The Development of the Gametophytes and Embryogeny in Cunninghamia sinensis[1]).

By

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With Plates I—V and 2 Figures in the Text.

Recent embryological studies of Arnoldi, Coker and Lawson have shown quite conclusively that the family *Taxodieae* is not at all a natural group, and that the present classification of the *Coniferae*, so far as this group is concerned, should be entirely rearranged. Arnoldi (1900) has already suggested that, of the genera of this family, *Taxodium, Cryptomeria* and perhaps also *Cunninghamia*[2]), should be united with the *Cupressineae* and that *Sequoia* and *Sciadopitys* should each constitute a family by itself. This suggestion, being followed by Coker (1903) and Lawson (1904), seems to be well founded. But before making any definite new arrangement of the group, it is very desirable that the life-history of all the genera should be thoroughly worked out.

Of eight genera[3]) belonging to the *Taxodieae*, we have only the fairly complete embryological records of three, namely those of *Sequoia* (Shaw, 1896; Lawson, 1904 a), *Taxodium* (Coker, 1903) and *Cryptomeria* (Lawson, 1904 b). Our knowledge about the life-history of the genus *Cunninghamia* is very meager, being based entirely on the fragmentary records of Arnoldi (1900) who has studied the genus incidentally, with insufficient material, in his comparative investigations of the *Taxodieae*. The present work was begun in the summer of 1905 with the hope of filling up the

[1]) A preliminary note was published in Bot. Mag. Tokyo. Vol. 22. (Miyake, 1908.)

[2]) He was not quite sure about *Cunninghamia* as the following statement shows: „Es wird also nach meiner Meinung die von Eichler als Pinoideae-Abietineae-Taxodiinae bezeichnete Gruppe in drei Familien geteilt. Die erste Familie Sequoiaceae — enthält zwei Gattungen: *Sequoia* and *Wellingtonia*; *Taxodium* und *Cryptomeria*, vielleicht auch *Cunninghamia (?)*, werden mit den Cupressineen vereinigt; *Sciadopitys* wird am besten als Repräsentant einer eigenen Familie Sciadopiteae — bezeichnet." (Arnoldi, 1900, p. 23.)

[3]) Seven genera have hitherto been included in the Taxodieae, and an another genus *Taiwania* was recently established by Hayata (1906) basing on a new Conifer from Formosa.

gaps left by Arnoldi and thus to complete, as far as possible, the history of the gametophytes and embryogeny of the genus.

Cunninghamia sinensis, which was the only member of the genus, until the recent discovery in Formosa, disclosed the presence of a sister species *C. Konishii* (Hayata, 1908), is a native of China and is found in Japan only in the cultivated condition. The material for the present study was collected chiefly by myself during the last four years 1905—1908 from plants growing in Kyoto and Tokyo. A few materials which had been collected by Prof. K. Shibata before the year 1905 and kindly given to me, were also examined.

The material was obtained from different trees, and from several cones of the same tree at each collection, and the fixing was done immediately after the collection. The staminate cones were fixed entire or cut into several pieces according to their sizes. In the early stages of development the ovules were fixed with a part of the scales, and later the ovules were entirely removed from the scales. For older stages, a part or the whole of the integument was removed before fixing.

Flemming's chrom-osmo-acetic acid solution of various concentrations was chiefly used for fixing, but chrom-acetic mixture was also occasionally used. After fixing, the material was washed, dehydrated and imbedded in paraffin in the usual way. The sections were cut usually from 5 to 10 μ in thickness. For staining, Flemming's safranin, gentian violet, and orange combination or Heidenhain's iron-alum haematoxylin were used.

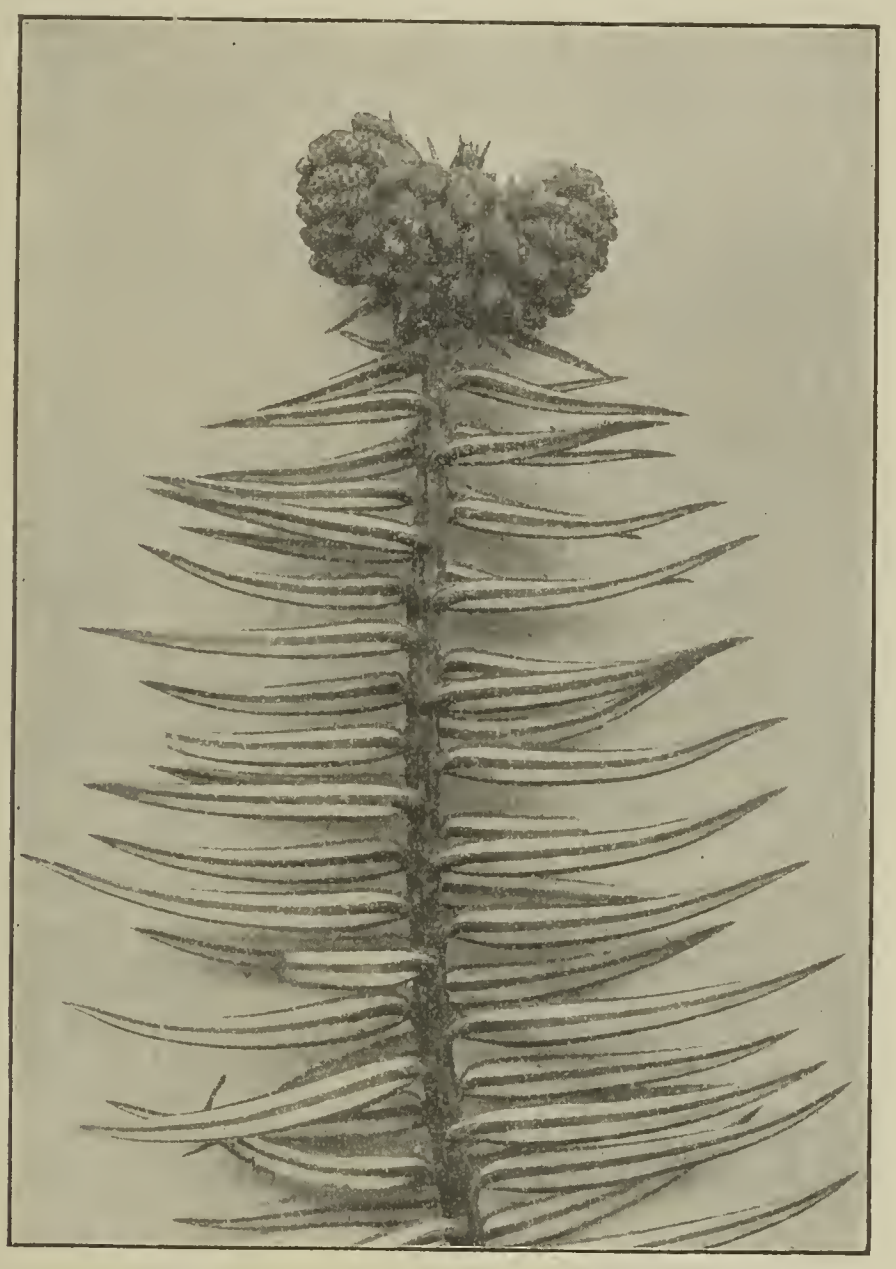
Development of the Pollen.

The staminate cones make their appearance, at or near the tips of the branches, as early as September of the year preceding pollination. The mature cones at the time of pollination is shown in text-figure 1. Young microsporangium, as observed in early November, has the wall consisting of two or three layers of cells and just within, in immediate contact with the archesporium, there is a layer of tapetum-cells. The archesporial cells are found in constant division during the autumn and the pollen mother-cells are formed before the end of the year.

The division of the pollen mother-cell takes place (in the middle part of Japan) about the end of February or the beginning of March. The pollen mother-cell, whose nucleus is in a resting condition, is shown in Fig. 1. As the cell prepares for division, the contents of the nucleus undergo a synaptic contraction (fig. 2). Then follows the spirem stage (fig. 3). The thin spirem thread gradually thickens itself and the double nature of the thread becomes more or less apparent (fig. 4). The thick spirem now segments into chromosomes (fig. 5). The stages between synapsis and chromosome-formation are now considered as the most important phases in the reduction-division, and have been investigated with special care by several cytologists during last few years. The

results and interpretations do not agree, and the opinions still differ among various investigators. In the present paper I have no intention to enter into a discussion of the disputed points; and the minute descriptions of these as well as other stages of the reduction-division are not to be given.

The chromosomes formed by the segmentation of the spirem are found in pairs. The bivalent chromosomes now become short and thick (fig. 5). With the disappearance of the nucleolus and the nuclear membrane, the formation of the spindle begins. The spindle is at



Text-fig. 1. Staminate cones ready for pollination, seen from the underside of a branch. About $\times$ $^3/_4$.

first multipolar and later assumes the bipolar structure (figs. 6—8). The chromosomes then arrange themselves at the equator of the spindle as shown in figs. 7 and 8. The number of the chromosomes can easily be counted at this stage, viewing from a pole of the spindle. Their number, i. e., that of the bivalent chromosomes, is twelve, as it is usually the case with other Conifers (fig. 9).

Each of the bivalent chromosomes, which are arranged at the equatorial plate, soon separates into two, and the daughter-chromosomes begin to move toward the poles. Very soon the chromosomes show the sign of longitudinal splitting and, as they approach

the poles, they appear as two thick and bent double rods (figs. 10, 11). Fig. 12 shows the chromosomes as they accumulate in both poles of the spindle; the daughter-nuclei are then organized as shown in fig. 13.

The daughter-nuclei, between which no wall is formed, soon become ready for the next division. The two spindles of the second division, are either parallel or perpendicular to each other. Various stages of the division are shown in figs. 14—18. After the four grand-daughter-nuclei are completely formed, walls appear between them and thus four pollen-cells or microspores are organized. Ultimately a fresh wall is formed around each cell, and the microspores then separate from each other by the dissolution of the original walls (fig. 19). After the microspores are separated from each other, they undergo a rapid growth and the walls become considerably thickened (fig. 20). The fully-formed microspore is a more or less spherical cell with a prominent nucleus; the nucleus usually contains a nucleolus. The wall of the microspore seems to consist of a thin exine and a thick intine (figs. 20, 21).

Development of the Male Gametophyte.

Pollination begins, as a rule, in the first few days of April, though the date differs somewhat by season as well as by locality. In Kyoto one tree has begun to shed the pollen on April 4 in 1906. In Tokyo pollination began in two different trees on April 1 in 1908. It usually lasts about ten days or two weeks. The pollen-grain, at the time of pollination, contains two nuclei unequal in size. The larger one represents the vegetative or tube-nucleus, and the smaller one corresponds to the generative nucleus. The two nuclei are separated from each other by a delicate plasmic membrane (fig. 26). The division in the formation of these two nuclei or cells seems to take place a few days before pollination. Various stages of the division are shown in figs. 22—25. The absence of the sterile prothallial cells seems to be the rule among the *Taxodieae* and the *Cupressineae*. The same thing is also found in the *Taxeae*[1]). In other groups of the *Coniferae*, one or two prothallial cells are usually formed.

A few days after pollination, the pollen found at the apex of the nucellus begins to send out tube. The pollen-tube, penetrating the tissue of the nucellus, grows gradually downward. The generative cell now divides into two, i. e., the so-called stalk- and body-cells. The process of the division itself has not been observed. The body-cell then assumes a more or less spherical shape, while the nucleus of the stalk-cell soon looses its own cytoplasm and lies free in the cytoplasm of the tube (fig. 27). Both the body-cell and the stalk-nucleus now enter into the pollen-tube, and move

[1]) The genus *Phyllocladus* which was formally placed in the *Taxeae*, having two or three prothallial cells in the pollen, seems to have more affinity to the *Podocarpeae*, and should better be excluded from the former (Pilger, 1905; Robertson, 1906; Kildahl, 1908).

slowly downward following the tube-nucleus which was previously found in the tube (figs. 28, 30, 31).

The stalk-nucleus now advances slightly ahead of the body-cell and comes very close to the tube-nucleus. The stalk-nucleus which was at first smaller than the tube-nucleus, soon approaches the latter in size, and as the both nuclei are of the same structure, it is almost impossible to distinguish one from the other in the later history of the pollen-tube (figs. 31—34). As the body-cell moves down the pollen-tube, following the stalk- and tube-nuclei, it becomes more or less elongated and increases much in size; it has no definite cell-wall and often assumes a more or less irregular outline (figs. 31, 32).

The downward growth of the pollen-tube is at first relatively slow and at the later stage of the development it is much accelerated. Fig. 31 shows the stage when the pollen-tube is about half way advanced the nucellar tissue in the middle of June, and in fig. 32 we have the tube which has almost completed the penetration of the nucellus in the end of June.

About the end of June, the pollen-tube reaches the female prothallium and soon penetrates into the depression just above the archegonial complex (figs. 70, 111). The body-cell, which is now very much enlarged and almost spherical in shape, lies at the enlarged tip of the pollen-tube. The tube- and stalk-nuclei are found just below the body-cell, being imbedded in the granular cytoplasm with numerous starch-granules (figs. 33, 34, 106 a, 106 b).

The body-cell has a large nucleus surrounded by the dense, finely granular cytoplasm. The nucleus has a prominent nucleolus and a well marked reticulum (figs. 34, 106 a, 106 b). As the cell prepares for the final division, it becomes more or less elliptical in outline. The figures showing the stages of the division were not found. The division takes place usually during the first few days of July. Figs. 35 and 107 show the sperm-cells which are completely organized. The two sperm-cells are of the same shape and of equal size. Each sperm-cell has a large nucleus and the latter is surrounded by a dense mass of cytoplasm containing abundant starch-granules. In the mature sperm-cell, as shown in fig. 35, the starch-granules are often arranged in a dense sheath immediately surrounding the nucleus and a clear area is seen in the periphery of the cell. A similar arrangement of the starch in the sperm-cell has been observed by Coker (1903, p. 11 and fig. 31) in *Taxodium*. When the sperm-cells are fully formed, fertilization takes place almost immediately.

The development and structure of the male gametophyte of *Cunninghamia* above described agree on the whole with those of *Taxodium* (Coker, 1903), *Cryptomeria* (Lawson, 1904 b) and the *Cupressineae* (Land, 1902; Lawson, 1907; Norén, 1907).

Formation of the Megaspore.

The pistillate cones appear as inconspicuous buds at or near the apex of shoots of the same year, in autumn. The ovules, as

they first appear, are seen as swellings on the axile of the sporophyll. Each sporophyll contains three ovules. The development of the integument and the formation of the megaspore occurs in early spring of the next year.

The megaspore mother-cell becomes differentiated shortly before pollination. Only one megaspore mother-cell is formed in each ovule and is situated about on the same level as the point of insertion of the integument (fig. 42). In this respect *Cunninghamia* agrees with *Taxodium* (Coker, 1903) and differs from *Cryptomeria*

Text-fig. 2. Pistillate cones at the time of pollination, seen from the upper side of a branch. About × $^3/_4$.

(Lawson, 1904b) where a group of three or four mother-cells is organized. *Cryptomeria,* on the other hand, resembles *Sequoia* (Shaw, 1896; Lawson, 1904a) where five or six mother-cells are formed.

The megaspore mother-cell is divided about the time of pollination, i. e., the beginning of April. The division commences with the sinaptic stage and is evidently the reduction-division. Various stages of the first division are shown in figs. 36—40. In *Taxodium,* according to Coker (1903), two cells unequal in size are formed as a result of the first division, but only the large lower one of these divides again. Thus there are only three

megaspores are formed from the mother-cell instead of four as it is the case with *Sequoia* (Shaw, 1896; Lawson, 1904a) and several other Conifers. As the megaspore mother-cell prepares for division, the nucleus is found in the upper part of the cell, and the accumulation of the starch-grains in the lower part of the cell is noticeable (figs. 37, 39). From this as well as from the position of the spindle, as shown in figs. 39 and 30, it can be inferred that the resulting daughter-cells would be unequal in size as was observed by Coker in *Taxodium*, although I was not able to find the later stages of the division. The second division was not studied. I am, therefore, not able to determine the number of the potential megaspores formed, but judging from the position of the spindle of the first division and the figure as shown in fig. 41 I am inclined to think that the number would usually be three as it is the case with *Taxodium*.

The number of potential megaspores formed from a single mother-cell is not constant even among the members of the same genus. Strasburger (1879) reports that, in *Larix europea*, there are usually three cells formed from the division of a mother-cell. Juel (1900) found four megaspores in *Larix sibirica*. In *Pinus laricio*, Coulter and Chamberlain (1901) find four megaspores, while Miss Ferguson (1904) states that among several species of *Pinus* the number varies from three to four and the variation may be found even in the same species, although there is a tendency in some species to form three and in others four cells. In *Juniperus communis* (Norén, 1907) the number seems to be usually three and rarely four. The formation of four potential megaspores seems to occur also normally in *Sequoia* (Shaw, 1896; Lawson 1904a), *Thuja* (Coker, 1904; Lawson, 1907), *Libocedrus* (Lawson, 1907) and *Taxus* (Strasburger, 1904; Coker, 1904).

Development of the Female Gametophyte.

The lowest of the megaspores now begins to enlarge, and develops into the female prothallium. The upper sister-cells gradually disintegrate and are crowded to the upper corner of the growing megaspore, remaining for a time as small deeply staining bodies which finally disappear altogether. As the megaspore grows to its full size, the cytoplasm seems to withdraw from the central portion of the cell by the formation of a large vacuole. The nucleus is found in one side of the cell and imbeds itself in the peripheral layer of cytoplasm (fig. 41). The organization of the parietal layer of cytoplasm in the one cell stage has been demonstrated by Miss Ferguson (1904) in *Pinus*. Norén (1907) seems to have observed a similar fact in *Juniperus*.

The nucleus of the megaspore then begins to divide. The free nuclei, formed by the successive divisions, are imbedded in the parietal layer of cytoplasm (figs. 43—46). The division takes place simultaneously, as it seems to be the rule in the free-nucleated young prothallium of the Gymnosperms (fig. 47). Miss

Carothers (1907) reports that in the early stages of the growing female prothallium of *Ginkgo,* the division of the free nuclei is simultaneous, but later free nuclear divisions proceed irregularly. The question whether this is normally so in *Ginkgo* or represents rather an abnormal case, remains to be investigated. Her material was subjected to "unnatural condition — growth after separation from the tree — although an effort was made to render conditions as natural as possible", and the author herself admits that the latter alternative is not impossible.

It is usual in the *Coniferae,* that in each ovule, a single megaspore develops into the female prothallium, and I have never found a case in *Cunninghamia* where the ovule contained more than one prothallium. Arnoldi (1900), however, found that more than one prothallium are sometimes developed in the ovule of *Cunninghamia,* and figures as many as five young prothallia of various sizes in a single ovule (Arnoldi, 1900, texfig. 5). In *Cryptomeria* Lawson (1904 b) found that only one out of twelve or sixteen potential megaspores germinates and there is consequently but one prothallium formed. Coker (1903) found that in *Taxodium* only a single prothallium is usually formed, but one case was found in which the nucellus contained two young prothallia. Hofmeister (1851) mentions the occasional presence of two prothallia in *Pinus sylvestris* and *Taxus baccata.* It was since confirmed by Farmer (1892) for *Pinus* and by Strasburger (1904) and Coker (1904) for *Taxus.* Coker (1902) has also found two prothallia in the young ovule of *Podocarpus.* In *Sequoia* (Arnoldi, 1899a, 1899b; Lawson, 1904a) there are usually more than one prothallium organized, and as many as eight has been counted in a single ovule; one of them enlarges more rapidly than others and form the primary prothallium, while one or two secondary prothallia, though failing to produce true prothallial tissue, nevertheless reach an advanced stage of development. In this respect *Sequoia* differs from other Conifers where the single prothallium seems to be the rule.

Early in the development of the young prothallium, it is surrounded by two to four layers of larger cells or tapetum. Each cell of the tapetum has a larger nucleus and denser cytoplasm when compared with the surrounding cells of the nucellus (figs. 46, 108). The presence of the tapetum-tissue in *Cunninghamia* has already been observed by Arnoldi and is figured in his paper (Arnoldi, 1900, textfigs. 4 and 5, Pl. XVII, fig. 2). A similar tapetum was found in *Taxodium* by Coker (1903), while it is reported by Lawson (1904b) to be absent in *Cryptomeria.*

The origin of the tapetal layer has been differently interpretted by various investigators. Lang (1901) designates a similar tissue in *Stangeria* as sporogenous cells. Thomson (1905) considers that in all Gymnosperms whose megaspore-membrane belongs to a normal type have the tapetum originated from the sporogenous tissue. Norén (1907) agrees with the above mentioned authors in regarding the tapetum-cells in *Juniperus* as sporogenous in origin. Coker (1903) on the other hand, is inclined to take the tapetal

tissue in *Taxodium* as not sporogenous in nature, but as formed from the nucellar cells surrounding the megaspore mother-cell. Miss Ferguson (1904) shares the same view in regard to the tapetum in *Pinus*, and attributes to the tissue the function of nourishing and protecting the growing prothallium. I have also observed that the similar tissue in *Cunninghamia* originates from undifferentiated cells of nucellus immediately surrounding the megaspore mother-cell, and am inclined to accept the latter interpretation.

The growth of the young prothallium is very slow during the first month of its development. Fig. 45 shows the stage reached on April 30, and on May 17 it has reached the stage shown in fig. 49. Growth then becomes more rapid and at the beginning of June, the prothallium which is still only a protoplasmic sac with an enormous vacuole surrounded by a cytoplasmic layer containing numerous free nuclei, attains to a considerable size (figs. 50, 109). Fig. 51 shows a part of the parietal layer of protoplasm in fig. 50 more highly magnified. Fig. 52 shows the surface view of the same. The free nuclear division now ceases and walls are developed between the nuclei. The manner of the wall-formation is shown in figs. 53—55. It seems to occur about between June 10 and 15.

The development of the prothallial tissue in Gymnosperms was first carefully investigated by Mlle. Sokolowa (1890) and her observations have, in general, been confirmed by most of the later investigators. The early stages of the prothallium-formation in *Cunninghamia* agrees on the whole with that described by Mlle. Sokolowa. No wall is formed on the inner side of the protoplasm facing the vacuole as first observed by Mlle. Sokolowa in other Conifers. A section, made parallel to the inner surface of the parietal layer of protoplasm, is shown in fig. 55. The cells are polygonal in outline and contain some starch-grains.

According to Mlle. Sokolowa, the first prothallial cells grow inward forming long open tubes which extend to the center without division; walls are then formed as the inner end of the tubes meet, and later on the cells become divided by cross-walls. A similar process of prothallium-formation was described by Arnoldi (1900) in *Sequoia* and the same was found to be the case with *Taxodium* studied by Coker (1903). Norén's (1907) observations on *Juniperus communis* agree on the whole with those of Mlle. Sokolowa.

Miss Ferguson's description (1904) of the prothallium-formation of *Pinus* differs somewhat from those of the above mentioned writers. She states that "no cell has ever been observed to extend from the circumference to the center of the prothallial cavity", and the first prothallial cells are divided by cross-walls before they reach the center of the vacuole. Lawson (1904 b) has observed a similar thing in the early stages of the prothallium-formation of *Cryptomeria*, although his statement of the later stages differs markedly from that given by any former investigators. My own observations on the formation of the prothallial tissue in *Cunning-*

hamia agree on the whole with the description of Miss Ferguson. As the first prothallial cells elongate toward the center of the vacuole, they divide several times by cross walls before reaching the center of the cavity. A part of the prothallial tissue as they half way advanced toward the center of the vacuole is shown in fig. 57.

The filling up of the central vacuole with growing prothallial tissue proceeds rather rapidly, and in about a week after the first wall-formation the whole megasporic or embryo-sac is filled with solid tissue. The stage just after the formation of the solid prothallial tissue is shown in fig. 58. Arnoldi (1900), Coker (1903), Lawson (1904) and several other recent investigators, confirming an earlier statement of Strasburger (1880), have noted many nuclei in each of the young prothallial cells. Miss Ferguson (1904), however, has "not observed multinucleated cells in the prothallium of *Pinus* up to the time when the suspensor has elongated and carried a several celled embryo to a considerable depth into the endosperm." She adds: "There is often an appearance of more than one nucleus in a cell, but careful study never fails to demonstrate a delicate cell-wall between the nuclei. At an early stage in prothallial development the cell-walls are very delicate, scarcely more than condensations of the ectoplasm so that they might easily be mistaken, in *Pinus*, for strands of cytoplasm. Doubtless the cells become plurinucleated during a more advanced stage in embryo formation." I have also failed to find multinucleated cells in young prothallium of *Cunninghamia*, but in the older prothallium, some of the cells seemed to contain more than one, usually two, nuclei in each. Careful study has sometimes proved that some of those cells are only apparently bi- or multinucleated, a very delicate wall being found between the nuclei. Thus most of the cells of the mature prothallium were found to be uninucleated.

It was often maintained that the nuclear division in the prothallium is sometimes amitotic. Mlle. Sokolowa (1890) makes a similar statement in her studies on the prothallium-formation of various Gymnosperms. Norén (1907) mentions that "Diese Teilung (division of the first prothallial cells) ist oft amitotisch, was auch von Sokolowa erwähnt wird". Coker (1903), on the other hand, states that "these nuclear divisions are generally, at least, of the mitotic type". So far as my observation goes, the division takes place mitotically, and no case was come across in which the nucleus showed a sign of amitosis.

The wall enclosing the female prothallium, or the megaspore-membrane is at first thin and delicate. During the growth of the prothallium the membrane becomes thicker and more conspicuous. Fig. 51 shows the female prothallium just before the wall-formation and the megaspore-membrane is found about half way thickened. In figs. 53 and 54 the double nature of the membrane, as clearly pointed out by Thomson (1905) in other Gymnosperms, is more prominent. In the mature prothallium, the exosporium shows characteristic radial striations and is several times as thick as the

endosporium. The megaspore-membrane is thicker at the lower part of the prothallium and becomes thinner toward the tip. Fig. 56 shows a part of the base of the female prothallium where the membrane is thickest and measures about 3 μ in thickness.

Thomson (1905) has made an extensive comparative study on the distribution and character of the megaspore-membrane of Gymnosperms. The thickness and structure of the membrane is considered by him to have great phylogenetic significance. Thus the coat is thick and well developed in the Cycadales, the group which is recognized as the most primitive of the modern Gymnosperms, while it is much thinner in the Gnetales, which is considered as being the highly specialized of the Gymnosperms. Among the members of the *Taxodieae*, *Sciadopitys* has the thickest megaspore-membrane and has more affinity to the *Abietineae*. Of two species of *Sequoia*, *S. sempervirens* has thicker membrane and measures about 2.5 μ in comparatively young stage, while in the mature seed of *S. gigantea* the coat is only 1.5 to 2 μ in thickness. According to Thomson (1905), in the mature seed of *Cryptomeria* the megaspore coat is not so thick as it is in *S. gigantea*, but otherwise is very similar to that of the latter". Thomson has also examined the megaspore-membrane of the mature seed of *Taxodium* and gives its thickness as about 2.5 μ. The megaspore-membrane of *Cunnighamia* seems to be as thick as that of any other member of the *Taxodieae*, except *Sciadopitys*, if not much thicker.

Development of the Archegonia.

The archegonial initials become apparent about the middle of June as a group of cells at the apex of the prothallium. They are the peripheral cells of the prothallial apex, and may be differentiated even before the prothallial tissue is thoroughly organized. When the archegonial initials are first formed, they are scarcely larger than the other cells of the prothallium and can only be distinguished from the neighboring cells by the larger nuclei and denser cytoplasm (figs. 58, 59). They soon become enlarged and elongate to three or four times of the original size before they divide. The fully formed archegonial initial has a prominent nucleus situated at the tip of the cell and most of the cytoplasm is collected around it. A very large vacuole occupies the greater part of the cell (figs. 60, 61). Fig. 62 shows the upper part of an archegonial initial whose nucleus is just dividing. This division results in the organization of a smaller upper cell, the primary neck-cell, and a large lower cell, the central cell of the archegonium (fig. 63).

The primary neck-cell soon divides into two cells by an anticlinal wall (figs. 64, 65). The two cells then divide again by walls which are perpendicular to the first and the four cells thus formed all lie in the same plane. The neck-cells usually divide no more, and the neck of the full-grown archegonium consists typically of a single tier of four cells. The neck, therefore, shows

but two cells in longitudinal section and it is only in the cross-section that all the four cells come under one view (fig. 76). Variation in the number and arrangement of the neck-cells has sometimes been observed. Figs. 73—75 show the diversity that may occur in the neck as seen in longitudinal section. Variation in the number of neck-cells has often been noticed in other Conifers. According to Coker (1903) they may vary from two to sixteen or more in *Taxodium*. In *Sequoia* (Arnoldi, 1899; Lawson, 1904a) they are typically two and sometimes four. In *Cryptomeria* (Lawson, 1904b) the neck consists normally of a single tier of four cells, and it was only in one preparation that Lawson found "a variation from this number and that was in a longitudinal section where four were observed, suggesting that there may have been eight altogether." In *Tsuja* (Land, 1902), the neck-cells seem to vary from two to six, and they may vary from four to six in *Libocedrus* (Lawson, 1907).

The rapid growth of the central cell takes place soon after its formation. The stages of its development are shown in figs. 63—68. The cytoplasm at first contains a very big vacuole beside a number of smaller ones, and as the cell continues to grow, the amount of cytoplasm increases much more rapidly. When the archegonium reaches to its full size, number of vacuoles of various sizes are found imbedded in the more or less finely granular cytoplasm (fig. 68). The nucleus of the central cell is, from the first, always situated near the apex of the cell and contains a prominent nucleolus.

Coker (1903) noticed two dense area in the cytoplasm of the central cell in *Taxodium*, one at the tip and the other near the base. According to him "these areas are of dense fibrous material", and "from them fibers radiate to the surface of the cell". Norén (1904, 1907) found a similar structure in *Juniperus* and named it "Strahlungscentrum". I have also found such a dense cytoplasmic mass at the tip of the full-grown central cell very near or almost in contact with the nucleus. But I failed to observe a similar structure near the base of the cell.

The development of the archegonia agrees, on the whole, with that observed in *Taxodium* and *Cryptomeria*, and of the Cupressineae type. The process is rather rapid, and at the end of June the archegonial complex is fully formed (figs. 69, 70, 110). It is usual that a single archegonial complex is located at the tip of each female prothallium, but a case was found in which two archegonial groups were present near the apex of a prothallium (fig. 105). The number of archegonia in a complex varies usually from thirteen to sixteen though smaller and larger numbers may sometimes occur. Unlike that of *Cryptomeria* and *Taxodium* the archegonial complex has a sterile prothallial tissue at the center, while the archegonia are arranged around it, completely enclosing the former (figs. 71, 72). Such an arrangement of the archegonia around a sterile tissue has not been found in any member of the *Taxodieae* and the *Cupressineae* so far investigated.

Enveloping the archegonial complex, there is a single layer of sheath- or jacket-cells. At first they are poor in contents and can scarcely be distinguished from the neighboring cells of the prothallium, but later on the cells become rich in cytoplasm and the nuclei more prominent (figs. 69, 70, 72, 87). In the full-grown archegonia, many of the jacket-cells are binucleate. The same was found to be the case with *Libocedrus* by Lawson (1907). Coker (1903) also mentions that the jacket-cells of the mature archegonia in *Taxodium* generally contain two nuclei. In *Cryptomeria,* according to Lawson (1904b), nearly all of them are multinucleate. It is to be noticed that the jacket-cells near the apex of the archegonia are generally poor in contents and resembles closely the adjacent prothallial cells. Judging from the figures of Lawson (1904b, figs. 39, 40) this seems to be true also in *Cryptomeria.*

As the central cell of the archegonium reaches its full size, the cytoplasm becomes densely granular and most of the smaller vacuoles disappear, leaving usually one big vacuole at the center. The nucleus now undergoes division. The early stages of the division were not found. Various stages of the karyokinetic spindle are shown in figs. 77—81. All of the nuclei of a single archegonial complex seem to divide almost simultaneously. Of the two nuclei thus formed, the upper one, the ventral canal-nucleus, usually soon degenerates and its remnant may, for a time, be seen as deeply staining body at the tip of the egg (fig. 82).

Arnoldi (1900) denies the formation of a ventral canal-nucleus in *Sequoia, Taxodium, Cryptomeria* and *Cunninghamia.* His conclusion was not confirmed by the later researches of Coker (1903) in *Taxodium* and those of Lawson (1904) in *Sequoia* and *Cryptomeria.* Coker has studied carefully the division in the ventral canal-nucleus in *Taxodium.* Although Lawson did not observe any division-figure in the two genera above mentioned, he seems to have enough evidence for the existence of such division. My observations now put the formation of the ventral canal-nucleus in *Cunninghamia* beyond doubt. A doubt was also expressed by Arnoldi (1899b) as to the presence of a ventral canal-nucleus in the *Cupressineae*; but the recent Investigators (Land, 1902; Norén, 1907; Lawson, 1907) all agree in the existence of such nucleus in that group. Only other species of the *Coniferae*, in which the absence of a ventral canal-nucleus was reported, is *Torreya taxifolia* (Coulter and Land, 1905). It was, however, found in *Torreya californica* by Miss Robertson (1904), and it is not improbable that the later researches may reveal the existence of the nucleus in the former species. It seems, therefore, that the formation of the ventral canal-cell or nucleus is a rule among the Coniferae and also among the rest of the Gymnosperms.

The lower of the two nuclei, resulted from the division of the central nucleus, the egg-nucleus, immediately begins to enlarge, and at the same time moves downward (figs. 82, 83). In the mature egg, the nucleus is usually found about one third below

the tip, and right below it, is one large vacuole occupying the center of the egg (figs. 84—87). The structure of the egg-nucleus does not seem to differ much from that of the other Conifers. It contains a more or less interrupted reticulum, which appears somewhat granular and may be composed of an irregular network of linin in which chromatic granules are imbedded (figs. 84—86, 88—90, 112).

The cytoplasm of the egg presents a finely granular appearance and contains no such bodies as can be compared with the proteid-vacuoles of the Abietineae. Only some more or less deeply-staining granules are often found scattered in the cytoplasm (figs. 84—86).

Fertilization.

Fertilization seems to take place one or two days after the cutting off of the ventral canal-nucleus and immediately after the egg-nucleus reaches its mature size. The date varies by season and locality. It differs also even in the same tree. In my material, most of the fusing nuclei were found between July 3 and 5. Generally speaking, we may say that in the middle part of Japan, the fertilization of *Cunninghamia sinensis* occurs during the first week of July, i. e., about three months after pollination.

As was stated before, the pollen-tube reaches the depression, the archegonial chamber, above the archegonial complex at the end of June. The division of the generative cell, which is situated at the tip of the tube, seems to take place about the same time as that of the central cell of the archegonium. The two sperm-cells, formed as the result of the division, become somewhat enlarged and filled with starch, as was already described. Now the sperm-cells are ready for fertilization, and the stalk- and tube-nuclei are found more or less disorganizing right below them (figs. 35, 87).

Only one sperm-cell enters each archegonium. No case was found in which two sperm-cells entered into the same egg as it appears sometimes to be the case with *Taxodium* (Coker, 1903). The sperm-cell advances toward the egg-nucleus, and its nucleus soon comes in contact with the egg-nucleus. The sperm-nucleus now flattens itself against the egg-nucleus (figs. 88—90). The diameter of the sperm-nucleus, before entering into the egg, is about half of the egg-nucleus (figs. 35, 90, 107, 112). The former seems to enlarge somewhat after entering the egg, and at the time of conjugation, it approaches the egg-nucleus in size, though somewhat smaller than the latter (figs. 88 – 90). A similar enlarging of the sperm-nucleus in the egg was also described by Coker (1903) in *Taxodium* and by Lawson (1904b) in *Cryptomeria*.

The fusing nuclei retain their identity for sometime, the two nuclei being separated by a membrane. They are surrounded by a dense sheath of starch-granules (figs. 89, 90, 113). There is no doubt that the greater part of the substance of the sheath is derived from the sperm-cell. A similar starch-sheath was observed in *Taxodium* (Coker, 1903) and *Cryptomeria* (Lawson, 1904b).

Strasburger (1884) has noticed the starch-sheath around the fusing nuclei of *Juniperus* and expressed a surprise at the sudden appearance of starch, as he has not found any starch in the sperm-cell, while the pollen-tube contains very little starch at the time of fertilization. Later students of the *Cupressineae* (Lawson, 1907; Norén, 1907) have also observed the presence of the starch-sheath, except Land (1902) who makes no mention about it in *Thuja*. The presence of starch in the sperm-cells were mentioned by Norén in *Juniperus* and by Lawson in *Libocedrus*. *Taxodium*, *Cryptomeria* and *Cunninghamia* have then these points in common with the Cupressineae.

The fusing nuclei are usually found at the center of the egg, occupying the cavity of the vacuole, into which they have probably dropped in after both nuclei came in contact. They may not fill up the entire space of the vacuole, and there is often a space left between them and the rest of the egg-cytoplasm (figs. 89, 113). This space is, however, gradually filled up by the surrounding cytoplasm, and almost disappear at the time of the first spindle-formation.

The outline of the female and male nuclei remains distinct until the fusing nuclei prepare themselves for division. It has been reported that in *Taxodium* (Coker, 1903) and *Taxus* (Jäger, 1899) the fertilized nucleus passes down to the base of the egg before the first division occurs. This is evidently not the case in *Cunninghamia*, for the first division-figure was always found at or near the center of the egg, just about the point where the fertilized nucleus is usually located (fig. 114). Most investigators of the *Coniferae* agree that the first division of the fertilized nucleus occurs near the center of the egg. So it was found by Lawson to be the case in *Cryptomeria* and several other members of the *Cupressineae*. Arnoldi (1900), on the other hand, states that in *Sequoia* the first division takes place at the base of the egg, but the fact was not confirmed by Lawson (1904 a).

Embryo-formation.

The fertilized nucleus soon prepares for division, while the outline of each sexual nuclei is still distinct. The nuclear membrane becomes indistinct and the spindle originates at the point where the walls of two nuclei meet. The process of division does not seem to differ much from that observed in other Conifers. Miss Ferguson (1901, 1904) made a very minute study of the division in *Pinus* and the results of her study can, in the main, be also applied to *Cunninghamia*. An early stage in the spindle-formation of *Cunninghamia* is shown in fig. 91 which resembles Miss Ferguson's (1901) fig. 56 with the exception that the latter has no starch-sheath. The sindle is intra-nuclear and seems to originate as a multipolar structure. The chromosomes are found in two groups and each group has evidently derived from one of the sexual nuclei.

In the conjugating nuclei of *Juniperus communis* Norén (1907) observed the decrease of the chromatic substance and the corresponding increase of the nucleolus-like bodies the "Pseudo-nuclei" which he thinks contribute the greater part of the substance of the chromosomes. Although I do not have enough evidence to speak for or against Norén's view, some of my preparations like fig. 90 may suggest the possibility of such a process. In figs. 92 and 114 are shown completely formed spindle, with chromosomes accumulating near the equator of the former; the paternal and maternal elements can no longer be distinguished.

The result of the first division is shown in fig. 94; two daughter-nuclei are surrounded by dense mass of starch-granules, and they travel toward the base of the egg. As they reach the base of the archegonium (figs. 95, 96), both nuclei divide simultaneously, and four free nuclei are formed as shown in fig. 97. The third division which now follows is also simultaneous and results in the formation of eight nuclei, which are arranged in tiers as shown in fig. 98. Walls are then formed between the nuclei, the upper tier remaining open at the top.

In *Cryptomeria*, Lawson (1904b) finds that the continuous fibrils of the spindle persists, and the first cell-membranes of the embryo are formed between the nuclei. According to Miss Ferguson (1901, 1904), who has studied the first wall-formation of the proembryo in *Pinus*, the spindle fibers of the third division seem to disappear before the walls are formed between the nuclei. Although I have not followed the process of the first wall-formation in detail, I am inclined to accept the view of the latter investigator on this point. The stage as shown in fig. 98, in which no trace of spindle fibers is visible between the eight free nuclei, can hardly be interpretted in accordance with Lawson's view.

While in the *Abietineae*, the eight nuclei of the proembryo, as a rule, arrange themselves in two tiers of four nuclei each, the same stage of *Cunninghamia*, *Cryptomeria* and *Taxodium* does not show such regularity in the arrangement of the nuclei. Coker (1903) states that in *Taxodium* two nuclei are situated at the base, and six nuclei lie above them in one plane. He adds: "while this is the usual arrangement, it is not uncommon to find only one at the base, while the other seven are arranged above it. In a few cases there were three below and five above." In *Cunninghamia* the number of cells in each of the two tiers is also not constant; it seems, however, that the upper tier generally contains five nuclei and the lower three, as shown in fig. 98. It was sometimes found that there are two below and six above. The other combinations of cell-arrangement are also possible. The lower group of completely-walled cells may not lie in the same plane; they are sometimes found one above the other instead of side by side.

The nuclei of the upper tier then divide simultaneously and the walls are formed between the daughter-nuclei (figs. 99—102). We have now in the proembryo two tiers of cells and one tier of the incompletely-walled cells (figs. 101, 102). Lawson (1904b)

states that in *Cryptomeria* the upper tier or the rosette consists of only free nuclei, no wall being formed between them, but I have found that in *Cunninghamia* the nuclei are all surrounded by walls at the sides, being open only at the top.

The middle tier of cells now elongates into the suspensors while the lower tier or group of cells forms the embryo (figs. 103, 104). The cells of the young embryo may divide before the suspensors elongate.

The process of the proembryo-formation in *Cunninghamia* agree, on the whole, with that of *Taxodium*, *Cryptomeria* and the *Cupressineae*, while it differs considerably from *Sequoia* in which no free nuclei are formed in the proembryo.

There is often found, in the fertilized egg, an extra nucleus above the proembryonal nuclei. It is derived probably either from one of the two free nuclei previously found in the pollen-tube or from the ventral canal-nucleus (figs. 95, 99, 101). In one preparation, I have found it in division. The division-figure, however, seemed to be more or less abnormal (fig. 93). Such abnormal or abortive karyokinetic figures are not uncommon in the fertilized egg of the *Abietineae* (Ferguson, 1901, 1904; Miyake, 1903).

Systematic Position of Cunninghamia.

The present study shows that the gametophytes and embryogeny of *Cunninghamia* show a close affinity with *Taxodium* and *Cryptomeria*, and are distinctly of the *Cupressineae* type. So far as the embryological evidences go, I can only confirm the suggestion of Arnoldi that these three genera should better be removed from the *Taxodieae* and placed with the *Cupressineae*. A new sub-group the *Taxodinae*, may perhaps be established in the *Cupressineae*, to receive these new comers.

According to Arnoldi (1900) the archegonia of *Cryptomeria*, *Taxodium* and *Cunninghamia* are not always arranged in a compact complex as in the *Cupressineae*, but they are rather loosely arranged, having often some sterile prothallial cells inserted between themselves, and with less distinct sheath-layer. He, therefore, proposes to put these genera in the *Cupressineae*, as the more primitive member of the group [1]). The presence of a sterile

[1]) "Die bis jetzt mehr oder weniger entwicklungsgeschichtlich bekannten Gattungen der Cupressineen sind *Juniperus*, *Thuja*, *Biota*, *Cupressus* und *Callitris*. Bei allen diesen Pflanzen bilden die Archegonien scharf ausgesprochene Complexe, welche mit einer auch scharf ausgesprochenen Deckschicht umgeben sind, während das für *Cryptomeria*, *Taxodium* und *Cunninghamia* nicht immer der Fall ist, hier, wie es gezeigt worden ist, kommen sehr oft unvollständige Complexe vor, indem die Archegonien locker verbunden werden und zwischen ihnen auch Endospermschichten sich befinden, es wird auch die Deckschicht nicht immer scharf gebildet, indem ihre Zellen nicht viel von denen des Endosperms abweichen. Das gibt uns aber Recht, solche Archegoniencomplexe nur als älteren noch nicht fixierten Typus zu bezeichnen, welcher später bei etwas weiter in der Entwicklung fortgeschrittener Gruppe zu vollkommener Ausbildung gekommen ist. Wir können also diese drei Gattungen in die Familie der Cupressineen stellen und zwar sie als ältere Formen derselben bezeichnen." (Arnoldi, 1900, p. 23.)

prothallial tissue in the center of the archegonial complex of *Cunninghamia* is certainly a primitive character. So far as *Cunninghamia* is concerned, I can agree with Arnoldi in considering it as more primitive than other members of the *Cupressineae* so far investigated.

From the presence of a steril prothallial tissue in the archegonial complex, I consider *Cunninghamia* as the most primitive of the three genera. Otherwise, *Cunninghamia* agrees with *Taxodium* in the essential characters of the gametophytes. *Cryptomeria* is considered as of more modern origin than the other two, as the tapetum of the female gametophyte seems to be less developed than the others. Lawson (1904) was not able to detect any tapetum in *Cryptomeria,* but Thomson (1905) has found a poorly developed tapetum around the young female prothallium.

Summary.

The staminate cones begin to develop in the autumn of the year preceding pollination. The pollen mother-cells are formed before the end of the year.

The division of the pollen mother-cell takes place about the end of February or the beginning of March. The division shows clearly the stages characteristic of the reduction-division, the reduced number of chromosomes being twelve.

The mature pollen-grain contains two cells, the larger tube-cell and smaller generative cell. Pollination takes place during the first half of April.

As the pollen begins to send out tube, shortly after pollination, the generative cell divides into the body- and stalk-cells, and these move down toward the tube-nucleus which has previously found in the tube.

The stalk-cell soon looses its own cytoplasm and its nucleus then passes the body-cell and lies near the tube-nucleus.

The downward growth of the pollen-tube is at first relatively slow and at the later stage it is much accelerated. The pollen-tube reaches the female prothallium about the end of June and penetrates into the depression just above the archegonial complex.

The body-cell which is very much enlarged and almost spherical, now lies at the enlarged tip of the pollen-tube. It then divides to form two sperm-cells which are equal in size. The sperm-cell contains numerous starch-granules in its cytoplasm, and is ready to fertilize soon after its formation.

The pistillate cones begin their development in the autumn, and a single megaspore mother-cell is formed in each ovule shortly before pollination, in the following spring.

The megaspore mother-cell divides about the time of pollination. The division commences with the synapsis stage and is evidently the reduction-division.

The usual number of the potential megaspores is probably three. One of the megaspores, the lowest of the row, develops into the female gametophyte.

The female prothallium develops, as in the other Conifers, at first by free cell formation in a parietal layer of protoplasm enclosing the central vacuole. The division of the free nuclei goes hand in hand with the enlargment of the protoplasmic sac.

The wall-formation between the nuclei begins about June 10—15. The cells then elongate toward the center, and cross-walls are laid down before they meet.

The filling up of the central vacuole with prothallial tissue proceeds rather rapidly, and in about a week after the first wall-formation the whole megasporic sac is filled with solid tissue.

The young female prothallium is surrounded by two to four layers of tapetum-cells, while in the older stages the tapetum is reduced to a single layer of cells. In the mature prothallium, the tapetum shows the sign of disorganization.

The megaspore-membrane is fairly well developed in the mature prothallium, and consists of two layers. It is thicker toward the base of the prothallium.

The archegonia develop from peripheral cells at the micropylar end of the prothallium. They are arranged in a group, and the entire complex is surrounded by a single layer of sheath-cells. The number of archegonia in a complex varies usually from thirteen to sixteen. The number of the neck-cells in each archegonium is usually four.

The archegonial complex has a sterile prothallial tissue at the center and the archegonia are arranged around it completely enclosing the former. The archegonia are also surrounded by a common layer of sheath-cells.

A ventral canal-nucleus is cut off just before fertilization, and it, as a rule, soon disintegrates, being usually absent in the mature egg. The mature egg has a large nucleus situated somewhat above the center and a vacuole just below it.

Fertilization occurs about the first week of July. A single sperm-cell enters the archegonium and its nucleus soon fuses with the egg-nucleus. The fusing nuclei are surrounded by a dense sheath of starch-granules. The outline of the female and male nuclei remains distinct until the fertilized nucleus prepares itself for division.

The division of the fertilized nucleus takes place at or near the center of the egg. The two segmentation-nuclei then move down toward the base of the archegonium, where the succeeding two free nuclear divisions take place.

Eight free nuclei arrange themselves in two tiers at the base of the archegonium, and the walls are then formed between them, but the upper side of the upper tier is left open. The nuclei of the upper tier now divide. The proembryo consists of three tiers of cells, and the middle tier elongates into the suspensors while the upper tier, which is open above, forms a rossette, and the lower tier develops into the embryo.

The gametophytes and embryogeny of *Cunninghamia* shows a close affinity with *Taxodium* and *Cryptomeria*, and are distinctly

of the Cupressineae type. These three genera, therefore, should better be placed in the Cupressineae.

In conclusion, I wish to express my sincere thanks to my friend Prof. K. Shibata, who took trouble in making photomicrographs for me and also gave me some fixed ovules of *Cunninghamia*.

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Explanation of Plates.

All figures, except those of Plate V. were drawn with the aid of a camera lucida. Plate V is a reproduction of photomicrographs and is reduced to $^2/_3$ of

the original size in reproduction. The photomicrographs are taken with the following oculars and objektives:

Figs. 106 a, 106 b, 107, 112—114: Zeiß Apochromat 2 mm, Comp. Oc. 4.
Figs. 108—111: Zeiß Obj. B, Comp. Oc. 4.

Plate I.

Fig. 1. Microspore mother-cell. × 1330.
Fig. 2. The same in sinapsis. × 1330.
Fig. 3. Spirem stage. × 1330.
Fig. 4. Late spirem stage just before the formation of chromosomes. × 1330.
Fig. 5. Diakinesis stage. × 1330.
Fig. 6. Multipolar spindle. × 1330.
Figs. 7—8. Equatorial plate stage of spindle. × 1330.
Fig. 9. The same viewed from pole; twelve bivalent chromosomes are seen. × 1330.
Figs. 10—11. Spindle in anaphase. × 1330.
Fig. 12. Telophase. × 1330.
Fig. 13. Daughter-nuclei formed. × 1330.
Fig. 14. Spindle of second division in metaphase. × 1330.
Figs. 15—16. The same in telophase. × 1330.
Fig. 17. Late telophase. × 1330.
Fig. 18. Four grand-daughter-nuclei formed. × 1330.
Fig. 19. Young microspores. × 1330.
Fig. 20. Microspore much more enlarged. × 1330.
Fig. 21. Mature microspore. × 660.
Figs. 22—25. Successive stages in first division of microspore. × 660.
Fig. 26. Mature pollen-grain, containing generative and tube-cells. × 660.
Fig. 27. Generative cell little after division; stalk-nucleus still attached to the body-cell. × 660.
Fig. 28. Body-cell, stalk- and tube-nuclei, which are found in young pollen-tube. × 660.
Fig. 29. Body-cell and one of two nuclei. × 660.
Fig. 30. A part of young pollen-tube with body-cell and two nuclei. × 660.
Fig. 31. Pollen-tube about half way penetrated the nucellar cap, with body-cell and two nuclei × 660.
Fig. 32. Lower part of pollen-tube which has almost completed the penetration of nucellus. × 660.
Fig. 33. A still later stage. × 660.
Fig. 34. Fully-formed body-cell with two nuclei. × 660.
Fig. 35. Two sperm-cells; a free nucleus found below them shows sign of degeneration. × 660.

Plate II.

Fig. 36. Oblique cross-section of a megaspore mother-cell, in synapsis. × 1330.
Fig. 37. Longitudinal section of megaspore mother-cell, in spirem stage. × 1330.
Fig. 38. Nucleus of megaspore mother-cell in diakinesis. × 1330.

Fig. 39. The same, in spindle of first division; equatorial-plate stage. × 1330.

Fig. 40. The same, in telophase. × 1330.

Fig. 41. Functional megaspore with two disorganizing spores above. × 660.

Fig. 42. Outline of longitudinal section of young ovule; megaspore mother-cell in metaphase of first division. Fig. 39 is sketched from the same preparation. × 50.

Fig. 43. The same at a later stage; integument is not shown. Female prothallium probably in four-nucleated stage. Tapetum is shaded. × 50.

Fig. 44. Four-nucleated stage of female gametophyte. × 660.

Fig. 45. Longitudinal section of ovule slightly older than that of fig. 43. Tapetum is shaded. April 30. × 50.

Fig. 46. Longitudinal section of central portion of young ovule, showing the arrangement of tapetum-cells around young gametophyte. × 240.

Fig. 47. A portion of young prothallium whose nuclei are in division. × 660.

Fig. 48. Longitudinal section of nucellar part of ovule, showing young gametophyte and well-developed tapetum. Tapetum is shaded. × 50.

Fig. 49. The same, in a later stage. May 17. × 50.

Fig. 50. The same, in a much later stage. Tapetum, which now consists mostly of a single layer of cells, is not shown. June 8. × 50.

Fig. 51. A part of fig. 50 highly magnified, showing a parietal layer of free nuclei, tapetum and nucellar cells. × 660.

Fig. 52. Surface view of free-nucleated prothallium as shown in fig. 51. × 660.

Fig. 53. Slightly later stage than in fig. 51; beginning of wall-formation between the nuclei. × 660.

Fig. 54. Still later stage. × 660.

Fig. 55. Surface view of the same. × 660.

Fig. 56. A part of the base of mature prothallium, showing the structure of megaspore-membrane at this stage. × 660.

Plate III.

Fig. 57. A part of growing prothallial tissue as it half way advanced toward the center of vacuole. × 160.

Fig. 58. Solid prothallial tissue just formed. × 50.

Fig. 59. Tip of the same more highly magnified. × 160.

Fig. 60. Archegonial initials. × 160.

Fig. 61. Upper part of the same more highly magnified. × 660.

Fig. 62. The same, with dividing nucleus. × 660.

Fig. 63. Young archegonium with a single primary neck-cell. × 160.

Fig. 64. Upper part of a similar archegonium, more highly magnified; neck-cell is dividing. × 660.

Fig. 65 a. A little later stage. × 160.

Fig. 65 b. Upper part of the above. × 660.

Figs. 66—68. Later stages in the development of archegonia. Fig. 68 represents an archegonium which has nearly reached to its full size. × 160.

Fig. 69. Upper part of female prothallium, showing a young archegonial complex. × 50.

Fig. 70. A same stage as above, showing a pollen-tube just reached the archegonia. × 50.

Fig. 71. Outline of longitudinal section of a similar prothallium as the preceding; position of sterile prothallial tissue is shown. × 50.

Fig. 72. Cross-section of upper part of mature prothallium; sterile prothallial tissue in the center of archegonial complex is shown. × 90.

Fig. 73—75. Abnormal neck-cells. × 270.

Fig. 76. A neck seen from above. × 270.

Fig. 77. Central cell of archegonium in division. × 160.

Figs. 78—81. Successive stages in division of central cells of archegonium. × 660.

Fig. 82. Young egg with remnants of ventral canal-nucleus above. × 160.

Plate IV.

Fig. 83. Young egg. × 160.

Figs. 84—86. Mature or nearly mature eggs. × 160.

Fig. 87. Longitudinal section of upper part of mature prothallium, showing mature eggs and sperm-cells ready for fertilization. × 50.

Fig. 88. Two sexual nuclei little before conjugation. × 270.

Fig. 89. Sexual nuclei in fusion. × 270.

Fig. 90. The same. × 660.

Fig. 91. Beginning of first spindle-formation after fertilization. × 660.

Fig. 92. Spindle fully established. × 660.

Fig. 93. Telophase of first division; an extra karyokinetic figure at the upper part of egg. × 330.

Fig. 94. Two daughter-nuclei of first division. × 660.

Fig. 95. The same at the base of archegonium; an extra nucleus near the tip. × 160.

Fig. 96. The same stage as above. × 160.

Fig. 97. Four free nuclei at the base of archegonium; only three of them are shown. × 160.

Fig. 98. Eight free nuclei at the base of archegonium; five of them are shown. × 160.

Fig. 99. A slightly later stage. Walls are formed between the nuclei and nuclei of upper tier are dividing. × 160.

Fig. 100. Division of upper tier of eight-celled proembryo. × 660.

Figs. 100—102. Proembryo after division of nuclei of upper tier. × 160.

Fig. 103. A little later stage; suspensors somewhat elongated. × 160

Fig. 104. A still later stage, with longer suspensors. × 160.

Fig. 105. Abnormal female prothallium in which two archegonial complex are present. × 50.

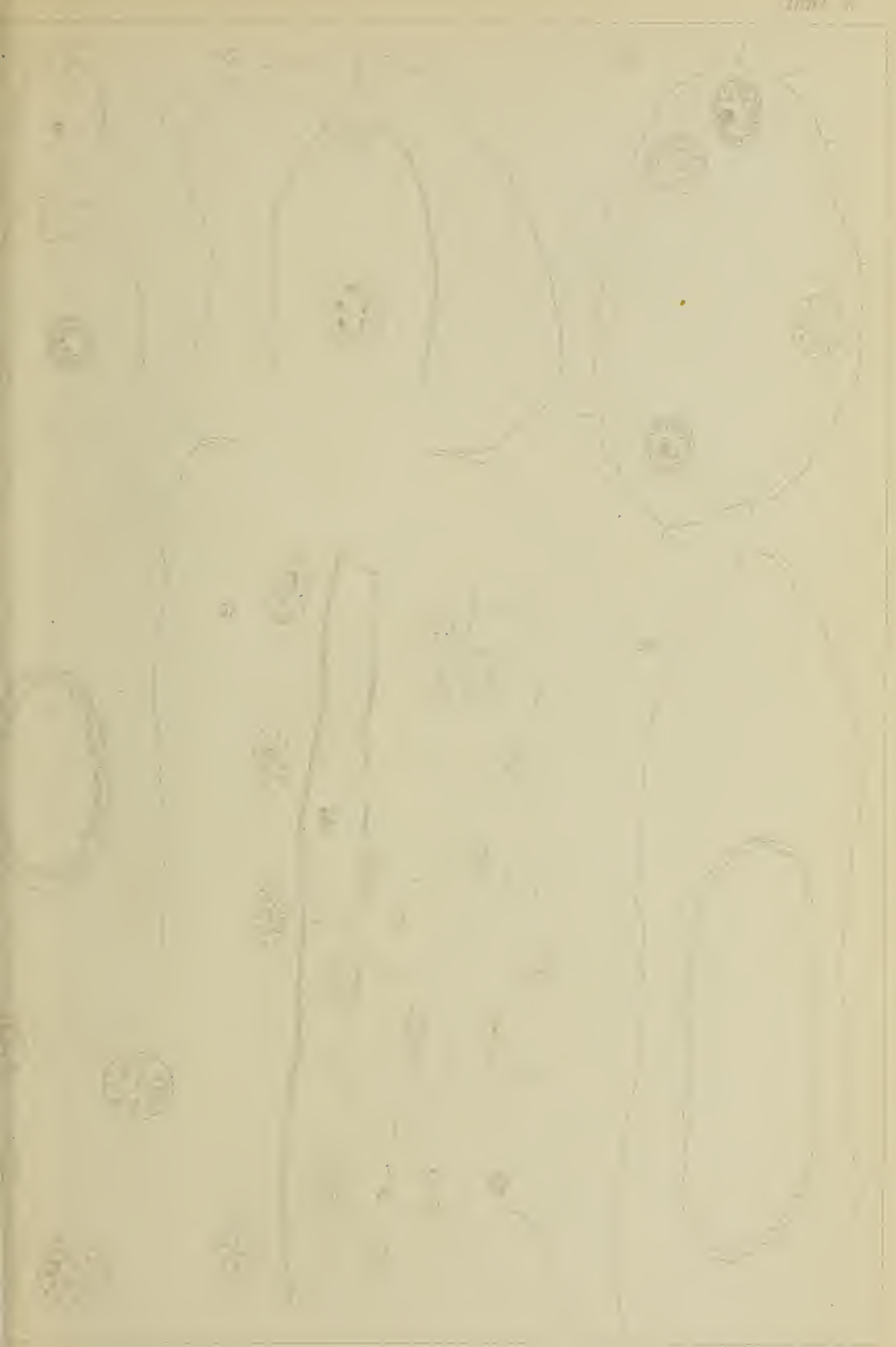
Plate V.

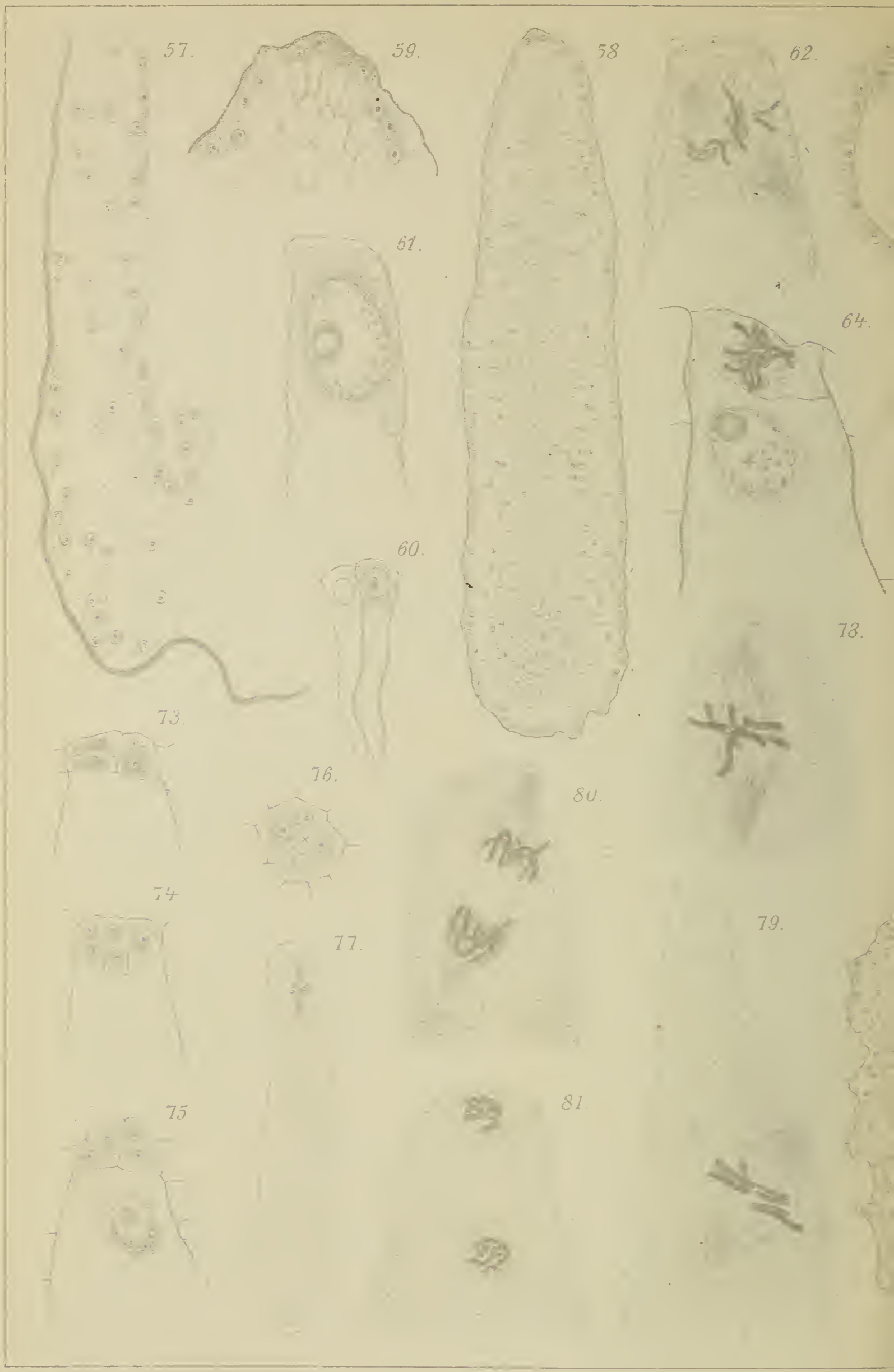
Fig. 106 a. Mature body-cell.

Fig. 106 b. Another section of the same, showing two free nuclei below

Fig. 107. Two sperm-cells.

Fig. 108. Longitudinal section of a young ovule, showing young female prothallium surrounded by several layers of tapetum cells. Several pollen-tubes are seen at the tip of nucellus.





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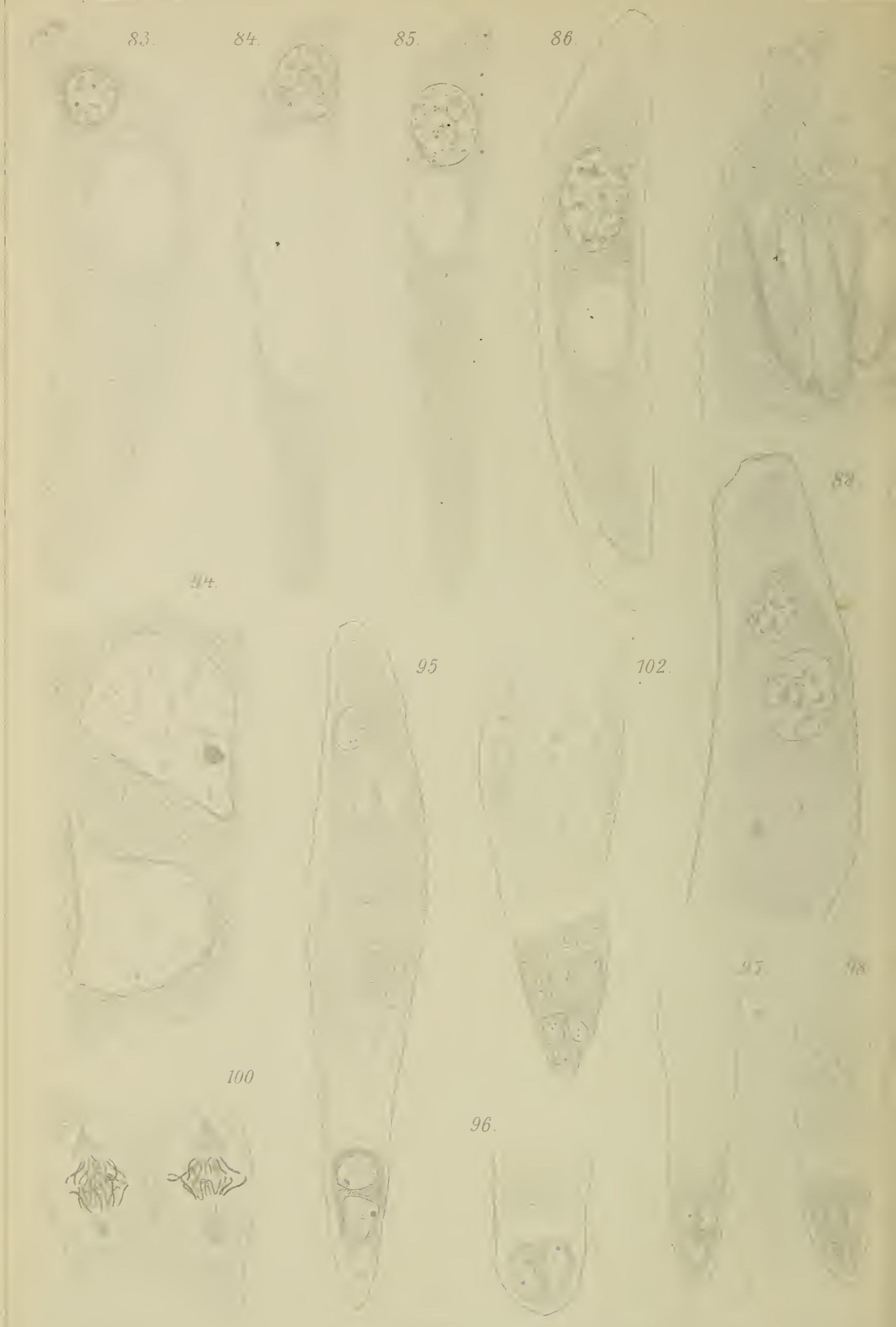

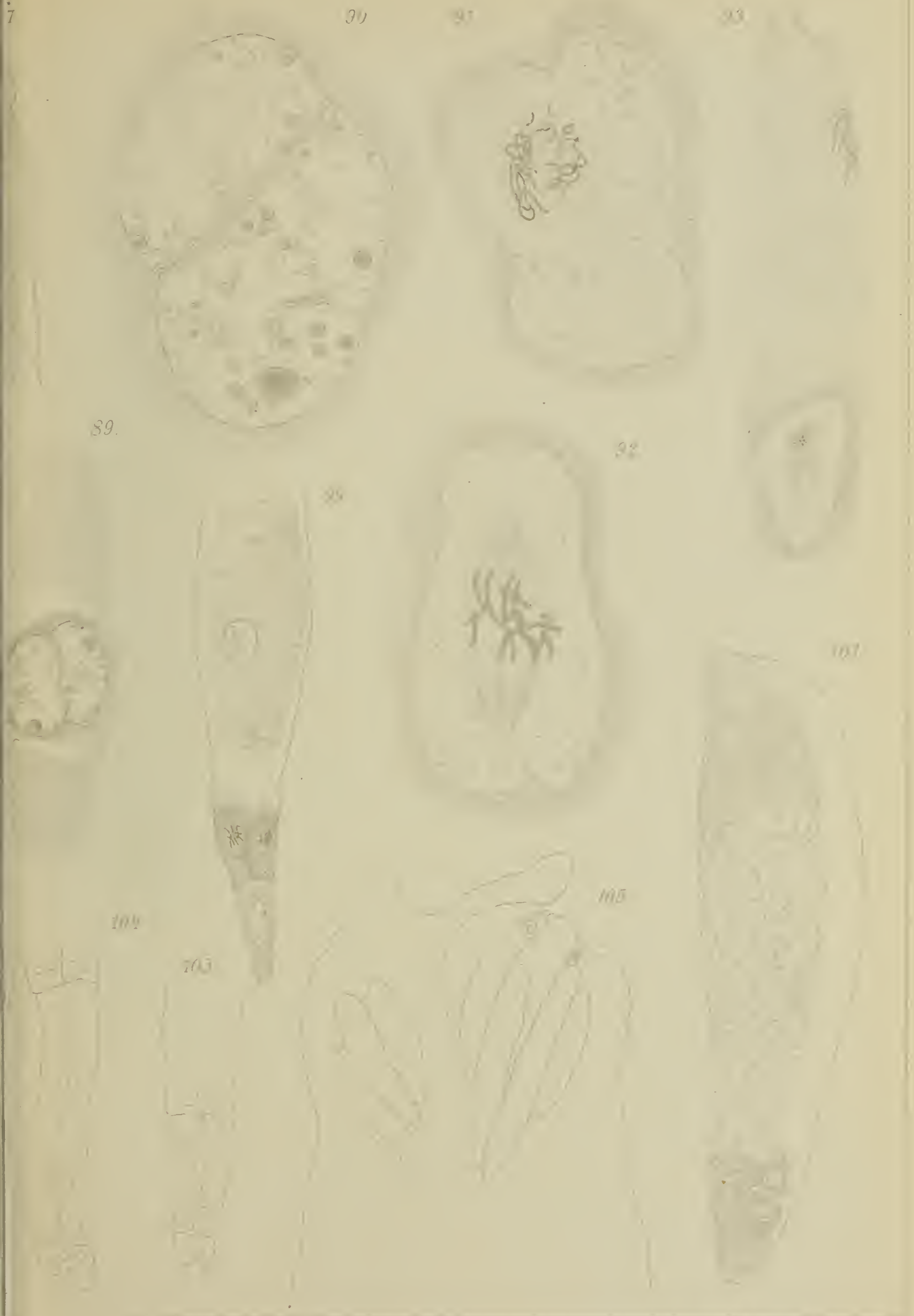

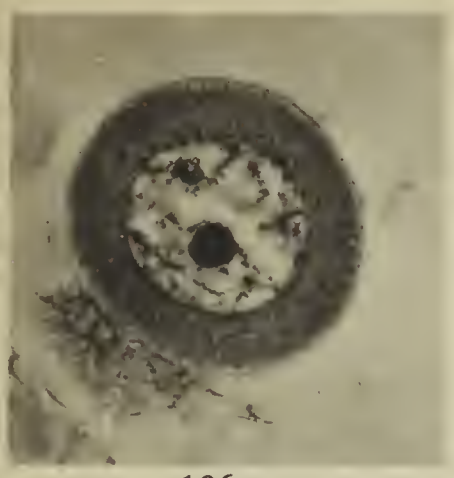

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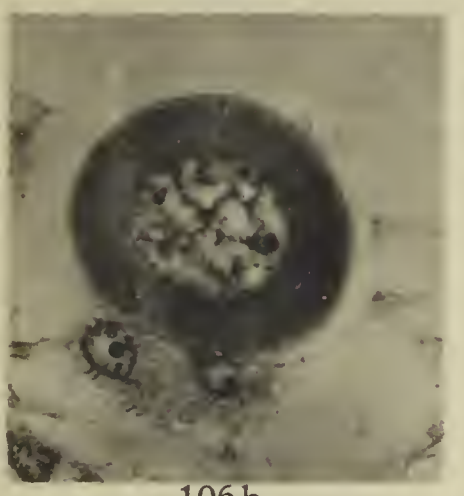

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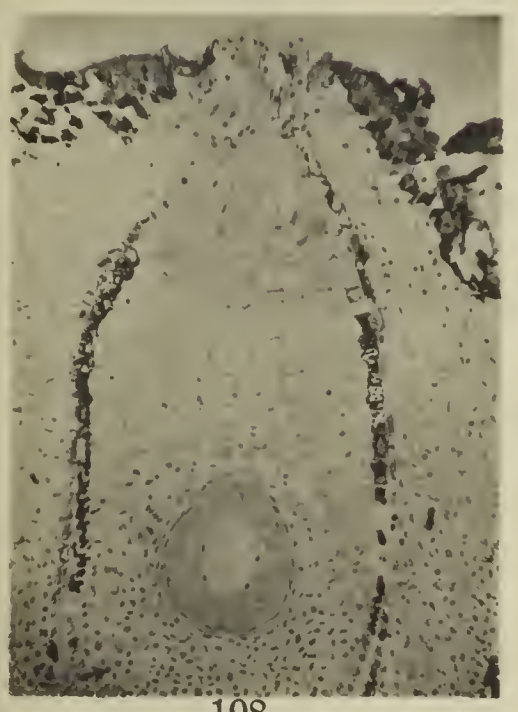

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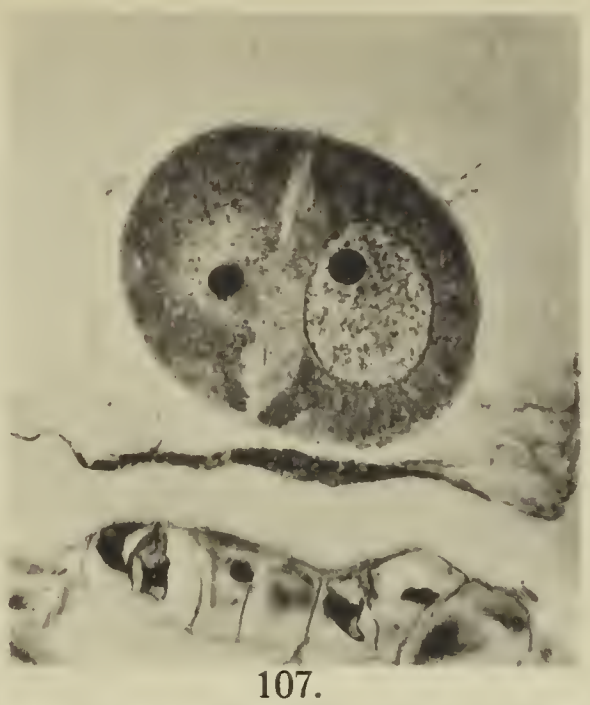

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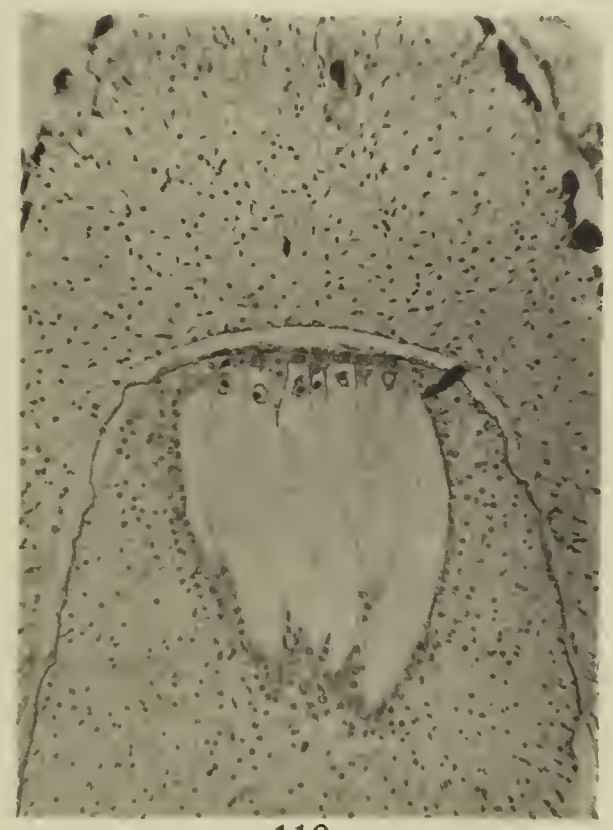

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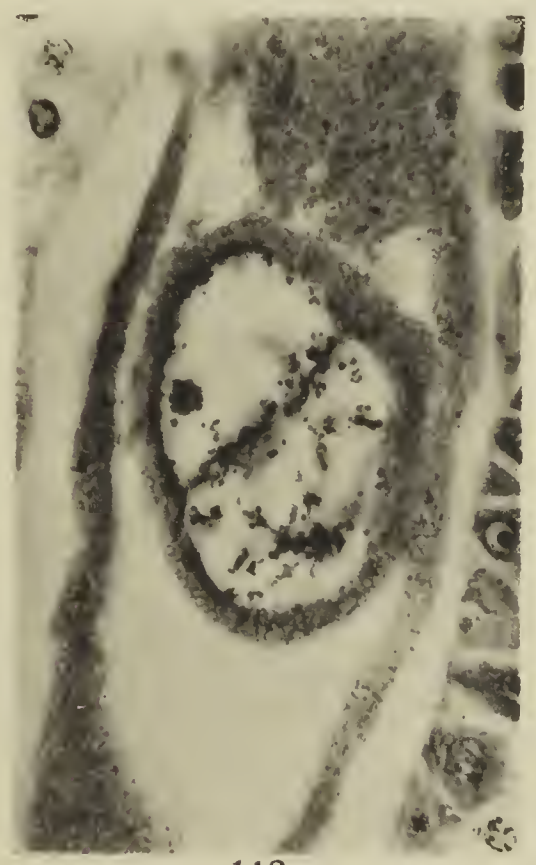

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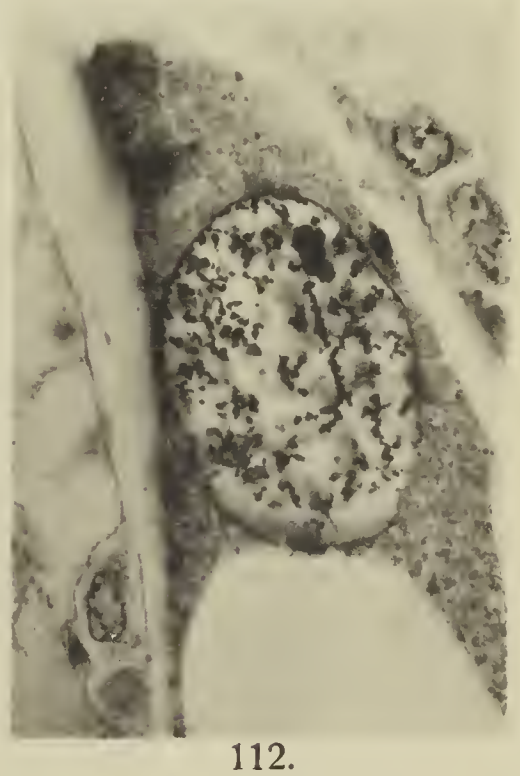

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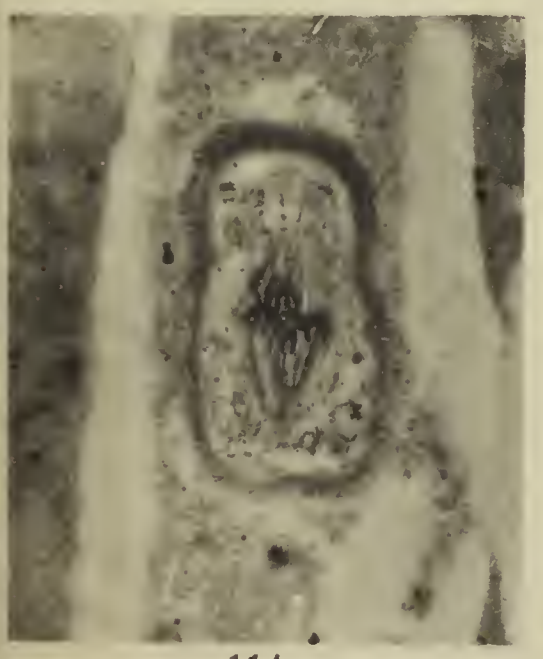

114.

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Fig. 109. Longitudinal section of a free nucleated prothallium, shortly before wall-formation. From the same preparation as fig. 50.

Fig. 110. Longitudinal section of upper part of a female prothallium, showing young archegonial complex; archegonial chamber is not yet formed. In upper part of nucellar cap a pollen-tube with a dark body, probably the body cell, is seen.

Fig. 111. The same in a later stage; tip of a pollen-tube with body-cell is found in archegonial chamber.

Fig. 112. Upper part of a mature egg, showing the egg-nucleus and a part of the vacuole.

Fig. 113. Conjugating sexual nuclei.

Fig. 114. Spindle of first division of fertilized nucleus.