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EMBRYOLOGY AND SEED DEVELOPMENT IN *MAYACA FLUVIATILIS* (MAYACACEAE)

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SUMMARY

The embryology and seed structure of *Mayaca fluviatilis* was studied. The embryological and structural seed characters clearly support the assumption of a separate familial status of the Mayacaceae. Most of the embryological and seed characters fit well in with those of the other commelinaceous families, but also suggest more reticulate relationships. The Mayacaceae deviates from the other families by the absence of differentiated siliciferous endotestal and sclerotic exotegmic layers. The presence of an exotesta is probably an adaptation to the hydrochorous mode of dispersal.

1. INTRODUCTION

The small, monotypic monocotyledonous family of the Mayacaceae contains one genus: *Mayaca*. In the latest monograph (LOURTEIG 1952) the ten species recognised by PILGER (1930) and the twelve by HORN AF RANTZIEN (1946) have been reduced to four, most of them falling in the synonymy of the most common and wide-spread species *Mayaca fluviatilis* Aublet and *M. sellowiana* Kunth. *Mayaca* is found in warm, temperate and tropical America, with one species (*M. baumii* Gürke) in S-W. tropical Africa. All species are perennial, aquatic or amphibious herbs living in fresh water.

The stems are usually branched, the plants forming vegetation mats. The leaves are helically arranged, simple, linear and commonly with a bifurcate apex. The actinomorphous flowers are born solitarily. The perianth is trimerous and biseriate. The outer whorl is sepaloid and persistent in the fruit, the inner one petaloid with broad, delicate segments. The three stamens alternate with the inner whorl. The anthers are tetrasporangiate and usually open with an apical pore or pore-like slit, sometimes with a tubular, apical appendage. The superior ovary is three-carpellate and unilocular, with three parietal placentae. The orthotropous, bitegmic ovules are arranged in two rows. The fruit is capsular and three-valved. The seeds are ovoid to globose, minutely beaked and scrobiculate. The small embryo is situated at the top end. The endosperm is farinaceous (HEYWOOD 1978, DAHLGREN *et al.* 1984).

The extensive literature on the anatomy has been reviewed and augmented by TOMLINSON (1969). Although *Mayaca* shares several characters with the Commelinaceae and Eriocaulaceae, the singular anatomy of *Mayaca* is largely

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an adaption to its aquatic way of life, so that any information about its systematic position and possible phylogeny is wholly obscured. According to TOMLINSON (1969) it is probable that *Mayaca* originated from the same stock that produced the Commelinaceae, Eriocaulaceae and Xyridaceae. From a more recent study on the occurrence and specialization of vessels in Commelinales, CHEADLE & KOSAKI (1980) concluded that *Mayaca* could possibly fit into Commelinaceae at one extreme of the family, or stands close to it.

The pollen grains are monosulcate with a finely reticulate exine sculpturing. The pollen wall texture of *M. fluviatilis* is tectate to intectate and exhibits many similarities to the pollen wall structure found in some Commelinaceae (ZAVADA 1983).

The embryology of the Mayacaceae had not previously been studied (DAVIS 1966), except for some scattered data: tenuinucellate ovules, a monosporic embryo sac, anthers with exothecium (POULSEN 1886, HORN AF RANTZIEN 1946, STEVENSON 1983). The seed coat is mainly formed by the outer layer of the outer integument. The presence of an operculum (stopper or embryostega) has erroneously been recorded for the seed by several authors (HAMANN 1961).

2. MATERIALS AND METHODS

Young flower buds, gynoecia and developing fruits of *Mayaca fluviatilis* Aublet were collected in a small stream in Serra do Cipó, MG, Brazil. The material was fixed in FAA 50%, dehydrated in a normal butyl alcohol series, embedded in glycolmetacrylate, sectioned at 5 μm with glass knives, stained with periodic acid-Schiff (PAS) reagent and counter-stained with Eastman's hematoxylin. Phloroglucinol-HCl, Sudan IV, ruthenium red, iodine in potassium iodide solution, absolute alcohol saturated with picric acid and nigrosin dye were used for specific tests.

For the SEM studies mature seeds were sputter-coated with gold-palladium for 3 min. Ovules, buds and some of the seeds were first critically point-dried with liquid CO_2. Mature seeds were investigated for the presence of silica by X-ray microanalysis.

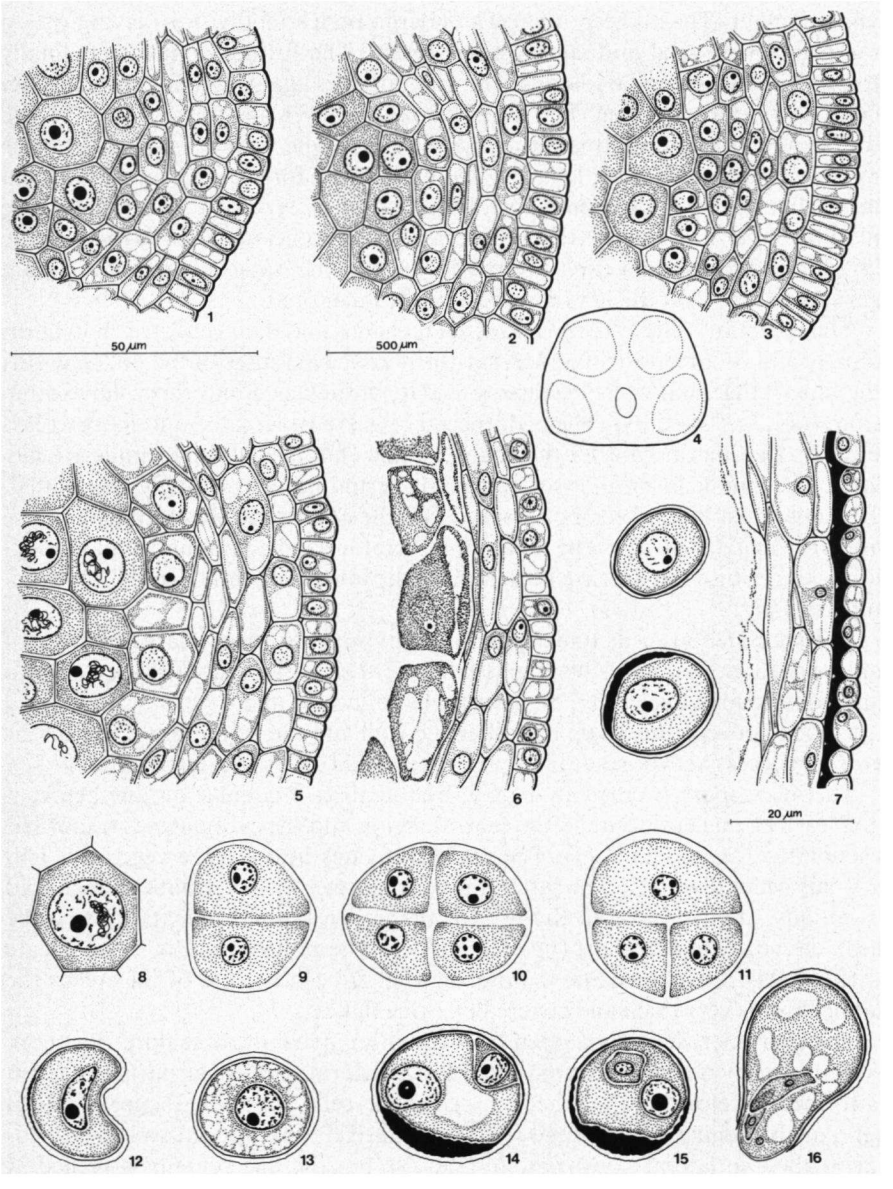
Voucher material of *M. fluviatilis* was collected by Amaral and Venturelli CFSC 9261, 9264 and deposited in SPF.

Mature seeds were obtained from herbarium sheets of *M. baumii* Gürke, Lisowski 191 (BR); *M. longipes* Mart. ex Seubert, de Granville 666 (U) and *M. sellowiana* Kunth, Lindeman, Haas & Hatschbach 13762 (U).

3. RESULTS

3.1. Microsporangium, microsporogenesis and male gametophyte

The anther is tetrasporangiate with a single amphicribral vascular bundle. The young anther consists of meristematic tissue with a well-defined dermal layer. A group of hypodermal cells differentiates near each of the four corners of the



Figs. 1–16. *Mayaca fluviatilis*, microsporangium and male gametophyte.
Figs. 1–7: transections of parts of anthers at various stages of development, to show formation of wall layers, Fig. 4 is a transect of mature anther. Fig. 8: microspore mother cell; Fig. 9: dyad; Figs. 10–11: isobilateral and decussate tetrads, respectively; Figs. 12–13: microspores; Figs. 14–15: pollen grains at successive stages of development; Fig. 16: germinating pollen grain (50 µm scale for figs. 1–3 and 5–7, 500 µm for fig. 4 and 20 µm for figs. 8–16, respectively).

young anther. The archesporial cells undergo periclinal divisions giving rise to the primary parietal and sporogenous layers. The former divides periclinally to produce two secondary layers. The outer divides again, forming the endothecium and a middle layer. The inner secondary layer also divides to give rise to the tapetal layer and to another middle layer. One final division takes place mainly in one of the middle layers resulting in the formation of an additional layer. The anther wall formation follows the Basic type of DAVIS (1966). The mature anther wall consists of an epidermis, an endothecium, two to four middle layers and the tapetum (*figs. 1–7*). The walls of the outer microsporangia are generally somewhat thicker than those of the inner ones.

The epidermis cells undergo anticlinal divisions and their cells stretch longitudinally and become vacuolated. After the uninucleate stage of the pollen grains the inner tangential walls become reticulately thickened and form the exothecium (*figs. 7,19* and *29*). The endothecial cells are vacuolated and more or less tabular. In a mature anther the characteristic endothecial thickenings are lacking. The middle layers elongate tangentially and become strongly vacuolated. The innermost layers become resorbed and the outermost persist in the mature anther (*figs. 6, 7* and *19*). The tapetum is secretory and compounded of uninucleate cells. Ubisch bodies are present near the inner tangential wall of the tapetum cells (*figs. 5–7* and *31*).

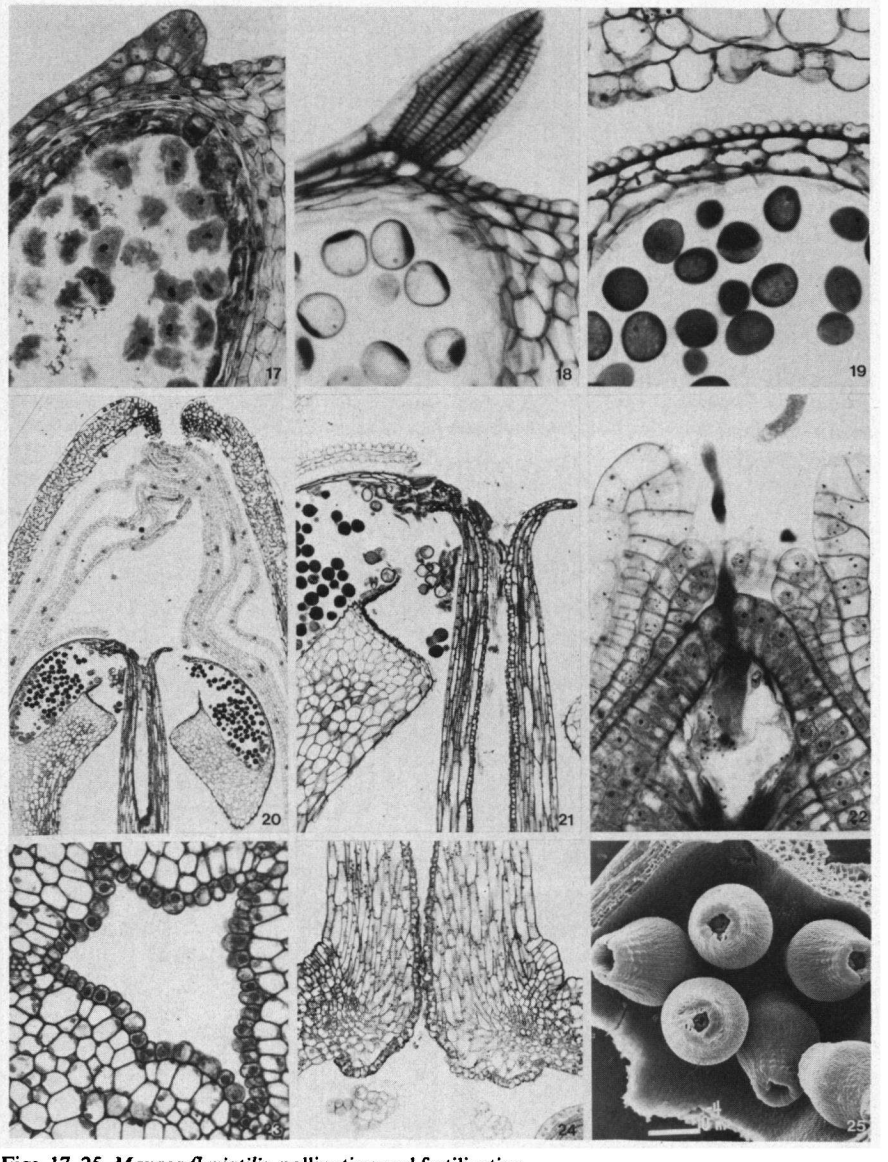
The sporogenous cells undergo mitotic divisions to form a large number of microspore mother cells, distinguishable by their large size, dense cytoplasm and conspicuous nuclei (*fig. 8*). These cells round off and a special callose wall is formed between the original mother cell wall and the cytoplasm. Cytokinesis is of the successive type resulting in isobilateral and decussate tetrads (*figs. 9–11*).

The microspore is crescent-shaped, but acquires a circular outline before it divides. The nucleus occupies the central region and the cytoplasm is somewhat vacuolated (*figs. 12* and *13*). The nucleus divides into a large vegetative and a small generative cell. The latter shows a distinct cytoplasmatic sheath and is initially situated close to the wall of the pollen grain. Later it comes to lie near the vegetative nucleus (*figs. 14* and *15*) The pollen grain is monosulcate with a finely reticulate exine sculpturing (*fig. 30*). At the site of the sulcus the intine layer is very thick and covered by exine flakes.

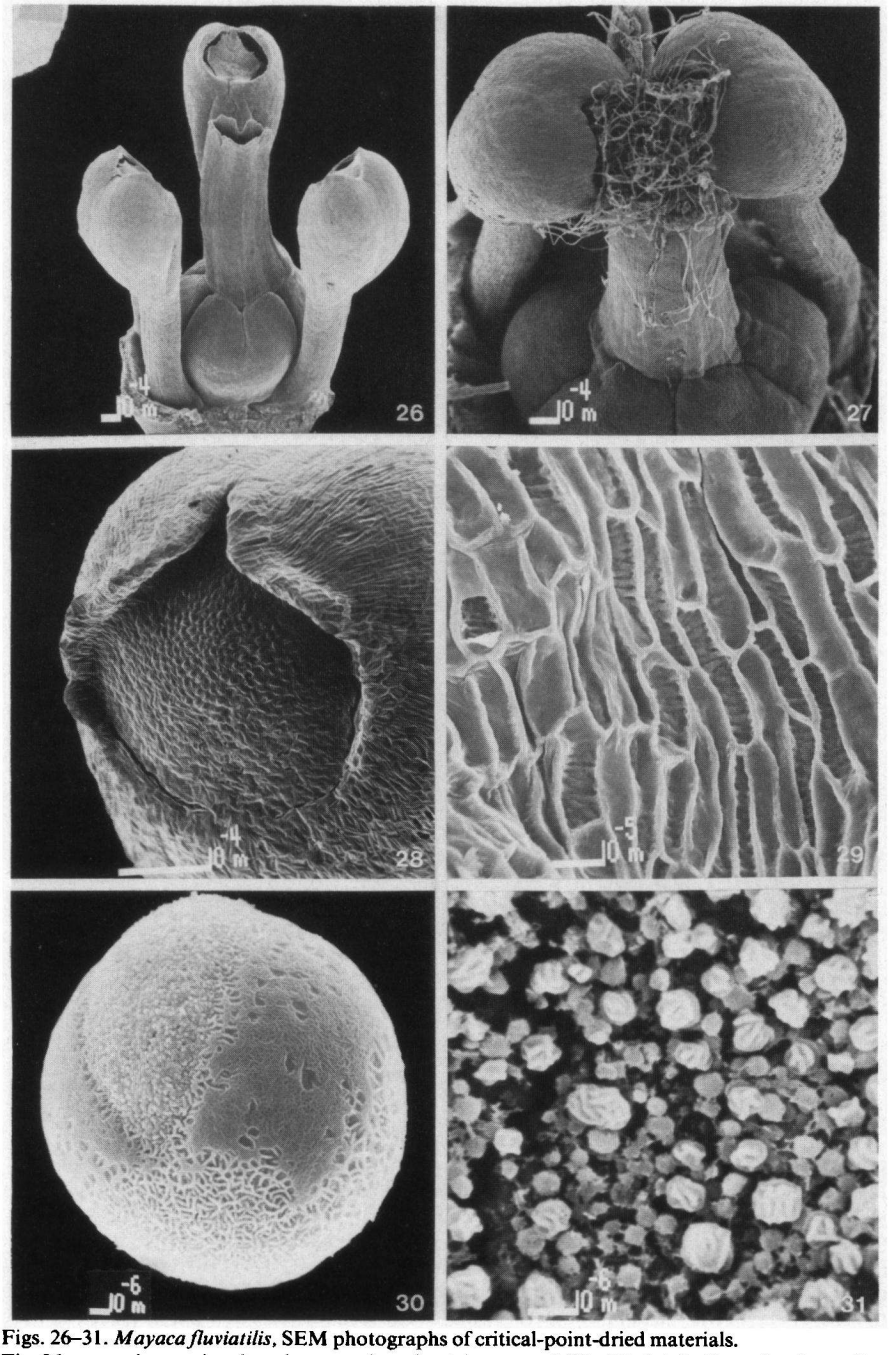
The four microsporangia open by a common apical introrse pore. The pore is bordered by a rim most prominent in the lateral and upper parts. This rim is formed by elongated exothecium cells. The cells are strengthened by local cell wall thickenings (*figs. 17* and *18*). The mature, desiccating anther starts opening at the two lateral sides (*figs. 26* and *28*), but the flap remains attached at the base and moves inwards. The epidermal cells of the flap differ from those of the exothecium; they are polygonal and their walls are not thickened.

3.2. Pollination

The flowers are partly cleistogamous (*figs. 20, 21* and *27*). Pollen germination starts inside the anther locule more or less simultaneously with the mitotic division of the generative cell. The sperm cells are fusiform with a condensed nucleus



Figs. 17–25. *Mayaca fluviatilis*, pollination and fertilization.
Figs. 17–18: longisections of the young and mature anther, respectively, showing the differentiation of the rim structure of the pore (× 130); Fig. 19: photographic evidence in support of fig. 7, transection of the mature microsporangium. The exothecium is clearly discernible (× 130); Fig. 20: longisection of the flower showing the cleistogamous condition (× 15); Fig. 21: detail of fig. 20 with germinating pollen tubes (× 35); Fig. 22: fertilization, the persistent synergid is visible at the right side (× 165); Fig. 23: transection through the stylar canal, showing the transmitting tissue (× 130); Fig. 24: longisection of the flower, showing the structure of the transmitting tissue at the top of the ovary (× 40); Fig. 25: SEM of critical-point-dried ovules.



Figs. 26–31. *Mayaca fluviatilis*, SEM photographs of critical-point-dried materials.
Fig. 26: gynoecium and androecium, sepals and petals removed; Fig. 27: detail of germinating pollen tubes; Fig. 28: closed pore; Fig. 29: exothecium, the outer walls collapsed; Fig. 30: pollen grain; Fig. 31: Ubisch bodies.

surrounded by a layer of cytoplasm (*fig. 16*). The germinating pollen grows in the direction of the tip of the style, the tubes subsequently growing ectotrophically along the stylar transmitting tissue (*figs. 20* and *21*).

The linear style is divided into three small stigmatic lobes. The style is hollow by being traversed by an in cross-section triangular to circular canal tapering towards the base. The stylar canal is lined with a glandular, papillate epidermis extending towards the apices of the stigmatic lobes (*figs. 20, 21, 23* and *26*). At the top of the ovary, where the stylar canal enters the ovary locule, the tissue of the ovary wall is swollen and projects into the locule. In these regions the subdermal cells appear as a spongy parenchyma and are covered by a continuous transmitting tissue (*fig. 24*). Also the epidermal cells of the placenta between the ovules and the funicle base look rather similar to those of the stylar transmitting tissue. The outer layer of the ovule does not show these characters.

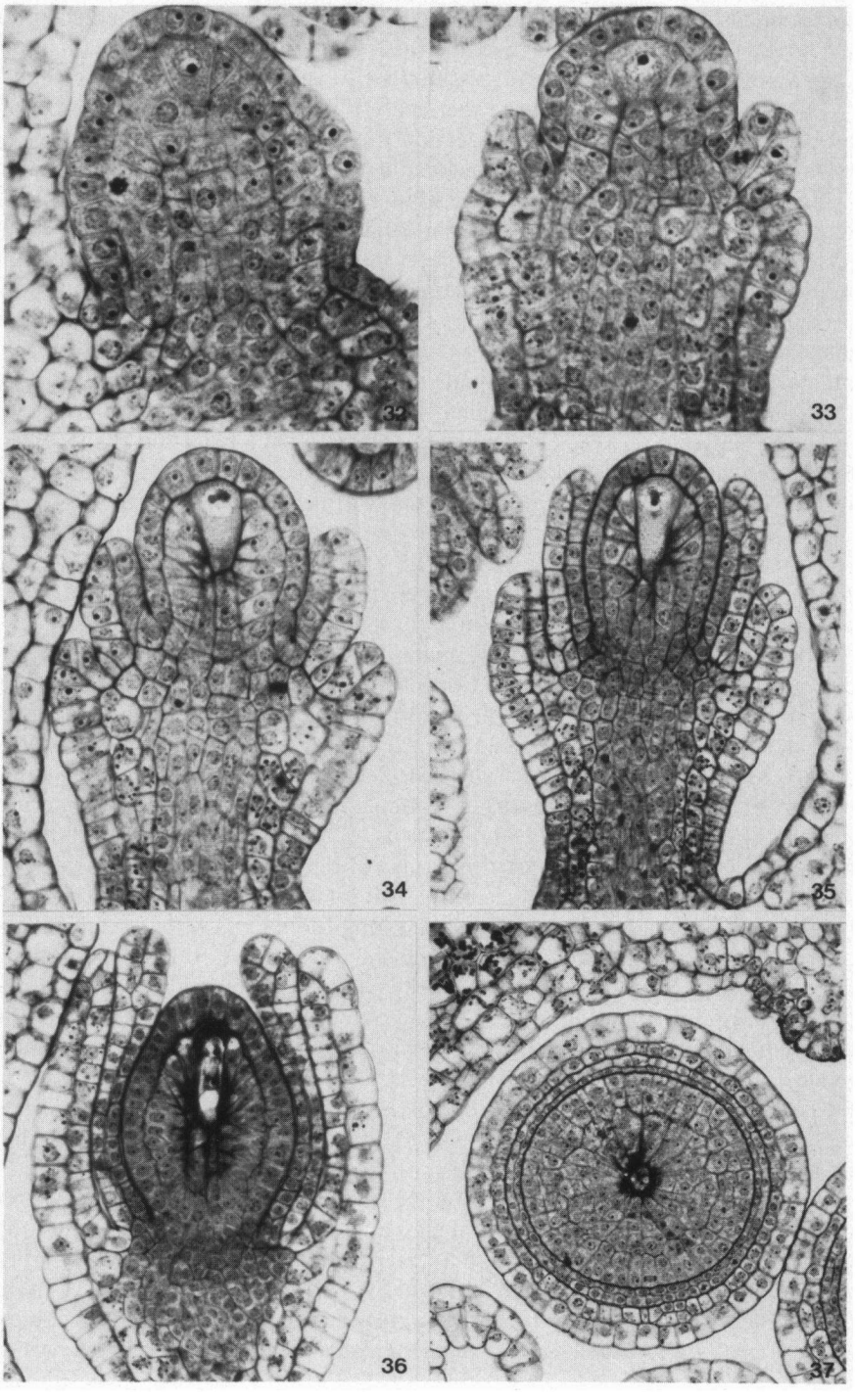
Within the ovary locule the pollen tubes do not follow a fixed pathway as might be suggested by the cytological characters of the placental tissue. From the place where the stylar canal opens into the locule the pollen tubes spread inside the space between the ovules and the ovary wall.

3.3. Ovule ontogeny

Mayaca fluviatilis has a superior, tricarpellary and unilocular ovary. The ovules are arranged in two or sometimes three rows on three parietal placentae.

The ovule primordium has a trizonate build-up. The two tunica layers, the dermatogen and subdermatogen, respectively, enclose the corpus. The archesporial cell is already discernible at an early ontogenetic stage by its fairly large size, rich cytoplasm and conspicuous nucleus. It does not divide and functions directly as megaspore mother cell (*fig. 32*). The initiation of the inner integument begins as a slight swelling resulting from a radial enlargement of the dermal initials. The inner integument originates as a complete ring wall by periclinal and oblique divisions in two or three rows of dermal cells and starts growing as a two-layered structure, but occasionally it is locally three-layered in its lower half (*figs. 32–37*). The cells are small and longitudinally a little stretched. The outer integument is initiated shortly after the inner one, also by divisions confined to dermal cells. It is mainly two cell layers thick, sometimes locally three-layered in its lower half. The cells of the outer layer have clear vacuoles and are relatively large. The outer integument overgrows the inner one during the ovule development (*figs. 33–36* and *45*). Already before the divisions of the initials of the inner integument, periclinal divisions occur in the underlying subdermal cells. The subdermal cells only lift the base of both integuments but they do not take part in their formation.

The nucellus is initially covered by a one-layered epidermis. During megasporogenesis the subdermal layer stretches and undergoes periclinal divisions, especially in the middle part and to a lesser extent at the base. The subdermal cells adjoining the embryo sac remain undivided. In this way a massive tissue consisting of radially oriented cells originates and the nucellus assumes a conical shape (*figs. 35* and *36*). The cells of the corpus occupying the central part of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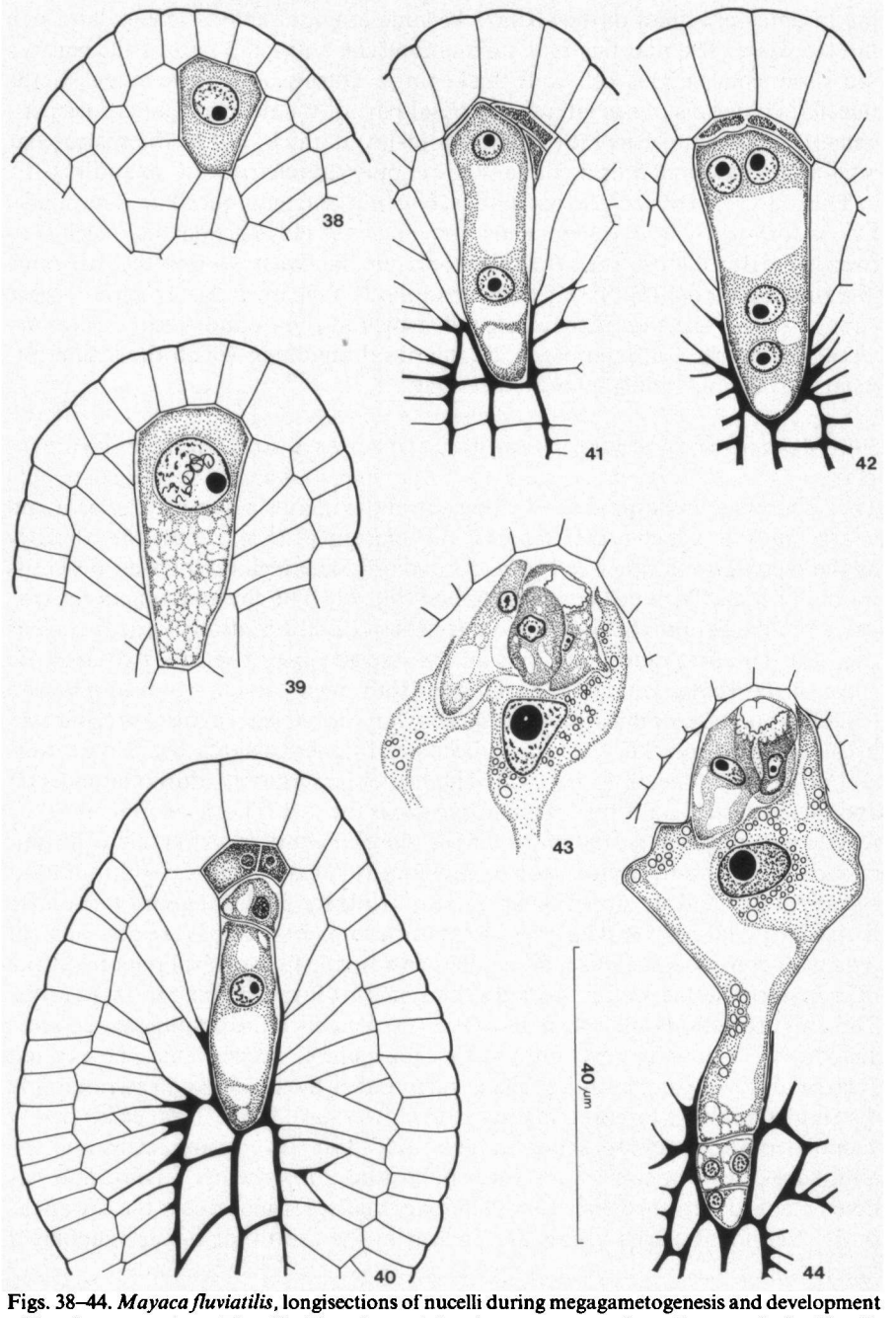
the nucellus proximal to the chalaza become longitudinally stretched and rich in cytoplasm. The nucellus cells surrounding the antipodal part of the embryo sac show conspicuous cell wall thickenings. During megagametogenesis the nucellar epidermis of the lateral and basal part of the nucellus undergoes periclinal divisions and becomes three to four-layers thick, but in the micropylar region the epidermal cells do not divide but only stretch (*figs. 35, 36* and *45*).

The full-grown ovule is orthotropous, bitegmic and tenuinucellate. The micropyle is formed by both integuments, the inner one closing over the nucellus to form a narrow endostome. The outer one remains open, so that the exostome is a broad channel (*fig. 25*). The endostome is Y-shaped; the exostome more circular in cross-section. The funicle is conspicuous, thick and, like the placenta, already contains a differentiated amphicribral bundle in which the xylem elements have annular and helical thickenings.

3.4. Megasporogenesis, megagametogenesis and female gametophyte

The subdermal archesporial cell elongates and contains a dense cytoplasm and a large nucleus, which is situated near the micropylar pole. It functions directly as the megaspore mother cell (*figs. 38* and *39*). The meiotic division proceeds normally and a T-shaped tetrad is formed (*fig. 40*). The chalazal functional megaspore enlarges and shows a vacuole at each side of the centrally located nucleus (*fig. 40*). The first mitotic division of the nucleus gives rise to a two-nucleate stage of the young embryo sac (*fig. 41*). Both nuclei divide again to give rise to a four-nucleate embryo sac. The micropylar and chalazal nuclei are separated by a large vacuole. The cytoplasm becomes thinner towards the lateral walls and denser near the poles (*fig. 42*). The nuclei undergo an additional division and ultimately an eight-nucleate embryo sac is formed. The development of the female gametophyte is, therefore, of the *Polygonum* type. After the eight-nucleate stage the micropylar quartet becomes re-arranged into the three-celled egg apparatus and the upper polar nucleus, while the chalazal quartet gives rise to the antipodal cells and the lower polar nucleus. The synergids are pear-shaped cells with conspicuous nuclei and a distinct vacuole in the basal region. At the micropylar pole the wall is strongly thickened to form the filiform apparatus. The wall projections are in two distinct layers, of which the peripheral one gives a strong PAS-positive reaction and the inner one is translucent. The egg cell is large and elongate, with a nucleus surrounded by small vacuoles randomly distributed in the cytoplasm, mainly in the lower part. The central cell is highly vacuolated and its cytoplasm is concentrated near the egg apparatus and the antipodal cells. The upper and lower polar nuclei fuse before fertilization has taken place to form a conspicuous secondary nucleus situated near the egg apparatus. Abundant starch grains are present in the central cell. The vacuolated

Figs. 32–37. *Mayaca fluviatilis*, LM photographs of ovule primordia and young ovules showing initiation of integuments, both are formed out of the dermal cells (figs. 32–33: × 390; fig. 34: × 325, figs. 35–36 × 260). Fig. 37: transection of the mature ovule with mainly two-layered integuments (× 160).



Figs. 38–44. *Mayaca fluviatilis*, longisections of nucelli during megagametogenesis and development of female gametophyte. Figs. 38–39: archesporial and megaspore mother cell, respectively; Fig. 40: T-shaped tetrad; Figs. 41–42: two- and four-nucleate embryo sacs; Fig. 43: egg apparatus and secondary nucleus; Fig. 44: organized female gametophyte.

antipodal cells are arranged in a T-shape fashion and degenerate during endosperm development. The antipodals are enclosed in a pouch formed by thickened nucellar cells (*figs. 43* and *44*).

The pollen tube enters the ovule through the micropyle and subsequently moves along the nucellar epidermis to penetrate one of the synergids through the filiform apparatus (*figs. 22* and *45*). The pollen tube persists till after a few free endosperm nuclei are formed.

3.5. Seed development and mature seed structure

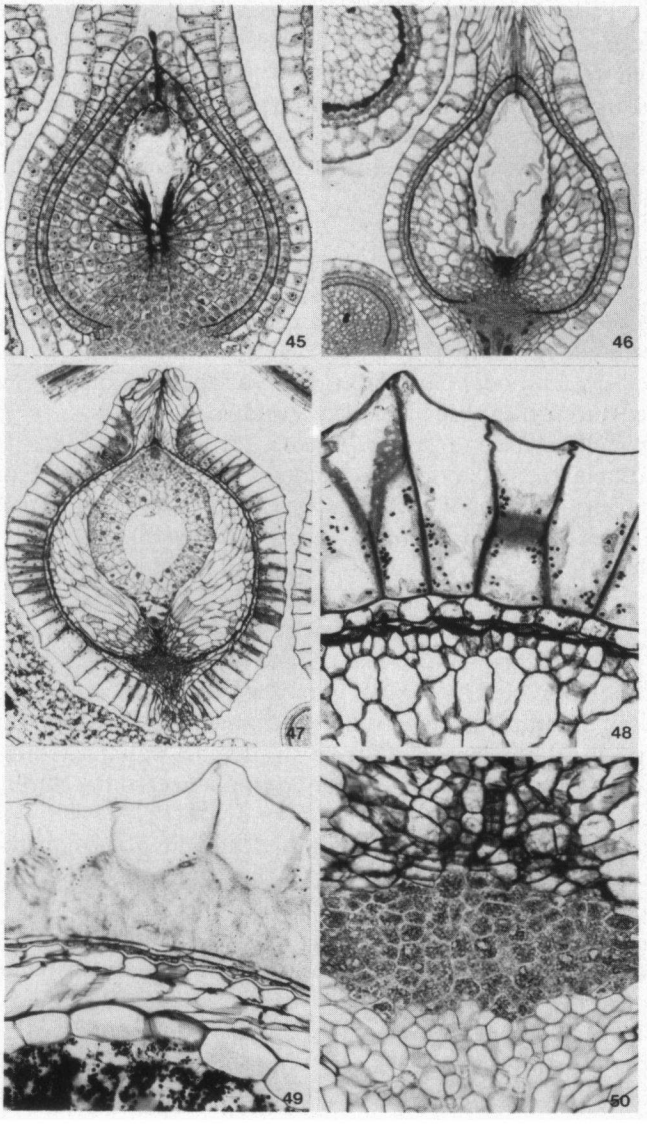
The growth of the embryo sac is accompanied by a stretching of the peripheral nucellar cells. The nucellar cells elongate radially. The outermost dermal cells of the nucellus do not stretch (*figs. 46* and *47*). The thickened nucellar cells which surrounded the antipodal part of the embryo sac in the mature ovule can still be observed during the subsequent seed development. This tissue seems to inhibit the enlargement of the endosperm (*figs. 45–47*). During the later phases of endosperm development the nucellus becomes partly compressed and resorbed. At the periphery of the embryo the nucellus disappears completely. At the lateral and basal sides of the endosperm the nucellar remnants are clearly discernible, also in the mature seed. The nucellus has a manifest cuticle. The cells remain thin-walled and highly vacuolated and do not accumulate storage material (*fig. 49*). The nucellar cells between the chalaza and the embryo sac are of a somewhat different nature by remaining smaller, and by having strongly PAS-positive walls. They are bordered by the tanniniferous hypostase.

The cells of the inner integument elongate tangentially but remain very narrow in the radial direction; they do not form any distinct wall thickenings. The inner layer becomes tanniniferous. The outer layer is squeezed flat during the further development (*figs. 47–49*).

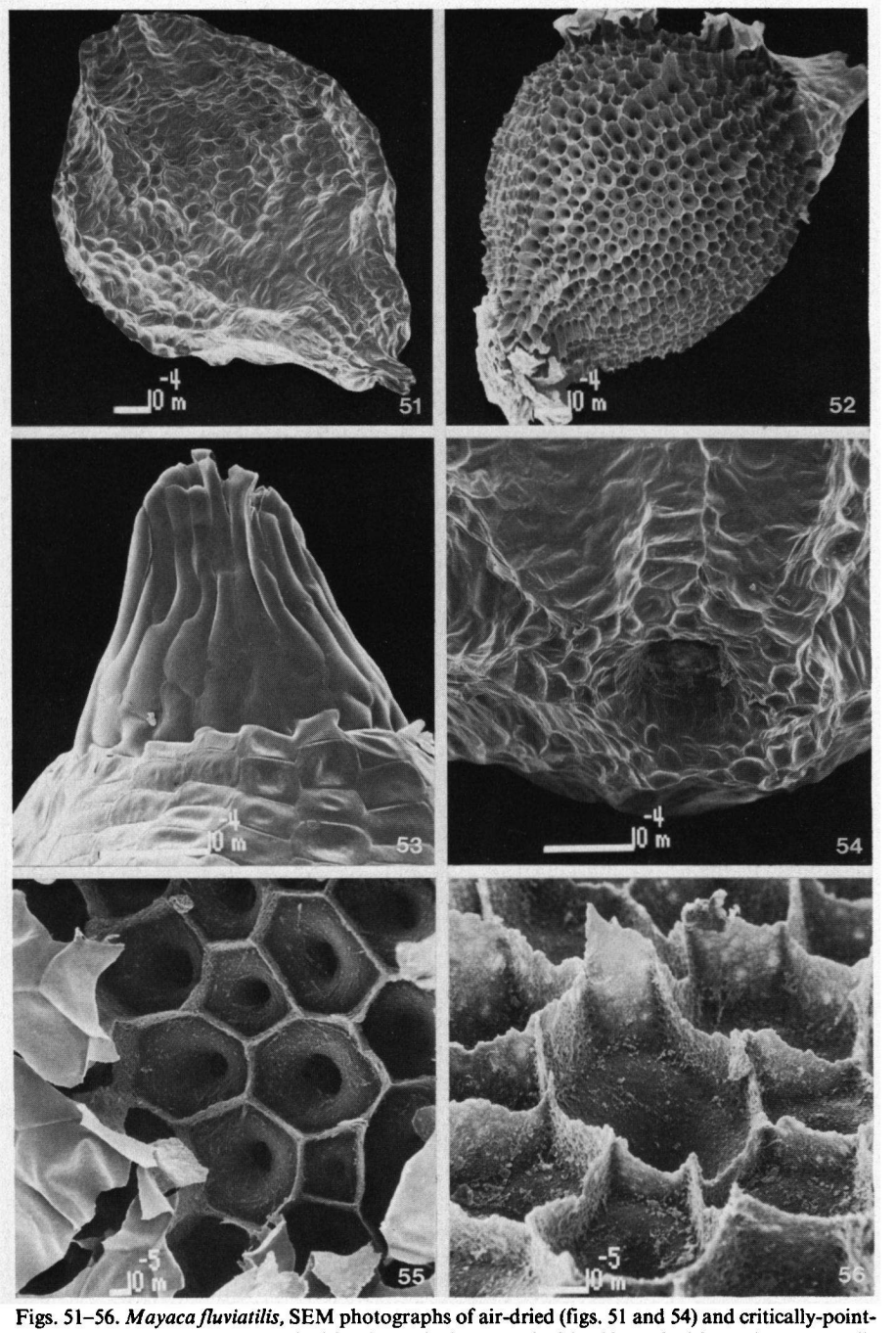
The cells of the outer layer of the outer integument do not divide any longer; they increase greatly in size, especially in the radial direction and accumulate starch grains. The lateral and inner tangential walls become strongly thickened. The strongly pitted cell walls contain lignin. The distal part of the lateral walls are less strongly thickened (*figs. 55* and *56*). The outer walls remain very thin and do not show a distinct cuticular layer (*figs. 47–49*). The inner layer of the outer integument is still preserved in the mature seed. The cells are longitudinally elongate but do not exhibit any special characters. There is no manifest cuticle between the inner and outer integument (*figs. 48* and *49*). No silica could be detected in the seed coat.

The tegumentary layers show a special differentiation in the micropylar region. The inner layer of the outer, the outer layer of the inner and to a lesser extent also the inner layer of the inner integument undergo an appreciable radial elongation to close the exo- and endostome. Only the cells of the inner layer of the outer integument have slightly thickened walls (*figs. 45-47*).

Within the chalazal tissue a hypostase consisting of several layers of tanniniferous cells is formed (*fig. 50*). This tissue is continuous with the tanniniferous endotegmen.



Figs. 45–50. *Mayaca fluviatilis*, LM photograph of seed coat development.
Fig. 45: ovule at the time of fertilization, the nucellar epidermis remains one-layered at the micropylar region and is three-four cells thick at the lateral and basal part (× 120); Fig. 46: young seed shortly after fertilization, endosperm is nuclear, the endo- and exostome are closed (× 50). Fig. 47: developing seed, the following structures are discernible from outside: outer and inner integument; nucellus; cellular endosperm and globular embryo (× 6). Fig. 48: longisection of the seed coat and nucellus (× 120), corresponding to the fig. 47. Fig. 49: transection of the mature seed coat, showing the thickened exotestal cells, the cuticle between nucellus and inner integument is clearly discernible (× 120); Fig. 50: detail of chalazal region with tanniniferous hypostase (× 190).



Figs. 51–56. *Mayaca fluviatilis*, SEM photographs of air-dried (figs. 51 and 54) and critically-point-dried seeds. Fig. 51: mature seed with micropyle downwards; Fig. 52: seed without the outer walls of the exotestal cells; Fig. 53: micropylar region; Fig. 54: hilar region; Fig. 55: surface view of exotesta after partly removal of the outer tangential walls by ultra sonoric treatment; Fig. 56: detail showing thin upper part of the radial walls.

The ellipsoid to broadly ellipsoid, black to brown mature seeds measure about 0.5 × 1.3 mm and bear a micropylar beak consisting of a cylindrical part representing the micropylar region covered by exotestal cells and a conical part formed by the enlongated, protruding cells of the inner layer of the outer integument (*figs. 51* and *53*). The round and sunken hilar scar lies opposite to the micropyle (*fig. 54*). The seed surface has irregular, longitudinal ridges rendering the seed scrobiculate. The ridges originate from more radially stretched exotesta cells. The testa consists of, in surface view, polygonal cells with sunken anticlinal boundaries. In the mature seed the thin outer cell walls are flat or slightly bulging outwardly. In the dry seed the outer walls collapse. The exotestal cells can hold air and are thus instrumental in keeping the mature seeds afloat for some time.

The seeds of *Mayaca baumii, M. longipes* and *M. sellowiana* confirm to the above-mentioned description and differ from those of *M. fluviatilis* only in some minor details.

The capsular and three-valved fruit remains enclosed by the persistent sepals and dehisces between the placentae. Its size varies with the number of mature seeds (usually there are three to fifteen). Most fruits in addition contain a number of abortive ovules and/or seeds. The fruit well is membraneaceous, about eight -twelve cells thick and parenchymatous throughout.

3.6. Endosperm

The large primary endosperm nucleus has a very conspicuous nucleolus. It is situated laterally, more or less in the middle region of the embryo sac. The endosperm is *ab initio* nuclear and the divisions of the primary endosperm nucleus precede that of the zygote. The free nuclei remain embedded in a folded cytoplasm sheath distributed near the periphery of the embryo sac. Later the number of folds increases and the cytoplasm fills the whole embryo sac. At the chalazal end the cytoplasm is more densely stained and penetrates the region initially occupied by the antipodal cells (*figs. 57* and *58*). At the stage of the globular embryo the endosperm becomes cellular. Wall formation commences at the periphery and extends into the interior ultimately to fill the entire embryo sac with cellular tissue (*figs. 47, 59, 61* and *63*). The chalazal part is histologically distinct. During the cellularization of the endosperm it remains for some time a multinucleate chalazal chamber (*fig. 60*). The nuclei undergo some divisions to produce several chromatine-rich, irregularly shaped nuclei. Somewhat later this chamber undergoes a cellularization and forms several cells with densely staining cytoplasm and nuclei of an irregular form. These cells do not store starch grains and degenerate later (*fig. 63*). The mature endosperm consists of polyhedral, radially elongated and thin-walled cells with one to four nuclei (or occasionally

Figs. 57–63. *Mayaca fluviatilis*, LM photographs of endosperm and embryo. Figs. 57, 59, 61 and 62 photographic evidence of the embryogenesis in support of figures 67, 71, 75 and 78, respectively. Fig. 57: bicellular proembryo and folded free nuclear endosperm (× 200); Fig. 58: longisection of a developing seed, showing the nuclear endosperm and details of the chalazal region (× 160). Fig. 59: globular embryo showing the derivatives of the apical and basal cells (× 200); Fig. 60: longisection of the seed showing the multinucleate chalazal chamber of the endosperm (× 160);

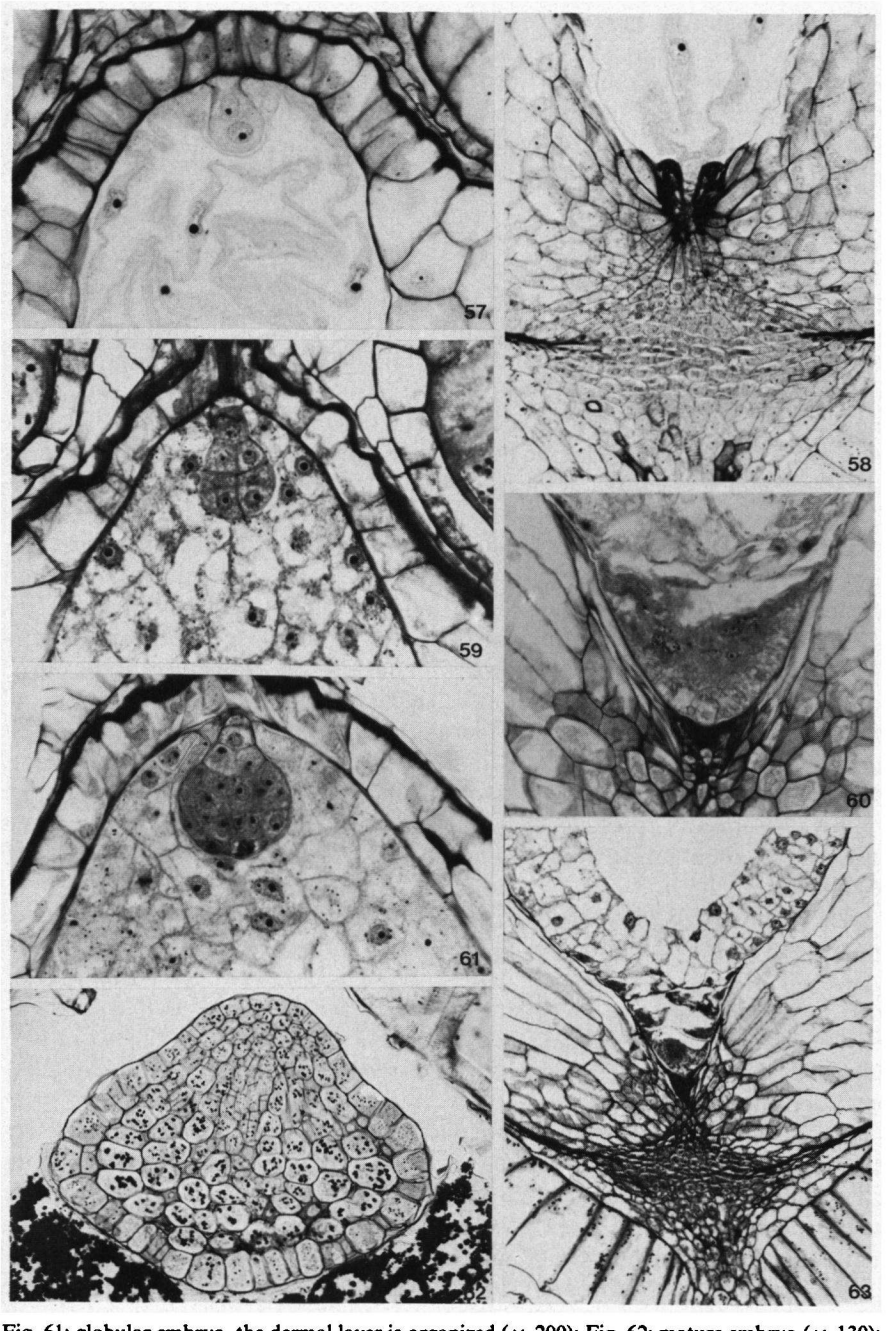


Fig. 61: globular embryo, the dermal layer is organized (× 200); Fig. 62: mature embryo (× 130); Fig. 63: longisection of the chalazal region of a developing seed at the time of cellularization of the endosperm, note their histologically distinct cells at the lower part (× 65).

with more than four in the outermost central cells). The peripheral cells form a layer of more or less rectangular, usually uninucleate cells. The cells of the central endosperm are mainly filled with closely-packed simple starch grains. The outermost layer of the endosperm is present as an aleuron layer which stores spherical protein bodies and lipids. The subaleurone layers contain starch grains, protein cristalline bodies and lipids (*fig. 49*).

3.7. Embryogeny

The zygote is initially globose. Its cytoplasm shows small vacuoles and is surrounded by a wall that gives a positive PAS reaction. Before the first mitotic division it elongates and assumes a characteristic bipolar shape, with a vacuolated micropylar pole, the chalazal one containing the nucleus and most of the cytoplasm (*figs. 64–66*). The first division is transverse and results in two superposed cells, of which the apical (ca) is smaller than the basal one (cb) (*figs. 57* and *67*). By a transverse division the basal cell gives rise to two superposed cells the intermediate one (m) and the basally situated one (ci) (*figs. 68* and *69*). This division is followed by a vertical one in the apical cell to form two cells in juxtaposition (q), so that an invertedly T-shaped, four-celled proembryo is formed (*fig. 70*). A vertical division in 'm' results in two cells that subsequently undergo further divisions and contribute to the initials of the future root meristem (*figs. 59, 71–77*). The 'ci' divides only once again by a transverse or vertical wall and forms a two-celled suspensor (*figs. 61, 72* and *73*). The derivatives of the apical cell divide transversely so that four cells are formed arranged in two tiers of two cells each (*figs. 59* and *71*). This stage is followed by one with numerous cell divisions in diverse planes, so that the embryo acquires a globular shape. Epidermis initials are separated by periclinal walls (*figs. 73–77*). The mature embryo assumes a depressed-ovoid shape, and remains small and undifferentiated. The cells contain amyloplasts and may form small intercellular spaces (*figs. 62* and *78*).

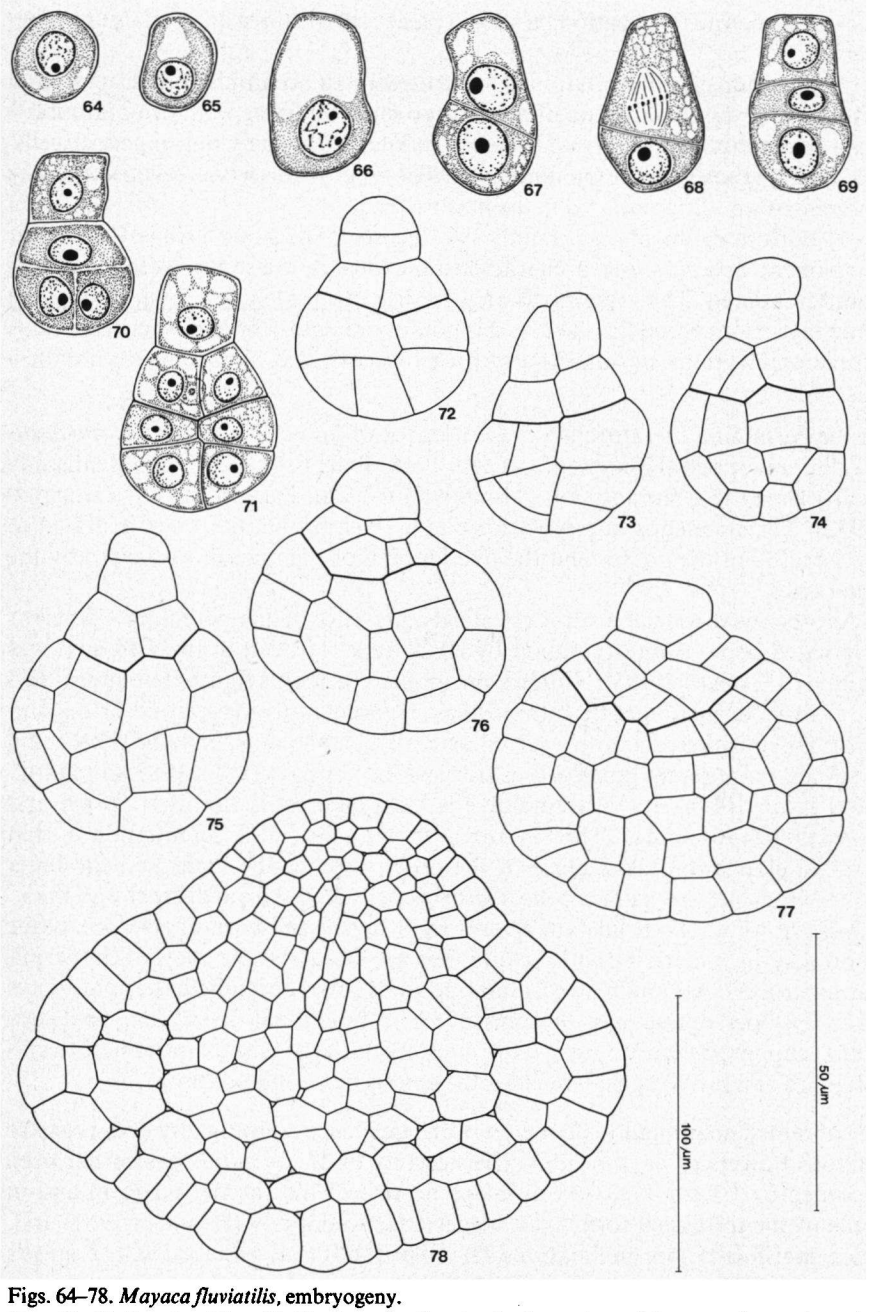
The minor participation of the basal cell in the formation of the embryo proper means that the embryogeny proceeds according to the Onagrad type.

4. DISCUSSION

The principal embryological and structural seed coat characters of the Mayacaceae appear to be:

(1) The anther is tetrasporangiate with an exothecium and its wall development is according to the Basic type. The endothecium fails to develop fibrous thickenings. The glandular tapetum is uninucleate. Successive cytokinesis in the microspore mother cells result in isobilateral and decussate tetrads. The pollen grains are shed at the binucleate stage.

(2) The ovule is orthotropous, bitegmic and tenuinucellate with a micropyle formed by both integuments. The archesporial cell functions directly as the megaspore mother cell. The tetrads are T-shaped, the chalazal megaspore developing into a *Polygonum* type of embryo sac. The synergids are pyriform, the polar



Figs. 64–78. *Mayaca fluviatilis*, embryogeny.
Figs. 64–66: Zygotes; Figs. 67–70: stages leading to the formation of the proembryo, the apical cell has divided longitudinally (fig. 70); Fig. 71: globular embryo, the derivatives of the apical cell have undergone transverse division; Figs. 72–77: organization of the globular embryo; Fig. 78: embryo in a mature seed (50 µm scale for figs. 64–77 and 100 µm for fig. 78).

nuclei fuse before fertilization has taken place and the three antipodals are ephemerous.

(3) The endosperm formation is nuclear, with an accumulation of nuclei and cytoplasm at the chalazal end of the embryo sac. Following wall formation starting at the periphery, the tissue becomes cellular with partly multinucleate cells. The embryogeny is of the Onagrad type. The embryo has a two-celled suspensor and remains undifferentiated in the mature seed.

(4) Both integuments are mainly two-layered. The outer layer of the outer integument develops into a characteristic exotesta, the inner layer remaining undifferentiated. The inner layer of the inner integument is tanniniferous, the outer layer is squeezed flat. The seed is non-operculate. The endosperm contains simple starch grains; its outer layer is proteinaceous. The hypostase is tanniniferous.

In the pertaining literature the exact number of anther locules in *M. fluviatilis* was left undecided (HORN AF RANTZIEN 1946, THIERET 1975). The present study clearly shows that the anther is definitely four-loculed as reported by LOURTEIG (1952). The erroneous interpretations have most probably to be attributed to the bending of the anther and the overlapping of the innermost locules by the outer ones.

Another wide-spread error is the alleged presence of an operculum. The error originated by a misinterpretation by HUTCHINSON (1934) of the seed as it was figured in SEUBERT (1885). Hutchinson adapted Seubert's figures but interpreted the embryo as a stopper or embryostegia (synonymous for seed lid or operculum). His misinterpretation was followed by HORN AF RANTZIEN (1946) and in several more recent publications (for instance by STEVENSON 1983 and DAHLGREN *et al.* 1984), notwithstanding the clear figures of POULSEN (1886) and HAMANN's (1961) notes. The micropyle shows a special differentiation in that a radial elongation takes place of the outer layer of the inner and the inner layer of the outer integument; the micropyle cells do not form distinct wall thickenings, however. Seed lids are always rigid by the presence of sclerotic tissue to protect the underlying embryo and they are lifted at some stage of the germination process. Although no germinating seeds could be studied, the anatomical structure does not suggest the presence of a functional operculum. HAMANN (1961) supposed that in *Mayaca* the micropylar tissue desintegrates before germination and provides a channel for the emergence of the seedling.

We obtained unambiguous evidence of the incidence of autogamy in the cleistogamous flowers of *M. fluviatilis*. Cleistogamy of *M. fluviatilis* has earlier been recorded by UPHOF (1933) both in the flowers of the submerged form and in some of the terrestrial form. The suggested entomophily (HAMANN 1961) and, more specifically, bee pollination (TIEMANN 1985) is at variance with UPHOF's (1933) and DAHLGREN & CLIFFORD's (1982) remarks. UPHOF reports that no insect pollinators were seen visiting the chasmogamous flowers of the land form and that wind pollination is unlikely to occur. The pollination syndrome of such flowers remains unclear and requires field studies.

In *M. baumii, M. longipes* and some forms of *M. fluviatilis* the pedicels may become reflexed after flowering and, depending on the water level, the fruits may ripen under water. This phenomenon is also known from other aquatic plants. The fruits may open above or below the water surface (UPHOF 1933). Mature seeds float with the help of their air-filled exotesta. Dispersal by water is most likely, as suggested by UPHOF (1933) and THIERET (1975). UPHOF could not detect a distinct mode of dispersal in the land form. The seeds germinate in the immediate vicinity of the parental plant. On the other hand seeds seem to need a dry period before being able to germinate (LUDWIG 1886).

At the superfamilial level the family of the Mayacaceae has been classified in different ways, e.g., by LINDLEY (1853) in the Xyridales; by BENTHAM & HOOKER (1883) in the Coronariae; by VAN TIEGHEM (1898) in his Liliinées; by ENGLER (1982) in the Farinosae, by WETTSTEIN (1901) in the Enantioblastae, by HUTCHINSON (1934) in the Commelinales. In all these cases the authors agree in that the taxon concerned always includes the Commelinaceae and often also the Xyridaceae and Eriocaulaceae. In all leading classifications proposed after 1965 the Mayacaceae are placed in the Commelinales (DAHLGREN & CLIFFORD 1982). The principal differences between the various systems are the numbers of families included in each recognised order and whether or not a separate order of the Eriocaulales has been distinguished. In the latest version of DAHLGREN'S system (DAHGREN *et al.* 1984) the Eriocaulaceae were included in the Commelinales.

The taxonomic position and the family status of the Mayacaceae have been discussed by several authors. GRISEBACH (1866) and VAN TIEGHEM (1898) included *Mayaca* in the Xyridaceae. HUTCHINSON (1934) suggested that the Mayacaceae are no more than depauperate representatives of the Commelinaceae and might be included in that family.

The embryological and structural seed characters clearly support a separate familial status of the Mayacaceae. Although the family cannot be linked directly with any other monocotyledonous taxon, it shares most characters with members of the Commelinales. Next to some characters of its own, the family has some characters in common with different combinations of other commelinalean families. The order of the Commelinales *sensu* Dahlgren comprises the Commelinaceae, Mayacaceae, Rapateaceae, Xyridaceae and Eriocaulaceae. The families have in common: a successive type of microsporogenesis (the Rapateaceae excepted), the *Polygonum* type of embryo sac formation, a nuclear endosperm (*Abolboda* excepted), the Asterad type of embryo (except in Mayacaceae), a broad embryo in the mature seed and starchy endosperm, but other embryological characters suggest more reticulate relationships with families of the Commelinales. Poricidal anthers are found in Mayacaceae, Rapateaceae and some Commelinaceae; the absence of a fibrous endothecium in Mayacaceae and some Xyridaceae; the Basic type of anther wall formation in Mayacaceae and Rapateaceae, a glandular tapetum in Mayacaceae, Rapateaceae, Eriocaulaceae and some Xyridaceae; orthotropous and tenuinucellate ovules in Mayacaceae, Eriocaulaceae and some Xyridaceae and Commelinaceae.

The characters of the mature seed are of but little use in establishing interfamiliar relationships. Barring the presence of a tanniniferous inner layer of the inner integument, the Mayacaceae clearly deviate from the other commelinalean taxa by the absence of differentiated siliciferous endotestal, and sclerotic exotegmic layers. The presence of a well-differentiated exotesta is probably an adaptation to the hydrochorous mode of dispersal.

The family Mayacaceae is one of the more advanced and specialized ones among the Commelinales and seems to be closest to the Eriocaulaceae and especially the Xyridaceae.

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