THE DEVELOPMENT OF THE FEMALE GAMETOPHYTE IN *ISOETES TAIWANENSIS* DEVOL

by

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ABSTRACT

The development of the female gametophyte in *Isoetes taiwanensis* DeVol is described. In the early stage of endosporic gametophyte development, the primary nucleus moves from base to apex and undergoes repeated nuclear divisions to form a free nuclear structure in the apical region. When there are only a few nuclei, the wall formation proceeds around the nuclei both basipetally and centripetally. Karyogenesis and cell wall formation could occur at the same time resulted in binucleate or multinucleate cells. In the full cellular stage, the relatively sharp border between the upper small celled and lower large celled area can be recognized. The lower large cells are rich in food particles. The first archegonial initial arises from a single superficial cell beneath the tri-ridge of the spore wall. The number of archegonia formed in each gametophyte is not constant ranging from 5 to 20. Rhizoids originated also from the superficial cells of the apical region are always on the exposed area after the spore wall breaks. The outgrowth of gametophytic tissue from megaspore wall mainly takes place in December to February indicating it might need cooler weather.

INTRODUCTION

Considerable work has been done on the genus *Isoetes*, especially on sporophyte (Chiang, 1976; Lang, 1915; Paollillo, 1963; Scott and Hill, 1900; Stokey, 1909; West, and Takeda 1915). Several membrs of *Isoetes* have also been studied from the standpoint of female gametogenesis occurring inside the spore wall. The development of female gametophyte has been described in *I. lacustris* (Hofmeister, 1862; Goebel, 1880-1881; Farmer, 1890), *I. echinospora* (Campbell, 1891), *I. malinverniana* (Arnoldi, 1896) and *I. lithophila* (LaMotte. 1933, 1937). These workers have presented the detailed reports on the development of female gametophyte. They showed that the developmental pattern in female gametophyte was not constant for the genus, especially in the early stage. The nucleus of a mature megaspore is found to be located near the apex (proximal end of the spore) in *I. malinverniana* (Arnoldi, 1896) and *I. lacustris* (Farmer, 1890). But it is not the case in *I. echinospora* (Campbell, 1891) and *I. lithophila* (LaMotte, 1933), it is located at the base of spore (distal end of the spore). Besides, different number of archegonium in a gametophyte ranging from one to thirty was reported (Hofmeister, 1862; Kienitz-Gerloff, 1881). However, the position and the cellular organization of an archegonium in different species of *Isoetes* seemed to quite uniform.

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The sporogenesis in ***Isoetes taiwanensis*** has been described in a previous report (Huang and Chiang, 1983). The present paper deals with the development of female gametophyte and the formation of archegonium.

MATERIAL AND METHODS

The spores ***Isoetes taiwanensis*** DeVol used in the present investigation were obtained from the plants grown in the aquaria of green house in the Department of Botany, National Taiwan University. Spores were collected at monthly intervals from September, 1980 to February, 1984 spread and grown in petri-dishes. Each petri-dish contained 20 ml distilled water or ¼ strength of Hoagland solution (Hoagland and Armon, 1938), and was renewed periodically. All petri-dishes were put on the bench in an controlled laboratory. Gross observations were made with stero-microscope every week.

Some developing spores were harvested from each petri-dish every week for sectioning. The materials were fixed in 3% glutaraldehyde in 0.1M phosphate buffer (pH=7.2) at 0-4°C for 3 hrs, rinsed with the same buffer twice in 30 min., and dehydrated through tertiary-butanol (TBA) series (Johansen, 1940). The dehydrated material was infiltrated and embedded in low viscosity resin (Spurr, 1969). The orientation of spores has been carefully identified in the embedding processes. Sections 1-2 μm thick were cut on a Sorval MT 5000 Ultramicrotome, and stained with 1% crystal violet, 5 min. for microscopic observation (Dawes, 1979).

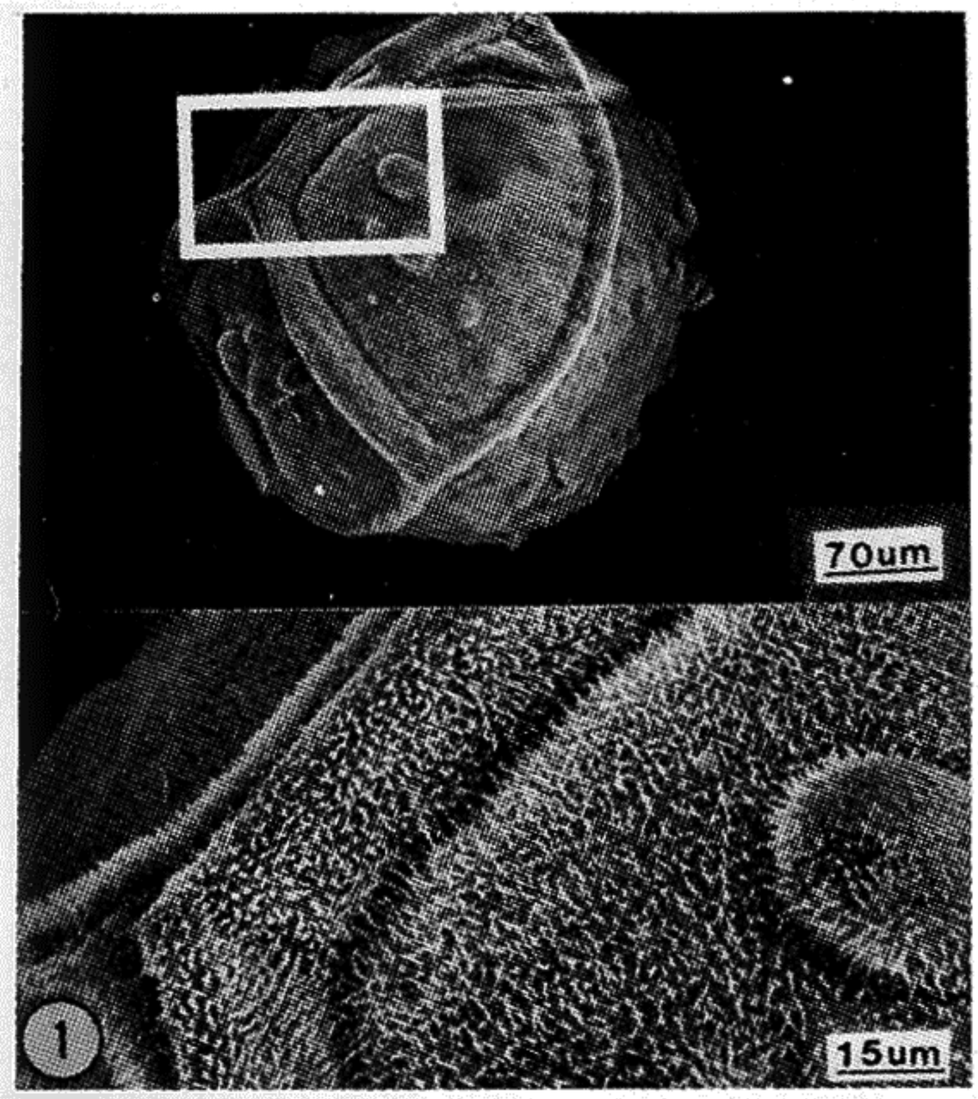
Part of the material was fixed and rinsed as previously described followed by ethanol series dehydration, acetone substitution, critical point dried with Hitachi Critical Point Dryer (HCP-1), coated with IB-2 ion coater, observation were made with Hitachi S-550 Scanning Electron Microscope (Dawes, 1979).

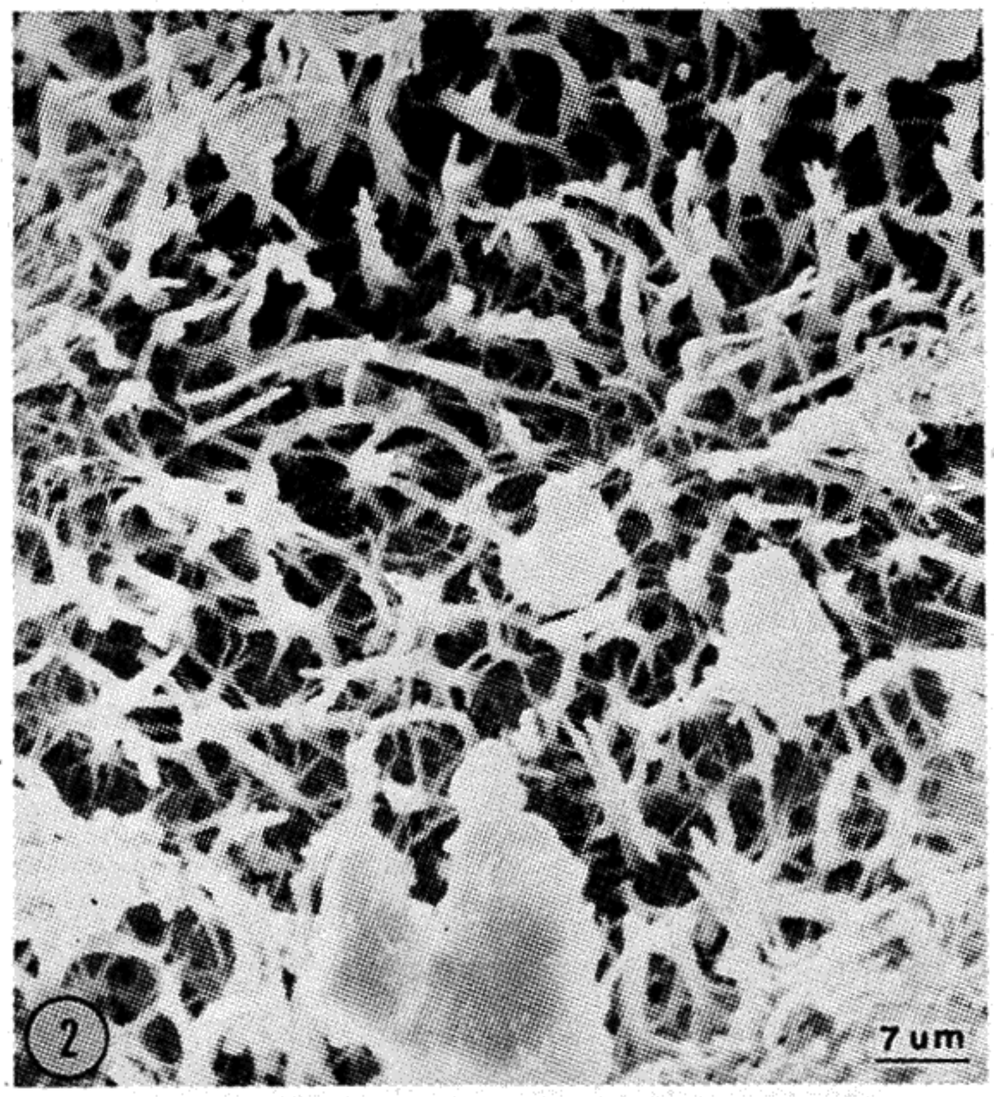
RESULTS

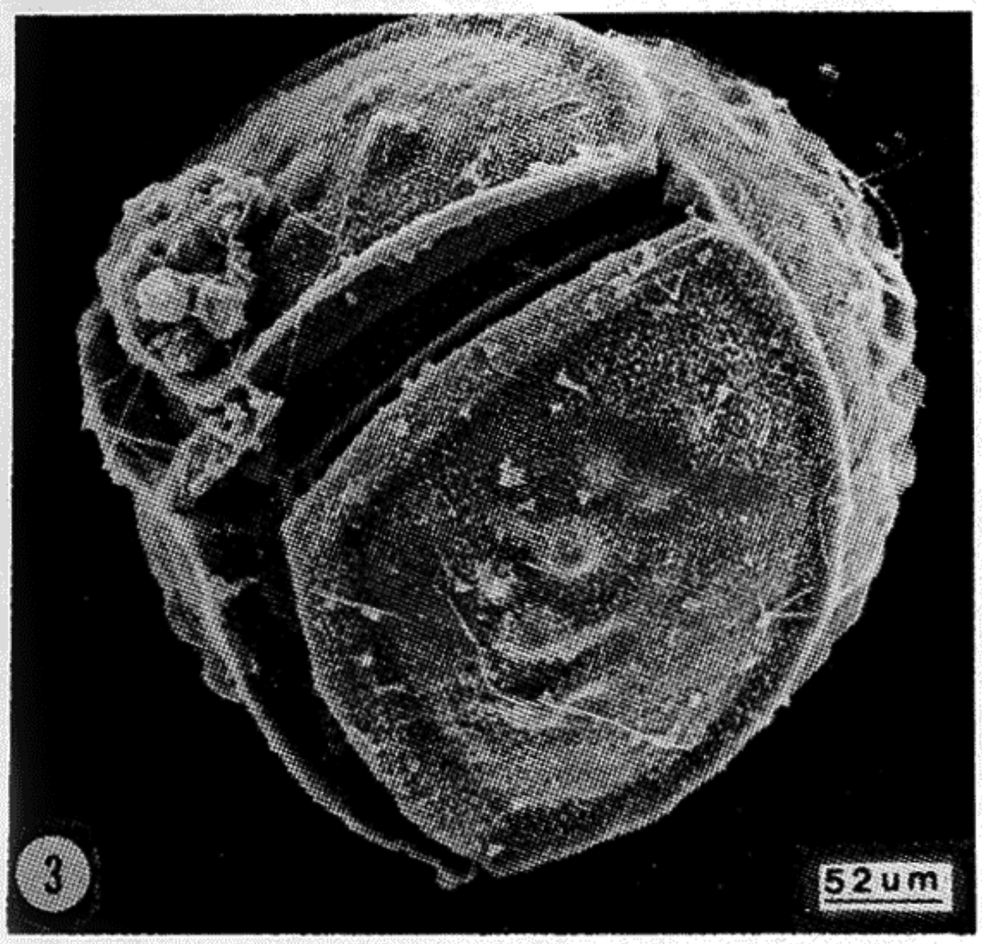
(A). External morphology:

The gametophytic tissue of a developing plant (gametophyte) protrudes slightly from the triradiate crack of the spore wall (Figs. 1-4). The most conspicuous tissue on the surface happens to be the archegonial neck cells. Rhizoids are also seen along the wall crack (Figs. 5, 8, 9). The distribution pattern of rhizoids in the intra-crack region varies in each plant. Most rhizoids become accumulated at three corners of the crack in some plants, whereas they are randomly developed over the entire surface in others. The archegonia are somewhat concentrated at the apex (central region of crack) in formal type of gametophyte, and they intermingle with rhizoids in the latter type. Some rhizoids in a well-developed gametophyte are very long, and appear to be two to three times of spore diameter in length.

In germinating spore, after being released from the sporangium, most spores become bigger and rounder gradually, and the spore wall splits along the triradiate ridge at the apex (Figs. 1, 3, 4). A few days after the formation of wall crack, the four neck cells in a cruciate set become visible (Figs. 7-9). The color of neck cell wall is always darker than the other gametophytic tissues. It is yellowish to golden brown at maturity. The germination of the spores which shed from the same sporangium do not always take place simultaneously.







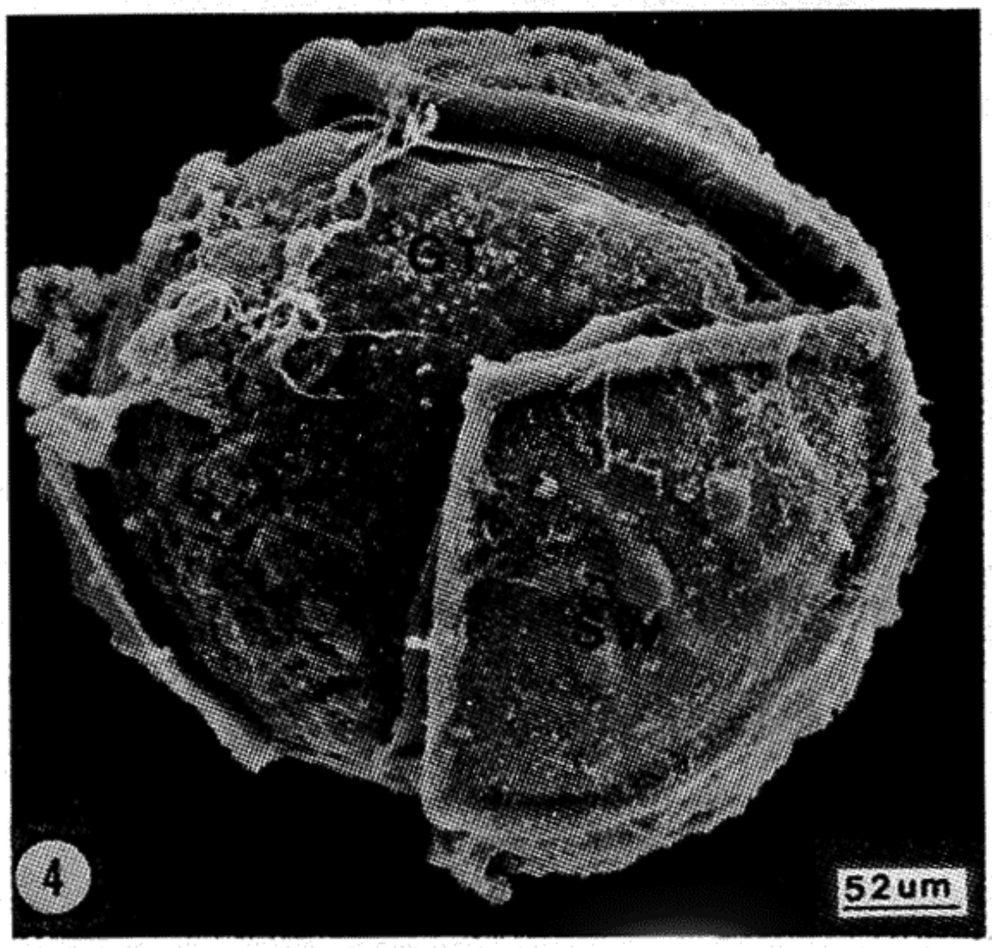
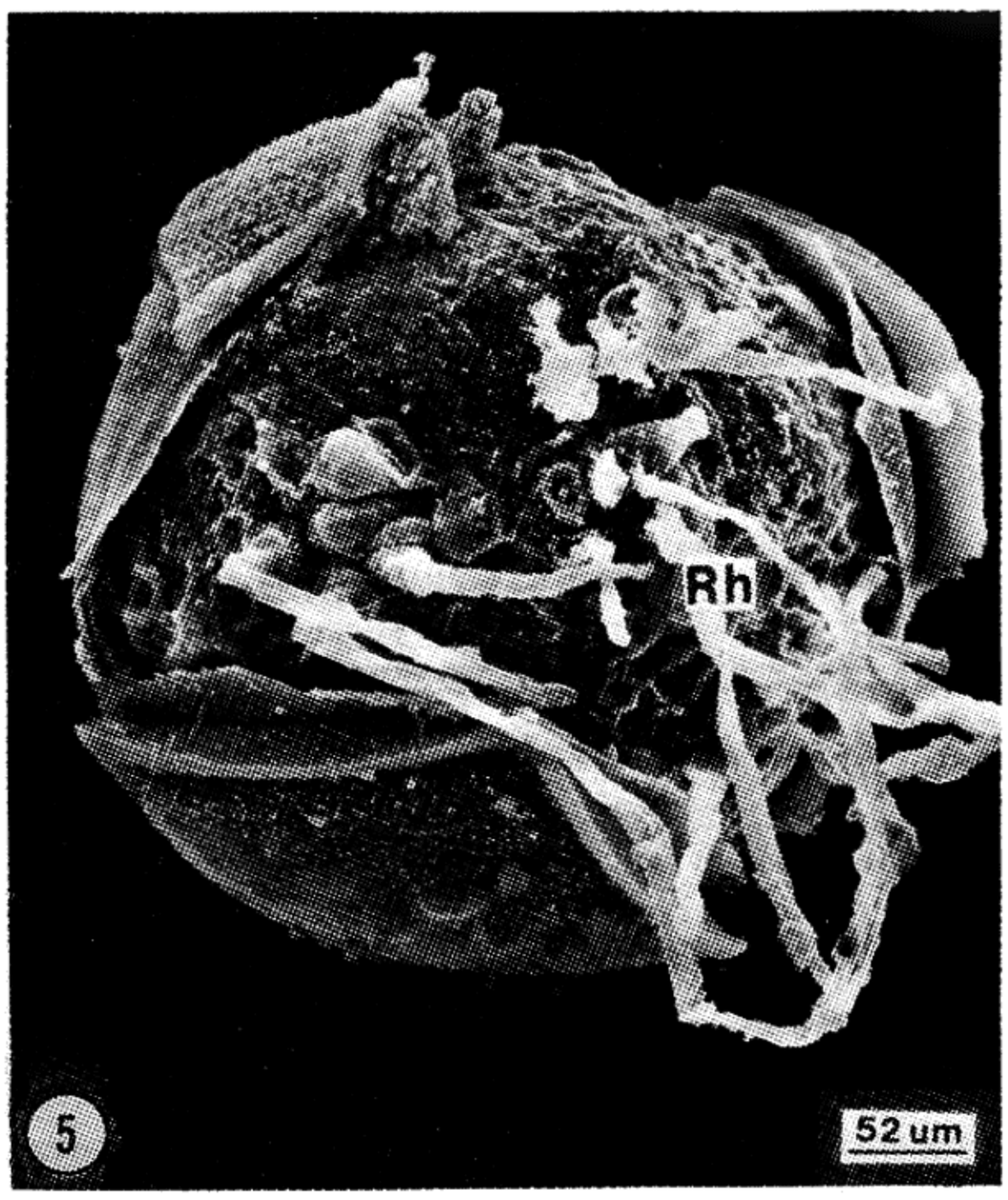
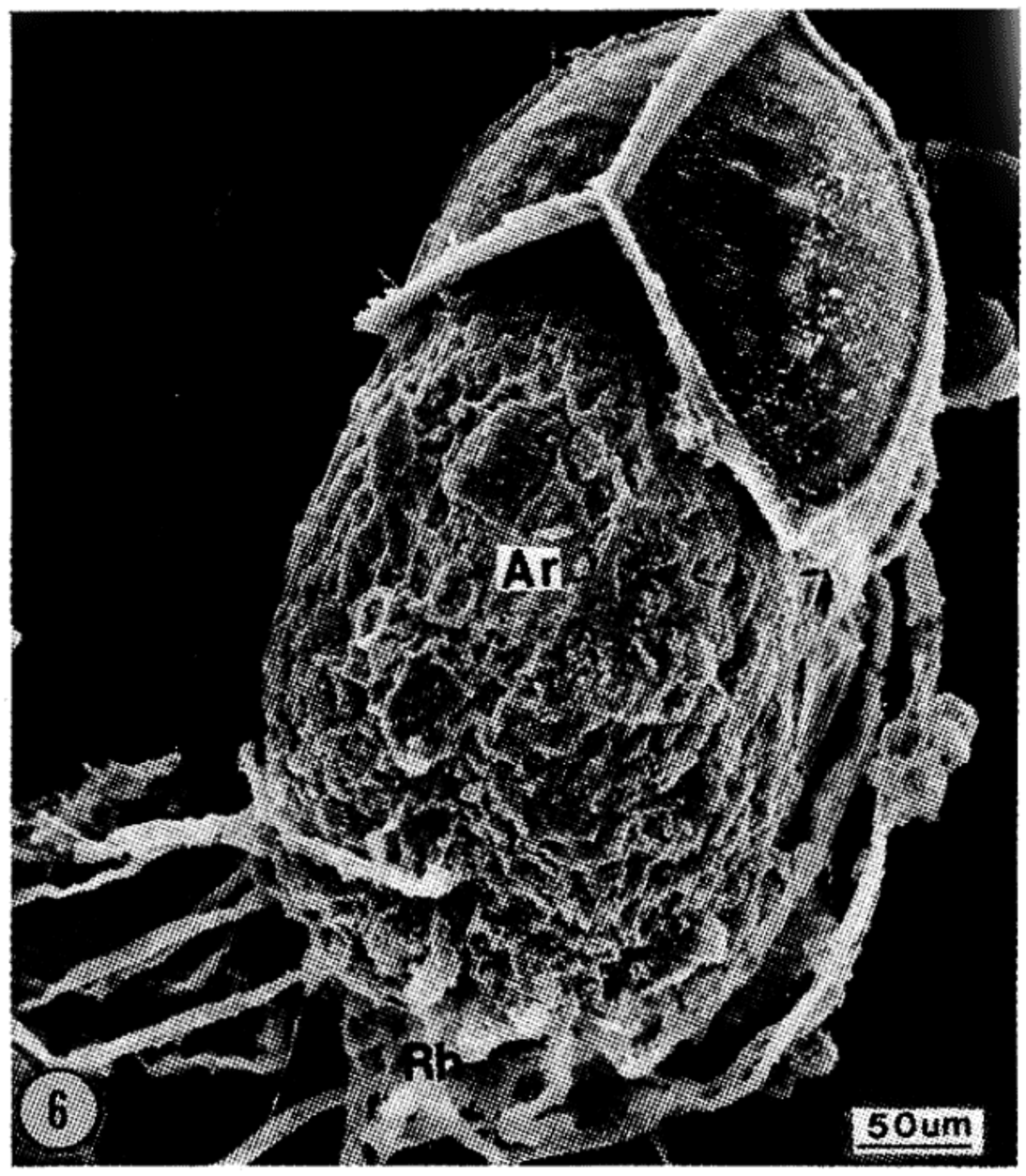
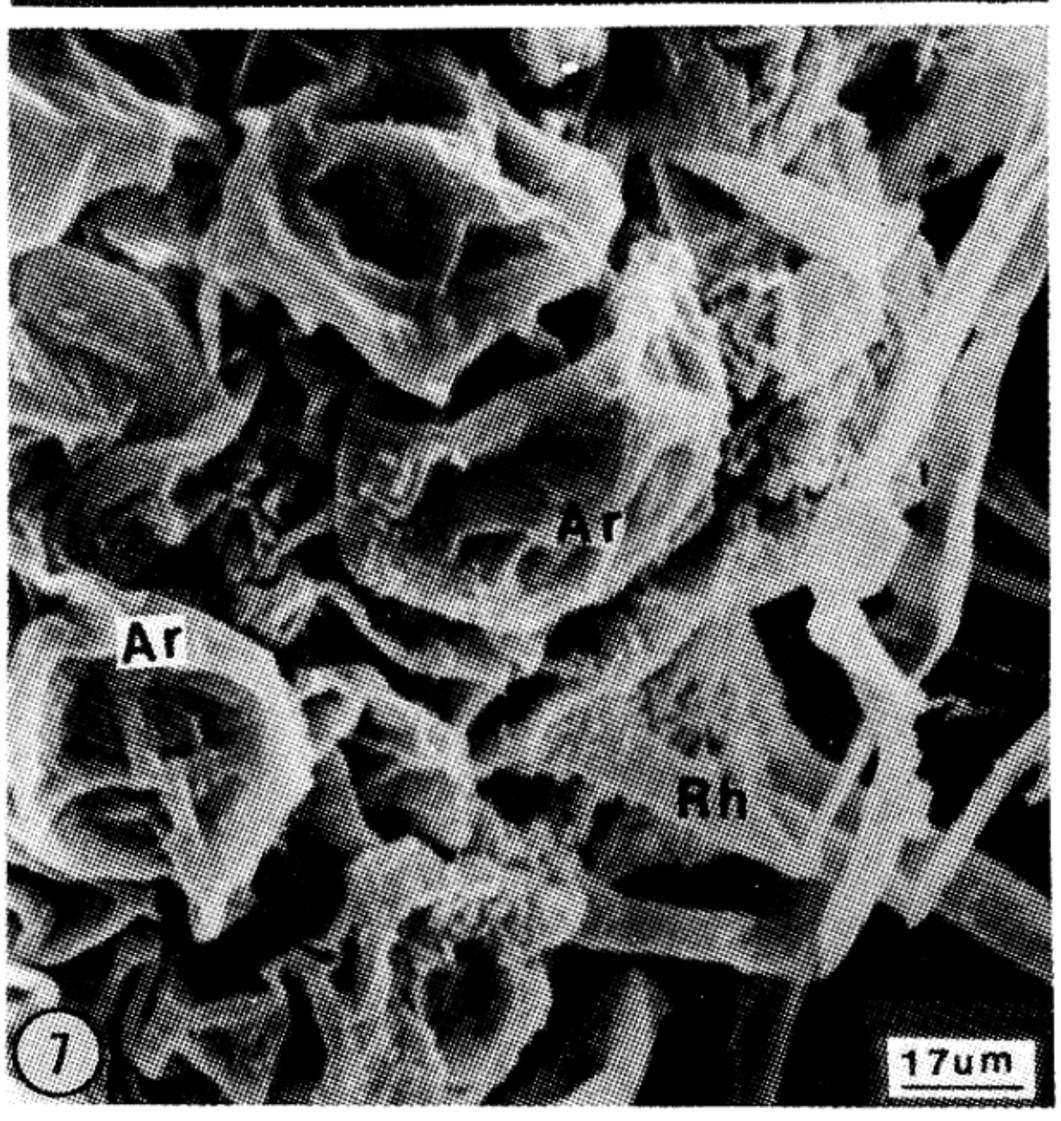


Fig. 1: Megaspore under SEM.
Fig. 2: Enlargement of Fig. 1, showing the spore surface.
Fig. 3: The spore wall splits along the triradiate ridge at the apex (SEM).
Fig. 4: The gametophytic tissue (GT) protruded from the triradiate crack of the spore wall (SW) (SEM).







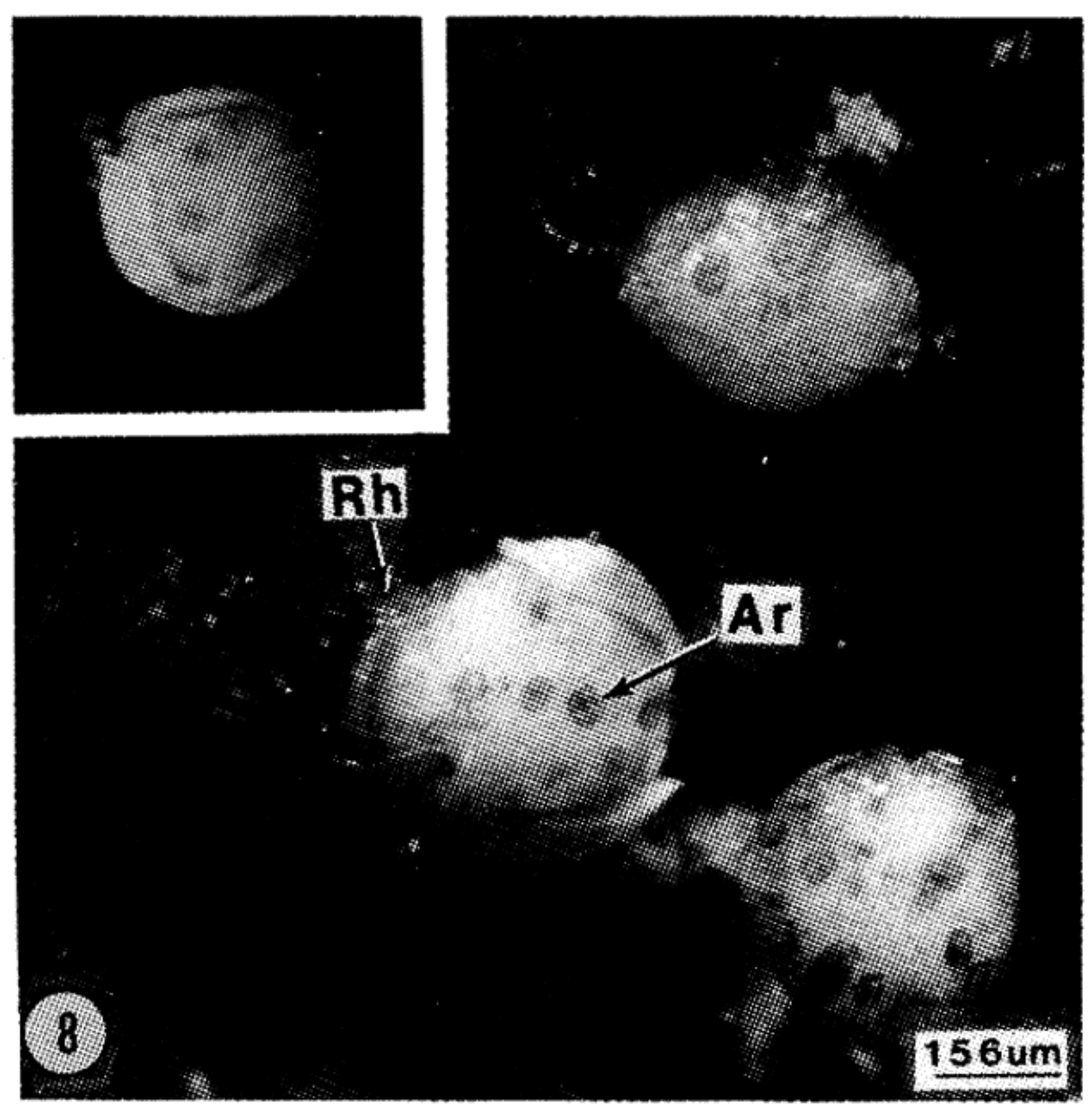


Fig. 5: Rhizoids distribute along the wall crack (SEM).
Fig. 6: The outgrowth of female gametophyte bearing numerous rhizoids (Rh) and archegonia (Ar) (SEM).
Fig. 7: Showing the tubular rhizoids and the four neck cells of archegonia in a cruciate set (SEM).
Fig. 8: Top views of spores with stero-microscope, showing the various stages of wall splitting.

(B). Germination time:

The mature sporangia were mostly found in summer to autumn. Spores obtained from the mature sporangia were sowed mainly from June to October. The spores collected and sowed in June and July, did not germinate until October or November (Table 1). Only a few of them germinated in next March or April. But some of the spores collected and sowed in September and October, germinated immediately after sowing. In general, most spores germinated in December to February. No germinating spores were found in July. The remained spores died out in May to July. The highest germination frequency was seen in the spores collected and sowed in August to October. It was also shown that the germination frequency of distilled water culture members was almost the same as that grown in Hoagland solution. It seems to that inorganic nutrient is not necessory for the gametogenesis to happen in vitro.

Most of the germinating spores completed the sporophyte formation. The first individual sporophytic leaf tip was observed protruding from a rather large number of the gametophytes in January to February (Table 1). This result showed an apparent influence of weather on the time course of spore germinaion as well as gametogenesis. It neeeds cooler weather.

(C). The development of female gametophyte:

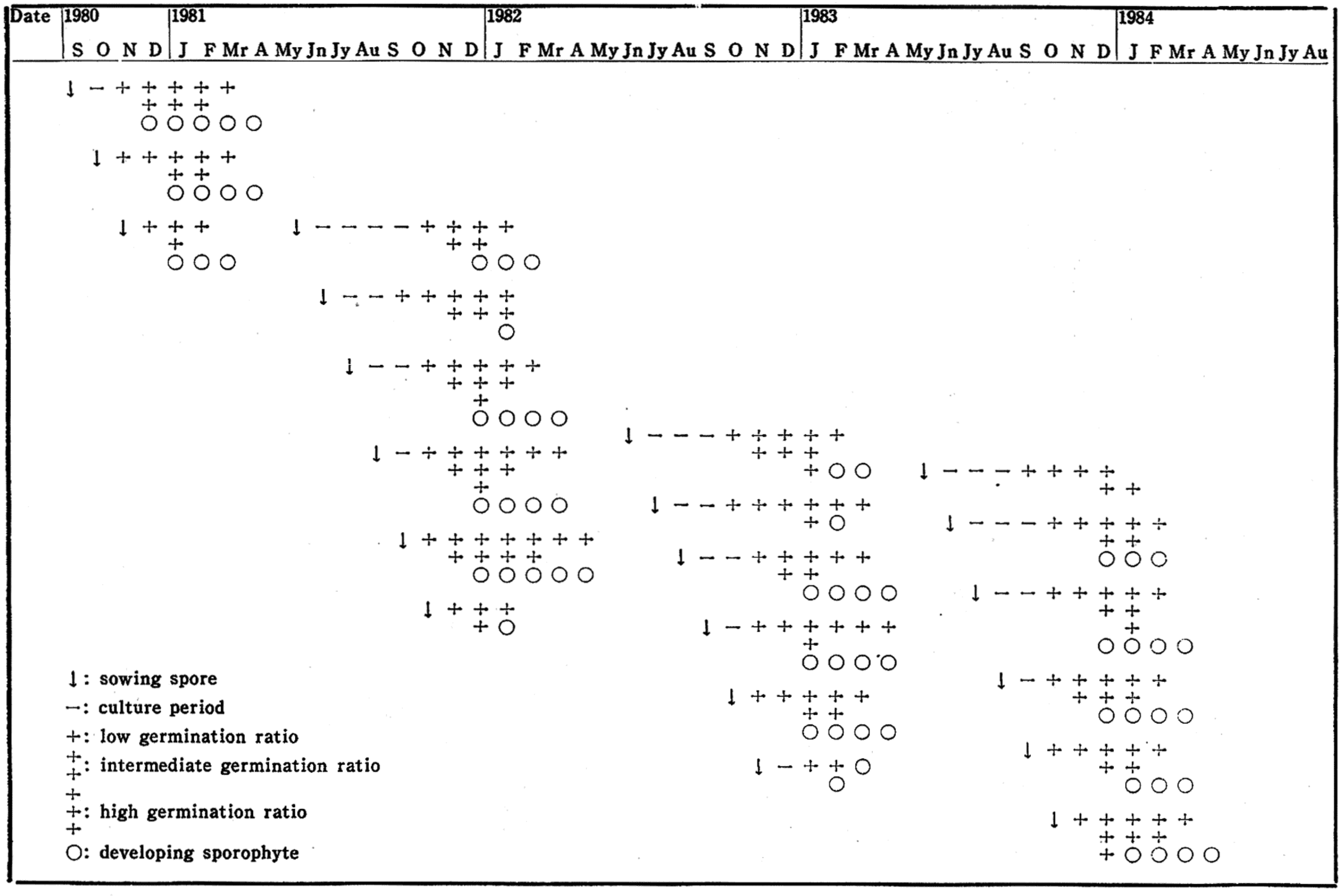
The megaspore wall is made of three layers (Figs. 13, 18). The outer layer is much thicker than the others, and is characterized by the presence of hard recurved bristle materials (Figs. 1, 2). The megaspore contains one nucleus (i. e. primary nucleus) when sheded from the sporangium, and is filled with food particles such as: starch grains, oil drops and protein bodies etc. The primary nucleus is located in the distal (basal) region (Figs. 10, 11).

In the early stage, there are a few free nuclei appearing in the apex of the developing endosporic gametophyte (Figs. 12, 13). These daughter nuclei are much smaller than the primary nucleus, and rich in chromic substance. It is suggested that the primary nucleus probably move to apex, and then proceeds the free nuclear division in apex. When there are still only a few nuclei in a developing gametophyte, the first cell wall formation occurs around the nuclei at the apical region, and proceeds both basipetally and centripetally (Figs. 14-16). During the early stage of wall formaton, these nuclei are still in the process of karyogenesis. Then the entire structure inside the spore wall becomes cellular (Figs. 17, 18). Still in some gametophytes, the wall formation in the basal region is very slow, and it fails to become a complete cellular gametophyte as late as in embryo bearing stage. However, the binucleate situation is commonly observed in the well developed cellular gametophyte (Fig. 17). The cells in the basal zone appear to be larger than those in the apicals. The food particles are more abundant in the basal cells than that in the apicals. The changes in the amount of the food particles as well ar the size of cell in the apical region toward the basal region are abrupt rather than gradual. But there is no clear boundary between these two regions (Figs. 15, 31, 32). At the cellular level the transition from one area to the other is spread out over a distance 3 to 6 cells wide.

ARCHEGONIA:

The first archegonium always forms immediately inside the triradiate intra-crack (Figs. 19-24, 30). One superficial cell in the apical region acts as archegonial initial which is greatly enlarged and becomes slightly denser in cell contents over its contiguous superficial cells (Fig. 19). The archegonial initial first divides transversely

Table 1: The time of sowing and germination



and gives rise to the outer primary cover cell and the inner central cell (Figs. 20, 33B). These two daughter cells are almost equal in size. The primary cover cell will form neck cells only, and the central cell will give rise to all the rest of cells in an archegonium such as: neck canal cells, ventral canal cell and the egg (Fig. 33). After the first segmentation in archegonial initial, the central cell divides prior to the primary cover cell by forming a transverse wall. Thus formed the 3-celled stage of a developing archegonium which consists of the outer primary cover cell, middle neck canal cell and the inner primary ventral cell (Figs. 21, right; 33-C). The neck canal cell does not divide again. Then the primary cover cell undergoes two vertical divisions at right angles to each other forming four neck initial cells (Figs. 21, left; 33-D). The primary ventral cell divides transversely to form one ventral canal cell and an egg (Figs. 22; 33-E). Finally the four neck initial cells undergo repeated transverse divisions to produce sixteen neck cells arranging in four tiers (Figs. 23, 24). A fully developed archegonium consists of sixteen neck cells, a neck canal cell, a ventral canal cell, and an egg cell (Figs. 24, 25, 33-G). Occasionally the further vertical divisions in the neck cells occur in a few archegonia to form more than sixteen neck cells (Fig. 28). The neck canal cell is usually pushed out and injected between the lower tiers of neck cells (Figs. 24, 25). The binucleate neck canal cell has been seen in several specimens (Fig. 24). Ultimately the upper one to two tiers of the neck cells protrude above the surface (Figs. 25-28). The archegonial necks are straight.

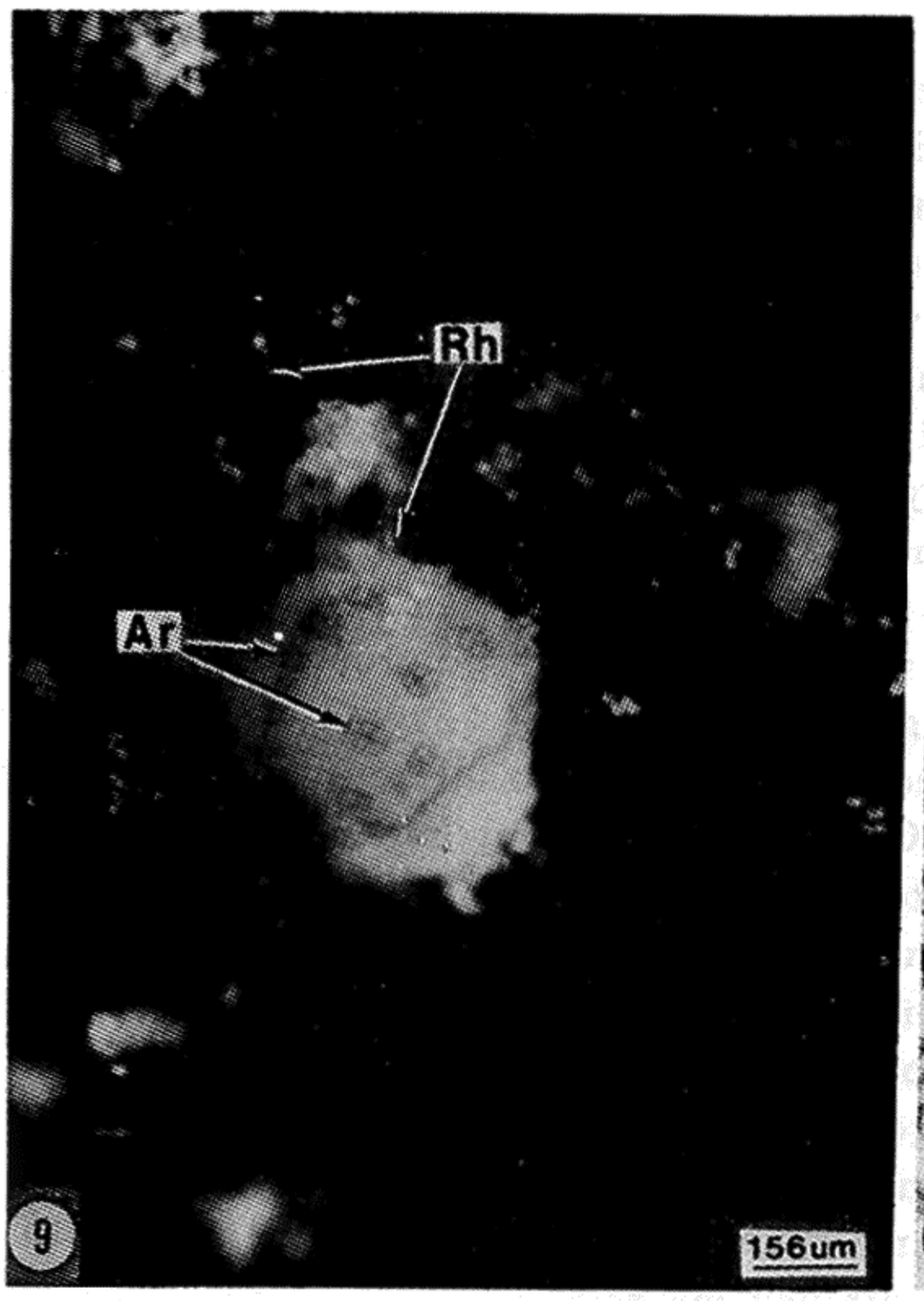
After the upper tiers of neck cells become projecting above the surface, the cell wall of the neck cells, neck canal cell, ventral canal cell and the egg cell increase in thickness. The accumulation of some darkly stained material appears surrounding both neck canal cell and ventral canal cell (Fig. 25). Both neck canal cell and ventral canal cell are short lived. As the egg cell enlarges and becomes round, both neck canal cell and ventral canal cell become flattened and disintergrated gradually (Figs. 26, 27). Their cell contents condense and lyse to become the mucilaginous mass and disintegrate gradually. Consequently the enlarged egg cell occupies the original position of the neck canal cell and ventral canal cell (Fig. 28). The neck of the archegonium opens up for the sperm entrance.

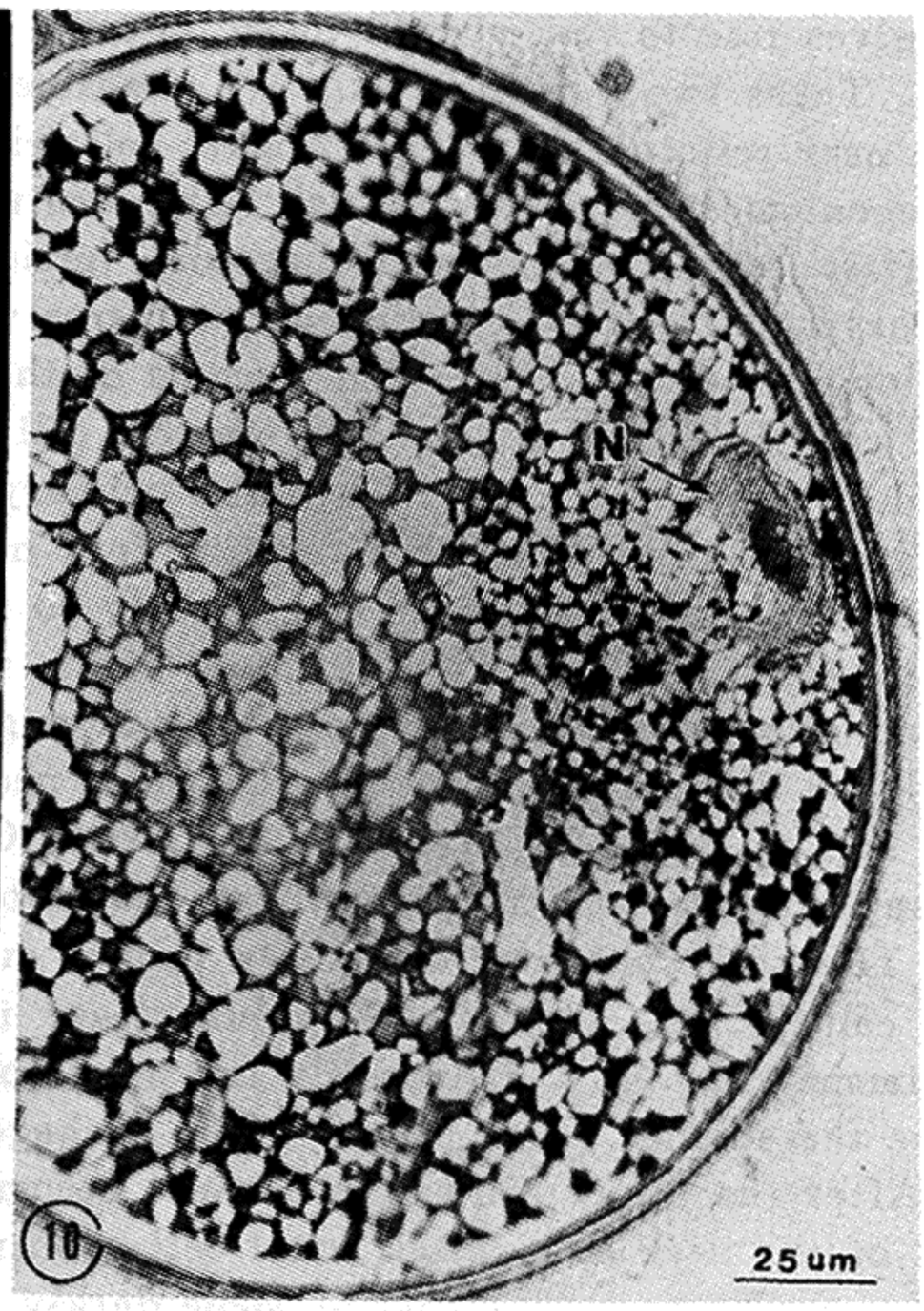
The nucleus of a fully mature egg cell increases in volume, and exhibits rich chromatic substance. By this stage, the cytoplasm of the egg cell appears to be dense and is fulled of reticulated fibrous substance (Fig. 28). Its apex projects into the base of neck canal slightly as the receptive spot. After this stage, if the fertilization was delayed or did not occur, the egg cell became collapsed (Fig. 29). The number of archegonia in an individual gametophyte varies from five to twelve. But it may reach as many as twenty in some (Fig. 9). Only one sporophyte can be obtained from an individual gametophyte, through two embryos developed in the separate archegonia are occasionally seen. One of them is in a few celled stage and the other quite elaborated.

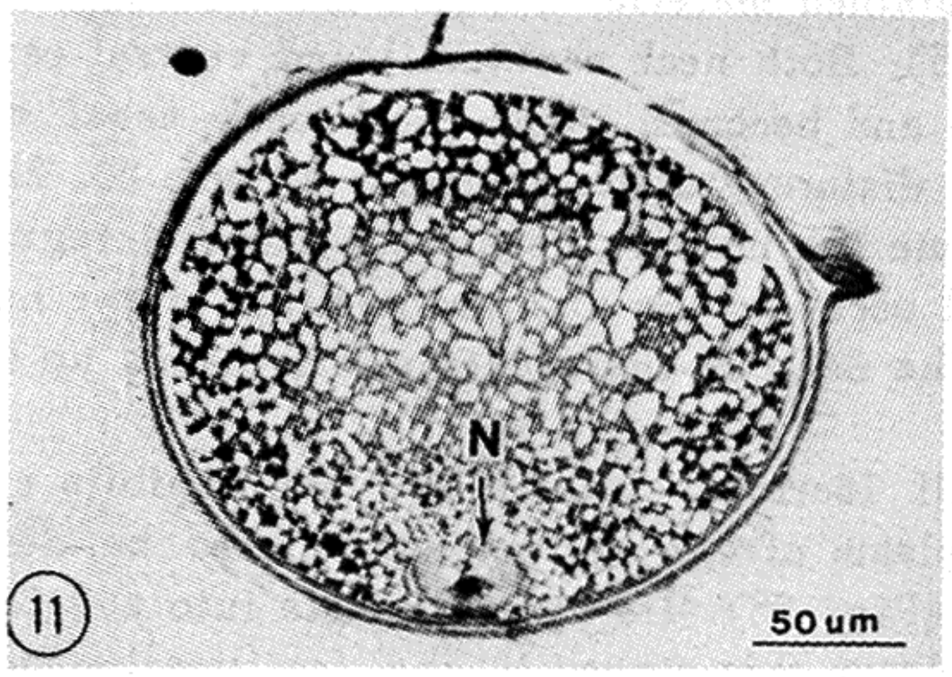
RHIZOID:

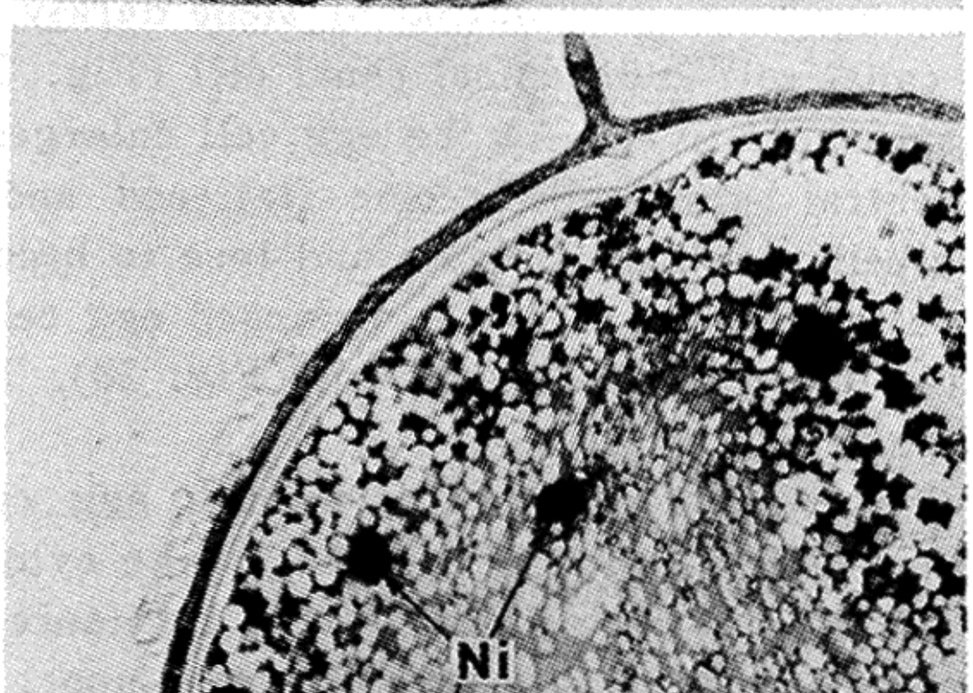
The rhizoids are restricted to the apical surface and are merely the tubular extension of superficial cells (Figs. 34-36). No septa have been found. They probably function as both absorbing and anchoraging structure. They remain as long as the gametophyte bears sporophyte, and usually become firmly attached to the petri-dish or substrates at the end of this investigation.

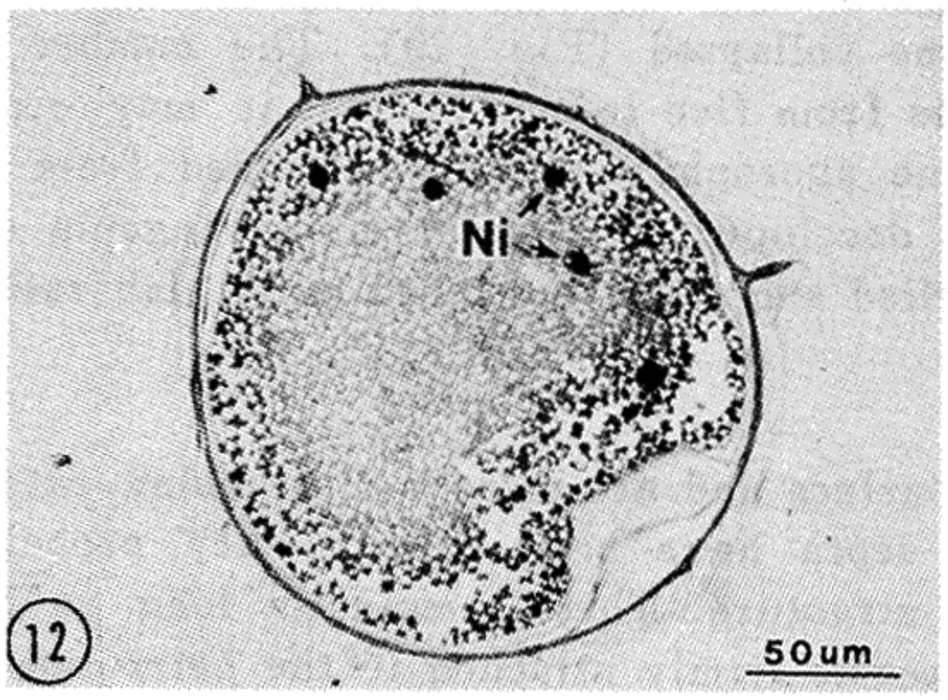
Like the archegonia, the rhizoid initials also have their origin in the superficial cells in the intra-crack region (Fig. 34). The rhizoid initials appear at the time when most of the archegonia are well organized in the same gametophyte. As mentioned











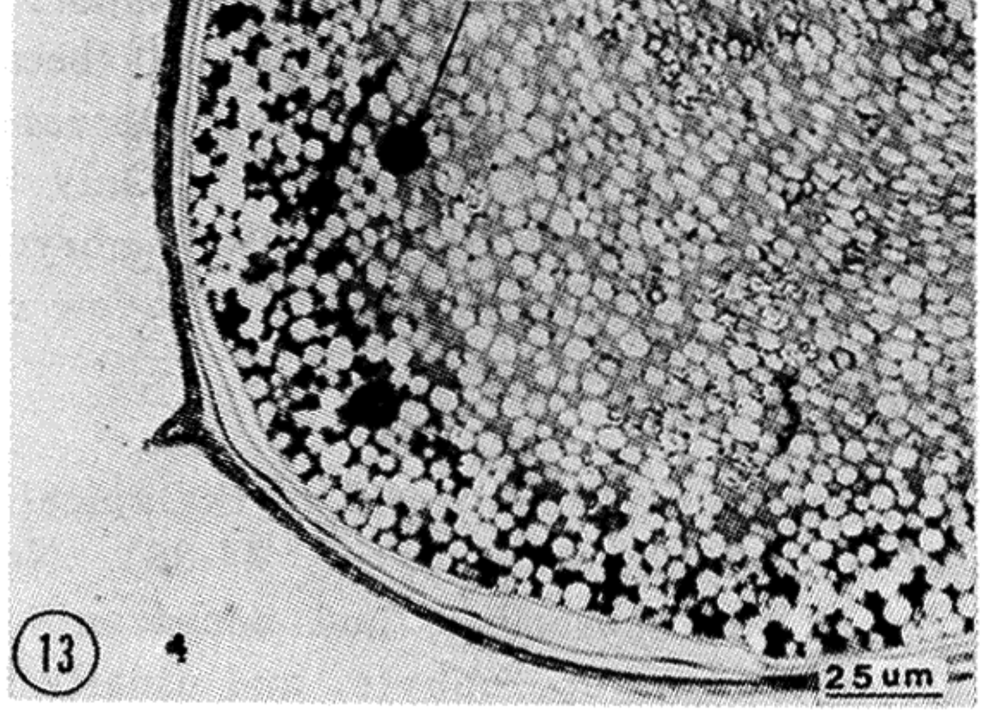
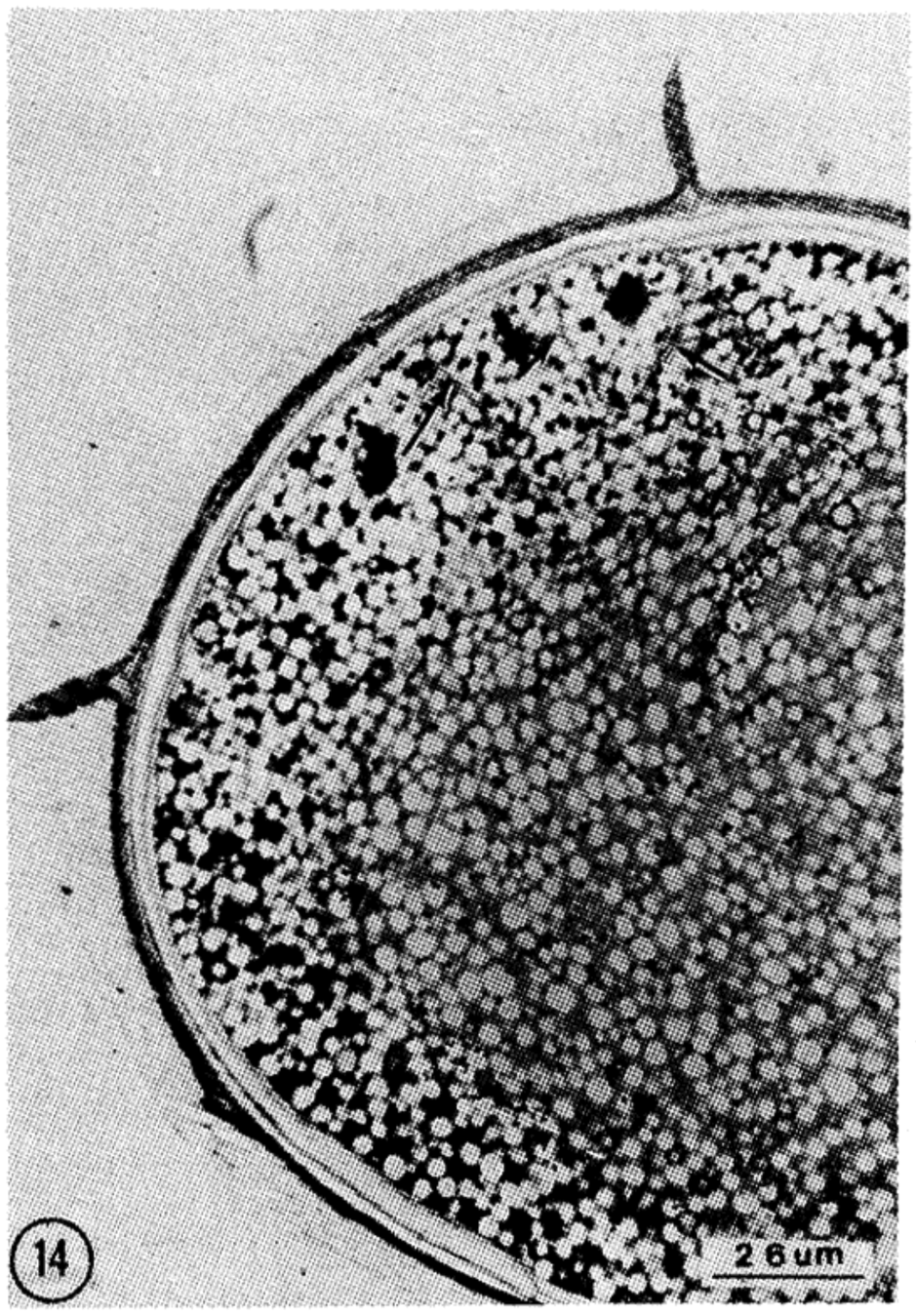
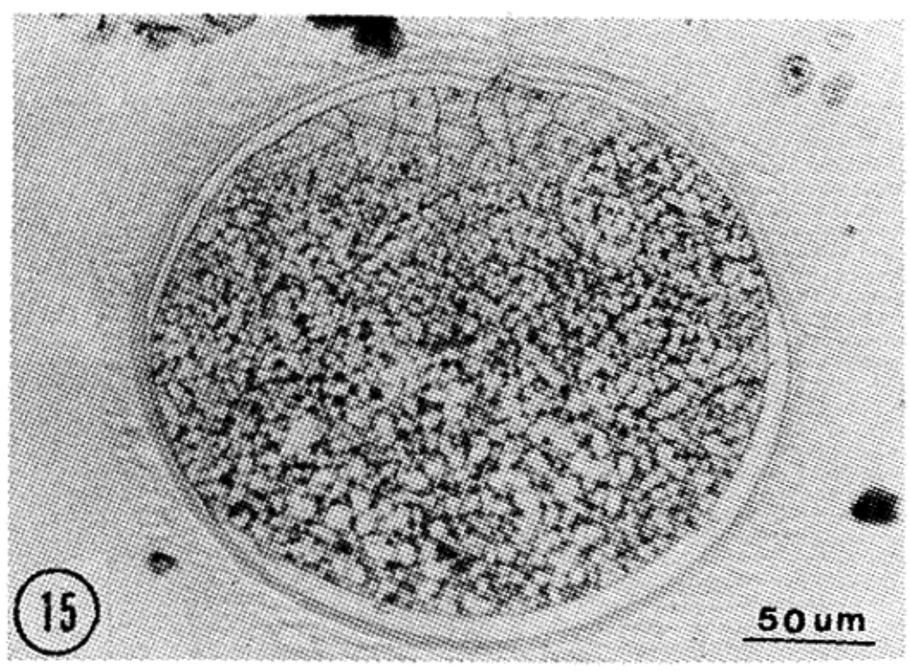
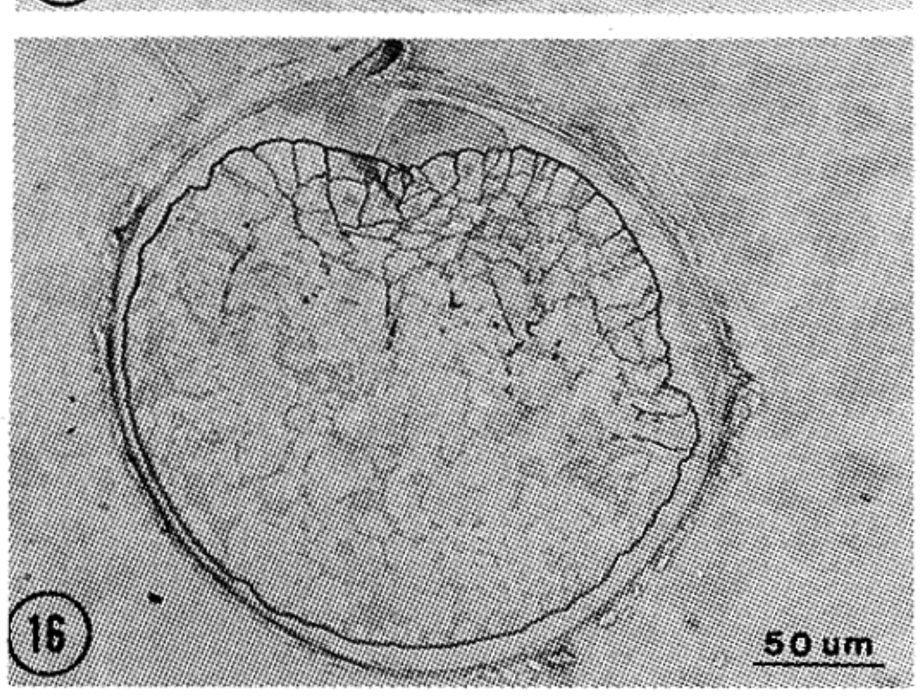
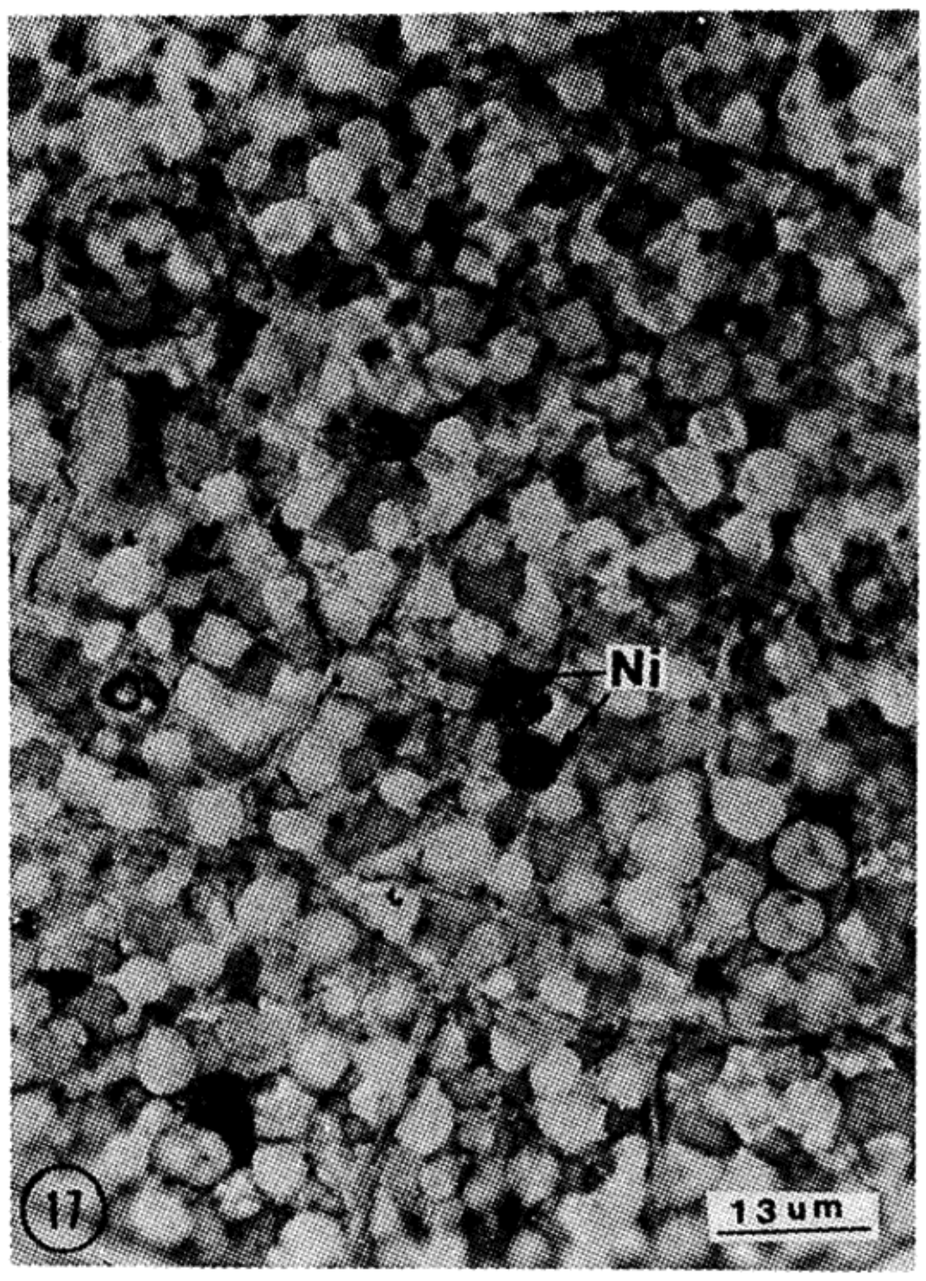


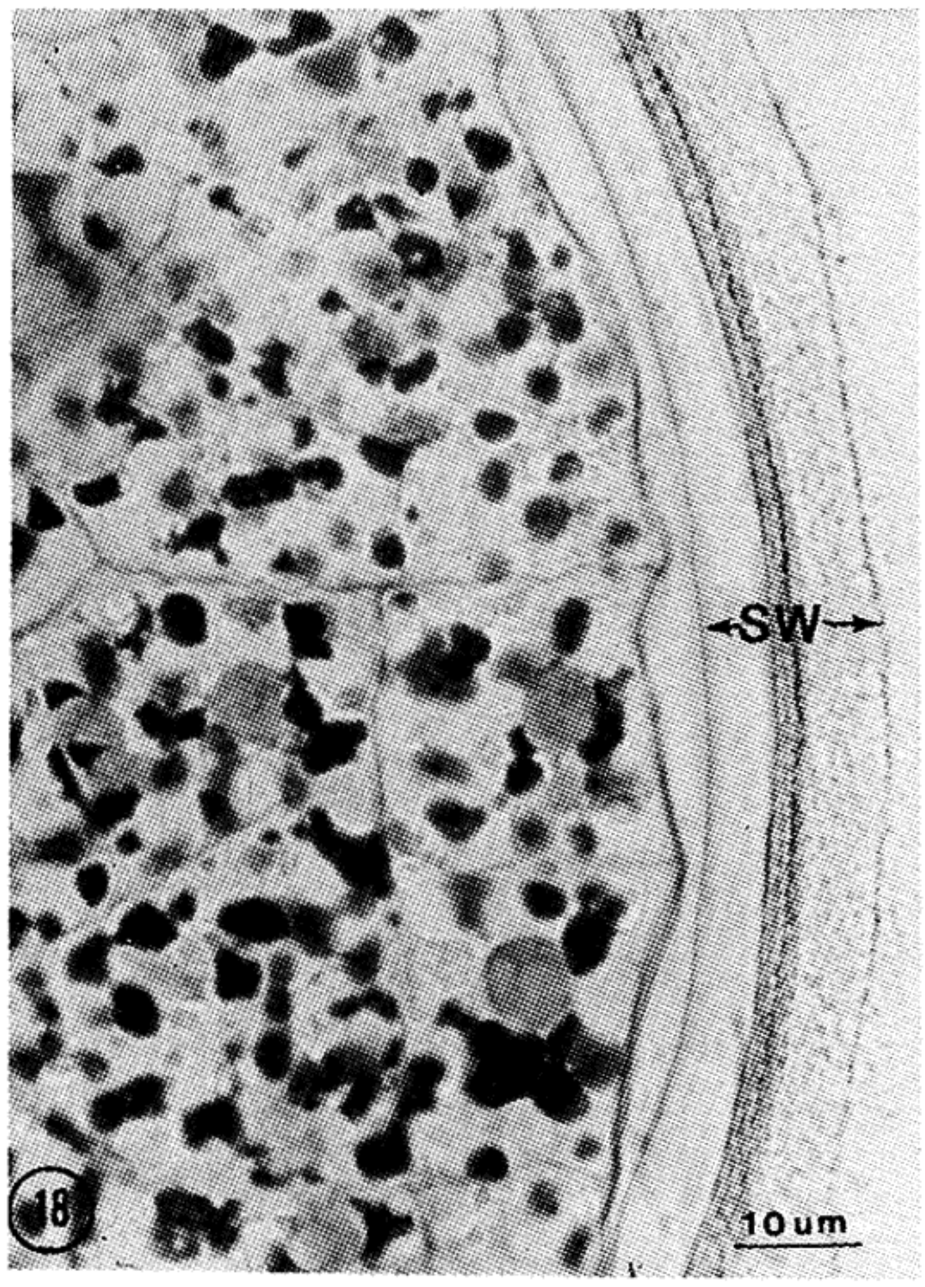
Fig. 9: Top view of a spore, showing the archegonia (Ar) intermingled with rhizoids (Rh).
Figs. 10, 11: The ripen megaspore with a single nucleus (N) located in the base.
Figs. 12, 13: The free nuclei (Ni) stage of female gametophyte development.



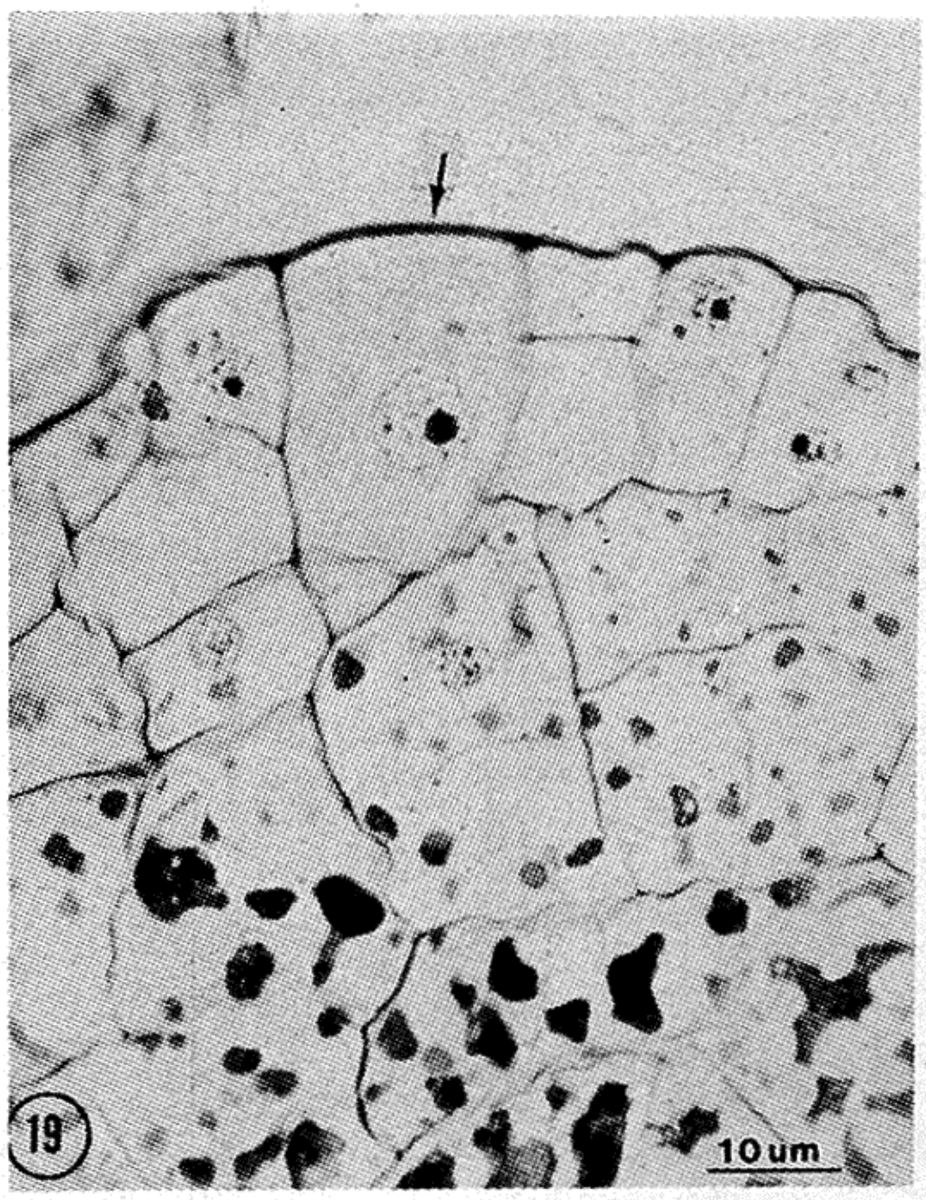


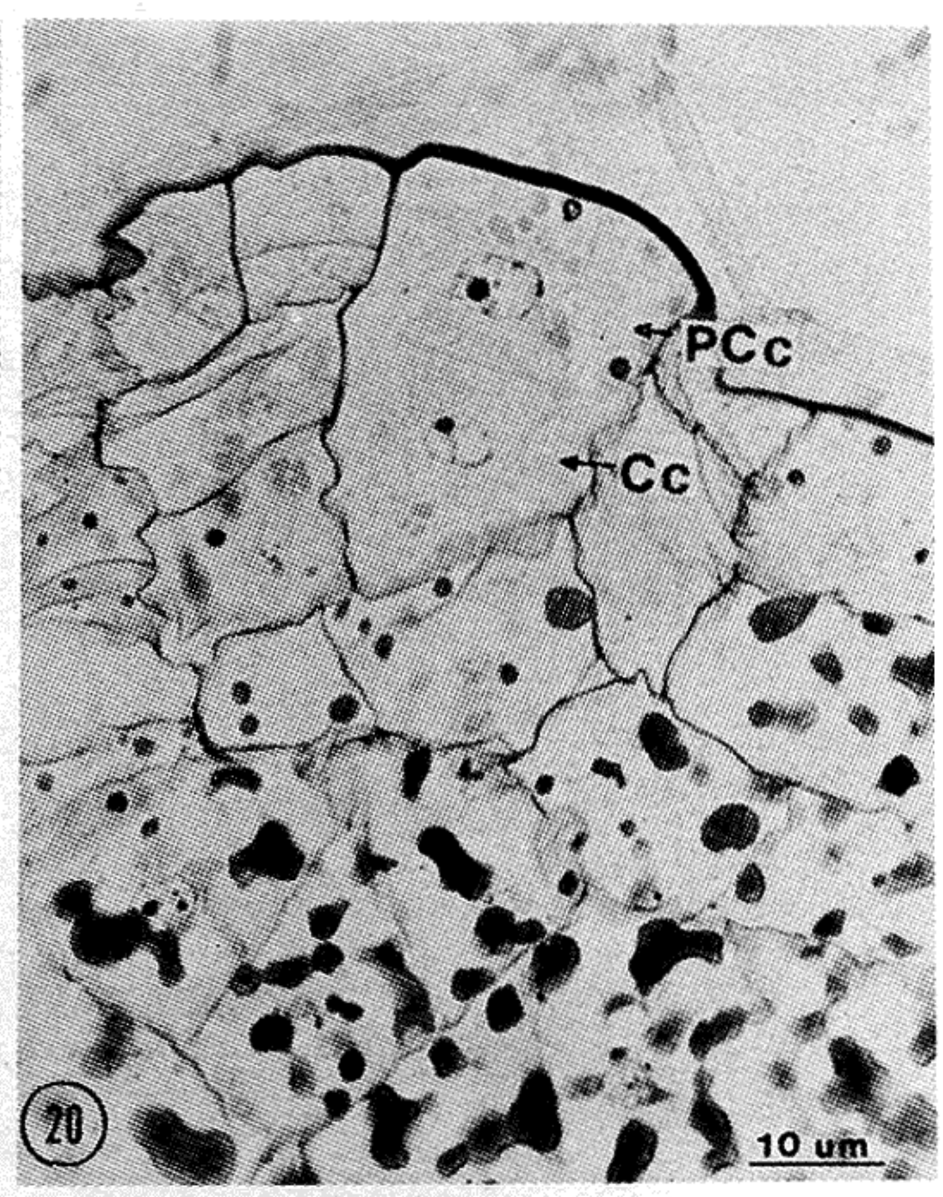


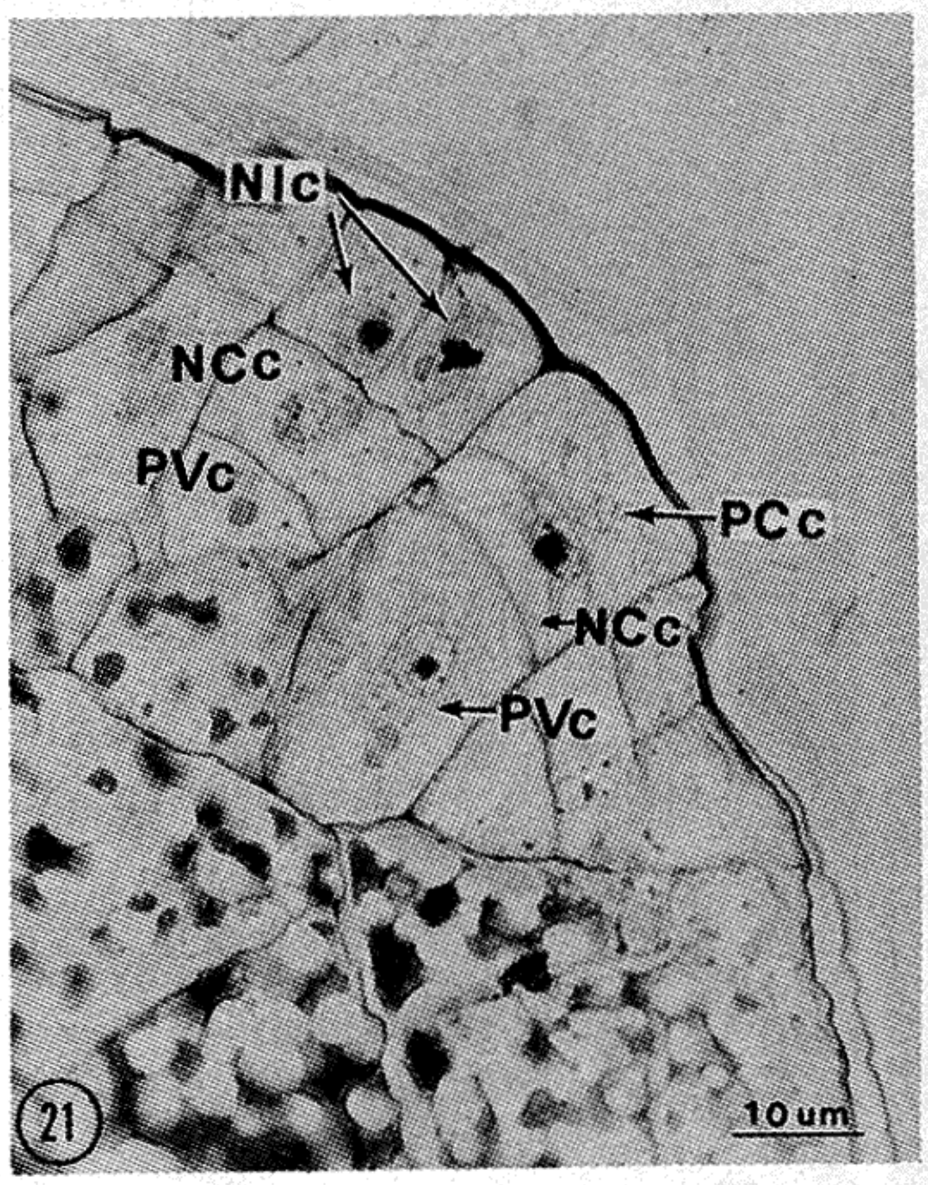


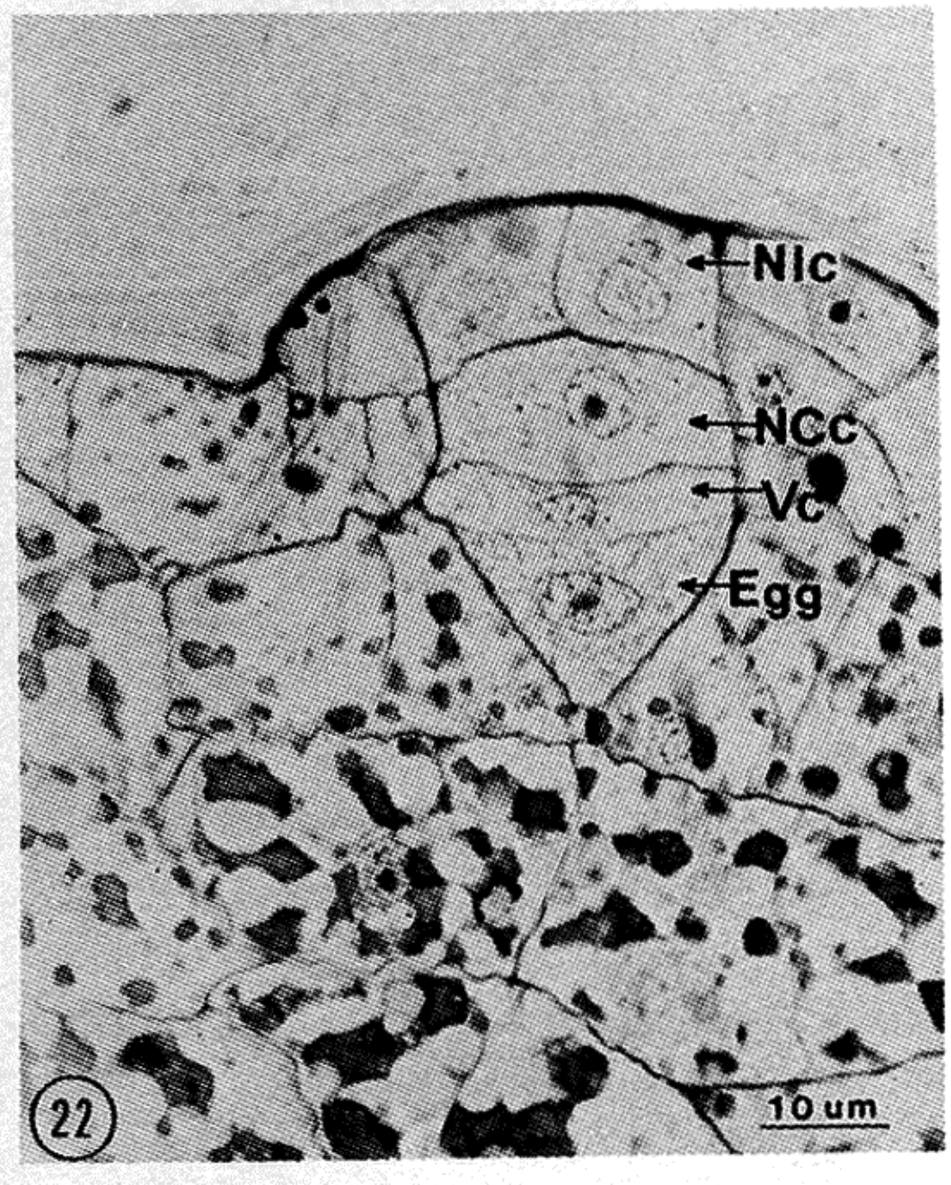


Figs. 14-18: The successive stages of cell wall formation in female gametophyte.
14: The first cell wall (arrows) formation occurs around the nuclei at the apical region.
15, 16: The cell wall formation proceeds both basipetally and centripetally.
17: The binucleate cell in the basal region of female gametophyte.
18: Abundance of food particles in the basal portion of cellular female gametophyte. Note the three layers of megaspore wall (SW).









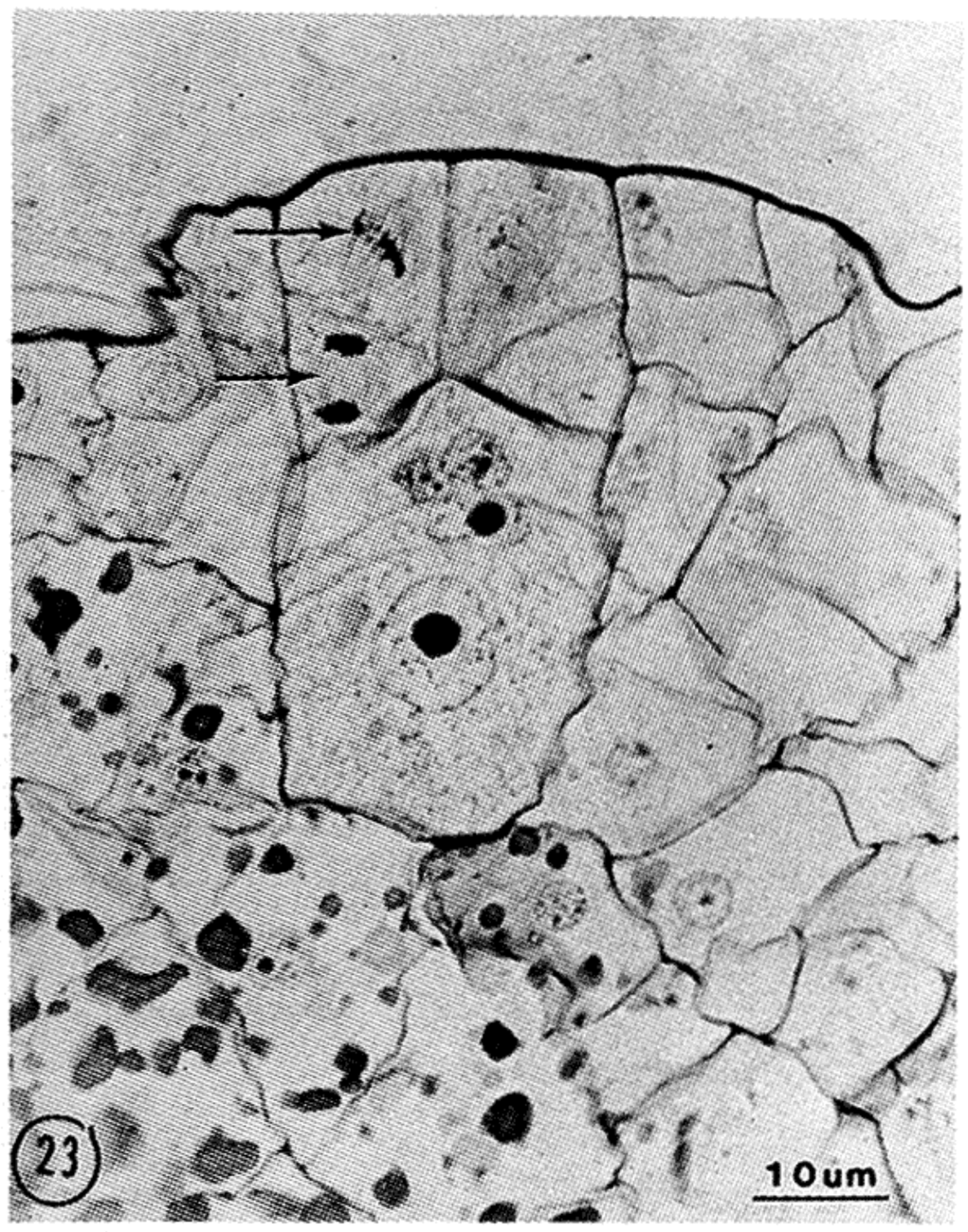
Figs. 19–28: The successive stages of archegonial formation.

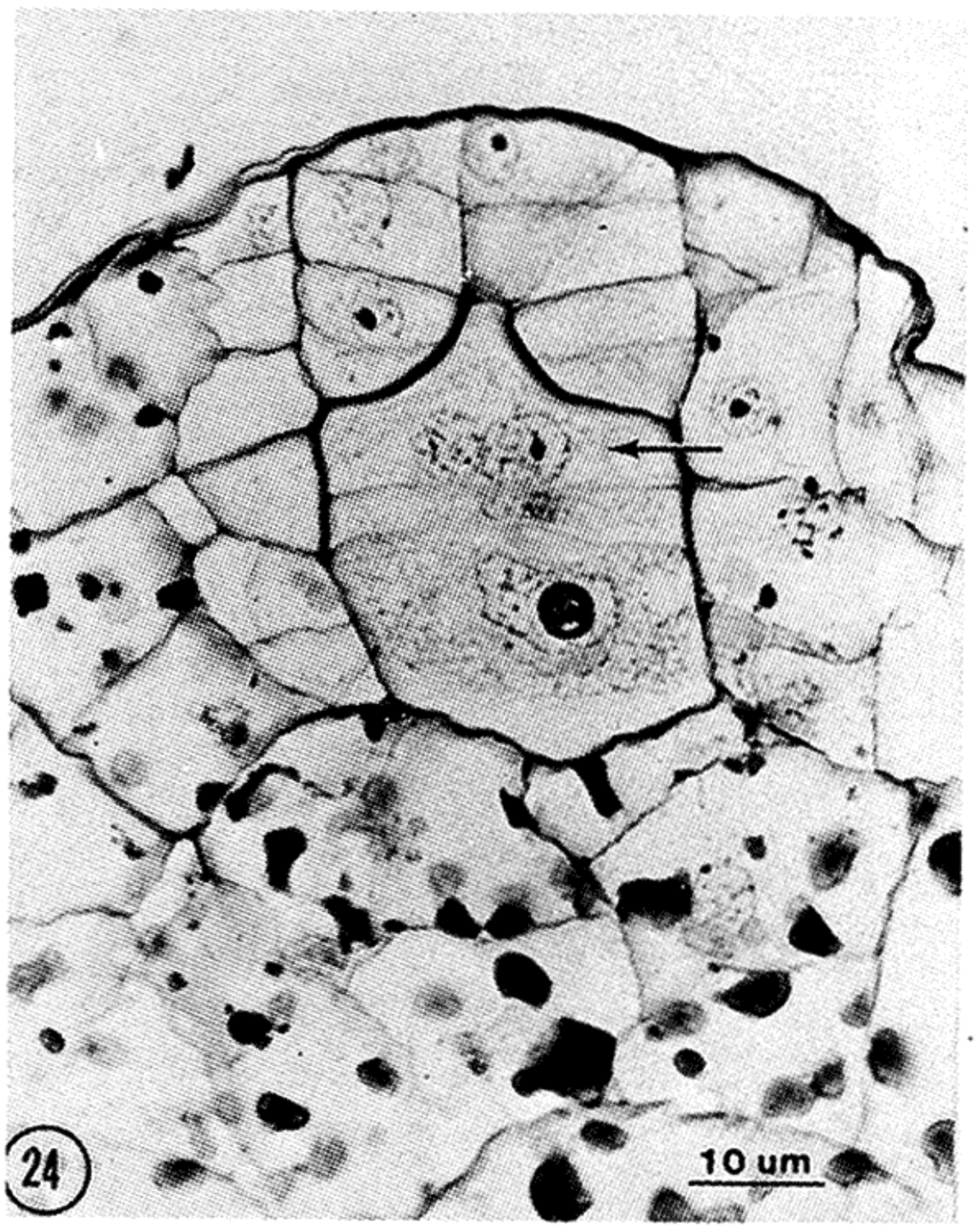
19: Enlarged archegonial initial (arrow) in surface of the female gametophyte.

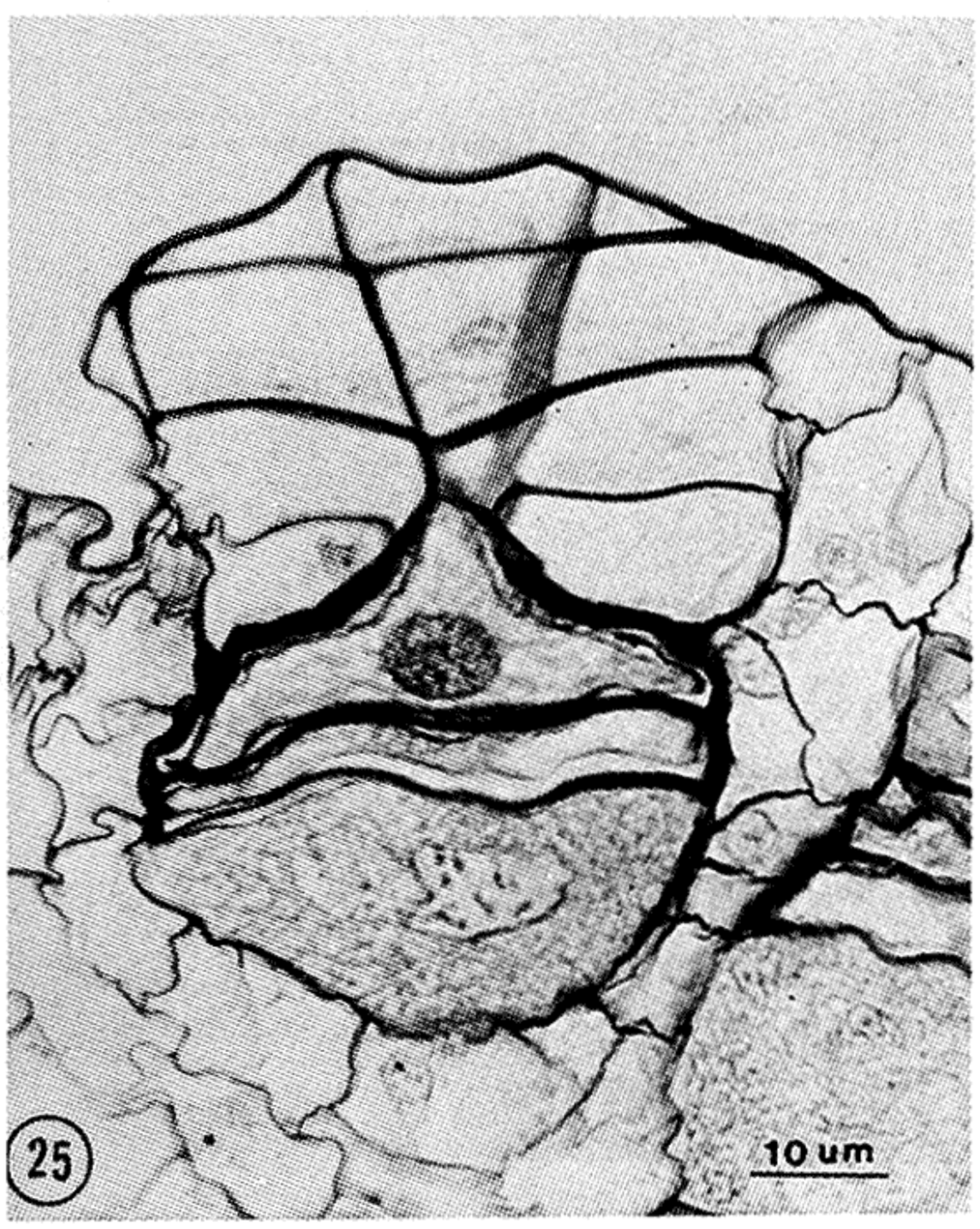
20: Formation of outer primary cover cell (PCc) and inner central cell (Cc). They are still in late telophase note the cell plate in between.

21: Two stages of developing archegonia.
Right: 3-celled stage. The outer primary cover cell (PCc), middle neck canal cell (NCc) and the inner primary ventral cell (PVc).
Left: 6-celled stage.
There are four neck initial cells (NIc), a neck canal cell (NCc) and a primary ventral cell.

22: 7-celled stage, with four neck initial cells (NIc), a neck canal cell (NCc), a ventral cell (Vc) and an egg cell.







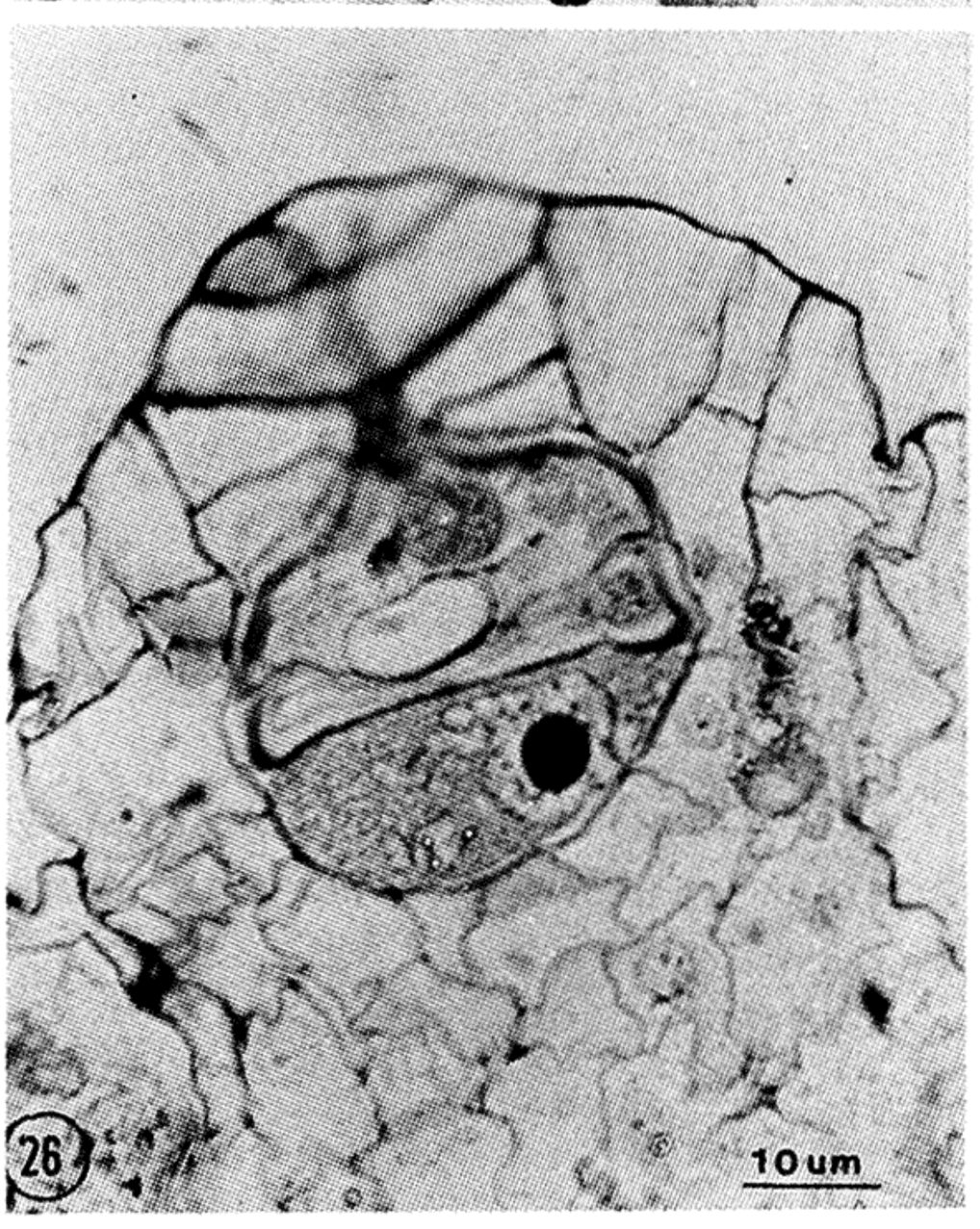
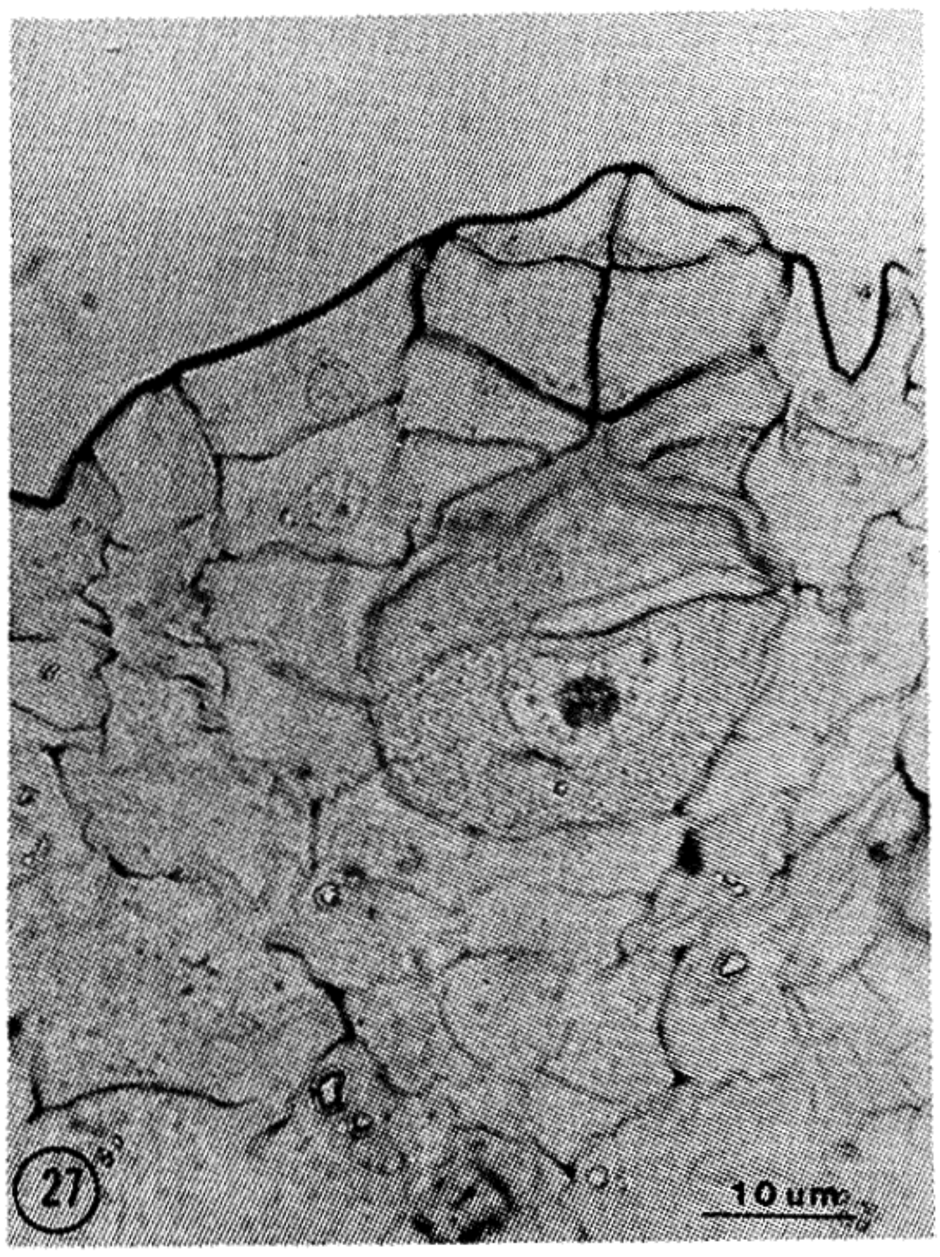
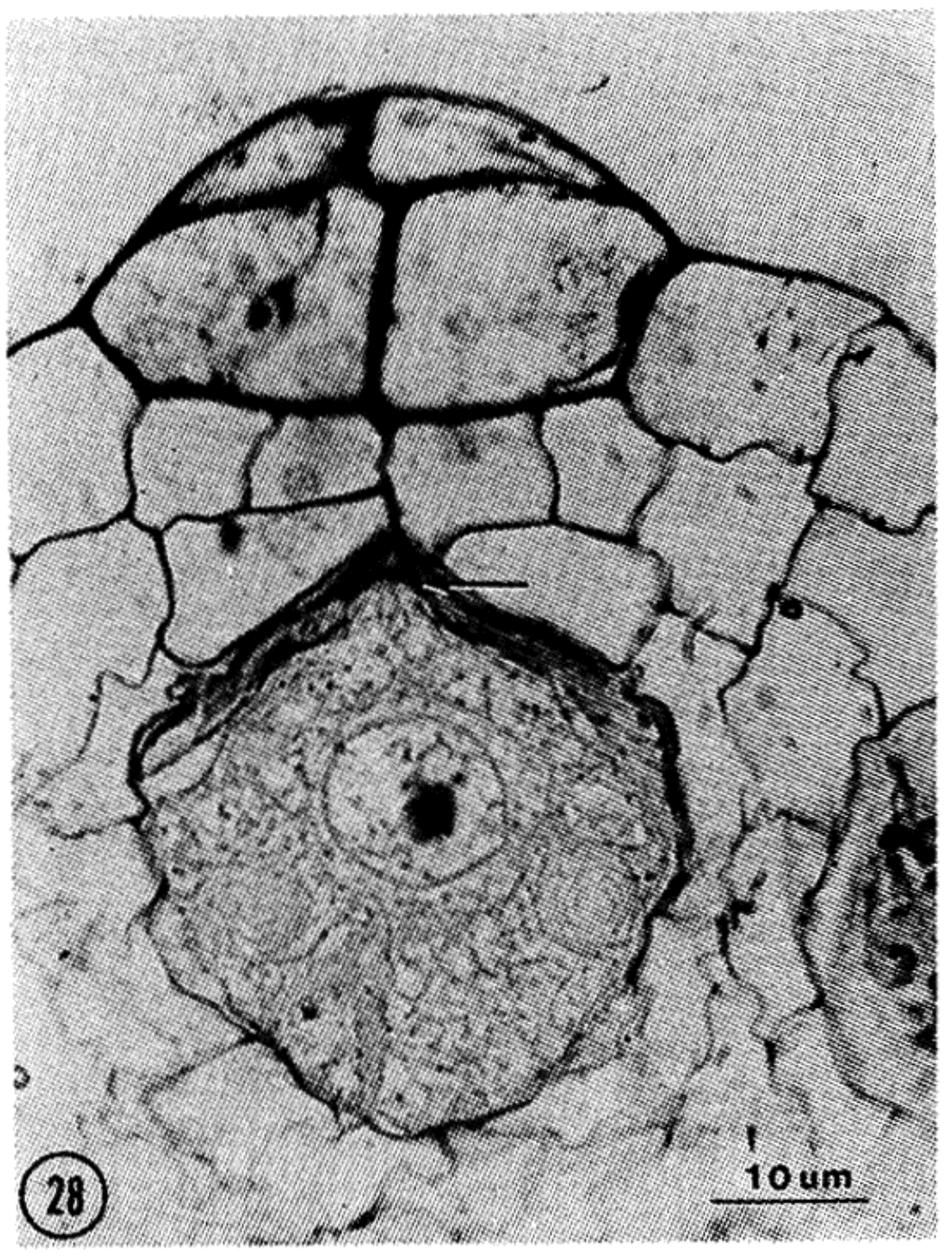
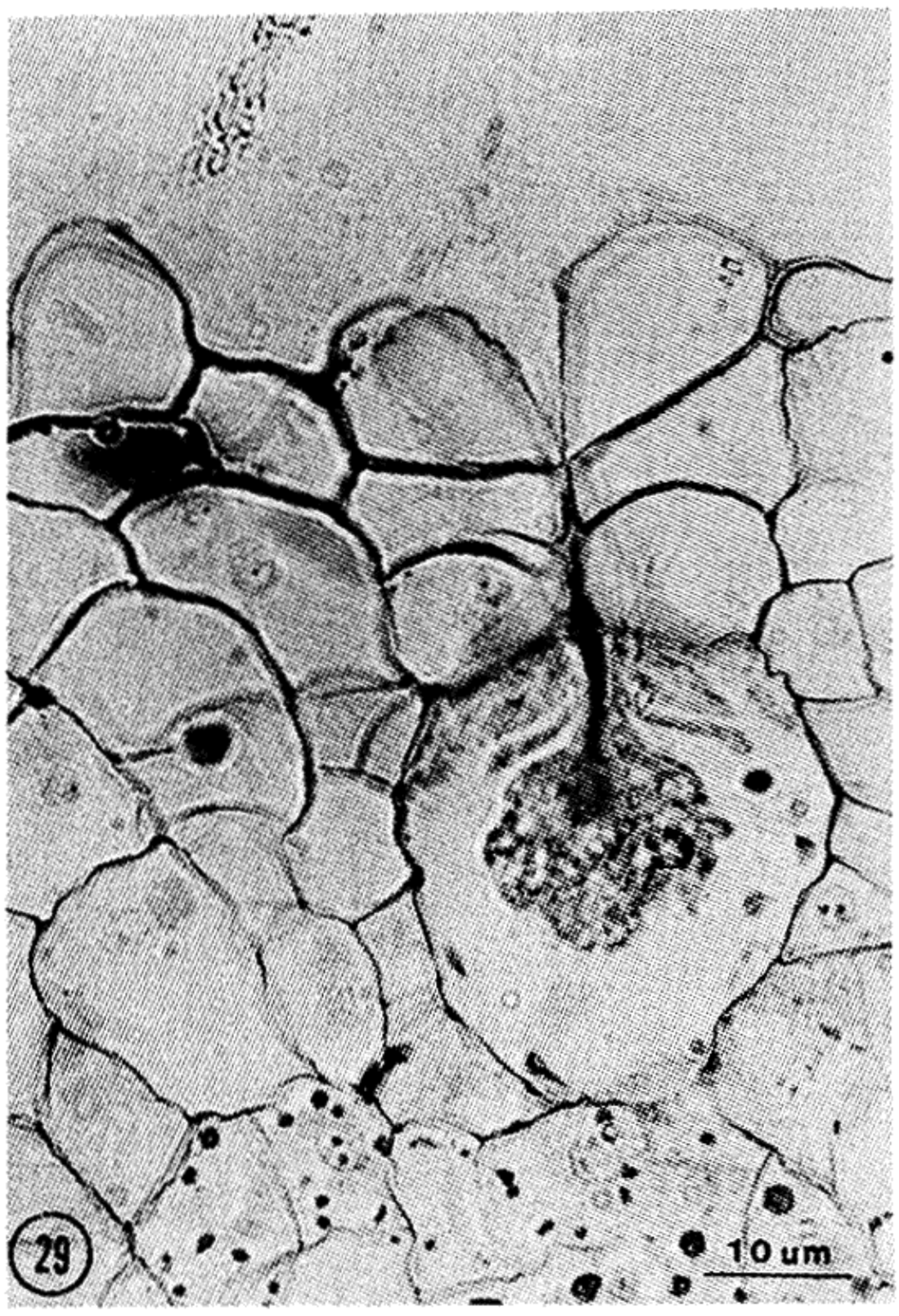


Fig. 23: Arrows indicate the periclinal divisions of neck cells into four tiers of neck cells.
Fig. 24: A well developed archegonium with binucleate neck canal cell (arrow).
Fig. 25: Median section through a well developed archegonium.
Fig. 26: Neck canal cell and ventral canal cell are in the process of disintegration.







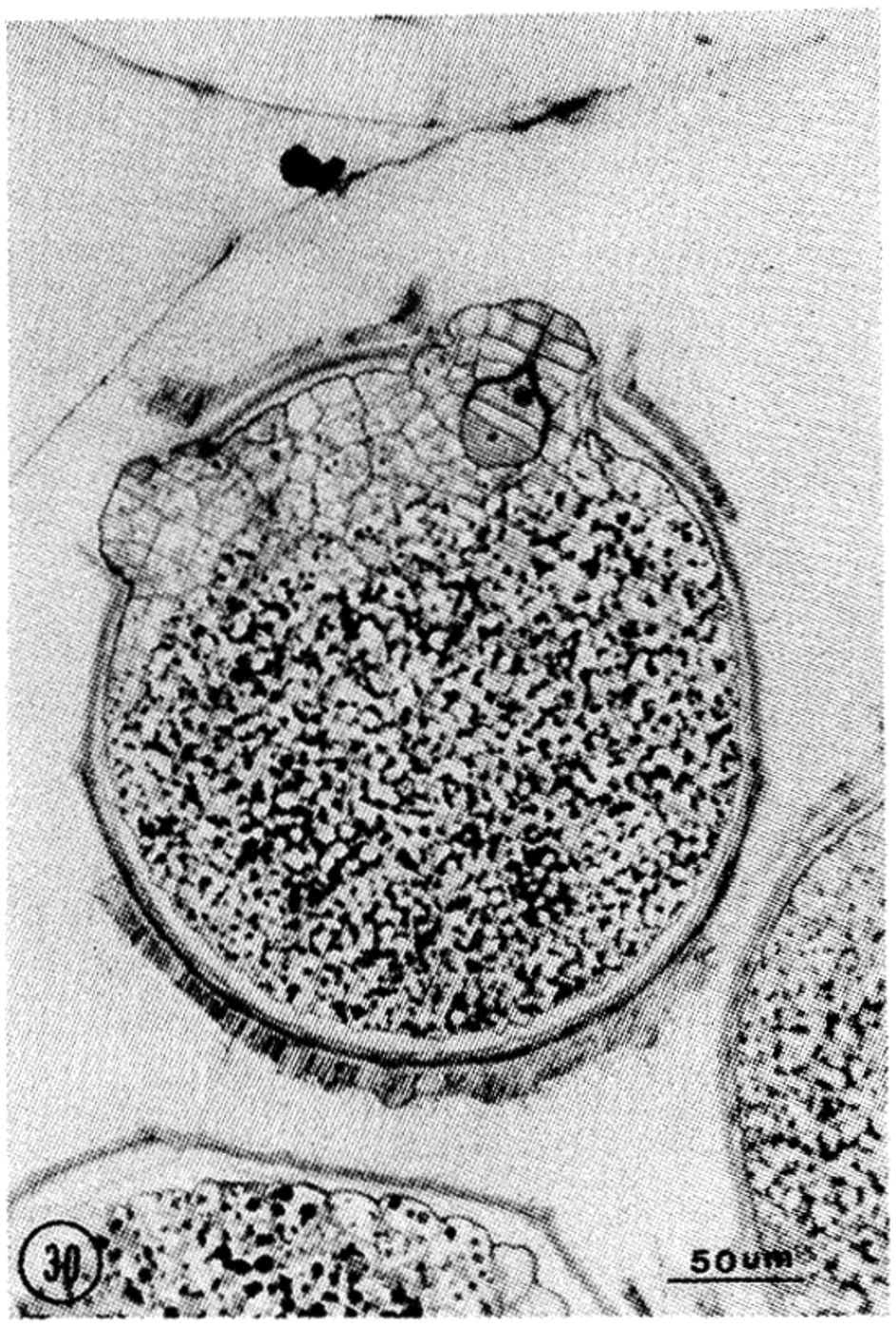
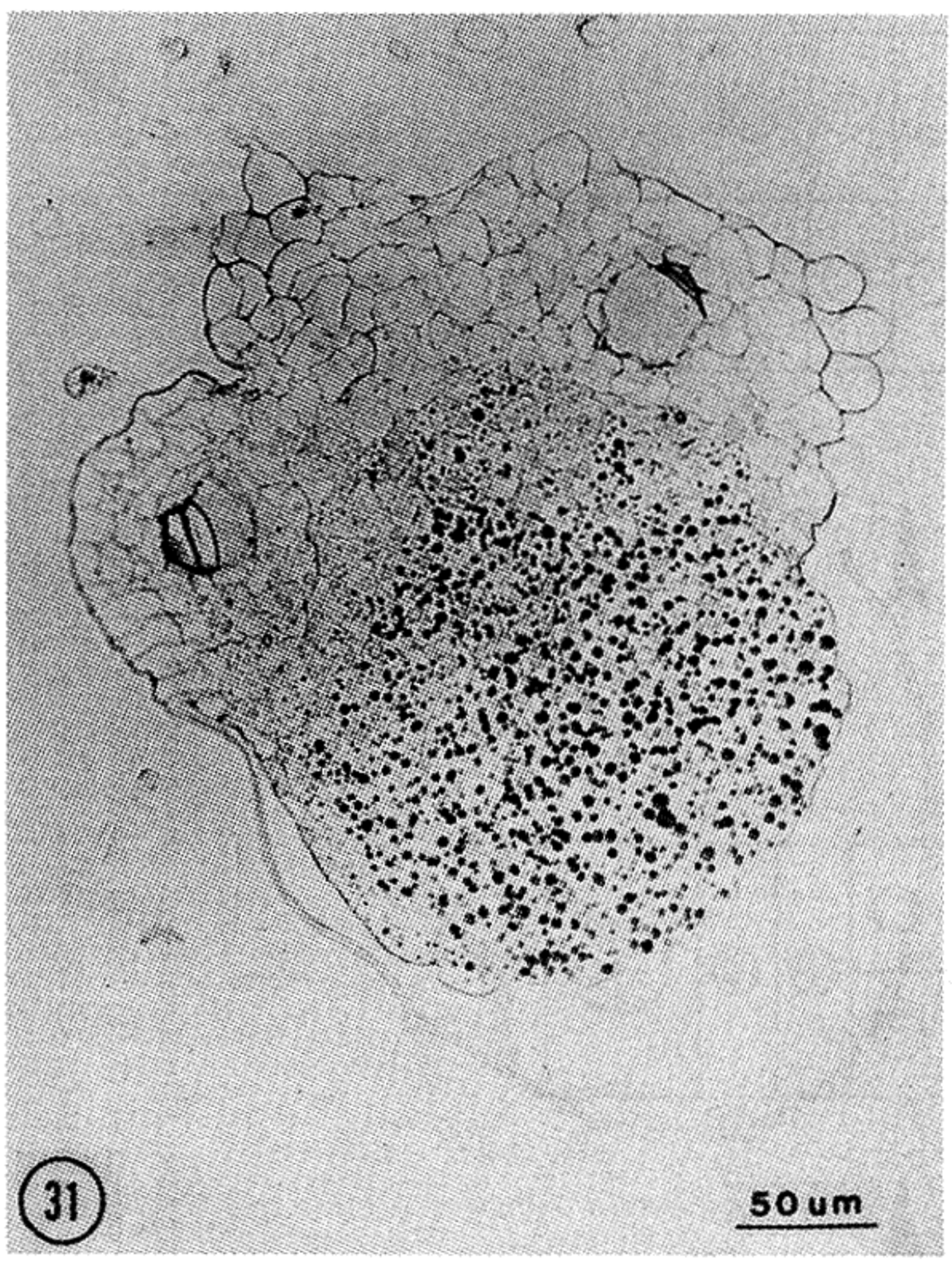
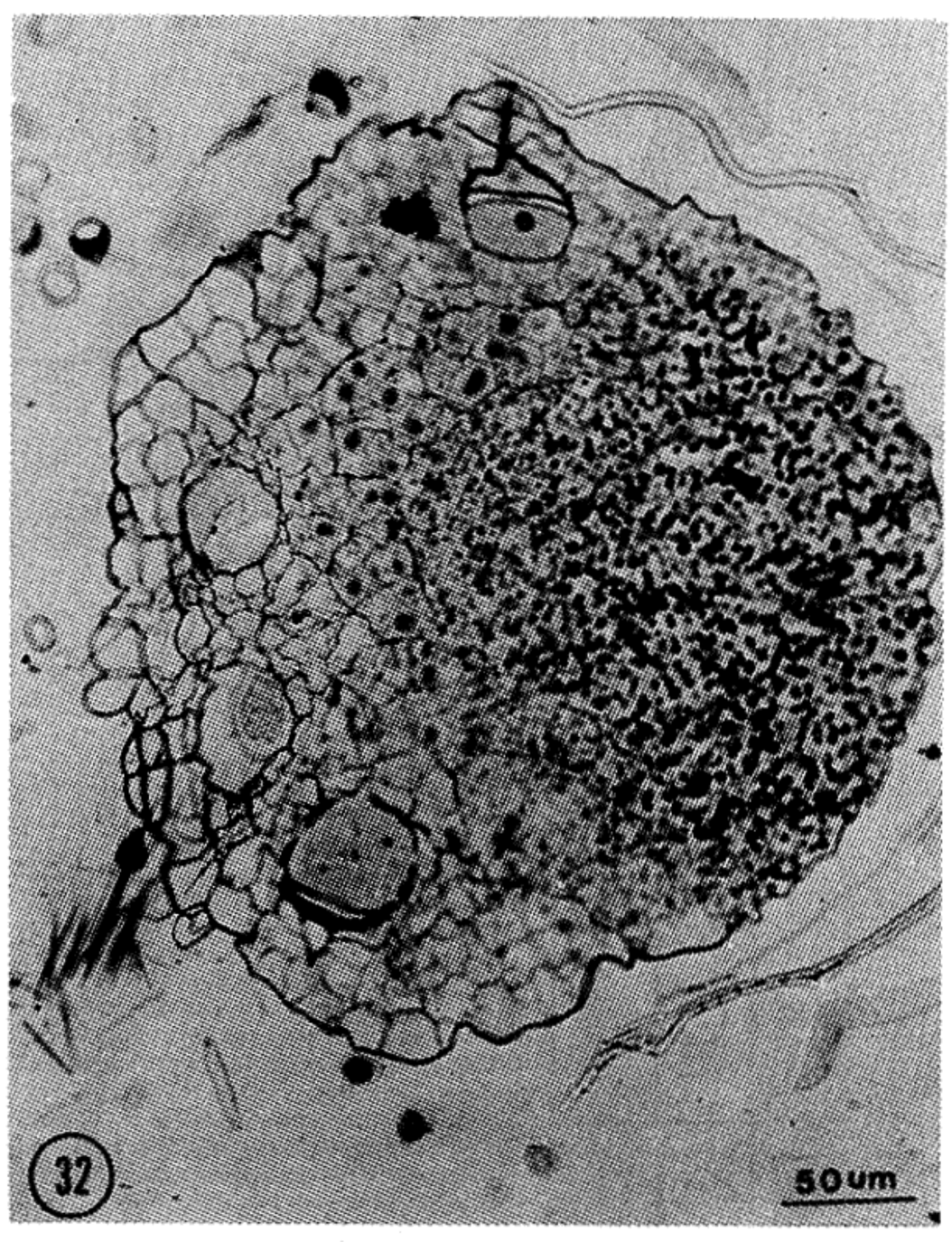


Fig. 27: An archegonium after the degeneration of neck canal cell.
Fig. 28: Mature archegonium after neck canal cell and ventral canal cell have disintegrated.
Arrow shows the receptive spot in mature egg.
Fig. 29: The collapse of unfertilized egg.
Fig. 30: Showing the early formed archegonia are located beneath the triradiate intra-crack.





Figs. 31-32: Sectional view of mature female gametophytes.

31: Note the difference in the amount of food particle and the basal region.

32: A poly-archegonia bearing gametophyte.

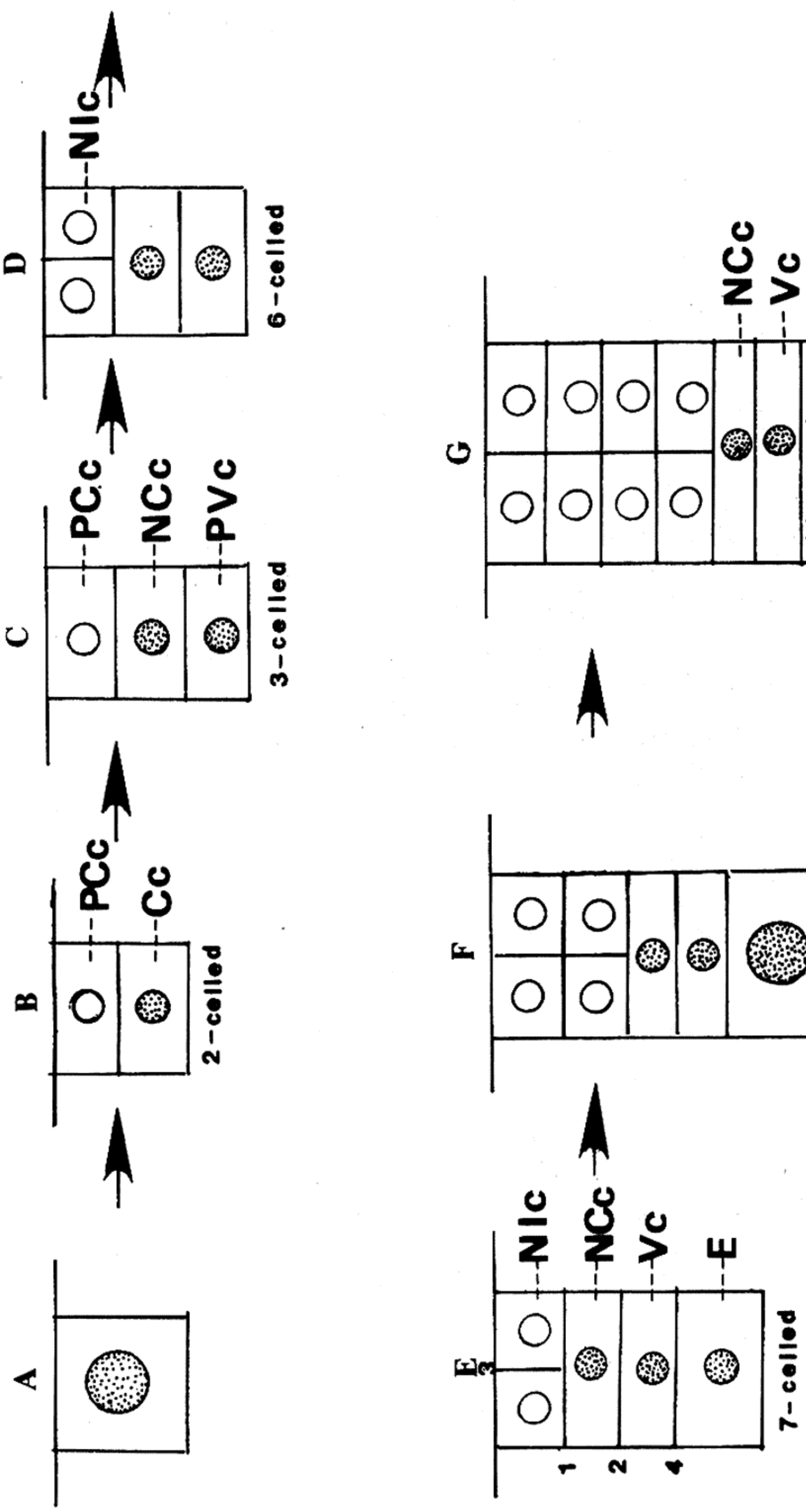
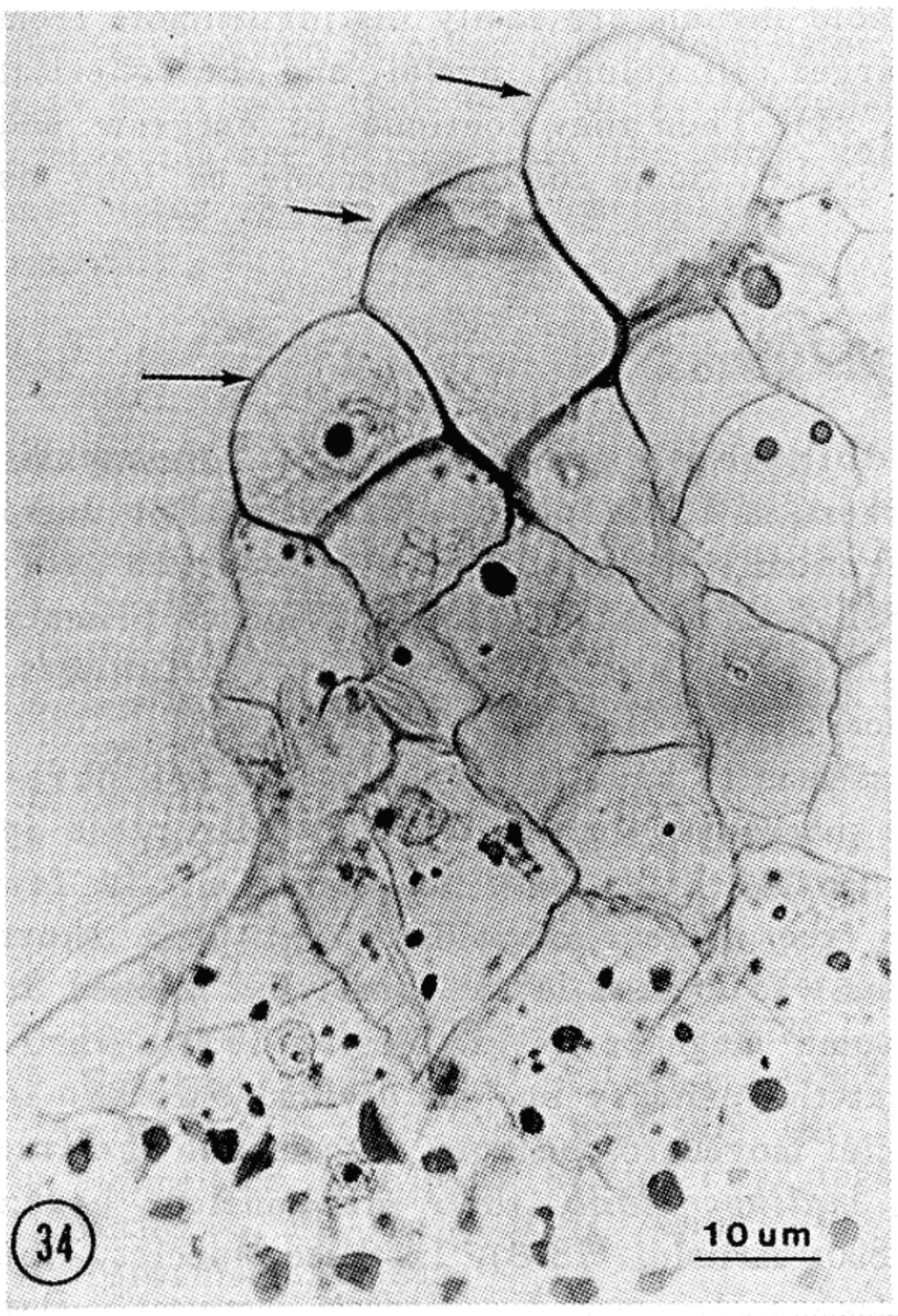
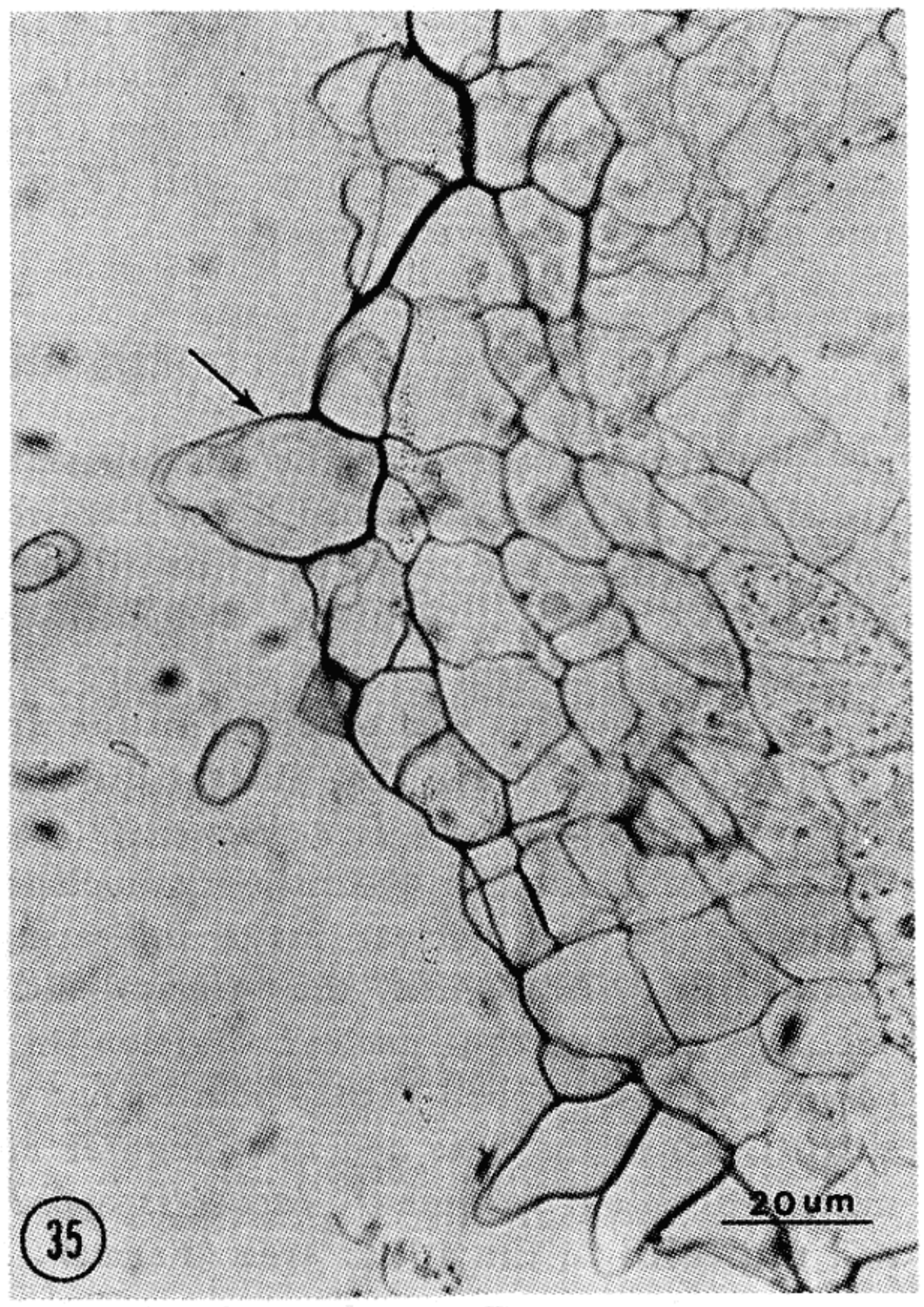
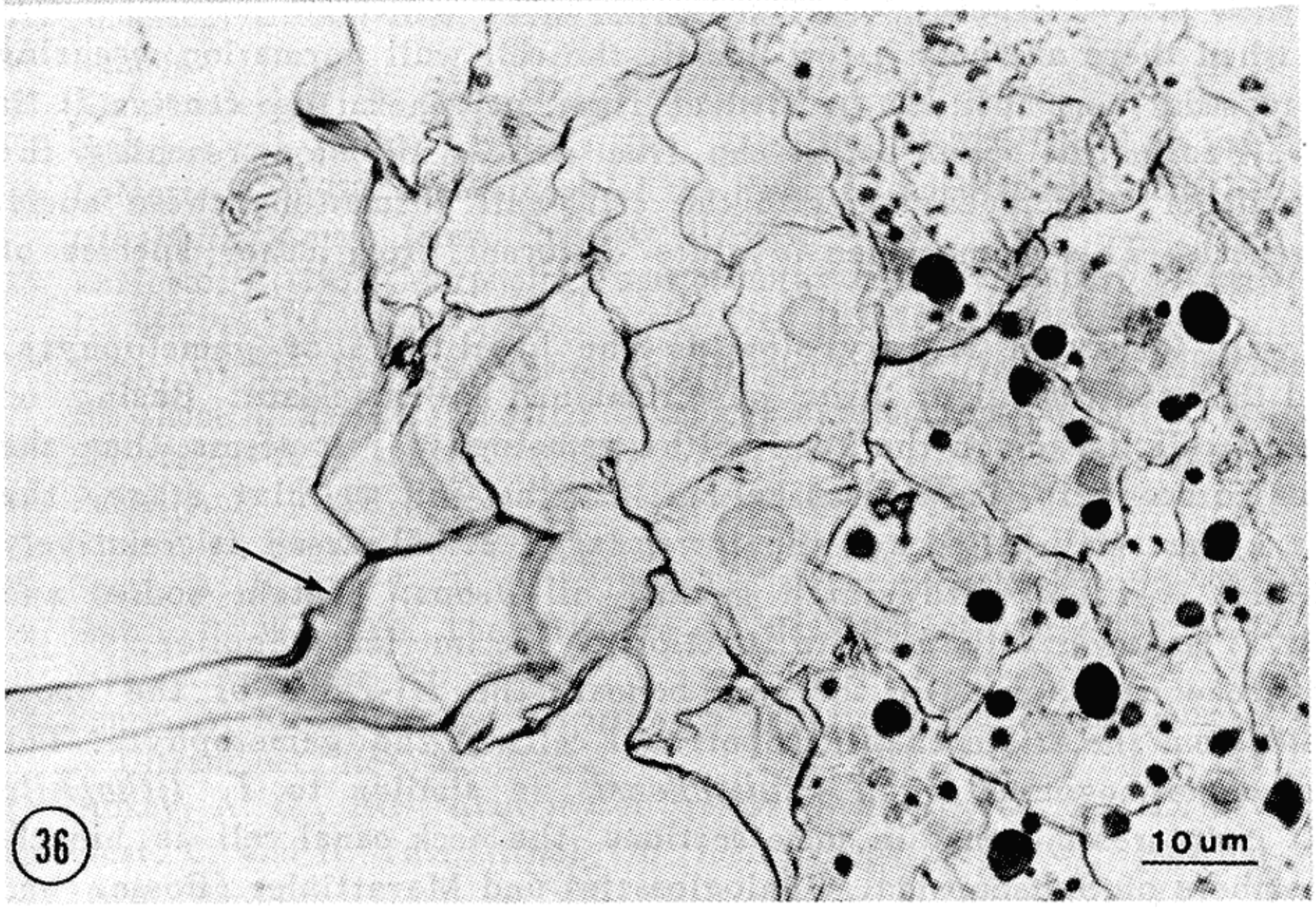


Fig. 33: Schematic illustrations showing the stages in development of archegonium. Cc: Central cell; E: Egg; NCc: Neck canal cell; NIc: Neck initial cell; PCc: Primary cover cell; PVc: Primary ventral cell; Vc: Ventral cell.







Figs. 34-36: Formation of rhizoids.
34: The rhizoid initials (arrows) occur from the superficial cell in the apical region.
35: The outgrowth of rhizoids (arrow).
36: The rhizoid bulges at the base (arrow).

above, the superficial cells in the apical region exhibit lighter in cell contents than that in the lower region (Fig. 31). The rhizoid initials are easily distinguishable from adjacent superficial cells by their slightly larger cell volume, nuclei, and the spherical extended protrution of the cell surface (Fig. 34). Then they continue to outgrow and emerge from the crack of spore wall (Figs. 5, 35). Some rhizoids become as long as one millimeter, or even longer.

DISCUSSION

The general courses of the development of the female gametophyte in *I. taiwanensis* are similar to those described by earlier workers in other species of *Isoetes*. But some detailed patterns are observed to be different from some of them. As reported in *I. echinospora* and *I. lithophila* (Campbell, 1891; LaMotte, 1933), the position of primary nucleus in the present species was found in the basal area. Whereas in *I. lacustris*, the primary nucleus was observed in the apical region (Farmer, 1890).

The location of the free nuclear division seems to have a morphological and physiological importance. In *I. taiwanensis*, the subsequent nuclear divisions take place in the apical region after the primary nucleus moving from base to apex. Only a few sections show free nuclear stage indicating free nuclear division might be a short process. In *I. lithophila*, the primary nucleus was located in the base, but moved to the apex prior to the nuclear division in some megaspores (LaMotte, 1933). In *I. echinospora*, Campbell (1891) pointed out that the primary nucleus divided only two to three times in the base and the daughter nuclei moved to the apex to continue the free nuclear division. In both *I. lacustris* and *I. malinverniana*, their free nuclear began and proceeded in the apical area (Farmer, 1890; Arnoldi, 1896). In these species, the cell wall formation does not occur until the free nuclei reachs a certain amount. But in *I. taiwanensis*, when there are only a few nuclei, the cell wall formation occuring around the nuclei proceeds from apex to base, and from peripheral to center. It is apparent that the wall formation starts before the free nuclear division reaching the maximum original number. Accompanying with the cell wall formation, these nuclei still proceed karyogenesis. This phenomenon is quite different from other species of *Isoetes*.

The process of wall formation is very slow in the basal part of gametophyte. Sometimes we found that the central or lower part remained multinucleate. Basing on the compact pattern of cell lineage in the apex of the gametophyte, it seems that the cell division continue to occur in the apical region. In the full cellular stage, the border between the upper small celled and the lower large celled areas is relatively sharp. The basal region is filled with starch grains, oil drops, protein bodies and other food materials. The lower cells seem to function as the nutrient supplier.

In general, the archeqonia appear in the apex beneath the tri-ridge of the spore wall. They originate from the triate shaped superficial cells of the gametophyte. The overall development of archegonium in *I. taiwanensis* is similar to *I. lithophila* (LaMotte, 1933). It is interesting that in some sections, the neck canal cell is binucleated as in some members of Sphenopsida, Ophioglossales and Marattiales (Foster and Gifford, 1974). The mumber of archegonia formed in a gametophyte is not constant. Before development of the first archegonium is completed, the female gametophyte may continue to produce more archegonia till the supply of food in the spore becomes exhausted or fertilization occurs. Whether or not all superficial cells of the gametophyte are potential archegonial or rhizoidal initials is not certained.

Although it was reported that the fertilization in a given archegonium inhibited the fertilization in other ones (Hofmeister, 1862), ferililzation in a single gametophyte may occur more than once in ***I. taiwanensis***. Sometimes, two embryos in separate archegonia are found within the same gametophyte. It seems that poly-fertilization may occur in the same gametophyte for ***Isoetes***, but only one embryo can reach maturity.

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臺灣水韭的雌性配子體發育

黃淑芳　江蔡淑華

摘　　要

本篇描述臺灣水韭（*Isoetes taiwanensis* DeVol）的雌性配子體發育和藏卵器生成過程。在早期發育，成熟孢子的核由基部移到上端，並在上端進行多次核分裂。只分裂至十數個核時，其核四周開始細胞壁之形成，細胞壁形成次序是由上向下，由四周向中心。隨同細胞壁形成，這些核仍不斷進行核分裂。所以，在發育完全的配子體，仍可看到多核現象。在一發育完全的配子體中，其上部細胞較小，而底部細胞較大，二者的差別非常顯著。其底部較大的細胞通常富含澱粉、蛋白質、油脂之類物質。藏卵器通常位於孢子壁開裂口下，起源自早期形成的頂部表面細胞，每一配子體生成藏卵器的數目不定，約5～20個左右，假根也來自配子體的表面細胞，通常在孢子壁開裂後露出的配子體部分生成，長可達1公分左右。配子體由孢子壁向外生長時期，大多在十二月至次年二月，顯示配子體的成熟需一較低溫度。