The embryology of *Prionium serratum* (L.f.) Drege ex E.Mey. (Juncaceae:Cyperales) and its implications for the phylogeny of Cyperales

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Abstract

This study investigates the phylogenetic placement of *Prionium serratum*, a monotypic genus in the family Juncaceae and the consequences of this on the evolutionary relationships of Cyperales using embryology, morphology and *rbc*L sequence data.

The embryology of *Prionium* conforms to that found in Juncaceae. The tapetum is glandular-secretory and microsporogenesis is simultaneous. Pollen is united in tetrahedral and cross tetrads and is tri-nucleate at release. The ovary is trilocular, there are three stigmata and the style is absent. There are many ovules per locule and placentation is axile. The ovule is anatropous, bitegmic and crassinucellate. The embryo sac conforms to the *Polygonum* type of development. Endosperm formation is helobial and embryogeny is of the *Onagrad* type, *Juncus* variation. The mature embryo is linear in shape and is embedded within the endosperm. The seed is trigonal, lacks processes and the seed coat is formed from both integuments. Germination is epigeal and the seedlings are of Type A.

Phylogenetic analyses using morphological data (including embryology) and total evidence (*rbc*L sequence data combined with morphological data) revealed that Juncaceae are paraphyletic with *Prionium* basal to Juncaceae plus Cyperaceae within Cyperales in both instances. In addition, *Oxychloë* is basal to Cyperaceae in the total evidence analysis.

The basal placement of *Prionium* within Cyperales is supported by its woody habit, carpels fused in the ovary region chlorenchymatous air canals in the leaf and the absence of air canals between vascular bundles.

The total evidence phylogeny is in agreement with Plunkett et al.'s (1995) *rbc*L topology and this study also supports Cutler's (1969) contention that *Prionium* should be excluded from Juncaceae. Therefore, it is suggested that *Prionium* be elevated to family status.

INTRODUCTION

Phylogenetic relationships among monocotyledonous families have posed a series of difficult taxonomic problems for many years. The placement of many families has been problematic, particularly in the commelinanean monocotyledons where reduction in floral morphology due to anemophily has created convergence among many taxa (Cronquist 1968, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Plunkett *et al.* 1995). This confusion is nowhere more apparent than in Juncaceae. The placement of this family has been uncertain, possibly because the classification of both Juncaceae and Cyperaceae has been based largely on floral morphology. Juncaceae is presently placed with Thurniaceae and Cyperaceae in Cyperales *sensu* Dahlgren *et al.* (1985). Hallier (1905) also proposed this classification, although Thurniaceae was still included in Juncaceae. Thorne (1992) also follows this classification although he refers to the order as Juncales/Cyperales. However, Juncaceae has also been placed in Liliflorae (Engler 1892, Bessey 1915 places Juncaceae in Liliales) and also with Restionaceae in Juncales (Hutchinson 1934, 1959, Cutler 1969) or with Thurniaceae only in Juncales (Hamann 1961, Melchior 1964, Cronquist 1968, Takhtajan 1969, Stebbins 1974, Dahlgren 1975, Dahlgren & Clifford 1982). Cyperaceae was usually included with the grasses (Engler 1892, Bessey 1915, Cronquist 1968) or in Cyperales on its own (Hutchinson 1934, 1959, Melchior 1964, Takhtajan 1969, Stebbins 1974).

Juncaceae is a small family in which eight to ten genera may be included, although the delimitation of some of the genera appears to be unclear. The genera are *Distichia* (3 spp.), *Juncus* (c. 300 spp.), *Luzula* (80 spp.), *Marsippospermum* (3 spp.), *Oxychloë* (?4 spp.), *Patosia* (1 sp.), *Prionium* (1 sp.) and *Rostkovia* (2 spp.). *Andesia* is often included in *Oxychloë* and

Cutler (1969) has suggested that the status of *Voladeria* is uncertain since the original description was made from male material only. The gross morphology of *Voladeria* is similar to both *Oxychloë* and *Distichia*, however, the number of stamens are reduced to three.

Juncaceae is restricted mainly to the southern hemisphere, except for *Juncus* and *Luzula* which are cosmopolitan. *Distichia*, *Oxychloë* and *Patosia* are all restricted to South America, while *Prionium* is restricted to South Africa occurring on oligotrophic soils growing in rivers from the South Western Cape and extending along the coast into southern Kwazulu/Natal. *Marsippospermum* and *Rostkovia* occur mainly in South America and New Zealand. It is likely that Juncaceae had its origin in the southern hemisphere (Weimarck 1946) and Dahlgren *et al.* (1985) have suggested a South American centre of divergence.

Prionium has always been included in the family Juncaceae. *Prionium* has a woody rhizome that grows to several metres in height and the serrate leaves are tristichously inserted. The inflorescence is paniculate and the flowers are bisexual with a trilocular pistil. Cutler (1969) however, suggested that based on its peculiar anatomical features *Prionium* should be excluded from Juncaceae. Recent research has shown conflict in the placement of *Prionium* (Chase *et al.* 1993, Linder & Kellogg 1995, Plunkett *et al.* 1995). At present two possible topological arrangements exist for *Prionium*. In the first arrangement *Prionium* is embedded in the Juncaceae with *Thurnia* as the sister taxon (see Simpson 1995). This topology is based exclusively on morphological data. In the second instance, *Prionium* is excluded from Juncaceae and is placed basally to Cyperaceae plus the remaining Juncaceae (see Chase *et al.* 1993, Plunkett *et al.* 1995). This topology is based exclusively on *rbc*L sequence data. These results indicate firstly that *Prionium* is not a member of Juncaceae and secondly that Juncaceae is paraphyletic.

The implications of these current findings to Juncaceae and Cyperaceae systematics have as yet not been addressed. Although, recent research based on morphological (Dahlgren *et al.* 1985, Thorne 1992, Linder & Kellogg 1995, Simpson 1995) and molecular (*rbc*L) data (Chase *et al.* 1993, Duvall *et al.* 1993, Chase *et al.* 1995, Linder & Kellogg 1995, Plunkett *et al.* 1995) separately as well as using total evidence (Chase *et al.* 1995, Linder & Kellogg 1995)

at the interfamilial level indicates that Juncaceae (*sensu lato*) and Cyperaceae are closely related and that these two families should be included within a single order. The position of Thurniaceae relative to these two families remains unsolved since Thurniaceae is largely unknown for many characteristics including *rbc*L.

The aims of this thesis are:

(i) to test the basal position of *Prionium* in Cyperales

(ii) to evaluate the status of Juncaceae and Cyperaceae

(iii) to determine whether Cyperales is monophyletic.

In order to establish interfamilial relationships certain types of data are preferred over others. These are discrete morphological characters (i.e. not measurement data), palynological characters, embryological characters and molecular data such as *rbc*L sequence data. This thesis emphasises embryological techniques for several reasons. The embryology of *Prionium serratum* has up until now been unknown. In addition embryology has been shown to be informative at the family level since, embryological characteristics are thought to be most consistent at the family level rather than at other taxonomic ranks (Buss & Lersten 1975, Palser 1975, Kapil & Bhatnagar 1991). Embryological characteristics are also often preferred over other morphological characteristics particularly where the environment is responsible for shaping the phenotype of a particular organism. As embryology is generally genetically predetermined and is less prone to environmental constraints it may be more informative than morphology in some circumstances (Kapil & Bhatnagar 1991). Furthermore, in situations where morphological features are confounded by convergence embryology may be better able to provide insight into relationships (Kapil & Bhatnagar 1991). Thus, in the commelinanean monocotyledons and in Juncaceae and Cyperaceae in particular, where reduction in floral morphology is confounded by convergence, it follows that embryological characters may provide insights into the relationships between these taxa.

METHODS

SAMPLING

Specimens of *Prionium serratum* were collected from three localities in the South Western Cape, South Africa: Silvermine Dam (34° 04,5'S; 18° 23,8'E) on the Cape Peninsula, the Palmiet River (34° 18,5'S; 18° 55,5' E) in the Kogelberg and the Rondegatrivier bridge (32° 22,3'S; 19° 03,5'E) on the road to the Algeria Forest Station in the Clanwilliam region. Inflorescence samples were fixed in formalin acetic alcohol (FAA) for 48 hours, and later transferred to 70 % ethanol (EtoH) and stored. In addition seed was collected from the Palmiet River population and stored in brown paper packets. Voucher specimens are in the Bolus Herbarium at the University of Cape Town and a voucher list of all material photographed is in Appendix 3.

Observations of whole flowers, anthers, ovules, and seed were made from each of the populations to ensure that the characteristics observed are widespread and not unique to a single population. A number of flowers from different inflorescences were sampled from each of the different populations and processed for particular embryological stages. Unfortunately, there was no standard/numerical way of deducing what stage the individual flowers were at. Initially, each floret was measured, but there was little variation in the size of the flower before anthesis and at or after anthesis. In addition, measurements of anther size were an unreliable indication of the age of the flower, there was little variation in the length of the anther at the different stages of development. The appearance of the anther and the length of the filament were better indicators of the age of the flower. The size of the ovule and the degree of

development/ageing of the stigmata were also more reliable indicators of flower age then flower size and anther length.

GENERAL PREPARATION TECHNIQUES

SECTIONING

Where paraplast embedding and sectioning were used for the different types of embryological material, a standard technique was applied. The embryological material was passed through a mechanical dehydration series using a Sukura tissue processor containing: two baths of 70% alcohol; two baths of 100% alcohol; two baths of N-propanol; two baths of N-butanol and two wax baths. The floral material was left in each bath for eight hours except in the second wax bath where it was left for a minimum of 24 hours. After wax soaking the embryological material was embedded in wax moulds. The wax blocks were trimmed and the embryological material sectioned to 10 μm on a Leitz Wetzlar rotary microtome. Wax ribbons containing sections were stretched in a tissue float water bath at 55°C before being placed on microscope slides prepared with Haupt's adhesive (Johansen 1940). Following drying, the microscope slides were manually taken through a staining procedure comprising the following chemicals in Coplin jars (Johansen 1940): two jars of xylene (five minutes each); one jar of 2-methoxy-ethanol (two minutes), two jars of 96% (two minutes each), one jar of safranin (30 minutes), one jar of distilled water (rinse), one jar of 2-methoxy-ethanol (two minutes), one jar of fast green (one minute), one jar of clove oil (50 parts clove oil, 25 parts 100% alcohol, 25 parts xylene) (rinse), three jars of 96% alcohol (rinse), two jars of n-butanol (one to two minutes each), two jars of xylene (five minutes each). Following staining, the sections were mounted on glass slides in DPX™ and a cover slip placed over the microscope slide.

CLEARING TECHNIQUES

The clearing technique described by Herr (1971) was used for whole ovules and for seeds. A modified Raj slide (Raj & Herr 1970, Radford *et al.* 1974), consisting of a microscope slide with two coverslips placed approximately 1-2 mm apart affixed to the slide with a small amount of Herr's (1971) clearing agent (2 lactic acid: 2 chloral hydrate: 2 phenol: 2 clove oil: 1 xylene made up by weight) was constructed. A drop of Herr's clearing agent was placed in the channel between the two coverslips and the plant material transferred into the channel and a coverslip placed on top. Ovules and seeds were allowed to clear for six to 48 hours.

MICROSCOPY AND PHOTOGRAPHY

Slides were viewed using both normal transmission and differential interference (DIC) optics on a Zeiss Axioskop microscope at magnifications 200x, 400x and 1000x (oil immersion). Photographs were taken on the Zeiss Axioskop using Ilford PanF ASA 100 (bright field viewing) black and white print film and Kodak Technical PanF ASA 25, high contrast black and white print film (DIC optics viewing). Ilford PanF ASA 100 black and white print film was developed using Ilford ID11 developer for six minutes at 20°C. The Kodak technical PanF ASA 25 high contrast black and white film was developed using Kodak HC-110 developer for eight minutes at 20°C. Negatives were printed using a Beseler 45MX enlarger.

SPECIFIC PREPARATION OF PLANT MATERIAL

ANTHER

Sections of anther sporangia

Whole flowers were used for the observations of all component parts of the male

reproductive structure and ontogeny. Ontogeny was observed at three stages:

(i) anther buds

(ii) anther and filament shorter than floral bracts

(iii) at anthesis

The flowers were removed from the panicles and dehydrated. After wax soaking, the flowers were embedded into wax moulds with the floral buds placed upright in preparation for transverse sections. On occasion, when the floral material was extremely tough, the wax blocks were softened in Mollifex™ for several hours prior to sectioning. The correct stage of the section of the flower was selected by serially sectioning the bud and observing the wax ribbons containing the sections under a Zeiss dissecting microscope. The region of the sections showing the correct stage in the sequence were then chosen for mounting and staining.

Pollen nuclei

Anthers that were beginning to release pollen were removed from flowers. A drop of Herr's clearing agent was placed on a microscope slide and an anther was submerged into the solution. The basal portion of each anther was removed, and the anther contents were forced out of the locules by rolling a pair of fine forceps' tips along the length of each locule viewed under a Zeiss dissecting microscope. A coverslip was placed over the Herr's fluid and pollen grains and the material was left to clear for 12-18 hours. Then pollen grains were observed at 1000x and the nuclei counted using DIC optics.

Pollen

Pollen from both fixed material and herbarium sheets was removed from anthers and prepared for acetolysis using standard techniques. Once acetolysed, the pollen was transferred to a stub covered in glue and sputter coated with Gold/Palladium for scanning electron

microscopy (SEM). Fresh pollen was also collected and transferred directly to the stub and sputter coated with Gold/Palladium for SEM.

OVULE

Ovary and ovule morphology

Flowers were sampled from different branches of the panicle and different regions of the inflorescence: top, middle and base. Each flower was dissected using a pair of fine forceps under a Zeiss dissecting microscope and the ovary removed. Ovaries were hand sectioned, both in a longitudinal and transverse plane and observed using a Zeiss dissecting microscope.

Sections of ovaries

A range of florets were sampled from different inflorescences to provide all the developmental stages (from initiation of development of the female gametophyte through to embryo formation) from the different populations. Each flower was dissected and the whole ovary removed, without breaking the ovary wall. This prevented air penetration into the ovary which would otherwise hinder successful embedding.

Each ovary was passed through the dehydration series. Soaking the ovaries in the final wax bath for more than 48 hours produced optimal results when sectioning. The wax soaked ovaries were then embedded into wax moulds with two ovaries in each mould, one placed upright for transverse sections and the other placed flat for longitudinal sections. This ensured that the greatest number of ovules would be sectioned through a longitudinal plane. The ovaries were serially sectioned.

The ovary, ovule and embryo sac sections were viewed using brightfield optics and DIC optics. When extremely contrasting sections were photographed, a developing time of 6 minutes at 20°C yielded better results.

Whole ovules

Herr's Clearing agent

Florets at different stages of development were selected from panicles from the different populations. Each floret was dissected and the ovary removed. The ovaries were then transferred to a hollowed out microscope slide containing a solution of Herr's clearing agent. Each ovule was removed from the ovary using size 00 insect pins, and allowed to soak in the Herr's clearing agent for 10 minutes. Ovules (up to 10) were placed onto the modified Raj slide and allowed to clear. Cleared ovules were examined using DIC optics only.

Clearing technique for tanniniferous material: Stockwell's bleaching agent

Florets were selected from the different populations and the ovaries dissected out. Ovules were removed from the ovaries using size 00 insect pins and transferred to size 4 McCartney bottles containing a modified version (Palser *et al.* 1989) of Stockwell's bleaching agent. Schmid (1977) outlines the tannin clearing technique mainly for sectioned material. The technique is attributed to Dr Palmer Stockwell (Foster & Gifford 1947) and is also outlined by Johansen (1940).

The modified Stockwell's bleaching agent (Palser *et al.* 1989) consists of 90ml distilled water, 1g potassium dichromate, 1g chromic acid, 10ml glacial acetic acid made up by weight. The Stockwell's bleaching agent degenerates with age and with increasing oxidation and should therefore be prepared freshly for each clearing event (Foster & Gifford 1947). This is usually noticeable when the solution changes from pale orange to deep russet orange. Ovules were placed in the McCartney bottles containing the Stockwell's bleaching agent for 20 hours at room temperature.

After 20 hours ovules were removed (following Palser *et al.* 1989) with a fine paint brush, placed in a funnel lined with fine muslin and rinsed with 0.1% sodium bicarbonate

followed by distilled water. Ovules were removed from the funnel with a fine artists paint brush and transferred to a watch glass filled with distilled water. Ovules were dehydrated to 70% so that they were chemically compatible with Herr's clearing agent, using the following dehydration procedure in watch glasses: one to two minutes in 50 % ethanol, one to two minutes in 60 % ethanol, one to two minutes in 70 % ethanol.

Following dehydration, ovules were transferred onto a modified Raj slide with Herr's clearing agent and left to clear for at least 24 hours. The ovules were examined using DIC optics.

SEED AND SEEDLINGS

Some of the seed collected was germinated to observe seedling growth and to obtain root tips for chromosome counts. Seeds were sprinkled onto filter paper circles placed in perspex petri dishes and watered with distilled water and a weak solution of benlate anti fungal agent. The petri dishes were placed in a plastic packet in a germination chamber programmed on a 10°C/20°C cycle. The petri-dishes were observed on a daily basis. Seeds were also sprinkled into river sand in a germination punnet and placed on a tray containing a shallow level of water on a windowsill and covered with shade cloth. The germination tray was observed daily and seedlings removed at desired developmental stages and fixed in FAA for 48 hours before being transferred to 70% alcohol for storage. Seeds and seedlings for sectioning were similarly fixed in FAA and stored in 70% alcohol.

Sections of whole seeds and seedlings

Fixed seeds and seedlings were taken manually through a dehydration series using a fine artists paint brush and size 4 McCartney bottles with screw on lids containing two changes of: 70% alcohol; 100% alcohol; N-propanol and N-butanol. The seeds and seedlings were placed for two hours in each treatment i.e. an hour in each McCartney bottle. The seeds were then

transferred into a metal wax-mould basket containing wax preheated to 58°C. The wax filled moulds were placed in an oven at 58°C for ten to 16 hours. The wax soaked seeds were then embedded into wax moulds with two seeds in each mould, one placed upright for transverse sections and the other placed flat for longitudinal sections.

Clearing technique

The seed coats from all of the seeds were carefully removed using fine forceps and size 00 insect pins at 50x magnification on a Zeiss dissecting microscope. The seeds (endosperm plus embryo) were placed in Herr's clearing agent on a modified Raj slide and allowed to clear (15-20 minutes). The cleared material was observed using DIC optics.

Seedlings were placed on a modified Raj microscope slide and allowed to clear (approx. 1 hour) in Herr's clearing agent. The cleared seedlings were observed using DIC optics.

CYTOLOGY

Seedlings were allowed to grow until the radicle of the seedlings was more than 1 cm in length. The seedlings were carefully removed from the soil and the roots rinsed in distilled water to remove any sand. Root tips from the apex of the radicle were removed using a scalpel blade under a Zeiss dissecting microscope.

A number of fixation and staining techniques were attempted, but due to the small size of the chromosomes, all pretreatment and heating techniques were abandoned. The most successful fixation and staining technique was to fix the root tips in a 9:1 solution of glacial acetic acid (GAA) and hydrochloric acid (HCl) from 10 minutes to two hours. Following fixation the root tips were rinsed in 70% EtoH and then transferred to distilled water. After maceration in a 1M solution of HCl at 60°C for 5 minutes (Dyer 1979) the root tips were transferred into distilled water.

The root tip was removed using a scalpel and placed on a slide containing a small drop

of acetocarmin. In order to allow for the ferric acetocarmin smear technique (Belling 1926, Johansen 1940, Thomas 1945, Darlington & La Cour 1960) to work, the solution of acetocarmin containing the root tip on the slide was manipulated with a pair of rusty tweezers. When the reaction proceeded, the acetocarmin turned from a pale red colour to dark purple (30-60 seconds), at this stage the coverslip was placed on the slide and the slide squeezed between two pieces of blotting paper until the excess liquid was removed. The slide was heated briefly over a spirit flame until the iron-acetocarmin solution became an even darker purple. This serves to flatten the cells and also to spread them over the slide and to allow them to stick to the slide and coverslip (Darlington & La Cour 1960). The root tip was squashed by tapping the cover slip with the back of a wooden pencil a few times (usually not more than 10 times) until a nice spread of the cells was obtained. The smears were observed using DIC optics and normal bright field optics.

PHYLOGENETIC ANALYSES

Morphological data

Several morphological characters from Simpson's (1995) Cyperales data set for the genera of Juncaceae and Cyperaceae, *Thurnia* and *Typha* and *Sparganium* were rearranged and reinterpreted (Appendix 1). Three other outgroups i.e. *Ananas*, *Flagellaria* and *Eriocaulon* were included in the data set. Extra information for some of the taxa and the outgroups was included from Cutler (1969), Dahlgren & Clifford (1982) and Dahlgren *et al.* (1985). In addition, several embryological characters were incorporated into the data set. The interpretation of the characters and the primary data sources are discussed in Appendix 1, while the list of taxa and the data matrix used in the phylogenetic analysis is provided in Appendix 2.

A morphological data matrix was constructed using MacClade vers. 3.0 (Maddison & Maddison 1992). Polymorphic characters were coded with respect to the number of states each character had. Missing data were coded with "?" (unknown). The data were analyzed with

PAUP vers. 3.1 (Swofford 1993) using the heuristic search option with tree bisection-reconnection (TBR) and the character states optimised by accelerated transformation (ACCTRAN). Both a strict consensus and a 50% majority rule tree were calculated. The degree of support in the data set for the morphological tree was investigated using the bootstrap re-sampling technique of Felsenstein (1985). Although this method has been criticised, it does provide a quick method of assessment of support for particular nodes within a topology (Sanderson 1995). Bootstrap percentiles for 200 replicates were calculated for all nodes using PAUP vers. 3.1 (Swofford 1993). Decay indices were not calculated due to insufficient memory.

To investigate the placement of *Oxychloë*, *Prionium* and *Luzula* in both the morphological tree and the *rbc*L tree of Plunkett *et al.* (1995), 100 bootstrap trees were generated with only one tree retained at each step and without collapsing zero length branches. The position of each genus in 100 trees gives a probability measure for a particular placement of a particular genus being due to chance. Thus, incongruence between cladograms derived from two different data sets can be assessed to determine whether the incongruence is due to chance and ultimately which data set will reflect the true phylogeny. This method was selected over a method described by Rodrigo *et al.* (1993) which uses Component Version 2.0 (Page 1993). A number of random bootstrap trees are generated for a particular data set and the greatest agreement subtrees (GAS) are found. However, the method does require that all nodes in 100 bootstrap replicates are resolved, and for this reason could not be used in this study due to a large proportion of the trees having unresolved nodes.

Combined data

The *rbc*L sequence data of Plunkett *et al.* 1995 (see Appendix 4 for citations of obtained sequences) for selected taxa was incorporated into a data matrix constructed in PAUP vers. 3.1 (Swofford 1993). Missing data were coded as "-" or "?" and invariant codons as "." in accordance to the first taxon (*Nicotiniana*). The data were analyzed with PAUP vers. 3.1 (Swofford 1993) using the heuristic search option with tree bisection-reconnection (TBR) and

the character states optimised by accelerated transformation (ACCTRAN). A 50% majority rule tree plus bootstrap percentiles were calculated using PAUP vers. 3.1 (Swofford 1993). The bootstrap percentiles were calculated using 200 replicates. Decay indices up to six steps were calculated for all nodes with greater than 50% occurrence.

RESULTS

DEVELOPMENT OF MALE REPRODUCTIVE STRUCTURES

Anther wall formation

Early development of the anther was not observed due to a lack of material at the appropriate stage. Therefore, anther wall formation could not be determined. The anther is tetrasporangiate, consisting of four clearly separated locules (Figure 1).

Differentiation into sporogenous tissue

Although several sets of very young buds of *Prionium serratum* were sectioned and observed, it was not possible to observe all the stages of development of the sporogenous tissue. For the initial stages of differentiation into sporogenous tissue, two age classes of bud were sampled. Firstly, flowers containing anthers that were developed and visible, but without stamens and, secondly, flowers containing anther buds that were just starting to become visible (primordia) were used. However, closer inspection revealed that neither sampling procedure was adequate and anthers were either too old or too young to record all stages of microsporogenesis. Anthers that were too old already had two to three cell layers of sporogenous tissue. In addition, the anther wall components had already developed in these anthers. Thus, it is possible that the anther wall components differentiate first from the cells closest to the epidermis and cells towards the inner region differentiate later into sporogenous tissue. In anthers that were too young, there was no differentiation into sporogenous tissue. Serial

sections of the anther primordia showed that a primordium consists of a well developed single layer of epidermis and an inner region of undifferentiated cells.

Pre-sporogenesis

At the stage prior to sporogenesis all the wall layers are well developed and the locules filled with sporogenous tissue (Figure 1). Each anther locule is surrounded externally and entirely by a single layer of epidermal cells (Figure 2). The epidermis is well defined with very symmetrical cells, all approximately the same size and shape. The endothecium which is well developed at this stage is directly below the endothecium (Figure 2). It is a single layer consisting of elongated cells, which are rectangular to spindle shaped, containing large, centrally placed nuclei, which are darkly staining. The cells of the endothecium are uni-nucleate. The next layer, which is the middle layer, consists of a single layer of cells and is well developed at this stage (Figure 2). Each cell is clearly visible, although the nuclei are not always prominent. Internal to this layer is the tapetum, which is closely associated with the sporogenous tissue (Figure 2). The cells of the tapetum are often irregularly placed and integrate with other tapetal cells around the locule. The tapetum is generally bi-layered and uni-nucleate, and the cells can be distinguished by their almost cuboidal shape and prominent, darkly staining nuclei. The sporogenous tissue is undifferentiated at this stage and is composed of several very large cells, almost square in shape, which are loosely arranged in the locule, with extremely bright nuclei and a clearly distinguishable nucleolus (Figure 1).

Sporogenesis

During sporogenesis, the anther locule itself is enlarged to accommodate the increasing volume of sporogenous tissue. The cells of the middle layer are less prominent and show signs of being squashed by the enlarging locule. As the cells are crushed, the cell shape and outline is no longer apparent. The tapetum is very clear at this stage, consisting of two clearly defined

layers with each cell containing a single nucleus.

The sporogenous tissue undergoes several mitotic divisions (not recorded here) to form microspore mother cells. The microspore mother cells undergo meiosis and the cells become two nucleate (two nucleate cells are common throughout the sporogenous tissue), but no evidence of cell plates or wall formation can be observed (Figure 3). Meiosis I was possibly observed in the sporogenous tissue (already well developed with an anther locule defined). During subsequent divisions i.e. Meiosis II, the cells become four nucleate, but no walls are formed (Figure 3 and 4). Cells apparently containing three nuclei can easily be observed simultaneously (within the same plane of focus) (Figure 5). This probably reflects the typical development of the tetrahedral tetrad where the fourth nucleus lies underneath and is obscured by the three superficial nuclei. Cell plate formation and quadripartition was not seen and was therefore not recorded.

Pre-dispersal stage

The epidermis is present as a single layer, but the cell structure is not as clear as in the earlier stages (Figure 6). The endothecium is still well defined and appears to be the most persistent of all the wall layers (Figure 6). The cell structure is still retained and the nuclei are prominent and visually clear. The middle layer is in the process of late degeneration, consisting of a few crushed pieces of tissue and has been replaced by an air space (Figure 6). The tapetum is beginning to show signs of degeneration, cell structure is not clearly discernable and no nuclei are visible. The anther locule consists largely of a sac containing tetrads of pollen grains (Figure 6). The pollen grains are uni-nucleate at this stage.

Dispersal stage (pollen)

The pollen is closely united in tetrahedral (Figure 7) or cross (Figure 8) tetrads. The surface of the grains are slightly granular (Figure 7). The pollen grains are without pores and

furrows are difficult to distinguish and may be very reduced (or absent). The grains were destroyed by acetolysis and in addition fresh grains collapsed prior to sputter coating. At the dispersal stage, the single celled pollen grain divides and each pollen grain becomes binucleate (Figure 9). Just before pollen release the second nucleus divides and a tri-nucleate pollen grain is dispersed (Figure 10 and 11).

SUMMARY

The anther wall consists of a single layered epidermis, a single endothecial layer, a middle layer and a bi-layered, uni-nucleate tapetum which retains cellular structure throughout microsporogenesis, without producing intrusions into the microspores, and is therefore glandular. Microsporogenesis is simultaneous, as no cell walls are laid down between the nuclei in the microspores. The middle layer is ephemeral, degenerating shortly after meiosis II before the microspores develop into pollen grains. The tapetum also degenerates, but is still visible prior to the stage when the pollen is shed. The pollen is released in tetrahedral and cross tetrads and all of the pollen grains are developed. The pollen is tri-nucleate at release.

DEVELOPMENT OF FEMALE REPRODUCTIVE STRUCTURES

Ovary morphology

The ovary is trilocular with complete septa between the locules (Figure 12). In very young ovaries, viewed in transverse sections, the septa are often incomplete, but with age the septa close up or grow together. The placentation is axile, with two rows of anatropous ovules on each placenta (Figure 12 and 13). The number of ovules per locule varies, but is never less than three. The ovary has three stigmata which have a sessile, papillate surface and a style is absent in the fertile flower (Figure 14).

Ovule morphology

Nucellar tissue formation and development of integuments

In the initial stage of development of the nucellar tissue, the archesporial cell lies directly beneath a single layer of nucellar epidermis (Figure 15). The archesporial cell is the largest cell within the ovule and is embedded in undifferentiated tissue, from which it can be distinguished by the presence of a single, large nucleus in the centre of the cell (Figure 15). The inner integument primordium can be distinguished by a small number of cells at the edges of the ovule (Figure 15).

The archesporial cell divides, and a parietal cell is formed (Figure 16). The archesporial cell plus the parietal cell lie directly underneath the single layer of nucellar epidermis, with the parietal cell closest to the epidermis (Figure 16). The inner integument is more distinct than at the archesporial cell stage (Figure 16).

The archesporial cell cuts off the parietal cell which undergoes further divisions to form the nucellar tissue. Thus, the nucellar tissue formation is of the crassinucellate type (*sensu* Davis 1966). As the nucellar tissue develops, the archesporial cell gets pushed further and

further away from the nucellar epidermis, towards the chalazal end of the ovule. By the time the nucellar tissue is a single layer thick, the archesporial cell now referred to as the functional megaspore mother cell, is already centrally situated in the ovule (Figure 17). The inner integument, which is bi-layered, is clearly distinguishable and begins to grow upwards around the ovule, encircling at least three quarters of the outer surface of the ovule (Figure 17). In addition, the outer integument is beginning to develop and is also two cell layers thick.

When the megaspore mother cell increases in size, the nucellar tissue is one layer thick (Figure 18). The inner integument almost completely encircles the ovule, while the outer integument is still shorter than the inner integument (Figure 19).

When the megaspore mother cell undergoes the first division (megasporogenesis, meiosis I) the nucellus is two layers thick and the integuments are complete, with the inner integuments lying close together. In all subsequent stages of megasporogenesis and megagametogenesis, the nucellar tissue is three layers thick. However, at the eight nucleate stage the innermost layer of nucellus appears to be crushed by the enlarging embryo sac.

Megasporogenesis

Megaspore mother cell

The megaspore mother cell is large and prominent with a darkly staining, centrally placed nucleolus (Figure 18). As mentioned previously, the integuments are well developed (Figure 19). Both integuments are bi-layered and lie closely together. There is no tannin in the inner layer of the inner integument at this stage. The nucellus is only one layer thick at this stage as previously discussed (Figure 18).

Megasporogenesis (meiosis II)

Tetrad formation was not observed. However, a late stage of megasporogenesis meiosis

II was observed and at this stage the nucellus is one to two layers thick. The chalazal nucleus of the tetrad remains functional and develops into the megaspore, while the other three cells degenerate and are crushed at the micropylar end of the ovule (Figure 20). The region around the megaspore becomes vacuolated (Figure 20). The integuments are well developed, with the inner integument starting to show some swelling at the tips (Figure 20).

Megagametogenesis

Megagametogenesis (mitosis I)

At this stage the embryo sac has formed (the micropylar nucleus of the tetrad forms the embryo sac). The functional megaspore undergoes a mitotic division to give rise to two daughter nuclei which start to move to opposite ends of the embryo sac (Figure 21). The region between them is occupied by a large vacuole (Figure 21). The crushed cells of the original micropylar nuclei remain visible (Figure 21 and 22). The integuments are well developed, with the inner integument showing a pronounced swelling at the tip and the inner layer showing darkened cells, although the tannin is not well developed at this stage (Figure 21). The embryo sac is approximately one third of the size of the ovule and the nucellus at the micropylar end is two to three layers thick. The crushed cells are no longer visible and the two daughter cells with their prominent nuclei remain in the embryo sac separated by a large vacuole (Figure 23).

Megagametogenesis (mitosis II and III)

The two daughter nuclei divide and give rise to a four nucleate embryo sac (not observed here). This in turn gives rise to an eight nucleate stage which is still disorganised i.e. the chalazal and micropylar polarity has not been established.

The mature embryo sac

The nucellar tissue is reduced to two layers in the micropylar region, and some cells of the nucellar tissue appear to have been crushed by the expanding embryo sac. Although a few starch grains are visible in the nucellar tissue (1000x magnification only), there is not an abundance of starch at this stage.

The mature embryo sac consists of eight organised nuclei i.e. nuclei showing polarity as well as functional structure. At the chalazal end of the nucleus there are three, large antipodal cells (Figure 24). Each antipodal cell has a single, large, centrally placed nucleus which is clearly visible. The antipodal cells plus nuclei are just slightly larger than the egg apparatus. The antipodal cells are not ephemeral and persist up until fertilisation. In the centre of the embryo sac, are two prominent, brightly staining polar nuclei, which do not fuse to form a central cell at any stage of the development before fertilisation (Figure 25 and 26). The egg apparatus is at the micropylar end of the embryo sac, and consists of a large egg cell (Figure 27) above which are two synergid cells, with smaller nuclei than the egg cell (Figure 28). The synergids have a large, extremely well developed filiform apparatus associated with them, which is placed close to the apex of the embryo sac (Figure 28). The filiform apparatus remains in the same position even after the fertilisation event and is often visible even after the proembryo has formed. The embryo sac itself (Figure 29) is approximately two thirds the length of the ovule and is quite slender, not filling the ovule in girth.

The micropyle is formed at this stage only and not in any of the foregoing stages. The inner integument is constricted at the tips which grow together, forming a space in between which is the micropyle (Figure 29). Thus, the micropyle is formed only by the inner integument. The outer integument exceeds the inner integument in length and the tips are also slightly constricted. The outer integument grows above and over the inner integument and clearly shows no involvement in the formation of the micropyle (Figure 29). Both integuments are two cell layers thick at this stage, whereas the swollen tips of the inner integument are often three

cell layers thick. The cells of the inner layer of the inner integument are heavily laden with tannin, so that the inner layer stains heavily (Figure 29).

SUMMARY

The ovary is trilocular having more than three ovules per locule with axile placentation. The ovule is completely anatropous, bitegmic and crassinucellate, with an archesporial cell cutting off a parietal cell and the resultant megaspore mother cell becoming deep seated within the ovule. The integuments consist of two layers and the micropyle is endostomic. The innermost layer of the inner integument is filled with tannin at the later stages of megagametogenesis. The embryo sac follows the polygonum type of development. The antipodals are prominent and persist up until fertilisation. The polar nuclei do not fuse before fertilisation. The filiform apparatus persists well after fertilisation has taken place, being visible even at the proembryo developmental stage.

POST FERTILISATION DEVELOPMENT

Endosperm formation

Fusion of the male gamete with the polar nuclei produces the primary endosperm nucleus which subsequently divides to form the endosperm tissue. Endosperm formation is of the helobial type, in which the primary endosperm nucleus divides to form a small chalazal and a larger micropylar chamber (Figure 30). The number of nuclear divisions occurring in the micropylar chamber is greater than that in the chalazal chamber. The chalazal chamber nucleus undergoes several divisions, with subsequent nuclear degeneration. The endosperm in the micropylar chamber is acellular to begin with (Figure 30) and later becomes cellular at which time the chalazal chamber consists of a few crushed cells and several disorganised nuclei (Figure 31).

When the proembryo starts to develop, the chalazal chamber is completely crushed, showing only one to two nuclei and the remains of several cells. Once the endosperm has become cellular, a mucilage cap (probably nucellar in origin) is formed and the micropyle is blocked. The endosperm only becomes cellular at Stages 6-8 of the development of the embryo.

The ovule during endosperm formation

During the initial post fertilisation stages, while the micropylar chamber is enlarging and the endosperm nuclei are multiplying, the outer integument grows together closing the micropyle. The two parts of the inner integument at the region of constriction also grow closer together, narrowing the micropyle. The inner epidermis of the inner integument is highly tanniniferous at this stage (staining very darkly). The nucellar tissue diminishes to one or two layers at the micropylar end and becomes reduced to a number of degenerative layers at the chalazal end. As the micropylar endosperm chamber becomes cellular, and the proembryo starts to differentiate, cells of the outer integument become elongated and the outer integument starts to pull away from the inner integument, leaving a small air space between the two integuments.

Both layers of the outer integument are still intact and retain cellular structure at this stage.

Embryogeny

The fusion of the pollen sperm cell and the egg cell (not observed) results in the formation of a zygote (syngamy) (Figure 32). This zygote subsequently divides to form a proembryo which eventually differentiates into the mature embryo. Embryo development is of the *Onagrad* type, in which the basal cell does not contribute a significant proportion of tissue to the embryonal mass. The development of the embryo displays the *Juncus* variation where some of the basal cell initials contribute to the formation of the suspensor, the root cap and the piliferous layer and the epidermal initials are cut off directly after the quadrant stage has been reached.

Embryogenesis

The zygote undergoes a transverse division to form a terminal cell *ca* and a basal cell *cb* (stage 1) resulting in a two-tiered proembryo (Figure 33). The terminal cell, *ca*, undergoes a longitudinal division producing a three-celled, two-tiered proembryo (stage 2) (Figure 34 and 35). The basal cell *cb* of the three-celled proembryo divides transversely, producing daughter cells, *m* and *ci* (stage 3). The four-celled proembryo consists of three tiers, *ca* (two cells), *m* (one cell) and *ci* (one cell) (Figure 36 and 37). Once the initials of *m* and *ci* are formed, the basal cell plays no further role in embryo development. The basal cell gives rise only to some of the suspensor tissue, the piliferous layer (root cortex or periblem) and root cap.

The two cells of *ca*, divide longitudinally to form a quadrant, tier *q*. The middle cell, *m* divides longitudinally to form two cells. The tier *ci* divides transversely to form two superposed cells, *n* and *n'*. The four-celled proembryo (stage 4) consists of four tiers, *q*, *m*, *n*, and *n'* and eight nuclei (Figure 38, 39 and 40). At this stage, the cells of the epidermal initials (tier *q*) are cut off and octants are not formed, but tier q does divide (Figure 41 & 42).

Tier *q* undergoes several divisions and forms two parts, *l* and *l'*, so that the basal region of the proembryo enlarges and the proembryo becomes pear shaped. Tier *n'* divides transversely to form two superposed cells, *o* and *p*. The tiers *p*, *o* and n form the suspensor. The proembryo consists of 6 tiers (stage 6), *p, o, n* (one cell each), *m* (two to four cells), *l'* and *l* (4 or more cells each) (Figure 43). Tiers *m, l'* and *l* undergo several divisions and increase in size. Tier *m* becomes one to two cell layers thick, whereas tiers *l'* and *l* become two to three cell layers thick (stage 6) (Figure 43).

Tiers *p* and *o* of the suspensor are reduced by cellular degeneration, and a single cell remains (*n'*) (Figure 44 and 45). The cell in tier *n* divides transversely and the cells in tier *m* proliferate so that *m* becomes multicellular and consists of several layers (Figure 45). Tiers *l'* and *l* both enlarge to thicknesses of eight and six cells respectively (stage 7) (Figure 44).

Eventually, tiers *n'* and *n* become reduced by cellular degeneration and the suspensor disappears, so that only tier *m* remains in this region. The mature embryo consists of a root cap region and the piliferous layer (tissue originating from tier *m*) (Figure 46 and Figure 47), the plumule (tissue from *l'*) (Figure 48 and 50), the central cylinder and the cotyledon (tissue originating from some of tier *l'* and most of tier *l*) and the outer layer of the cotyledon (tissue originating from tier *q*) (stage 8) (Figure 50).

The ovule during early embryogenesis (stages 1-5)

The cells of the two layers of the outer integument decrease in volume and become elongated. While the outer integument is much more reduced than in previous stages (i.e. pre-fertilisation stages), the inner integument does not diminish in size, and is maintained as two distinct layers of rounded, rather than elongated, cells. At this stage, the inner layer of the inner integument seems to become even more tanniniferous than in any of the preceding stages (staining darker with each successive stage of embryo formation).

The embryo sac is much enlarged in the micropylar compartment and is filled with a fluid endosperm containing nuclei scattered throughout. At the early stages of proembryo formation

(stages 1-3), the nucellus is at least 2 layers thick at the micropylar end of the ovule. As the proembryo differentiates however, the nucellar tissue is crushed by the expanding embryo sac, leaving a few cellular remains by the time the embryo has formed.

The proembryo differentiates at the micropylar end of the ovule directly below the filiform apparatus. The filiform apparatus degenerates and the cells of the proembryo grow toward the margin of the endosperm. It is not until the endosperm becomes cellular that the embryo is lodged into the outermost layer of the endosperm through the differentiation of a suspensor (stage 5).

The ovule during late embryogenesis (stages 6-8)

The outer integument separates from the inner integument, leaving a space between them. The cells of the outer layer of the inner integument become reduced in volume, and are now elongated, similar to the cells of the outer integument. The cells of the inner layer of the inner integument, however, maintain their size and tannin content until the final stages of embryo formation (stages 7 & 8), at which point they become elongated.

The nucellus remains as a collection of disorganised cells and a mucilage cap at the micropylar end of the ovule, but degenerates toward the chalazal region leaving an air space between the inner integument and the endosperm epidermis. At this point, the endosperm is completely cellular, and consists of uni-nucleate cells containing starch grains and several oil droplets.

The seed

Seed morphology and structure

The seed is trigonal, ovate to widely ovate in shape and very small (Figure 49). The seed contains the mature embryo which is linear in shape and is embedded within the endosperm

(Figure 50). The embryo takes up approximately half the volume of the seed.

The seed coat

The seed coat develops from both the integuments, the outer layer being loose around the seed, and almost transparent, separated by an air space from the inner layer of the seed coat (Figure 50). The cells are slightly elongated and loosely packed amongst one another (Figure 51). The inner layer of the seed coat, by contrast, is tough and envelopes the endosperm and embryo (Figure 50). The inner layer of the seed coat seems to be derived from the inner layer of the inner integument since this layer appears to retain the tannin that was formed at an earlier developmental stage. The cells of the inner layer of the seed coat are closely arranged and are elongated (Figure 52), so that the coat is continuous around the endosperm (Figure 50).

The endosperm

A thin layer between the inner layer of the seed coat and the endosperm appears to lack cellular structure and may be a cuticle (Figure 53). The endosperm tissue is enclosed by a distinct outer layer (possibly an aleurone layer as is found in grasses) which is composed of symmetrical cells that are larger than the endosperm matrix cells, and filled with granular inclusions (not starch) and some oil globules (Figure 53). The cells comprising the endosperm matrix are small, symmetrical, and almost spheroidal. They are filled with abundant starch grains and several oil droplets (Figure 53).

The cotyledon

The cotyledon occupies approximately three quarters of the embryo and is composed of a single, outer layer consisting of almost square cells and an inner portion, consisting of small, rounded cells which are five cell layers thick (Figure 50). When the embryo is mature, the cells

of the central cylinder of the cotyledon differentiate to form the vascular trace (Figure 50). The plumule lies directly above the vascular trace and consists of a region of small differentiated cells closely associated in a ball, and demarcated by a small elliptical space in the cotyledonary tissue (Figure 48 and 50).

Germination and seedling morphology

Germination is epigeal and takes place about one week after imbibition. To begin with, the base of the green cotyledon emerges from the seed, and following contact with the soil surface, grows upwards, raising the seed coat from the soil surface (Figure 55). Continued growth of the cotyledon is thus vertical, and the seed coat remains at the apex of the cotyledon. When the seedling is about one month old and several leaves have formed the cotyledon dies.

Towards the base of the embryo is the piliferous layer and the root cap region (Figure 46 and 47). From this tissue the primary root, or radicle, differentiates and grows downwards through a slit at the base of the cotyledon into the soil. The radicle divides and grows and is initially the major functioning root. Later, adventitious roots are also formed.

In a region immediately above the base of the cotyledon, the plumular bud differentiates to form the first foliar organ which emerges through a slit, giving rise to the primary leaf (Figure 55). The primary leaf is formed after one week. By the time the seedling is one month old, at least 3 leaves have been formed and the seedling is a miniature replica of the adult plant (Figure 55).

CYTOLOGY

The chromosomes in the root tips were visible although very small (Figure 54). No deductions could be made about the stages of mitosis and in addition no chromosome counts could be conducted due to the small size of the chromosomes.

SUMMARY

Endosperm formation is of the helobial type. The micropylar chamber is large and functional, while the chalazal chamber is crushed and becomes afunctional. The endosperm is abundant and is filled with oil droplets and starch grains. Embryogeny is of the *Onagrad* type (*sensu* Johansen 1950), the basal cell does not contribute significantly to the formation of the embryo, contributing instead to the short suspensor, the piliferous layer and the root cap. The epidermal initials are precociously cut off from the rest of the dividing proembryo directly after the quadrant stage, with octants not being formed. These characteristics are typical of the *Juncus* variation of the *Onagrad* type of embryogeny. The short suspensor is ephemeral, degenerating at late embryogenesis. The mature embryo consists of a root cap region and the piliferous layer, the cotyledon and central cylinder, the plumule and epidermis. The seed is trigonal, extremely small and is ovate to widely ovate. The seed coat is formed by both integuments; the outer seed coat is loose, the inner seed coat is thin and filled with tannin. The mature embryo is embedded in the endosperm and is linear in shape. Germination is epigeal, and the seedlings can be classified as type A (*sensu* Boyd 1932, Dahlgren & Clifford 1982).

PHYLOGENETIC ANALYSIS

Morphological data

The heuristic search found 221 trees and the shortest was 66 steps long. Each clade in the tree is referred to by name according to the most basally placed member. The 50% majority rule tree (Figure 56 and 57) for the revised morphological data set including embryological characters (Appendix 1) retrieved four major internested clades viz. the *Thurnia*, *Prionium*, *Distichia-Marsippospermum* and *Luzula* clades, while the position of *Juncus* was unresolved. The *Thurnia*, *Prionium* and *Luzula* clades have 68%, 29% and 37% bootstrap support respectively. The position of *Prionium* basal to *Juncus* plus the *Distichia-Marsippospermum* clade only has 18% support. The *Distichia-Marsippospermum* clade is weakly supported (43%). Within the *Distichia-Marsippospermum* clade, the *Distichia* clade has 44% bootstrap support, while the *Marsippospermum* clade has 68% support. Within the *Luzula* clade, the *Scleria-Rhynchospora-Oreobolus* clade has 93% support. The *Scleria* clade is weakly supported (44%) and within the *Scleria* clade the *Carex-Hypolytrum* clade is weakly supported (35%). The *Carex* and *Hypolytrum* clades are however relatively strongly supported (84% and 70% respectively). The *Rhynchospora* clade is weakly supported (40%) and within this clade the *Fuirena* and *Schoenoplectus* clades are also weakly supported (49% and 16% respectively), while the *Cyperus* clade is strongly supported (91%). The *Oreobolus* clade has quite strong bootstrap support (85%).

In 100 bootstrap replicates, *Luzula* was placed with Juncaceae (*Juncus* and/or the *Distichia-Marsippospermum* clade and/or *Prionium*) in only 24% of the trees and was placed to basally to Juncaceae (*Juncus* and/or the *Distichia-Marsippospermum* clade and/or *Prionium*) in 9% of the trees. *Prionium* was placed with Juncaceae (*Juncus* and/or the *Distichia-Marsippospermum* and/or *Luzula*) in 35% of the trees and was placed basally to Juncaceae (*Juncus* and/or the *Distichia-Marsippospermum* and/or *Luzula*) in 16% of the trees. *Oxychloë* was placed in the Cyperaceae (*Scleria-Rhynchospora-Oreobolus* clade) in only 4% of the trees,

and in each situation was never placed on its own, always with one or more of the other members of Juncaceae (usually members from the *Distichia-Marsippospermum* clade).

Combined data-total evidence

The combined analysis resulted in three trees, with the shortest 376 steps long. Four major clades were retrieved in the 50% majority rule tree (Figure 58) viz. the *Prionium* clade, the *Luzula-Oxychloë* clade, the *Luzula* clade and the *Oxychloë* clade. The *Prionium*, *Luzula-Oxychloë* and *Oxychloë* clades have strong bootstrap support (97%, 100% and 100% respectively), while the *Luzula* clade is weakly supported (38%). Within the *Luzula* clade the *Juncus* clade is weakly supported (53%), while the *Distichia- Marsippospermum* clade is strongly supported (94%). Within the *Distichia-Marsippospermum* clade, the *Distichia* clade has 100% support and the *Marsippospermum* clade 85%. In the *Oxychloë* clade both the *Carex* and *Cyperus* clades are strongly supported (92% and 94% respectively).

DISCUSSION

SYSTEMATIC IMPLICATIONS AND EVOLUTIONARY TRENDS IN MORPHOLOGY OF PARTICULAR EMBRYOLOGICAL STAGES IN SELECTED COMMELINANEAN MONOCOTYLEDONS

In the comparative embryology of selected families the classification of Thorne (1992) is followed for the commelinanean monocotyledons: except for Typhales, in which two families, Typhaceae and Sparganiaceae are recognised following Dahlgren *et al.* (1985). Thorne's treatment of *Anarthria* (included in Restionaceae) is followed here for simplicity although *Anarthria* is usually elevated to family status as suggested by Cutler & Airy Shaw (1965) and Linder & Rudall (1993). Thorne's Phylidrales, Zingiberales and Commelinineae (Commelinaceae and Mayaceae) are not considered in this study.

ANTHER DEVELOPMENT

Anther wall Formation

The development of the anther wall layers has been poorly studied in Juncaceae and thus no clear inference about the distribution of the type of wall formation in this taxon can be made. However, since wall formation has been reported to be of the monocotyledonous type in some Cyperaceae (see Dnyansagar & Tiwari 1956, Padhye & Moharir 1958, Padhye 1960, Davis 1966, Padhye 1971, Untawale & Makde 1971, Nagaraj & Nijalingappa 1973, Dahlgren & Clifford 1982, Nijalingappa 1986, Makde & Bhuskute 1987), it is possible that Juncaceae have the

monocotyledonous type of wall formation too. The monocotyledonous type of wall formation is approximately 13 times as common as the dicotyledonous type of wall formation within the monocotyledons (Kapil & Bhatnagar 1991)[1]. In fact Dahlgren *et al.* (1985) suggest that the monocotyledonous type of wall formation is found in almost all monocotyledons and may be a synapomorphy for the group. In addition it is highly probable that all of the commelinanean monocotyledons show this type of anther wall formation (see Weinzieher 1914, Begum 1966, 1968, Prakash 1969, Subramanyam & Narayana 1972, Hamann 1975, Rao & Wee 1979, Dahlgren & Clifford 1982, Bhanwra 1988, Johri *et al.* 1992).

The anther wall layers

Tapetum

The structure of the tapetum, i.e. the number of layers and the number of nuclei, are often thought to be taxonomically useful (Schnarf 1929, Davis 1966, Buss & Lersten 1975, Palser 1975, Dahlgren & Clifford 1982, Grayum 1991, Kapil & Bhatnagar 1991, Johri *et al.* 1992). This usefulness may however be limited since it is not always clear whether the data are recorded at microsporogenesis or at the stage of pollen release. Furthermore, the tapetum characteristics seem to vary considerably within genera and families (Table 1). However, it is important to stress that in most of the literature examined, only a limited number of studies have been undertaken at the family and even at the generic level. In legume subfamilies, Buss &

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[1] Kapil & Bhatnagar (1991) calculate the distribution of selected characters throughout the angiosperms and compare the proportions between dicotyledons and monocotyledons. For example, the distribution of the monocotyledonous type of wall formation is reported as 40% in monocotyledons and 60% in dicotyledons and the dicotyledonous type of wall formation as 3% in monocotyledons and 97% in dicotyledons. Thus, to obtain the distribution of a particular character within the monocotyledons, a ratio of the distribution of each character relative to others was calculated. For example, the ratio of the dicotyledonous wall type relative to the monocotyledonous type is 3:40 or that the monocotyledonous type of wall formation is 13 times more common than the dicotyledonous type.

Lersten (1975) surveyed 167 species from 89 genera, and were able to show that the tapetal nuclear number was taxonomically informative at the level of subfamily. This suggests that with an increased sample size, tapetal characters could be important in many other families.

In *Prionium serratum* the tapetum is uni-nucleate, as in *Juncus* and *Luzula* (Table 1). Cyperaceae however, has an inconsistent number of tapetum nuclei. The tapetum in Juncaceae usually consists of a single layer (Table 1). In contrast, although the tapetum in *P.serratum* is a single layer initially, it later becomes irregularly bi-layered. The tapetum is also irregularly bi-layered in *Oxychloë andina* Phil. (Table 1). This variation in tapetum structure is a feature present in several other Commelinanes e.g. *Flagellaria* and Typhales (Table 1). However, variation in the number of layers in the tapetum should be treated with caution. The number of layers in the tapetum may depend on the developmental stage of the anther and this is not usually indicated by researchers. In addition, the number of tapetal layers may not always be consistent among all species within a genus and therefore single species observations may be misleading. A single layered tapetum is typical of Cyperaceae (Table 1).

There seems to be no distinctive pattern in the number of layers and nuclei in tapeta throughout the commelinanean monocotyledons. However, the number of tapetal nuclei shows a general pattern of one for Juncaceae, one or two for Cyperaceae, mostly two for Poales, mostly two for Commelinales, one, two then eight for Typhales and mostly two to multi-nucleate in Bromeliaceae.

The type of tapetum

Generally, two types of tapetum are recognised in the land plants. These are the glandular-secretory type of tapetum and the amoeboid type which functions as a true periplasmodium.

Juncaceae is described as having a glandular-secretory anther tapetum (Davis 1966, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Johri *et al.* 1992), as does *P.serratum*. However, it is probably premature to say whether a glandular-secretory tapetum is a

characteristic of the family since only a few species have been investigated. A glandular-secretory tapetum is also typical of Thurniaceae, Cyperaceae, most of Poales and may be general in the commelinanean monocotyledons under consideration (Table 2). Exceptions are found in Bromeliales where both types of tapetum have been recorded; Typhales which always appears to have an amoeboid tapetum (but see Davis 1966) and *Abolboda* in Xyridaceae which also has an amoeboid tapetum (Table 2).

Dahlgren & Clifford (1982), suggest that the amoeboid tapetum in Typhales might indicate a closer relationship between this order and Haemodoraceae, Pontederiaceae and some Bromeliaceae in which an amoeboid tapetum predominates. The amoeboid type of tapetum also predominates in the Alismatiflorae and Ariflorae and is therefore likely to have evolved initially in these groups (Dahlgren & Clifford 1982). Pacini *et al.* (1985) propose that the amoeboid type of tapetum is widespread within the monocotyledons. This is consistent with Davis's (1966) contention. Kapil & Bhatnagar (1991) propose that the amoeboid type of tapetum is approximately five times more common than the secretory type of tapetum within the monocotyledons. In contrast, however, Dahlgren & Clifford's (1982) review suggests that the amoeboid type of tapetum is restricted to a smaller number of taxa. This conflict could be a result of different interpretations of "amoeboid". Dahlgren & Clifford's (1982) definition pertains to a true periplasmodium following the categories proposed by Carniel (1963), whereas Davis (1966), Pacini *et al.* (1985) and Kapil & Bhatnagar (1991) seem to have looser, if not slightly different interpretations of amoeboid tapetum types. The amoeboid tapetum type is generally defined by cells which retain their shape, but lose their cell walls. A number of subtypes within this category may also occur: e.g. cytoplasms fusing only after certain stages, and cytoplasms intruding between or enclosing the microspores (see Pacini *et al.* 1985 for a detailed account). However, Dahlgren & Clifford's categories of amoeboid types is dependent on when the tapetal cells form the periplasmodium and in which phase the nuclei multiply rather than whether the cytoplasms intrude or not. Thus in a "true periplasmodium" *sensu* Dahlgren & Clifford (1982) the cytoplasms of the tapetal cells must fuse at the beginning of the meiotic stage and the periplasmodium must undergo nuclear divisions. It seems that this situation occurs less

frequently than the other subtypes/categories which could account for the stark difference in numbers proposed by Dahlgren & Clifford.

It would appear that the evolution of an amoeboid tapetum may be a specialisation in response to a particular type of habitat, such as a moist one (Pacini *et al.* 1985). This hypothesis is supported by the data on tapetum types i.e. that the glandular-secretory tapetum is predominant in most of the Spermatophyta (see Pacini *et al.* 1985). However, an important finding is that the type of tapetum can be shown to have undergone phylogenesis which can be traced in extant taxa and therefore the development of different tapetum types may be phylogenetically informative. Both tapetum types are present in the commelinanean monocotyledons under consideration, however most of the families are characterised by a glandular-secretory type of tapetum. Pacini *et al.* (1985) propose that the secretory tapetum with walls (i.e. that found in bryophytes) is the most primitive type, which gives rise to the several other types, and the amoeboid tapetum is the most advanced type of tapetum in the Spermatophyta. The glandular-secretory tapetum is considered to be the ancestral state by Dahlgren *et al.* (1985) in the monocotyledons as it is in the dicotyledons, whereas the amoeboid tapetum is the derived condition in monocotyledons.

The endothecium

The endothecium in *P.serratum* is a single uni-nucleate layer and is also a single, fibrous layer in *Juncus bufonius* L. (Shah 1963). Zaman (1950) reports that the endothecium in *J.effusus* L. and *J.prismatocarpus* R.Br. is bi-layered along certain points in the anther.

The types of thickening patterns in monocotyledonous endothecia are usually classified as Girdle or Spiral. Noel (1983), developed a more sophisticated method of describing and classifying variation in endothecia, which Manning & Linder (1990) applied to Poales\Restionales, Manning & Goldblatt (1990) to Iridaceae and Freudenstein (1991) to Orchidaceae. In both Iridaceae and Orchidaceae, endothecial thickening types were consistent with proposed phylogenetic relationships within the families. However, Manning & Linder (1990) found that

the inclusion of endothecial thickening characters did not lead to a greater resolution of the relationships in Poales\Restionales.

Both Spiral and Girdle endothecial thickenings have been recorded within Juncaceae, Cyperaceae and other related families (see Untawale & Bhasin 1973) and therefore are unlikely to be useful in family level taxonomy and phylogeny. Manning & Linder (1990) suggest that previous recordings i.e. Spiral or Girdle types of endothecial characters have not been refined enough and therefore, could not be incorporated in a phylogenetic analysis. It therefore appears that the use of existing data on endothecial thickenings is questionable, and until such a time that these data are more refined they may not have taxonomic or phylogenetic value.

The middle layer(s)

The middle layer of the anther wall often varies with respect to the number of layers and whether it is ephemeral or persistent. The middle layer in *P.serratum* is a single layer which degenerates before pollen release. Similarly, the middle layer is single and ephemeral in *J.bufonius* (Shah 1963), indicating that this may be a general juncaceous feature. By contrast, Johri *et al.* (1992) report that the middle layer in *O.andina* develops fibrous thickenings and is therefore persistent. An ephemeral middle layer is a feature of Commelinales, Typhales and Bromeliales, the only exception being *O.andina* in which it is persistent (see Davis, 1966, Johri *et al.* 1992). The number of middle layers in the commelinanean monocotyledons under consideration does not seem to be a highly variable character (Table 3). Most of the families have a single middle layer, but Cyperaceae has one to two layers and only Flagellariaceae, some Poaceae and some Bromeliales having two middle layers, and Rapateaceae having one to four middle layers (Table 3).

It therefore appears that the presence of more than one middle layer is a feature specific to some genera, and may not be a family level character. Thus the middle layer may not elucidate phylogenetic relationships in the families of the commelinanean monocotyledons under consideration. In addition, the developmental sequence of the middle layer is not really known.

It is therefore not possible to determine whether the single layer is initially single in its development and remains so, or whether a single layer is a result of the degeneration of other layers at some time during the development.

POLLEN DEVELOPMENT

Microsporogenesis

Microsporogenesis has long been thought to be a useful systematic character which is able to elucidate family level relationships. Usually a family can be characterised by either the successive or simultaneous type of microsporogenesis. In situations where both types of microsporogenesis are recorded for a family, it is likely that microsporogenesis has been incorrectly determined (see Kapil & Bhatnagar 1991).

The simultaneous type of microsporogenesis seen in *Prionium serratum* has also been recorded in Juncaceae and Thurniaceae (Table 4). Schnarf (1929) suggests that simultaneous microsporogenesis should occur in the whole Juncaceae since the pollen is usually arranged in tetrahedral tetrads, although only the simultaneous type was reported in *Luzula pilosa* (L.) Willd and *L.multiflora* Lej.. Although microsporogenesis is simultaneous in Cyperaceae (Table 4), it is slightly different to that in Juncaceae (see Wulff 1939, Dnyansagar & Tiwari 1956, Padhye & Moharir 1958, Padhye 1960, Khanna 1963, Davis 1966, Padhye 1971, Nagaraj & Nijalingappa 1973, Meyer & Yaroshevskaya 1976, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Makde & Bhuskute 1987, Nijalingappa 1986, Johri *et al.* 1992). The first two meiotic divisions of the pollen mother cells are the same, with no cell plate formation occurring between the four nuclei. Three of the four nuclei are pushed toward the edge of the pollen mother cell and later degenerate. However, in some genera, the non-functional nuclei have been observed to divide (Padhye 1971, Johri *et al.* 1992). The single remaining nucleus enlarges, becomes separated from the degenerating nuclei by a septum and functions as the pollen grain nucleus.

In the commelinanean monocotyledons under consideration, simultaneous

microsporogenesis is restricted to Juncaceae, Cyperaceae, Thurniaceae and Rapateaceae while the rest of the families have successive microsporogenesis (Table 4). The ancestral condition in angiosperms is the successive type of microsporogenesis and this is also suggested as such in the monocotyledons (Dahlgren *et al.* 1985). However, Palser (1975) proposes that the simultaneous type of microsporogenesis predominates in dicotyledons and it is the more primitive type of microsporogenesis. Lowe (1961) proposes a similar argument for monocotyledons. In monocotyledons generally, successive microsporogenesis predominates, with only some Liliales, all Orchidales, most Dioscorales, some Asparagales and most Arecales having simultaneous microsporogenesis (Dahlgren & Clifford 1982). Thus, it is probable that simultaneous microsporogenesis evolved independently several times in monocotyledons.

The pollen tetrads in *P.serratum* are tetrahedral and less frequently cross tetrads. However, van Zinderen Bakker (1953) observed tetrahedral, cross and isobilateral tetrads in *P.serratum.* In *Juncus bufonius* (Shah 1963), tetrads are also tetrahedral, but less frequently isobilateral. Cranwell (1953) records tetrahedral pollen tetrads in *Juncus, Luzula* and *Rostkovia.* In general, the pollen in Juncaceae is reported to be united in tetrahedral tetrads (Wodehouse 1935, Erdtman 1945, Selling 1947, Cranwell 1953, van Zinderen Bakker 1953, Shah 1963, Dahlgren *et al.* 1985) although cross (Selling 1947, Cranwell 1953, van Zinderen Bakker 1953), isobilateral (Selling 1947, van Zinderen Bakker 1953) and rhomboidal (Selling 1947, Cranwell 1953) tetrads are also recorded. The pollen in Thurniaceae is also united in tetrahedral tetrads (Erdtman 1944, 1945, Dahlgren *et al.* 1985). Erdtman (1944, 1945) suggests that the pollen grains in Thurniaceae are morphologically the same as *Juncus, Luzula* and *Prionium.* Zavada (1983) also reports that Thurniaceae have pollen in tetrads, but does not elaborate on the arrangement of the tetrads. In Cyperaceae, the pollen is also arranged in tetrads, except that only the grain containing the functional nucleus is developed. The arrangement of the pollen in the tetrad in Cyperaceae has been termed a pseudomonad (Selling 1947, Cranwell 1953, Meyer & Yarashevskaya 1976, Dahlgren *et al.* 1985, Takhtajan 1991, Johri *et al.* 1992) or a cryptotetrad (see Davis 1966).

Pollen at dispersal

When pollen is dispersed, it is released either in the bi-nucleate condition, where the generative cell divides only on contact with the stigma, or in the tri-nucleate condition, where the generative cell divides just prior to pollen release. This phenomenon is often characteristic of particular families and thus, may be a very useful taxonomic character. In the monocotyledons, the occurrence of tri-nucleate pollen grains is approximately twice as abundant as bi-nucleate pollen grains (Kapil & Bhatnagar 1991).

Pollen is tri-nucleate at dispersal in *P.serratum* (Table 5). However, Brewbaker (1967) observed tri-nucleate pollen grains in only *Juncus* and *Luzula* and suggested that there were too few data available to make an assumption that Juncaceae could be characterised by tri-nucleate pollen grains. The available literature seems to indicate that tri-nucleate pollen grains are a feature of Juncaceae (Table 5), although this character has not been recorded in *Distichia*, *Marsippospermum*, *Patosia* and *Rostkovia*. Generally the pollen is tri-nucleate at anthesis in Cyperaceae (Table 5). Brewbaker (1967) has reported both binucleate and tri-nucleate pollen grains in some Cyperaceae (Table 5). Davis (1966) proposed that reports of binucleate pollen grains are on immature material. In Poales the pollen is bi and tri-nucleate at anthesis (Table 5). Flagellariaceae is the only family that has exclusively binucleate pollen, and Joinvilleaceae the only family that has exclusively tri-nucleate pollen grains at dispersal. The rest of the families have both binucleate and tri-nucleate pollen. In addition Restionaceae may have four nucleate pollen grains (Table 5). Pollen at dispersal is binucleate in Rapateaceae and Xyridaceae, but is tri-nucleate in *Xyris indica* L. (Table 5). In Eriocaulaceae, pollen is typically tri-nucleate, but is binucleate in *E.tribolum* (Table 5). In Typhaceae and Sparganiaceae pollen is binucleate at dispersal (Table 5). Pollen is generally binucleate at dispersal in Bromeliaceae, except *Schlumbergeria* which has tri-nucleate pollen (Table 5). The disparity in the number of nuclei in pollen grains within particular genera and/or families may be a result of observation at different stages in the pollen grain as suggested by Davis (1966). In *P.serratum*, the pollen was tri-nucleate only at the stage where the anther was already open and the pollen being released.

Thus, it is difficult to assess whether all reports of pollen nuclei numbers have been made at exactly the same stage, casting doubt on the reliability of reports of nuclei numbers.

It is postulated that binucleate pollen grains gave rise to tri-nucleate pollen grains (see Brewbaker 1967, Dahlgren & Clifford 1982) and therefore, that binucleate pollen grains are more primitive (Lowe 1961). Dahlgren *et al.* (1985) propose that the binucleate condition is plesiomorphic in angiosperms as well as in monocotyledons and that tri-nucleate pollen grains have evolved repeatedly in both dicotyledons and monocotyledons. Generally in monocotyledons, tri-nucleate pollen grains are found mainly in the Alismatiflorae, Commeliniflorae and Ariflorae (Dahlgren *et al.* 1985). In addition, tri-nucleate pollen grains in monocotyledons are often found in anemophilous families with reduced flowers (e.g. Cyperaceae and Juncaceae) and in those families adapted to aquatic habitats, but tri-nucleate pollen does not appear to have evolved as a result of these adaptations (Dahlgren 1975).

Pollen morphology

Although pollen wall ultrastructure of *P.serratum* has not been studied in detail, preliminary investigation reveals that the surface of the pollen grain has granulations. Some pollen morphological features are reported for Juncaceae and Thurniaceae which are potentially useful as phylogenetic characters (used later in the phylogenetic analysis). Van Zinderen Bakker (1953) records the exine of the pollen grain as being psilate or flecked with granules in Juncaceae, but as vaguely reticulate in *Prionium*. Wodehouse (1935) also recorded a granular exine in *J.campestris* L. and Selling (1947), a minutely granular exine in *L.hawaiiensis*. Zavada (1983) reports the exine sculpturing as psilate to scabrate in Juncaceae, whereas Meyer & Yaroshevskaya (1976) record the pollen surface as being finely rugulate in Juncaceae. The exine of Cyperaceae pollen is also granular or psilate as it is in Juncaceae (Selling 1947, Zavada 1983). The exine in Flagellariaceae and Joinvilleaceae is verrucate and minutely punctate, whereas in Restionaceae it is verrucate to verrucate-rugulate and psilate (Chanda 1966, Zavada 1983). In Centrolepidaceae the exine is verrucate and in Poaceae it is psilate, spinulose or

minutely scabrate (Chanda 1966, Zavada 1983). The exine in Eriocaulaceae is echinate (Zavada 1983), while in Rapateaceae it is reticulate, psilate or scabrate and is similar in Xyridaceae (Zavada 1983). The exine in Typhales is finely reticulate (Zavada 1983).

Pollen is ulcerate in Juncaceae (Selling 1947, Dahlgren & Clifford 1982, Zavada 1983) and also in Thurniaceae (Zavada 1983). Erdtman (1944) reports that the pollen in Thurniaceae has a three slit opening and that this is also similar in *Juncus* and *Luzula*. Cyperaceae pollen grains are thought to have several obscure pores (Selling 1947, Meyer & Yaroshevskaya 1976). However, Dahlgren & Clifford (1982) and Zavada (1983) report that in Cyperaceae, some genera have mono-apertures, whereas others have a few obscure pores e.g. *Calyptrocarya* which has four obscure pores. Pollen in Flagellariaceae, Joinvilleaceae, Restionaceae, Centrolepidaceae and Poaceae is spheroidal and monoporate, with the morphology of the pore varying slightly in the different families (Erdtman 1944, Chanda 1966, Dahlgren & Clifford 1982, Zavada 1983, Linder & Ferguson 1985). Linder & Ferguson (1985) suggest that the pore in Centrolepidaceae is similar to that found in Cyperales and based on other pollen morphology characters propose that Centrolepidaceae cannot be closely allied with Restionaceae. Pollen is spiraperturate in Eriocaulaceae (Zavada 1983). The different tribes in Rapateaceae are characterised by either monosulcate, disulcate or zonasulcate pollen (Zavada 1983). In Xyridaceae, the pollen is monosulcate (*Xyris*) or inaperturate (*Abolboda*) (Zavada 1983). Pollen in Typhales is monoporate and ulceroid (Zavada 1983). The pollen grain in Bromeliaceae can be monosulcate or multi-aperturate depending on subfamily (Zavada 1983). Rao & Wee (1979) report that pollen is di-aperturate in *Ananas comosus* Merrill.

The sporopollenin wall in Juncaceae is thin, atectate and is composed of a globular ektexine (Hesse 1980). Endexine is recorded as absent in Juncaceae (Hesse 1980). The pollen wall is not known in Thurniaceae (see Zavada 1983). The pollen wall in *Carex* (Hesse 1980) and *Eleocharis* (Zavada 1983) is tectate-collumelate with a perforate tectum and thick foot layer and is without endexine. In Restionaceae, Centrolepidaceae and Poaceae the tectum is scrobiculate (Chanda 1966, Zavada 1983, Linder & Ferguson 1985).

The development of the pollen and the pollen exine in Cyperaceae is similar to that of

Juncaceae. However, the arrangement of the pollen in the tetrad (i.e. pseudomonad in Cyperaceae) and the pollen pore morphology in Cyperaceae differs to that found in Juncaceae, and may be regarded as an advanced state to that in Juncaceae (Dahlgren & Clifford 1982, Dahlgren *et al.* 1985). Furthermore, the atectate (or reduced tectum) pollen in Juncaceae and Cyperaceae may be a synapomorphy for these two families and the ultrastructure of the pollen wall in Thurniaceae needs to be studied to determine whether this is a synapomorphy for Cyperales.

Although the pollen development in Cyperaceae differs slightly in the later stages to that in Juncaceae it is completely different to the development of the pollen in Poales. In addition the pollen morphology in Cyperaceae and Poales is also different. Thus, based on pollen characters, Cyperaceae are more closely allied to Juncaceae than to any of the Poales.

The pollen morphology and development in the three families of Commelinales under consideration differs substantially, with Rapateaceae possessing simultaneous microsporogenesis and also having pollen in tetrahedral tetrads (Dahlgren & Clifford 1982, Venturelli & Bouman 1988), whereas in Eriocaulaceae and Xyridaceae pollen is in monads (Dahlgren & Clifford 1982). Thus, Rapateaceae shows similar pollen development and exine sculpturing to Juncaceae and Cyperaceae. However, both Rapateaceae and Xyridaceae have sulcate pollen grains, indicating that these families may not be closely allied to Juncaceae and Cyperaceae. Moreover, pollen development and morphology in Eriocaulaceae is quite different to that in Juncaceae and Cyperaceae and therefore based on pollen development, no close relationship apparently exists between these families.

Along with Flagellariaceae, Typhaceae and Sparganiaceae are the only other families in the commelinanean monocotyledons under consideration that exclusively have binucleate pollen grains at dispersal. The pollen morphology is similar to Poales, Juncaceae and Cyperaceae in that it is ulceroid. However, the reticulate sculpturing and the arrangement of the pollen tetrads differs significantly to any of the poalean families and to Juncaceae and Cyperaceae.

The pollen development and morphology in Bromeliaceae is very different to that in Juncaceae and Cyperaceae and thus, based on these characters, Juncaceae and Cyperaceae are

not closely related to Bromeliaceae.

In monocotyledons and in angiosperms in general, the sulcate type of pollen grain is primitive (Zavada 1983, Dahlgren *et al.* 1985). Thus, the trichotomosulcate, sulculate, zonisulculate, ulcerate, foraminate, spiraperturate and inaperturate types of pollen grain are advanced (Dahlgren *et al.* 1985). With the loss of the sulcus, a reduction in thickness and complexity of the exine is often seen (Zavada 1983). Thus, pollen exhibiting the loss of a sulcus and a reduced exine are often present in more advanced taxa (Zavada 1983, Dahlgren *et al.* 1985). In the commelinanean monocotyledons, the sulcus is reduced to a pore via the ulcerate aperture type (Zavada 1983). Thus, in less derived families in the Commelinales e.g. Rapateaceae and Xyridaceae the monosulcate aperture prevails, while in more derived families e.g. Eriocaulaceae the ulcerate condition is present (Zavada 1983). The derived monoporate condition is seen in the derived orders of the Commelinales e.g. Juncales, Cyperales and Poales (Zavada 1983). Zavada (1983) suggested that the evolution of aperture types in the commelinanean monocotyledons coincides with a shift from insect pollination to wind pollination.

NUCELLAR TISSUE DEVELOPMENT

The formation of a nucellar tissue, or the absence of nucellar tissue, are important characteristics in angiosperm systematics (Davis 1966, Dahlgren 1975, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Kapil & Bhatnagar 1991, Johri *et al.* 1992). Many families can be characterised by their nucellar tissue development, but the terminology surrounding nucellar development has been confusing. Ovules are crassinucellate when the archesporial cell cuts off a parietal cell which then divides to form the nucellar tissue. Tenuinucellate refers to the condition that results when the archesporial cell functions directly as the megaspore mother cell without cutting off a parietal cell. In addition, ovules may have nucellar tissue which is formed when the nucellar epidermis divides forming a tissue cap or a nucellar cap, but these ovules are tenuinucellate, since the nucellar cap is not of parietal cell origin. Davis (1966) refers to these type of ovules as pseudo-crassinucellate. Furthermore, crassinucellate ovules may also form

nucellar caps, but no name is given to these types of ovules. In monocotyledons, the crassinucellate ovule is approximately twice as common as the tenuinucellate ovule (Kapil & Bhatnagar 1991).

The nucellar tissue in *Prionium serratum* is derived from a parietal cell, so the ovules are crassinucellar. This is typical of Juncaceae (Table 6). However, Eleuterius (1984) reports the nucellus as being crassinucellate in *Juncus roemerianus* Scheele, but later records the archesporial cell enlarging to form the megaspore mother cell directly, with no mention of a parietal cell. Zaman (1950) does not actually say that the ovule in *J.effusus* and *J.prismatocarpus* is crassinucellate, but does refer to several layers of nucellus and a deeply embedded archesporial cell differentiating into the megaspore mother cell. In *J.bufonius* (Shah 1963), *J.effusus* (Zaman 1950) and *J.prismatocarpus* (Zaman 1950) several archesporial cells are initiated, but only one becomes functional. The number of nucellar tissue layers formed in *P.serratum* is dependant on the stage of development of the ovule. If the ovule of *P.serratum* is compared at the mature megagametophyte stage, then there are 2 layers of nucellar tissue. In *J.roemerianus* (Eleuterius 1984) the nucellus is also two layered at the mature megagametophyte stage.

The ovule in Cyperaceae is of the crassinucellate type (Table 6). Generally, only one archesporial cell takes part in the nucellar tissue formation, but sometimes two archesporial cells are seen (Padhye 1971, Nagaraj & Nijalingappa 1973). This is a similar situation to that in *J.bufonius*, *J.effusus* and *J.prismatocarpus*. In *Cyperus rotundus* L. and *C.triceps* Endl., Khanna (1963) reported a crassinucellate ovule, but did not describe the formation of the nucellar tissue. The number of layers of nucellar tissue that are formed in Cyperaceae ovules is variable, usually being two to three (Padhye 1971, Untawale & Makde 1971, Nagaraj & Nijalingappa 1973, Makde & Bhuskute 1987) but in ovules of *Kyllinga triceps* Rottb. (Padhye 1960) the nucellar tissue consists of two to four layers and in *Scleria foliosa* Hochst ex A. Rich (Nijalingappa 1986) is five to six layered.

The crassinucellate condition is fairly widespread within the commelinanean monocotyledons under consideration, with only Poales (except Flagellariaceae), Eriocaulaceae

and Xyridaceae having tenuinucellate ovules (Table 6). In addition some Poaceae and Restionaceae (and possibly Xyridaceae, see Dahlgren *et al.* 1985) have ovules in which the nucellar epidermis proliferates to form a nucellar cap. There are conflicting reports in nucellar tissue types: for example, in Restionaceae, Johri *et al.* (1992) report the crassinucellate type of nucellus, while Goldberg (1989) reports both tenuinucellate and crassinucellate ovules, Goldberg (1989) also reports the crassinucellate type of ovule in Centrolepidaceae which is contrary to all other authors (Table 6).

The crassinucellate ovule is considered to be the more primitive of the two types in both dicotyledons and in monocotyledons (Lowe 1961, Sporne 1969, Dahlgren *et al.* 1985, Goldberg 1989, Kapil & Bhatnagar 1991). The crassinucellate condition is more widespread (particularly in dicotyledons); it is common in the Magnoliidae; and the developmental pathway indicates that this condition is less derived than the tenuinucellate condition. The tenuinucellate condition generally groups large numbers of families and is therefore of great phylogenetic significance (Dahlgren *et al.* 1985).

FEMALE GAMETOPHYTE DEVELOPMENT

Several types of embryo sac have been recognised by Maheshwari (1950) which are dependent on the number of spores involved in megagametogenesis to form the mature embryo sac. The *Polygonum* type of embryo sac is found in *P.serratum* and is common among the commelinanean monocotyledons; the only exceptions being Flagellariaceae which has the *Allium* type, Ecdeiocoleaceae which has the *Drusa* type (Rudall 1990) and Xyridaceae which has both *Polygonum* and *Allium* embryo sacs (Table 7). Variations of the *Polygonum* type also occur and these are found in some Poaceae which have a Poaceae variant of the *Polygonum* type (Anton & Cocucci 1984) and some Restionaceae which have proliferated antipodals (Rudall & Linder 1988) (Table 7).

Dahlgren *et al.* (1985) propose that because the *Polygonum* type of embryo sac is widespread in both monocotyledons and dicotyledons and possibly in the ancestor to

angiosperms as well as to the monocotyledons it is the general state, with all other embryo sac types derived. Gvaladze & Akhalkatsi (1990) and Takhtajan (1991) have suggested that monosporic embryo sacs e.g. the *Polygonum* type have given rise to the various other types of embryo sacs. However, Gvaladze & Akhalkatsi (1990) propose that the *Peperomia* type of embryo sac is more primitive than the *Polygonum* embryo sac and that mono, bi and tetrasporic embryo sacs may have had independent origins in angiosperms. Haig (1990) proposes, in contrast, that because of the independent origin of embryo sac types in a number of groups, the phylogenetic derivation of one type from another is not possible unless referred to in the context of that type's taxonomic distribution. Stebbins (1974) has argued that because the *Polygonum* type of embryo sac is so widespread throughout angiosperms and that the other types have only sporadic appearances in certain families, this character has no value in determining evolutionary trends.

ENDOSPERM DEVELOPMENT

The fusion of a male gamete with the polar nuclei or the secondary nucleus results in the formation of the primary endosperm nucleus. The division of this nucleus and the succeeding daughter nuclei result in the formation of endosperm tissue (Johri *et al.* 1992). Davis (1966) documents two types of endosperm formation, nuclear and helobial. The difference between the two types of endosperm can only be distinguished at the very early stages of formation where in the helobial type, the chalazal chamber becomes constricted and much reduced as free nuclear divisions occur in the micropylar chamber which becomes much larger as a result, while in the nuclear type no chalazal chamber is formed. Others (e.g. Swamy & Parameswaran 1962, Swamy 1973, Palser 1975, Dahlgren & Clifford 1982, Kapil & Bhatnagar 1991, Takhtajan 1991, Johri *et al.* 1992, Friedman 1994) distinguish between three types of endosperm formation, nuclear (the primary endosperm nucleus divides followed by free nuclear divisions which aggregate in either the chalazal or micropylar region without the formation of walls between the two chambers), helobial (the primary endosperm nucleus polarises to the chalazal region of the

embryo sac where it divides and wall formation (or a membrane) separates the two chambers) and cellular (the primary endosperm nucleus divides and wall formation occurs directly thereafter; further divisions may be followed by transverse or crosswise wall formation). In the commelinanean monocotyledons under consideration, no reports of the cellular type have been made and so this type will not be further considered here. The type of endosperm formation is poorly documented, with conflicting results, thus it is difficult to say whether reports in some of the older literature are accurate. In the monocotyledons, the ratio of endosperm types is 1 cellular:5 nuclear:28 helobial and thus the helobial type of endosperm is most common (Kapil & Bhatnagar 1991).

The helobial type of endosperm formation observed in *Prionium serratum* is characteristic for Juncaceae (Table 8). Laurent (1904) recorded the nuclear type of endosperm formation in *Luzula campestris* (L.) DC., *Juncus bufonius* and *J.glaucus* Ehrh.. Schnarf (1929) however, reports the helobial endosperm formation in these species and in other species of *Juncus*, *Luzula* and *Distichia*. The author suggests that Laurent (1904) incorrectly determined the nuclear type of endosperm by misinterpreting the chalazal cell nucleus. Laurent (1904) refers to this as a persistent median antipodal cell. In the commelinanean monocotyledons under consideration, nuclear endosperm is general while helobial endosperm occurs additionally (to Juncaceae) only in Typhales, *Abolboda* and in Bromeliaceae (Table 8). There are presumably mistaken reports of helobial endosperm formation in Eriocaulaceae and nuclear endosperm in Sparganiaceae (see Dahlgren *et al.* 1985, Müller-Doblies 1969, Asplund 1973).

There is no consensus on which endosperm type is ancestral in either the monocotyledons or dicotyledons (see Swamy & Parameswaran 1962, Dahlgren *et al.* 1985, Kapil & Bhatnagar 1991, Takhtajan 1991, Friedman 1994). Dahlgren *et al.* (1985) argue that the most plausible ancestral type of endosperm formation is the nuclear type because of its general distribution and that both the cellular and helobial types of endosperm are special or derived conditions in monocotyledons. Kapil & Bhatnagar (1991) on the other hand postulate that evolution of endosperm occurs through either the cellular or helobial type in monocotyledons. Whichever type is ancestral, the evolution of the tissue has been highly homoplasious. However,

for phylogenetic reconstruction local conditions are pertinent and that the occurrence of parallel evolution elsewhere is of little relevance.

ZYGOTE DEVELOPMENT (EMBRYOGENY)

Of the several different types of embryogeny (see Johansen 1950), only two types are present in the commelinanean monocotyledons under consideration. These are the *Onagrad* and *Asterad* types, with the different variations of these types present in different families (Table 9). In the *Asterad* type the basal cell contributes significantly to the rest of the embryonal tissue while in the *Onagrad* type it does not. Within the monocotyledons, the *Asterad* type of embryogeny is approximately three times as common as the *Onagrad* embryogeny (Kapil & Bhatnagar 1991).

The development of the embryo in *Prionium serratum* follows the *Juncus* variation of the *Onagrad* type which is characteristic of both Juncaceae and Cyperaceae (Table 9). Thus, these two families appear to have close affinities where the development of the zygote is considered. The *Juncus* variation of the *Onagrad* type of embryogeny is also seen in Centrolepidaceae, Sparganiaceae and possibly Restionaceae. The *Asterad* embryology and its variants have been recorded in Poaceae. Unfortunately, the type of embryogeny has not been recorded for Flagellariaceae which means that little inference about the general type of embryogeny in Poales can be made. Campbell and Kellogg (1986) propose that the embryogeny may be of the *Asterad* type in Joinvilleaceae, linking this family closely to Poaceae. Out of all of the embryological characters investigated thus far, there has been little or no conflict in characters between Typhaceae and Sparganiaceae except in the embryogeny, since Typhaceae is characterised by *Asterad* embryogeny, while Sparganiaceae has *Onagrad Juncus* variation. Eriocaulaceae, Xyridaceae and Rapateaceae are characterised by *Asterad* embryogeny of the *Penea* variation. Bromeliaceae are characterised by the *Muscari* variation of the *Asterad* embryogeny. The phylogenetic significance of the embryo is obscure due to the large amount of missing information in certain orders (e.g. Poales) and due to the fact that there is no plausible

hypothesis in the evolutionary pathway of the different embryo types.

In addition to the course of development of the embryo as a systematic character, the final stage of development of the mature embryo is different for many genera and is often elucidates relationships between genera (e.g. Shah 1965, Swamy 1966, 1979, Vanhecke 1974, Padhye & Makde 1982, Ramaswamy & Arekal 1982, Guignard 1984). Embryogeny in monocotyledons in general is *Asterad* and less often *Onagrad* (Dahlgren *et al.* 1985). Dahlgren & Clifford (1982) suggest that both types are closely related and either one is likely to have occurred in the monocotyledonous ancestor. However, Stebbins (1974) argues that embryogeny is not able to elucidate evolutionary trends in angiosperms because there is no correlation to other characters and because embryogeny is still unknown for many taxa. Stebbins (1974) does however consider that later stages of embryogeny in monocotyledons can be of evolutionary importance.

SEEDLING DEVELOPMENT

Seedling types are classified according to whether the cotyledon is green or not and where the plumular bud emerges from the cotyledon (Dahlgren & Clifford 1982). Dahlgren & Clifford (1982) have based their classification of the different seedling types on Boyd's (1932) seedling work. In Type A, the cotyledon is green and the plumule emerges at the base of the cotyledon and this type of germination is equivalent to epigeal germination as observed in dicotyledons. In seedling Type B, the cotyledon is non-photosynthetic and the plumular bud emerges through the sheathing base of the cotyledon. In seedling Type C, the cotyledon is also non-photosynthetic but the plumular bud emerges through a collar around the cotyledonary pore. Seedling types B and C are equivalent to hypogeal germination as observed in dicotyledons.

Seedling types appear to be rather specific for particular families (Table 10). However, only two major literature sources (Boyd 1932 and Dahlgren & Clifford 1982) were consulted for the seedling types, and little information is available on most genera and families within the commelinanean monocotyledons under consideration. Apparently seedling types were previously

not considered as potentially useful in systematics (e.g. Schnarf 1929, Davis 1966) and as a result few embryological studies have investigated germination characteristics. However, Thongpukdee (1989) suggests that in *Laxmannia* (Antheriaceae), seedling types are important phylogenetically. Furthermore, Thongpukdee was able to show some variation in a type A seedling, both within and between genera, thus indicating that seedling types are an important character.

Juncaceae have seedlings of type A where the germination is epigeal and the cotyledon is photosynthetic. This type of germination also occurs in *Prionium serratum* (Table 10). Cyperaceae on the other hand, have seedlings of type C, where the germination is hypogeal and the cotyledon is haustorial (Table 10). In Poales, seedling types are variable with Restionaceae having both Types A and C, Centrolepidaceae having Type A, and Poaceae having either type B or C, depending on how the grass embryonic organs are interpreted (Table 10). However, Hoshikawa (1969) has shown that germination in Poaceae is hypogeal and the cotyledon (scutellum) is haustorial. In Commelinales, data was only available for Eriocaulaceae which has seedlings of Type A (Table 10). Typhaceae also has seedlings of Type A (Table 10). The seedling types seem to vary considerably in Bromeliaceae (Table 10) and Boyd (1932) attributes this to the different habitat and growth form strategies of the particular genera and/or subfamilies. Most genera in Bromeliaceae do have seedlings of type A with the exceptions of *Pitcairnia* possibly representing a transitional type (Table 10) and *Aechmea* having seedlings of Type B.

The primitive condition in monocotyledonous seedling types is where the cotyledon is an undifferentiated sucker (Boyd 1932). The sucker is assumed to refer to a haustorial cotyledon. The epigeal type of seedling evolved from the sucker type and the hypogeal may have evolved from the epigeal or independently (Boyd 1932). Boyd (1932) contends that the sucker in Cyperaceae is a secondarily attained condition. In monocotyledons in general (also Juncaceae) there are relatively few Type A seedlings (only in Alismatiflorae, Ariflorae and Liliiflorae) (Dahlgren & Clifford 1982). If the seedling of Poaceae is interpreted as Type C, then it is actually a modified form of Type C as seen in Cyperaceae, because the prolongation of the

axis (i.e. mesocotyl) between the coleoptile and the scutellum leads to elongation of the collar (Dahlgren & Clifford 1982). There are several suggestions that seedling types may evolve in response to habitat types, but there is still a large amount of information required for many genera and families (see Boyd 1932, Dahlgren & Clifford 1982).

ONTOGENY OF EMBRYOLOGICAL STAGES IN PRIONIUM SERRATUM

Another approach as opposed to comparative embryology is to interpret embryology as a series of ontogenetic sequences. Each embryological stage is often a distinct ontogenetic trajectory and therefore may reflect a succession from a primitive character state to a more advanced one. Thus, ontogenetic sequences may be useful as a method of determining the direction of evolution. In addition the ontogeny of various tissues may also elucidate the origin of various tissue layers.

DEVELOPMENTAL ONTOGENY OF EMBRYOLOGICAL STAGES IN *PRIONIUM SERRATUM*

Anther ontogeny

During anther development, there are basically two ontogenetic stages: the differentiation of the anther wall into several layers and the differentiation of the tapetum so that it functions as the nourishing tissue for the developing microspores. The structure and formation of each of the tissue types within the wall layers is predetermined. The development of each tissue type is specific in particular anther wall types and the final structural arrangement of the tissues can be seen at the stage of anther wall formation. However, the function of particular tissues such as the tapetum require specialisation in the development of the tissue. Thus, the developmental specialisation of the tapetum occurs after the process of wall formation. Therefore, wall formation and tapetum specialisation may be viewed as two separate

developmental events, even though the initial formation of tapetal tissue is determined by the type of wall formation.

Anther wall ontogeny

There are four types of anther wall formation, the basic type from which the dicotyledonous, monocotyledonous and reduced types are derived (Davis 1966). In the basic type (Figure 59.A) the primary parietal layer divides to form two secondary parietal layers, an outer and an inner. The outer secondary parietal layer gives rise to the endothecium and one middle layer, while the inner secondary parietal layer gives rise to another middle layer and the tapetum. In the dicotyledonous type (Figure 59.C), the only inner secondary parietal layer gives rise to the tapetum. In the monocotyledonous type (Figure 59.D), the outer secondary parietal layer only gives rise to the endothecium. In the reduced type of wall formation (Figure 59.B), the primary parietal layer divides to form the endothecium and tapetum directly. Davis (1966) suggests that the reduced wall type is the most advanced type of wall formation.

Although wall formation was not observed in *Prionium serratum* it is likely to be of the monocotyledonous type. This is because the structure of the anther wall (Figure 2) (i.e. a single middle and endothecial layer and an initially single tapetal layer) is the same as the arrangement formed by the monocotyledonous type of wall formation (Figure 59.D). In addition, the widespread distribution of the monocotyledonous type of wall formation in related genera e.g. *Cyperus* (Padhye & Moharir 1958, Untawale & Makde 1971, Nagaraj & Nijalingappa 1973), *Scleria* (Nijalingappa 1986), *Kyllinga* (Padhye 1960, 1971, Makde & Bhuskute 1987) and *Fimbristylis* (Dnyansagar & Tiwari 1956) and monocotyledons in general (see Davis 1966, Dahlgren & Clifford 1982) points to this type being present in *P.serratum* too.

Tapetum ontogeny

Two tapetum types (viz. amoeboid and glandular-secretory) are present in the

commelinanean monocotyledons under consideration (Table 2). For both types, the tapetum structure is identical in the initial stages of microspore formation (Figure 60.A & B). It is only at microsporogenesis that the difference in both structure and function of the tapetum types becomes apparent. Thus, it appears as though the development of the two types of tapeta is divergent, with each type of tapetum being initiated early in development and following an independent ontogeny. For this reason, it is difficult to interpret the evolution of tapetum types, because it appears as though they follow mutually exclusive pathways which diverge at the start and do not consist of a continuous series of sequential stages. The development of the glandular secretory type of tapetum observed in *P.serratum* (Figure 60.C_2 & D_2) differs from the amoeboid type (Figure 60.C_1 & $D._1$) in that the cells, once initiated, remain intact throughout microsporogenesis. In the amoeboid type, however, the cells lose their structure and intrude between the developing microspore mother cells.

Pollen ontogeny

Generally, pollen grain ontogeny begins when a group of archesporial cells enlarge and differentiate into microspore mother cells (Figure 61.A). The microspore mother cells then undergo cytokinesis along one of two possible routes (Figure 61.B_1 or B_2). In simultaneous cytokinesis (Figure 61.B_1-J_1) as observed in *P.serratum*, wall formation does not occur after Meiosis I (Figure 61.B_1 and C_1), and the mother cell is separated simultaneously into four parts (tetrahedrally arranged) after Meiosis I and II (Figure 61.D_1). Separation is effected by advancing centripetal furrows across the middle of the mother cells (Figure 61.F_1-J_1).

In *P.serratum*, uni-nucleate microspore mother cells were observed (Figure 61.A, Figure 1), but Meiosis I was not seen, although the bi-nucleate cells succeeding Meiosis I were (Figure 61.C_1, Figure 5). At this stage, no cell plates could be distinguished between the nuclei. Meiosis II was possibly observed (Figure 3 & 4), and the microspore resting stage was, with the three nuclei in the tetrad visible simultaneously (Figure 61.E_1, Figure 5). No cell plates were observed in between the nuclei. The events leading to centripetal furrowing (Figure 61.F_1-J_1)

in the tetrad were not observed in *P.serratum*, but the fully formed microspore stage was (Figure 61.J_1, Figure 5). In *P.serratum* the uni-nucleate pollen cells divide to become bi-nucleate just prior to pollen release (Figure 9). At pollen release, the second nucleus divides to form a tri-nucleate pollen grain (Figure 10 & 11).

In successive cytokinesis (Figure 61.B_2-J_2), the cell plate is formed directly after meiosis I (Figure 61.C_2 & D_2), and again in each of the daughter cells after Meiosis II (Figure 61.F_2). The cell plate is centrifugal and divides the mother cell into two equal halves. Each cell dissociates from the tetrad and develops into a separate pollen grain (Figure 61.G_2-J2).

Integument ontogeny

In *P.serratum* growth of the inner integument is initiated when the archesporial cell differentiates (Figure 62.A). The inner integument begins to grow and encircle the ovule when the parietal cell is initiated and starts to divide to form the nucellar tissue (Figure 62.B). By the time that the megaspore mother cell has differentiated, the inner integument has developed into two layers and has grown to encircle about 3/4 of the ovule (Figure 62.C). At this time the outer integument is initiated and also differentiates into two layers and begins to grow around the ovule (Figure 62.C). When the megaspore divides, both integuments are complete and bi-layered, and lie close together (Figure 62.D). At the onset of megagametogenesis, the inner integument begins to swell at the apex, and the inner layer begins to show darkened cells, which are probably tannin cells (Figure 62.E). The micropyle is initiated at the eight nucleate stage when the inner integument becomes constricted at the apices (Figure 62.F). The outer integument is longer than the inner, but does not contribute to the formation of the micropyle. The cells of the inner layer of the inner integument are darkly stained with tannin cells at this stage.

The integument ontogeny as described above is useful in identifying the developmental stage of the ovule in *P.serratum* and may be useful in other species and/or genera too. Moreover, integument initiation and formation may be different in tenuinucellate or pseudo-

crassinucellate ovules relative to crassinucellate ovules. In a crassinucellate ovule (such as in *P.serratum*) the initiation of the integument coincides with the division of the archesporial cell to form the parietal cell. In both the tenuinucellate and pseudo-crassinucellate ovules the parietal cell has been lost and thus the initiation stage of the integuments might occur at a different stage of development in these ovules. Unfortunately integument ontogeny is rarely reported on in embryological studies (but see Dnyansagar & Tiwari 1956, Padhye 1960, Makde & Bhuskute 1987) and thus no comparisons between integument ontogenies in the commelinanean monocotyledons can be made at this stage.

Often, the stages in the growth of the integument coincide with particular stages in ovule curvature, nucellar tissue and female gametophyte formation (see Bouman 1971, Grootjen & Bouman 1988, Venturelli & Bouman 1988). In addition, the initiation of integuments and their ontogeny may be of paramount importance in understanding and defining seed coat characteristics in many taxa (Bouman 1971, Grootjen & Bouman 1988, Venturelli & Bouman 1988). For example, many layered integuments are often derived from different tissues initially in their development and therefore the later structures cannot be compared (Bouman 1971). The number of integuments and their mode of initiation may also be an important ontogenetic character which is useful in predicting phylogenetic relationships (e.g. Tobe & Raven 1985). Often the number of integuments is consistent at the family level and is an important systematic character, although this is truer of dicotyledons than monocotyledons (Davis 1966, Bouman & Calis 1977). Bitegmy is thought to be ancestral in angiosperms (Bouman & Calis 1977). The change from bitegmy to unitegmy can be explained by the developmental sequence i.e. either by (1) the disappearance of one of the integuments or (2) by the fusion of integuments or (3) by arrested development of the inner integument. Thus unitegmic integuments may not be homologous throughout the angiosperms, further emphasising the need to investigate developmental patterns.

Nucellar tissue ontogeny

In general, nucellar tissue ontogeny begins when the archesporial cell is initiated (Figure 63.A). At this stage, the development can proceed along two possible routes. The first route is where the archesporial cell enlarges and functions directly as the megaspore mother cell without undergoing any cellular divisions to form nucellar tissue (Figure 63.C_1). As a result, the formation of nucellar tissue may not occur. This type of developmental pattern is termed tenuinucellar. In addition, a modification of this type may also occur (Figure 63.C_2), where the nucellar epidermis multiplies to form a nucellar cap. Davis (1966) refers to this condition as pseudo-crassinucellate (Davis 1966).

The second possible route for nucellar tissue development is where the archesporial cell divides, forming a parietal cell and a sporogenous cell (Figure 63.B). The parietal cell undergoes several divisions to form the nucellar tissue, while the sporogenous cell is pushed into the ovule, where it enlarges to form the megaspore mother cell (Figure 63.C_3). This type of developmental pattern is termed crassinucellar and is the condition observed in *P.serratum*. In addition, in a crassinucellate ovule, the nucellar epidermis may also divide, forming a nucellar cap (Figure 63.C_4). Davis (1966) offers no terminology for this condition.

Thus, a crassinucellate ovule can have nucellar tissue formed only from the parietal cell, or from both the parietal cell and the nucellar epidermis. Since nucellar tissue formed from both a parietal cell and the nucellar epidermis can be present in an ovule at the same time, these two types of nucellar tissue cannot be homologous because homologous tissues cannot occur in the same ontogenetic stage at the same time (Patterson 1982). The two types of nucellus, each of which has separate developmental origin must therefore be described in a separate ontogenetic trajectory. The pseudo-crassinucellate condition is best described as a modification of the tenuinucellate type of ovule rather than a separate type of nucellus.

Female gametophyte ontogeny

In general, female gametophyte ontogeny is initiated when the megaspore mother cell undergoes the first division (Figure 64.A & B). Embryo sac type is determined by the number of spores that participate in the formation of the embryo sac. The developmental sequence of each of the different embryo sac types is modified after the first division of the megaspore mother cell. In *P.serratum*, the mature embryo sac is of the *Polygonum* type and thus is a monosporic embryo sac. The second division of the megaspore mother cell (Figure 64.B) results in a functional chalazal nucleus, while the three other cells degenerate (Figure 64.C). The third division results in two functional nuclei (Figure 64.D) which then divide, forming the four nucleate embryo sac (Figure 64.E). Each of the two nuclei at each pole of the embryo sac then divides forming an eight nucleate embryo sac (Figure 64.F) which later becomes organised as the mature embryo sac (Figure 64.G).

Endosperm ontogeny

Once fusion of the male gamete and the polar nuclei has occurred, the behaviour of the primary endosperm nucleus determines what type of endosperm tissue is formed (Figure 65). In *P.serratum* endosperm formation is of the helobial type in which the primary endosperm nucleus polarises to the chalazal chamber (Figure 65.B_1). Thereafter, the primary endosperm nucleus divides, producing a nucleus in both the micropylar and chalazal chambers (Figure 65.C_1). A wall is laid down between the two chambers and the chalazal cell may undergo several divisions. However, a greater number of nuclear divisions occur in the micropylar chamber, which enlarges and crushes the smaller chalazal chamber (Figure 65.D_1). Cell walls are laid down in the micropylar chamber late in the developmental sequence (Figure 65.E_1).

Zygote ontogeny

In general, zygote ontogeny is initiated when the zygote divides and begins to form the embryo. In angiosperms, the different types of embryogenesis are initially identical. The zygote usually divides transversely to begin with, except in the Piperad type, where the initial division of the zygote is longitudinal. Each particular type of embryogenesis can be characterised by quadrat formation, the degree of involvement that either the basal or apical cell plays and whether or not the suspensor is developed, ephemeral or persistent.

In *P.serratum* the trajectory of zygote development can be illustrated as in Figure 66. The *Juncus* variation of the *Onagrad* embryogeny is characterised by the basal cell only contributing to the formation of the initials of the root cortex and root cap (Figure 66.B-K), the early initiation of epidermal initials (Figure 66.F) and that octants are not formed after the eight celled stage (Figure 66.E).

Seedling ontogeny

Generally, seedling type is dependent on the developmental characteristics of the cotyledon, i.e. whether it is photosynthetic or haustorial, and this in turn determines how the seedling will germinate. The seedling type in *P.serratum* is of Type A and therefore germination is epigeal (Figure 67). Following germination (i.e. extension of the cotyledon) the radicle emerges from the base of the cotyledon, at which time the cotyledon starts to bend and grow upwards (Figure 67.C). Several adventitious roots are initiated once the radicle has formed and the cotyledon continues to grow upwards (Figure 67.D). The first leaf is initiated at the base of the cotyledon, where it grows from the plumular bud through a slit in the cotyledonary sheath (Figure 67.E). Once several leaves have formed, the cotyledon shrivels (Figure 67.F) and the young plant with permanent leaves is a replica of the adult plant (Figure 67.G). Tillich (1995) examined the seedlings of *Prionium* but does not give a description nor any illustrations of their development.

INTERPRETATION OF DEVELOPMENTAL ONTOGENY IN *PRIONIUM SERRATUM*

Developmental sequences

Generally, any ontogenetic sequence should go through a series of events beginning with a basic structure (or character state) that is modified as development proceeds into a more complex structure (see Nelson 1978, Kluge 1988, Roth 1988, Weston 1988). In addition, each stage should follow on from the preceding stage without interruption or loss of any of the stages (see Nelson 1978). Stated simply this means that any ontogenetic sequence should ideally be directional, law like and possibly hierarchical (e.g. Nelson 1978, Weston 1988, Wheeler 1990). However, several ontogenetic sequences differ with respect to many of these criteria. For example, ontogenies can be directional, but do not always proceed from the basic to the more complex. Furthermore, stages in the developmental sequence may be gained or lost i.e. deletions, additions, reversals and abbreviations (see Kluge & Strauss 1985, Kluge 1988, Kraus 1988, Mabee 1989, Wheeler 1990). The proposed hierarchical nature of ontogenies may also be questionable, since this is dependent on many of the foregoing criteria, such as whether ontogenies are directional, and whether stages have been gained or deleted etc. Thus, if these criteria are not met, then it may not be possible to resolve hierarchical relationships using ontogenetic sequences.

On examining the developmental sequences in *P.serratum*, it becomes apparent that few proceed from a general state (initial stage) through a series of intermediate states to an advanced state (final stage) within a single developmental sequence in one individual. Thus, a comparison between taxa shows that only the initial stages are common between the developmental sequences, while the final stages are different. This is characteristic of most developmental sequences considered in this discussion (e.g. tapetum types, microsporogenesis, nucellus formation, female gametophyte development, endosperm and zygote development). Consequently, these developmental pathways are entirely independent and by their very nature cannot reflect phylogenetic pattern.

All types of anther wall development are derived from the basic type (Davis 1966). Consequently, it initially appears that during the development of the anther wall, some hierarchical developmental pattern occurs. However, in the monocotyledonous type for example, the outer secondary parietal layer develops only into the endothecium. Thus, this differs from the basic type in that the development of one of the middle layers is bypassed. If this can be shown in the development of the anther wall i.e. that one of the middle layers is never formed, then the monocotyledonous type of wall formation is an example of a deletion of one of the middle layers (i.e. terminal deletion). Otherwise, if both of the middle layers are formed, but the development of one is terminated, then it may also be an example of an abbreviation of the middle layer (i.e. terminal abbreviation). A similar situation occurs in the dicotyledonous type of wall formation, except in the development of the inner secondary parietal layer. However, in the reduced type of wall formation, both the outer and inner secondary parietal layers do not form, and thus the reduced type may be an example of non-terminal abbreviation or deletion. Consequently, based on the nature of the ontogeny of anther wall types, phylogenetic patterns cannot be determined because of the abbreviation/deletion of various stages. However, the developmental sequences involved in anther wall formation may nevertheless offer insight into the derivation of particular components of the wall. For example in *P.serratum*, *Oxychloë andina*, *Flagellaria indica* L. and Sparganiaceae where the tapetum may be composed of two layers, this must occur after wall formation and therefore is a multiplication of the tapetum after the tapetum is initiated in the developmental sequence. Similarly, where particular families have two middle layers (e.g. Flagellariaceae, Poaceae and Bromeliaceae) the multiplication of the middle layers must have occurred after wall formation and the initial middle layer was formed. These types of developmental sequences are examples of terminal additions.

Another developmental sequence which may offer insight into phylogenetic relationships is pollen development. Of the commelinanean monocotyledon families under consideration only Cyperaceae has a pollen developmental sequence that proceeds through the general state to an advanced and specialised state. Pollen formation in Cyperaceae begins when the microspore mother cells undergo simultaneous microsporogenesis (Figure 68.B_1 and B_2). Initially four nuclei

are formed in the tetrad (Figure 68.B_3). At this stage the sequence of developmental events begins to differ to that found in the usual development of a tetrad following simultaneous microsporogenesis (Figure 68.A). Three of the four nuclei then migrate to the proximal end of the pollen grain (Figure 68.B_4) where they degenerate and the remaining nucleus becomes the functional nucleus (Figure 68.B_{4-6}). This developmental sequence should directly reflect some phylogenetic pattern since in the development of Cyperaceae pollen, the more general state (i.e. simultaneous microsporogenesis and development of the tetrad) is observed early in the ontogeny of the pollen grain. Rapateaceae, Thurniaceae, *P.serratum*, Juncaceae and Cyperaceae possess the general state at some time during pollen development, but only Cyperaceae exhibit the less general state i.e. degeneration of three of the nuclei. Such an ontogenetic sequence would suggest that Cyperaceae pollen are derived from Rapateaceae, Thurniaceae and Juncaceae and that the pollen in Cyperaceae is apomorphic relative to Rapateaceae, Thurniaceae and Juncaceae. This result (i.e. Cyperaceae pollen apomorphic relative to Juncaceae pollen) is congruent with that in the morphological cladogram (Figure 56 & 57). Furthermore, because Cyperaceae pollen has a common developmental origin with Rapateaceae, Thurniaceae and Juncaceae pollen, the developmental sequence is not an independent trajectory. Therefore, microsporogenesis cannot be referred to as "Cyperaceae-type" (see Simpson 1995 and discussion in Appendix 1) since microsporogenesis is simultaneous and the specialisation of the pollen development only occurs after that time.

Another ontogeny which may reflect hierarchical patterns in development is seedling ontogeny (see however Stevens 1980). However, only a few studies (e.g. Boyd 1932, Laurent 1904, Hoshikawa 1969, Eleuterius 1975) include seedling ontogeny from the initial stages through to the adult plant. Seedlings which are different to the adult early in their development may reflect phylogenetic relationships. For example Boyd (1932) vaguely refers to some of the aquatic monocotyledonous seedlings that show a tubular leaf which later develops into a wide floating leaf. In this case the tubular leaf seen in the seedlings is considered general (i.e. the ancestors had this type of leaf both in the seedling and adult). Thus, it is possible to determine

the polarity of the states directly. However, in *P.serratum* there is no difference between the seedling and the adult (Figure 67).

Ontogeny and phylogeny

Ontogeny is thought to be useful in determining the direction of evolution (Nelson 1978, Patterson 1982, Maddison *et al.* 1984, Brooks & Wiley 1985, Nelson 1985, Kluge 1985, Kluge & Strauss 1985, O'Grady 1985, Kluge 1988, Kraus 1988, Weston 1988, Mabee 1989, de Pinna 1991) and the origin of homologous structures (Nelson 1978, Patterson 1982, Alberch 1985, Nelson 1985, Kluge 1985, Kluge & Strauss 1985, O'Grady 1985, Blackmore & Crane 1988, Kluge 1988, Roth 1988, Weston 1988, de Pinna 1991). Consequently, it may be useful in reconstructing phylogenies. The concept that ontogenetic sequences directly reflect phylogenetic relationships is one that was modified and advanced by Nelson (1978, 1985) as a reformulation of Von Baer's Law. For successful delineation of phylogenetic relationships using Nelson's direct method, for example, each embryological sequence of a particular gametophytic or sporophytic stage must proceed from and include a more primitive character state. From this point, the sequence must proceed through intermediate developmental stages, with the most advanced state being expressed as the final stage of the sequence. Taxa which possess the primitive character state only are then said to be less derived than taxa which possess the intermediate or advanced state, but taxa possessing the advanced states must show the development of the primitive state early in their ontogeny. This forms the basis of Nelson's (1978) generality argument which is extended by Weston (1988) who postulates that the more general character must be possessed by all of the taxa that possess the less general character and also by some that don't. Nelson's law has the advantage of being applicable to both particular developmental states or entire ontogenies (Kluge & Strauss 1985) and therefore may give an idea of the direction of evolution in ontogenetic sequences. Thus, it is possible to take a pattern and propose direction of evolution without having an *a priori* hypothesis of relationship among taxa. However, the hypothesis of evolutionary direction is applicable only to that

ontogenetic sequence.

The direct method is particularly useful in determining character phylogenies where for example outgroups cannot be clearly defined (Kraus 1988, Weston 1988). The direct method is also appealing in that it deals exclusively with pattern and no *a priori* process assumptions are incorporated (Wheeler 1990). However, the direct method is not infallible, and there are several instances under which it fails, as Nelson himself notes. There are several characteristics of ontogenetic sequences which do not fit into the law-like, orderly nature predicted by Nelson's direct law (see Stevens 1980, Kluge 1985, Kluge & Strauss 1985, Kluge 1988, Kraus 1988, O'Grady 1985, Weston 1988, Mabee 1989, Wheeler 1990). These are subterminal modifications, such as additions, deletions and substitutions. Moreover, abbreviations (leading to neoteny or paedomorphosis) of particular stages also result in ontogenetic sequences that cannot be resolved using the direct method.

There are few botanical examples of ontogenies that can be resolved using the direct method. By contrast, in animals where the structural organisation of the organisms is far more complex, more examples are apparent (e.g. in vertebrate gill slits, Nelson (1973, 1978), in organs in salamanders Kraus, (1988), bones in Teleost fish, Mabee (1989) and in slime-mould beetles, Wheeler (1990)). Mabee (1989) found, that although the direct method was able to polarise and resolve character phylogeny, it yielded an incorrect polarity prediction. This was partly due to paedomorphosis (neotenic development or abbreviation). On the other hand, Wheeler (1990) found that the direct method yielded good predictions of character polarity and that paedomorphosis did not adversely affect the results. Wheeler does however suggest that this phenomenon might be a unique condition to his data set.

Weston (1988) uses *Acacia* leaf development as an example of where the direct method can be applied in plants. The development of Acacia leaves involves the initial formation of a compound leaf. The mature "leaves" are phyllodes which are petiolate in origin. Thus, this example incorporates serial homology, which strictly speaking does not fit into the criteria outlined for the successful use of the direct method. In the strict sense, phyllodes are not modifications of compound leaves, at the same time phyllodes are not a separate ontogenetic

trajectory since they are initially derived from the same meristematic precursor. Thus, Weston (1988) uses the direct method in the sense of generality to polarise the character phylogeny of *Acacia* leaf development. Other than the example using Cyperaceae pollen discussed earlier, other examples in embryological developmental sequences in the commelinanean monocotyledons considered are not apparent and generally seem to be lacking in the literature.

If structural complexity is required in ontogenetic sequences to provide a series of character transformations which can be used to show the direction of evolution, there is no reason why the ontogeny of complex floral organs cannot offer insight into phylogeny. Rasmussen (1986) proposes that development in orchid flowers may offer the possibility of elucidating phylogenetic history since, the flowers form complex reproductive structures. In addition, Rasmussen (1986) suggests that the ontogeny of the rostellum in some groups shows mature stages that have simpler rostella, indicative of other orchid groups. Thus the direct method may be of use in elucidating the relationships between orchid groups based on the rostellum. However, Rasmussen (1986) suggests that these characters should be polarised using outgroup comparison to understand evolution within the family. Another example in orchids which may offer insight into evolution within the family is the process of floral resupination (H.P. Linder *pers. comm.*). Initially in the floral development, the flowers are straight and only begin resupination later in the ontogeny. Thus, if any adults had straight flowers in Orchidaceae they would be less derived than those with resupinate flowers, assuming that no subterminal modifications have taken place. However, almost all orchids have resupinate flowers in the adult stage, thus indicating that this feature is apomorphic for Orchidaceae.

Linder & Kurzweil (in press) were able to show that in *Brownleea* the origin of the stigma is critical in understanding the phylogeny of the genus. In *Brownleea parviflora* Harv. ex Lindl. and *B.mulanjiensis* H.P. Linder the stigma is derived from all three carpel apices as it is in other orchids, while in *B.coerulea Harv. ex Lindl.*, *B.galpinii* H.Bol., *B.macroceras* Sond., *B.recurvata* Sond. and *B.maculata* P.J.Cribb the stigma is derived from a single carpel apex. This suggests, that a stigma derived from three carpel apices is the general condition and would be seen to develop early in ontogeny, while the stigma derived from a single carpel apex is the less general

condition, probably a result of arrested development of two of the carpel apices. This would imply that *B.coerulea*, *B.galpinii*, *B.macroceras*, *B.recurvata* and *B.maculata* are more derived than *B.parviflora* and *B.mulanjiensis*, or that *Brownleea* is not monophyletic.

Another example of where the direct method is applied is in the ontogeny of stylar appendages in Xyridaceae and Eriocaulaceae (although the author (Stützel 1990) does not actually say that he is using it). Stützel (1990) was able to show that initially the development of the stylar appendages is similar in both families. Three stylar appendages are formed in both families, but in *Abolboda* (Xyridaceae) the median appendage stops developing early in the ontogeny and in *Eriocaulon* and *Mesanthemum* (Eriocaulaceae) the stylar appendages are lost altogether (Stützel 1990). Thus, in both Xyridaceae and Eriocaulaceae, the general state is the presence of three stylar appendages. However, Stützel (1990) suggests that appendages in Xyridaceae are a derived character. This view however, is contradicted by the early development of stylar appendages in the ontogeny, and the abortion of the median appendage later in the developmental sequence in *Abolboda*.

Mishler (1986, 1988) also found that a direct observation of ontogeny in Bryophytes (e.g. *Tortula* gametophores) was able to elucidate character polarities, even though complex structures (such as floral organs) were not part of the developmental sequence. This suggests that there are examples of plant developmental sequences that can be analyzed using the direct method, although few ontogenetic sequences seem to meet the criteria required for the direct method.

Few studies however, actually use the direct method as an alternative to outgroup methods even when the ontogenies meet the criteria for the direct method. For example in *Eucalyptus* Drinnan & Ladiges (1988, 1989a, 1989b, 1989c) studied the ontogeny of floral organs, but analyzed the relationships (Drinnan & Ladiges 1991a, 1991b) between the species based on floral development using a phylogeny presented by Ladiges & Humphries (1983). Further, Tucker *et al.* (1993), used ontogenetic characters in addition to morphological characters to generate a phylogeny of Piperales to elucidate relationships between Saururaceae and Piperaceae. Other workers (e.g. Ramírez-Domenech & Tucker 1990, Han-Xing & Tucker

1990), tend to use comparative ontogeny (i.e. particular stages of development of the perianth) rather than directly observed ontogenies to elucidate relationships between taxa. Several other studies involving floral ontogeny and the use of outgroup criteria have successfully elucidated evolutionary relationships e.g. in *Delphinium* (Guerrant 1982) and in the evolution of cleistogamy e.g. in *Lamium* (Lord 1982).

These uses of other methods may largely be due to the fact that the direct method is problematic in that it can only be applied under certain circumstances. In addition its use has been met with criticism by several authors (see Brooks & Wiley 1985, Kluge 1985). The examples discussed here illustrate that the method may not be as easily applied as proposed by Nelson. In addition some authors (e.g. Wheeler 1990), perceive that the direct method proposed by Nelson is not in its strictest sense direct, since it involves the use of more than one taxon. This may be a valid supposition, but it is direct in the sense that it does not rely on any pre-existing phylogeny and thus may be used in instances where it is difficult to elucidate outgroups. The direct method, albeit problematic, may be a useful tool in predicting character polarities and ultimately can offer insight into phylogenetic relationships (see for e.g. Kraus 1988, Wheeler 1990).

PHYLOGENETIC ANALYSIS

The most parsimonious cladogram based on morphology does not corroborate the monophyly of the Juncaceae, despite the fact that the members of the Juncaceae have several characters in common *viz.* the absence of silica, more than one ovule per locule, axile, parietal or central placentation and helobial endosperm formation. Cyperaceae on the other hand is monophyletic and is defined by one to three fertile stamens, the presence of pseudomonads, a fruit that is indehiscent formed by a tricarpellary uni-ovulate ovary, the presence of silica bodies in its tissues and nuclear endosperm formation.

The morphological cladogram (Figure 56 & 57) is quite different to that of Simpson (1995) (Figure 69) even though the same genera were used. In particular, the position of *Juncus*, *Luzula*, *Prionium* and *Thurnia* are markedly different to their placement in Simpson's topology. *Prionium* is placed as sister to the remainder of Juncaceae and to Cyperaceae; while *Thurnia* is sister to *Prionium*, Juncaceae and Cyperaceae. *Juncus* is unresolved at the base of the *Marsippospermum-Distichia* clade, while *Luzula* is basal to Cyperaceae. Despite this divergence, the *Marsippospermum-Distichia* clade and a number of sedge clades of Simpson were retrieved. The *Distichia* clade is characterised by solitary, unisexual flowers, but unisexual flowers are also present in other commelinanean monocotyledons as well as in *Typha* and *Sparganium* and therefore are not unique to this group. The *Marsippospermum* clade is defined by having longer outer perianth segments than inner perianth segments. However, Heywood (1978) proposes that *Rostkovia* has perianth segments that are equal in length, a feature that would render *Rostkovia* and *Marsippospermum* invalid as sisters.

The morphological tree in this study is generally congruent with the *rbc*L tree of Plunkett *et al.* (1995) (Figure 70) except for the groupings of *Oxychloë* (cyperaceous in *rbc*L, juncaceous in morphological) and *Luzula* (juncaceous in *rbc*L, cyperaceous in morphological). These conflicts may be 'real' in that *rbc*L (plastid genome) and morphology (nuclear genome) actually track different phylogenies, or may be due to 'chance', in that the data sets may not be large enough to resolve the nodes confidently.

To determine whether the conflict between the morphological tree and the *rbc*L tree of Plunkett *et al.* (1995) may be due to chance, a method similar to that of Rodrigo *et al.* (1993) was used. The placement of *Oxychloë* in 100 replicates of the morphological and *rbc*L data sets was analyzed and the number of common nodes for Oxychloë determined. All 100 replicates of the *rbc*L data set retrieved *Oxychloë* as cyperaceous, while only four replicates of the morphological data set retrieved *Oxychloë* as cyperaceous. This suggests that the incongruence is not due to chance and that the placement of *Oxychloë* in Juncaceae in the morphological tree is also not due to chance. This suggests that although the morphological data are less robust than the *rbc*L, there is still a less than five percent probability that it would support grouping *Oxychloë* with Cyperaceae, and that the placement of *Oxychloë* in Juncaceae in the morphological trees is probably not due to chance.

The placement of *Luzula* is in conflict in the morphological and *rbc*L trees. The position of *Luzula* basal to Cyperaceae has weak bootstrap support and is supported by only a single character which is shared with Cyperaceae, namely basal placentation. The fact that *Luzula* was placed in Juncaceae in 24% of the morphological bootstrap replicates suggests that the position of *Luzula* is labile and that the conflict in its placement between the two trees may be due to chance. Several characters support this statistical evidence. *Luzula* lacks silica and has helobial endosperm formation, characters which are shared with other Juncaceae. However, in addition to basal placentation, the reduction in the number of ovules in *Luzula* may provide further support for the basal position of *Luzula* to Cyperaceae. The reduction in the number of ovules in *Luzula* may suggest that *Luzula* has moved from a trilocular multi-ovular situation with axile placentation to a unilocular situation with only three ovules, indicating a progenitor-derivative link between Juncaceae and Cyperaceae.

Juncus is in an unresolved position basal to Juncaceae and Cyperaceae in the morphological tree. The genus lacks synapomorphies and is variable for several characters, and also lacks the characters which define the *Marsipposperma-Distichia* clade thereby excluding it from this clade. *Juncus* may be paraphyletic or even polyphyletic; this would account for its ambiguous placement in the morphological tree. The *rbc*L tree in which *Juncus* is represented

by a single specimen, would not be affected by paraphyly.

The incongruence between the morphological and *rbc*L trees with respect to the placement of *Oxychloë* is apparently not due to chance in either case. Plunkett *et al.* (1995) suggest that the placement in the *rbc*L tree could not have been due to contamination since the juncaceous material was collected and sequenced long before any cyperaceous material. In addition, the voucher material for the sequence data was confirmed to be *O.andina*. Furthermore, they are confident that this topology reflects the true phylogenetic relationship between *Oxychloë* and Cyperaceae. There are three possible explanations as to why *Oxychloë* is included in the sedges in Plunkett *et al.*'s *rbc*L tree. The first being that *Oxychloë* is incorrectly determined as juncaceous, as Plunkett *et al.* (1995) suggest. The second is that the voucher material/herbarium material is not *O.andina* and may possibly be a sedge. The third is that *Oxychloë* is of hybrid origin, having both a juncaceous and cyperaceous origin (H.P. Linder pers. comm.). If there was a hybrid that formed from a paternal juncaceous member and a maternal cyperaceous member, and then back-crossed with another/same juncaceous member (i.e. introgression) the hybrid would have a juncaceous nuclear genome, but plastid sedge characteristics. Since *rbc*L is plastid genetic material it is only maternally inherited and this could offer an explanation for maternally inherited *rbc*L characteristics in *Oxychloë* and its placement in Cyperaceae in the *rbc*L tree, while the biparentally inherited characteristics such as morphological features place *Oxychloë* in Juncaceae as seen in the morphological tree. This phenomenon of "plastid capturing" is not uncommon in the plant kingdom and has been demonstrated in a number of plants using cpDNA (chloroplast-DNA)(e.g. Soltis *et al.* 1991a, 1991b, 1992, Soltis & Soltis 1995).

In the combined analysis (Figure 58), the topology obtained is in agreement with both the morphological topology and Plunkett *et al.*'s *rbc*L topology except for the position of *Oxychloë* in the morphological topology. The placement of *Oxychloë* in the combined tree suggests that the *rbc*L data is driving this arrangement due to the fact that *Oxychloë* was only placed with sedges in 4% of the random morphological bootstrap trees. The position of both *Juncus* and *Luzula* in the combined analysis is weakly supported (as they were in both the

morphological tree and Plunkett *et al.*'s *rbc*L tree) suggesting that the exact relationship of these two genera within Juncaceae and relative to Cyperaceae is interesting and warrants further research. The combined analysis also supports the notion of two clades in Juncaceae (excluding *Prionium* and *Oxychloë*).

The position of *Prionium* basal to Cyperaceae plus the rest of Juncaceae in the morphological tree, Plunkett *et al.*'s *rbc*L tree and the total evidence tree agrees with Cutler's (1969) contention that *Prionium* should be excluded from Juncaceae. This basal position of *Prionium* is supported by a number of morphological and anatomical features (i.e. the woody habit, carpels fused in the ovary region, chlorenchymatous air canals in the leaves and the absence of air canals between the vascular bundles (usually in parenchymatous tissue). The woody growth form is, however, not exclusive to *Prionium* within Cyperales, since *Microdracoides* and *Ghania* in Cyperaceae are also woody. The fusion of the carpels in the ovary region i.e. lack of a style is a feature rarely seen in Juncaceae although, some members of *Juncus* also lack styles i.e. *Juncus* is variable for this character. The placement of *Prionium* in Juncaceae in 35% of the 100 random morphological bootstrap trees suggests that based on morphology the basal position of *Prionium* is weakly supported (corroborated by weak bootstrap support). This indicates that the juncaceous characters which *Prionium* has (e.g. lack of silica and helobial endosperm formation) do support some relationship with Juncaceae. However, based on the unique features that *Prionium* has and the topology obtained from total evidence (100% bootstrap support for *Prionium* basal to Juncaceae and Cyperaceae) it is apparent that *Prionium* should be elevated to family status.

Cyperales may now consist of three families [Thurniaceae, 'Prioniaceae' and Cyperaceae (including Juncaceae *sensu strictum*)] or four [Thurniaceae, 'Prioniaceae', Juncaceae (excluding *Prionium* and *Oxychloë)* and Cyperaceae (including *Oxychloë*)]. It is impossible to recognise Juncaceae without raising *Prionium* to family level; however if Juncaceae are included in Cyperaceae then the recognition of Thurniaceae and 'Prioniaceae' would be arbitrary. Both *Thurnia* and *Prionium* have several apomorphies, but the residue (remaining Juncaceae and Cyperaceae) is poorly defined morphologically. Cyperaceae is monophyletic, except for the

inclusion of *Oxychloë*. Similarly the 'core' of Juncaceae is monophyletic. Problematic taxa are *Juncus*, *Luzula* and *Oxychloë*, which require further study for clear resolution.

Conclusions

The total evidence topology illustrated a number of features within Cyperales;

(i) *Prionium* is basal to Juncaceae plus Cyperaceae.

(ii) *Oxychloë* is basal to Cyperaceae

(iii) Juncaceae is paraphyletic

(iv) Cyperaceae is monophyletic

(v) Cyperales is monophyletic

The basal placement of *Prionium* within the Cyperales in the morphological and combined analyses indicates that the classification of *Prionium* may need to be reconsidered. This result is in agreement with Plunkett et al.'s (1995) *rbc*L analysis in which *Prionium* is also basal within Cyperales. This study also supports Cutler's (1969) contention that *Prionium* should be excluded from Juncaceae.

Although the embryology of *Prionium* conforms to that found in Juncaceae, *Prionium* has several unique characters viz. the woody habit, carpels fused in the ovary region, chlorenchymatous air canals in the leaf and the absence of air canals between vascular bundles. Based on these characters and the 100% bootstrap support for *Prionium* basal to Cyperaceae plus the paraphyletic Juncaceae in the total evidence analysis, it is suggested that *Prionium* be recognised as a distinct family.

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Figure 1: TS anther showing four sporangia (arrows) containing sporogenous tissue (sp) at sporogenesis. **Figure 2:** TS anther sporangium showing wall layers at sporogenesis. Epidermis (ep) distinct, endothecium (en) distinct but nuclei not prominent, middle layer (ml) well developed with nuclei visible, tapetum (t) irregularly bi-layered with prominent nuclei. **Figure 3 and 4:** TS anther sporangium at sporogenesis at two focal levels showing the division of nuclei (arrows) to form a cross tetrad. **Figure 5:** TS anther sporangium at sporogenesis showing bi-nucleate cells and tri-nucleate cells. Nuclei indicated by arrows. **Figure 6:** TS anther sporangium at pre-dispersal stage. Pollen tetrads (p) fill the sporangium. Epidermis (ep) degenerating, endothecium (en) persistent, middle layer crushed and degenerated, tapetum (t) showing signs of degeneration. **Figure 7:** SEM image of tetrahedral tetrad showing arrangement of pollen grains (pg) and granular surface of grains. **Figure 8:** DIC image of cross tetrad showing arrangement of pollen grains (pg). Bars = 1μm, except Figures 1, 6 & 7 bars = 5μm.

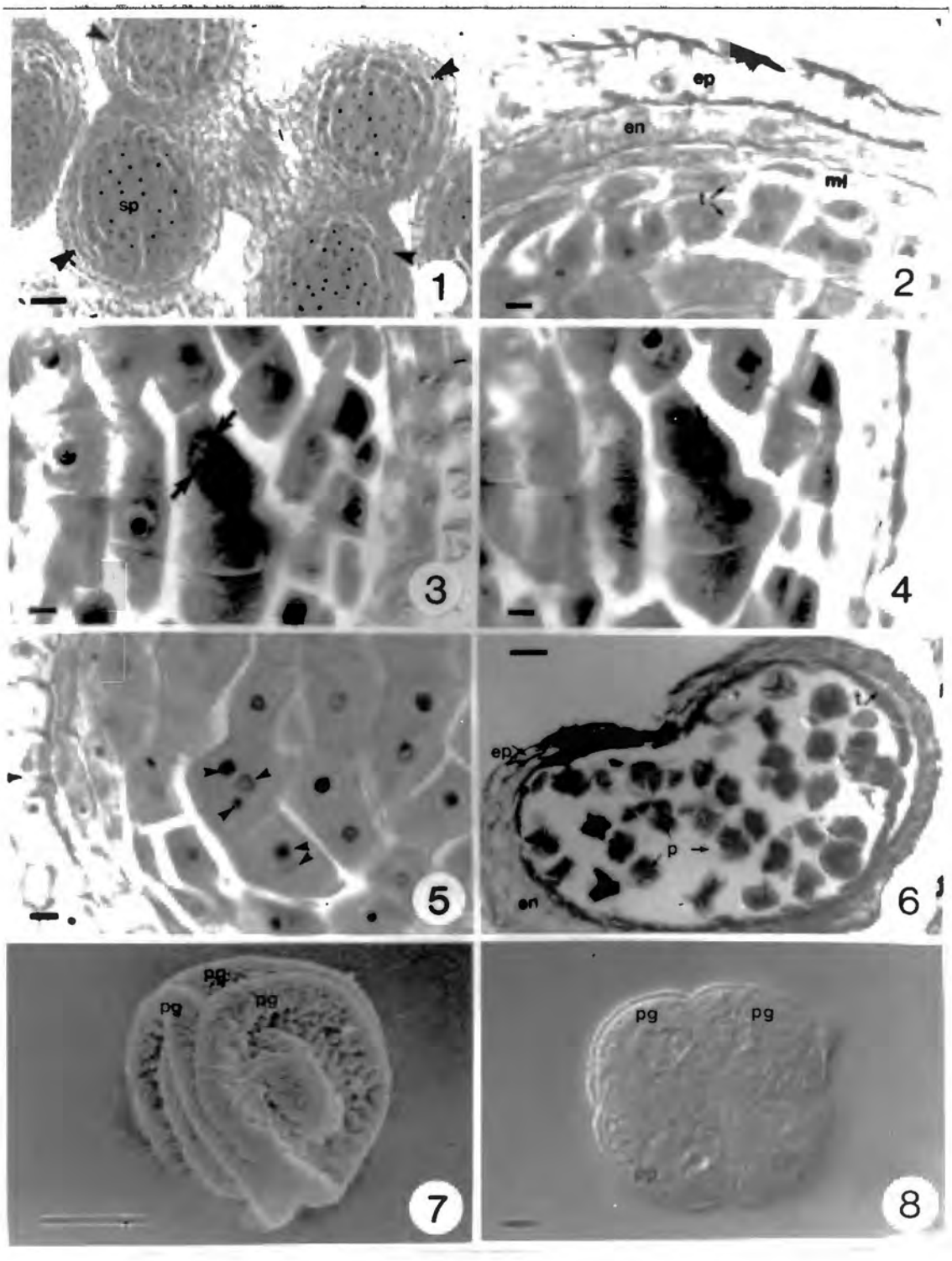
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Figure 9: DIC image of pollen grain at bi-nucleate stage (Nuclei indicated by arrows). **Figure 10:** DIC image of pollen grains with first nucleus and second nucleus dividing (arrows). **Figure 11:** DIC image of pollen grain nearly at tri-nucleate stage with first nucleus and second nucleus divided, daughter cells at opposite poles (arrows). **Figure 12:** TS ovary showing three locules (arrows) with ovules (o) borne axially on placenta (p) with complete septa (s) between locules. **Figure 13:** TS ovary showing anatropous curvature of ovules (arrows) on the placenta. **Figure 14:** Whole fruit showing ovary (o) and three papillate stigmata (s) (third beneath). Arrow indicates absence of style. **Figure 15:** LS ovule showing the archesporial cell (a) with prominent nucleus (arrow) embedded in undifferentiated tissue directly beneath the nucellar epidermis (ne). Inner integument primordium (ii) only just distinguishable. **Figure 16:** LS ovule showing archesporial cell (a) and parietal cell (pc) both with prominent nuclei (arrows), lying directly beneath the nucellar epidermis (ne). Inner integument (ii) beginning to develop. Bars = 1μm except Figures 12 & 13 bars = 10μm and Figure 14 bar = 100μm.

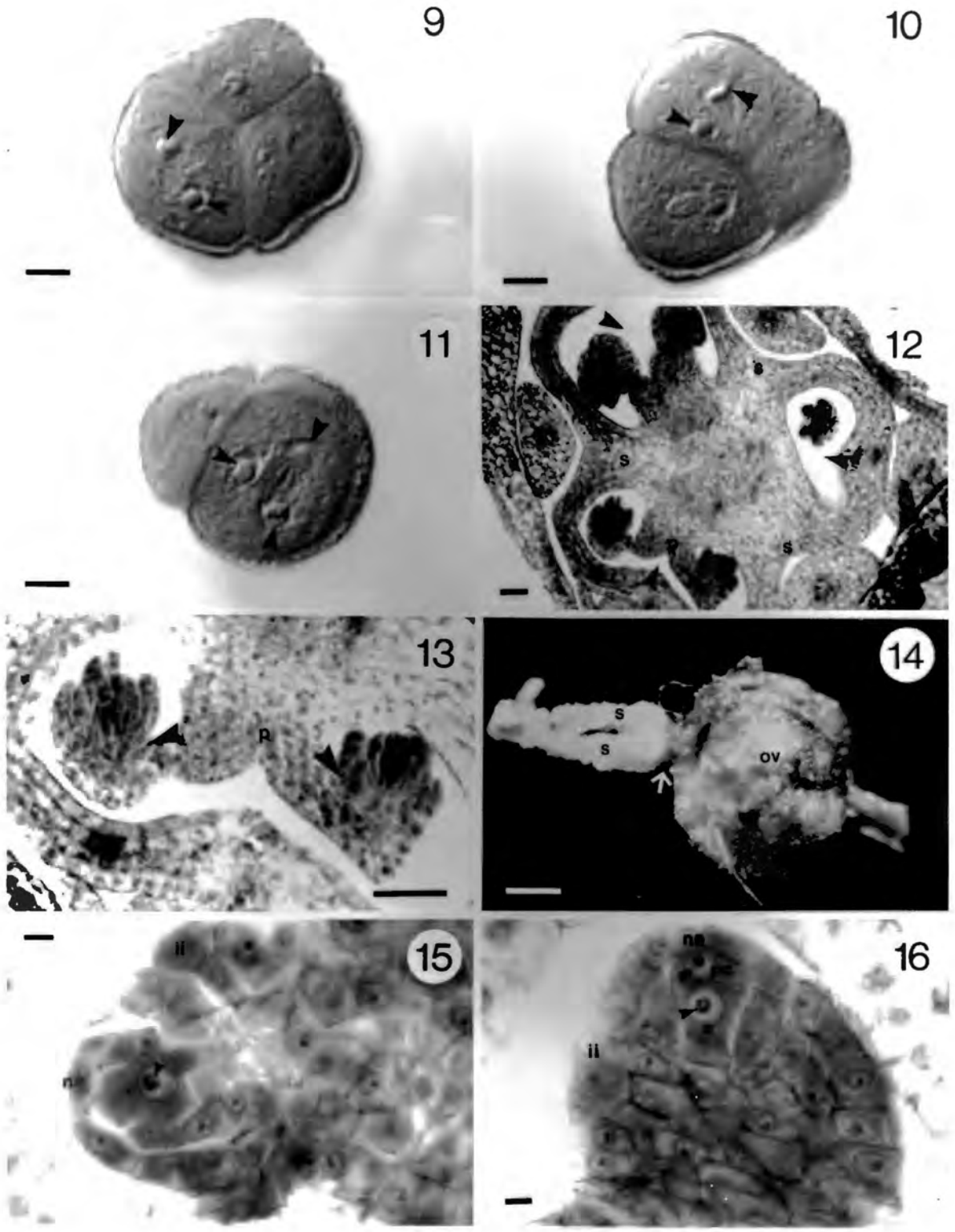
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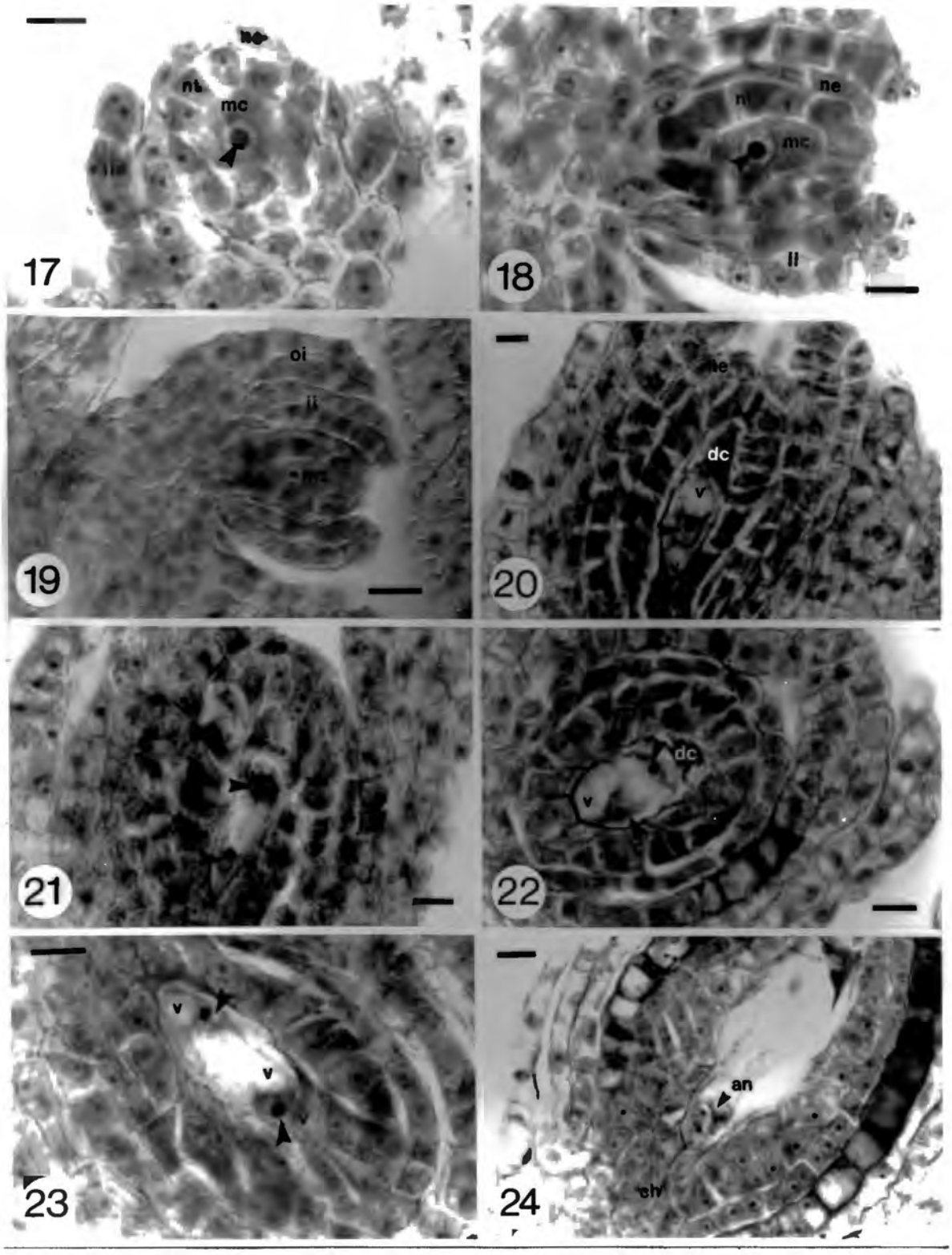
Figure 17: LS ovule just as megaspore mother cell (mc) has differentiated. Nucellar tissue (nt) one layer beneath the nucellar epidermis (ne). Inner integument (ii) bi-layered. **Figure 18:** LS ovule showing megaspore mother cell (mc) enlarging with prominent nucleus (arrow) with one layer of nucellar tissue (nt) beneath the nucellar epidermis (ne) and the inner integument well developed (ii). **Figure 19:** LS ovule at megaspore mother cell stage showing outer integument (oi) less developed than inner integument (ii), both bi-layered. **Figure 20:** LS ovule showing late megasporogenesis meiosis II. Chalazal cell nucleus (arrow) functional and the cell becoming vacuolated (v). with the other three cells degenerating (dc). Nucellar tissue (nt) one to two layers beneath the nucellar epidermis (ne). **Figure 21:** LS ovule megagametogenesis mitosis I. The functional megaspore divides mitotically forming two daughter cells which move to opposite poles of the ovule (arrows). Crushed cells (cc) visible at micropylar end of ovule. Nucellar tissue (nt) two layers beneath nucellar epidermis (ne). **Figure 22:** LS ovule megagametogenesis mitosis I. Daughter cells (arrows) and crushed cells degenerating (dc) at micropylar end of ovule. Nucellar tissue (nt) two layers beneath nucellar epidermis (ne). **Figure 23:** Composite photograph of LS ovule megagametogenesis mitosis I with only daughter cells remaining (arrows) and becoming vacuolated (v). **Figure 24:** LS ovule at 8-nucleate stage showing the three antipodal cells (an) at the chalazal end (ch) of the ovule. Bars = 2μm except Figure 19 bar = 10μm.


17
nt
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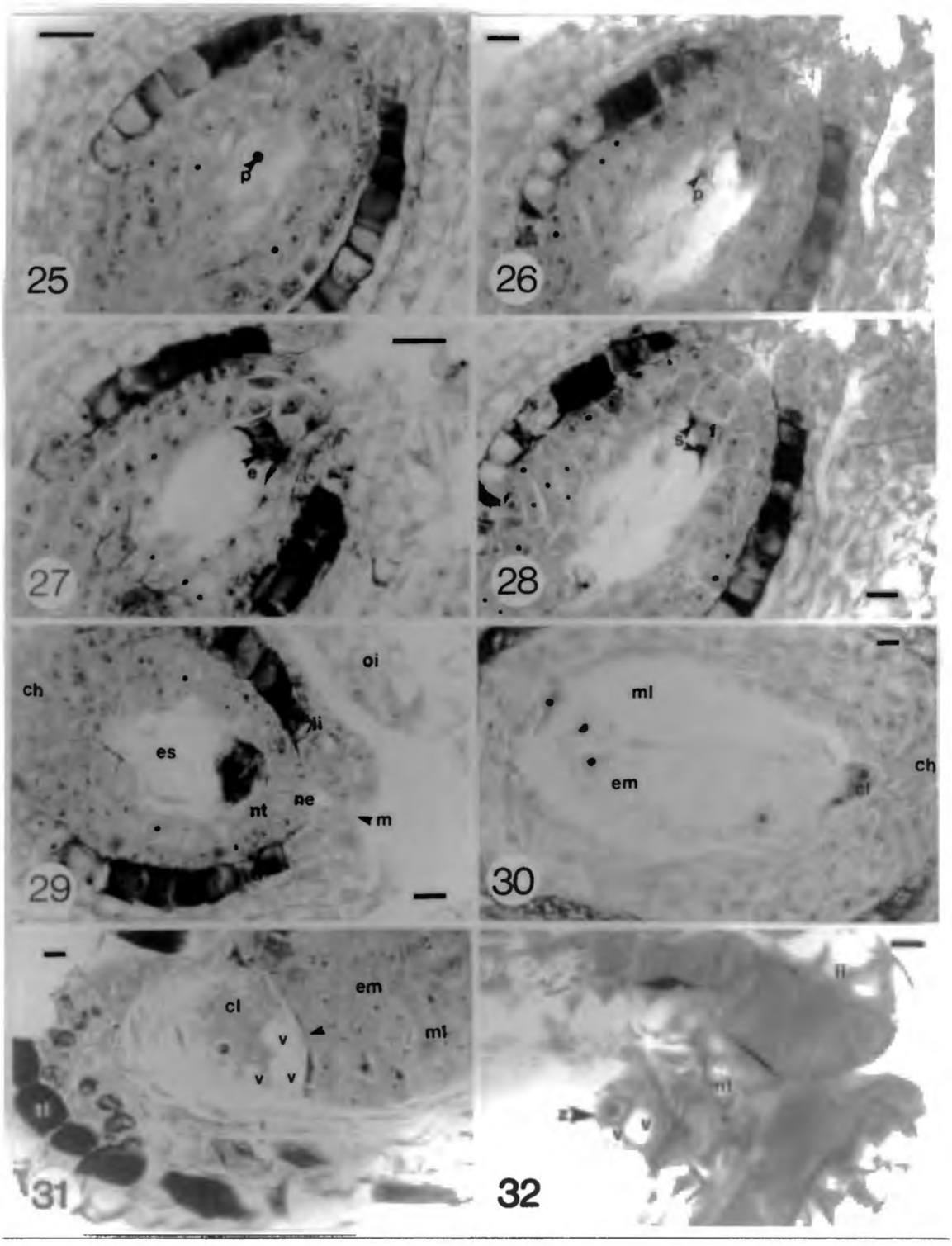
sh

Figure 25 & 26: LS ovule at 8-nucleate stage showing the two polar nuclei (p) at two focal levels. **Figure 27 & 28:** LS ovule at 8-nucleate stage showing the egg apparatus at two focal levels, egg cell (e), the two synergids (s) and the associated filiform apparatus (f). **Figure 29:** LS ovule at 8-nucleate stage showing micropyle (m), the inner and outer integuments (ii and oi) with the tannin layer (tl) in inner layer of inner integument, embryo sac (es) and chalazal region (ch). **Figure 30:** LS post fertilisation ovule showing helobial endosperm formation. Endosperm (em) acellular consisting of several scattered nuclei in a larger micropylar chamber (ml). The crushed chalazal chamber (cl) vacuolated (v) and containing a single nucleus. **Figure 31:** LS post fertilisation ovule showing cellular endosperm (em) in the micropylar chamber (ml). The smaller chalazal chamber (cl) vacuolated (v) containing a single nucleus. Arrow indicates membrane between micropylar and chalazal chambers. **Figure 32:** LS post fertilisation ovule showing zygote (z) with vacuoles (v) lodged beneath the nucellar tissue (nt), inner integument (ii) with tannin layer (tl). All bars = 2μm.


25
p
26
p
27
e
28
e
29
ch
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li
es
nt
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m
30
ml
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ch
cl
31
em
cl
v
ml
v
v
ll
32
ll
ml
v

Figure 33: LS ovule showing the two celled proembryo (stage 1) with terminal cell (ca) and basal cell (cb). **Figure 34-35:** LS ovule showing the three celled, two tiered proembryo (stage 2) over two sections with basal cell (cb) and terminal cell which has divided to form two daughter cells (ca). **Figure 36:** Composite photograph of LS ovule showing the three tiered proembryo at stage 3. The basal cell has divided to form two daughter cells (m) and (ci), terminal cell (ca). **Figure 37:** as for figure 36, but on a single plane of focus. **Figure 38-40:** LS ovule showing proembryo at stage 4 over three sections. Tier (ca) divides to form a quadrant (q), the middle cell divides to form to cells (m), tier (ci) divides to form two cells (n) and (n'). All bars = 2μm.

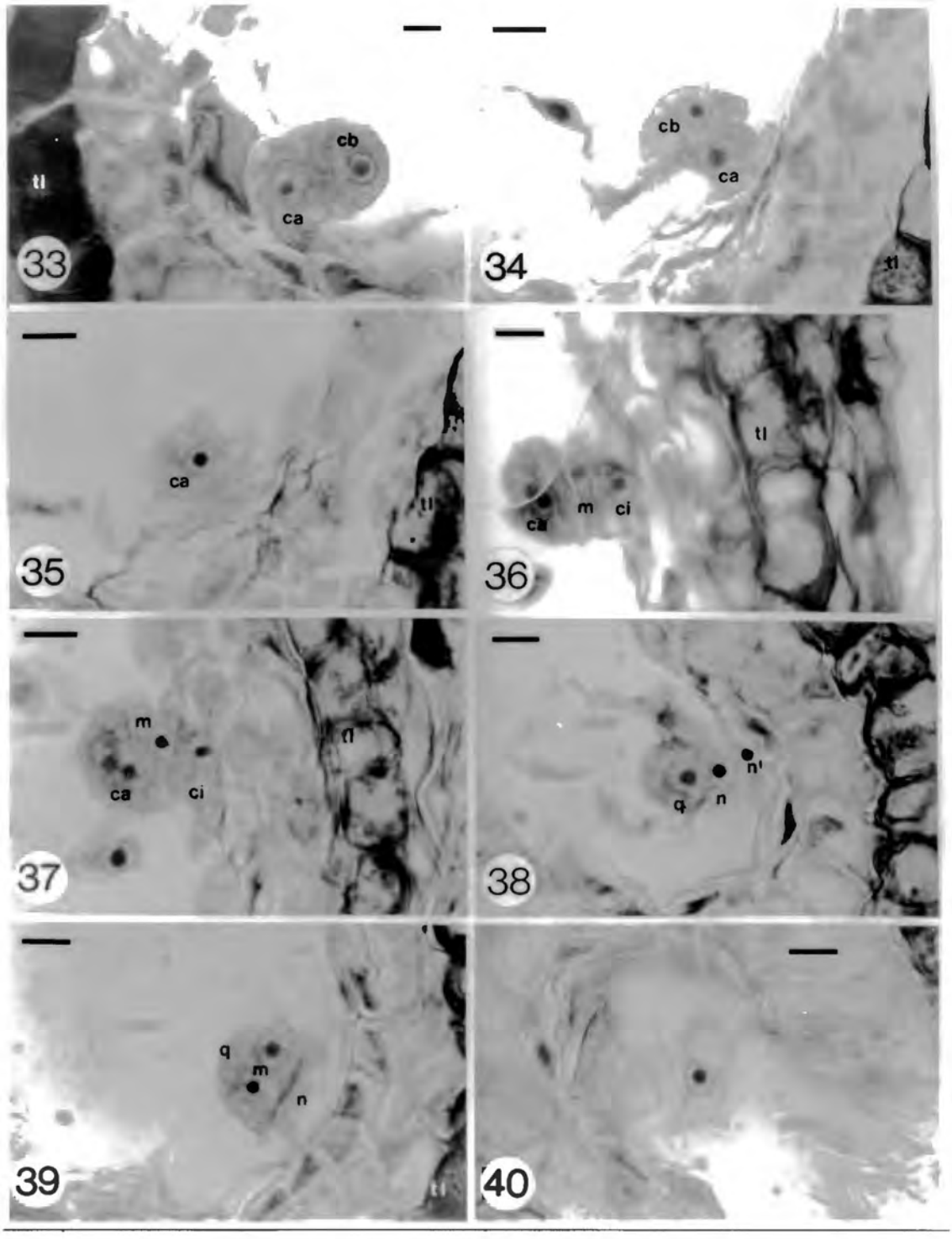
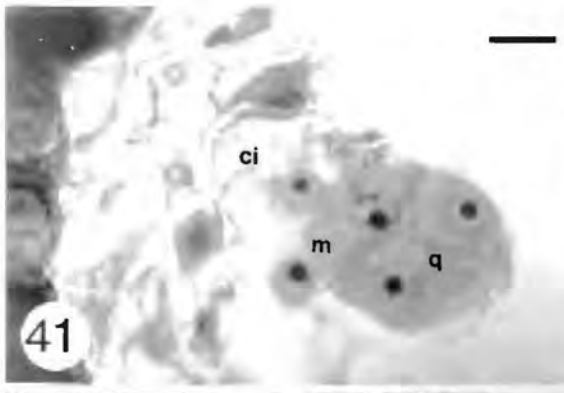
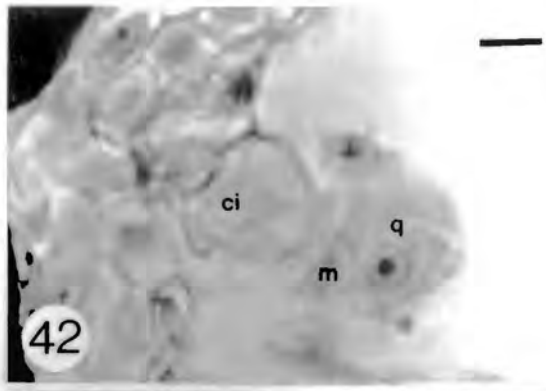
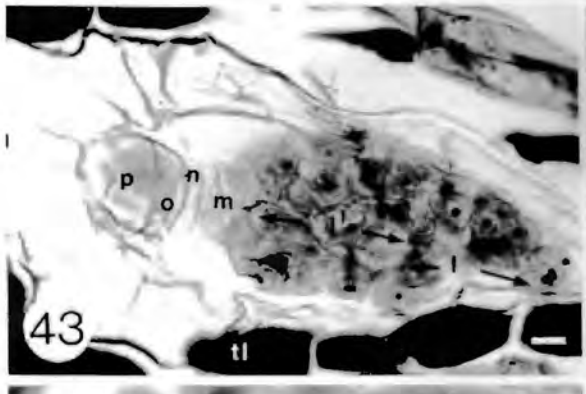
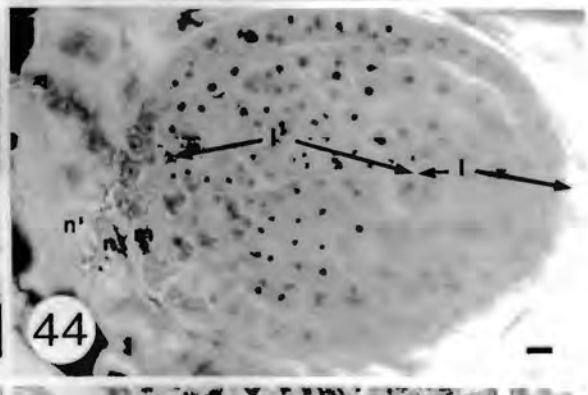

cb
tl
ca
33
cb
ca
34
ca
tl
35
tl
m
ci
ca
36
m
tl
ca
ci
37
n'
q
n
38
q
m
n
39
40

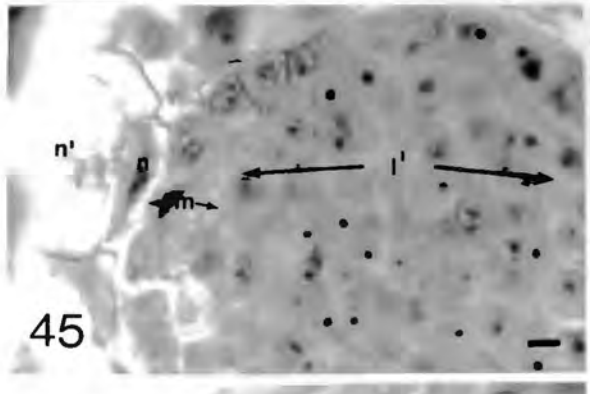
Figure 41-42: LS ovule showing proembryo at stage 5, where tier (q) has undergone several divisions so that the basal region of the proembryo has expanded in size. **Figure 43:** LS ovule showing proembryo at stage 6. Tier (q) forms two parts (l) and (l'), tier (n') forms two cells (o) and (p). The proembryo consist of six tiers, p,o,n,m,l' and l. **Figure 44-45:** LS ovule showing the proembryo at stage 7. Tiers (p) and (o) of the suspensor are reduced and a single cell remains (n'). Tier (m) becomes several layers thick, tier (l') becomes 8-10 layers thick while (l) is 5-6 layers thick. **Figure 46:** LS seed showing the mature embryo, root cap region (rc) and cotyledon (co). **Figure 47:** DIC image of embryo showing root cap region (rc) and cotyledon (co). **Figure 48:** LS seed showing mature embryo, plumule (pl), outer seed coat (oc) and inner seed coat (ic). All bars = 2μm.

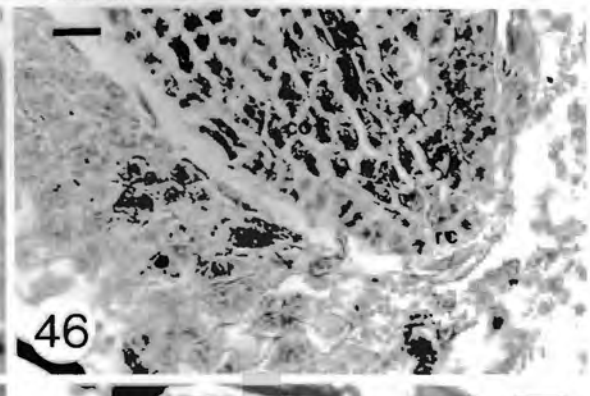

ci
m
q
41

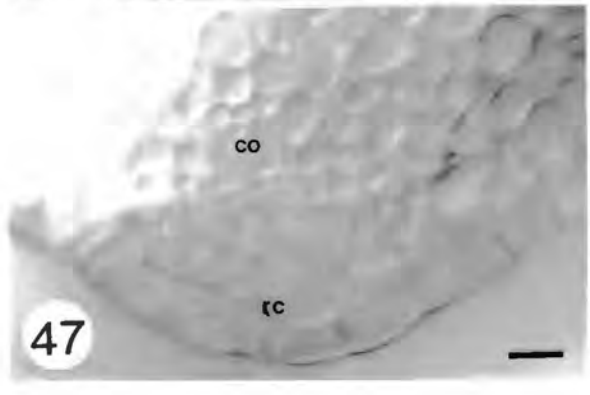

ci
q
m
42


p
o
n
m
l
43
tl


l'
l
n'
44


n'
p
m
l'
45


co
rc
46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47

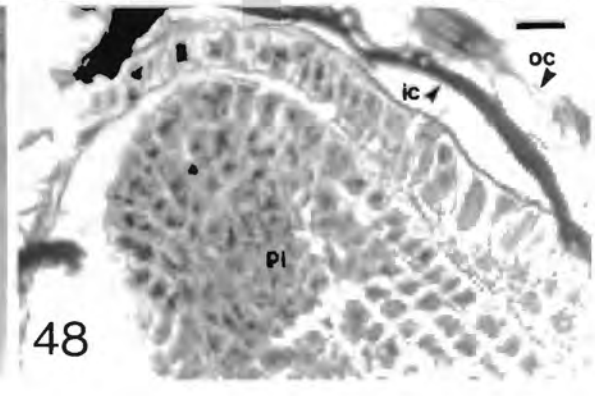
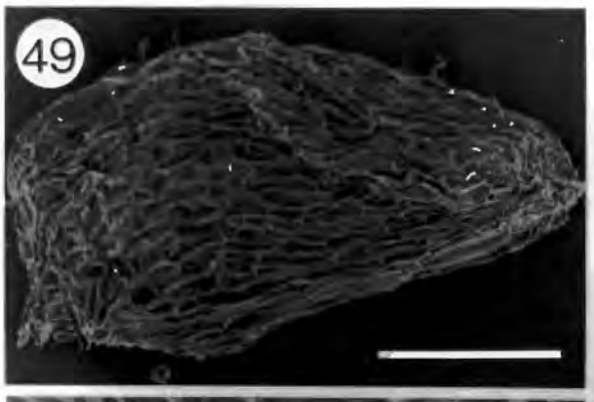

oc
ic
pl
48

Figure 49: SEM image of seed showing loose outer seed coat and shape of seed. Bar = 100μm. **Figure 50:** LS seed showing outer seed coat (oc), inner seed coat (ic) and embryo embedded within the endosperm (em), plumule (p), cotyledon (co) and vascular trace (vt). Bar = 5μm. **Figure 51:** SEM image of outer seed coat showing loosely clustered cells. Bar = 10μm. **Figure 52:** SEM image of inner seed coat showing elongated, closely arranged cells. Bar = 10μm. **Figure 53:** LS seed showing inner seed coat (ic), an outer acellular layer of the endosperm (cuticle?) indicated by large arrow, the outer layer (ol) of the endosperm (aleurone layer?) and small arrow indicating oil droplet within the endosperm. Bar = 1μm. **Figure 54:** Root tip cell squash showing very small, unclear chromosomes (arrows). Bar = 1μm.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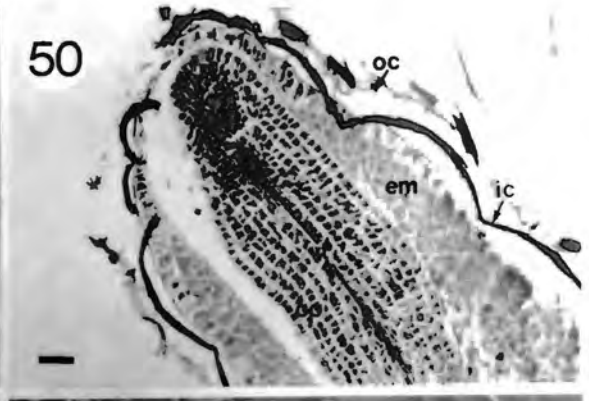
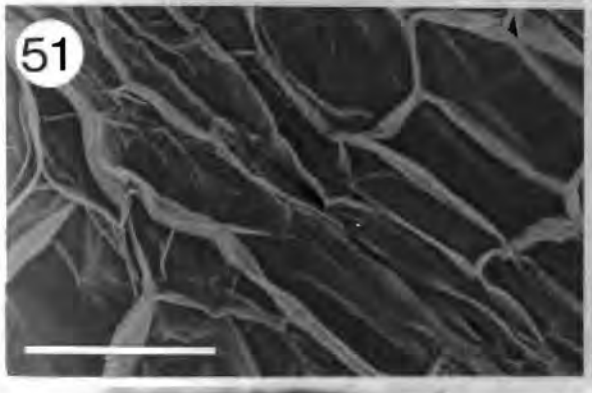

50
oc
em
ic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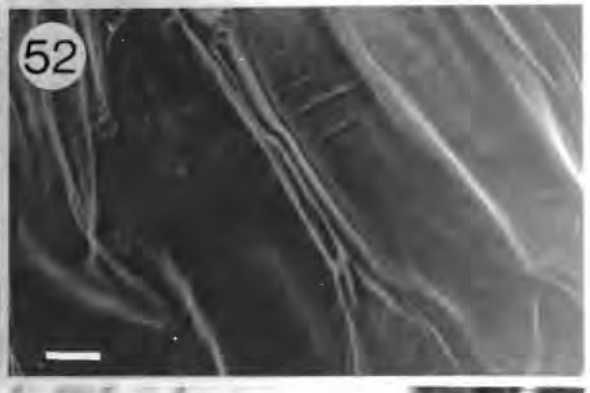
52


53
ic
ol

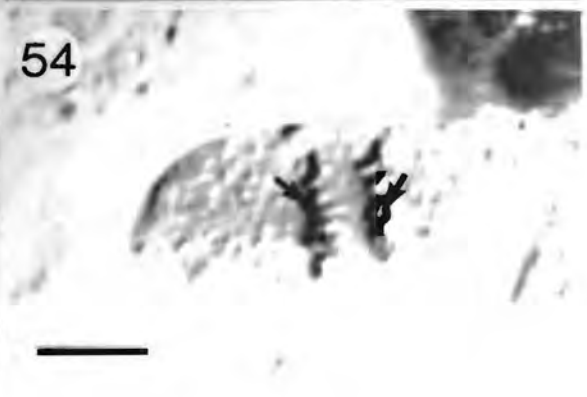
54

Figure 55: Germination sequence of seedlings. Seed coat (s), cotyledon (co), radicle (ra), secondary roots (r), first leaf (1), second leaf (2), third leaf (3). Arrow indicates new plant sprouting from adult rhizome.


78mm
17mm
5mm
2.5mm
s
co
ra
1
2
3
55

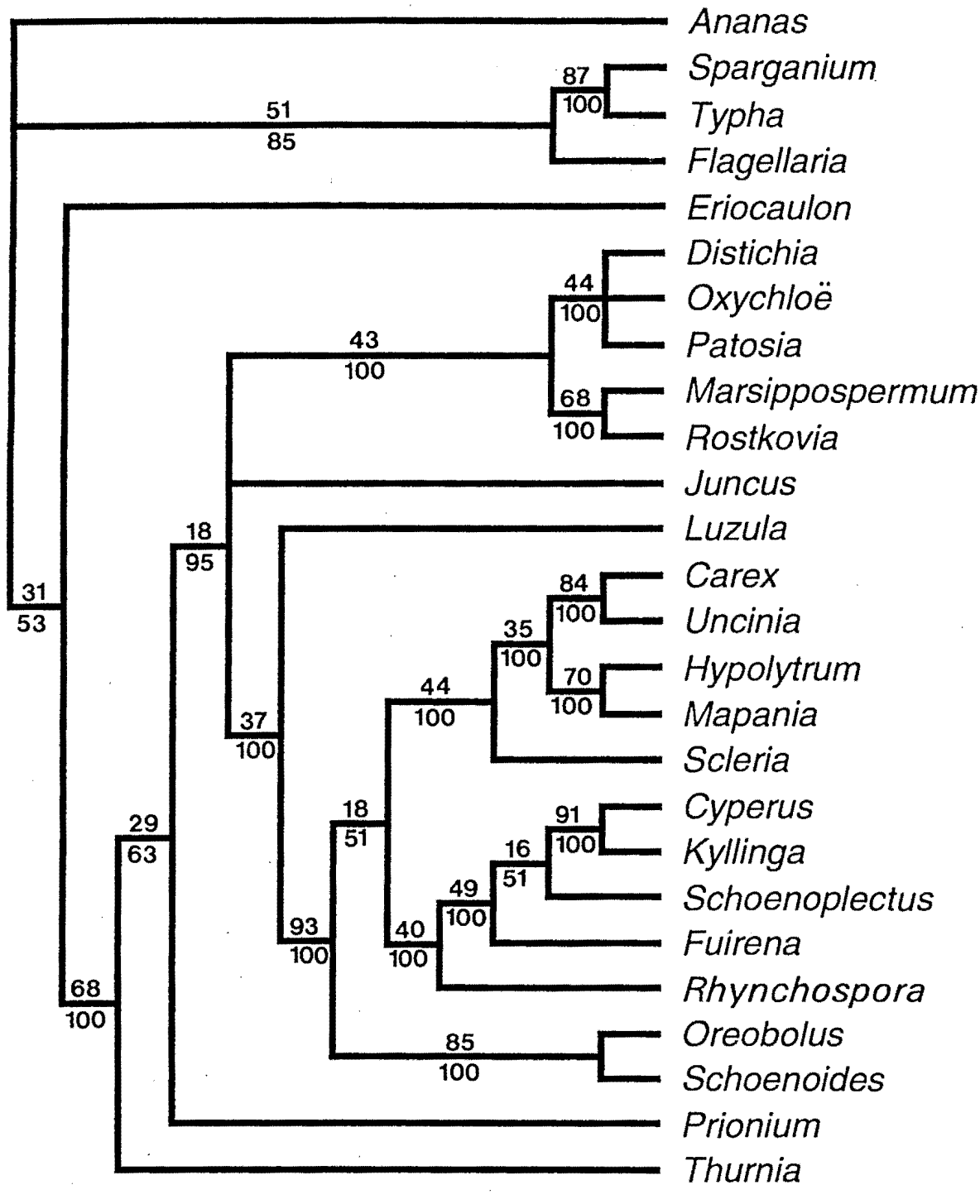

Figure 56: 50% majority rule tree of 221 trees based on morphological data (ci = 0.61, ri = 0.79). Numbers above branches refer to bootstrap percentages and numbers below refer to the percentage of trees supporting each node.

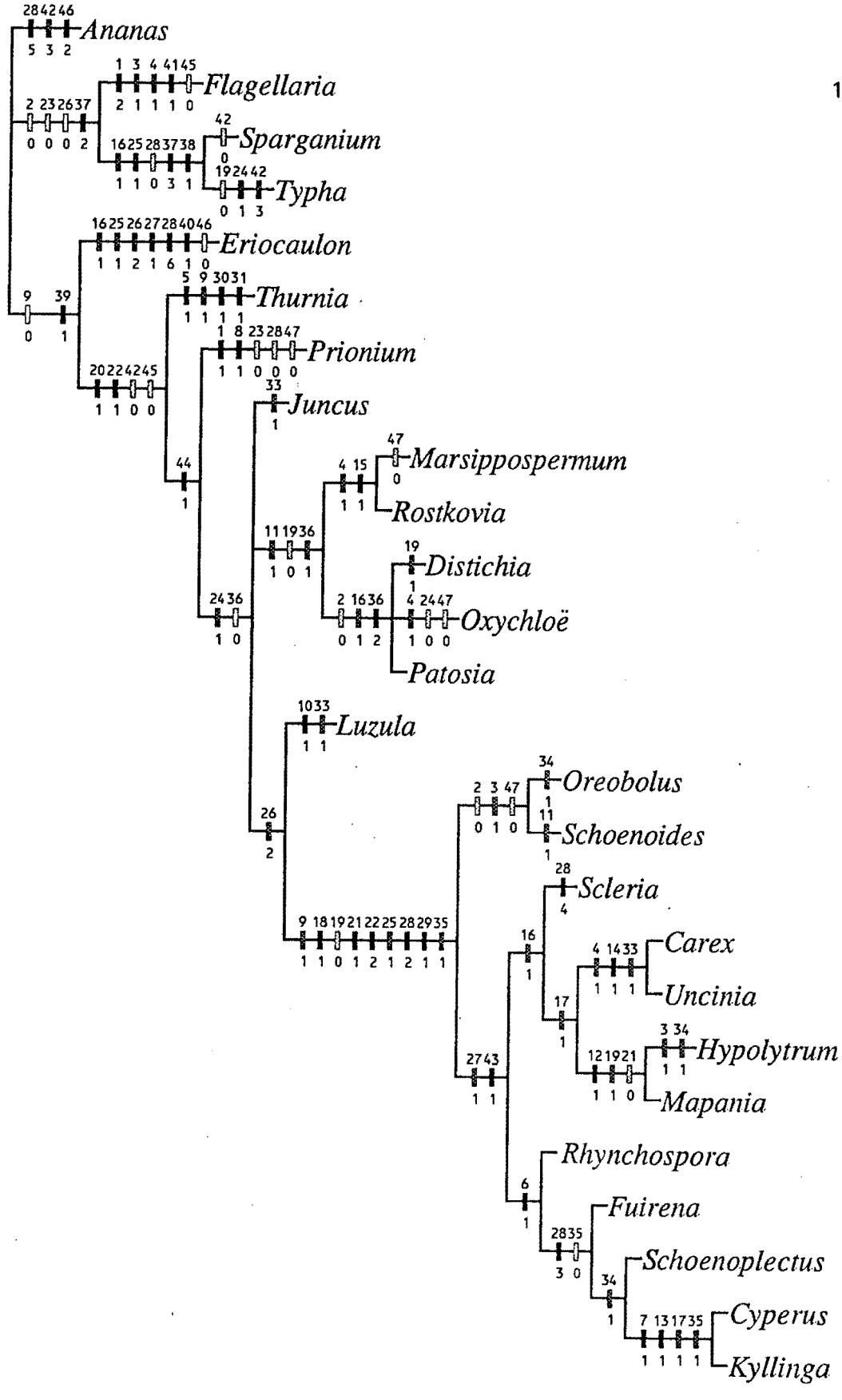

Figure 57: 50% majority rule tree of 221 trees based on morphological data. Solid bars indicate apomorphies, shaded bars indicate homoplasies. Numbers above the branches refer to the characters while numbers below the branches refer to the character states.

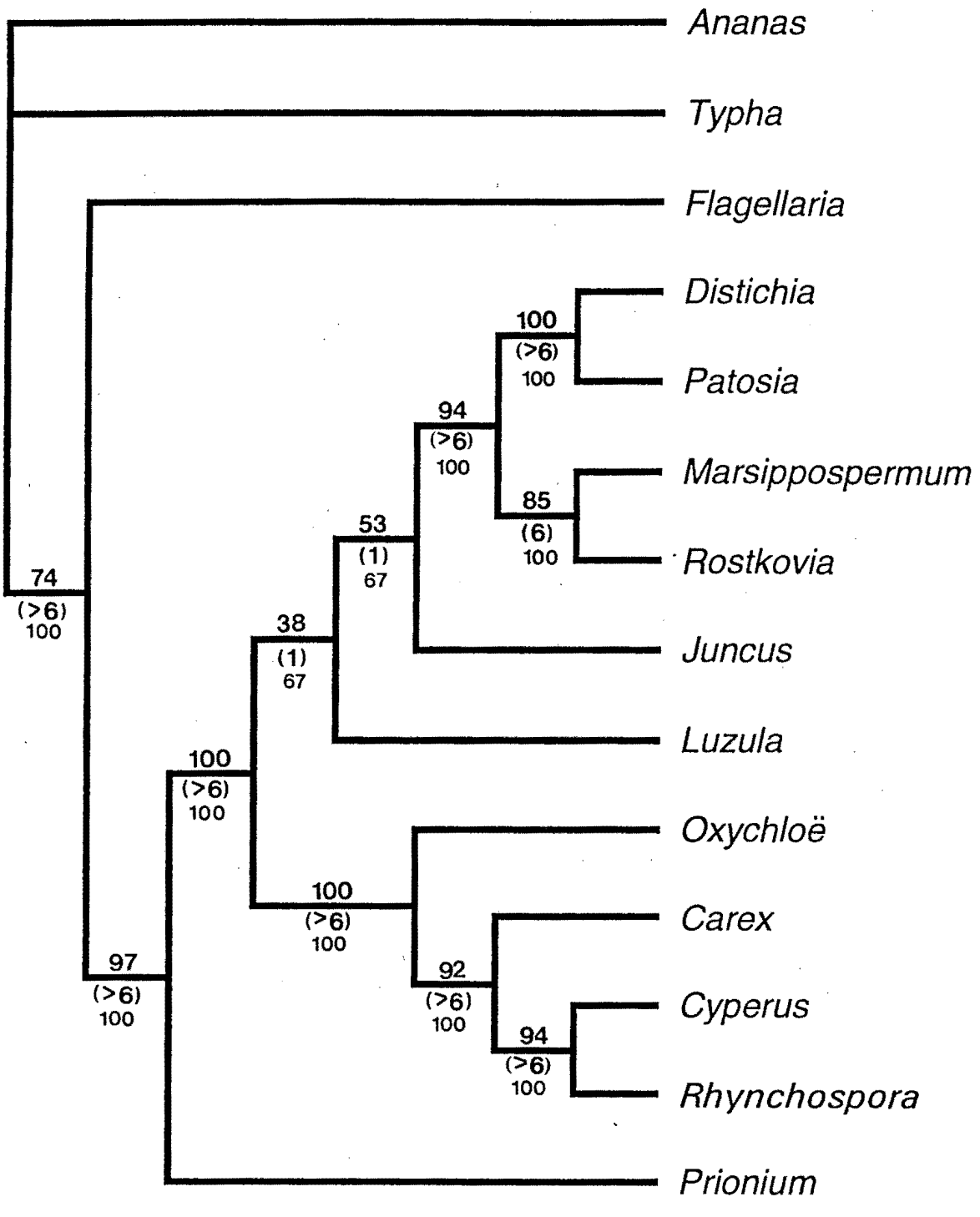

Figure 58: 50% majority rule tree of 3 trees based on both morphological and *rbc*L sequence data (ci = 0.71, ri = 0.69). Numbers above the branches refer to bootstrap percentages, numbers below branches in parentheses are decay indices, while numbers below these are the percentage of trees supporting each node.

Table 1: The number of layers and nuclei in the tapetum in selected commelinanean monocotyledons. (1-2) is number of layers\nuclei initially 1, becoming 2 later; (1&2) is one and two layers\nuclei recorded for the taxon; (2-1) is number of layers\nuclei initially 2, later becoming 1 by fusion.

ORDER	FAMILY	GENUS	SPECIES	NO. TAPETAL LAYERS	NO. TAPETAL NUCLEI
Juncales	Juncaceae	*Juncus* (Schnarf 1929, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985)		1	1
			bufonius L. (Shah 1963)	1	1
			effusus L.(Zaman 1950)	1	1
		Luzula (Dahlgren & Clifford 1982, Dahlgren *et al.* 1985)		1	1
		Prionium	*serratum*	1-2	1
		Oxychloë	*andina* Phil. (Schnarf 1929, Johri *et al.* 1992)	2	1
Cyperales	Cyperaceae (Johri *et al.* 1992)			1	1-many
	Cyperaceae (Davis 1966)				1-2
		Cyperus	*alopecuroides* Rottb.(Nagaraj & Nijalingappa 1972)	1	1
			alternifolius L. (Untawale & Makde 1971)	1	1
			tegetum Roxb. (Padhye & Moharir 1958)	1	1-2
		Fimbristylis	*quinquangularis* Kunth. (Dnyansagar & Tiwari 1956)	1	1
		Kyllinga	*brevifolia* Rottb. (Padhye 1971)	1	1-2
			monocephala Rottb. (Makde & Bhuskute 1987)	1	1
			triceps Rottb. (Padhye 1960)	1	1

Table 1 Cont.

ORDER	FAMILY	GENUS	SPECIES	NO. TAPETAL LAYERS	NO. TAPETAL NUCLEI
		Scleria	*foliosa* Hochst ex A. Rich (Nijalingappa 1986)	1	1
Poales (Dahlgren *et al.* 1985)					2
	Flagellariaceae (Johri *et al.* 1992)				2
		Flagellaria (Dahlgren & Clifford 1982)			2
		Flagellaria	*indica* L. (Subramanyam & Narayana 1972)	1-2	2
	Joinvilleaceae			-	-
	Restionaceae (Davis 1966, Johri *et al.* 1992)				2
		Elegia (Dahlgren & Clifford 1982)			2
		Hypodiscus (Dahlgren & Clifford 1982)			2
		Leptocarpus (Dahlgren & Clifford 1982)			2
		Restio (Dahlgren & Clifford 1982)			2
		Hypodiscus	*aristatus* Nees (Johri *et al.* 1992)		3-4
			aristatus (Krupko 1962)	1	2
	Ecdeiocoleaceae			-	-
	Centrolepidaceae (Hamann 1975, Dahlgren & Clifford 1982)				2

Table 1 Cont.

ORDER	FAMILY	GENUS	SPECIES	NO. TAPETAL LAYERS	NO. TAPETAL NUCLEI
		Centrolepis (Prakash 1969)			2
			aristata Roem. & Schult. (Hamann 1962)	1	2
			fascicularis Labill. (Prakash 1969)		1 & 3
			fascicularis (Johri *et al.* 1992)		2
	Poaceae (Davis 1966, Bhanwra 1988, Johri *et al.* 1992)				1 & 2
	Poaceae (Dahlgren & Clifford 1982)				1, 2, 4 & many
Commelinales	Eriocaulaceae				
		Eriocaulon	*cinerum* (Johri *et al.* 1992)		1
			hookerianum Stapf (Johri *et al.* 1992)		1
			septangulare With. (Johri *et al.* 1992)		1
			quinquangulare L. (Johri *et al.* 1992)		2
			quinquangulare (Begum 1966, 1968)		1-2
	Xyridaceae (Davis 1966)				2-1
		Xyris (Dahlgren & Clifford 1982)			2
			indica L. (Weinzieher 1914)		2
			pauciflora (Johri *et al.* 1992)		1-2

Table 1 Cont.

ORDER	FAMILY	GENUS	SPECIES	NO. TAPETAL LAYERS	NO. TAPETAL NUCLEI
		Abolboda	*grandis* Griseb. (Johri *et al.* 1992)		2
	Rapateaceae (Venturelli & Bouman 1988)			1	1-2
	Rapateaceae (Johri *et al.* 1992)				2
		Spathanus (Dahlgren & Clifford 1982)			2
		Rapatea (Dahlgren & Clifford 1982)			2
Typhales	Sparganiaceae (Davis 1966, Johri *et al.* 1992)			2	1-many
		Sparganium (Dahlgren & Clifford 1982)			8
			erectum L. (Muller-Doblies 1969, Asplund 1973)		1-2-8
			minimum Wallr.(Muller-Doblies 1969)		1-2-8
			simplex Huds. (Muller-Doblies 1969, Asplund 1973)		1-2-8
	Typhaceae (Davis 1966)				2
		Typha (Dahlgren & Clifford 1982)			many
			latifolia L. (Asplund 1972)		1-2-8
Bromeliales	Bromeliaceae	*Ananas*	*comosus* Merrill (Johri *et al.* 1992)		2
		Ananas	*comosus* (Rao & Wee 1979)		1-2

Table 1 Cont.

ORDER	FAMILY	GENUS	SPECIES	NO. TAPETAL LAYERS	NO. TAPETAL NUCLEI
		Cryptanthes (Dahlgren & Clifford 1982)			1-2
		Pitcairnia (Dahlgren & Clifford 1982)			2-4
		Billbergia (Dahlgren & Clifford 1982)			many

Table 2: Type of tapetum - whether glandular-secretory or amoeboid in selected commelinanean monocots. (g-s) = glandular-secretory tapetum, (a) = amoeboid tapetum, and (g-s to a) represents a transition from glandular-secretory to amoeboid.

ORDER	FAMILY	GENUS	SPECIES	TAPETUM TYPE
Juncales	Thurniaceae (Dahlgren *et al.* 1985)			g-s
	Juncaceae (Davis 1966, Dahlgren *et al.* 1985, Johri *et al.* 1992)			g-s
		Juncus	*bufonius* (Shah 1963)	g-s
		Prionium	*serratum*	g-s
Cyperales	Cyperaceae (Davis 1966, Dahlgren *et al.* 1985, Johri *et al.* 1992)			g-s
		Cyperus	*alopecuroides* (Nagaraj & Nijalingappa 1973)	g-s
			alternifolius (Untawale & Makde 1971)	g-s
			rotundus L. (Khanna 1963)	g-s
			tegetum (Padhye & Moharir 1958)	g-s
			triceps Endl. (Khanna 1963)	g-s
		Fimbristylis	*quinquangularis* (Dnyansagar & Tiwari 1956)	g-s
		Kyllinga	*brevifolia* (Padhye 1971)	g-s
			monocephala (Makde & Bhuskute 1987)	g-s
			triceps (Padhye 1960)	g-s
		Scleria	*foliosa* (Nijalingappa 1986)	g-s
Poales (Dahlgren *et al.* 1985)				g-s

Table 2 Cont.

ORDER	FAMILY	GENUS	SPECIES	TAPETUM TYPE
	Flagellariaceae (Johri *et al.* 1992)			g-s
	Joinvilleaceae			-
	Restionaceae (Davis 1966, Johri *et al.* 1992)			g-s
		Hypodiscus	*aristatus* (Krupko 1962)	g-s
	Ecdeiocoleaceae			-
	Centrolepidaceae (Hamann 1975, Johri *et al.* 1992)			g-s
		Centrolepis	*aristata* (Hamann 1962)	g-s
			fascicularis (Prakash 1969)	g-s
	Poaceae (Davis 1966, Bhanwra 1988, Johri *et al.* 1992)			g-s
Commelinales	Eriocaulaceae (Ramaswamy & Arekal 1982, Dahlgren *et al.* 1985, Johri *et al.* 1992)			g-s
		Eriocaulon	*quinquangulare* (Begum 1968)	g-s
	Xyridaceae (Davis 1966, Johri *et al.* 1992)			g-s
		Xyris	*indica* (Weinzieher 1914)	g-s
			pauciflora (Johri *et al.* 1992)	g-s
		Abolboda (Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Johri *et al.* 1992)		a
	Rapateaceae (Dahlgren *et al.* 1985, Venturelli & Bouman 1988, Johri *et al.* 1992)			g-s

Table 2 Cont.

ORDER	FAMILY	GENUS	SPECIES	TAPETUM TYPE
Typhales (Dahlgren *et al.* 1985)				g-s to a
	Sparganiaceae (Davis 1966, Johri *et al.* 1992)			a
		Sparganium (Dahlgren & Clifford 1982)		a
			erectum (Muller-Doblies 1969, Asplund 1973)	a
			minimum (Muller-Doblies 1969)	a
			simplex (Muller-Doblies 1969, Asplund 1973)	a
	Typhaceae (Pacini *et al.* 1985)			a
	Typhaceae (Davis 1966)			g-s
		Typha (Dahlgren & Clifford 1982)		a
			latifolia (Asplund 1972, Johri *et al.* 1992)	a
Bromeliales (Dahlgren *et al.* 1985)				gs
	Bromeliaceae (Davis 1966)			g-s
	Bromeliaceae (Dahlgren & Clifford 1982)			g-s & a
		Ananas	*comosus* (Rao & Wee 1979, Johri *et al.* 1992)	g-s

Table 3: The number of middle layers in the anther wall in selected commelinanean monocots. (1 or 2) = both one and two layered middle layers occur, and (1-4) = one up to four middle layers occur.

ORDER	FAMILY	GENUS	SPECIES	NO. OF MIDDLE LAYERS
Juncales	Juncaceae (Johri *et al.* 1992)			1
		Juncus	*bufonius* (Shah 1963)	1
			effusus (Zaman 1950)	1
		Prionium	*serratum*	1
	Cyperaceae (Johri *et al.* 1992)			1 or 2
		Cyperus	*alopecuroides* (Nagaraj & Nijalingappa 1973)	1
			alterniflolius (Untawale & Makde 1971)	1
			tegetum (Padhye & Moharir 1958)	1
		Fimbristylis	*quinquangularis* (Dnyansagar & Tiwari 1956)	1
		Kyllinga	*brevifolia* (Padhye 1971)	1
			monocephala (Makde & Bhuskute 1987)	1
			triceps (Padhye 1971)	1
		Scleria	*foliosa* (Nijalingappa 1986)	1
Poales	Flagellariaceae (Johri *et al.* 1992)			2
		Flagellaria	*indica* (Subramanyam & Narayana 1972)	1 or 2
	Joinvilleaceae			-
	Restionaceae (Johri *et al.* 1992)			1
		Hypodiscus	*aristatus* (Krupko 1962)	1
	Ecdeiocoleaceae			-

Table 3 Cont.

ORDER	FAMILY	GENUS	SPECIES	NO. OF MIDDLE LAYERS
	Centrolepidaceae (Hamann 1972, Johri *et al.* 1992)			1
			aristata (Hamann 1962)	1
		Centrolepis	*fascicularis* (Prakash 1969)	1
	Poaceae (Bhanwra 1988)			1
	Poaceae (Johri *et al.* 1992)			1 or 2
Commelinales	Eriocaulaceae (Johri *et al.* 1992)			1
		Eriocaulon	*quinquangulare* (Begum 1966, 1968)	1
	Xyridaceae (Johri *et al.* 1992)			1
	Rapateaceae (Venturelli & Bouman 1988)			3
	Rapateaceae (Johri *et al.* 1992)			1-4
Typhales	Sparganiaceae (Johri *et al.* 1992)			1
	Typhaceae (Johri *et al.* 1992)			1
Bromeliales	Bromeliaceae (Johri *et al.* 1992)			2
		Ananas	*comosus* (Wee & Rao 1974, Rao & Wee 1979)	2

Table 4: Microsporogenesis in selected commelinanean monocots. (sim) = simultaneous microsporogenesis and (succ) = successive microsporogenesis.

ORDER	FAMILY	GENUS	SPECIES	MICROSPOROGENESIS
Juncales	Thurniaceae (Dahlgren & Clifford 1982)			sim
	Juncaceae (Schnarf 1929, Cranwell 1953, Davis 1966, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Johri *et al.* 1992)			sim
		Juncus	*bufonius* (Wulff 1939, Shah 1963)	sim
			effusus (Zaman 1950)	sim
			filiformis L. (Wulff 1939)	sim
			squarrosus L. (Wulff 1939)	sim
		Luzula	*multiflora* (Schnarf 1929)	sim
			pilosa (L.) Willd (Schnarf 1929)	sim
Cyperales	Cyperaceae (Schnarf 1929, Wulff 1939, Cranwell 1953, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Johri *et al.* 1992)			sim
		Cyperus	*alopecuroides* (Nagaraj & Nijalingappa 1973)	sim
			alterniflolius (Untawale & Makde 1971)	sim
			tegetum (Padhye & Moharir 1958)	sim
		Fimbristylis	*quinquangularis* (Dnyansagar & Tiwari 1956)	sim

Table 4 Cont.

ORDER	FAMILY	GENUS	SPECIES	MICROSPOROGENESIS
		Kyllinga	*brevifolia* (Padhye 1971)	sim
			monocephala (Makde & Bhuskute 1987)	sim
			triceps (Padhye 1971)	sim
		Scleria	*foliosa* (Nijalingappa 1986)	sim
Poales (Dahlgren *et al.* 1985)				succ
	Flagellariaceae (Davis 1966, Dahlgren & Clifford 1982)			succ
		Flagellaria	*indica* (Johri *et al.* 1992)	succ & sim
	Joinvilleaceae			-
	Restionaceae (Johri *et al.* 1992)			succ
		Hypodiscus (Dahlgren & Clifford 1982)		succ
			aristatus (Krupko 1962)	succ
		Elegia (Dahlgren & Clifford 1982)		succ
		Leptocarpus (Dahlgren & Clifford 1982)		succ
		Restio (Dahlgren & Clifford 1982)		succ
	Ecdeiocoleaceae			-
	Centrolepidaceae (Hamann 1975, Dahlgren & Clifford 1982, Johri *et al.* 1992)			succ
		Centrolepis	*fascicularis* (Prakash 1969)	succ

Table 4 Cont.

ORDER	FAMILY	GENUS	SPECIES	MICROSPOROGENESIS
	Poaceae (Schnarf 1929, Davis 1966, Dahlgren & Clifford 1982, Bhanwra 1988, Johri *et al.* 1992)			succ
Commelinales	Eriocaulaceae (Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Johri *et al.* 1992)			succ
	Eriocaulaceae (Davis 1966)			sim
		Eriocaulon	*quinquangulare* (Begum 1966, 1968)	succ
	Xyridaceae (Davis 1966, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Johri *et al.* 1992)			succ
		Xyris	*indica* (Weinzieher 1914)	succ
	Rapateaceae (Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Venturelli & Bouman 1988, Johri *et al.* 1992)			sim
Typhales (Dahlgren *et al.* 1985)				succ
	Sparganiaceae (Dahlgren & Clifford 1982, Johri *et al.* 1992)			succ
		Sparganium	*erectum* (Muller-Doblies 1969, Asplund 1973)	succ
			minimum (Muller-Doblies 1969)	succ
			simplex (Muller-Doblies 1969, Asplund 1973)	succ

Table 4 Cont.

ORDER	FAMILY	GENUS	SPECIES	MICROSPOROGENESIS
	Typhaceae (Davis 1966, Dahlgren & Clifford 1982, Johri *et al.* 1992)			succ
		Typha	*latifolia* (Asplund 1972)	succ
Bromeliales (Dahlgren *et al.* 1985)				succ
	Bromeliaceae (Davis 1966, Johri *et al.* 1992, Dahlgren & Clifford 1982)			succ
		Ananas	*comosus* (Wee & Rao 1974, Rao & Wee 1979)	succ

Table 5: The number of nuclei in pollen grains at dispersal in selected commelinanean monocotyledons.

ORDER	FAMILY	GENUS	SPECIES	NO. NUCLEI IN POLLEN AT DISPERSAL
Juncales	Thurniaceae (Dahlgren *et al.* 1985)			3
	Juncaceae (Davis 1966, Dahlgren *et al.* 1995, Goldberg 1989, Johri *et al.* 1992,)			3
		Juncus (Brewbaker 1967, Dahlgren & Clifford 1982)		3
		Luzula (Brewbaker 1967, Dahlgren & Clifford 1982)		3
			campestris (L.) DC. (Schnarf 1929)	3
			bufonius (Wulff 1939, Shah 1963)	3
			effusus (Zaman 1950)	3
			filiformis (Wulff 1939)	3
			squarrosus (Wulff 1939)	3
		Oxychloë	*andina* (Schnarf 1929)	3
		Prionium	*serratum*	3
Cyperales	Cyperaceae (Brewbaker 1967, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985)			2 & 3
	Cyperaceae (Davis 1966, Goldberg 1989, Johri *et al.* 1992)			3
		Carex (Brewbaker 1967, Dahlgren & Clifford 1982)		3
		Cyperus (Brewbaker 1967, Dahlgren & Clifford 1982)		3

Table 5 Cont.

ORDER	FAMILY	GENUS	SPECIES	NO. NUCLEI IN POLLEN AT DISPERSAL
			alopecuroides (Nagaraj & Nijalingappa 1973)	3
			alternifolius (Untawale & Makde 1971)	3
			tegetum (Padhye & Moharir 1958)	3
			rotundus (Khanna 1963)	3
		Eleocharis (Brewbaker 1967, Dahlgren & Clifford 1982)		3
		Fimbristylis (Brewbaker 1967, Dahlgren & Clifford 1982)		2
			quinquangularis (Dnyansagar & Tiwari 1956)	2 & 3
		Kyllinga	*brevifolia* (Padhye 1971)	3
			monocephala (Makde & Bhuskute 1987)	3
			triceps (Padhye 1960)	3
		Rynchospora (Brewbaker 1967, Dahlgren & Clifford 1982)		3
		Scirpus (Brewbaker 1967, Dahlgren & Clifford 1982)		2
		Scleria	*foliosa* (Nijalingappa 1986)	3
		Schoenoplectus (Brewbaker 1967, Dahlgren & Clifford 1982)		3
Poales (Dahlgren *et al.* 1985)				2 & 3

Table 5 Cont.

ORDER	FAMILY	GENUS	SPECIES	NO. NUCLEI IN POLLEN AT DISPERSAL
	Flagellariaceae (Brewbaker 1967, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Campbell & Kellogg 1986, Goldberg 1989, Johri *et al.* 1992)			2
		Flagellaria (Brewbaker 1967)		2
			indica (Subramanyan & Narayana 1972)	2
	Joinvilleaceae (Campbell & Kellogg 1986)			3
	Restionaceae (Dahlgren *et al.* 1985, Campbell & Kellogg 1986, Goldberg 1989)			2 & 3
	Restionaceae (Brewbaker 1967)			3
	Restionaceae (Johri *et al.* 1992)			3 & 4
		Chondropetalum (Dahlgren & Clifford 1982)		2
		Elegia (Dahlgren & Clifford 1982)		3
		Hypodiscus (Brewbaker 1967, Dahlgren & Clifford 1982)		3
			aristatus (Krupko 1962)	3
		Leptocarpus (Dahlgren & Clifford 1982)		3
		Restio (Dahlgren & Clifford 1982)		3
	Ecdeiocoleaceae			-

Table 5 Cont.

ORDER	FAMILY	GENUS	SPECIES	NO. NUCLEI IN POLLEN AT DISPERSAL
	Centrolepidaceae (Hamann 1975, Johri *et al.* 1992, Campbell & Kellogg 1986, Goldberg 1989)			3
	Centrolepidaceae (Brewbaker 1967)			2
		Aphelia (Dahlgren & Clifford 1982)		3
		Brizula (Dahlgren & Clifford 1982)		3
		Centrolepis (Brewbaker 1967)		2
		Centrolepis (Dahlgren & Clifford 1982)		3
			aristata (Hamann 1962)	3
			fascicularis (Prakash 1969)	3
	Poaceae (Davis 1966, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Campbell & Kellogg 1986, Bhanwra 1988, Johri *et al.* 1992)			3
		Spinifex	*littoreus* Merrill (Johri *et al.* 1992)	2
Commelinales (Dahlgren *et al.* 1985)				2 & 3
	Eriocaulaceae (Brewbaker 1967, Ramaswamy & Arekal 1982, Johri *et al.* 1992)			3
	Eriocaulaceae (Dahlgren & Clifford 1982, Dahlgren *et al.* 1985)			2 & 3
		Eriocaulon (Brewbaker 1967, Dahlgren & Clifford 1982)		3

Table 5 Cont.

ORDER	FAMILY	GENUS	SPECIES	NO. NUCLEI IN POLLEN AT DISPERSAL
			tribolum (Johri *et al.* 1992)	2
			quinquangulare (Begum 1966, 1968)	2
	Rapateaceae (Dahlgren *et al.* 1985, Venturelli & Bouman 1988, Johri *et al.* 1992)			2
		Cephalostemon (Dahlgren & Clifford 1982)		2
		Spathanthus (Dahlgren & Clifford 1982)		2
	Xyridaceae (Brewbaker 1967)			2
	Xyridaceae (Dahlgren *et al.* 1985)			2 & 3
		Xyris (Brewbaker 1967)		2
		Xyris (Dahlgren & Clifford 1982)		2 & 3
			grandis Ridley (Johri *et al.* 1992)	2
			indica (Weinzieher 1914, Davis 1966, Johri *et al.* 1992)	3
			pauciflora (Davis 1966, Johri *et al.* 1992)	2
Typhales (Dahlgren *et al.* 1985)				2 & 3
	Sparganiaceae (Dahlgren *et al.* 1985)			2 & 3
	Sparganiaceae (Brewbaker 1967, Johri *et al.* 1992)			2
	Sparganiaceae (Goldberg 1989)			

Table 5 Cont.

ORDER	FAMILY	GENUS	SPECIES	NO. NUCLEI IN POLLEN AT DISPERSAL
		Sparganium (Brewbaker 1967, Dahlgren & Clifford 1982)		2
			erectum (Muller-Doblies 1969, Asplund 1973)	2
			minimum (Muller-Doblies 1969)	2
			simplex (Muller Doblies 1969, Asplund 1973)	2
	Typhaceae (Davis 1966, Brewbaker 1967, Dahlgren *et al.* 1985, Goldberg 1989, Johri *et al.* 1992)			2
		Typha (Brewbaker 1967, Dahlgren & Clifford 1982)		2
			latifolia (Asplund 1972)	2
Bromeliales (Dahlgren *et al.* 1985)				2 & 3
	Bromeliaceae (Brewbaker 1967)			2 & 3
	Bromeliaceae (Davis 1966, Dahlgren & Clifford 1982, Goldberg 1989, Johri *et al.* 1992)			2
		Aechmea (Brewbaker 1967)		2
		Ananas (Brewbaker 1967)		2
			comosus (Wee & Rao, 1974, Rao & Wee, 1979)	2
		Aregelia (Brewbaker 1967)		2
		Billbergia (Brewbaker 1967)		2
		Cryptanthus (Brewbaker 1967)		2

Table 5 Cont.

ORDER	FAMILY	GENUS	SPECIES	NO. NUCLEI IN POLLEN AT DISPERSAL
		Guzmania (Brewbaker 1967)		2
		Nidularium (Brewbaker 1967)		2
		Pitcairnia (Brewbaker 1967)		2
		Schlumbergeria (Brewbaker 1967, Dahlgren & Clifford 1982)		3
		Tillandsia (Brewbaker 1967)		2
		Vriesia (Brewbaker 1967)		2

Table 6: Nucellar tissue formation in selected commelinanean monocotyledons. Where c = crassinucellate ie. parietal cell cut off from archesporial cell & dividing to form nucellar tissue; t = tenuinucellate ie. archesporial cell becomes megaspore mother cell and parietal cell never formed; nc = nucellar cap ie. tissue formed from divisions of nucellar epidermis.

ORDER	FAMILY	GENUS	SPECIES	NUCELLUS
Juncales	Juncaceae (Schnarf 1929, Davis 1966, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Goldberg 1989, Johri *et al.* 1992)			c
		Juncus	*bufonius* (Shah 1963)	c
			effusus (Zaman 1950)	c
			prismatocarpus R.Br. (Zaman 1950)	c
			roemerianus Scheele (Eleuterius 1984)	c
		Prionium	*serratum*	c
Cyperales	Cyperaceae (Schnarf 1929, Davis 1966, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Goldberg 1989, Johri *et al.* 1992)			c
		Cyperus	*alopecuroides* (Nagaraj & Nijalingappa 1973)	c
			alternifolius (Untawale & Makde 1971)	c
			rotundus (Khanna 1963)	c
			tegetum (Padhye & Moharir 1958)	c
			triceps (Khanna 1963)	c
		Fimbristylis	*diphylla* Vahl. (Murty & Kumar 1967)	c

Table 6 Cont.

ORDER	FAMILY	GENUS	SPECIES	NUCELLUS
			quinquangularis (Dnyansagar & Tiwari 1956)	c
		Kyllinga	*brevifolia* (Padhye 1971)	c
			monocephala (Makde & Bhuskute 1987)	c
			triceps (Padhye 1960)	c
		Scleria	*foliosa* (Nijalingappa 1986)	c
Poales (Dahlgren *et al.* 1985)				t & nc
	Flagellariaceae (Campbell & Kellogg 1986, Dahlgren *et al.* 1985, Johri *et al.* 1992)			c
		Flagellaria (Dahlgren & Clifford 1982)		c
			indica (Subramanyam & Narayana 1972)	c
	Joinvilleaceae			?
	Restionaceae (Johri *et al.* 1992)			c
	Restionaceae (Davis 1966, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Campbell & Kellogg 1986, Rudall & Linder 1988)			t
	Restionaceae (Goldberg 1989)			t & c
		Anarthria	*gracilis* R.Br. (Linder & Rudall 1993)	t

Table 6 Cont.

ORDER	FAMILY	GENUS	SPECIES	NUCELLUS
			humilis Nees (Linder & Rudall 1993)	t
			laevis R.Br. (Linder & Rudall 1993)	t
			prolifera R.Br. (Linder & Rudall 1993	t
			scabra R.Br. (Linder & Rudall 1993)	t
		Chondropetalum	*tectorum* (L.f.) Rafin. (Rudall & Linder 1988)	t
		Lyginia	*barbata* R.Br. (Rudall & Linder 1988)	t
		Elegia	*capensis* (Burm. f.) Schelpe (Rudall & Linder 1988)	t
	Ecdeiocoleaceae	*Ecdeiocolea*	*monostachya* F.Muell. (Rudall 1990)	t
	Centrolepidaceae (Schnarf 1929, Davis 1966, Hamann 1975, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Campbell & Kellogg 1986, Johri *et al.* 1992, Linder & Rudall 1993)			t
	Centrolepidaceae (Goldberg 1989)			c
		Aphelia	*brizula* F.Muell. (Linder & Rudall 1993)	t
		Centrolepis	*aristata* (Hamann 1962)	t
			fascicularis (Prakash 1969)	t

Table 6 Cont.

ORDER	FAMILY	GENUS	SPECIES	NUCELLUS
			pilosa Hieron. (Linder & Rudall 1993)	t
			glabra Hieron. (Linder & Rudall 1993)	t
	Poaceae (Campbell & Kellogg 1986, Bhanwra 1988)			t
	Poaceae (Schnarf 1929, Chandra 1963, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Goldberg 1989, Johri *et al.* 1992)			t & nc
	Poaceae (Davis 1966)			nc
Commelinales	Eriocaulaceae (Schnarf 1929, Davis 1966, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Goldberg 1989, Johri *et al.* 1992)			t
		Eriocaulon	*quinquangulare* (Begum 1966, 1968)	t
	Xyridaceae (Davis 1966, Dahlgren & Clifford 1992, Goldberg 1989, Johri *et al.* 1992)			t
	Xyridaceae (Dahlgren *et al.* 1985)			t & nc
		Xyris	*indica* (Weinzieher 1914, Schnarf 1929)	t

Table 6 Cont.

ORDER	FAMILY	GENUS	SPECIES	NUCELLUS
	Rapateaceae (Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Venturelli & Bouman 1988, Johri *et al.* 1992)			c
Typhales (Dahlgren *et al.* 1985)				c
	Typhaceae (Davis 1966, Dahlgren & Clifford 1982, Goldberg 1989, Johri *et al.* 1992)			c
		Typha	*latifolia* (Asplund 1972)	c
		Typha	*angustifolia* L. var *elongata* (Asplund 1972)	c
	Sparganiaceae (Davis 1966, Dahlgren & Clifford 1982, Goldberg 1989, Johri *et al.* 1992)			c
		Sparganium	*angustifolium* Michx. (Asplund 1973)	c
			erectum (Muller-Doblies 1969, Asplund 1973)	c
			glomeratum Laest. ex Beurl. (Asplund 1973)	c
			simplex (Muller-Doblies 1969, Asplund 1973)	c
			minimum (Muller-Doblies 1969, Asplund 1973)	c
Bromeliales (Dahlgren *et al.* 1985)				c

Table 6 Cont.

ORDER	FAMILY	GENUS	SPECIES	NUCELLUS
	Bromeliaceae (Davis 1966, Dahlgren & Clifford 1982, Goldberg 1989, Johri *et al.* 1992)			c
		Ananas	*comosus* (Wee & Rao 1974, Rao & Wee 1979)	c
		Billbergia	*coppei* (Schnarf 1929)	c
		Tillandsia	*usneoides* L. (Schnarf 1929)	c

Table 7: Embryo sac type in selected commelinanean monocotyledons. Terminology after Maheshwari (1950), except *Polygonum*-Poaceae variant, after Anton & Cocucci (1984).

ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES	EMBRYO SAC TYPE
Juncales	Juncaceae (Davis 1966, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Johri *et al.* 1992)				*Polygonum*
			Distichia	*muscoides* Nees & Meyen. (Schnarf 1929)	*Polygonum*
			Juncus	*bufonius* (Schnarf 1929, Shah 1963)	*Polygonum*
				compressus Jacq. (Schnarf 1929)	*Polygonum*
				effusus (Zaman 1950)	*Polygonum*
				filiformis (Schnarf 1929)	*Polygonum*
				lamprocarpus Reichb. (Schnarf 1929)	*Polygonum*
				prismatocarpus R.Br. (Zaman 1950)	*Polygonum*
				roemerianus (Eleuterius 1984)	*Polygonum*
				squarrosus (Schnarf 1929)	*Polygonum*
			Luzula	*campestris* (Schnarf 1929)	*Polygonum*
				multiflora Lej. (Schnarf 1929)	*Polygonum*
				pilosa (Schnarf 1929)	*Polygonum*
			Prionium	*serratum*	*Polygonum*

Table 7 (Cont.)

ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES	EMBRYO SAC TYPE
Cyperales	Cyperaceae (Davis 1966, Dahlgren & Clifford 1982, Johri *et al.* 1992)				*Polygonum*
			Cyperus	*alopecuroides* (Nagaraj & Nijalingappa 1973)	*Polygonum*
				alternifolius (Untawale & Makde 1971)	*Polygonum*
				natalensis Hochst. ex Krauzs (Schnarf 1929)	*Polygonum*
				tegetum (Padhye & Moharir 1958)	*Polygonum*
			Carex	*caryophyllea* Lateur (Schnarf 1929)	*Polygonum*
				digitata L. (Schnarf 1929)	*Polygonum*
				ericetorum Poll. (Schnarf 1929)	*Polygonum*
				panicea L. (Schnarf 1929)	*Polygonum*
				pilulifera L. (Schnarf 1929)	*Polygonum*
				praecox (Schnarf 1929)	*Polygonum*
			Fimbristylis	*diphylla* (Murty & Kumar 1967)	*Polygonum*
				quinquangularis (Dnyansagar & Tiwari 1956)	*Polygonum*
			Kyllinga	*brevifolia* (Padhye 1971)	*Polygonum*
				monocephala (Makde & Bhuskute 1987)	*Polygonum*

Table 7 (Cont.)

ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES	EMBRYO SAC TYPE
				triceps (Padhye 1960)	*Polygonum*
			Scleria	*foliosa* (Nijalingappa 1986)	*Polygonum*
Poales	Flagellariaceae				
			Flagellaria (Dahlgren & Clifford 1982)		*Allium*
				indica (Subramanyam & Narayana 1972, Anton & Cocucci 1984, Rudall & Linder 1988, Johri *et al.* 1992)	*Allium*
	Joinvilleaceae (Campbell & Kellogg 1986)				*Polygonum*
	Restionaceae (Davis 1966, Dahlgren & Clifford 1982, Johri *et al.* 1992)				*Polygonum*
	Restionaceae (Anton & Cocucci 1984)				*Polygonum*-Poaceae variant
	Restionaceae (Rudall & Linder 1988)				*Polygonum* & *Polygonum*-Poaceae variant
			Hypodiscus	*aristatus* (Krupko 1962)	*Polygonum*
			Thamnochortus	*fruticosus* Berg (Mlodzianowski 1964)	*Polygonum*-Poaceae variant
			Anarthria	*laevis* (Linder & Rudall 1993)	*Polygonum*
	Ecdeiocoleaceae		*Ecdeiocolea*	*monostachya* (Rudall 1990)	*Drusa*?

Table 7 (Cont.)

ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES	EMBRYO SAC TYPE
	Centrolepidaceae (Schnarf 1929, Hamann 1962, 1975, Dahlgren & Clifford 1982, Johri *et al.* 1992, Linder & Rudall 1993)				*Polygonum*
			Centrolepis	*fascicularis* (Prakash 1969)	*Polygonum*
	Poaceae (Schnarf 1929, Davis 1966, Dahlgren *et al.* 1992, Johri *et al.* 1992)				*Polygonum*
	Poaceae (Anton & Cocucci 1984)				*Polygonum* & *Polygonum*-Poaceae variant
		Bambusoideae (Bhanwra 1988)			*Polygonum*-Poaceae variant
		Arundinoideae (Bhanwra 1988, Verboom *et al.* 1994)			*Polygonum*-Poaceae variant
		Pooideae (Bhanwra 1988)			*Polygonum* & *Polygonum*-Poaceae variant
		Chloridoideae (Bhanwra 1988)			*Polygonum* & *Polygonum*-Poaceae variant
		Panicoideae (Bhanwra 1988)			*Polygonum* & *Polygonum*-Poaceae variant

Table 7 (Cont.)

ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES	EMBRYO SAC TYPE
Commelinales	Eriocaulaceae (Schnarf 1929, Davis 1966, Dahlgren & Clifford 1982, Ramaswamy & Arekal 1982, Johri *et al.* 1992)				*Polygonum*
			Eriocaulon	*quinquangulare* (Begum 1968)	*Polygonum*
	Xyridaceae (Schnarf 1929, Davis 1966)				*Polygonum*
			Abolboda (Dahlgren & Clifford 1982)		*Polygonum*
			Xyris (Dahlgren & Clifford 1982)		*Polygonum*
				indica (Weinzieher 1914, Johri *et al.* 1992)	*Polygonum*
				pauciflora (Johri *et al.* 1992)	*Allium*
				schoenoides Mart. (Johri *et al.* 1992)	*Allium*
	Rapateaceae (Venturelli & Bouman 1988, Johri *et al.* 1992)				*Polygonum*
			Cephalostomon (Dahlgren & Clifford 1982)		*Polygonum*
			Rapatea (Dahlgren & Clifford 1982)		*Polygonum*
			Spathanthus (Dahlgren & Clifford 1982)		*Polygonum*

Table 7 (Cont.)

ORDER	FAMILY	SUBFAMILY	GENUS	SPECIES	EMBRYO SAC TYPE
Typhales	Sparganiaceae (Schnarf 1929, Dahlgren & Clifford 1982, Johri *et al.* 1992)				*Polygonum*
			Sparganium	*angustifolium* (Asplund 1973)	*Polygonum*
				erectum (Muller-Doblies 1969, Asplund 1973)	*Polygonum*
				glomeratum (Asplund 1973)	*Polygonum*
				minimum (Muller-Doblies 1969, Asplund 1973)	*Polygonum*
				simplex (Muller-Doblies 1969, Asplund 1973)	*Polygonum*
	Typhaceae (Sparganiaceae, Davis 1966, Dahlgren & Clifford 1982, Johri *et al.* 1992)				*Polygonum*
			Typha	*latifolia* (Asplund 1972)	*Polygonum*
Bromeliales	Bromeliaceae (Schnarf 1929, Davis 1966, Dahlgren & Clifford 1982, Johri *et al.* 1992)				*Polygonum*
			Ananas	*comosus* (Wee & Rao 1974, Rao & Wee 1979)	*Polygonum*

Table 8: Endosperm formation in selected commelinanean monocotyledons.

ORDER	FAMILY	GENUS	SPECIES	ENDOSPERM
Juncales	Juncaceae (Swamy & Parameswaran 1962, Swamy & Krishnamurthy 1973, Davis 1966, Dahlgren *et al.* 1985, Goldberg 1989, Johri *et al.* 1992)			helobial
		Distichia (Dahlgren & Clifford 1982)		helobial
			muscoides (Schnarf 1929)	helobial
		Luzula (Dahlgren & Clifford 1982)		helobial
			campestris (Schnarf 1929)	helobial
			multiflora (Schnarf 1929)	helobial
			pilosa (Schnarf 1929)	helobial
		Juncus (Dahlgren & Clifford 1982)		helobial
			bufonius (Schnarf 1929, Shah 1963, Swamy & Krishnamurthy 1973)	helobial
			compressus (Schnarf 1929)	helobial
			effusus (Zaman 1950)	helobial
			filiformis (Schnarf 1929)	helobial
			lamprocarpus (Schnarf 1929)	helobial
			prismatocarpus (Zaman 1950)	helobial
			squarrosus (Schnarf 1929)	helobial
		Prionium	*serratum*	helobial

Table 8 Cont.

ORDER	FAMILY	GENUS	SPECIES	ENDOSPERM
Cyperales	Cyperaceae (Swamy & Parameswaran 1962, Davis 1966, Dahlgren *et al.* 1985, Goldberg 1989, Johri *et al.* 1992)			nuclear
		Carex (Dahlgren & Clifford 1982)		nuclear
			caryophyllacea (Schnarf 1929)	nuclear
			digitata (Schnarf 1929)	nuclear
			ericetorum (Schnarf 1929)	nuclear
			panicea (Schnarf 1929)	nuclear
			pulilifera (schnarf 1929)	nuclear
		Cyperus (Dahlgren & Clifford 1982)		nuclear
			alopecuroides (Nagaraj & Nijalingappa 1973)	nuclear
			alternifolius (Makde & Bhuskute 1984)	nuclear
			natalensis (Schnarf 1929)	nuclear
		Eleocharis	*atropurpurea* Kunth. (Makde & Bhuskute 1984)	nuclear
		Eriophorum	*comosum* Wall. (Makde & Bhuskute 1984)	nuclear
		Fimbristylis (Dahlgren & Clifford 1982)		nuclear
			cymosa R.Br. (Makde & Bhuskute 1984)	nuclear
		Fuirena	*ciliaris* (L.) Roxb. (Makde & Bhuskute 1984)	nuclear

Table 8 Cont.

ORDER	FAMILY	GENUS	SPECIES	ENDOSPERM
		Kyllinga	*brevifolia* (Padhye 1971)	nuclear
			monocephala (Makde & Bhuskute 1987)	nuclear
			triceps (Padhye 1960)	nuclear
		Mariscus	*paniceus* Vahl (Makde & Bhuskute 1984)	nuclear
		Pycreus	*pumilis* Nees. (Makde & Bhuskute 1984)	nuclear
		Remirea	*maritima* Aubl. (Makde & Bhuskute 1984)	nuclear
		Rhynchospora	*corymbosa* (L.) Britton (Nijalingappa & Tejavathi 1983)	nuclear
		Scirpus	*supinus* L. (Makde & Bhuskute 1984)	nuclear
		Scleria	*foliosa* (Nijalingappa & Devaki 1979, Nijalingappa 1986)	nuclear
			lithosperma Roxb. (Makde & Bhuskute 1984)	nuclear
Poales	Flagellariaceae			-
	Joinvilleaceae			-
	Restionaceae (Davis 1966, Dahlgren *et al.* 1985, Goldberg 1989)			nuclear
		Chondropetalum (Dahlgren & Clifford 1982)		nuclear
		Hypodiscus (Dahlgren & Clifford 1982)		nuclear
			aristatus (Krupko 1962)	nuclear

Table 8 Cont.

ORDER	FAMILY	GENUS	SPECIES	ENDOSPERM
		Leptocarpus (Dahlgren & Clifford 1982)		nuclear
		Thamnochortus (Dahlgren & Clifford 1982)		nuclear
			fruticosus (Mlodzianowski 1964, Johri *et al.* 1992)	nuclear
	Ecdeiocoleaceae			
	Centrolepidaceae (Schnarf 1929, Swamy & Parameswaran 1962, Hamann 1962, 1975, Dahlgren *et al.* 1985, Goldberg 1989, Johri *et al.* 1992)			nuclear
		Aphelia (Dahlgren & Clifford 1982)		nuclear
		Brizula (Dahlgren & Clifford 1982)		nuclear
		Centrolepis (Dahlgren & Clifford 1982)		nuclear
			fascicularis (Prakash 1969)	nuclear
		Gaimardia (Dahlgren & Clifford 1982)		nuclear
	Poaceae (Davis 1966, Schnarf 1929, Dahlgren *et al.* 1982, Bhanwra 1988, Goldberg 1989, Johri *et al.* 1992)			nuclear
Commelinales	Eriocaulaceae (Schnarf 1929)			helobial

Table 8 Cont.

ORDER	FAMILY	GENUS	SPECIES	ENDOSPERM
	Eriocaulaceae (Davis 1966, Goldberg 1989)			nuclear & helobial
	Eriocaulaceae (Dahlgren *et al.* 1985)			nuclear
		Eriocaulon (Dahlgren & Clifford 1982)		nuclear
			hookerianum Stapf (Johri *et al.* 1992)	nuclear
			quinquangulare (Begum 1968)	nuclear
		Leiothrix (Dahlgren & Clifford 1982)		nuclear
	Xyridaceae (Schnarf 1929, Swamy & Parameswaran 1962, Davis 1966, Goldberg 1989, Johri *et al.* 1992)			nuclear
		Xyris (Dahlgren & Clifford 1982, Dahlgren *et al.* 1985)		nuclear
			indica (Weinzieher 1914)	nuclear
		Abolboda (Dahlgren & Clifford 1982, Dahlgren *et al.* 1985)		helobial
	Rapateaceae (Dahlgren *et al.* 1985, Venturelli & Bouman 1988, Johri *et al.* 1992)			nuclear
		Cephalostemon (Dahlgren & Clifford 1982)		nuclear
		Rapatea (Dahlgren & Clifford 1982)		nuclear
		Spathanthus (Dahlgren & Clifford 1982)		nuclear

Table 8 Cont.

ORDER	FAMILY	GENUS	SPECIES	ENDOSPERM
Typhales	Sparganiaceae (Schnarf 1929, Swamy & Parameswaran 1962)			nuclear
	Sparganiaceae (Davis 1966, Goldberg 1989)			nuclear & helobial
	Sparganiaceae (Swamy & Krishnamurthy 1973, Dahlgren *et al.* 1985, Johri *et al.* 1992)			helobial
		Sparganium (Dahlgren & Clifford 1982)		helobial
			angustifolium (Asplund 1973)	helobial
			erectum (Muller-Doblies 1969, Asplund 1973)	helobial
			glomeratum (Asplund 1973)	helobial
			minimum (Muller-Doblies 1969, Asplund 1973)	helobial
			simplex (Muller-Doblies 1969, Asplund 1973)	helobial
	Typhaceae (Schnarf 1929, Swamy & Parameswaran 1962, Goldberg 1989)			nuclear
	Typhaceae (Dahlgren *et al.* 1985, Johri *et al.* 1992)			helobial
		Typha (Dahlgren & Clifford 1982)		helobial
			latifolia (Asplund 1972)	helobial

Table 8 Cont.

ORDER	FAMILY	GENUS	SPECIES	ENDOSPERM
Bromeliales	Bromeliaceae (Schnarf 1929, Davis 1966, Swamy & Krishnamurthy 1973, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985, Goldberg 1989, Johri *et al.* 1992)			helobial
		Ananas	*comosus* (Wee & Rao 1974, Rao & Wee, 1979)	helobial

Table 9: Embryo types in selected commelinanean monocotyledons. Terminology after Johansen (1950). (*) = where author does not use the terminology, but clearly describes and illustrates the particular type of embryogeny.

ORDER	FAMILY	GENUS	SPECIES	EMBRYOGENY
Juncales	Juncaceae (Davis 1966, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985)			*Onagrad*, *Juncus*-variation
	Juncaceae (Johri *et al.* 1992)			*Onagrad*
		Juncus	*bufonius* (*Laurent 1904, Shah 1963)	*Onagrad*, *Juncus*-variation
			effusus (*Souegès 1933, Johansen 1950, Zaman 1950)	*Onagrad*, *Juncus*-variation
			lamprocarpus (*Laurent 1904)	*Onagrad*, *Juncus*-variation
			prismatocarpus (*Zaman 1950)	*Onagrad*, *Juncus*-variation
		Luzula (Laurent 1904)		*Onagrad*, *Juncus*-variation
			forsteri (Sm.) DC. (*Souegès 1923, 1933, Johansen 1950)	*Onagrad*, *Juncus*-variation
		Prionium	*serratum*	*Onagrad*, *Juncus*-variation
Cyperales	Cyperaceae (Davis 1966, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985)			*Onagrad*
	Cyperaceae (Johansen 1950, Johri *et al.* 1992)			*Onagrad*, *Juncus*-variation
		Bulbostylis	*barbata* C.B. Clarke (Shah 1965)	*Onagrad*, *Juncus*-variation
		Carex	*wallichiana* Presc.(Shah 1965)	*Onagrad*, *Juncus*-variation
		Cyperus	*alternifolius* (Padhye & Makde 1982)	*Onagrad*, *Juncus*-variation
			alopecuroides (Nagaraj & Nijalingappa 1973)	*Onagrad*, *Juncus*-variation
			articulatus L. (Shah 1965)	*Onagrad*, *Juncus*-variation

Table 9 Cont.

ORDER	FAMILY	GENUS	SPECIES	EMBRYOGENY
		Fimbristylis	*diphylla* (Murty & Kumar 1967)	*Onagrad*, *Juncus*-variation
		Kyllinga	*brevifolia* (Padhye 1971)	*Onagrad*, *Juncus*-variation
			monocephala (Makde & Bhuskute 1987)	*Onagrad*, *Juncus*-variation
			triceps (Padhye 1960)	*Onagrad*, *Juncus*-variation
		Mariscus	*paniceus* (Padhye & Makde 1982)	*Onagrad*, *Juncus*-variation
		Pycreus	*pumilis* (Padhye & Makde 1982)	*Onagrad*, *Juncus*-variation
		Scirpus	*mucronatus* L. (Nijalingappa & Tejavathi 1977)	*Onagrad*, *Juncus*-variation
		Remirea	*maritima* (Padhye & Makde 1982)	*Onagrad*, *Juncus*-variation
Poales	Flagellariaceae (Johansen 1950, Campbell & Kellog 1986, Johri *et al*. 1992)			?
	Joinvilleaceae (Campbell & Kellog 1986)			*Asterad*?
	Restionaceae (Johri *et al*. 1992)			*Onagrad*, *Juncus*-variation?
	Restionaceae (Campbell & Kellog 1986)			*Onagrad*?
	Ecdeiocoleaceae			-
	Centrolepidaceae (Dahlgren & Clifford 1982, Dahlgren *et al*. 1985, Campbell & Kellogg 1986)			*Onagrad*
	Centrolepidaceae (Hamann 1975, Johri *et al*. 1992)			*Onagrad*, *Juncus* variation
		Centrolepis	*fascicularis* (Prakash 1969)	*Onagrad*, *Juncus* variation

Table 9 Cont.

ORDER	FAMILY	GENUS	SPECIES	EMBRYOGENY
	Poaceae (Davis 1966, Campbell & Kellog 1986, Bhanwra 1988, Johri *et al.* 1992)			*Asterad*
	Poaceae (Johansen 1950, Dahlgren & Clifford 1982, Dahlgren *et al.* 1985)			*Asterad*, *Poa* variation
Commelinales (Dahlgren *et al.* 1985)				*Asterad*
	Eriocaulaceae (Johansen 1950)			*Asterad*, *Penea* variation
		Eriocaulon	*quinquangulare* (Davis 1966, Dahlgren & Clifford 1982, Johri *et al.* 1992)	*Asterad*
			quinquangulare (Begum 1968)	*Asterad*, *Penea* variation
	Xyridaceae (Dahlgren & Clifford 1982)			*Asterad*
	Xyridaceae (Johansen 1950, Davis 1966)			*Asterad*, *Penea* variation
		Abolboda	*grandis* (Johri *et al.* 1992)	*Asterad*, *Penea* variation
	Rapateaceae (Dahlgren & Clifford 1982)			*Asterad*?
	Rapateaceae (Johri *et al.* 1992)			*Asterad*, *Penea* variation
Typhales	Typhaceae (Davis 1966, Dahlren & Clifford 1982)			*Asterad*
		Typha	*latifolia* (Johri *et al.* 1992)	*Asterad*
	Sparganiaceae (Davis 1966, Dahlgren & Clifford 1982)			*Onagrad*
			ramosum Curt. (Johansen 1950)	*Onagrad*, *Juncus* variation
		Sparganium	*simplex* (Johri *et al.* 1992)	*Onagrad*

Table 9 Cont.

ORDER	FAMILY	GENUS	SPECIES	EMBRYOGENY
			simplex (Johansen 1950)	*Onagrad*, *Juncus* variation
Bromeliales	Bromeliaceae (Davis 1966, Dahlgren & Clifford 1982, Johri *et al.* 1992)			*Asterad*
		Ananas	*comosus* (Wee & Rao 1974, Rao & Wee 1979)	*Asterad*
		Tillandsia	*usneoides* (Johansen 1950)	*Asterad*, *Muscari* variation

Table 10: Seedling types in selected commelinanean monocotyledons. Terminology after Boyd (1932).

ORDER	FAMILY	GENUS	SPECIES	SEEDLING TYPE
Juncales	Juncaceae			
		Juncus (*Laurent 1904, Dahlgren & Clifford 1982)		A
			arcticus (Boyd 1932)	A
			effusus (Boyd 1932)	A
			gerardi (Boyd 1932)	A
			niveus (Boyd 1932)	A
			**roemerianus* (Eleuterius 1975)	A
		Luzula (*Laurent 1904, Dahlgren & Clifford 1982)		A
			campestris (Boyd 1932)	A
			sylvaticus (Boyd 1932)	A
		Prionium	*serratum*	A
Cyperales	Cyperaceae (Dahlgren & Clifford 1982)			C
		Cyperus	*alternifolius* (Boyd 1932)	C
			natalensis (Boyd 1932)	C
		Scirpus	*setaceus* (Boyd 1932)	C
Poales	Restionaceae (H.P. Linder pers. comm.)			A & C
		Restio (Dahlgren & Clifford 1982)		A
		Alexgeorgia (Dahlgren & Clifford 1982)		A
	Centrolepidaceae			

Table 10 Cont.

ORDER	FAMILY	GENUS	SPECIES	SEEDLING TYPE
		Centrolepis (Dahlgren & Clifford 1982)		A
	Poaceae (Dahlgren & Clifford 1982)			B or C
	Poaceae (Boyd 1932, Hoshikawa 1969)			C
Commelinales	Eriocaulaceae	*Eriocaulon* (Boyd 1932, Dahlgren & Clifford 1982)		A
Typhales	Typhaceae	*Typha* (Dahlgren & Clifford 1982)		A
Bromeliales	Bromeliaceae	*Aechmea* (Dahlgren & Clifford 1982)		B
		Dyckia (Boyd 1932, Dahlgren & Clifford 1982)		A
		Pitcairnia (Dahlgren & Clifford 1982)		A
		Pitcairnia (Boyd 1932)		transitional A
		Puya (Dahlgren & Clifford 1982)		A
		Tillandsia (Boyd 1932)		A

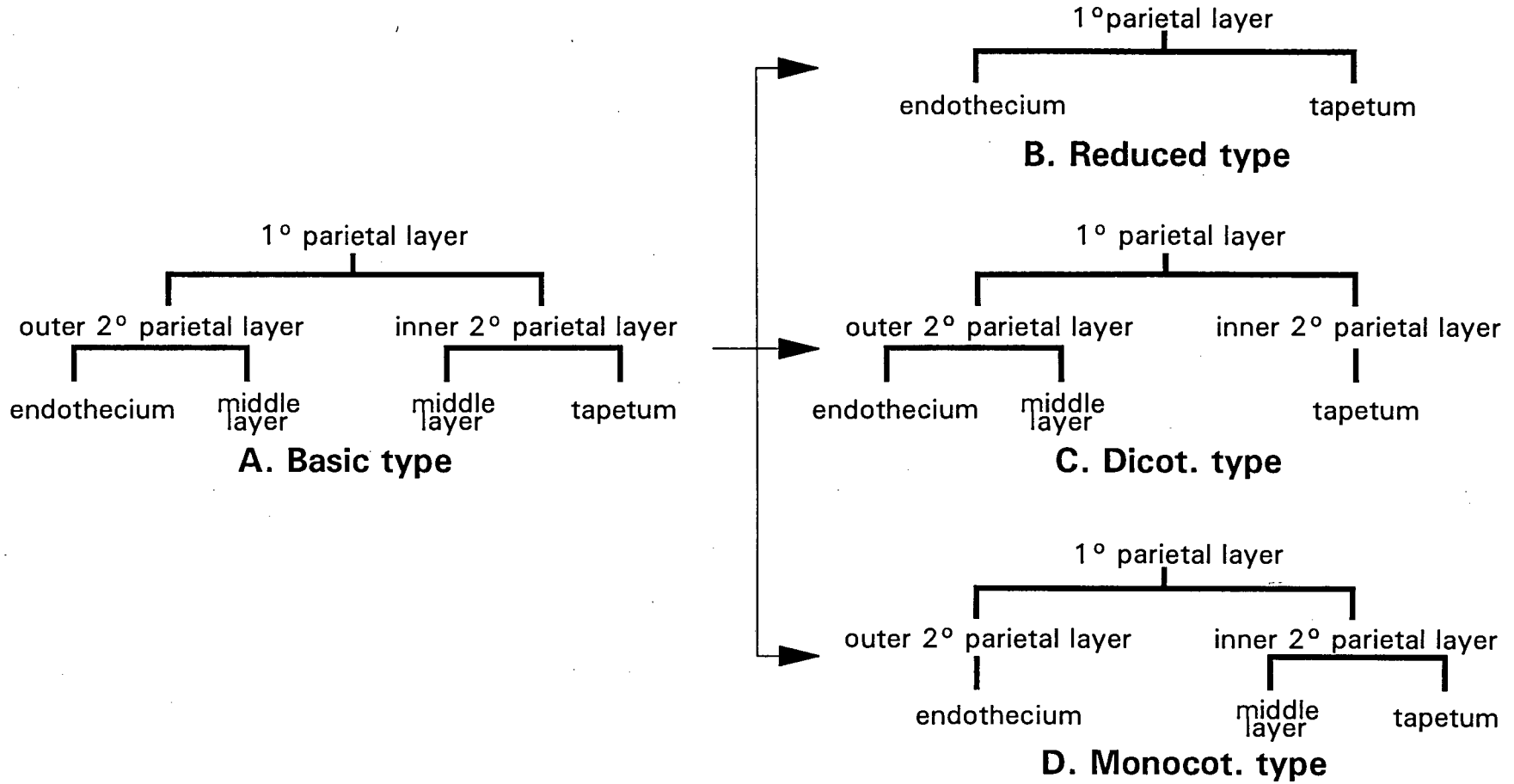

Figure 59: Proposed ontogeny of anther wall types in which the Basic type gives rise to the Reduced, Dicotyledonous and Monocotyledonous types. (A) Basic type, (B) Reduced type, (C) Dicotyledonous type and (D) Monocotyledonous type. (Modified from Davis 1966).

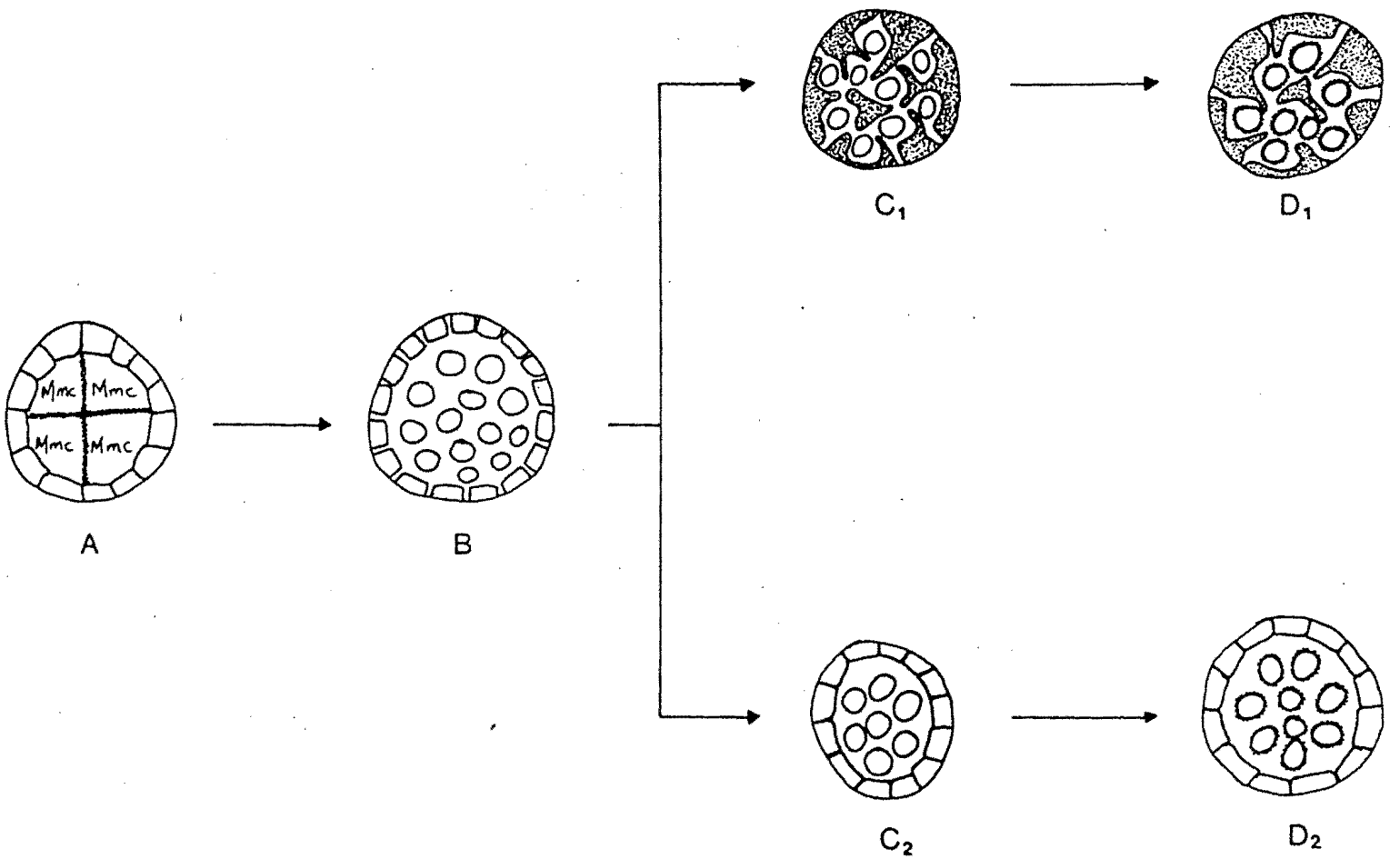

Figure 60: Proposed ontogenies of the amoeboid (C_1 & D_1) and glandular-secretory (C_2 & D_2) tapetum types. In the amoeboid type the tapetum breaks down and intrudes between the microspores, whereas in the glandular-secretory type the tapetum remains intact right up until pollen release. (Modified from Pacini *et al.* 1985).

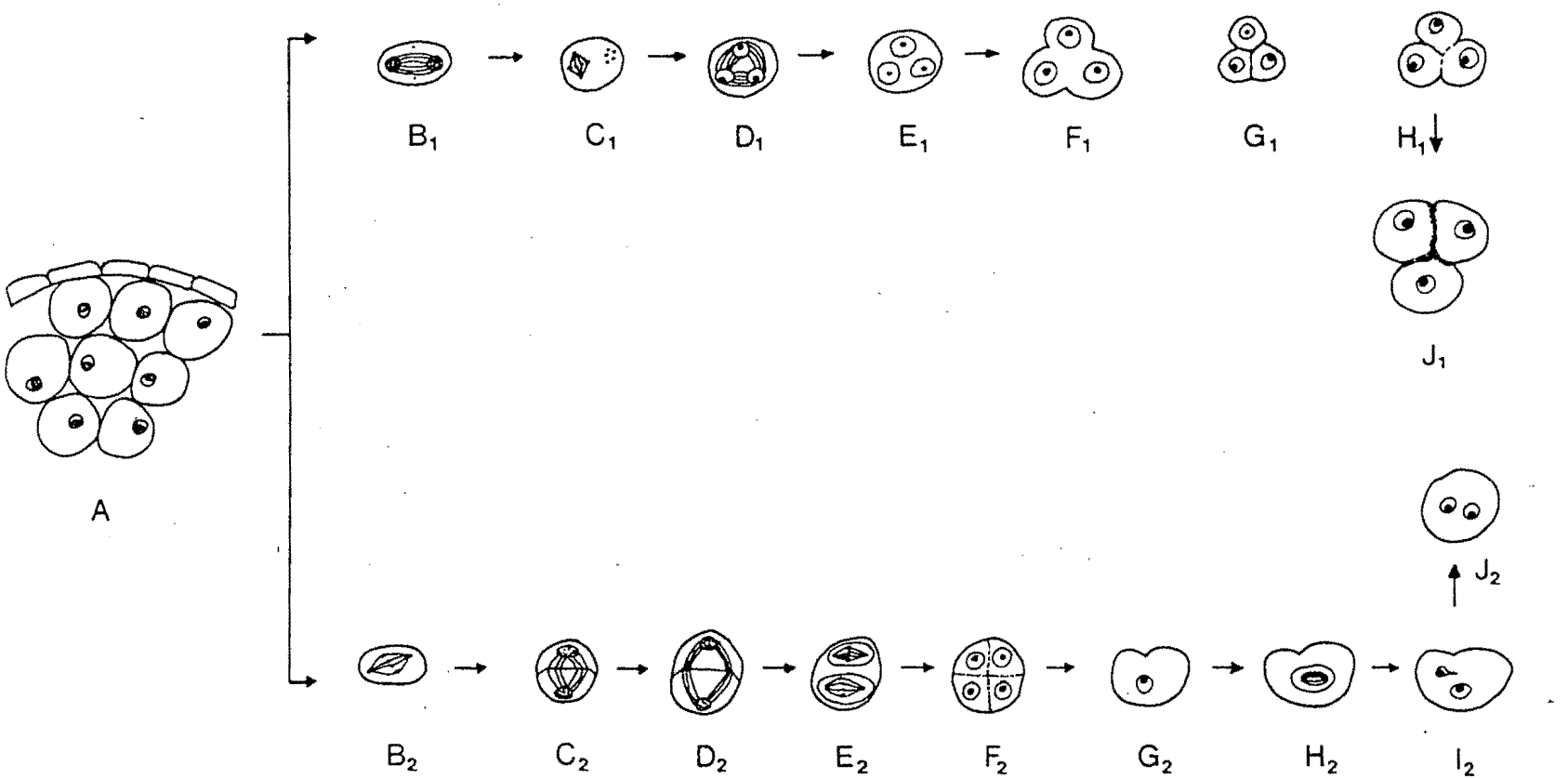

Figure 61: Pollen grain ontogeny showing the divergence between the simultaneous (B_1-J_1) and the successive (B_2-J_2) types of microsporogenesis. (Modified from Dahlgren & Clifford 1982)

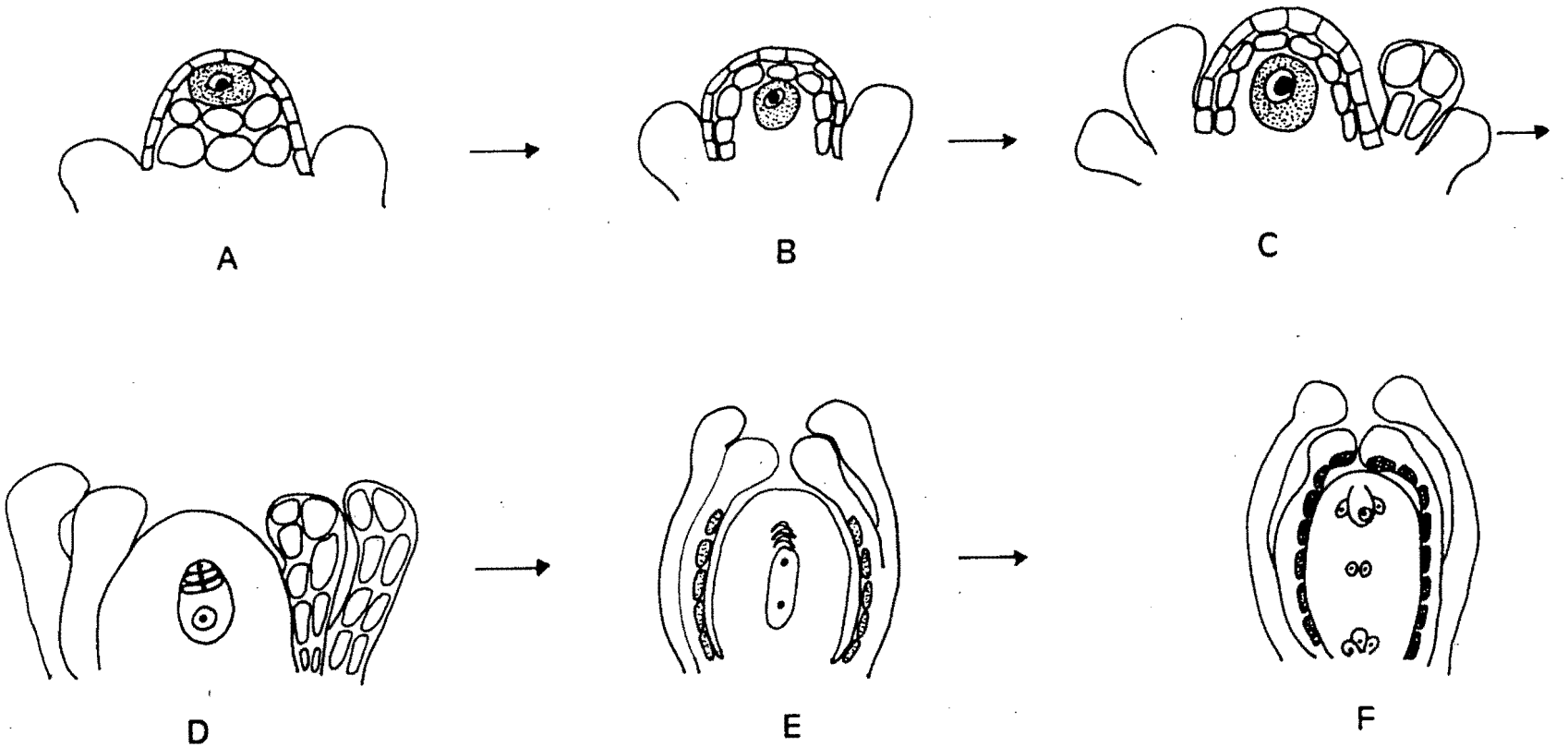

Figure 62: Integument ontogeny in *Prionium serratum*. (A) archesporial cell stage in ovule development, (B) nucellar tissue formation stage, (C) megaspore mother cell stage, (D) megasporogenesis (meiosis II), (E) megagametogenesis (mitosis I), (F) eight nucleate stage.

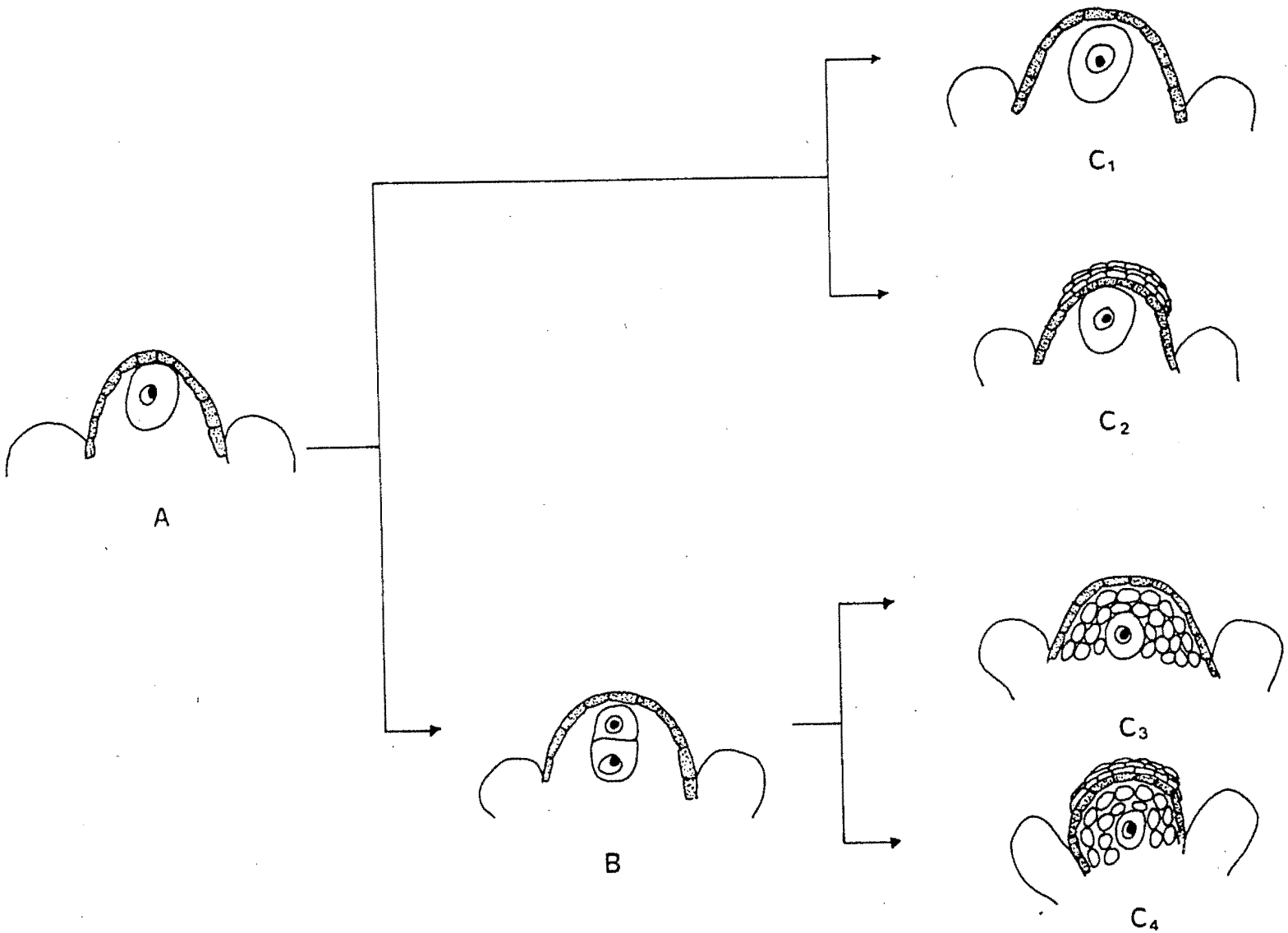

Figure 63: Ontogeny of nucellar tissue. (A) archesporial cell stage, (B) archesporial and parietal cell stage (C_1) tenuinucellate condition where the archesporial cell functions directly as the megaspore mother cell (parietal cell formation has been lost), (C_2) tenuinucellate condition with nucellar cap formation, (C_3) crassinucellate condition where the archesporial cell cuts of the parietal cell so that it divides, forming several layers of nucellar tissue and C^4 crassinucellate condition with nucellar cap formation.

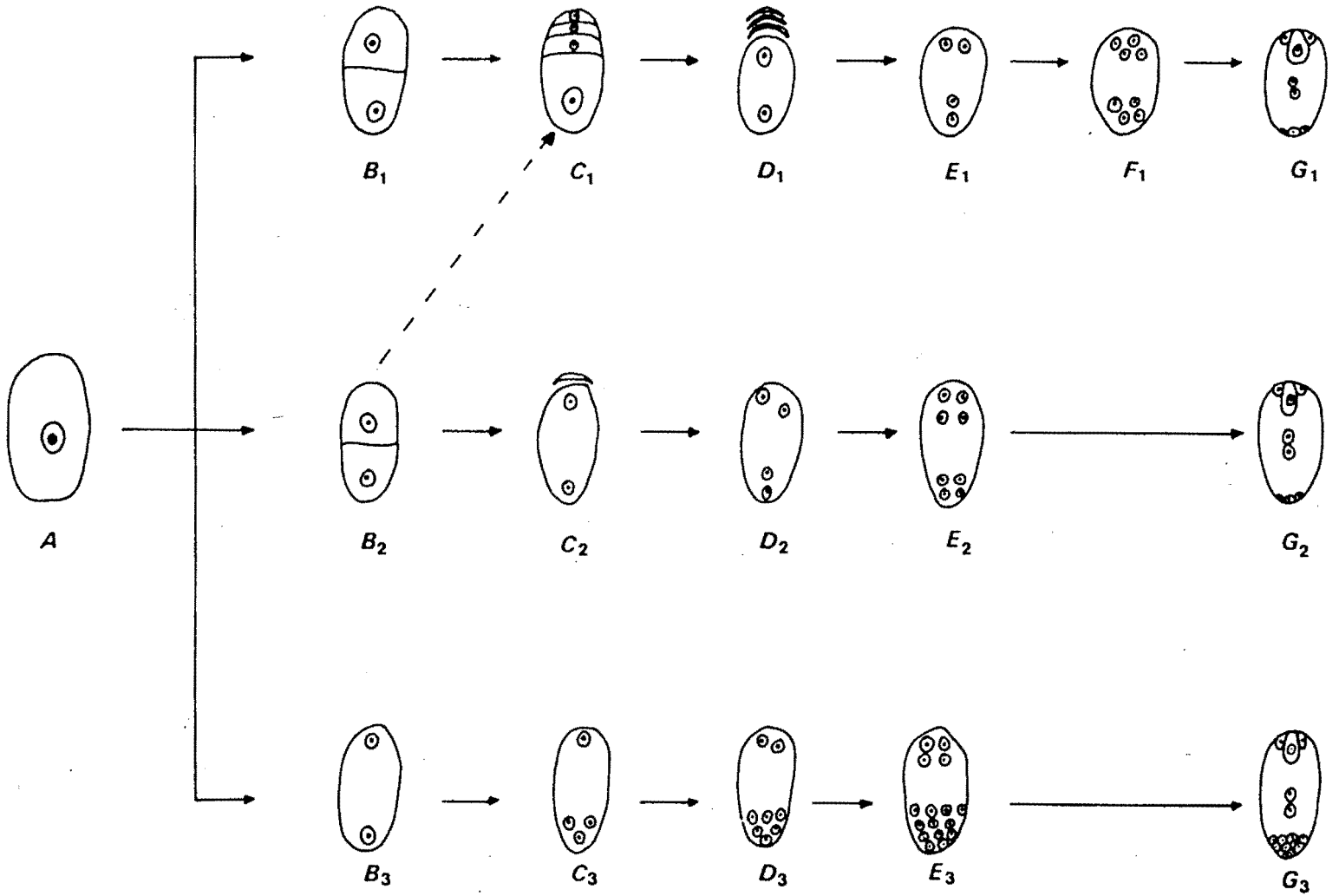

Figure 64: Female gametophyte ontogeny in three different types of gametophyte formation; *Polygonum* (B_1-G_1), *Allium* (B_2-G_2), *Drusa* (B_3-G_3). If B_1 and B_2 are the same at this stage then, C_1-G_1 would be an ontogenetic sequence following on from B_2 (indicated by dotted line). However, it is not clear whether the two stages are the same. (Modified from Maheshwari 1950).

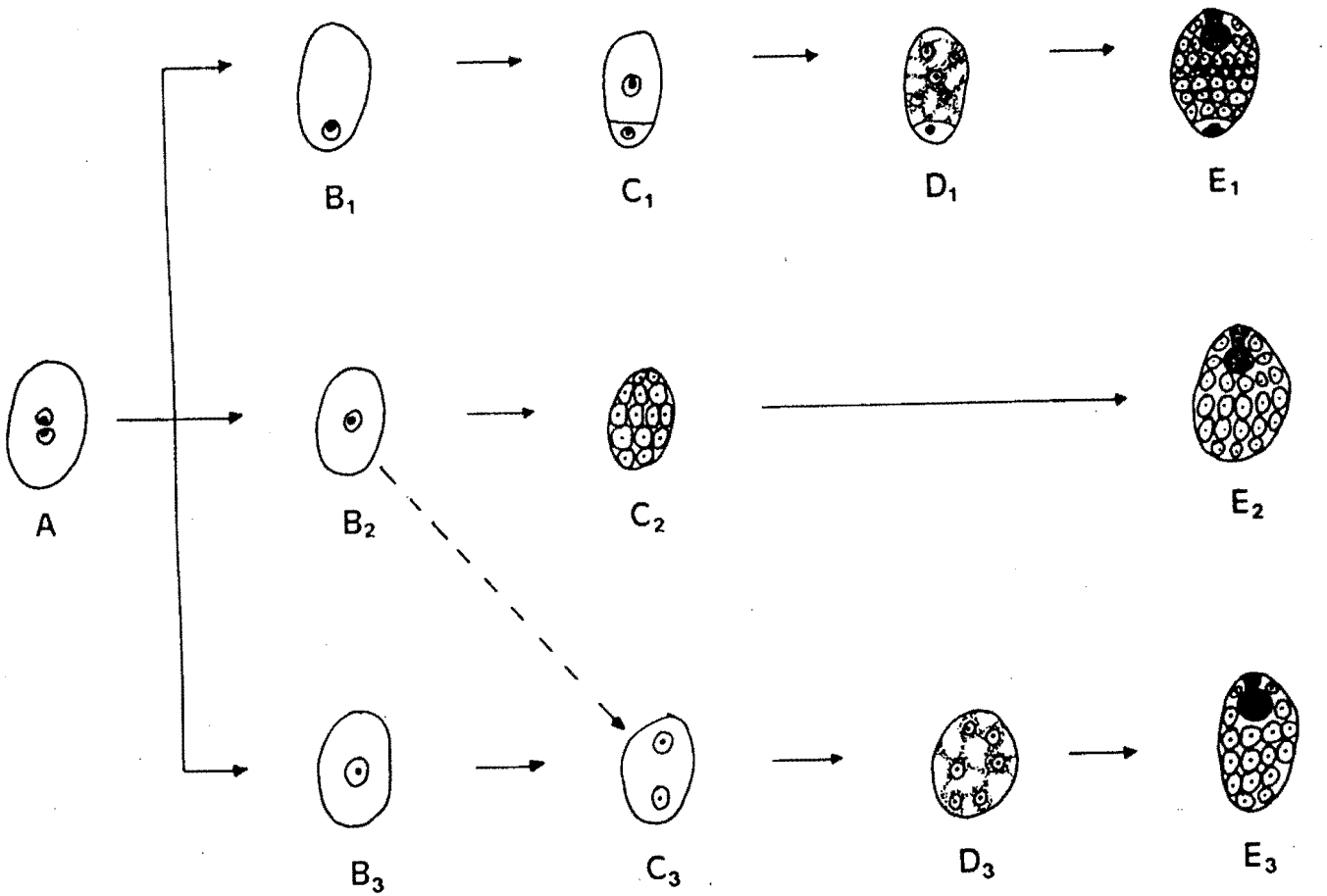

Figure 65: Endosperm ontogeny. (B_1-E_1) helobial endosperm formation, (B_2-E_2) cellular endosperm formation, (B_3-E_3) nuclear endosperm formation. If B_2 and B_3 are the same at this stage then, C_3-E_3 would be an ontogenetic sequence following on from B_2 (indicated by dotted line). However, it is not clear whether the two stages are the same.

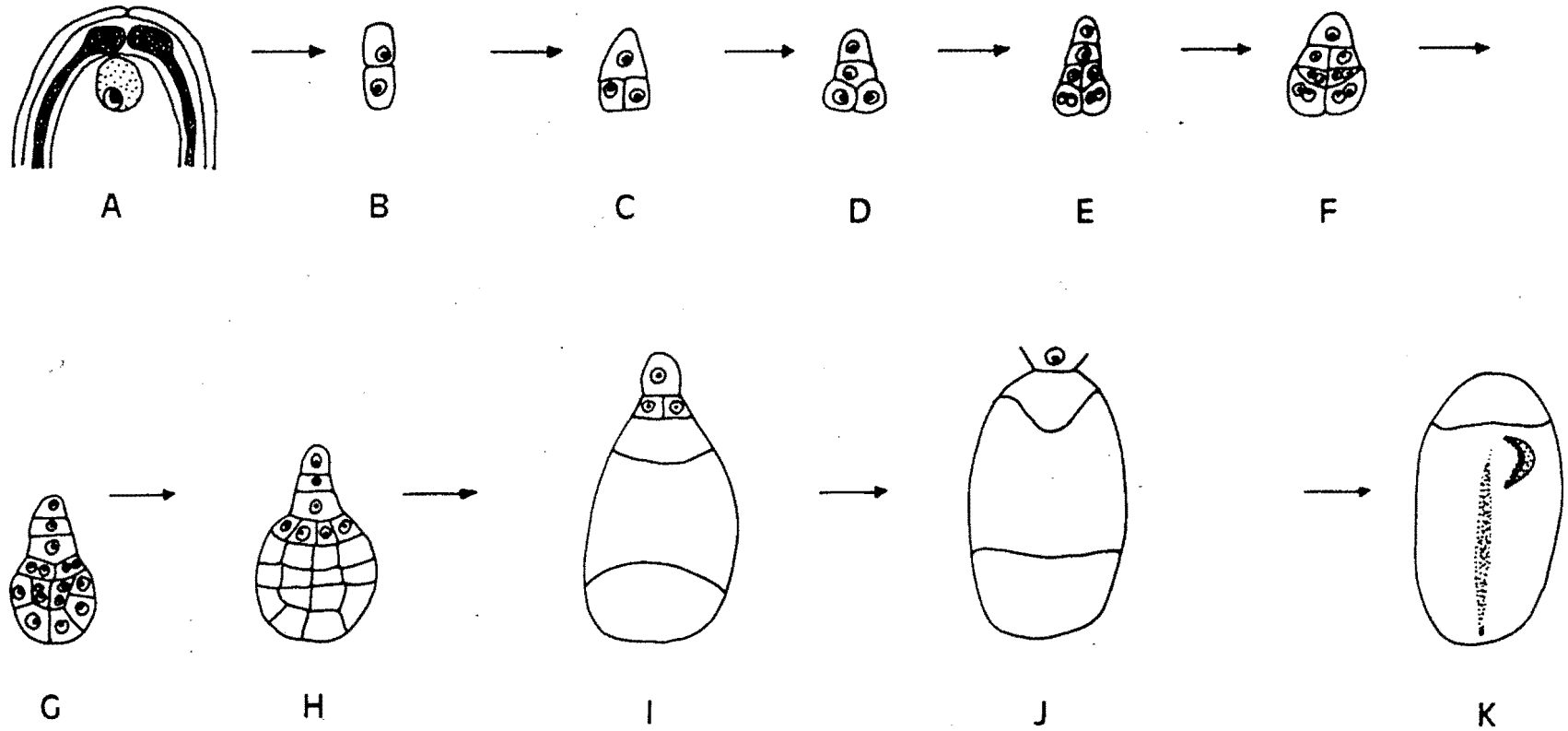

Figure 66: Zygote ontogeny in *Prionium serratum*. (A) zygote, (B-J) embryo formation, (K) mature embryo.

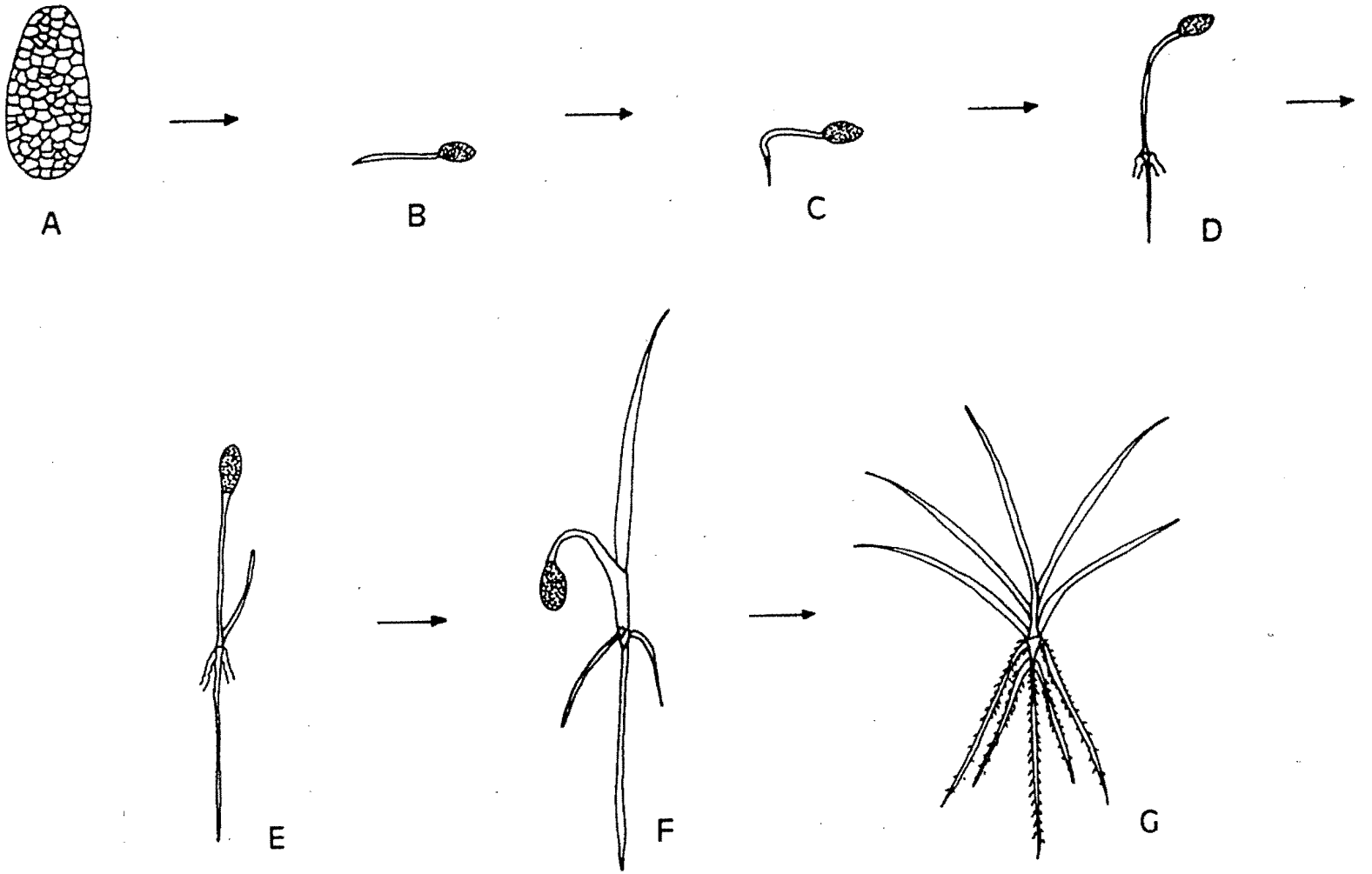

Figure 67: Seedling ontogeny in *Prionium serratum*. (A) seed, (B) germination, (C-F) seedling development and (G) adult plant.

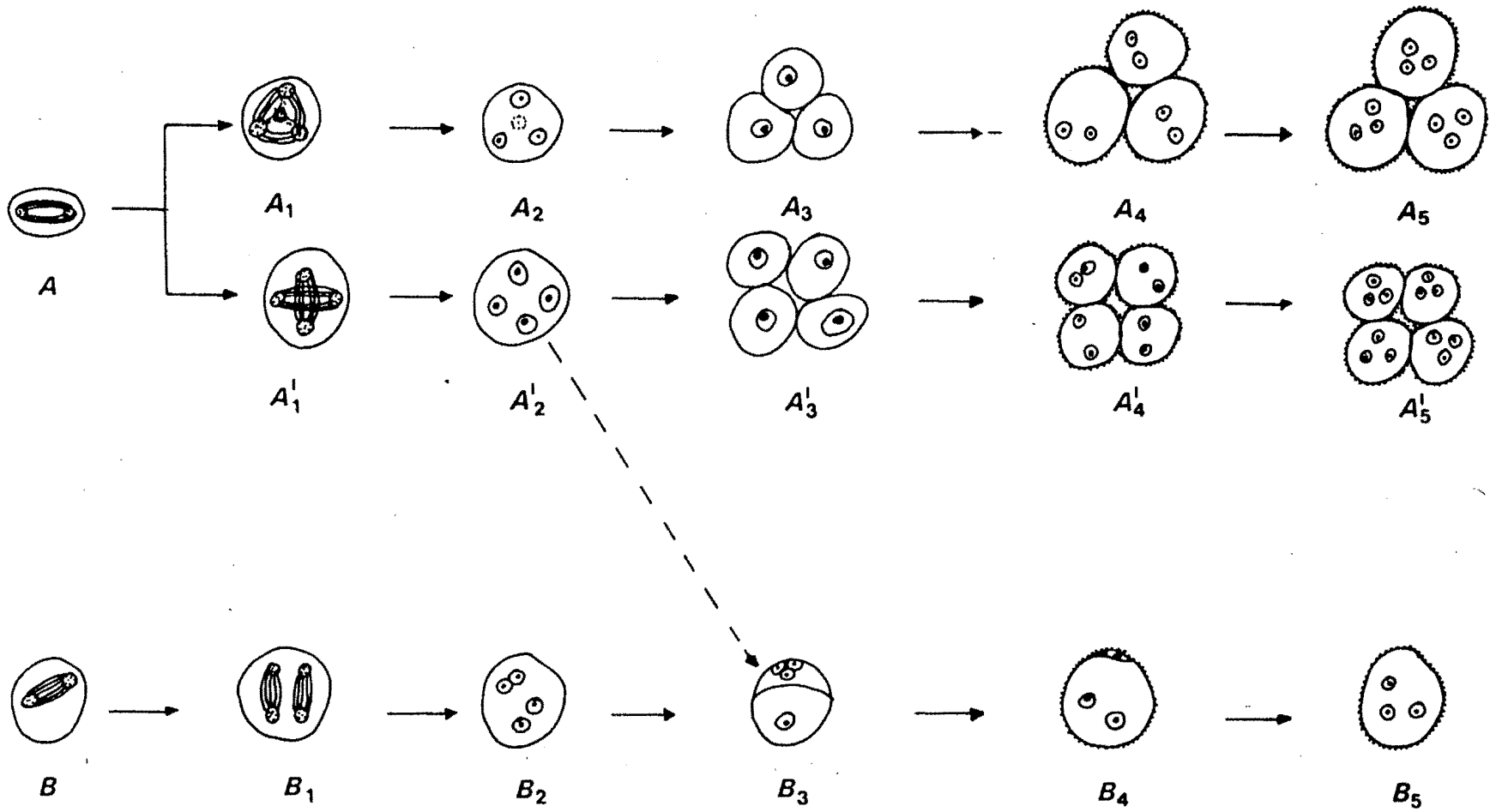

Figure 68: Ontogenetic development of pollen tetrads in Juncaceae (A) and Cyperaceae (B). (A_1-A_5) tetrahedral tetrad formation, (A'_1-A'_5) cross tetrad formation, (B_1-B_5) formation of pseudomonad. The formation of the Cyperaceae pseudomonad may follow on from stage A_2 as in Juncaceae (indicated by dotted line), however it is not clear whether cross and tetrahedral arrangements of the tetrad stage are found in the Cyperaceae pollen developmental sequence.

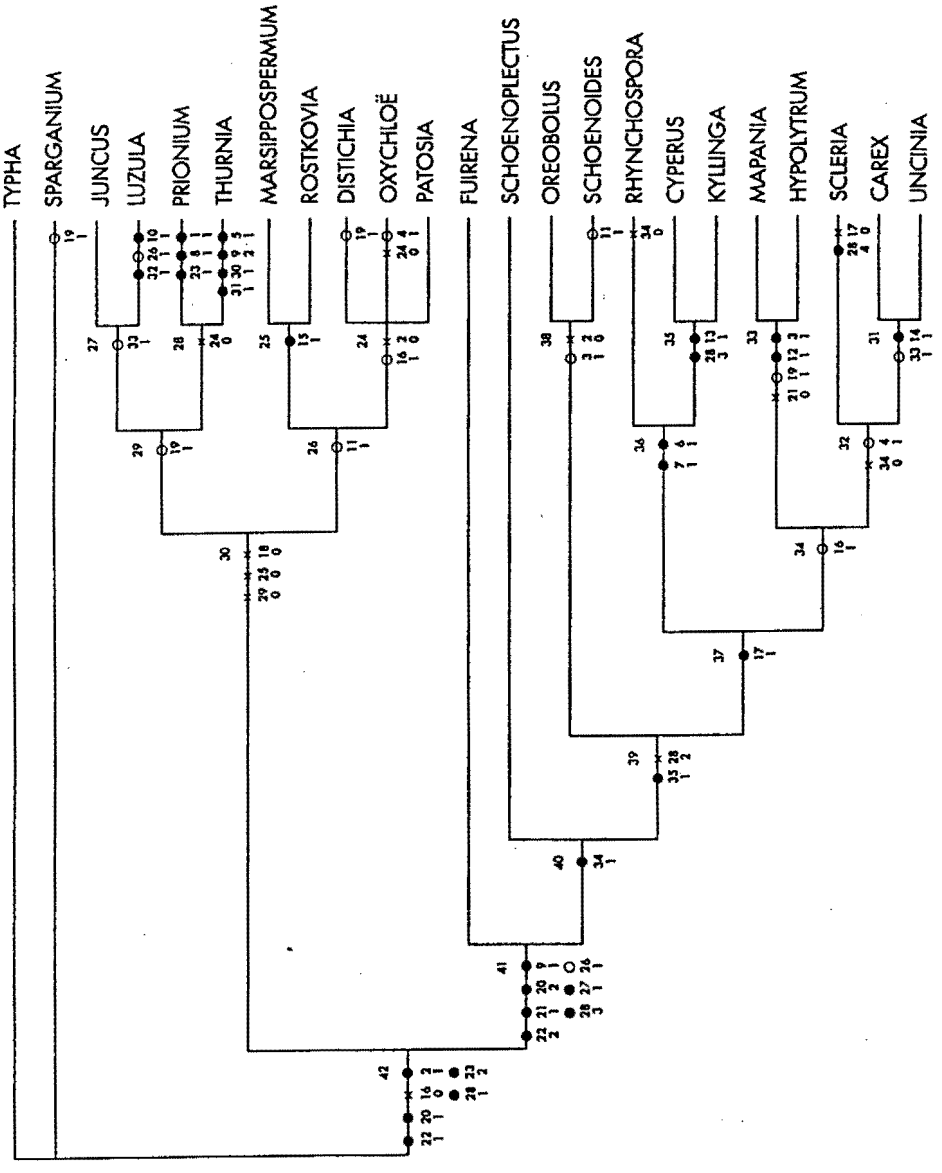

Figure 69: Simpson's (1995) Cyperales topology based on morphological characters. Solid circle = synapomorphy, open circle = parallelism, x = reversal (From Simpson 1995).

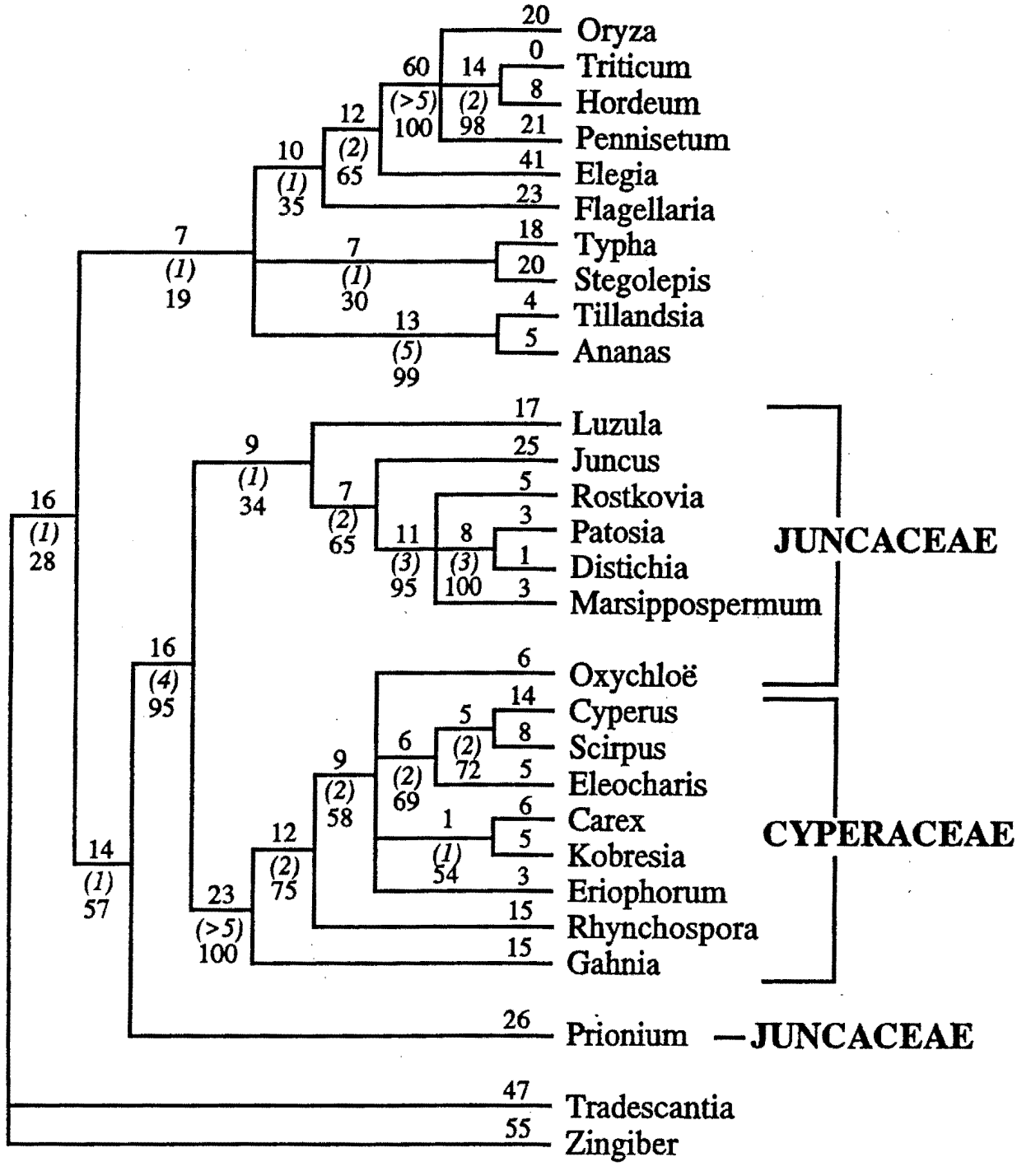

Figure 70: Plunkett *et al.*'s (1995) Cyperales topology based on *rbc*L sequence data. Numbers above the branches refer to the number of apomorphies. Numbers below nodes in parentheses are decay values and below these bootstrap percentages. (From Plunkett *et al.* 1995)

APPENDICES

Appendix 1. Modifications to Simpson's (1995) Cyperales data set.

The Simpson (1995) Cyperales data set was entered into MacClade vers. 3.0 (Maddison & Maddison 1992) as published. Due to an error in his character matrix (viz. Thurnia has an extra character (no. 31) and character 30 (seeds glabrous (state 0), seeds hispid (state 1) is published as state 0 for *Thurnia* which is incorrect since the seeds in *Thurnia* are hispid which Simpson has discussed as an apomorphy for *Thurnia*) the same tree for the data set could not be obtained when analyzed in PAUP vers. 3.1 (Swofford 1993). The data set was therefore corrected for *Thurnia*, incorporating the correct state for character 30 and excluding the extra character (positional character 31). A number of characters were also reinterpreted and in addition to Simpson's outgroups of *Typha* and *Sparganium*, several other outgroups were coded up for the same characters of the Cyperales data set, namely *Ananas*, *Eriocaulon* and *Flagellaria*. A number of embryological characters were incorporated into Simpson's data set as well as the new outgroups. For *Prionium* the embryological characters used were gained from observations in this study. For the other taxa the embryological information was taken from Tables 1-9, using the relevant literature sources as cited.

A list of modifications to Simpson's Cyperales data set and the source of the data follows:

Character 2 (leaves distichous or tristichous), spiral leaf insertion was included under the tristichous condition.

Character 6 (scalariform perforation plates in stems) additional data were added from Cheadle & Kosakai (1973).

Character 20 (microsporogenesis). Simpson (1995) refers to microsporogenesis in Cyperaceae as a special type i.e. Cyperaceae-type. As discussed previously (see Discussion ontogeny section), however, it is the pollen formation that is of a special type in Cyperaceae, rather than microsporogenesis. Microsporogenesis is the same in both Cyperaceae and Juncaceae with the microspore mother cells dividing simultaneously. It is only after microsporogenesis that a differentiation in the type of pollen development

occurs. Thus, since Simpson has taken into account the different types of pollen arrangements (i.e. character 22 monads, tetrads or pseudomonads) he effectively codes the same character twice. Therefore, the data set was modified for character 20 (microsporogenesis) which was changed to a two state character i.e. simultaneous or successive and Cyperaceae were included as having simultaneous microsporogenesis. The special type of pollen formation in Cyperaceae is accounted for in character 22 (pollen arrangement at release, monads, tetrads or pseudomonads) which was left unchanged.

Character 23 (carpel fusion). Solitary carpels in *Typha* and *Sparganium* are difficult to interpret in the sense that they may have arisen by fusion or by the loss of the other carpels and for this reason cannot be categorised (H.P. Linder pers. comm.). Consequently, the carpels in *Typha* and *Sparganium* were coded as unknown for this character. Thus, character 23 (carpels solitary, fused in ovary region only, fused with single style) was changed from three states to two states (carpels fused in ovary region only, fused with single style) and ordered.

All characters are ordered except characters 1 (plants herbaceous, or with woody, shrub-like base, or climber), 28 (embryo type), 36 (number of tapetal layers), 37 (Number of tapetal nuclei), 42 (embryogeny), and 43 (seedling type) which are unordered. Several characters from Simpson's data set were also excluded from the analysis:

Character 19 (anther connective tip prominent or not) was excluded since it may easily be misinterpreted and may be uninformative from an evolutionary point of view.

Character 21 (pollen grain shape) was excluded from the data set since the evolution of shape in pollen grains appears to be evolutionarily uninformative. Instead, pollen aperture was used which has been shown to be evolutionarily informative (Dahlgren & Clifford 1982, Zavada 1983, Linder & Ferguson 1985).

Character 25 (number of ovules per pistil) was excluded since it provides no insight into the evolutionary reduction/fusion of carpels and the reduction in the number of ovules. A more informative character would be the number of fertile ovules per carpel

which was incorporated into the data set.

Simpson's fruit character (character 29) of dehiscence/indehiscence was excluded for the reasons that indehiscence has evolved independently several times in the monocotyledons (H.P. Linder pers. comm.) and that indehiscence in the Cyperaceae cannot be coded equally/the same as indehiscence in *Typha* and *Sparganium*. The new character (number of fertile carpels involved in fruit formation, three carpellate, multi-ovulate/three carpellate uni-ovulate) is coded as state 0 for all taxa which do not have Cyperaceae indehiscence.

Simpson's character 33 (parasitism by advanced rusts) appears to have been obtained from Savile's (1990) study. This character was excluded because Savile's (1990) conclusions that Juncaceae evolved from Cyperaceae strongly contradicts current thinking in the evolution of Juncaceae/Cyperaceae (e.g. Dahlgren *et al.* 1985, Chase *et al.* 1993, Plunkett *et al.* 1995).

Simpson's character 32 (eliaosome present or absent) was excluded since Dahlgren *et al.* (1985) report that an eliaosome is present in only some species of *Luzula* and thus the character becomes uninformative since it is variable for *Luzula*.

Simpson's characters 34 (presence/absence of aurones) and 35 (presence/absence of tricin) were excluded since these chemicals seem to be a special feature of Cyperaceae but little information is available for the juncaceous genera e.g. *Marsippospermum*, *Patosia* and *Rostkovia* and also *Thurnia* and the outgroup genera. Some embryological characters that were excluded are 36 (number of tapetal layers) and 43 (seedling type) since little information was available for many of the taxa under consideration.

Additional characters that were included into the data set are the presence/absence of diffuse centromeres (data from Berger 1946, La Cour 1952, Battaglia 1955, Nordenskiöld 1961, Braselton 1981) and the presence/absence of calcium oxalate raphides (data from Cutler 1969) and the presence/absence of parenchymatous air canals (data from Cutler 1969).

Appendix 2. List of characters and data matrix.

Character list

[1] plants - herbaceous (0)/woody shrub-like base (1)/climber (2)
[2] leaf insertion - distichous (0)/spiral or tristichous (1)
[3] pseudopetiole - abs (0)/pres (1)
[4] ligule - abs (0)/pres (1)
[5] inverted vascular bundles in leaf - abs (0)/pres (1)
[6] scalariform perforation plates in stem vessels - pres (0)/abs (1)
[7] c4 photosynthesis - abs (0)/pres (1)
[8] chlorenchymatous air canals - abs (0)/pres (1)
[9] silica bodies - abs (0)/pres (1)
[10] multicellular hairs - abs (0)/pres (1)
[11] flowers - more than one (0)/solitary (1)
[12] spicoid-type inflorescence - abs (0)/pres (1)
[13] distichously arranged flowers - abs (0)/pres (1)
[14] utricle - abs (0)/pres (1)
[15] outer perianth segments - 0-7mm long (0)/over 7mm long (1)
[16] flowers - bisexual (0)/unisexual (1)
[17] perianth segments - pres (0)/abs (1)
[18] fertile stamens - more than three (0)/one to three (1)
[19] connective tip - prominent (0)/not prominent (1)*
[20] microsporogenesis - successive (0)/simultaneous(1)
[21] pollen grains - spheroidal (0)/pear-shaped (1)*
[22] pollen grains at release - monads (0)/tetrads (1)/pseudomonads (2)
[23] carpels - fused in ovary region only (0)/fused with single style (1)
[24] ovary - multi-locular (0)/uni-locular (1)
[25] no. ovules per pistil - more than one (0)/one (1)*
[26] placentation - apical (0)/axile (1)/basal (2)
[27] endosperm formation - helobial (0)/nuclear (1)
[28] embryo type - linear (0)/*Juncus*-type (1)/*Carex*-type (2)/*Cyperus* type (3)/*Fimbristylis* type (4)/cylindrical (5)/lens-shaped (6)
[29] no. of fertile carpels involved in fruit formation - three carpellate multi-ovulate (0)/three carpellate uni-ovulate (1)
[30] seeds - glabrous (0)/hispid (1)
[31] subulate processes on seed - abs (0)/pres (1)
[32] Eliaosome - abs (0)/pres (1)*
[33] parasitism by advanced rusts - no (0)/yes (1)*
[34] aurones - abs (0)/pres (1)*
[35] tricin - abs (0)/pres (1)*
[36] no. tapetal layers - one (0)/one becoming two (1)/two (2)*
[37] no. tapetal nuclei - one (0)/one becoming two (1)/two (2)/Typhaceae type (3)
[38] tapetum type - glandular (0)/amoeboid (1)
[39] pollen at dispersal - bi-nucleate (0)/tri-nucleate (1)
[40] nucellus - crassinucellate (0)/tenuinucellate (1)

[41] embryo sac - *Polygonum* (0)/*Allium* (1)/*Drusa* (2)
[42] embryogeny - *Onagrad, Juncus* variation (0)/*Asterad Poa* variation (1)/*Asterad, Penea* variation (2)/*Asterad* (3)
[43] seedling type - a (0)/c (1)/b (2)*
[44] diffuse centromeres - abs (0)/pres (1)
[45] Calcium oxalate - abs (0)/pres (1)
[46] pollen - spiraperturate (0)/ulcerate (1)/sulcate (2)
[47] parenchymatous air canals - abs (0)/pres (1)

* = Excluded characters

Table 1. Cyperales data matrix.

Ananas	0100000010000?000?0?0100105000???00000370121
Sparganium	00000000{01}00000010{01}1000?{01}100?0000000?3100007011?
Typha	00000000{01}00000010{01}0000?1100000000007310003001 1?
Eriocaulon	{01}1?000000{01}{01}000?100?000101216000?????{02}0{01}10200101
Flagellaria	2011000?1?0000?00??0000000??0000????120001??001?
Distichia	00000?0000100001001101110107?0000000?????0??1011
Juncus	0100000000000000001101{01}{01}0101000010000010000101 1
Luzula	0100000001000000001101110201000{01}100000170001011
Marsippospermum	0101000000100010000?011101??00000???????????1010
Oxychloë	0001000000100001000101100 1??0000000207?1????1010
Patosia	0000000100001000?0111010?00000???????????1 0??
Prionium	1100000100000000001101000100000000010010000 1010
Rostkovia	0101000000100010000?011101??00000???????????1011
Thurnia	0100100010000000001101100107011 0?????01?????011
Carex	0101000010000101110112111212100010 1????00?1011
Cyperus	01{01}{01}01101000100011{01}1121111213100001100010001101{01}
Fuirena	1000?001000000001011211112131000000?????????101{01}
Hypolytrum	0110000010010001111102111 2?2100?11?????????101{01}
Kyllinga	0100011010001000110112111213100001100010007101{01}
Mapania	01{01}0000010010001111102111 2?2100?????????????1011
Oreobolus	0010000010000000010112111 2?2100001 1????????1010
Rhynchospora	10001{01}01000000{01}{01}1{01}1121111212000001???1????1011
Schoenoides	0100000101000000101121111 2?2100007????????????101?
Schoenoplectus	010{01}0?0010000000{01}10112111 2?3100001 0???1????1011
Scleria	010{01}0000010000001010112111214100000100010 0??101{01}
Uncinia	01010?0010000101110112111 2?2100010 1????????101{01}

Appendix 3. Voucher List for Figures 1-55.

Figure 1: Linder 5771, Palmiet River.
Figure 2: Linder 5771, Palmiet River.
Figure 3: Linder 5771, Palmiet River.
Figure 4: Linder 5771, Palmiet River.
Figure 5: Linder 5771, Palmiet River.
Figure 6: Linder 5771, Palmiet River.
Figure 7: Linder 5771, Palmiet River.
Figure 8: Linder 5771, Palmiet River.
Figure 9: Linder 5771, Palmiet River.
Figure 10: Linder 5771, Palmiet River.
Figure 11: Linder 5771, Palmiet River.
Figure 12: Linder 5770, Palmiet River.
Figure 13: Linder 5770, Palmiet River.
Figure 14: Linder 5771, Palmiet River.
Figure 15: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 16: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 17: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 18: Linder 5770, Palmiet River.
Figure 19: Linder 5770, Palmiet River.
Figure 20: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 21: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 22: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 23: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 24: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 25: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 26: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 27: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 28: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 29: Munro 1, bridge on road to Algeria Forest station, Clanwilliam.
Figure 30: Linder 5773, Silvermine Nature Reserve.
Figure 31: Linder 5773, Silvermine Nature Reserve.
Figure 32: Linder 5773, Silvermine Nature Reserve.
Figure 33: Linder 5773, Silvermine Nature Reserve.
Figure 34: Linder 5773, Silvermine Nature Reserve.
Figure 35: Linder 5773, Silvermine Nature Reserve.
Figure 36: Linder 5773, Silvermine Nature Reserve.
Figure 37: Linder 5773, Silvermine Nature Reserve.
Figure 38: Linder 5773, Silvermine Nature Reserve.
Figure 39: Linder 5773, Silvermine Nature Reserve.
Figure 40: Linder 5773, Silvermine Nature Reserve.
Figure 41: Linder 5773, Silvermine Nature Reserve.
Figure 42: Linder 5773, Silvermine Nature Reserve.
Figure 43: Linder 5773, Silvermine Nature Reserve.
Figure 44: Linder 5773, Silvermine Nature Reserve.
Figure 45: Linder 5773, Silvermine Nature Reserve.
Figure 46: Linder 5771, Palmiet River.
Figure 47: Linder 5771, Palmiet River.
Figure 48: Linder 5771, Palmiet River.
Figure 49: Linder 5771, Palmiet River.
Figure 50: Linder 5771, Palmiet River.
Figure 51: Linder 5771, Palmiet River.
Figure 52: Linder 5771, Palmiet River.
Figure 53: Linder 5771, Palmiet River.
Figure 54: Linder 5771, Palmiet River.
Figure 55: Linder 5771, Palmiet River.

Appendix 4: Author citations for *rbc*L sequence data.

All sequence data courtesy of Greg Plunkett.

Sequences of *Juncus* (Juncaceae), *Carex* and *Cyperus* (Cyperaceae), *Ananas* (Bromeliaceae), *Typha* (Typhaceae) and *Flagellaria* (Flagellariaceae) are all from Chase *et al.* (1993) (see Appendix in Chase *et al.* 1993 for individual researchers)

Sequences of *Distichia*, *Luzula*, *Marsippospermum*, *Oxychloë*, *Patosia*, *Prionium* and *Rostkovia* (Juncaceae) and *Rhynchospora* (Cyperaceae) all from Plunkett *et al.* (1995), sequenced by Greg Plunkett.