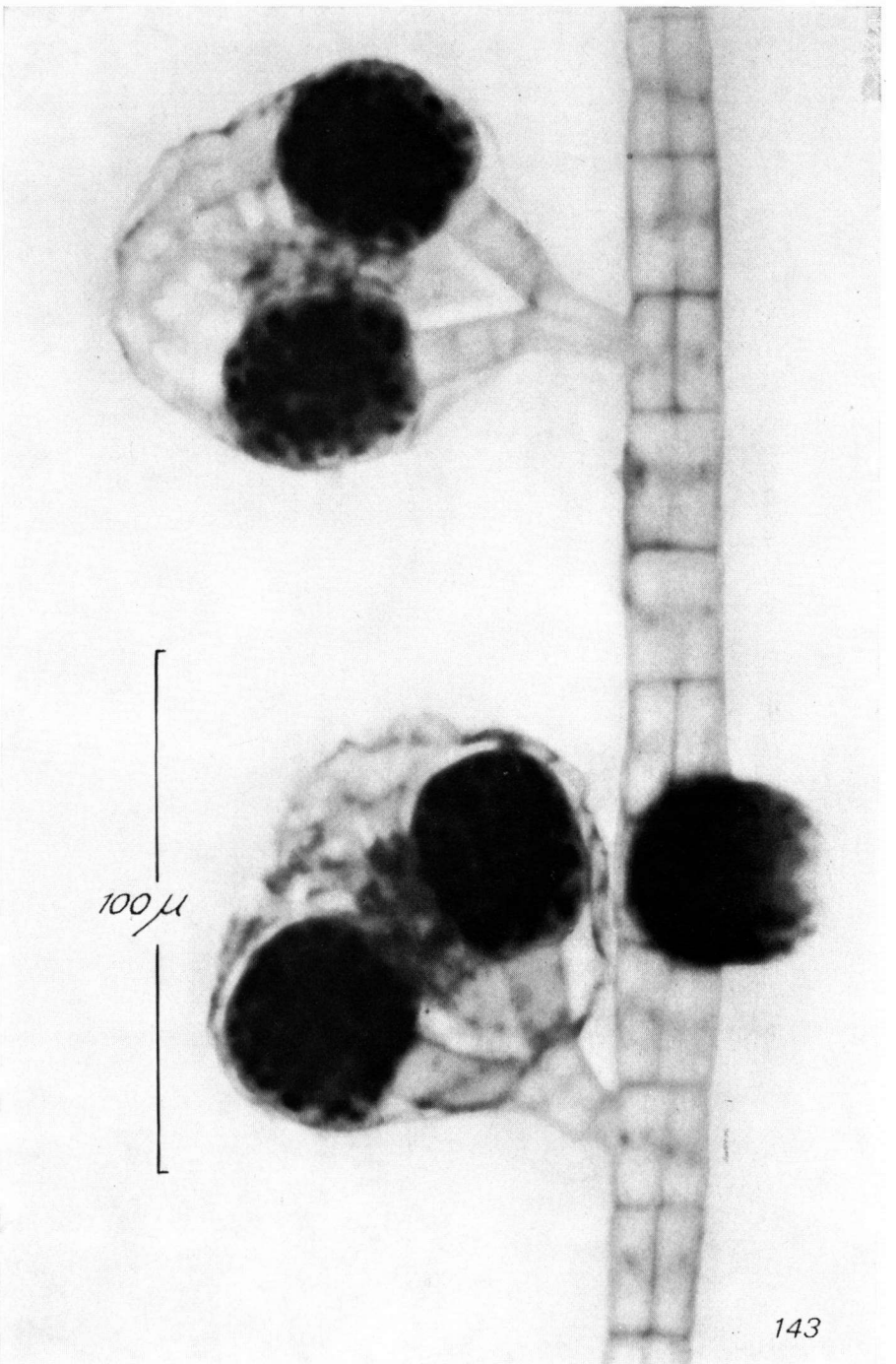
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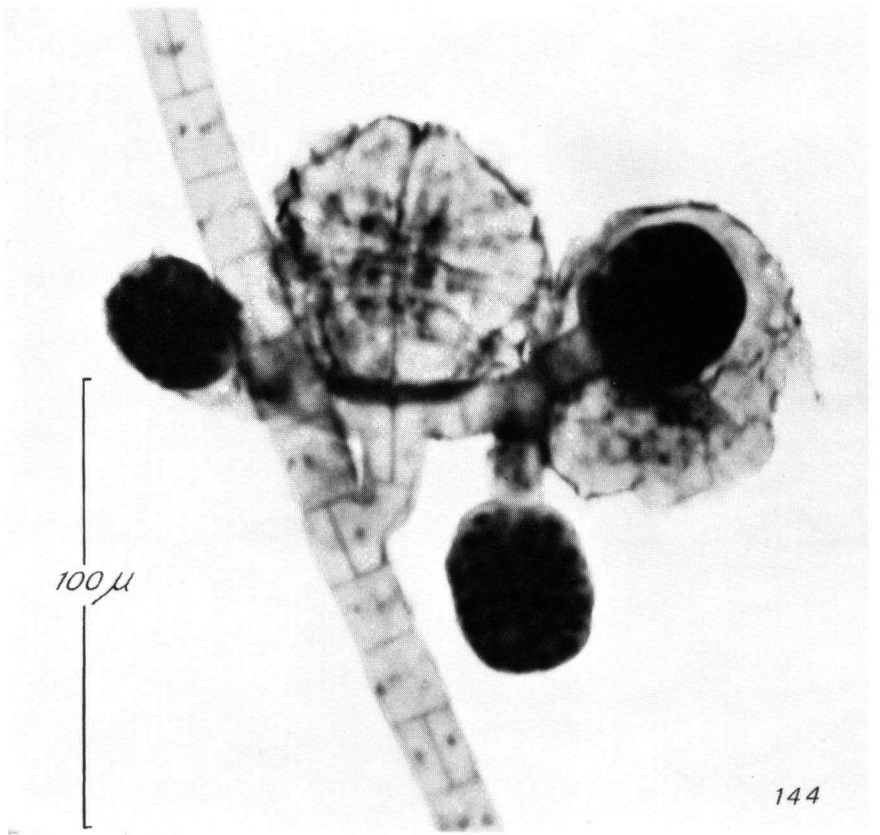
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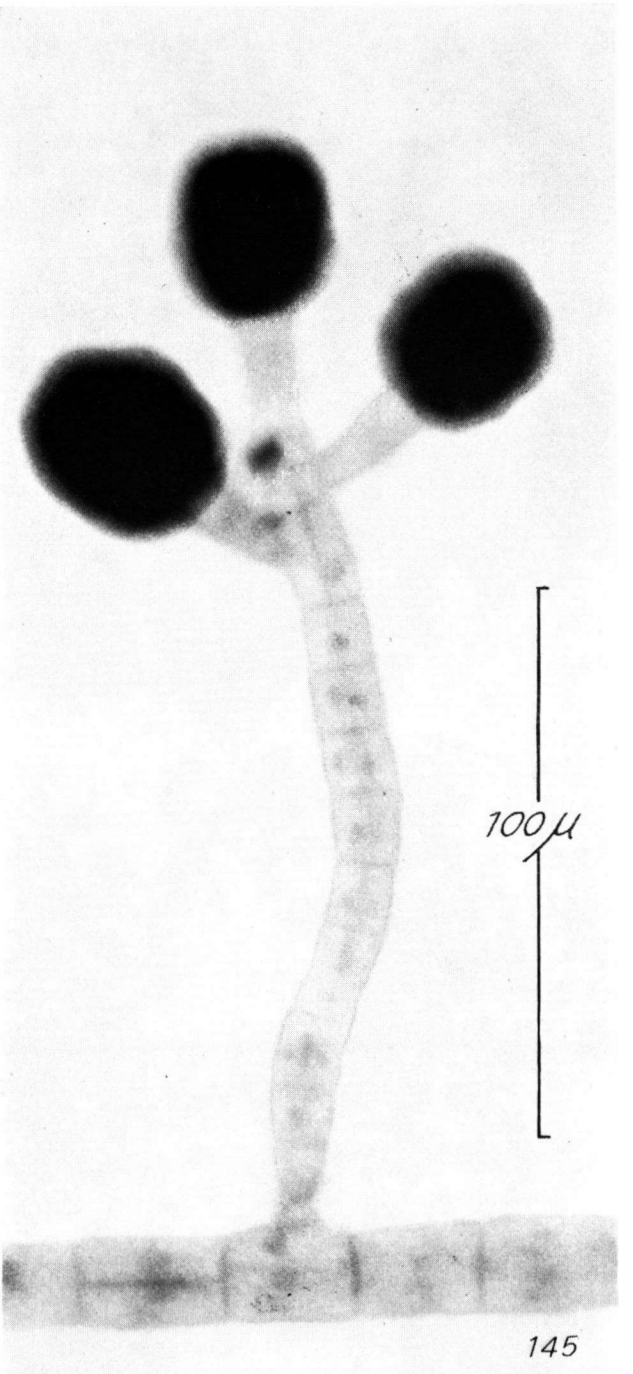
144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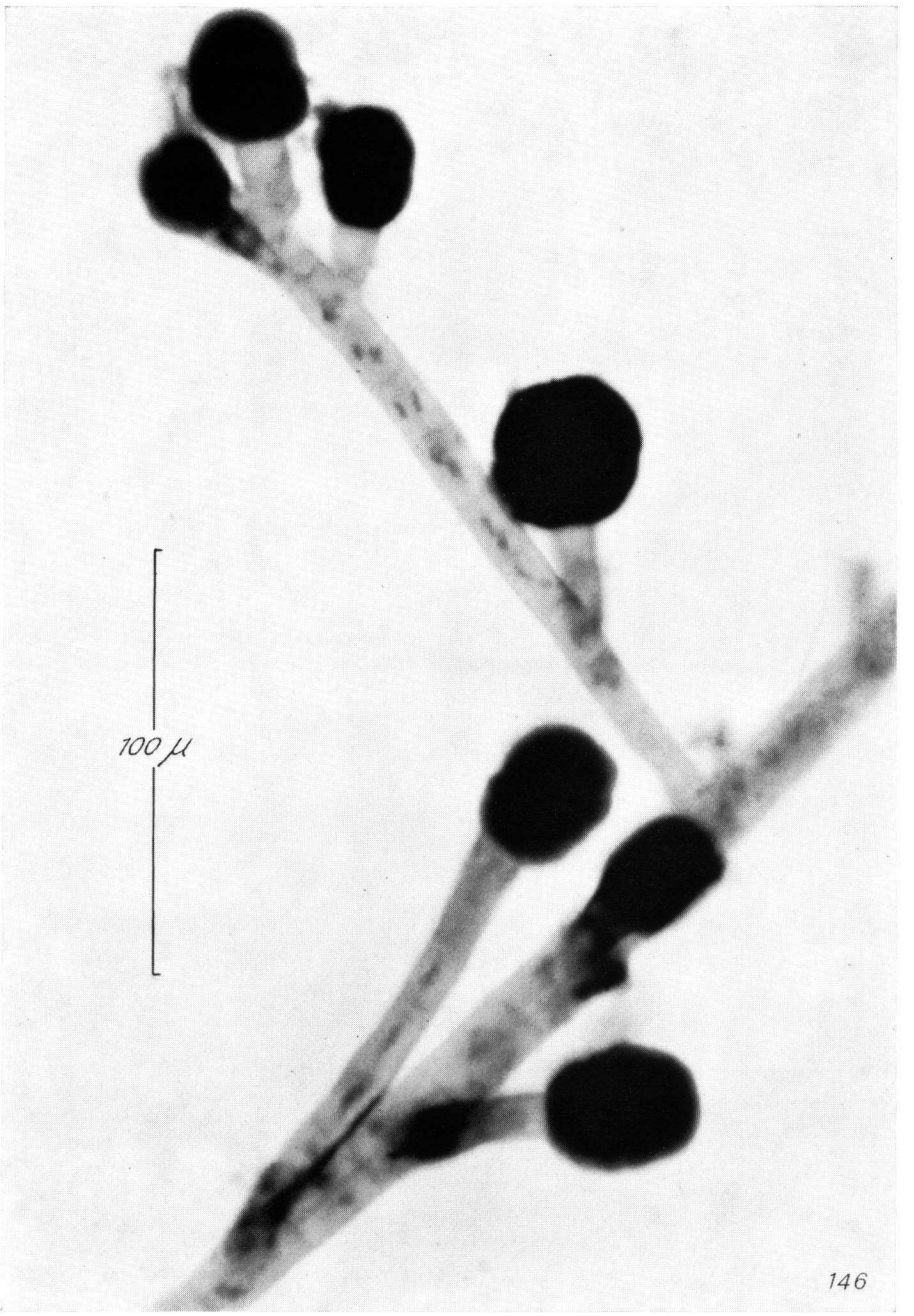
145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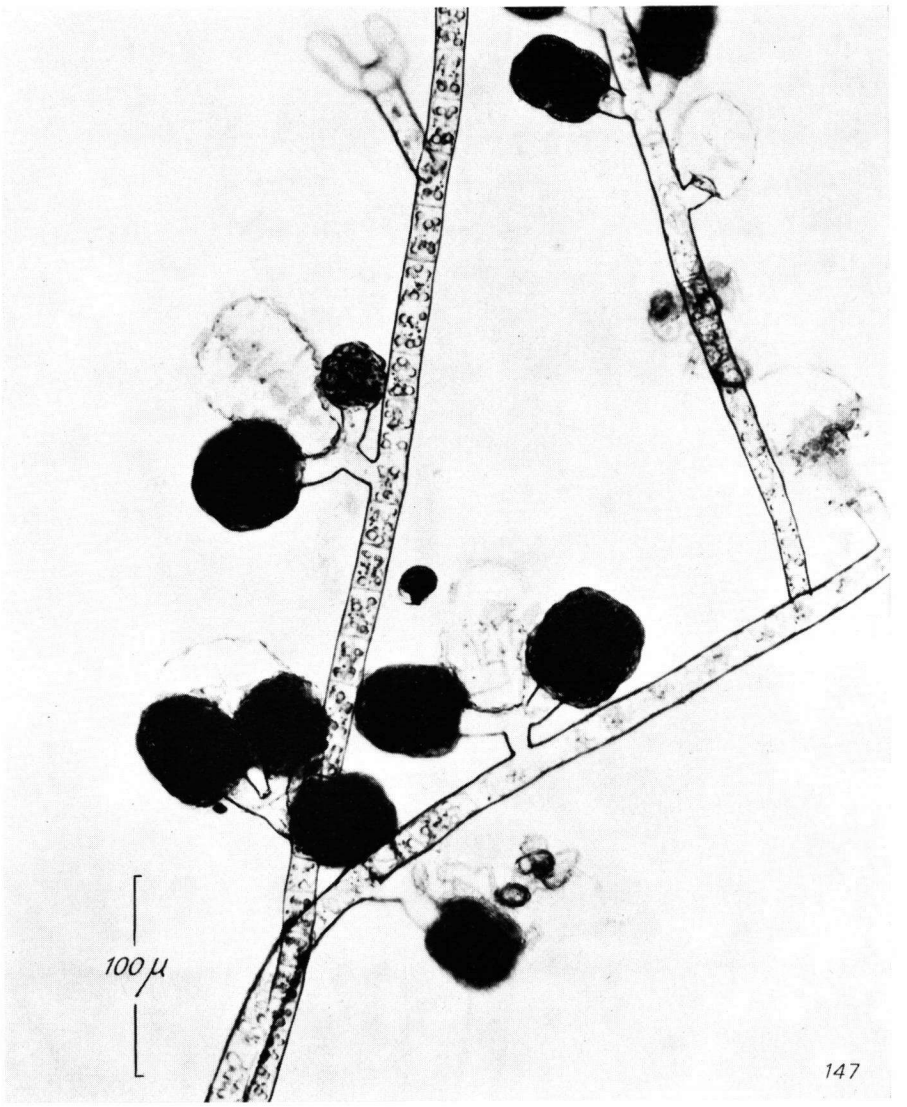
146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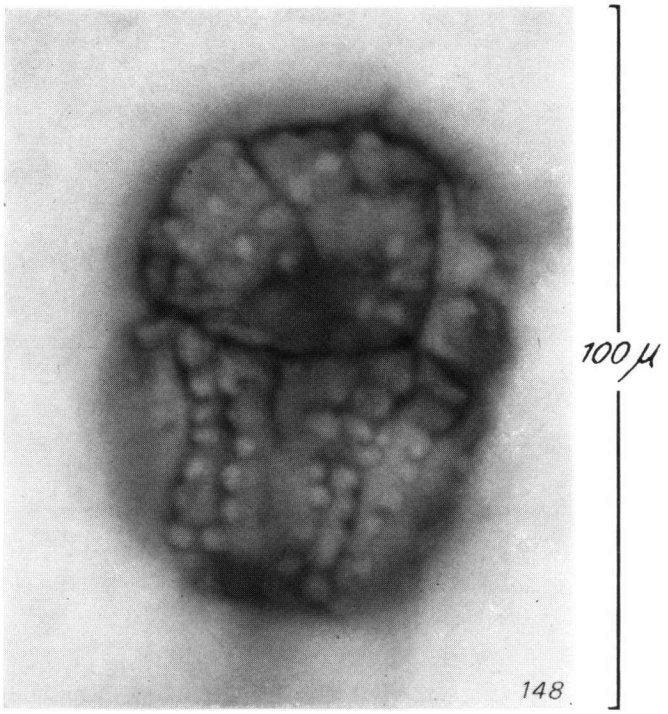
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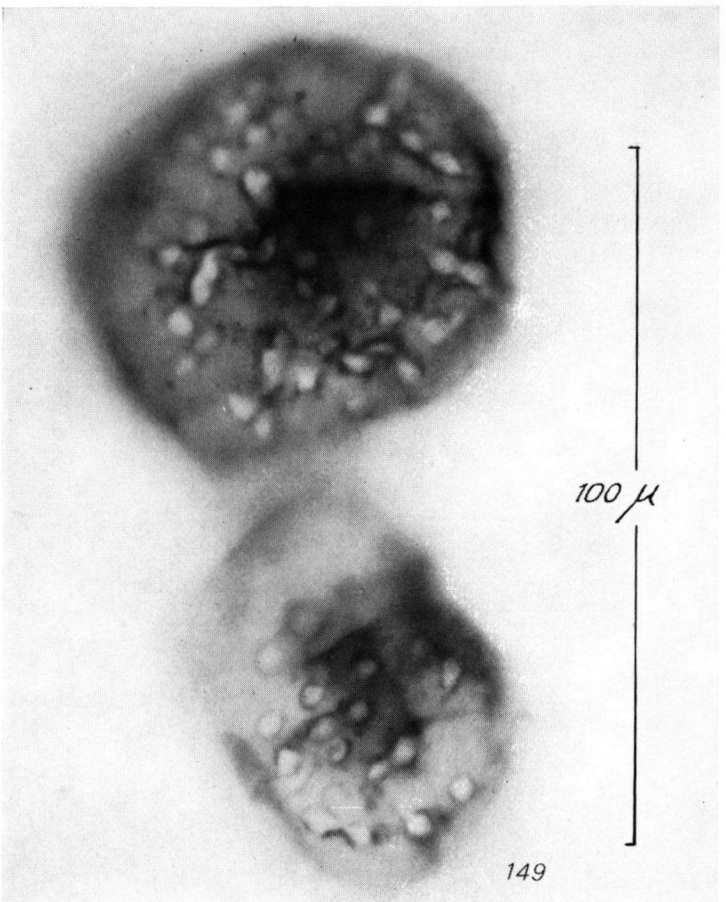
148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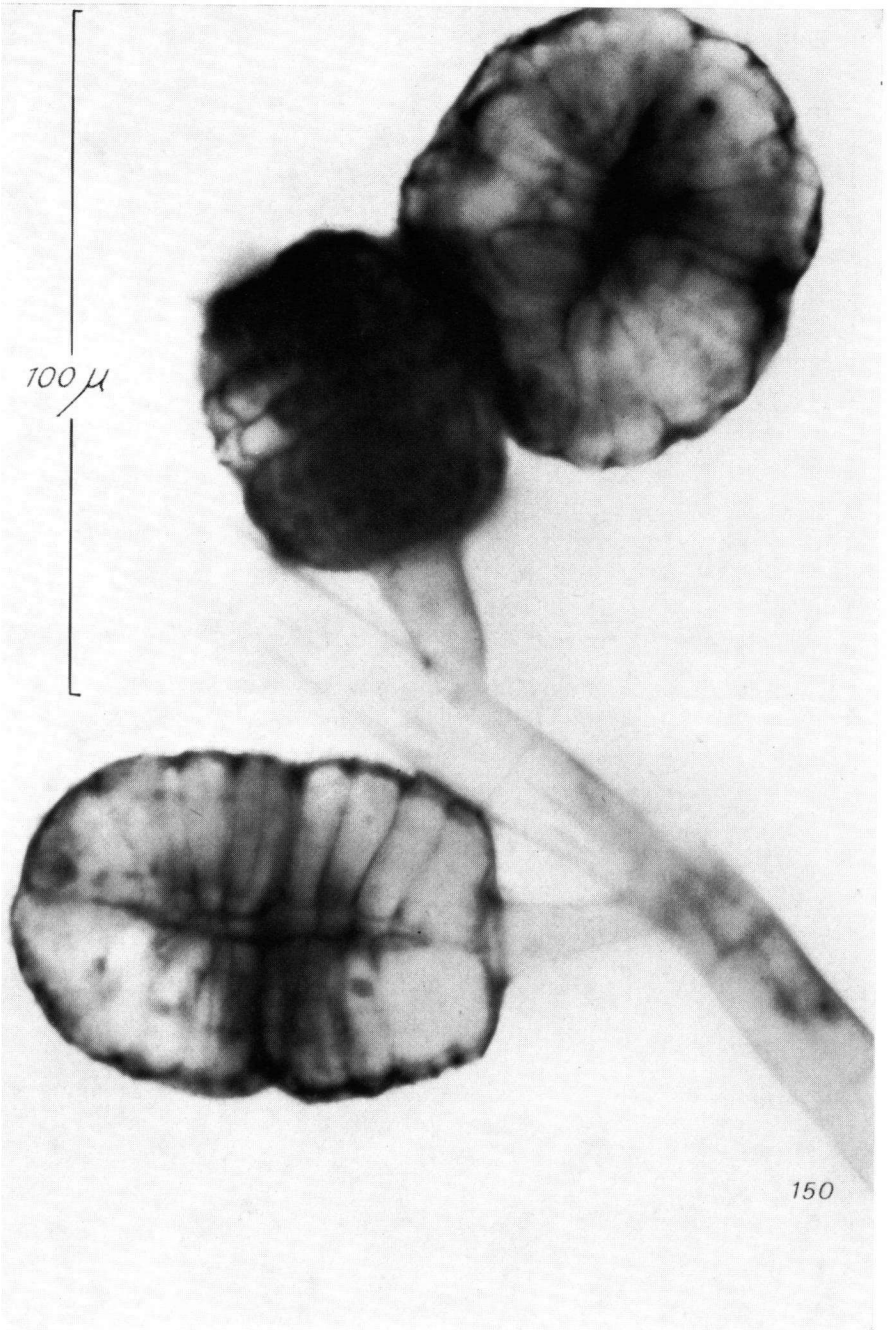
149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150