CSIRO PUBLISHING
www.publish.csiro.au/journals/ajb *Australian Journal of Botany*, 2010, **58**, 597–604

Microsporogenesis and microgametogenesis of *Cardiospermum grandiflorum* and *Urvillea chacoensis* (Sapindaceae, Paullinieae)

Stella M. Solís[A], *Beatriz Galati*[B] *and María S. Ferrucci*[A,C]

[A]Instituto de Botánica del Nordeste (UNNE-CONICET), C. C. 209, W3400CBL, Corrientes, Argentina.
[B]Cátedra de Botánica Agrícola, Facultad de Agronomía, Universidad de Buenos Aires, C1417DSE, Buenos Aires, Argentina.
[C]Corresponding author. Email: msferrucci@yahoo.com.ar

Abstract. Microsporogenesis and microgametogenesis of two species, *Cardiospermum grandiflorum* Sw. and *Urvillea chacoensis* Hunz. (Sapindaceae, Paullinieae), were studied using light and transmission electron microscopy. Both species are monoecious, with staminate and hermaphrodite, although functionally pistillate, flowers. A comparative pollen-development study of these two floral morphs is reported. For the present study, five stages of pollen ontogeny were identified. The development of the anther wall is of basic type. Its wall consists of epidermis, endothecium, two middle layers and a uninucleate secretory tapetum. The microspore tetrads are tetrahedral. The mature anther in staminate flowers presents the endothecium with well developed fibrillar thickenings, remains of tapetal cells, a single locule formed in the theca by dissolution of the septum before anther dehiscence and two-celled pollen grains when shed. In functionally pistillate flowers, the mature anthers present remnants of the middle layers, tapetal cells without signs of degradation, the theca with two locules and pollen grains uni- or bicellular, some of them with the cytoplasm collapsed. These anthers are not dehiscent. It can be concluded that male sterility is characterised by failure to produce functional pollen grains, an event that would be associated with the persistence of tapetal cells. Ultrastructural analysis clearly shows the difference in tapetal cells between the two flower morphs.

Introduction

Cardiospermum grandiflorum Sw. and *Urvillea chacoensis* Hunz. belong to the tribe *Paullinieae* (Sapindaceae), and both species are climbers. This tribe is characterised by the presence of monoecy, the most common condition in the family. The family Sapindaceae consists of 14 tribes (Radlkofer 1934), of which the most evolved is Paullinieae (Solís and Ferrucci 2009). The climbing habit and development of tendrils and stipules are synapomorphies for Paullinieae (Buerki *et al*. 2009). The species analysed here belong to very closely related genera of the tribe; however, small differences in the fruit make them recognisable (Ferrucci and Acevedo-Rodríguez 1998). *Cardiospermum* L. comprises 16 species, all in America, with three of its species, including *C. grandiflorum*, with a nearly cosmopolitan distribution (Ferrucci 2000*a*). This genus is the most heterogeneous within the tribe and shows well marked differences in growth habit, morphological characters and chromosome numbers (Ferrucci 2000*a*, 2000*b*; Urdampilleta *et al*. 2006). Meanwhile, *Urvillea* Kunth has 17 species with a tropical or subtropical distribution in the New World (Ferrucci 2000*a*, 2006). *U. chacoensis* occurs in Brazil, western Mato Grosso, south-eastern Bolivia, western Paraguay and north-central Argentina.

Although some embryological research has been conducted in species of the family Sapindaceae, no reports have provided ultrastructural details. In the tribe Paullinieae, the only record is of *Cardiospermum halicacabum* L. (Kadry 1946; Nair and Joseph 1960). Within the other tribes, there are descriptions of *Filicium decipiens* (Wight & Arn.) Thwaites (Gulati and Mathur 1977), *Allophylus alnifolius* (Baker) Radlk. (Mathur and Gulati 1980), *Lepidopetalum jackianum* Hiern (Mathur and Gulati 1981), *Allophylus zeylanicus* L. (Mathur and Gulati 1989), *Xerospermum intermedium* Radlk., *Nephelium lappaceum* L., *Pometia pinnata* Forst. (Ha *et al*. 1988) and *Handeliodendron bodinieri* (Cao *et al*. 2008). Ha *et al*. (1988) described the embryology of *X. intermedium* and emphasised that *N. lappaceum* and *P. pinnata* exhibit similar patterns, with minor differences. Mathur (1989) provided an overview of embryology in Sapindaceae.

Among pollen studies in Sapindaceae, the most complete is that of Muller and Leenhouts (1976), who surveyed pollen at the family level. More recently, Ferrucci and Anzótegui (1993) reported a detailed analysis of 31 species of the tribe Paullinieae, where the pollen grains of *C. grandiflorum* and *U. chacoensis* were described.

The aim of the current paper is to present a comparative analysis of anther-wall development, microsporogenesis and microgametogenesis, with an emphasis on ultrastructure of tapetal cells, so as to describe the cytological events of secretory tapetum in relation to the development of pollen

10.1071/BT10162 0067-1924/10/070597

grains. The study will contribute to the morphological and functional characterisation of both types of flowers, staminate and functionally pistillate, hermaphrodite, in both *C. grandiflorum* and *U. chacoensis*.

Materials and methods

Anthers of staminate and pistillate flowers in different stages of development were fixed in formaldehyde–acetic acid–alcohol (FAA). The voucher specimens were deposited in the herbarium of the Instituto de Botánica del Nordeste (CTES), Argentina.

Transverse serial sections of buds and pre-anthesis flowers in different stages were prepared according to standard techniques. Permanent microscope slides were made by dehydration through an ethanol series, with a rinsing pre-impregnant (González and Cristóbal 1997). For infiltration in paraffin, the technique of Johansen (1940) was applied, and the material was later embedded in 'Histoplast®' (Biopack, Buenos Aires, Argentina). Sections of 10–15 µm were made with a rotary microtome and stained with astra blue-safranin (Luque *et al.* 1996) before mounting with synthetic Canada Balsam (Biopur, Buenos Aires, Argentina). Observations and photographs were performed with a Leica MZ6 stereomicroscope and a Leica DM LB2 binocular microscope (Leica, Wetzlar, Germany), both equipped with a digital camera.

Scanning electron microscope (SEM) micrographs were obtained with a JEOL 5800 LV scanning electron microscope (JEOL USA, Peabody, MA, USA) operating at 20 kV. Preserved material was dehydrated and then immersed in CO_2 for critical-point drying before coating, and sputter-coated with gold palladium.

For transmission electron microscopy (TEM) studies, the anthers were pre-fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) overnight and then post-fixed in OsO_4 at 2°C in the same buffer for 3 h. Following dehydration in an ethanol series, the material was embedded in Spurr's resin. Ultrathin sections (750–900 nm) were cut on a Sorvall ultramicrotome (Thermo Scientific, Asheville, NC, US) and then stained with uranyl acetate and lead citrate (O'Brien and McCully 1981). The sections were observed and photographed with a JEOL 100c TEM (JEOL USA, Peabody, MA, USA).

Material examined

Cardiospermum grandiflorum

ARGENTINA. Province of Corrientes: Department Berón de Astrada, 10.i.2007, *Ferrucci et al. 2711*; Department Capital, 25.viii.1978, *Ferrucci 32*; Department San Cosme, 30.i.2006, *Ferrucci 2217*; Idem, 06.ii.2008, *Ferrucci et al. 2829*. Province of Formosa: Department Formosa, 1.ii.2007, *Ferrucci et al. 2745*. BOLIVIA. Department Santa Cruz, 4.iv.2006, *Ferrucci et al. 2524*.

Urvillea chacoensis

ARGENTINA. Province of Salta: Department San Martín, 8.iv.2004, *Meza Torres et al. 214*. BOLIVIA. Department Santa Cruz, 19.vii.2003, *Ferrucci et al. 1785*; Idem, 29.iii.2006, *Ferrucci et al. 2258*; Idem, 12.iv.2006, *Ferrucci et al. 2680*; Idem, 14.iv.2006, *Ferrucci et al. 2701*.

Results

Floral morphology

The inflorescences in *C. grandiflorum* and *U. chacoensis* are subracemiform thyrses with staminate and functionally pistillate hermaphrodite flowers. The most common floral pattern in the inflorescence shows this sequence; the first flowers are staminate, pistillate and subsequently staminate. Flowers are obliquely monosymmetric; the androecium has eight stamens of unequal lengths. In staminate flowers, the gynoecium is reduced to a rudimentary pistillode and the anthers show longitudinal dehiscence (Fig. 1*A*, *C*), whereas the pistillate flowers have eight short stamens with indehiscent anthers (Fig. 1*B*, *D*).

Development of the anther wall

At an early stage of development, the anthers of *C. grandiflorum* and *U. chacoensis* have four lobes. The archesporial cells are a relative homogeneous group of isodiametric cells with dense cytoplasm and conspicuous nuclei. These divide periclinally to generate primary sporogenous cells and primary parietal cells. The latter divides and forms two layers, one internal and one external. Periclinal and anticlinal divisions of the inner layer give

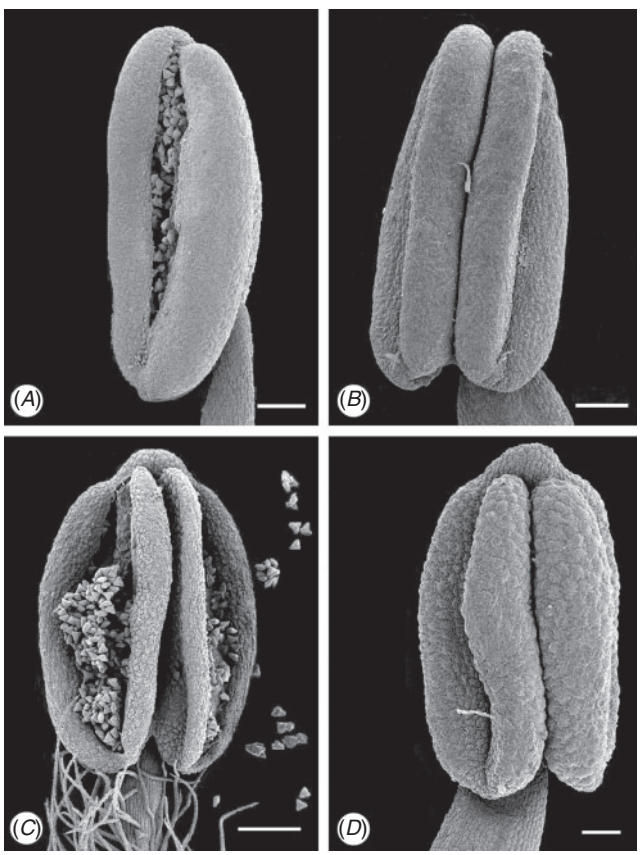


Fig. 1. Scanning electron micrographs of mature anthers. *Cardiospermum grandiflorum*. (*A*) Fertile anther of staminate flower, showing longitudinal dehiscence. (*B*) Sterile anther of hermaphrodite flower, functionally pistillate. *Urvillea chacoensis*. (*C*) Fertile anther of staminate flower. (*D*) Sterile anther of hermaphrodite flower, functionally pistillate. Scale bars = 250 µm (*A*–*C*), and 100 µm (*D*).

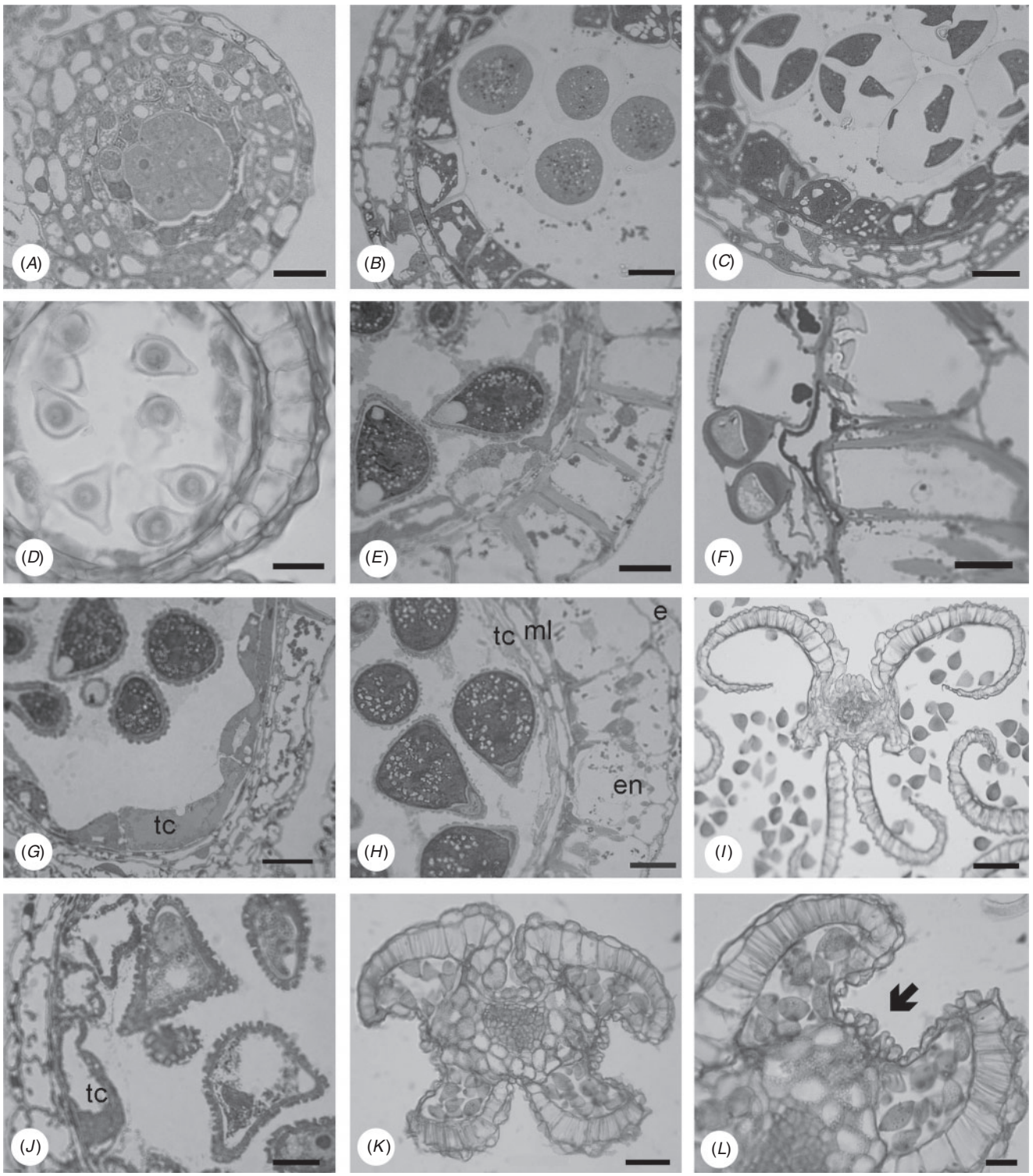


Fig. 2. Light micrographs of microsporangium tissues in different stages of development. *Urvillea chacoensis*. (*A*) Cross-section of the young anther, showing wall differentiation and sporogenous cells. *Cardiospermum grandiflorum*, features shared by both flower morphs. (*B*) The microspore mother cells enclosed in callose walls. (*C*) Microspore tetrads. (*D*) Young free microspores. (*E*) Mature anther wall, showing the endothecium cells with fibrous thickenings, tapetal cells and pollen grains. (*F*) Cross-section of mature anther wall, showing one stoma in the epidermis. *C. grandiflorum*, staminate flower. (*G*) Pollen grains and tapetal cells (tc). (*H*) Detail of mature anther-wall layers: epidermis (e), endothecium cells, showing fibrillar secondary wall thickenings (en), remnants of middle layer (ml) and tapetal cells (tc), and mature bicellular pollen grains. (*I*) Dehiscent anther, with fertile pollen grains. *U. chacoensis*, pistillate flower. (*J*) Late-microspore stage, with a well developed wall and collapsed cytoplasm. (*K*) Indehiscent anther, showing epidermal cells, endothecium with wall thickenings, tapetal cells and pollen grains with collapsed cytoplasm. (*L*) Detail showing non-functional stomium (arrow). Scale bars = 20 µm (*A*–*J*, *L*), and 50 µm (*K*).

rise to a middle layer and the tapetum, and divisions of the outer layer form the endothecium and the second middle layer. The wall development corresponds to basic type (Davis 1966).

The young anther is bithecal and tetrasporangiate. The four lobes are separated by connective tissue. The anther wall consists of a unistratose epidermis, with cells that have thin walls and striated cuticles, a thin-walled endothecium with conspicuous nuclei, two middle layers and a uninucleate secretory tapetum (Fig. 2*A*).

Microsporogenesis and microgametogenesis

Both species share developmental patterns in microsporogenesis and microgametogenesis. The following five stages were recognised during the process of development of the anther and pollen-grain formation:

Stage 1: microspore mother cell

The primary sporogenous tissue consists of a large mass of undifferentiated cells with thin walls and dense cytoplasm (Fig. 2*A*). Later, mitotic divisions give rise to microspore mother cells (MMC), in which cell walls become thicker because of the deposition of callose between the plasmalemma and the primary wall. The MMCs are large, spherical to polygonal in shape, with an unevenly thickened callose wall (Fig. 2*B*). Subsequently, they come apart by dissolution of the middle lamella and primary walls that keep the sporogenous tissue together.

The secretory tapetum cells present thin walls, dense cytoplasm with small vacuoles, mitochondria, rough endoplasmic reticulum, active dictyosomes with numerous vesicles, small plastids in division and prominent nuclei. Plasmodesmata were observed in the radial walls (Fig. 3*A*, *B*).

Stage 2: tetrad formation

The anther wall maintains its structure, except for the tapetum, whose cells are the first to enlarge; the cytoplasm shows high activity, and the vacuoles have displaced the nucleus to the periphery. The same ultrastructural characteristics are preserved from the earlier stage. Each microspore mother cell undergoes simultaneous reductive divisions and gives rise to microspore tetrads with tetrahedral arrangement (Fig. 2*C*). The callose wall of the young microspores is thick and completely uniform. Deposition of the primexine between the callose wall and the plasmalemma begins when the tetrad is formed. The ultrastructure of the microspore cytoplasm consists of abundant mitochondria, many ribosomes, dividing plastids and scattered elaioplasts. The nucleus shows a prominent concentration of chromatin and a conspicuous nucleolus (Fig. 3*C*).

Stage 3: microspores just after their release

The tapetal cells' cytoplasm shows abundant rough endoplasmic reticulum, prominent vacuoles, numerous mitochondria, ribosomes and dictyosomes (Fig. 3*D*). The callose walls of the tetrads have been dissolved and the microspores are free in the anther loculus. They exhibit spherical to triangular shapes, depending on the view (Fig. 2*D*). Prominent details of the ultrastructure are the dense cytoplasm with ribosomes, plastids and small vacuoles. At this point, the microspores are uninucleate and will remain so until the exine is formed.

Stage 4: microspore with well formed exine

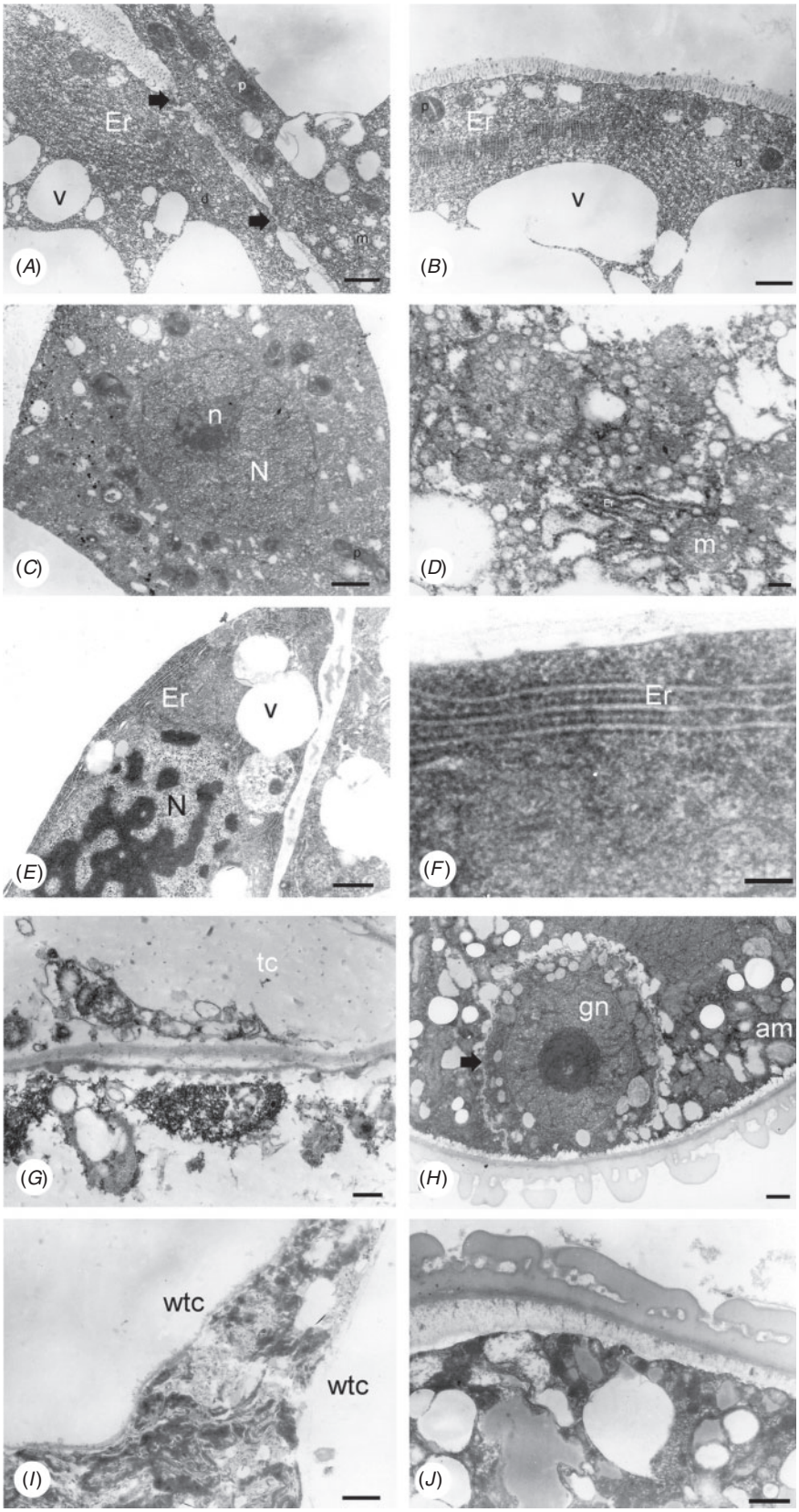
At this stage, the anther wall shows some distinctive changes. Epidermal cells become compressed, although non-functional stomata were still observed (Fig. 2*F*). In the endothecium, fibrous secondary thickenings develop from the inner tangential wall, where they form a base plate, and extend outwards and upwards, terminating near the outer tangential wall; such thickenings are more developed in staminate anthers than in the pistillate ones (Fig. 2*E*). The cells of the middle layers show signs of degradation. Tapetal cells retain their shape, and the cytoplasm has some mitochondria, dictyosomes and endoplasmic reticulum cisternae (Fig. 3*E*, *F*). The microspore cytoplasm presents a large central vacuole and the nucleus is displaced to a parietal position.

Stage 5

Pollen grains of staminate flower. In mature anthers, the epidermal cells have reduced in size and are somewhat flattened. Endothecium cells have enlarged, elongated radially and preserved their thickenings. The two middle layers have been degraded and tapetal cells elongate tangentially (Fig. 2*G*, *H*). The cytoplasm of the tapetal cells shows less activity, with remnants of endoplasmic reticulum, numerous vesicles, mitochondria and plastids (Fig. 3*G*). The walls of the tapetal cells are no longer present and their nuclei are obliterated when pollen grains reach the bicellular stage.

Just before the mature anther is ready to dehisce, the parenchymal septum that separates both locules of an anther theca breaks down, and consequently a single locule is formed (Fig. 2*I*). Before anther dehiscence, in the free microspore, the nucleus divides by mitosis, giving rise to a small generative cell and a large vegetative one, and the walls of the pollen grain complete the formation of the intine and the exine. The vegetative cell contains cytoplasm, with an extensive rough endoplasmic reticulum, small vesicles, numerous mitochondria and abundant amyloplastids. The generative cell has a conspicuous electron-translucent wall, a prominent nucleus and reduced cytoplasm with

Fig. 3. Transmission electron micrographs of tapetal cells and pollen grains in staminate and pistillate flowers of *Cardiospermum grandiflorum*. (*A*) Plasmodesmata (arrow) between tapetal cells. (*B*) Details of tapetal-cell cytoplasm, showing proplastids (p), vacuoles (v), proplastids (p), dictyosomes (d) and endoplasmic reticulum (Er). (*C*) A free microspore with a prominent nucleus (N) and nucleolus (n), and cytoplasm with proplastids (p). (*D*) Details of tapetal-cell cytoplasm in the free-microspore stage, showing plastids, mitochondria and Er. (*E*) Tapetal cells in the anther in the late-microspore stage. (*F*) Detail of tapetal-cell cytoplasm, showing abundant Er of rough type. (*G*) Remains of tapetal cells (tc) in anther dehiscence in a staminate flower. (*H*) Mature pollen-grain stage in a staminate flower, showing generative cell (arrow), nucleus generative (gn) and cytoplasm with amyloplastids (am). (*I*) Detail of tapetal cells of an indehiscent anther in a pistillate flower (arrows), tapetal cell wall. (*J*) Detail of a mature pollen grain of a pistillate flower, showing numerous lipid globules and a well developed wall. Scale bars = 1 μm (*A*, *B*, *E*, *G*–*J*), 500 nm (*C*, *F*), and 200 nm (*D*).


Er
v
p
g
m
(A)
Er
v
p
g
(B)
n
N
p
(C)
Er
m
(D)
Er
v
N
(E)
Er
(F)
tc
(G)
gn
am
(H)
wtc
wtc
(I)
(J)

some mitochondria and few plastids; this cell is enclosed by the vegetative cell (Fig. 3*H*).

Pollen grains are two-celled when shed. The mature pollen grain is hemitrisyncolporate, peroblate or oblate, and the exine is semitectate, reticulate and heterobrochate.

Pollen grains of pistillate flower. Development of the anther is similar to that described for staminate flowers. The transverse section of the mature anther shows small epidermal cells, and an endothecium with large rectangular cells and wall thickenings comparatively less developed than in the staminate flowers. These thickenings are absent in the endothecium cells along the stomium margins. Remnants of the two middle layers were observed and the parenchymal septum that separates both locules of a theca persists, and so remain tetraloculate anthers. Features common to both species are the presence of druses in the parenchymal septum and the non-functional stomium (Fig. 2*J–L*).

Tapetal cells maintain their wall. The cytoplasm has a reduced number of organelles, a small amount of endoplasmic reticulum and a collapsed membrane system. The walls of these cells persist even when the pollen grains are apparently mature (Fig. 3*I*). The pollen grain has completed the formation of the walls, intine and exine, although the cytoplasm is collapsed and shows abundant lipid inclusions (Fig. 3*J*). In some pollen grains, mitosis takes place and forms generative and vegetative cells, whereas in others, the nucleus does not divide, and so the pollen grains can be uni- or bicellular. The wall of the anther does not open; therefore, the anther is indehiscent.

Discussion

The species studied here, *C. grandiflorum* and *U. chacoensis*, have unisexual flowers, the staminate flowers with a reduced gynoecium and the pistillate flowers with indehiscent stamens. This type of floral structure is characteristic of the tribe and dominant in the family. Cao *et al*. (2008), in reference to this floral dimorphism in *Handeliodendron bodinieri*, used the term ‘pseudo-bisexual, functionally unisexual’. These authors considered that the character state ‘pseudo-bisexual flowers’ would be one of the two apomorphies of *Handeliodendron* and distinguished it from other species of Sapindaceae and Hippocastanaceae. However, to consider unisexual flowers as an apomorphy of Sapindaceae would not be correct, because this floral pattern is characteristic of the family. The sexual dimorphism in both dioecious and monoecious species is a feature that would ensure cross-pollination (Ha *et al*. 1988).

The microsporogenesis and microgametogenesis in *C. grandiflorum* and *U. chacoensis* did not present different characteristics, which would indicate the high affinity between both species. However, differences in the pollen development between the species studied in the present work and other species of Sapindaceae were found (Table 1).

In *C. grandiflorum* and *U. chacoensis*, the endothecium develop fibrillar thickenings after uninucleate pollen grains are formed, which is consistent with what was observed by Nair and Joseph (1960) and Ha *et al*. (1988). The endothecial cells are observed to be radially elongated when the pollen grains are mature; this feature coincides with that reported by Mathur and Gulati (1981, 1989). In pistillate flowers, fibrous thickenings of the endothecial cells are attenuated and they are not present in endothecial cells along the stomium margin, whereas in the staminate flowers they are more marked. The species analysed here correspond to the base plate type according to the different patterns of endothecium thickening recognised by Manning (1996). This author recorded two types of thickenings for Sapindaceae, namely U-shaped with basal anastomosis, and the base plate. Reddy and Reddi (1974) observed a positive correlation between pollen sterility and endothecium thickness at maturity; therefore, the resulting anthers are indehiscent and prevent pollen release.

Whereas remnants of middle layers remained to the end of the microgametogenesis in the pistillate flowers, both layers were completely destroyed at the time of microspore differentiation in the staminate flowers. This feature was also observed by Mathur and Gulati (1989).

The tapetum cells play the most important physiological role in pollen development (Pacini and Franchi 1993). In accordance with the classification proposed by Pacini (1997), the tapetum observed here belongs to the parietal type, the most common

Table 1. Summary of differential characters of microsporogenesis and microgametogenesis in some species of Sapindaceae

Species	Anther wall type	Epidermis of the anther	Tapetal cells	Tetrads	Pollen grain	References
Allophylus alnifolius	Dicotyledonous	Papillose	2-nucleate	Linear	2- or 3-cellular	Mathur and Gulati (1980)
A. zeylanicus	Dicotyledonous	Compressed	3-nucleate	Tetrahedral	3-cellular	Mathur and Gulati (1989)
Cardiospermum grandiflorum	Basic	Compressed	1-nucleate	Tetrahedral	2-cellular	Present paper
C. halicacabum	Basic	Compressed	1-nucleate	Terahedral	2-cellular	Nair and Joseph (1960)
Handeliodendron bodinieri	Basic	Compressed	1-nucleate	Tetrahedral	2-cellular	Cao *et al*. (2008)
Lepidopetalum jackianum	Dicotyledonous	Compressed	Multinucleate	Tetrahedral	2-cellular	Mathur and Gulati (1981)
Nephelium lappaceum	Basic	Compressed	1- or 2-nucleate	Terahedral	2-nucleate	Ha *et al*. (1988)
Pometia pinnata	Basic	Compressed	1- or 2-nucleate	Terahedral	2-cellular	Ha *et al*. (1988)
Urvillea chacoensis	Basic	Compressed	1-nucleate	Tetrahedral	2-cellular	Present paper
Xerospermum intermedium	Basic	Compressed	1- or 2-nucleate or 1-multinucleate in pistillate flower	Terahedral	2-cellular	Ha *et al*. (1988)

character state of eudicots and monocots. In staminate flowers of *C. grandiflorum* and *U. chacoensis*, the tapetum disintegrated before dehiscence occurred.

In pistillate flowers, tapetal cells show a clear difference in metabolic activity, the disruption of cytoplasmic organelles, and an early onset of vacuolation; these are initial differences compared with the fertile anthers. This abnormal activity of tapetal cells, combined with the presence of large vacuoles, would be related to the collapsed pollen grains. Previously, pollen sterility has been linked to tapetal-cell hypertrophy (Zenkteler 1962; Cruz *et al*. 1976). Persistence or delayed degeneration of the tapetum could occur as a result of changes in physical and physiological factors (Bhandari 1984). Laser and Lersten (1972) pioneered the use of TEM to identify early signs of degeneration of cell organelles. A review of the literature showed a greater variation in the patterns of anther development associated with male sterility than with fertile anthers (Cruz *et al*. 1976; Ilarslan *et al*. 1999; Cuevas García 2009).

The large number of mitochondria, endoplasmic reticulum and dictyosomes in the cytoplasm of the microspore mother cells suggest a high metabolic activity. Because of the activity of the dictyosomes, numerous vesicles in the cytoplasmic periphery are present, indicating the possible role of dictyosomes in the production of callose (Heslop-Harrison 1966). Microspore mother cells undergo simultaneous meiosis, forming tetrads with a tetrahedral arrangement; this finding is consistent with the report of *Filicium decipiens* (Gulati and Mathur 1977) and *Lepidopetalum jackianum* (Mathur and Gulati 1981). In other species of Sapindaceae, the tetrads can be tetrahedral or, less frequently, tetragonal, as occurs in *Cardiospermum halicacabum* (Nair and Joseph 1960) and *Xerospermum intermedium* (Ha *et al*. 1988). In *Allophylus alnifolius*, occasionally one or two of the microspore mother cells fail to separate and a linear octant is formed (Mathur and Gulati 1980). Nair and Joseph (1960) described degeneration of pollen grains occurring at the tetrad stage in pistillate flowers of *C. halicacabum*. The eudicots have simultaneous microsporogenesis, a state that is considered plesiomorphic in angiosperms (Furness *et al*. 2002). Although microsporogenesis is normally consistent within families, some families such as Aristolochiaceae (González *et al*. 2001) and Magnoliaceae (Wang *et al*. 2005) are especially variable in tetrad configuration.

In the staminate flower at the time of dehiscence, the septal cells separating each half of the anther into two locules were stretched and degenerating. Later, those cells were completely dissociated, producing a bilocular anther. The small cells in the stomium region apparently provide a weak area in the anther wall along which dehiscence occurs. The anther opens along a slit in both thecae and the dehiscence is longitudinal. Anthers do not dehisce in pistillate flowers, and the parenchymatous tissue between the adjacent pollen sacs reveals a well organised appearance without degenerated cells, maintaining their tetralocular form, a common feature among all embryological studies conducted in Sapindaceae. In the latter flower type, pollen appears to be normal, but these grains exhibit a protoplast retracted or with signs of degradation. These pollen grains do not germinate *in vitro* or *in vivo* (M. S. Ferrucci, unpubl. data). Our findings are consistent with those of Ha *et al*. (1988), but contradict data cited by Banerji and Chaudhury (1944) who recognised the formation of pollen tubes from both flower types. Bawa (1977), in a study of reproductive biology of *Cupania guatemalensis* Radlk., noted that, in the pistillate flower, pollen is not available for pollinators because anthers do not dehisce.

A distinctive feature in Sapindales is the presence of a nectar disk where nectar is hidden by the stamens (Endress 1994; Judd *et al*. 1999). In *C. grandiflorum* and *U. chacoensis*, the morpho-anatomical characteristics of the nectary are similar in staminate and pistillate flowers (Solís and Ferrucci 2009). These plants are compatible to both geitonogamous and xenogamous pollen; however, temporal dioecism enforces cross-pollination; flowers are visited for nectar and pollen, and the massive display of staminate flowers serves to attract pollinators.

In conclusion, the most pronounced cytological events accompanying male sterility in the pistillate flower are those concerning the tapetum. They involve disturbances at the time of programmed tapetum death, such as conservation of the cell wall, abnormal vacuolisation and reduction in the number of organelles and endoplasmic reticulum membranes.

Acknowledgements

We thank Dr M. Gotelli for reviewing the English. Financial support for our research was provided by the Consejo Nacional de Investigaciones Científicas y Técnicas (PIP N° 112-200801-02248), by the Universidad Nacional del Nordeste (PI A005-2009) and by the Agencia Nacional de Promoción Científica, Tecnológica y de Innovación (ANPCyT-UNNE, PICTO 00096).

References

Banerji I, Chaudhury KL (1944) A contribution to the life history of *Litchi chinensis* Sonn. *Proceedings of the Indian Academy of Sciences, Section B* **19**, 19–27.

Bawa KS (1977) The reproductive biology of *Cupania guatemalensis* Radlk. (Sapindaceae). *Evolution* **31**, 52–63. doi:10.2307/2407544

Bhandari NN (1984) The microsporangium. In 'Embryoloy of angiosperms'. (Ed. BM Johri) pp. 53–122. (Springer-Verlag: Berlin)

Buerki S, Forest F, Acevedo-Rodríguez P, Callmander MW, Nylander JAA, Harrington M, Sanmartín I, Küpfer P, Alvarez N (2009) Plastid and nuclear DNA markers reveal intricate relationships at subfamilial and tribal levels in the soapberry family (Sapindaceae). *Molecular Phylogenetics and Evolution* **51**, 238–258. doi:10.1016/j.ympev.2009.01.012

Cao LM, Xia NH, Deng YF (2008) Embryology of *Handeliodendron bodinieri* (Sapindaceae) and its systematic value: development of male and female gametophytes. *Plant Systematics and Evolution* **274**, 17–23. doi:10.1007/s00606-008-0024-0

Cruz ND, Ungaro MRG, Medina DM (1976) Observações citológicas em três espécies de *Erythrina* L. *Bragantia* **35**, 133–139. doi:10.1590/S0006-87051976000200030

Cuevas García E (2009) La esterilidad masculina en plantas, una perspective embriológica. *Revista Biológicas* **11**, 79–86.

Davis GL (1966) 'Systematic embryology of the angiosperms.' (Wiley: New York)

Endress PK (1994) 'Diversity and evolutionary biology of tropical flowers.' (University Press: Cambridge, UK)

Ferrucci MS (2000*a*) Revisión de los géneros *Cardiospermum* y *Urvillea* para el neotrópico (Sapindaceae). Doctoral Thesis, Universidad Nacional de Córdoba, Argentina.

Ferrucci MS (2000*b*) Cytotaxonomy of Sapindaceae with special reference to the tribe Paullinieae. *Genetics and Molecular Biology* **23**, 941–946. doi:10.1590/S1415-47572000000400039

Ferrucci MS (2006) A new species of *Urvillea* (Sapindaceae) from northwestern Venezuela. *Brittonia* **58**, 83–87. doi:10.1663/0007-196X(2006)58[83:ANSOUS]2.0.CO;2

Ferrucci MS, Acevedo-Rodríguez P (1998) *Cardiospermum cuchujaquense* (Sapindaceae), a new species from Sonora, Mexico. *Novon* **8**, 235–238. doi:10.2307/3392008

Ferrucci MS, Anzótegui LM (1993) El polen de Paullinieae (Sapindaceae). *Bonplandia* **6**, 211–243.

Furness CA, Rudall PJ, Sampson FB (2002) Evolution of microsporogenesis in angiosperms. *International Journal of Plant Sciences* **163**, 235–260. doi:10.1086/338322

González AM, Cristóbal CL (1997) Anatomía y ontogenia de semillas de *Helicteres Lhotzkyana* (Sterculiaceae). *Bonplandia* **9**, 287–294.

González F, Rudall PJ, Furness CL (2001) Microsporogenesis and systematics of Aristolochiaceae. *Botanical Journal of the Linnean Society* **137**, 221–242. doi:10.1111/j.1095-8339.2001.tb01119.x

Gulati N, Mathur S (1977) Embriology and taxonomy of *Filicium decipiens. Phytomorphology* **27**, 261–266.

Ha CO, Sands VE, Soepadmo E, Jong K (1988) Reproductive patterns of selected understorey trees in the Malaysian rain forest: the sexual species. *Botanical Journal of the Linnean Society* **97**, 295–316. doi:10.1111/j.1095-8339.1988.tb01585.x

Heslop-Harrison J (1966) Cytoplasmic connections during spore formation in flowering plants. *Endeavour* **25**, 65–72. doi:10.1016/0160-9327(66)90071-8

Ilarslan H, Horner HT, Palmer RG (1999) Genetics and cytology of a new male-sterile, female-fertile soybean mutant. *Crop Science* **39**, 58–63. doi:10.2135/cropsci1999.0011183X003900010009x

Johansen DA (1940) 'Plant microtechnique.' (McGraw-Hill Book Company, Inc.: New York)

Judd WS, Campbell CS, Kellog EE, Stevens PF (1999) 'Plant Systematics, a phylogenetic approach.' (Sinauer Associates: Sunderland, MA)

Kadry A (1946) Embriology of *Cardiospermum halicacabum* L. *Svensk Botanisk Tidskrift Utgifven af Svenska Botaniska Föreningen* **40**, 111–126.

Laser KD, Lersten NR (1972) Anatomy and cytology of microsporogenesis in cytoplasmic male sterile angiosperms. *Botanical Review* **38**, 425–454. doi:10.1007/BF02860010

Luque R, Sousa HC, Graus JE (1996) Métodos de coloração de Roeser (1972) – modificado – E. Kropp (1972), visando a substituição do azul de astra por azul de alcião 8GS ou 8GX. *Acta Botanica Brasilica* **10**, 199–212.

Manning JC (1996) Diversity of endothecial patterns in the angiosperms. In 'The anther, form, function and phylogeny'. (Eds WG D'Arcy, RC Keating) pp. 136–158. (Cambridge University Press: Cambridge, UK)

Mathur S (1989) Review on embryology of Sapindaceae. *Indian Journal of Botany* **12**, 35–38.

Mathur S, Gulati N (1980) Embryology and taxonomy of *Allophylus alnifolius* Radlk. ex Engl. (Sapindaceae). *Indian Journal of Botany* **3**, 103–112.

Mathur S, Gulati N (1981) Embryology of *Lepidopetalum jackianum* Hiern. *Indian Journal of Botany* **4**, 216–221.

Mathur S, Gulati N (1989) Embryological studies in *Allophylus zeylanicus* L. *Indian Journal of Botany* **12**, 62–65.

Muller J, Leenhouts PW (1976) A general survey of pollen types in Sapindaceae in relation to taxonomy. In 'The evolutionary significance of the exine'. Linnean Society symposium 1. (Eds IK Ferguson, J Muller) pp. 407–445. (Academic Press: London)

Nair NC, Joseph J (1960) Morphology and embryology of *Cardiospermum halicacabum. Journal of Indian Botanical Society* **39**, 176–194.

O'Brien TP, McCully ME (1981) 'The study of plant structure. Principles and selected methods.' (Termarcarphi Pty Ltd: Melbourne)

Pacini E (1997) Tapetum character states: analytical keys for tapetum types and activities. *Canadian Journal of Botany* **75**, 1448–1459. doi:10.1139/b97-859

Pacini E, Franchi G (1993) Role of the tapetum in pollen and spore dispersal. Plant systematics and evolution. In 'The tapetum, cytology, biochemistry and evolution'. (Eds M Hesse, E Pacini, M Wilense) pp. 1–11. (Springer Verlag: New York)

Radlkofer L (1934) Sapindaceae. In 'Das Pflanzenreich. Vol. 98'. (Ed. A Engler) pp. 1–1539. (Wilhelm Engelmann: Leipzig)

Reddy GB, Reddi MV (1974) Cytohistological studies on certain male sterile lines of pearl millet (*Pennisetum typhoides* S. & h.). *Cytologia* **39**, 585–589.

Solís SM, Ferrucci MS (2009) The floral nectary of *Cardiospermum grandiflorum* and *Urvillea chacoënsis* (Sapindaceae): morpho-anatomy and ontogeny. *Annales Botanici Fennici* **46**, 485–495.

Urdampilleta JD, Ferrucci MS, Torezan JMD, Vanzela ALL (2006) Karyotype relationships among four South American species of *Urvillea* (Sapindaceae: Paullinieae). *Plant Systematics and Evolution* **258**, 85–95. doi:10.1007/s00606-005-0393-6

Wang LL, Hu JQ, Pang JL, Xiang TH (2005) Studies on the megasporogénesis and microsporogenensis and the development of their female and male gametophyte in *Magnolia biloba. Shi Yan Sheng Wu Xue Bao* **38**, 490–500.

Zenkteler M (1962) Microsporogenesis and tapetal development in normal and male sterile carrot (*Daucus carota*). *American Journal of Botany* **49**, 341–348. doi:10.2307/2439072

Manuscript received 24 June 2010, accepted 12 August 2010