SPERMATOGENESIS IN *SELAGINELLA TAMARISCINA* (BEAUV.) SPRING[1]

I. From Microspore to Spermatid

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Abstract: The spermatogenesis in *Selaginella tamariscina* (Beauv.) Spring is studied from the mature microspore to the spermatid. The persistent prothallial cell produced by the first division of the mature microspore situates at the distal pole. The plane of the first division of the antheridial initial is parallel to the equator of the microspore in the majority of specimens. The spermatid is characterized by containing a pair of parallelly oriented centrioles and multilayered structure which is composed of four layers.

INTRODUCTION

The Selaginellaceae is the largest one among the only three living families of lycopods. It contains over 700 species in the world, 14 from Taiwan (DeVol, 1975). *Selaginella tamariscina* is the only native species with stout stem.

The studies on the structure and developmental pattern of microgametophyte in several species have been published very early (Belajeff, 1885; Campbell, 1902; Lyon, 1901; Millardet, 1869; Pfeffer, 1871; Slagg, 1932; Yuasa, 1933). A well-developed microgametophyte consists of one prothallial cell, a layer of jacket cells surrounding the central spermatid mass. In most cases the prothallial cell remains intact at the time while the spermatids are set free (Belajeff, 1885; Millardet, 1869; Pfeffer, 1871). On the other hand, no wall has been seen in *S. apus* (Lyon, 1901). About the fate of jacket cells, Belajeff (1885), Millardet (1869) and Slagg (1932) have mentioned that they disintegrated into slime and the antherozoid mother cells, in the later stage the antherozoids floated in it. Pfeffer (1871) though described there is a layer of slime between spore coat and antherozoids, he did not explain the origin of the slime. Pfeffer (1871) thought even some jacket cells are primary spermatogenous cells in nature. The developmental pattern of microspore to gametophyte may vary in different species. Belajeff (1885) has classified ten species of *Selaginella* into two groups on the basis of microgametogenesis.

For the following years, however, the microgametogenesis in this genus has not interested botanists for a long time. Since the knowledge of microgametogenesis is based on light microscopic observation, little is known at submicroscopic level until Robert (1973) worked on *S. kraussiana*. Under EM survey Robert (1973)

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has described the cell number in jacket layer and spermatozoids, as well as the shape of prothallial cell.

The present work investigates the microgametogenesis of *S. tamariscina* from a mature microspore to the spermatid. The succeeding developmental details including the maturation and liberation of the spermatozoids have been revealed in the next report.

MATERIALS AND METHODS

Living plants of *Selaginella tamariscina* (Beauv.) Spring were procured from Luh-Shui, Hualien in June, 1987. They were then planted in the shading house of Botany Department, NTU. Microspores were collected, sterilized and grown in axenic culture on 1% plain agar. Both cultured and naturally grown microspores containing endosporic gametophytes were fixed every two weeks in glutaraldehyde and postfixed in osmium tetraoxide, dehydrated in acetone series and embedded in Spurr's resin. Sections were stained with methanolic uranyl acetate and lead citrate, and observed with a Hitachi H-600 or JEOL JEM 1200 EX II TEM. The main purpose of the present study is to trace the cell lineage in the early gametogenesis. The spore wall is very hard and the newly formed walls are extremely thin. We have difficult time in preserving all the structures such as cytoplasm, cell wall and spore wall in the same preparation. Therefore we have showed some micrographs with emphasis merely on the newly formed cell wall and ignor the resolution of other structures.

For scanning electron microscopy, microspores were fixed in the same fixatives as for TEM study. After dehydration in acetone, critical point dried and coated with a thin layer of gold, they were viewed and photomicrographed with a Hitachi S-520 SEM.

RESULTS

The majority of microspores inside the sporangium have attained maturity. The microsporangia are oval in shape, 0.1×0.08 cm in size and reddish in color (Pl. I-1, 2). The sporangial wall exhibits two-layered. The outer wall comprises one to two cells, whereas there is one row of cells in inner sporangial wall (Pl. I-6, 7). The cells in outer sporangial wall are larger and more or less isodiametric in shape. They are characterized by containing several large amyloplasts, vacuoles and sparsely distributed small plastids (Pl. I-6, 7). The cells in inner wall are smaller and tabular, containing numerous plastids, mitochondria and small vacuoles. The tapetal cells have disintegrated as amorphous state and are almost empty in cellular contents (Pl. I-6, 7).

The mature microspores are monads, trilete, with radio-symmetry; 40-65×35-40 μm, exine reddish-orange, 0.5-1 μm thick (Pl. I-3, 4, 5).

At maturity, the nucleus of the microspore moves to the upper position near the proximal pole (Pl. II-1). Large and irregularly shaped vacuoles always occupy most of the lumen, but the rest of cytoplasm is still dense and composed of many organelles, such as ER, mitochondria, dictysomes, microbodies and plastids. Lipid droplets are numerous and very conspicuous (Pl. II-5).

As the spermatogenesis begins, the microspore divides into a smaller prothallial cell and a much larger antheridial initial (Pl. III-5, 6). The prothallial cell does

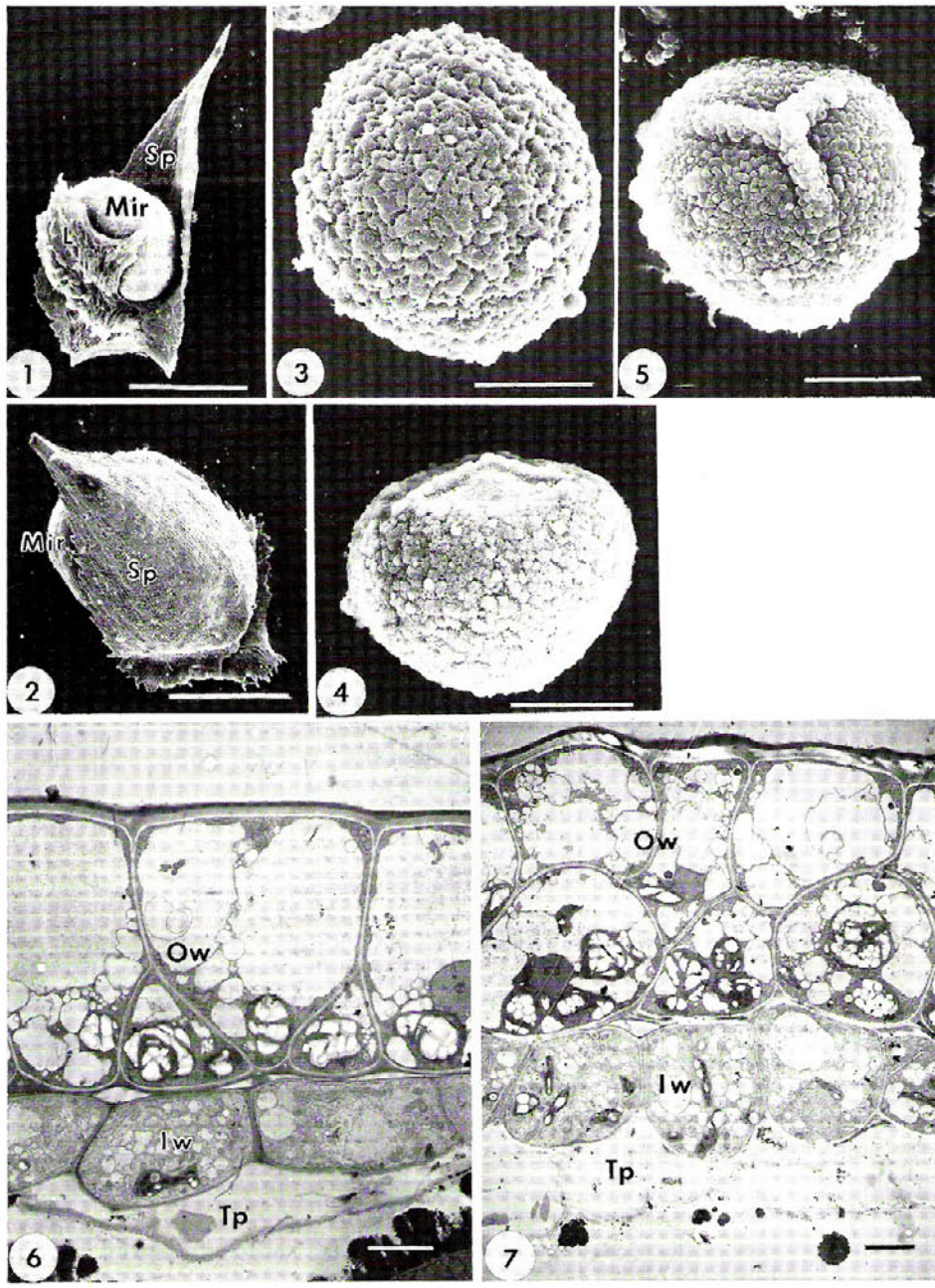


Plate I.

1: SEM. Adaxial view of a microsporangium (Mir) bearing microsporophyll (Sp). (L: ligule).
2: SEM. Abaxial view of a microsporophyll (Sp) and part of the microsporangium (Mir).
3-5: A Mature microspore under SEM. 3: dorsal view. 4: equatorial view. 5: proximal view.
6, 7: The sporangial wall of which the microspores inside have attained maturity.
Iw: inner wall, Ow: outer wall, Tp: tapetum.
1, 2: bar=380 μm; 3, 5: bar=20 μm; 4: bar=25 μm; 6, 7: bar=5 μm.

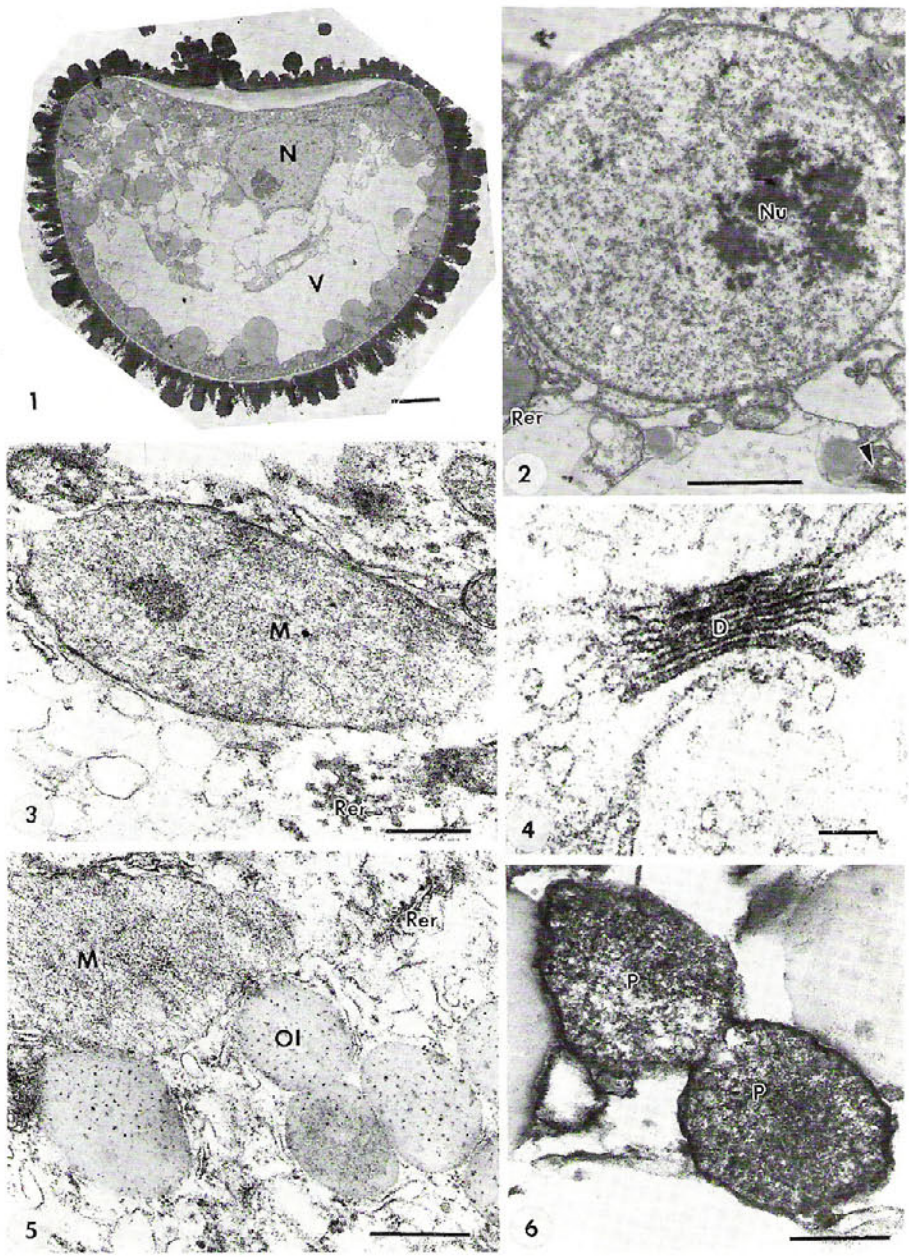


Plate II.

1: The sagittal section of a mature microspore (plane of section refer to Pl.IV-1). N: nucleus, V: vacuole.

2-6: The ultrastructure of the cellular contents of the mature microspore. 2: nucleus (N). Rer: rough endoplasmic reticulum, arrow head indicates dictyosome. 3: mitochondrion. 4: dictyosome (D). 5: oil droplets (Ol). 6: a dividing plastid (P).

1: bar=400 nm; 2: bar=250 nm; 4: bar=10 nm; 3, 5, 6=50 nm.

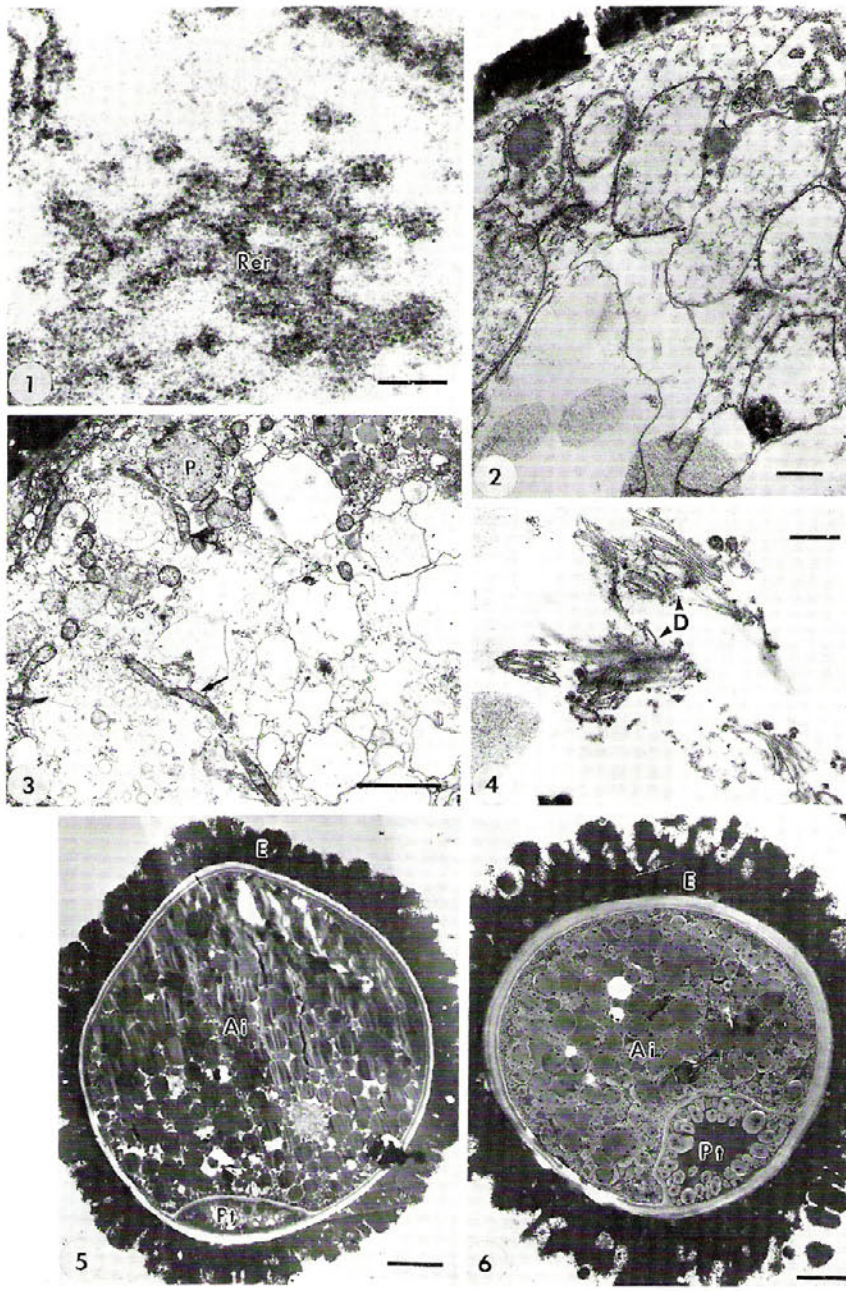
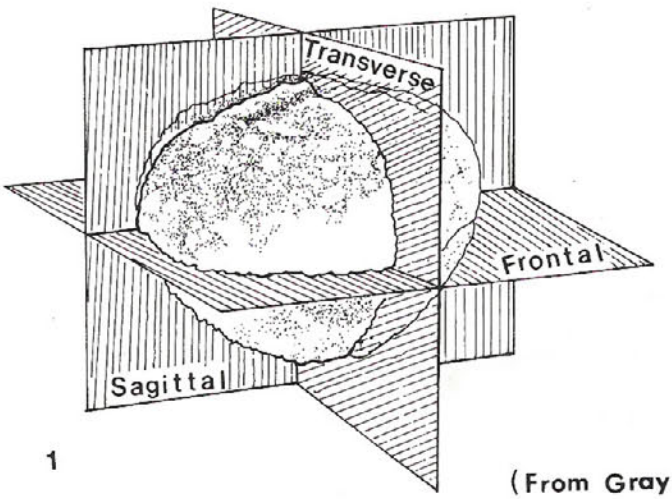


Plate III.

1-4: The ultrastructure of the cellular contents of the mature microspore.
1: rough endoplasmic reticulum (Rer). 2: vesicles. 3: numerous mitochondria (arrow) and plastid (P). 4: dictyosome (D).

5, 6: Young microgametophyte after the first division of microspore.
Ai: antheridial initial, Pt: prothallial cell, E: exine of spore wall.

1: bar=100 nm; 2, 4: bar=500 nm; 3: bar=2.5 μm; 5, 6: bar=5 μm.



1 (From Gray, 1964)

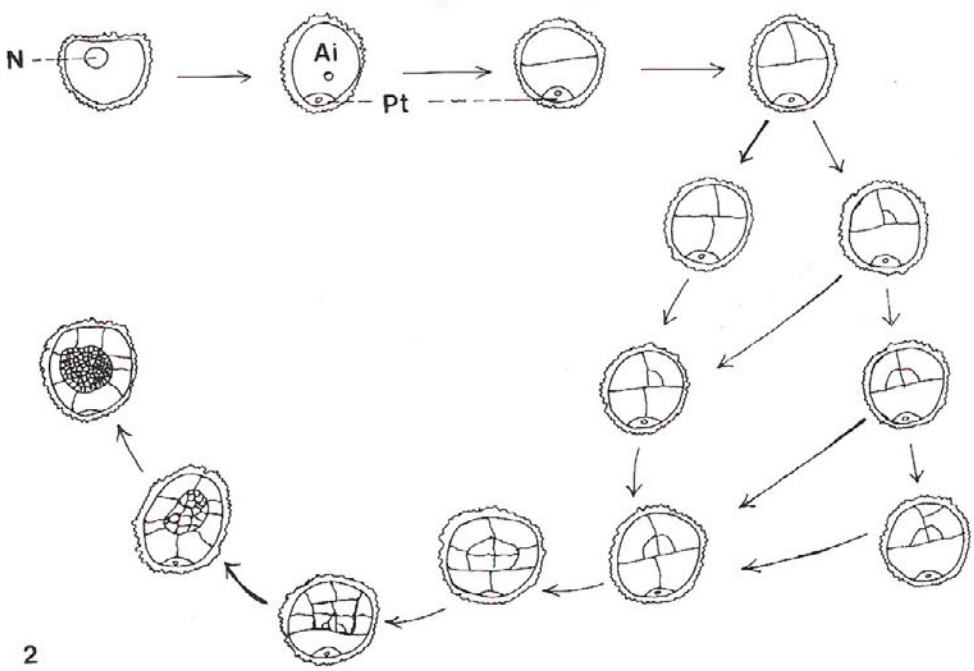


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Plate IV.

Drawings showing the series of sequential divisions in early microgametogenesis.

1: Diagram of different planes (from Gray, 1964).

2: The mature microspore in sagittal view showing the sequential stages of cell wall formation.

Ai: antheridial initial; N: nucleus; Pt: prothallial cell.

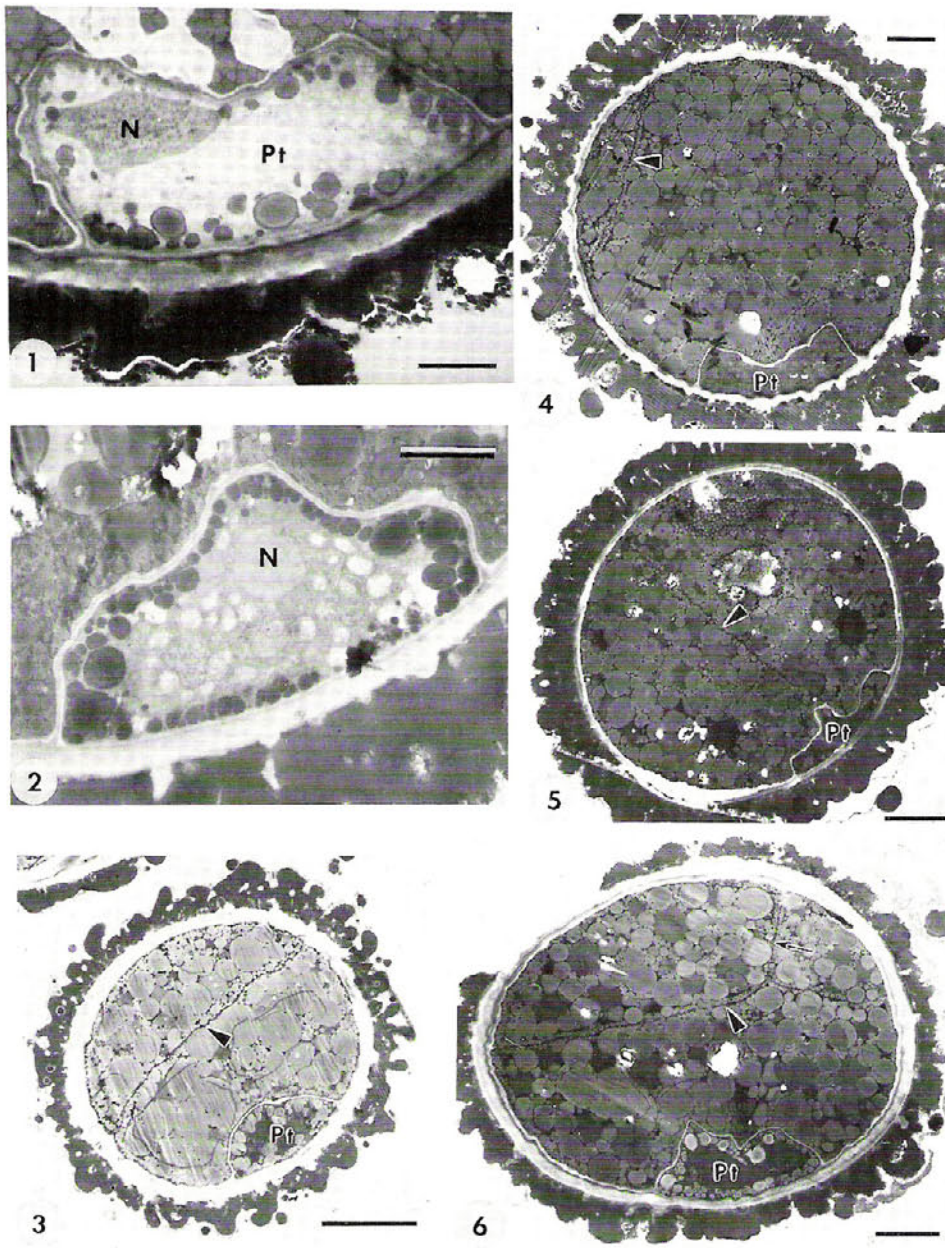


Plate V.

Sectional views of microspore.

1, 2: The prothallial (Pt) cell with different shapes. N: nucleus.

3-5: Showing the various planes of the first division (arrowhead) of antheridial initial. Mostly in parallel plane to the equator (3), sometimes oblique (4), or perpendicular and intersect the prothallial cell (Pt) wall (5).

6: Showing the second division (thin arrow) of antheridial initial (arrowhead: first wall).

1, 2: bar=2.5 μm; 3-6: bar=5 μm.

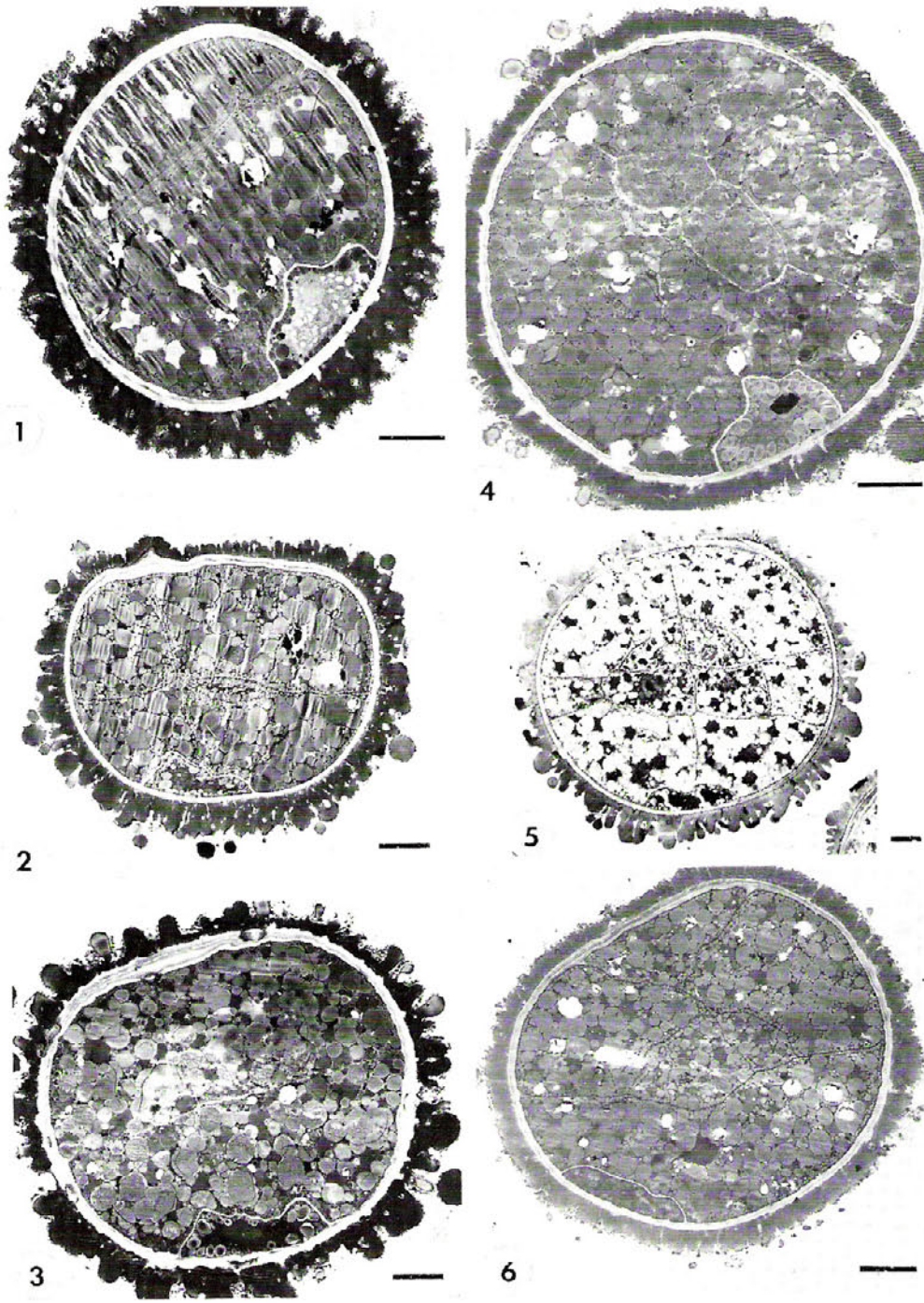


Plate VI.

Sagittal sections showing the early stages of microgametogenesis (refer to Pl. IV). bar=5 μm.

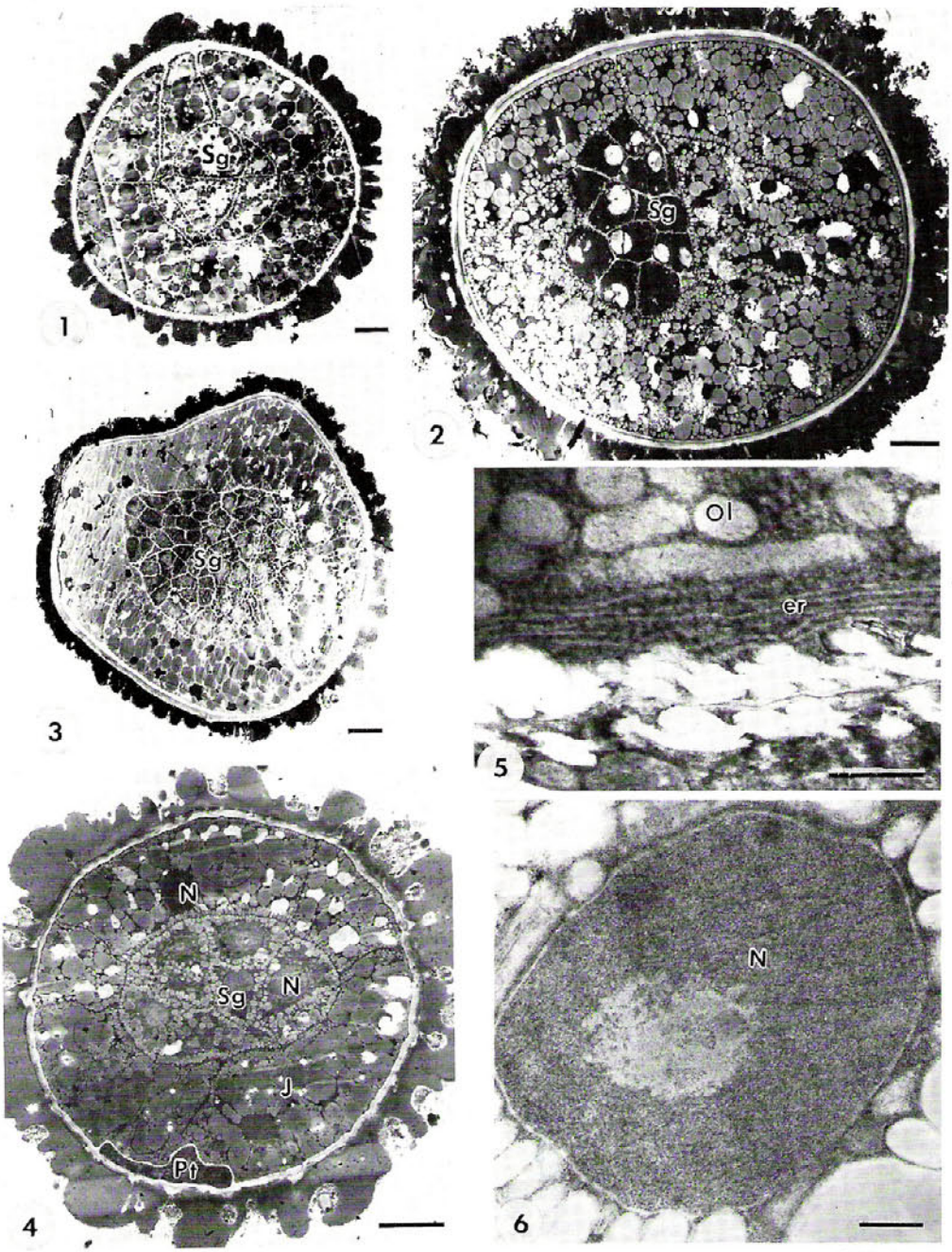
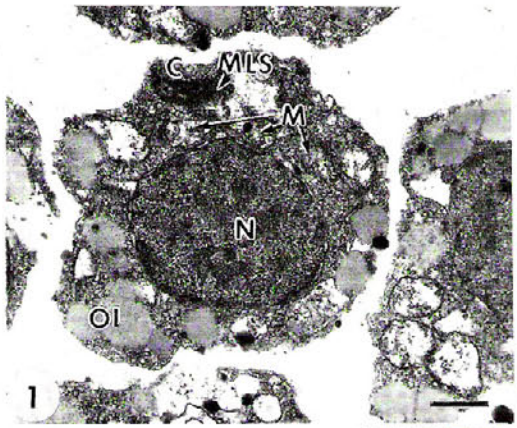


Plate VII.

1-3: Frontal sections.
4: Sagittal section of developing microgametophyte.
5: Endoplasmic reticulum (Er) and oil droplets of a spermatogenous cell (Sg).
6: Nucleus (N) of a jacket cell, Ol: oil droplet.
1-4: bar=5 μm; 5, 6: bar=0.5 μm.


C
MLS
M
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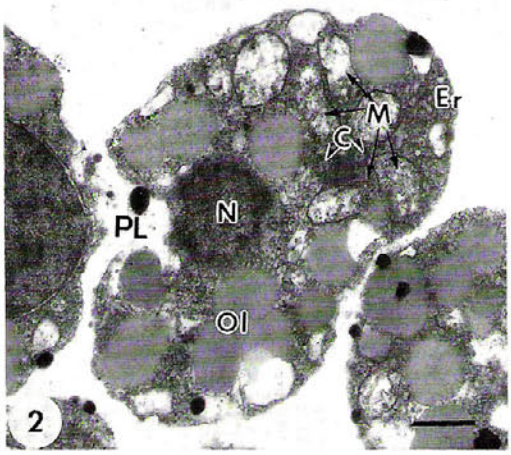
1


M
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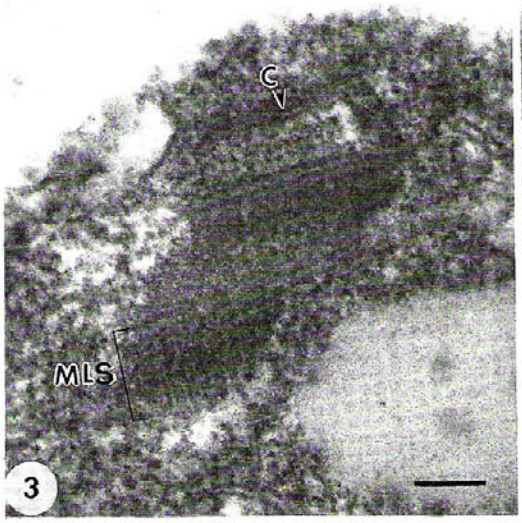
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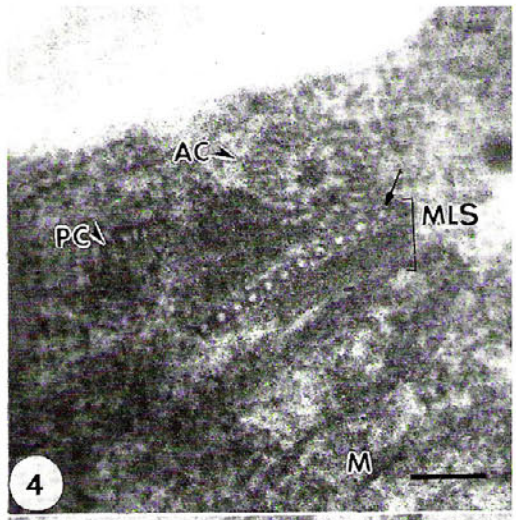
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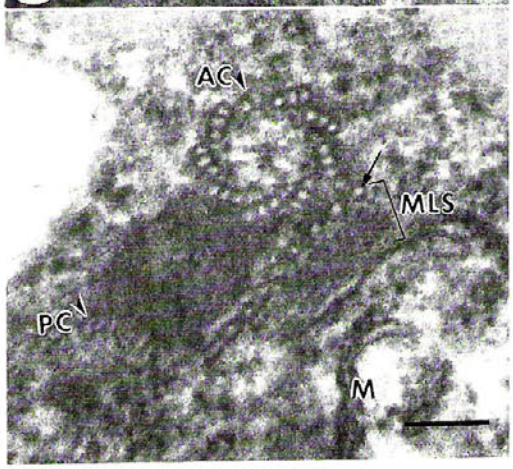
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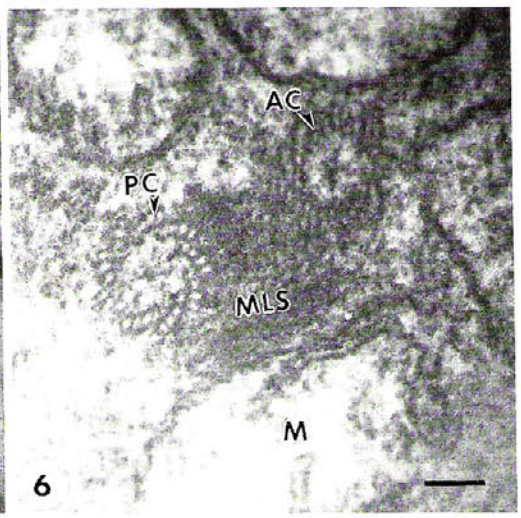
AC
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6

not divide any more. It remains intact throughout the stage while the majority of mature sperms are expelled and the jacket layer disintegrates. In other words, the prothallial cell maintains its figure during the entire stages of gametogenesis. The prothallial cell contains a nucleus, some plastids, protein crystals and lipid bodies. The lipid bodies always occupy the periphery of the prothallial cell (Pl. III-6, V). The prothallial cell in the majority of specimens appears to be lens-shaped, however different appearance has also been found (Pl. V). The antheridial initial divides very soon. In most cases, the new wall is formed parallel to the equator, accidentally oblique (Pl, V-4) or perpendicular to the equator, i.e. parallel to the polar axis, and meets the wall of the prothallial cell (Pl. V-5). In the present observation no further division takes place in the last pattern of antheridium. Generally, two daughter cells formed by the first division of the antheridial initial are somewhat unequal in size, with the basal (distal) one larger than the upper (proximal) one. The basal daughter cell may remain undivided for a long period or divide once to form two jacket cells. The upper one divides anticlinally resulting in three to four-celled male gametophyte (Pl. V-6; VI-1). The upper two newly formed daughter cells divide successively to form the primary spermatogenous cells and jacket initials (Pl, IV). A series of subsequent divisions occur rapidly in the primary spermatogenous cells and jacket initials, particularly the former. Consequently a large amount of smaller spermatogenous cells become surrounded by a layer of jacket cells. The cell number of the jacket layer is counted as eight and the size of each cell is much larger than the spermatogenous cell (Pl. VII). The total number of the spermatogenous cells has not been counted in this study. Both the spermatogenous cells and jacket cells are rich in food reserves. Protein bodies and lipid droplets are the main types of food reserves (Pl. VII-4). The hardness of the former always causes difficulty on sectioning. After a period of growth, some of the reserves are consumed so the nucleus and organelles become more evident (Pl. VII-4, 6).

Spermatid is cytoplasmic, with large spherical nucleus (as much as 50% of volume), several small mitochondria, a pair of centrioles and ER. Each centriole has nine imbricating triplets on the periphery (Pl. VIII). Its total length is about 40 nm and the diameter 17-20 nm. The distance between two centrioles is about 9 nm. The long axes of both centrioles run parallel with each other and are parallel to the cell surface as well. But one centriole exhibits slightly behind the other (Pl. VIII-4-6). The spermatid is abundant in mitochondria and some of which are located between the centrioles and nucleus (Pl. VIII-1). Lipid droplets are also abundant but always fewer than that in spermatogenous cell (Pl. VIII-1, 2).

Plate VIII.

1, 2: Young spermatids with centrioles (Ac: anterior centriole, Pc: posterior centriole) and multilayered structure (MLS). Note numerous mitochondria (M) located between the nucleus (N) and MLS. One of them will elongate in the subsequent stage.
Er: endoplasmic reticulum, Ol: oil droplet, Pl: plasmolemma.

8: Longisection through the MLS and centriole which is in the process of transforming into the basal body of flagellum.

4-6: Cross section through two centrioles and MLS, eleven microtubules arranging on S_1 layer (arrow); the clockwise imbricating triplet of two entrioles indicates their parallel orientation.

1, 2: bar=500 nm; 3: bar=125 nm; 4-6: bar=100 nm.

The multilayered structure (MLS) appears simultaneously beneath the centrioles and very close to it (Pl. VIII). The newly formed MLS comprises eleven microtubules in the S_1 layer (layer closest to centriole). The centrioles will soon act as the basal bodies of flagella (Pl. VIII-3). As two flagella emerge from these two basal bodies, spermatid gives rise to spermatozoid. The gametophyte is partly developed when the spores are shed. Various stages of development from two-celled gametophyte, the formation of prothallial cell to that of spermatids have been found at this time. The detailed metamorphosis of the spermatozoid will be presented in the succeeding paper in this series.

DISCUSSION

The position of prothallial cell seems to be a variable feature within this genus. The prothallial cell of *S. tamariscina* is constantly at the distal pole of microspore. This is not the case in *S. kraussiana* (Robert, 1973; Slagg, 1932). Slagg (1932) has observed the cell lineage in *S. kraussiana* and has also presented the detail drawings. Most of the later descriptions on the gametogenesis of this genus seemed to be based on Slagg's (1932) work (Eames, 1936; Gifford and Foster, 1988). The prothallial cell in *S. kraussiana* has been found to be at the lateral position (Slagg, 1932).

The longivity and the wall nature of the prothallial cell have also been discussed by some workers (Belajeff, 1885; Millardet, 1869; Pfeffer, 1871). Most of these workers have mentioned the presence of prothallial cell at the stage of spermatozoid discharge. On the other hand, no wall in prothallial cell has been seen in *S. apus* (Lyon, 1901). *S. kraussiana* possesses only a frail wall without cellulose (Slagg, 1932). In the present species the prothallial cell remains its figure during the entire stages of gametogenesis.

With a few exceptions, the early segmentation and the fate of each derivative of antheridial initial agree fairly with Slagg's (1932) report. However, the first wall formed in antheridial initial passes vertically through the main axis and meets the protherial cell in Slagg's (1932) material, whereas it does not come contact with the prothallial cell wall in the present species not only because of the different position of prothallial cell, but also the first division of antheridial initial goes transversely parallel to the equator. The proximal derivative formed by the first division of antherial initial gives rise to almost all the tissues in the mature antheridium except a part of jacket cells which have been derived from the distal derivative of antheridial initial. In several materials, the distal derivative divides once only, or it remains undivided to become one of jacket cells. Slagg (1932) stated that the cell number in the shedding time in the majority of microgametophytes has been counted as thirteen including one prothallial cell, eight jacket cells and four spermatogenous cells. The present observation shows that all stages of development from two-celled to flagellum-bearing spermatozoid materials are found. The resurrection nature of the plants of *S. tamariscina* is very distinct. Besides, the heterogenous population of tiny microgametophyte shows an excellent means for the species to live in the difficult enviroment.

As mentioned above, some developmental patterns are similar to that described by earlier workers. The present results reveal that it still lacks the developmental consistancy within the genus *Selaginella*.

Centrosomes occur in the spermatogenesis of bryophytes (Ikeno, 1897; Moser and Kreitner, 1970), pteridophytes (Duckett, 1973; Hepler, 1976; Robbins and Carothers, 1975; Robert, 1973; Sharp, 1912), gymnosperms (Guignard, 1898; Ikeno, 1897). Robert (1973) says that the intervention of centrosomes during spermatogenesis is a rule in archegoniates. The difference between spermatogenous cell and the subsequent spermatid mother cell (SMC) is the *de novo* generation of the paired centrosomes on the opposite side of the nucleus (Moser and Kreitner, 1970; Robbins and Carothers, 1975; Robert, 1973).

Centrosome of bryophytes (Moser and Kreitner, 1970) and lycopods (Robbins and Carothers, 1975; Robert, 1973) comprises a coaxial pair of centrioles, whereas a globe of procentrioles is characterized by multiflagellate-sperm-producing pteridophytes and gymnosperms. In bryophytes and lycopods, the last mitosis brings SMC into two daughter spermatids and each spermatid has a pair of coaxial centrioles. These two centrioles then reorient to have a parallel relation with their nine triplets imbricate in the same direction. Then they will transform into basal bodies of flagella and lead the spermatid to spermatozoid.

Beneath the contrioles there is multilayered structure (MLS) (Pl. VIII). It also generates *de novo* in the spermatid of those plants mentioned alone, i. e., bryophytes (Carothers, 1973; Carothers and Duckett, 1980; Carothers and Kreitner, 1967, 1968; Paolillo, Kreitner and Reighard, 1968a, b; Rushing and Carothers, 1986), lycopods (Robbins and Carothers, 1978; Robert, 1974), pteridophytes (Bell, 1974; Duckett, 1973; Duckett and Bell, 1969; Myles, 1975) and gymnosperms (Norstag, 1974). In *S. tamariscina* it is composed of four layers. Whether it presents in all the spermatids of the archegoniate is an interesting topic for further research.

The maturation of the spermatozoids, including the elongation of the nucleus, the elimination of cytoplasm, and the changing process of jacket cells for discharging spermatozoids, will be reserved for the succeeding paper in this series.

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萬年松雄配子之生成

I. 由小孢子至精原細胞的發育

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摘　要

本文研究萬年松 *Selaginella tamariscina* (Beauv.) Spring 由成熟的小孢子發育成精原細胞 (spermatid) 之過程。小孢子第一次分裂產生的原葉體細胞 (prothallial cell) 位於離心極(distal pole)，宿存。雄配子始源細胞 (antheridial initial) 第一次分裂之分裂面與小孢子之赤道面平行。精原細胞 (spermatid) 含有一對中心粒，中心粒下方有四層由微小管 (microtubule) 及微纖維 (microfibril) 組成之「多層結構」(multilayered structure)。