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Electron microscopic study of cell division and reproductive differentiation in male branches of Dasya baillouviana (Gmelin) Montagne (Rhodophyta)

Dawn Phillips Sigfred
College of William & Mary - Arts & Sciences

ELECTRON MICROSCOPIC STUDY OF CELL DIVISION AND REPRODUCTIVE DIFFERENTIATION IN MALE BRANCHES OF *DASYA BAILLOUVIANA* (GMELIN) MONTAGNE (RHODOPHYTA)

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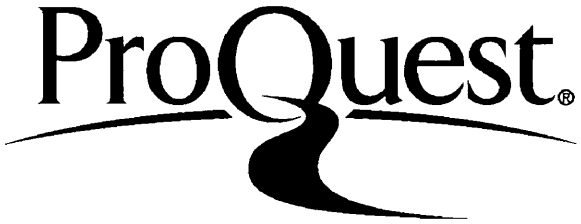

APPROVAL SHEET

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Dawn Phillips Siffred
Author

Approved, April 1980

Joseph L. Scott
Joseph L. Scott

Stanton F. Hoegerman
Stanton F. Hoegerman

Lawrence L. Wiseman
Lawrence L. Wiseman

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ABSTRACT

The purpose of this study is to describe the ultrastructure of cell division and gametogenesis in the male branches of Dasya baillouviana, a marine red alga.

During cell division, the nuclear envelope remains intact with the exception of polar fenestrations. Two cylindrical nucleus associated organelles, or polar rings, are found adjacent to the nucleus. During prophase, opposing spindle poles are established by polar ring migration. By the end of prophase, the nucleus is ensheathed by a highly organized array of microtubules external to the nuclear envelope. During prometaphase several layers of perinuclear endoplasmic reticulum develop around the nucleus and persist throughout mitosis. The perinuclear microtubules disappear during prometaphase. Metaphase is characterized by the alignment of well condensed chromosomes into an equatorial plate. Distinct kinetochores and both chromosomal and non-chromosomal microtubules are present. While the chromatids separate during anaphase, vacuoles begin to coalesce around the interzonal spindle. Two daughter nuclei are formed at telophase following dehiscence of the interzonal spindle. The vacuoles coalesced between the daughter nuclei keep them apart until cytokinesis is effected by a cleavage furrow which develops centripetally. The process of cytokinesis is completed by the formation of a pit connection between the two daughter cells.

Differentiation of a spermatangial cell into a spermatium involves lateral migration of two polar rings from their post-mitotic apical position on the spermatangial nucleus to opposite sides of the nucleus. This process is accompanied by proliferation of microtubules which ensheath the nucleus and persist through spermatial release. Formation of basal vacuoles begins with markedly enlarged endoplasmic reticulum, followed by aggregation of mitochondrial-dictyosome associations into a cup-like array immediately subjacent to the nucleus. Dictyosome activity then supplants that of endoplasmic reticulum in basal vacuole formation. The spermatangium is transformed into a spermatium when the contents of the vacuoles are secreted basally. Following reformation of the plasmalemma, the spermatium is no longer attached to its spermatangial mother cell by a pit connection. Spermatia are released from the plant by ruptures in the wall of the male branch. Chloroplasts were very rarely observed in either mother cells or spermatia.

ELECTRON MICROSCOPIC STUDY OF CELL DIVISION AND REPRODUCTIVE DIFFERENTIATION IN MALE BRANCHES OF *DASYA BAILLOUVIANA* (GMELIN) MONTAGNE (RHODOPHYTA)

INTRODUCTION

Cell division has long intrigued biologists. Electron microscopists have done much to elucidate this process, resulting in voluminous literature (see 5,6,19,22-24,27,33,35,36,43,44,49,53,66,67, 69,70,73,84 for selected reviews). Apart from interest in the process, features of cell division have been used to clarify taxonomic relationships. This approach has been particularly successful in the green algae, and has resulted in substantial alteration of opinion regarding the ancestry of land plants (66,69,72). However, cell division in two important groups, the red algae and the brown algae, has not been extensively studied.

The red algae, Division Rhodophyta, are distinguished from other algal groups by three characteristics. First, they contain the photosynthetic pigments phycocyanin, phycoerythrin, and chlorophylls a and d, the latter being unique to the division. Second, cilia, flagella, and centrioles have never been observed. Third, their reserve product, floridean starch, has been found in no other group. In addition, in most species a structure known as a pit connection, which is actually a membrane enclosed cytoplasmic plug, is formed between daughter cells at the end of cytokinesis. Most members of the group are marine.

There are two classes in the Rhodophyta. The Bangiophyceae includes unicells and simply organized multicellular thalli. The

more advanced Florideophyceae are multicellular plants with complex anatomies and life histories. The subject of this study, Dasya baillouviana, is a member of the most advanced order in the Florideophyceae, the Ceramiales, and has a Polysiphonia-type life history (16,87). Four plants are involved: male gametophytes, female gametophytes, tetrasporophytes, and carposporophytes. The first three are free-living and morphologically similar to one another. The fourth is parasitic upon the female gametophyte. Spermatia, produced mitotically by male plants in specialized reproductive branches, are carried passively by water currents to female plants. Fertilization follows adhesion of a spermatium to the trichogyne, an extension of the female gamete. The resulting zygote is retained upon the female plant and develops into a diploid carposporophyte which produces carpospores by mitosis. These are released and germinate into tetrasporophytes which produce tetraspores by meiosis. The tetraspores are released and germinate into haploid gametophytes, completing the sexual life cycle.

Red algae are generally considered to be one of the most primitive groups of eukaryotes. This judgement has been based primarily upon their pigmentation, which has much in common with that of blue-green algae, their simple chloroplast structure, and the complete absence of cilia, flagella, and centrioles. The lack of flagellation has been questioned by one worker (81,83), but her fixation quality was poor, and the work has been discounted repeatedly (88). Should flagellation ever be confirmed, which seems unlikely, taxonomic revisions would undoubtedly ensue.

Phylogenetic schemes based upon the primitiveness of red algae

have long been constructed without the benefit of detailed information concerning mitosis. Light microscopists have been hampered by a number of factors including small nuclear size (2-4 μm diameter) and thick cell walls. Very few electron microscopists have observed mitotic figures. Isolated stages of mitosis (11,61,63,64,76), meiosis (37,62, 65,79), and cytokinesis (13,26,75) at the ultrastructural level have been reported, but there is only one detailed account of mitosis in the literature to date. McDonald examined vegetative divisions in gametophyte germlings of Membranoptera platyphylla (48), a florideophycean alga. As had Peyriere in an earlier study on Griffithsia (63), McDonald noted a type of spindle pole body, for which he suggested the term "polar ring."

There are several possible reasons for the paucity of ultrastructural literature about mitosis in the red algae. Division apparently occurs very rapidly, and information about when it occurs in nature is scanty and conflicting (3,4,16,20,74). Red algae are difficult to fix for electron microscopy. In addition, there are very few workers in this field, and their selection of material has often not optimized the probability of observing dividing cells.

This study exploited the fact that hundreds of mitotic divisions occur in the compact spermatangial branches of male Dasya plants. These reproductive structures are small enough that one entire branch can be examined in a single thin section, thus making observation of many potentially mitotic cells very convenient. This, in conjunction with the fortuitous discovery during previous work in this laboratory of a method of inducing division in up to 50% of these cells in other red algal species (10,80), made possible the examination of many

division figures.

Spermatia, which are the ultimate result of the mitotic divisions in the fertile branches of male plants, are produced by the direct transformation of spermatangia. This process and the resulting spermatia have been studied in a number of species both with light microscopy (18,21,28) and electron microscopy (12,14,38,63,64,78,81-83). Because some of these reports are conflicting, several unresolved questions remained, including the fate of chloroplasts, origin of spermatangial vacuoles, arrangement of microtubules, and the possible breakdown of the spermatial nuclear envelope. In addition, previous reports have not described the behavior of polar rings in spermatangia. Since spermatial nuclei are generally regarded as being in a condition resembling late prophase or prometaphase, documentation of this aspect of differentiation is of interest.

MATERIALS AND METHODS

Specimens of Dasya baillouviana were collected at low tide from the York River a few hundred meters west of the York River Bridge at Yorktown, Virginia, in the fall of 1977 and summer of 1978. Both free-floating and attached plants were taken and either fixed immediately or transported to the laboratory in river water and maintained in culture incubators prior to fixation.

Male plants were grown in either 0.45 μm Millipore filtered river water or von Stosch's culture medium (85) diluted 1:1 with filtered river water. The former were aerated constantly and the medium was changed daily; the latter were incubated without aeration and medium changes. In both cases, Corning Pyrex No. 3250 deep storage dishes were used. The algae were acclimated for 2-3 days in Percival culture incubators at 21° C, light intensity 100 ft-c and a light-dark cycle of either 12:12 or 14:10 depending upon natural conditions at the time of collection.

Fixations were begun 2 hrs after the beginning of the light cycle because previous work in this laboratory using this method with two species of Polysiphonia (10,80) resulted in considerable numbers of dividing cells. Whether in the field or laboratory, the specimens were fixed in 2% glutaraldehyde for 1½-2 hrs at ambient temperature. Following three 15 min buffer rinses the specimens were post-fixed in 1% OsO_4 for 1½-2 hrs at room temperature. 0.1 M Sorensen's phosphate

buffer (31) (pH 6.6) with 0.15-0.25 M sucrose added to adjust osmolarity was used during fixation. After brief rinses in 50% and 70% acetone at room temperature, 1% uranyl acetate in 70% acetone was used as an en bloc stain for 12-20 hrs at 4° C. Dehydration was completed in a graded acetone series ending in 100% acetone which had been stored over molecular sieves. The tissue was infiltrated with 1:2, 1:1, and 2:1 mixtures of Epon 812 epoxy resin and acetone. After 2 days in pure resin with changes at least once a day, the material was flat-embedded in plastic petri dishes and polymerized at 60° C for 3 days. Serial thin sections were cut with a Dupont diamond knife on an LKB III ultramicrotome, collected on one hole slot copper grids coated with 1% formvar (25), poststained with either Venable and Coggleshall's lead citrate (86), or Sato's lead citrate (31), and examined and photographed with either a Zeiss EM 9S-2 or a Hitachi HU-11B-1 electron microscope. Thick (0.2 μm) sections of the epon-embedded material were cut with a Dupont diamond on an LKB I ultramicrotome, flattened with 10% acetone (29), and stained with toluidine blue. Both live and thick-sectioned material were examined with a Zeiss Photomicroscope II and photographed on 35 mm Panatomic X film.

OBSERVATIONS

Dasya baillouviana (Fig. 1) can be collected in early summer and fall from local sublittoral waters. Male thalli are consistently the least numerous. During a previous study in this laboratory (30) mitotic figures were not observed in field fixed material. In the course of this investigation, only one mitotic figure was seen in unaerated incubated plants. These plants frequently died within 48 hrs of collection. Aeration proved to be necessary for maintenance of Dasya in the laboratory. Fixation 2 hrs after incubator "sunrise" disclosed dividing cells. Mitotic figures were also found in a plant fixed in the field late in the day; nevertheless, incubated material was found to be more reliable.

A reproductively active male plant (Fig. 2) bears many spermatangial branches in various stages of development; each branch produces hundreds of spermatia. A reproductive branch is initiated when an apical cell undergoes a series of basipetal divisions, resulting in a column of axial, or central, cells (Figs. 3-5). Several of the axial cells at the tip of the branch and two at the base remain uniseriate (Fig. 4). The intervening cells of the filament divide laterally to produce a ring of five pericentral cells which are characteristically cut off in a clockwise manner. The pericentral cells can function as spermatangial mother cells (SMCs), or they can cut off pericentral derivatives which act as mother cells;

the derivative cells can also produce more mother cells. Each mother cell can produce at least three spermatangia (Figs. 5, 6). A spermatangial cell does not divide; its protoplast is eventually released as a spermatium.

This study encompasses two subjects: first, the mitotic divisions which occur in the cells of the male reproductive branches, and second, the developmental sequence which transforms a spermatangial cell into a spermatium.

Interphase

Interphase nuclei (Figs. 7-10) are essentially spherical, centrally located, with dispersed heterochromatin and a conspicuous nucleolus. In mature central cells (Figs. 7-9), two "nucleus associated organelles" (NAOs, 27), or polar rings (PRs), as they have been termed in the higher forms of red algae (48), are found very near one another and in close proximity to the nuclear envelope. There are no associated microtubules, and no zones of exclusion (areas free of ribosomes and packed with microtubules and/or microtubule precursors). The vacuolated cell shown in Fig. 7 has finished dividing.

The PRs in Dasya are quite similar in morphology to those found in Membranoptera (48) and Polysiphonia (10,80). They are short, hollow, electron dense cylinders with a diameter of 120-140 nm and a height of 35-45 nm. Their orientation is such that the axis of the cylinder is perpendicular to the nuclear envelope. In transverse section the ring-like nature of the organelle is immediately apparent (Fig. 11). In longitudinal section (Fig. 12) a distinct groove on the inner surface of the cylinder is apparent. In tangential section

(Fig. 13) the cylinder appears to be composed of two stacked rings. In some sections, struts attaching the base of the cylinder to the outer membrane of the nuclear envelope are seen (Figs. 12,13,15).

In young SMCs, very few vacuoles are present in the cytoplasm. Typical red algal chloroplasts, floridean starch, and pit connections (17) are seen, as are the mitochondria-dictyosome (Golgi apparatus) associations which have been previously reported in red algae (77). Ribosomes and endoplasmic reticulum (ER) are found throughout the cytoplasm.

Prophase

One of the most striking events of prophase is PR migration (Figs. 15,16). Based upon other studies of mitosis in this laboratory (10,80), it appears that one pole is first established by nuclear rotation. Following development of zones of exclusion, migration of one of the PRs to the opposite side of the nucleus establishes the second pole. During migration, struts attaching the PR to the outer nuclear envelope are especially apparent (Fig. 15). As migration progresses, perinuclear microtubules form an extranuclear spindle. Whether or not these microtubules are continuous from pole to pole was not determined. The spacing of these microtubules is fairly regular, both with respect to one another and to the nuclear envelope (Figs. 17,18).

Several layers of perinuclear endoplasmic reticulum (PER) are then formed and persist throughout the remaining stages of mitosis. The outer membrane of the nuclear envelope is involved in synthesis of at least some of the PER (Fig. 18). The nucleus assumes an elliptical

shape; the degree of elongation varies with cell type and is greatest in SMCs. The nuclear envelope does not break down, but nuclear pores become very numerous at the poles, with the conspicuous exception of the membrane immediately beneath the PRs. During late prophase, slight plateaus in the nuclear envelope beneath the PRs are formed (Fig. 14). The nucleolus remains intact.

Prometaphase

The nuclear envelope during prometaphase becomes flattened and fenestrated at the poles (Figs. 19,20). Microtubules were frequently observed penetrating the nucleoplasm through the polar fenestrations. Chromosomal condensation is nearly complete and the development of randomly oriented indistinct semicircular kinetochores can be discerned (Fig. 21). The nucleolus has dispersed and the perinuclear microtubules which were formed during prophase have disappeared.

Metaphase

Metaphase is characterized by completely condensed chromosomes aligned on an equatorial plate. Distinct pairs of kinetochores are oppositely oriented toward the poles (Fig. 22). Chromosomal microtubules still can be seen extending through the polar fenestrations; non-chromosomal microtubules are also present in the nucleoplasm. The nucleus is spindle shaped, bulging equatorially with marked polar flattening. Both PRs have now separated into proximal and distal halves, each remaining in the form of a ring (Figs. 22-24).

Anaphase

Only mid-anaphase was observed (Fig. 25). At this time, the

nucleus is dumbell shaped. The chromosomes are approximately the same distance from the poles as during metaphase. Polar fenestrations are still present. Vacuoles have begun to coalesce around the interzonal spindle.

Telophase

By the beginning of telophase, the chromosomes are at opposite ends of the nucleus with their kinetochores adjacent to the nuclear envelope. Following dehiscence of the interzonal spindle and nuclear envelope reformation, the daughter nuclei remain separated by the vacuoles which have coalesced between them (Fig. 26). As the chromosomes begin to disperse, kinetochores become less distinct. Remnants of the interzonal spindle break down quickly, while those of the PER persist into cytokinesis.

Cytokinesis

In the early stages of cytoplasmic division cleavage furrows grow centripetally into the vacuoles coalesced between the daughter nuclei (Figs. 27-30). This process is uneven. As a result, the furrows are not seen in every section. No microtubules or microfilaments are seen associated with the developing furrows, although ER is always seen adjacent to them (Fig. 30). Nucleolar reformation is noticeable. No tendency for SMC chloroplasts to be located only basally with respect to the SMC nucleus was noted. Nevertheless, chloroplasts were very rarely found partitioned into spermatangia.

The last event of cytokinesis is pit connection formation. These structures connect the two cells, but prevent cytoplasmic communication

between them (Fig. 31). The process of pit connection formation has been described previously (1,9,75) and was not explored in this study.

Spermatangial Differentiation

Spermatangia constitute the outermost cell layer in a mature reproductive branch. Young spermatangia are ellipsoidal cells, each attached to its SMC by a pit connection. ER is the first cytoplasmic component to undergo noticeable differentiation. The basal half of the cell is soon filled with cisternae expanded by their granular contents (Fig. 31). ER extends around the apically situated nucleus as well.

Two PRs are found in close proximity to the nuclear envelope in young spermatangia (Fig. 32). They migrate away from each other and the former pole toward opposite sides of the nucleus (Figs. 32, 34). During migration a zone of exclusion develops around each PR. Numerous nuclear pores are distributed throughout the nuclear envelope.

An unusual cup shaped aggregation of mitochondria-dictyosome associations develops immediately subjacent to the nucleus and partially surrounds it (Figs. 33, 34). The dictyosomes contribute the clear contents of their vesicles to the basal vacuoles (Fig. 35, arrow), supplanting ER as the most active secretory organelle in the spermatangium at this time. The basal vacuoles eventually comprise the greater part of the spermatangium.

By the time vacuole formation is complete, the PRs are located 90° from the former pole, at opposite sides of the nucleus (Figs. 36, 37). The PRs are no longer attached to the nuclear envelope and have moved slightly away from it. The nucleus is flattened beneath each

broad zone of exclusion and nuclear pores have become concentrated in this area. Perinuclear microtubules radiating from the poles ensheath the nucleus (Fig. 38). These microtubules are regularly spaced with respect to one another and to the nuclear envelope. Whether or not these microtubules are continuous was not determined. The spermatangium is transformed into a spermatium when the contents of the distended vacuoles are secreted basally (Figs. 39, 40). Following reformation of the plasmalemma, the young spermatium is no longer attached to its SMC by a pit connection. The spermatium's shape is at first somewhat distorted by the contents of the secreted vacuoles, but its ultimate form is spherical or ovoid.

The spermatial nucleus is in a condition resembling late prophase (Figs. 39-44). Marked chromatin condensation is evident and the nucleolus has dispersed. The extranuclear sheath of microtubules which formed during PR migration persists at least through release of the spermatia from the plant, but no evidence of nuclear envelope breakdown is seen.

As noted previously, chloroplasts are not usually incorporated into spermatangia, and are therefore seen only rarely in spermatia (Fig. 41). In some instances where chloroplasts were seen, they were surrounded by ER and the interior of the chloroplast appeared digested (Fig. 42). Some spermatial mitochondria were observed in a similar condition (Fig. 44).

The spermatia are ultimately released from the plant by ruptures in the wall of the reproductive branch (Fig. 43).

DISCUSSION

Through the use of laboratory acclimated material the environmental variables imposed by nature were eliminated, and some degree of mitotic synchrony, presumably mediated by photoperiod, was achieved. Over a hundred dividing cells were seen, most of which were in prophase. The subsequent stages of mitosis are evidently very rapid. Approximately three cells in prometaphase, ten in metaphase, one in anaphase, two in telophase, and two during cytokinesis were seen.

Significant differences from the only detailed published account of mitosis in a florideophycean alga (48) were found during the course of this investigation. The inconsistencies noted, however, are believed to be due to insufficient observation and/or misinterpretation in the previous study, rather than to real differences between the two species. The importance of examining serial sections in a study of this nature cannot be overstated. The main disparity involves PR behavior. McDonald observed PRs only during prophase and did not undertake an exhaustive search for them in later stages. Observations in Dasya of PRs during interphase, prophase migration, metaphase, and in spermatangia conform with those of Scott et al. in Polysiphonia and contradict McDonald's assertions that PRs "appear" at the poles during prophase and that a non-polar position is "unusual." Non-polar PRs are not unusual; PRs in a migratory position were frequently seen.

Whether PR migration is effected primarily by the action of microtubules, nuclear membrane, or nuclear envelope associated structures, e.g., actin (32), is not clear, but microtubular activity appears to be the least probable in that migration begins while the zones of exclusion are quite small and few microtubules are evident. PRs appear to function as microtubule organizing centers (MTOCs) during mitosis, and by the time the poles are established large zones have developed around each PR.

The pronounced nuclear envelope protrusions seen at the poles during late prophase in Polysiphonia (10,80) were not observed in Dasya; only slight plateaus were noted. Although none were reported in Membranoptera, they are present (McDonald, personal communication with Scott). The protrusions presumably result when the nucleus shifts from its elongated prophase shape to the flattened prometaphase condition. The PRs are attached to the nuclear envelope during prophase and are also embedded in the microtubule packed zones. As the nucleus retracts from the poles, the nuclear envelope immediately beneath the PRs is held in place temporarily, creating the protrusions observed. This same action may be involved in the splitting of the PRs into halves. As the flattening of the poles continues, the bonds between the two halves of each PR could conceivably be broken by simple mechanical force. This hypothesis implies that the bonds between the two halves are weaker than those of the proximal half to the nuclear envelope and the distal half to the zone of exclusion. Other mechanisms, i.e., enzymatic activity, may operate in addition to or instead of mechanical rupture. Half PRs were seen previously in Polysiphonia, but were overlooked in Membranoptera. In Polysiphonia the half rings were

found to remain at the poles through telophase, the proximal half being connected to the nuclear envelope, and the distal half ultimately settling down beside it. In both Dasya and Polysiphonia two entire PRs are found in close association with the nuclear envelope at the former poles, a new half having been formed for each of the pair during late telophase or early interphase.

The extranuclear spindle found in Dasya during prophase is also found in Membranoptera. PER was characteristic of all three genera. McDonald suggested that it might serve to sequester microtubule subunits.

Although the nuclear envelope does not break down, an intranuclear spindle forms. Two routes for the entry of spindle precursors exist: first, the nuclear pores which become concentrated at the poles during prophase, and second, the large polar fenestrations which develop at prometaphase.

Following formation of a well developed metaphase plate, the chromosomes separate during anaphase. McDonald interpreted the separation of daughter nuclei during telophase as occurring by constriction in the middle of the elongated nucleus. This was not found to be the case in either Polysiphonia or Dasya. Instead, it appears that in Dasya, as has been shown in Polysiphonia, the interzonal spindle dehisces, followed by reformation of the nuclear envelopes of the daughter nuclei. A similar mechanism is likely in Membranoptera; McDonald misinterpreted a micrograph in which the middle portion of the interzonal spindle was out of the plane of section. Examination of adjacent sections could have prevented this error. Vacuoles which coalesce around the interzonal spindle and

ultimately serve to keep the daughter nuclei separated during cytokinesis were not mentioned by McDonald, although they are present in his micrographs. Similar vacuoles have been seen in the red algae Polysiphonia (10,80) and Batrachospermum (2,13), and in the green algae Stichococcus (68) and Raphidonema (71).

While cytokinesis begins later in Dasya than in Polysiphonia, where evidence of its beginnings may be seen in prophase, the mechanism in both, centripetal growth of cleavage furrows, is the same as in Membranoptera. In each case, ER was found near the furrows, but no microtubules or microfilaments were seen.

While mitosis and the PRs in these three genera are basically similar, the PRs in Dasya are morphologically more like those in Membranoptera.

The second published description of a mitotic sequence in a red alga concerns Porphyridium (11), a unicell in the other, more primitive class, the Bangiophyceae. It was based upon very few observations and is not in agreement with extensive work done in this laboratory (76). While cell division in Dasya and Porphyridium are fundamentally similar, i.e., both have intranuclear spindles, polar fenestrations, and NAOs, or spindle pole bodies (SPBs), as they were called in Porphyridium, there are some notable differences. Porphyridium's SPB is not a PR, but does consist of two parts: a broad ellipse proximal to the nuclear envelope, and a smaller cylindrical distal portion. It is not known with certainty whether or not the SPBs in Porphyridium are persistent organelles. Microbodies become associated with the SPBs, and following migration upon the nuclear envelope, the elliptical portions disappear during prometaphase

and the distal portion is associated with the spindle which develops at that time. The extranuclear spindle and PER found in Dasya do not occur in Porphyridium, the chromosomes are not as condensed, and the kinetochores are much smaller and less distinct. Cytokinesis also involves centripetal cleavage furrows; however, microfilaments seem to be instrumental in this process in Porphyridium, as opposed to Dasya and Polysiphonia, since a conspicuous contractile band of what appear on morphological grounds to be microfilaments is seen near the furrow.

While the PRs and SPBs of Dasya and Porphyridium are morphologically dissimilar, there is a behavioral parallel unique to the Rhodophyta. In each case, the SPBs are radically modified by the end of prometaphase. While in Porphyridium one part of the SPB breaks down, both halves of PRs persist, and their behavior is completely unlike that of any other NAO. During telophase of one division, the templates of the PRs for the next division settle into place on the nuclear envelope. By late telophase or early interphase, replication has occurred, and two complete PRs are ready to function as MTOCs during either the next division or spermatangial differentiation.

Porphyridium is a member of the least advanced order in the Rhodophyta, the Porphyridiales, while Polysiphonia, Membranoptera, and Dasya are found in the most advanced order, the Ceramiales. PRs have been found in at least one representative of every family within that order: Ceramiaceae—Griffithsia (63), Ceramium (J. Scott, personal communication); Rhodomelaceae—Chondria (J. Scott, personal communication), Polysiphonia harveyi (80), Polysiphonia denudata (10);

Delesseriaceae—Membranoptera (48); and Dasyaceae—Dasya. PRs have also been seen in one genus in the third most advanced order, the Gigartinales—Solieria (J. Scott, personal communication). In the complete absence of information about mitosis and NAOs in both the more advanced bangiophycean orders and the more primitive florideophycean orders, one can only speculate as to whether it is more probable that the Bangiophyceae gave rise to the Florideophyceae, or that both were derived from a common ancestor, although morphological and biochemical evidence support the former hypothesis.

The Rhodophyta are generally thought to be among the most primitive eukaryotes, based upon the similarity of their pigments to those of blue-green algae, simplicity of chloroplast structure, and the complete absence of cilia, flagella, and centrioles (16). If mitosis is a conservative process, as has been stated (36), one would expect to find some evidence of primitive characteristics, for example, nuclear envelope mediated movement of chromosomes, lack of a typical metaphase plate, and incomplete condensation of chromatin (36,50) during mitosis in the Rhodophyta. To date, none has been found.

Cell division has been described in several members of the class Cryptophyceae, a group of unicellular flagellates with which the Rhodophyta share some similarities of pigmentation. These similarities do not seem to extend to mitosis. The nuclear envelope of cryptophytes disperses, and neither centrioles nor SPBs were reported (41,54-59).

Interestingly, the group in which features of cell division most closely resemble those of the Rhodophyta is not any other algal assemblage, but the fungi, particularly the Ascomycetes and Basidiomycetes, both of which also lack cilia, flagella, and

centrioles. As in the red algae, mitosis is characterized by an intranuclear spindle, NAOs, and in some of the Basidiomycetes, polar fenestrations (32). The term NAO, which was first used in reference to fungi, was proposed by Girbardt and Hadrich in 1975 (27) because it is more neutral in terms of function and location than SPB. Splitting of NAOs into persistent proximal and distal halves has never been reported outside of the Florideophyceae. Cylindrical NAOs have been reported in two species of Zygomycetes (32), but descriptions by different workers are conflicting and have not been reconciled as yet. Additionally, PER is found in both the Florideophyceae and in a number of fungal classes (32).

The Rhodophyta have more than mitotic similarities in common with the Ascomycetes and Basidiomycetes. There are striking resemblances between their life histories and reproductive morphology. Glycogen, stored by fungi, is similar to floridean starch. The septa formed between fungal cells (24) look very much like red algal pit connections, although the two result from somewhat different developmental processes. Uninuclear meiosis, in which both meiotic divisions occur within a single nuclear envelope, has been reported in both groups (16,36,79).

For a hundred years, the Rhodophyta have been proposed by a few investigators as the ancestors to the Ascomycetes and Basidiomycetes (8). Recently, this view has been supported by Chadefaud (36), Kohlmeyer (34), and Demoulin (15) based upon many of the similarities noted above. The evolutionary route envisioned is chloroplast loss in parasitic red algae, forms which are common among extant red algae. However, an alternative possibility exists, and has been discussed by a

few phycologists (K. Stewart, personal communication to J. Scott). It appears at least as probable that the Rhodophyta are derived from certain higher forms of fungi, the appearance of chloroplasts being explained by endosymbiosis of a blue-green alga (46).

Although an argument as fundamental as this cannot be settled by comparative ultrastructure of mitosis alone (60), the spindle apparatus in red algae is certainly no less complex than that found in higher fungi.

In comparing cell division in Dasya and other florideophycean species to what limited information is available concerning brown algae (7,40,42,47), one finds that while both have intranuclear spindles and polar fenestrations, the NAOs in brown algae are centrioles. A great deal is known about cell division in green algae and has been reviewed elsewhere (69). Intranuclear spindles, polar fenestrations, and PER are found in some members of this group. In some species centrioles function as NAOs, while in others NAOs are not present. The chromosomes, kinetochores, and spindles of most eukaryotic algae, regardless of pigmentation, do not appear to differ fundamentally from one another. The same can be said in comparing division in Dasya to the process in animals and vascular plants: The similarities are greater than the differences. The most notable features distinguishing mitosis in Dasya include PRs and PR behavior, a persistent, fenestrated nuclear envelope, differences in cytokinesis, and pit connection formation.

The process of spermatangial differentiation and spermatial release has been described in several other red algal species by both light (18,21,28), and electron microscopists (12,14,38,63,64,78,81-83).

The events observed in Dasya during this study are in general agreement both with these reports and the only previous investigation of Dasya (30), although both differences and previously unreported events were noted.

PRs were found to persist in spermatangial cells. Located initially at the former pole, both PRs migrate 90° to opposite sides of the nucleus. This contrasts with prophase migration, in which one PR migrates 180°. During PR migration in spermatangia, a conspicuous array of microtubules develops, surrounding the nucleus. These perinuclear microtubules, radiating from the region of the PRs and ensheathing the nucleus, persist at least until spermatial release. The development and arrangement of these spermatangial perinuclear microtubules is identical to that of the extranuclear spindle found during prophase in mitotic cells.

An association of microtubules with the nucleus during spermiogenesis has been reported in many plant and animal species and has been reviewed (51). An involvement of these microtubules in both nuclear shaping and chromatin condensation has been proposed (51,52). While perinuclear microtubules ensheathing the nucleus have been mentioned in Dasya spermatangia (30) and are apparent in published micrographs of Rhodomela spermatia (64), this is the first complete description of such an association in the Rhodophyta.

Contrary to some earlier reports (21,45,78), the spermatial nuclear envelope remains intact through release. Prominent nuclear pores and prophase-like chromatin condensation have been previously reported (30,61). The behavior of the chromosomes, nuclear envelope, and microtubules following release and during fertilization is unknown

at the EM level and cannot be resolved with light microscopy.

This study confirms Haskell's observation that although chloroplasts are present in SMCs in Dasya, they are rarely transmitted to spermatangia. Why they are not is uncertain since they are found throughout the SMC cytoplasm, and not aggregated basal to the SMC nucleus as noted in another species (39). Chloroplasts have also been noted in SMCs, but not spermatangia, of Griffithsia (63) and Ptilota (78). The presence or absence of chloroplasts in spermatangia and the resulting spermatia is significant in that it is involved with the question of paternal vs. maternal inheritance. This seems to vary with species among the Rhodophyta, but appears to be maternal in Dasya. Additional weight is given to this view by the possibility of autolysis of those rare chloroplasts which are incorporated into spermatangia. This idea, not suggested elsewhere, awaits unambiguous cytochemical confirmation.

The formation of the basal spermatangial vacuoles has been described in several other species (30,38,61,64,78), and in Dasya (30). Observations during this investigation conform in general to the earlier studies, although the previous work in Dasya gave little importance to the role of ER, which was found to be significant during this study.

The aggregation of mitochondria-dictyosome associations subjacent to the nucleus has been reported previously in Dasya (30). While mitochondria-dictyosome associations are typical of red algae, this highly organized array, which would appear to be physiologically efficient, has not been reported in any other species.

The contents of the spermatangial vacuoles are reported to include

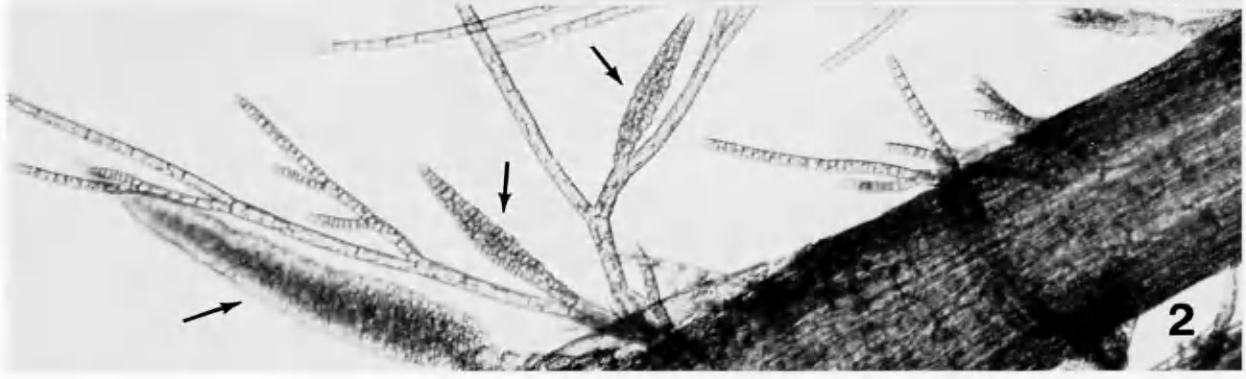
complex polysaccharides (61,63,78). A number of functions have been attributed to the spermatangial secretions, including rupture of the spermatangial wall through osmotic pressure, severance of the pit connection between SMC and spermatium (38), protection of the spermatium, which has no cellulosic wall, and adhesion of the spermatium to the trichogyne of the female gametophyte (12,63,78).

Several other unanswered questions remain. Although PRs have been shown to be persistent organelles in fertile branches of male Dasya, as in Polysiphonia, their fate in released spermatia and presence or absence in the rest of the male thallus and in female, carpospore and tetraspore thalli is undetermined, although PRs have been seen in tetraspore plants during meiosis I in two other species (J. Scott, personal communication; K. McDonald, personal communication to J. Scott). PR composition is unknown, and while there have been a few studies of NAO composition in fungi (32), the reports are variable, and there has been no comparable work in the Rhodophyta. How they split is uncertain and how they replicate is unknown.

Figure 1: Dasya baillouviana. Habit.

Figure 2: Light micrograph of a portion of an axis bearing several spermatangial branches in different developmental stages (arrows). (X 40).

1



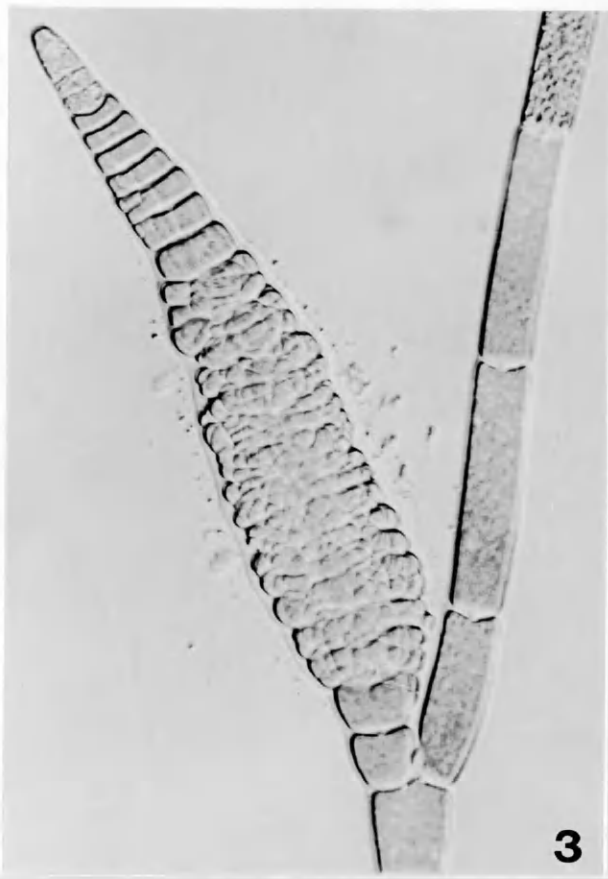
2

Figure 3: Light micrograph of a young male reproductive branch. Nomarski differential interference. (X 100).

Figure 4: Light micrograph of a mature spermatangial branch. (X 25).

Figure 5: Light micrograph of a longitudinal section through a spermatangial branch. The axial cells at the tip of the branch are out of the plane of section. Toluidine blue, phase contrast. (X 100).

Figure 6: Higher magnification of the same section shown in Figure 5. One spermatangial mother cell (single arrow) bears 3 spermatangia, while another (double arrow) is in telophase. Toluidine blue, phase contrast. (X 770).



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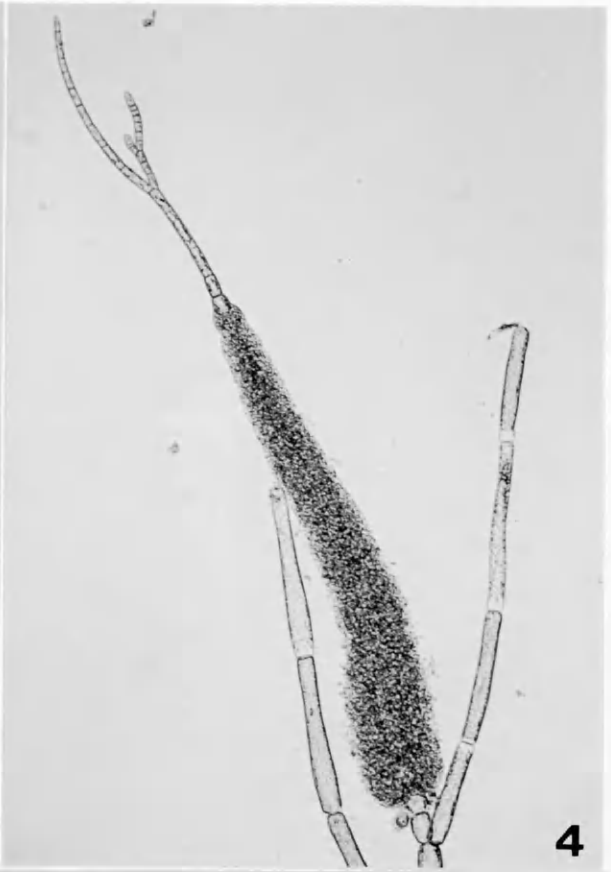

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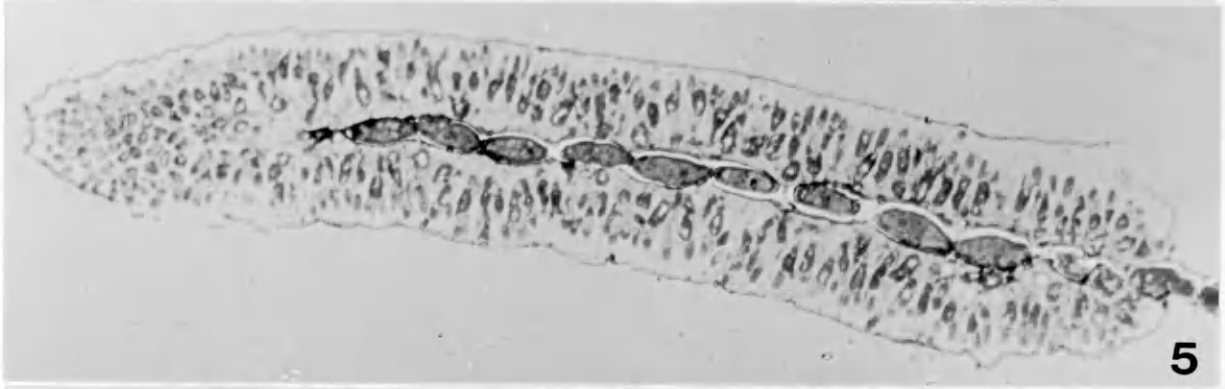

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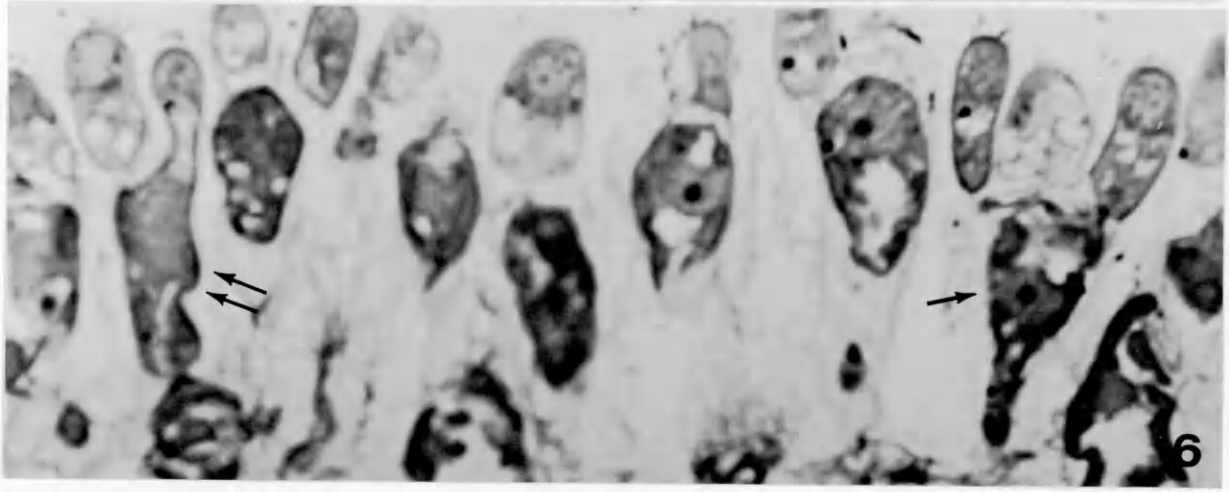


6

Figure 7: Longitudinal section through an interphase axial cell. Typical red algal chloroplasts (C) and pit connections (PC) are present. Large cytoplasmic vacuoles (V) have begun to develop. One polar ring (arrowhead) is in close association with the nucleus (N). (X 6,000).

Figure 8: Higher magnification of the nucleus in Figure 7. No zone of exclusion is present around the polar ring (arrowhead). (X 22,000).

Figure 9: The same axial cell nucleus a few sections away from Figures 7 and 8. The second polar ring (arrowhead) is located near the first one and is also in close association with the nuclear envelope. (X 22,000).

Figure 10: Longitudinal section through an interphase spermatangial mother cell. The nucleolus (NO) is prominent. Floridean starch (FS) and typical red algal mitochondria-dictyosome (MD) associations are present. (X 15,000).

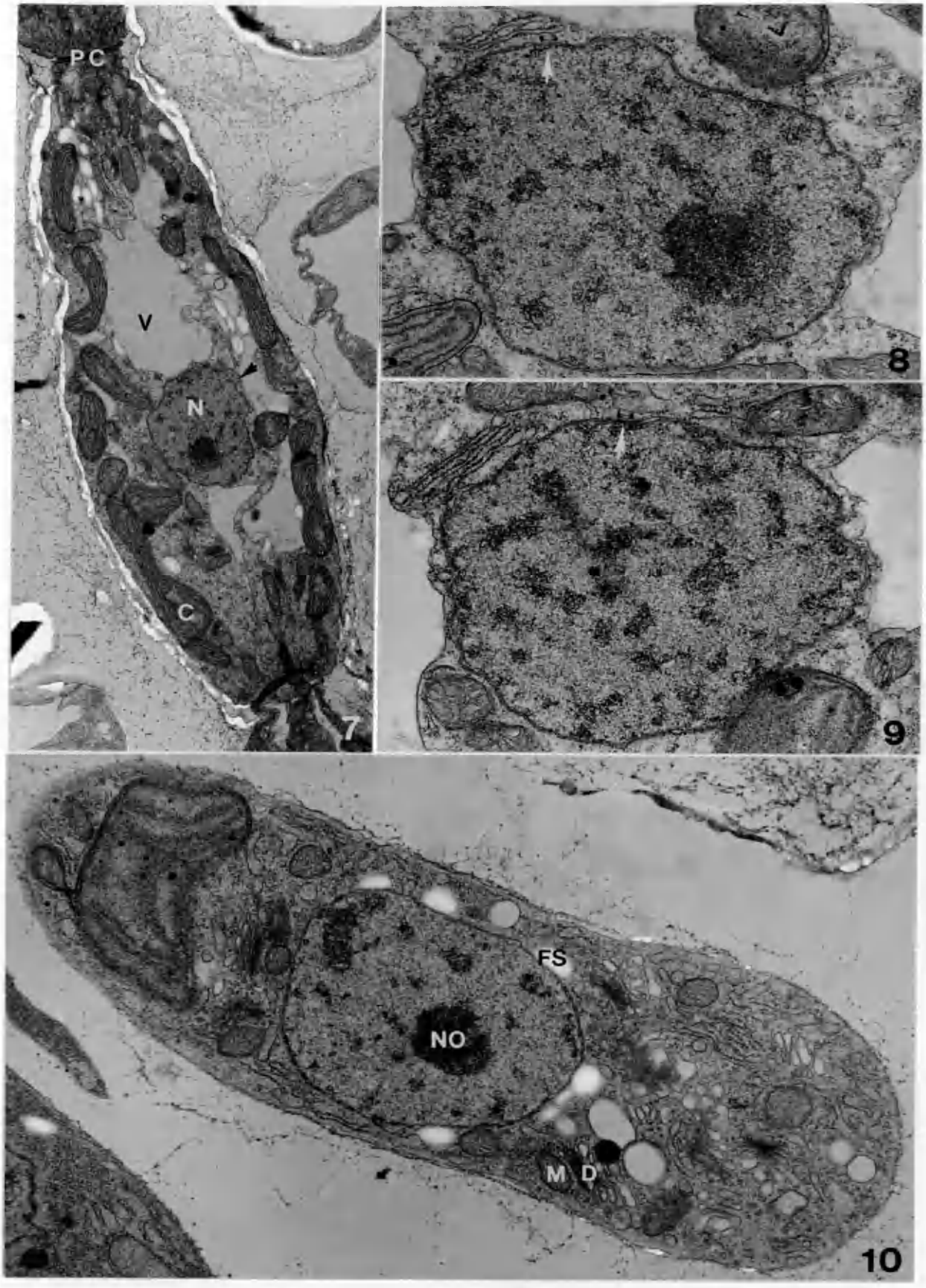

PC
V
N
C
7
8
9
FS
NO
M
D
10

Figure 11: Transverse section of a polar ring (PR). A nuclear pore (NP) is nearby. (X 62,500).

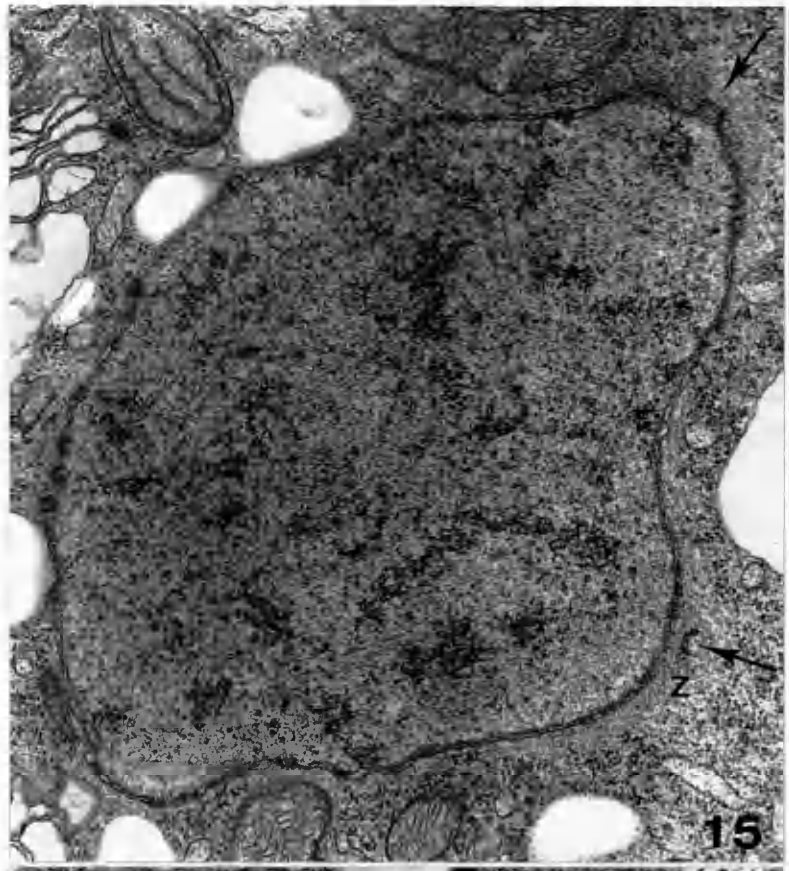
Figure 12: Longitudinal section of a polar ring. Note groove on inner surface and struts attaching the ring to the nuclear envelope. (X 62,500)

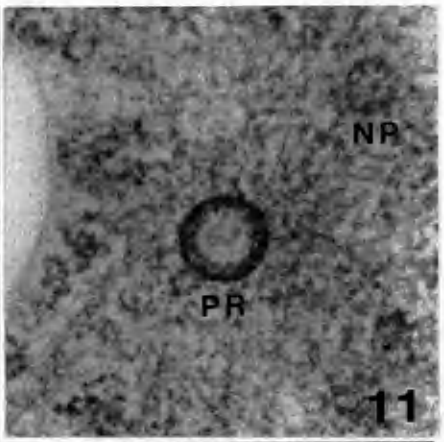
Figure 13: Tangential section of a polar ring showing double nature of the ring. (X 62,500)

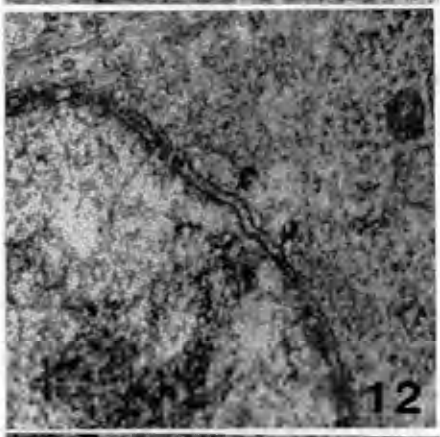
Figure 14: One pole of a late prophase nucleus. Note slight, nuclear pore free plateau in the nuclear envelope beneath the polar ring. (X 62,500).

Figure 15: Longitudinal section of a nucleus in early prophase showing migration of polar rings (arrows). Each PR is surrounded by a ribosome-free zone of exclusion (Z). (X 25,000).

Figure 16: Longitudinal section of a prophase nucleus in which polar ring (arrows) migration is almost completed. Nuclear pores are concentrated at the poles. (X 25,000).


Z
15


NP
PR
11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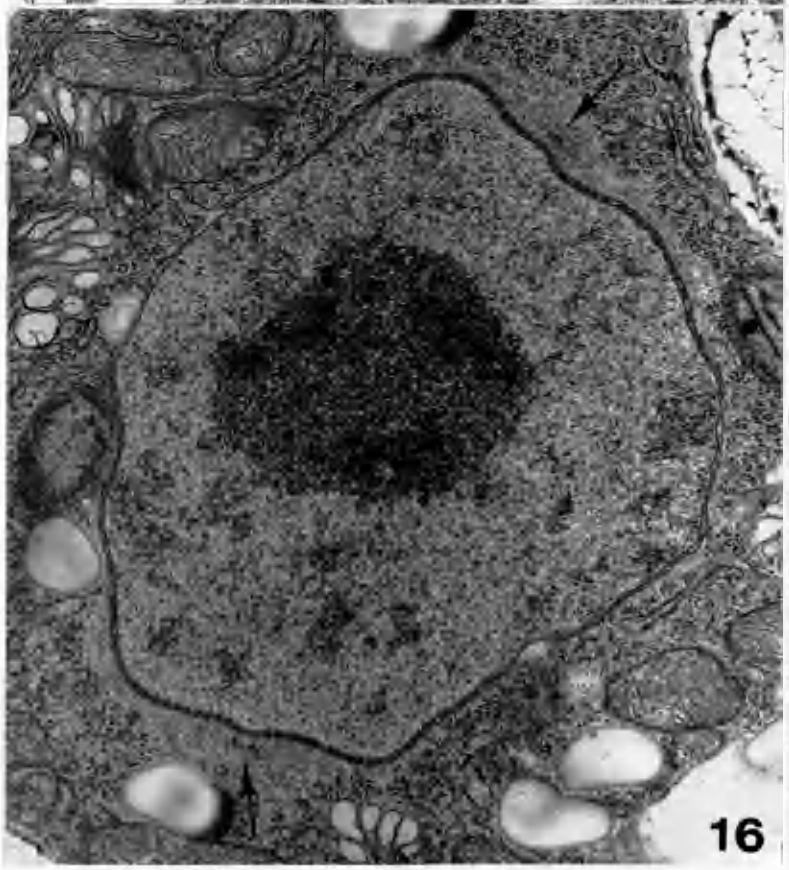
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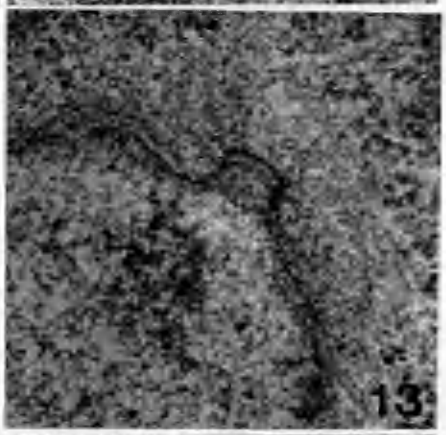
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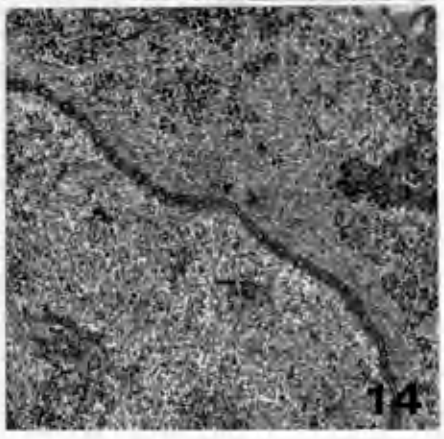
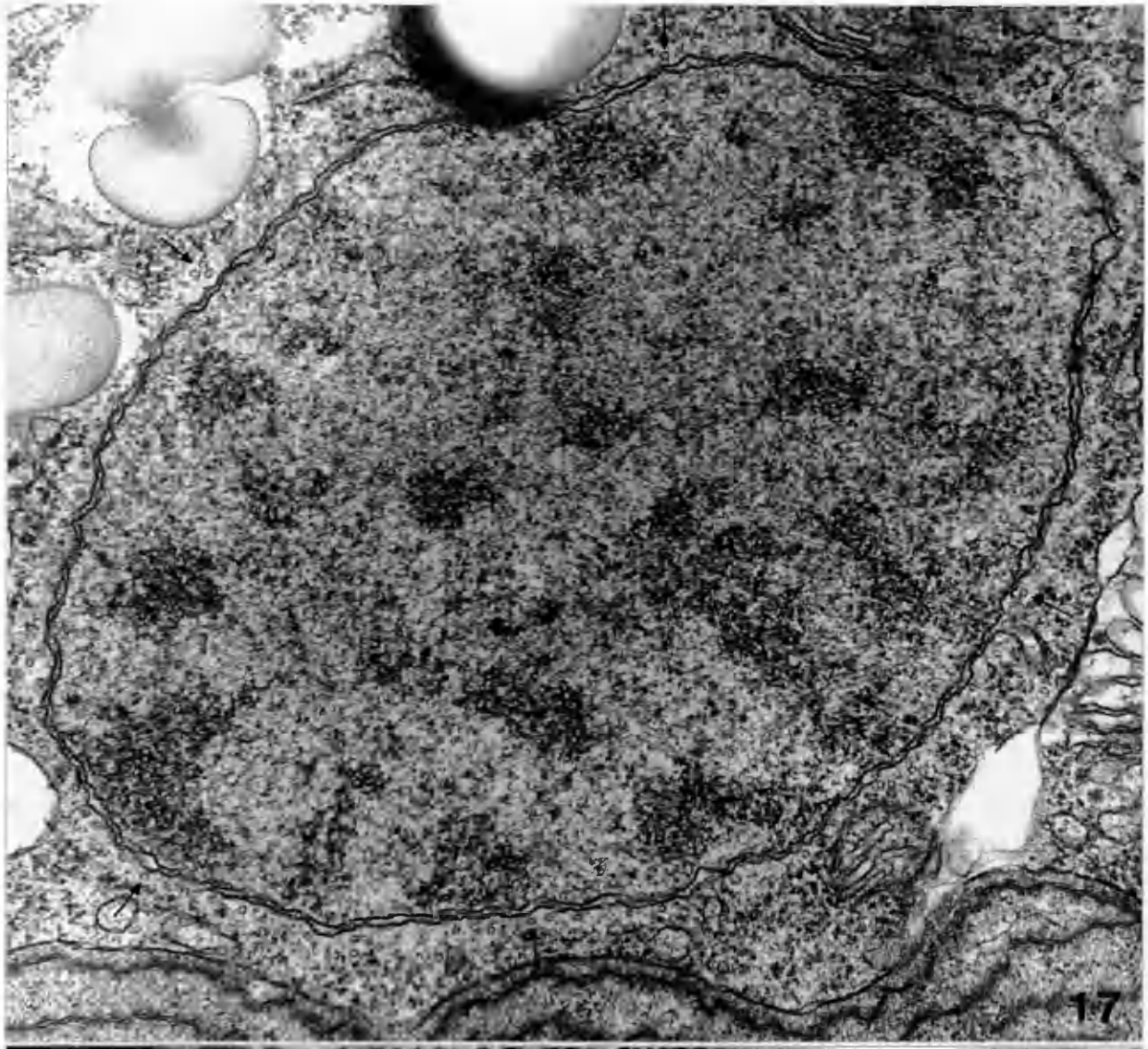

14

Figure 17: Cross section of a prophase nucleus. An extranuclear spindle of microtubules (arrows) ensheathes the nucleus. (X 43,500).

Figure 18: Cross section of a portion of a prophase nucleus at a later stage. Perinuclear endoplasmic reticulum (arrows) develops in part from the nuclear envelope. Perinuclear microtubules are still apparent. (X 89,600).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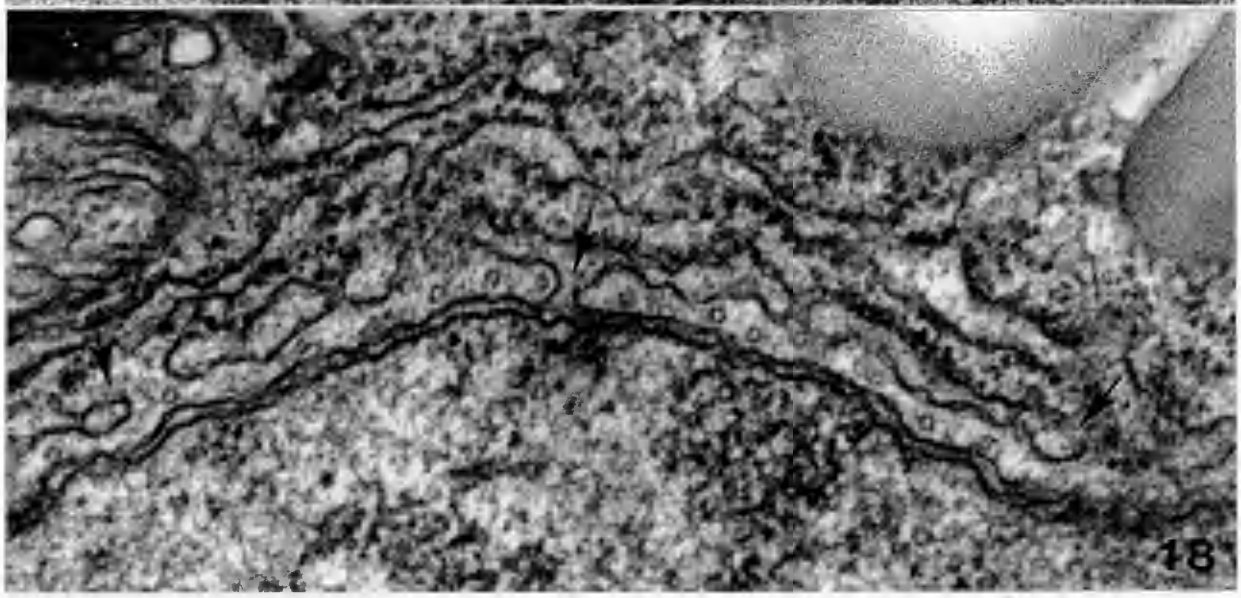
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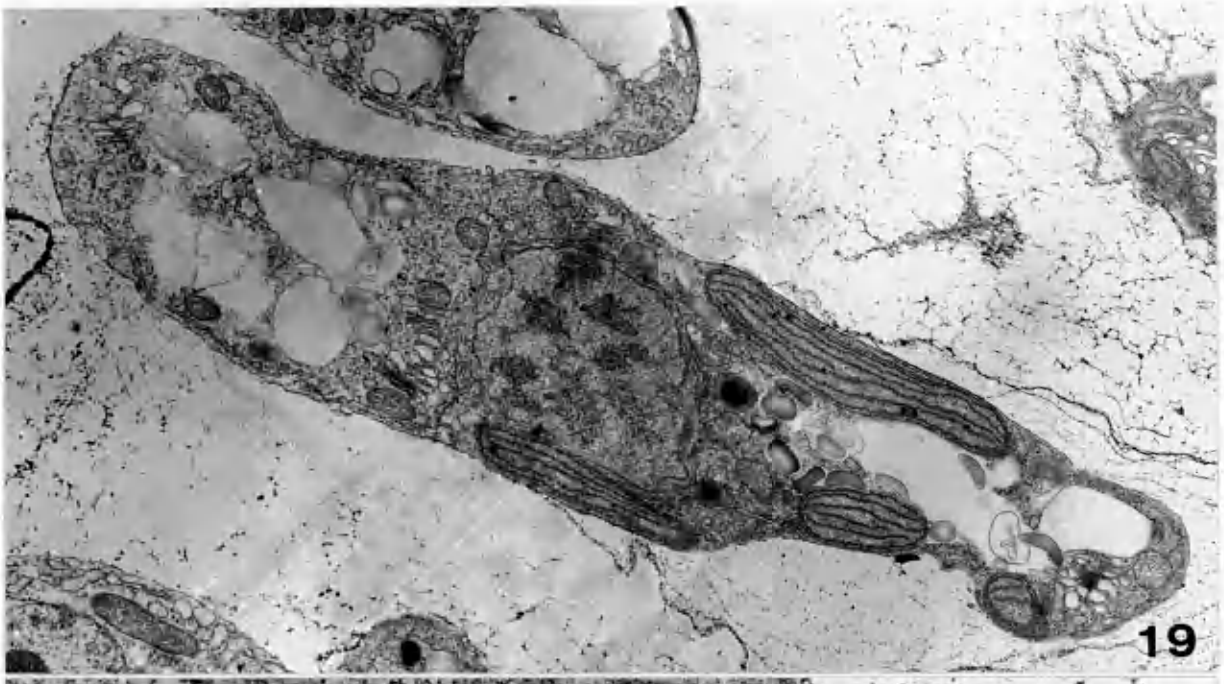
Figure 19: Longitudinal section of a prometaphase spermatangial mother cell. Chromosomal condensation is evident. (X 2,700).

Figure 20: Higher magnification of prometaphase nucleus in Figure 19. (X 40,000).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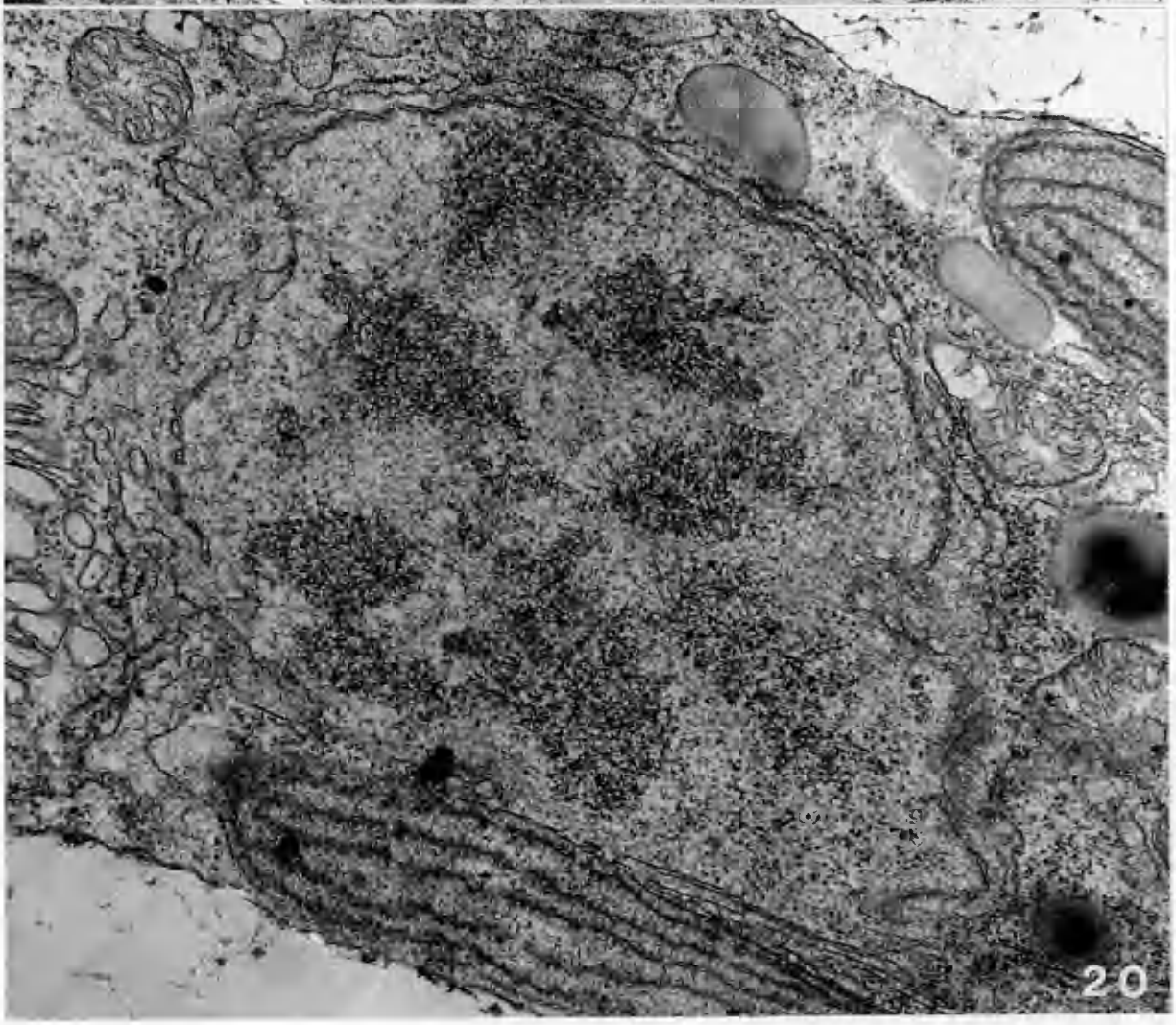
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Figure 21: Cross section of a prometaphase nucleus. Kinetochore (arrows) formation has begun. (X 25,200).

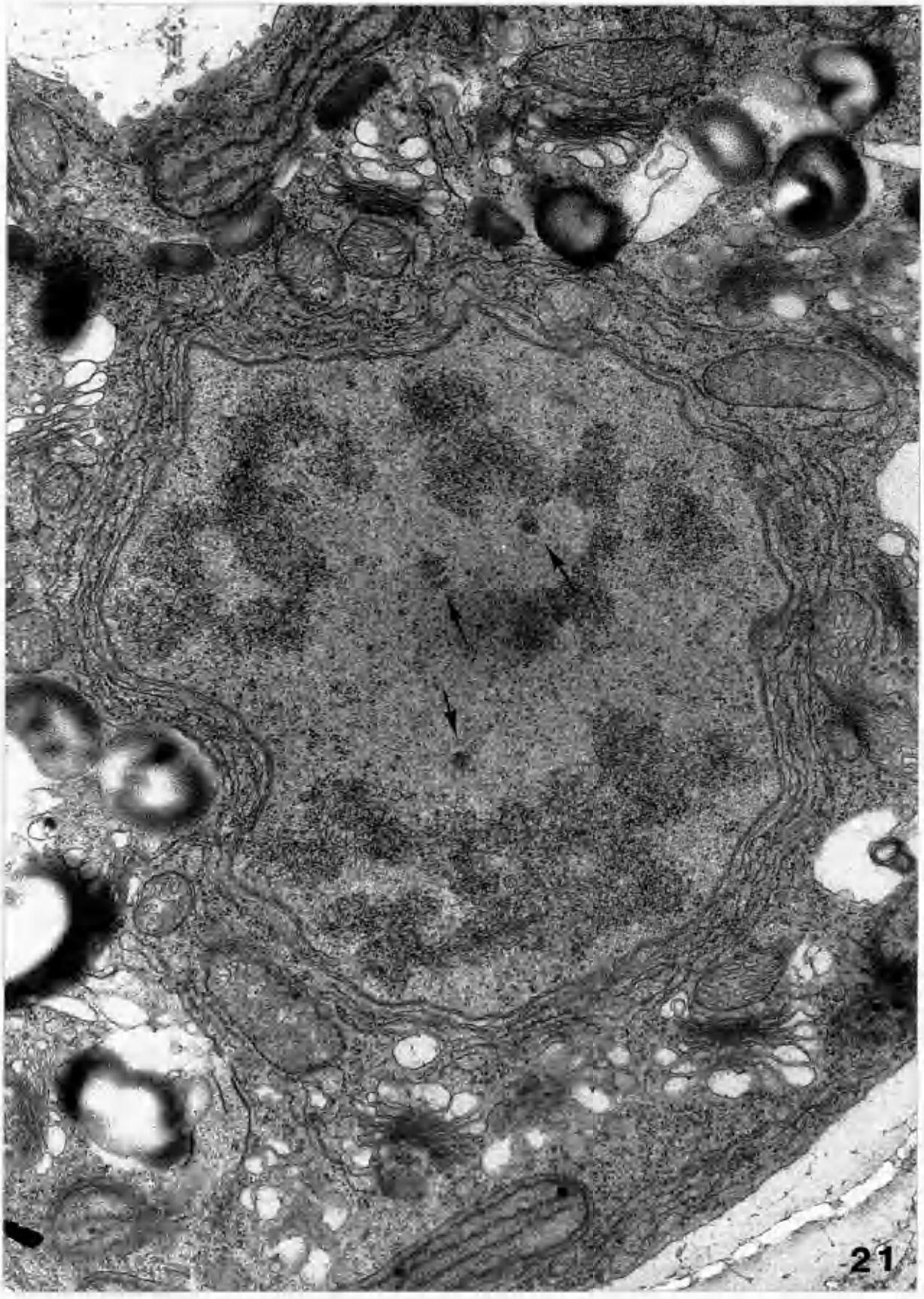

21

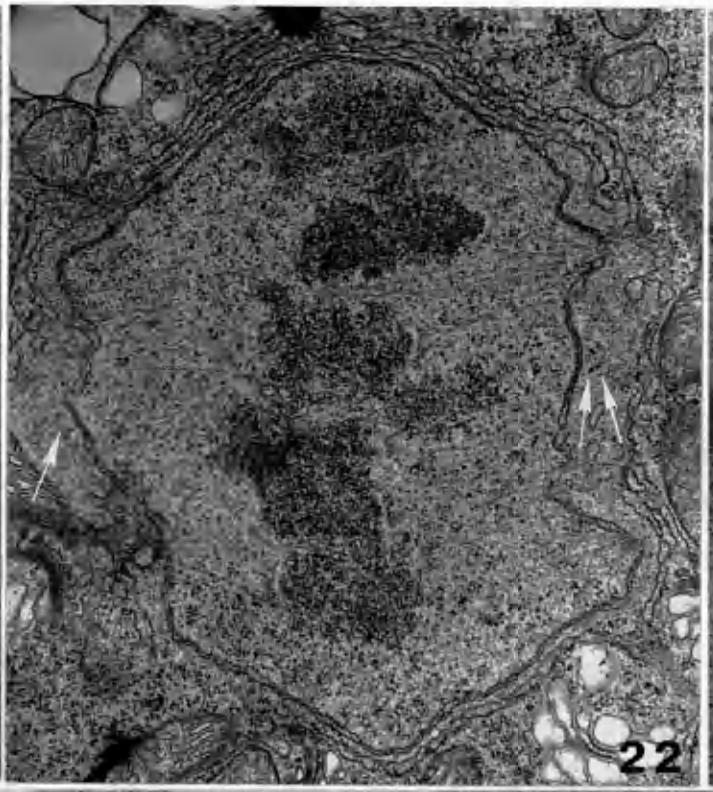
Figure 22: Longitudinal section of a metaphase nucleus. Proximal and distal halves of polar rings, three of which are seen in this section, are indicated by arrows. (X 25,000).

Figure 23: Adjacent section to that in Figure 22. One half (arrow) of a polar ring is present. (X 25,000).

Figure 24: Other adjacent section to that in Figure 22. Both halves (arrows) of a polar ring are present. (X 25,000).

Figure 25: Longitudinal section of mid-anaphase nucleus. (X 40,000).


23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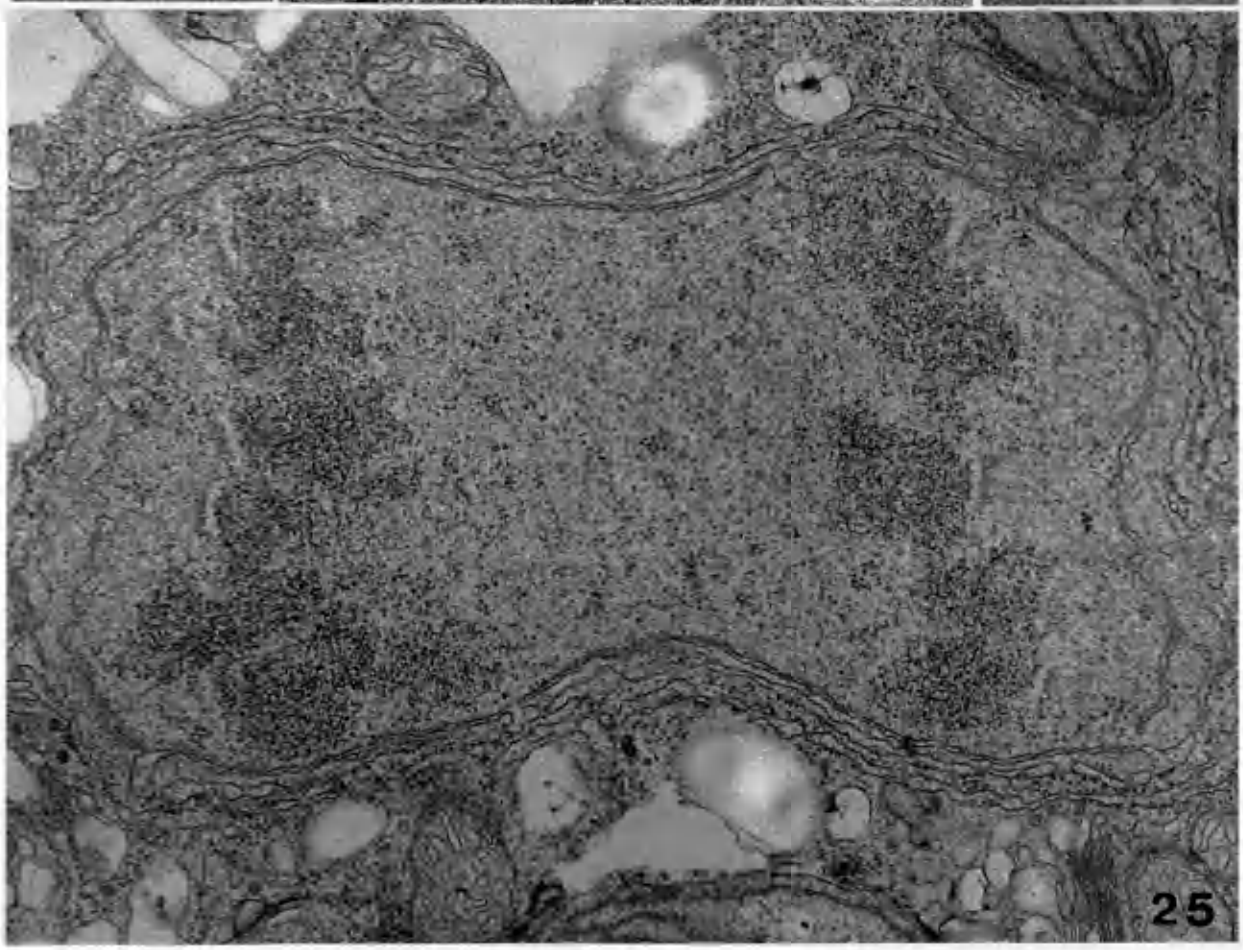
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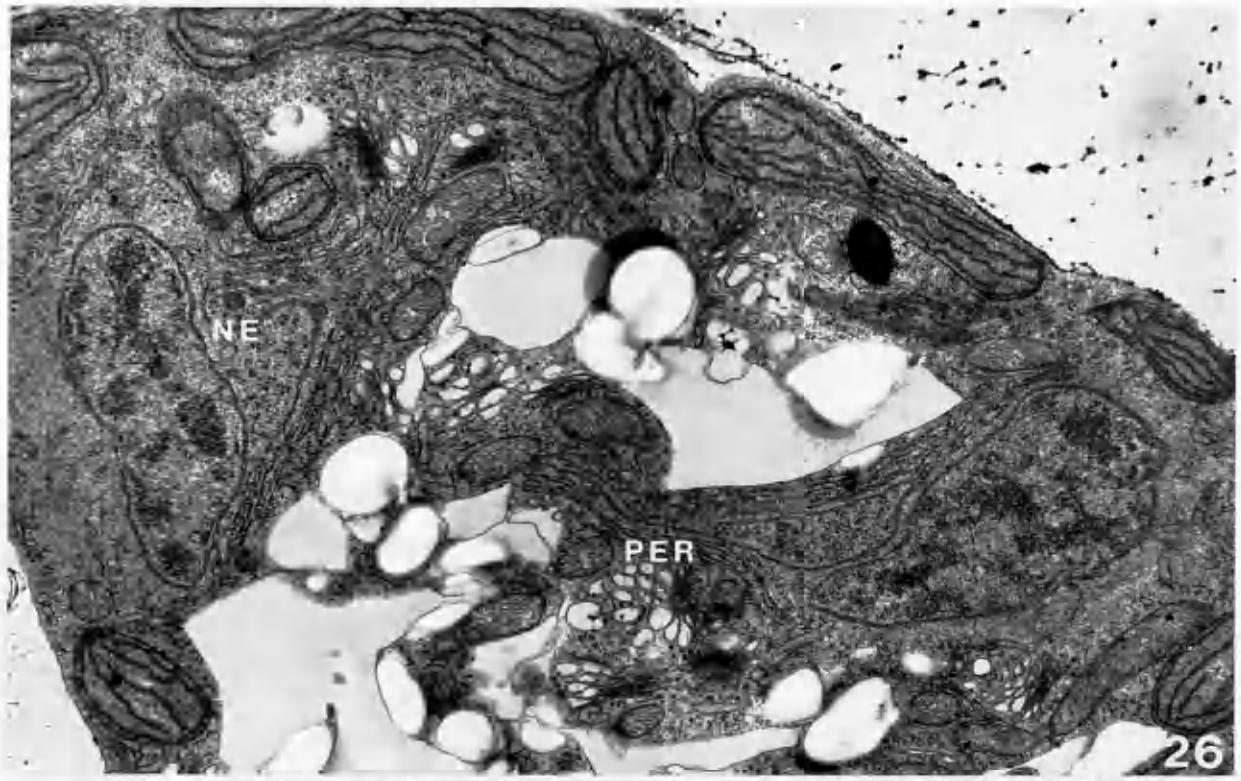
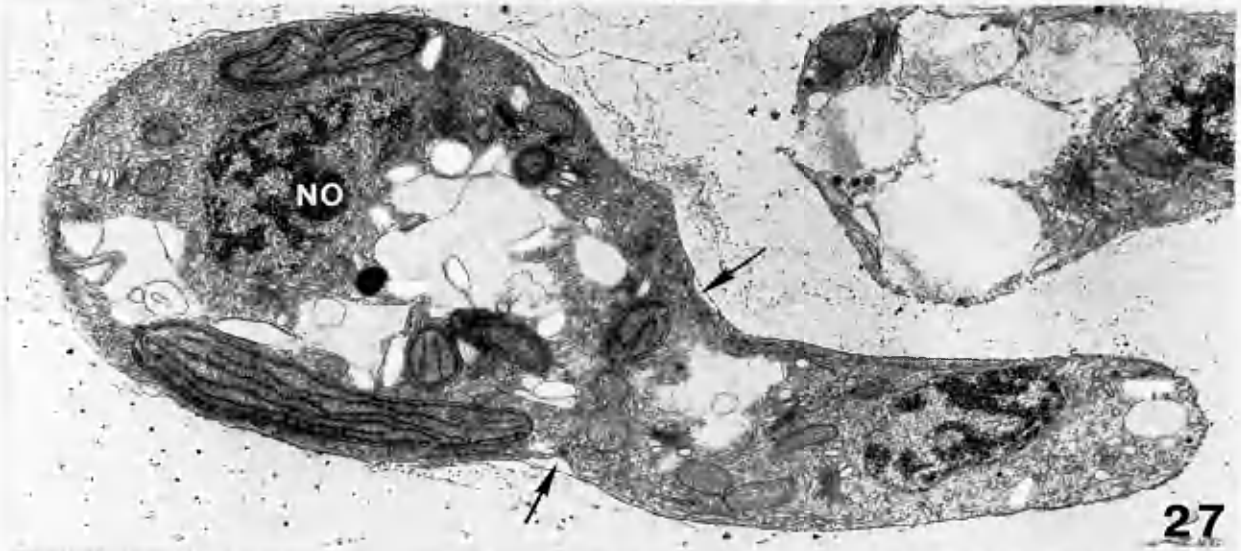
Figure 26: Late telophase nuclei separated by vacuoles (V). Perinuclear endoplasmic reticulum (PER) and nuclear envelope (NE) remnants are still present. (X 14,600).

Figure 27: Early cytokinesis in a spermatangial mother cell. Cleavage furrow formation has begun (arrows). The SMC nucleolus (NO) has reformed. (X 10,500).

Figure 28: Late cytokinesis. Note unevenness of furrow development. (X 13,200).


NE
PER
26


NO
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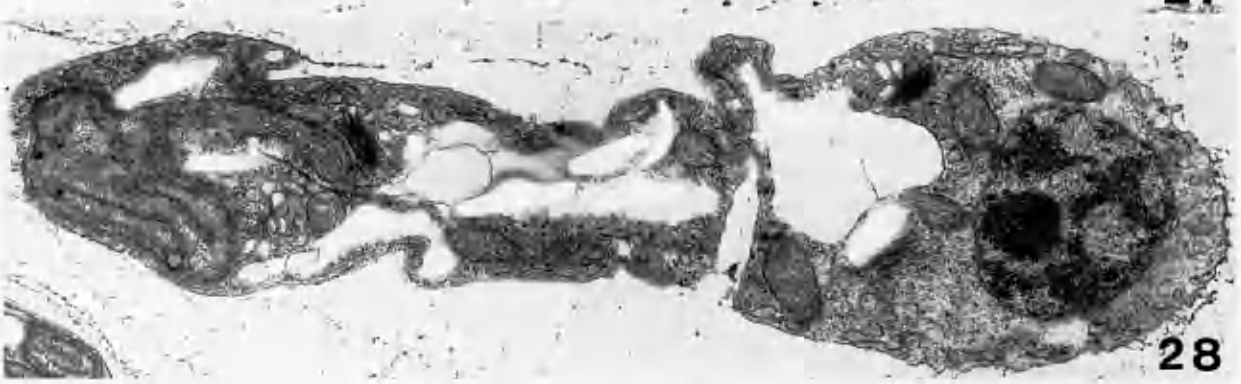
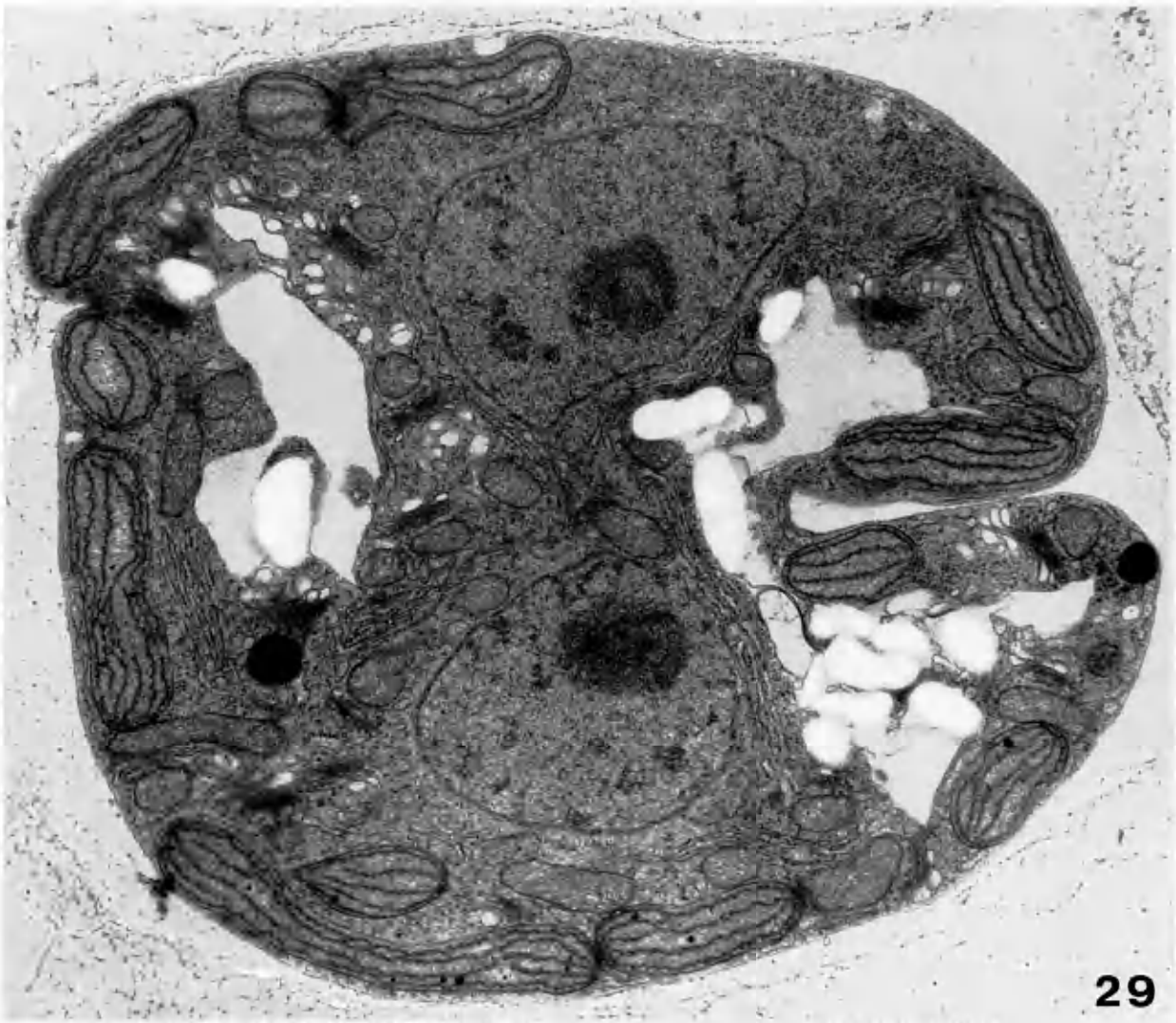

28

Figure 29: Cytokinesis in a vegetative cell of the male branch. Unevenness of furrow development is evident. (X 13,400).

Figure 30: Higher magnification of cleavage furrow in same cell in another section, oriented in the same direction. Note the absence of microtubules and microfilaments. Endoplasmic reticulum (ER) is found at the leading edge of the furrow. (X 63,000).



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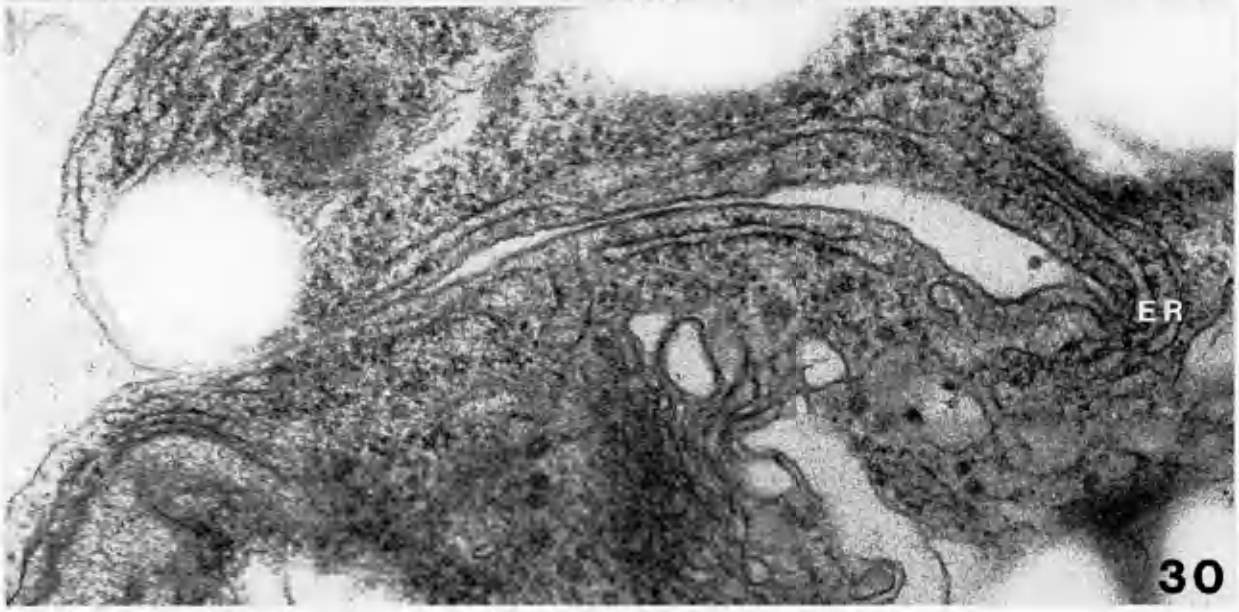
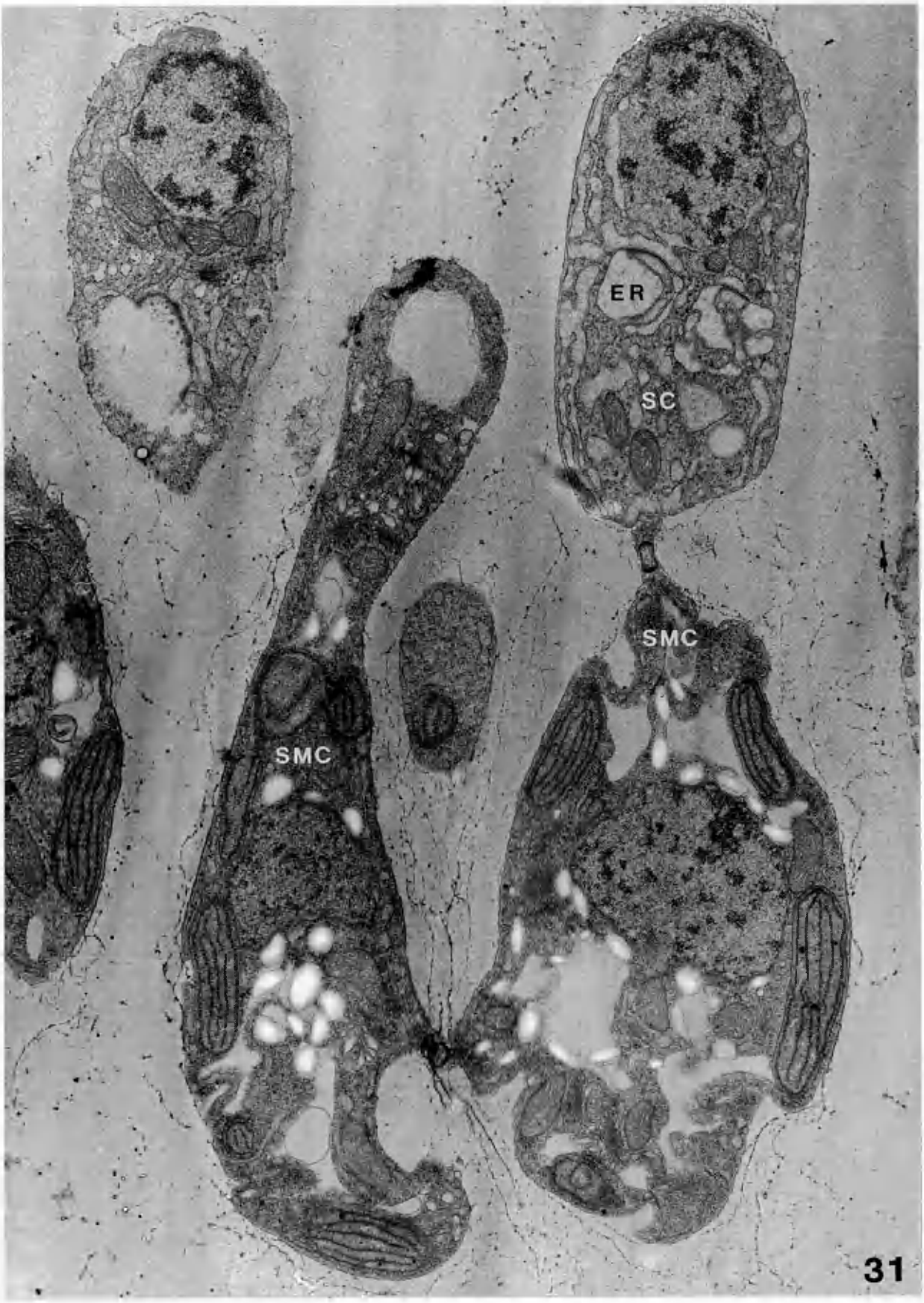


30

Figure 31: Two spermatangial mother cells (SMC) joined by a pit connection. One SMC has a young spermatangial cell (SC) still attached by a pit connection. Note distended endoplasmic reticulum (ER) basal to spermatangial nucleus. (X 13,800).

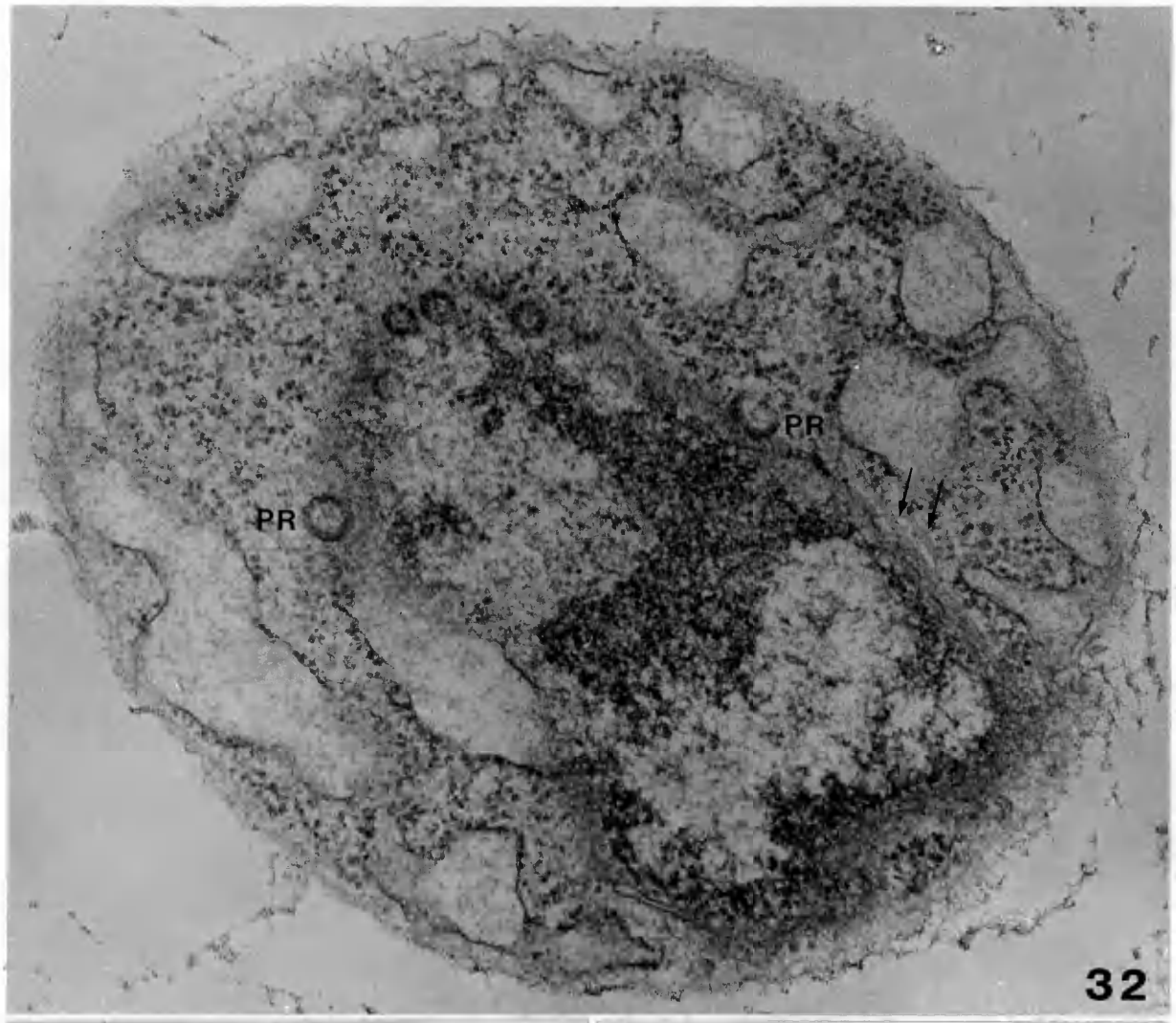


31

Figure 32: Apical cross section of a young spermatangial cell. Two polar rings (PR) in migratory positions are present. Nuclear pores are numerous. Formation of the microtubular (arrows) extranuclear spindle has begun. (X 54,250).

Figure 33: Cross section through the base of the nucleus of a young spermatangial cell. Mitochondria (M) - dictyosome (D) associations have aggregated. Nuclear pores are conspicuous. (X 23,000).

Figure 34: Tangential longitudinal section of a young spermatangial cell. One polar ring (arrow) is in a migratory position. The cup shaped nature of the mitochondria-dictyosome aggregation is apparent. (X 25,500).



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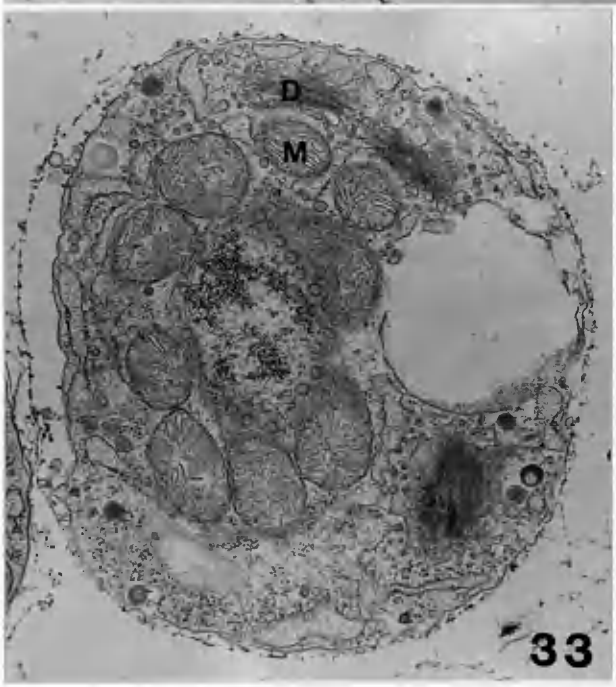

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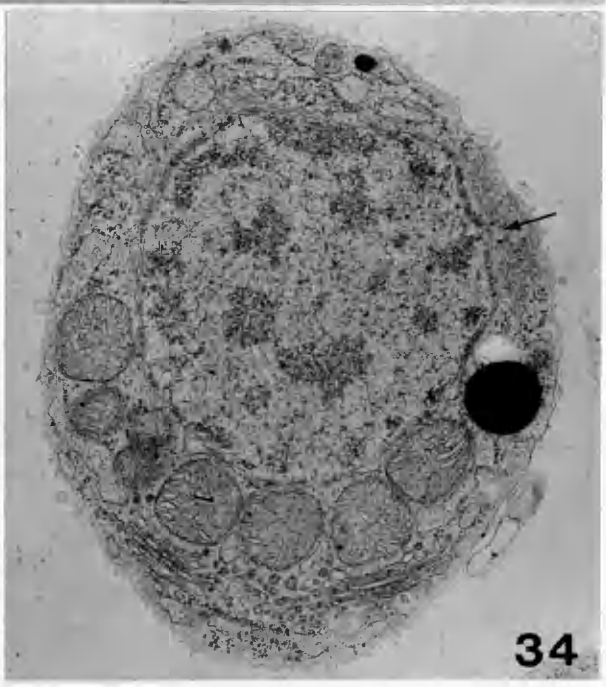


34

Figure 35: Tangential section of a spermatangial cell. The nucleus (N) is ensheathed by microtubules. A dictyosome vesicle (arrow) emptying its contents into a basal vacuole (V) is present. Several vesicles (arrowheads) of medium electron density are present. (X 28,000).

Figure 36: Longitudinal section of a spermatangial cell nucleus. The poles are noticeably flattened. One polar ring (arrow) is at the pole in this section. (X 29,500).

Figure 37: Same nucleus as in Figure 36. The other polar ring (arrow) is present at the opposite pole in this section. Microtubules radiating from the poles around the nucleus are evident. (X 29,500).

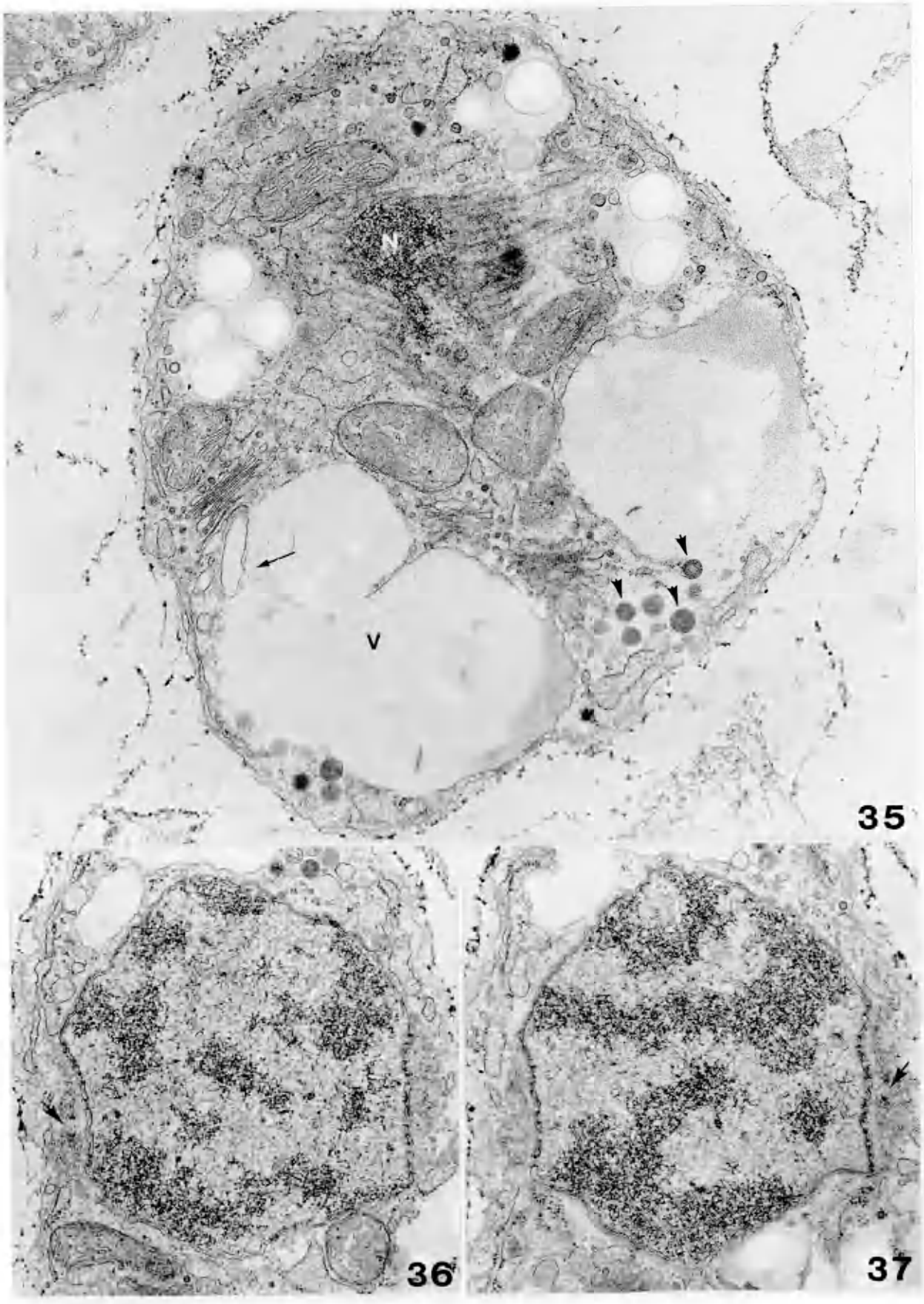
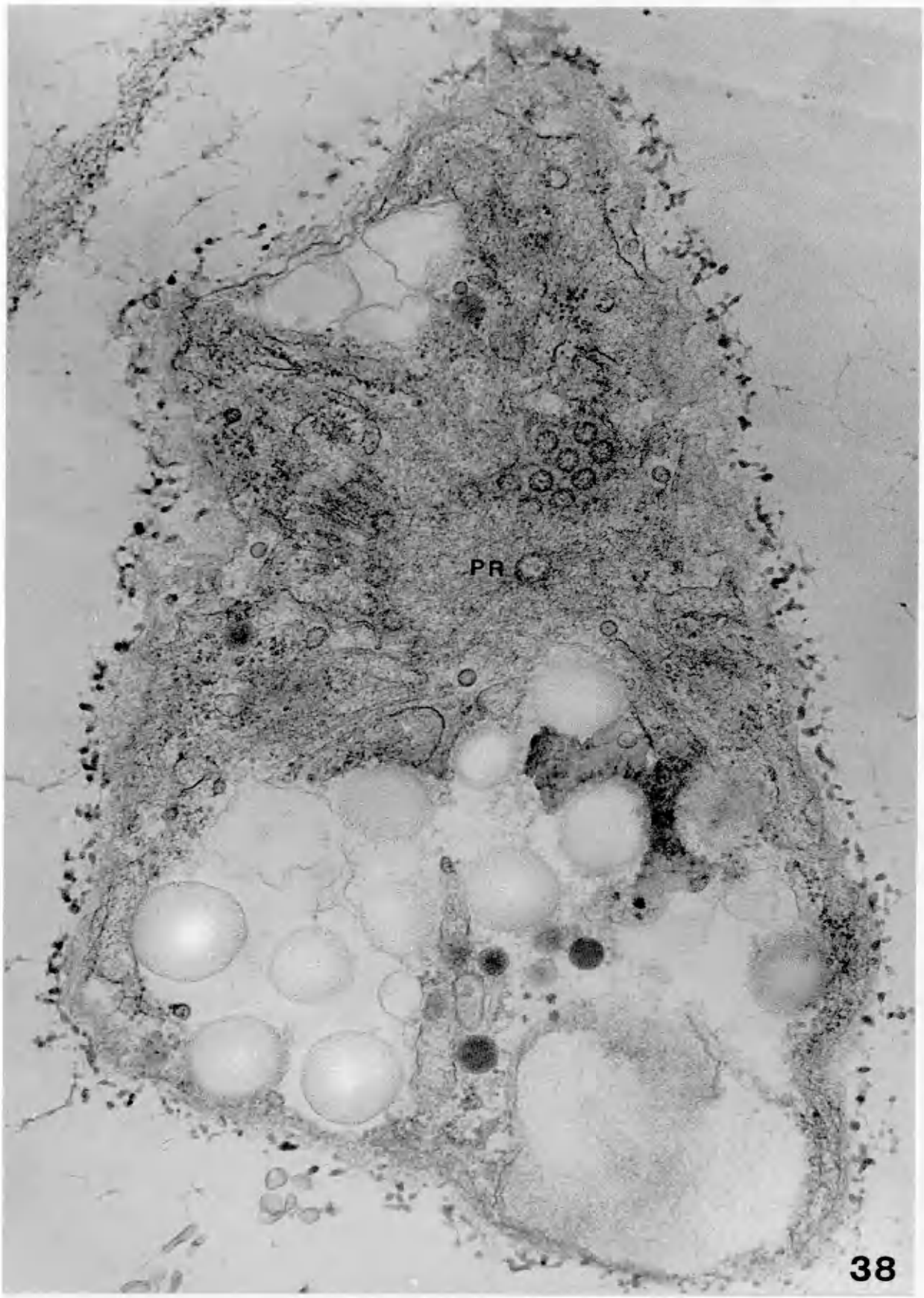

N
V
35
36
37

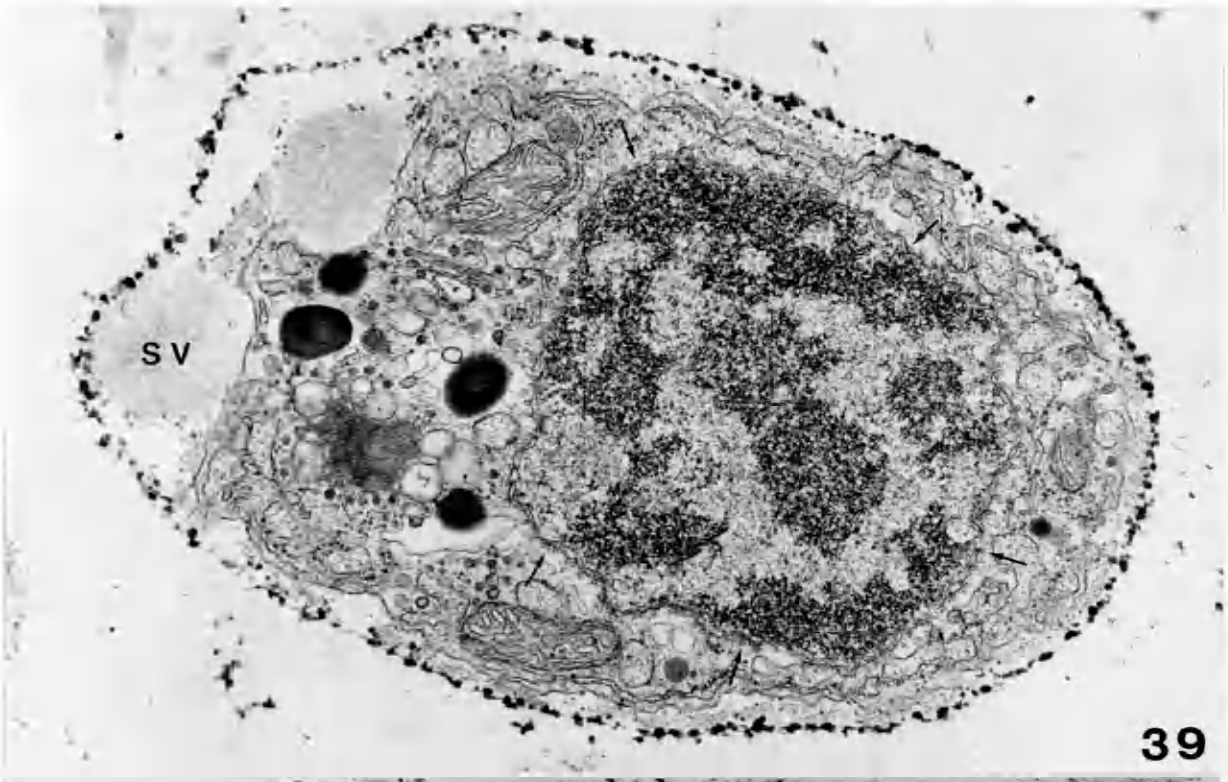
Figure 38: Tangential section of a spermatangial nucleus. Microtubules radiate around the nucleus from the vicinity of the polar ring (PR). Nuclear pores are numerous. (X 52,000).



38

Figure 39: Longitudinal section of a spermatium following release of the spermatangial vacuole contents (SV). Chromatin is condensed. The nuclear envelope is ensheathed in microtubules (arrows). (X 29,500).

Figure 40: Tangential section of a spermatium. Evenly spaced parallel microtubules adjacent to the nuclear envelope are obvious. Nuclear pores are numerous. (X 29,500).



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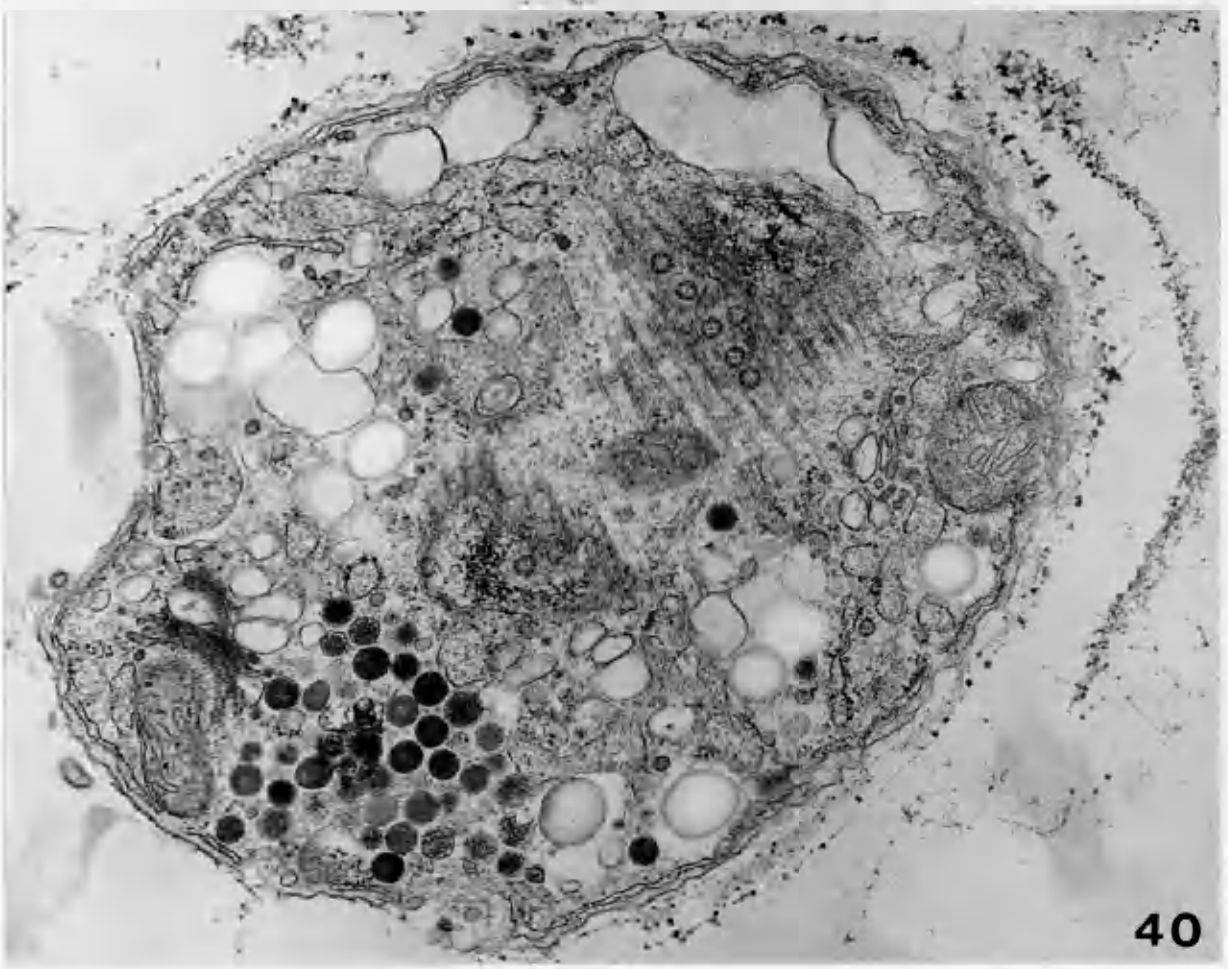


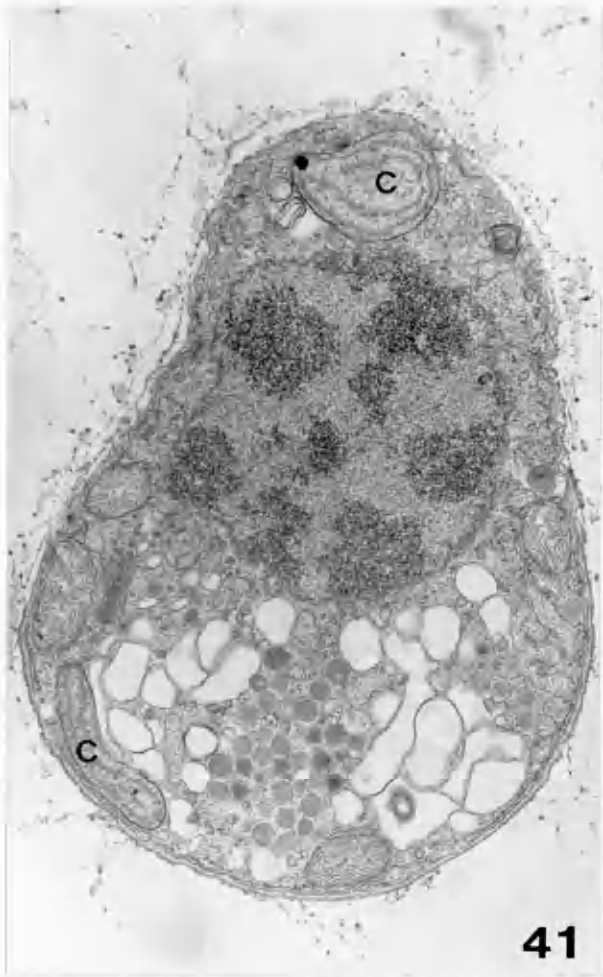
40

Figure 41: Longitudinal section of a very unusual spermatium in that it contains chloroplasts (C). (X 23,000).

Figure 42: Longitudinal section of another spermatium containing a chloroplast (C), which appears digested. (X 17,600).

Figure 43: A spermatium being released through the wall of the male branch (BW). (X 20,000).

Figure 44: A free spermatium. A mitochondrion (M) is enveloped by membranes and appears digested. (X 22,500).



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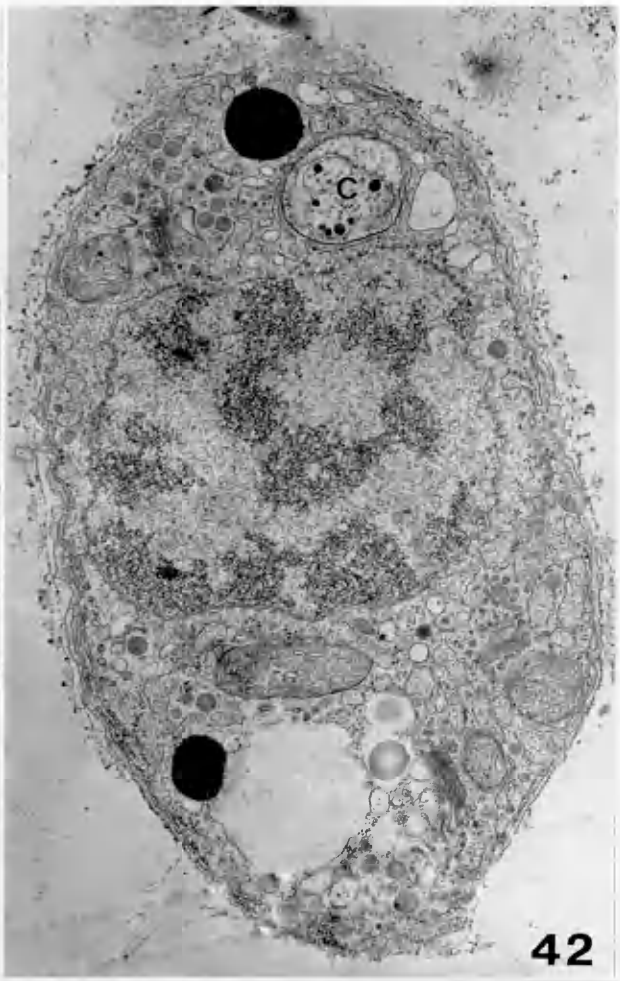

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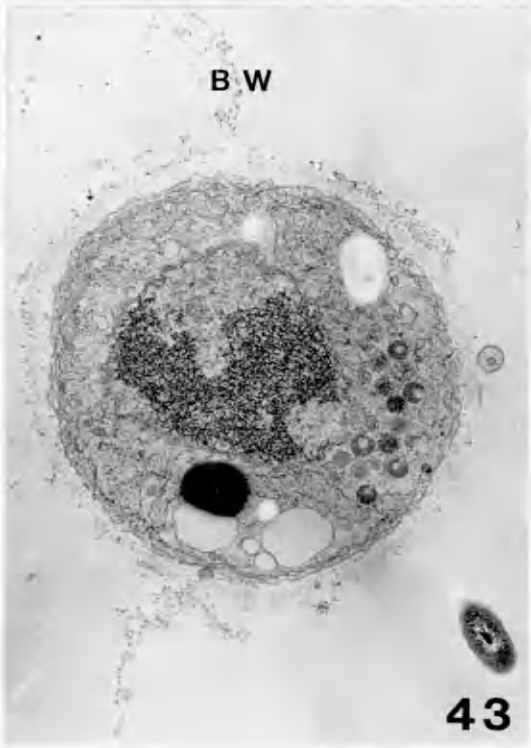

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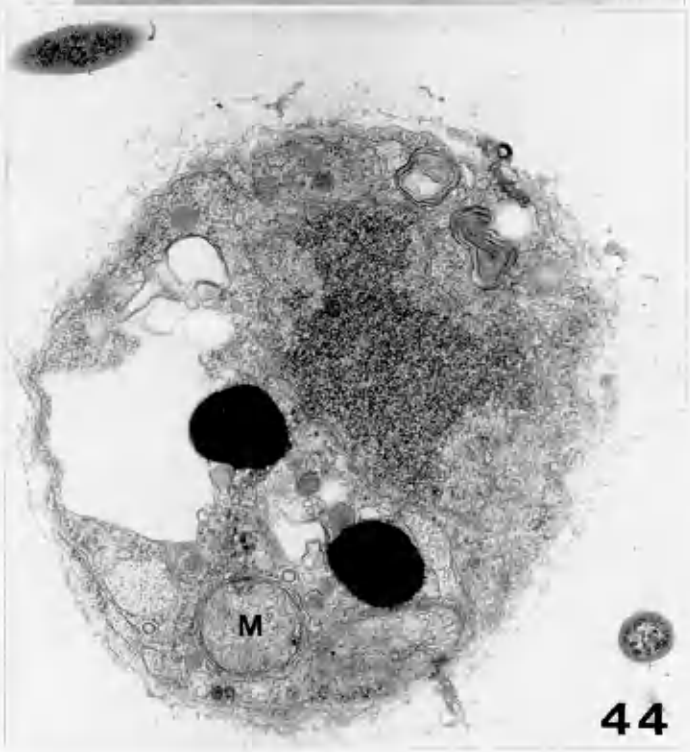

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VITA

Dawn Phillips Sigfred

Born in Berkeley, California, March 26, 1947. Graduated from Plant High School, Tampa, Florida, 1965. Attended the University of South Florida and the University of Florida. B.S. in Biology from Christopher Newport College of the College of William and Mary, 1974. Teaching assistantship in biology, College of William and Mary, 1976-78. M.A. candidate in biology, College of William and Mary, 1977-79. Ph.D. student at the School of Marine Science of the College of William and Mary 1979-present. Research assistantship, School of Marine Science, 1979-present.