EMBRYOLOGY AND SEED DEVELOPMENT IN *PERESKIA LYCHNIDIFLORA* (CACTACEAE)

KARINA JIMÉNEZ-DURÁN
Facultad de Química,
Universidad Nacional Autónoma de México,
Ciudad Universitaria, 04510 México, D. F. México.
email: jikary@unam.mx

SALVADOR ARIAS-MONTES
Jardín Botánico, Instituto de Biología,
Universidad Nacional Autónoma de México, Apartado Postal 70-614,
Ciudad Universitaria, 04510 México, D. F. México.
email: sarias@ibunam2.ibiología.unam.mx

AUREA CORTÉS-PALOMEC
Centro de Investigaciones en Ecosistemas,
Universidad Nacional Autónoma de México,
Antigua Carretera a Pátzcuaro # 8701 Col. Ex-hacienda de San José de la Huerta.
58190 Morelia, Michoacán, México.
email: ac399295@oak.cats.ohiou.edu

JUDITH MÁRQUEZ-GUZMÁN[1]
Facultad de Ciencias,
Universidad Nacional Autónoma de México, Ciudad Universitaria,
04510 México, D. F. México.
email: judith.marquez@ciencias.unam.mx

Abstract: In order to increase our embryological knowledge of *Pereskia*, a genus considered the earliest member of Cactaceae, we followed the development of the flowers and seeds of *Pereskia lychnidiflora*, a species from southeastern Mexico. We compared our data with that available from other closely related members in the family. The embryological characterization showed that basic features are homogeneous across the family and supported the placement of *P. lychnidiflora* as a member of one of two *Pereskia* clades, the one sister to remaining cacti. Our data also supports the hypothesis that within the family Cactaceae, the evolutionary tendency is towards a reduction of the perisperm. A multilayered seed coat was observed in *P. lychnidiflora.* A novel finding was the presence of stomata along the basal and central part of the ovary, a character that has never been reported before in other members of *Pereskia.*

Keywords: Cactaceae; megagametogenesis; megasporogenesis; microgametogenesis; microsporogenesis; *Pereskia*.

INTRODUCTION

The Cactaceae is a very diverse family, renowned for its large number of species (>1800), the different environments they inhabit, and the variations in the morphological and anatomical features associated with evolution of their floral diversity (Anderson 2001). Flowers range between 0.4 and 45 cm in length, are generally solitary, and mostly bisexual and actinomorphic, although there are some well documented exceptions, such as the presence of paniculate or corymbose inflorescences in *Pereskia aculeata* Mill., zygomorphic flowers in *Schlumbergera* spp. and unisexual flowers in *Opuntia stenopetala* Engelm. (dioecious), and *Pachycereus pringlei* (S. Watson) Britton & Rose (trioecious) (Leuenberger 1986; Anderson 2001; Hunt 2006).

An important characteristic of cactus flowers is their basal portion, which is inserted within a modified branch, or pericarpel, that covers the ovary and may extend upward to form the receptacular tube (Buxbaum 1953; Anderson 2001). The pericarpel is a trait that unites all the four recognized subfamilies within Cactaceae, the monogeneric Pereskioideae and Maihuenioideae, and the more genus-rich Cactoideae and Opuntioideae.

[1] Corresponding author

Among the diverse genera of cacti, study of the genus *Pereskia* Mill. has generated considerable interest for understanding some of the evolutionary trends in the family since it is in *Pereskia* where some of the most ancestral states are found, especially with flower development. Some of these attributes include the nature of the pericarpel, the position of the gynoecium, and the parietal versus axial placentation (Boke 1963, 1966, 1968; Leuenberger 1986). *Pereskia* is distributed mainly in the neotropics, and includes approximately 18 species native to the West Indies, Central and South America. Species of *Pereskia* can be described as trees, shrubs, or climbing plants (vines), characterized by having alternate, wide, flattened, deciduous leaves. The flowers emerge from the terminal or axillary regions of the branches, and may appear alone or in clusters opening during the daytime (Leuenberger 1986; Hunt 2006). Recent phylogenetic analyses using molecular data, have revealed that *Pereskia* forms a paraphyletic assemblage of two groups of species at the base of the cactus lineage (Butterworth and Wallace 2005; Edwards et al. 2005).

Evolutionary interest in *Pereskia* within the Cactaceae, as well as its relationship to other families of the Caryophyllales, mainly the Portulacaceae, has centered on anatomy and morphology (Buxbaum 1953; Bailey 1963; Leuenberger 1986; Mauseth and Landrum 1997), and more recently on the establishment of phylogenetic hypotheses (Wallace and Gibson 2002; Butterworth and Wallace 2005; Edwards et al. 2005) and physiological processes (Edwards 2006; Edwards and Donoghue 2006), highlighting the relevance of development of structural characters in the evolution of this group. Notwithstanding, embryological information is still scarce and incomplete for the genus.

Boke (1963, 1966, 1968) showed that *P. lychnidiflora, P. aculeata* and *P. diaz-romeroana* have perigynous flowers and superior ovaries. The placentation in *P. lychnidiflora* and *P. diaz-romeroana* is axillary and in *P. aculeata* is basal.

In order to improve our understanding of character changes early in the evolution of the Cactaceae, we present an embryological assessment of flower and seed development in *P. lychnidiflora,* a species native to the dry-warm regions of Mexico and Central America, using light and scanning electron microscopy (SEM). Additionally, we compare our data on megasporogenesis, megagametogenesis and embryogenesis with other existing studies for *Pereskia* and other members of the family.

MATERIALS AND METHODS

Pereskia lychnidiflora is a large shrub to small tree that reaches up to 10 m in height. It bears deciduous, slightly succulent leaves up to 7 cm long, and produces solitary showy orange flowers up to 6 cm broad. It is a common element in the tropical deciduous forest from southern Mexico to Costa Rica, and within Mexico it is found in the states of Guerrero and Oaxaca (Leuenberger 1986). The sampled population is located in the Isthmus of Tehuantepec, near the Zapotec ruins of Guiengola, 10 km northwest of Santo Domingo Tehuantepec (16°22'12.4" N; 95° 19'09.3" W, elevation: 79 m), Oaxaca, Mexico.

During four different visits to the site, flower buds in different developmental stages, flowers in anthesis and post-anthesis, and fruits were collected and fixed in FAA fixative (formaldehyde, acetic acid, 96% ethanol, and water; 1:0.5:5:3.5). Material was later dehydrated through a graded ethanol series and embedded in paraplast (McCormick Scientific) and LR-White (SPI Supplies). Samples embedded in paraplast were cut using a rotary microtome at 5-10 µm, and stained with safranin-fast green. Naphthol blue black was used to identify proteins. Samples embedded in LR-white were cut using an ultramicrotome (RMC-MT990) at 1-2 µm and stained with toluidine blue. Due to the hardness that characterize the seeds of Cactaceae, and in order to obtain good sections of the material, a subset of the seeds were submerged in a 6% solution of sodium hypochlorite (NaClO) for up to 3 days, while the remaining seeds had their seed coat removed before being embedded in paraplast. We analyzed a total of 200 seeds.

Tissue was additionally processed for observation under SEM. Those samples—mainly styles, ovaries, and pollen grains—were dehydrated through a graded ethanol series, critically point dried in CO_2, and mounted on aluminum stubs. Mature seeds and pollen grains were placed directly on aluminum stubs. All samples were coated with gold and observed on a JEOL 5310 LV SEM.

To describe the development of the seed coat, we chose a particular region of the seed for close observation. Two imaginary lines were traced, one crossing the micropyle perpendicularly and a second line dividing the seed at its widest point; the intersection zone of these two imaginary lines was the region used to describe the seed coat development.

RESULTS

Microsporogenesis, microgametogenesis and anther wall development. Anther development in *P. lychnidiflora* is centrifugal. During the primordial stage, anthers are comprised of archesporial cells surrounded by one layer of protodermic cells. In a later stage, the subepidermal cells divide periclinally giving rise to the primary parietal layer and sporogenous tissue. The primary parietal layer divides, forming an external secondary parietal layer that then will become the endothecium and an inner secondary parietal layer that after periclinal divisions will form the middle layer and the tapetum (Fig. 1A). The development of the anther wall follows a monocotyledonean type. The mature anther wall is composed of an epidermis, endothecium, middle layer, and tapetum, all of which have only one layer of cells each (Fig. 1B). Anthers have four microsporangia arranged in pairs in two lobes, and druses are common in the connective tissue (Fig. 1B). Simultaneously with anther wall development, the cells of the sporogenous tissue divide mitotically increasing

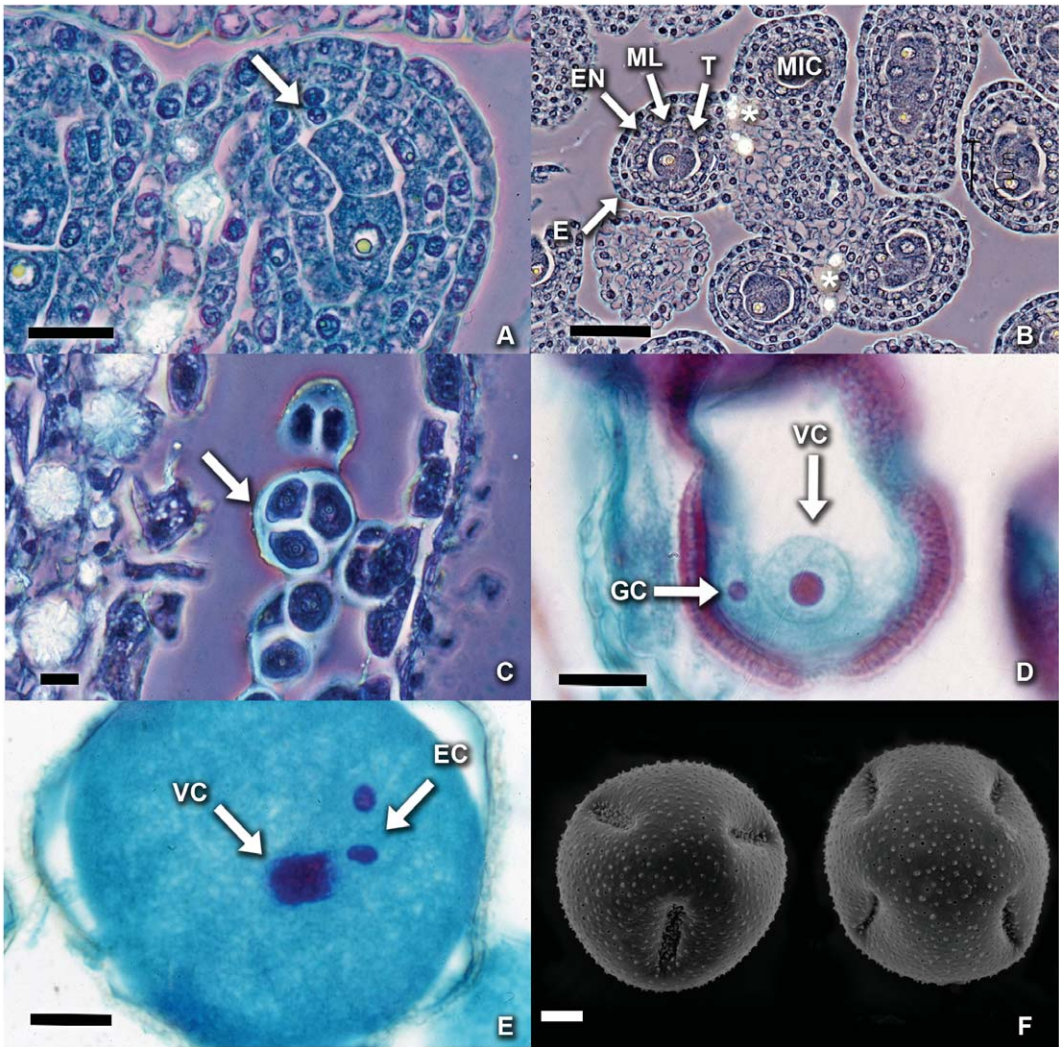

Figure 1. Microsporogenesis and microgametogenesis in *Pereskia lychnidiflora*. Transversal sections of anther development. **A** Periclinal mitotic division of the inner secondary parietal layer (arrow) gives rise to the cells of the middle layer and the tapetum. *Scale bar* 10 µm. **B** Anther with four microsporangia. Druses are common in the connective tissue (*). Mature anther wall is formed by an epidermis (E), endothecium (EN), middle layer (ML), and tapetum (T), microspore mother cells (MIC) are surrounded by these layers. *Scale bar* 50 µm. **C** Microspore tetrads surrounded by callose (arrow). *Scale bar* 10 µm. **D** Bi-nucleated pollen grain with a vegetative cell (VC) and a generative cell (GC). *Scale bar* 10 µm. **E** Tricellular pollen grain with one vegetative cell (VC) and two spermatic cells (EC). *Scale bar* 10 µm. **F** Tricolpate and tetracolpate pollen grains with perforations and spinules. *Scale bar* 10 µm.

their size; the nucleus of the cells become more conspicuous and develops into microspore mother cells (Fig. 1B). At the same time, the cells of the tapetum undergo karyokinesis without cytokinesis, thus producing bi-nucleated cells. Microspore mother cells, which at this point are surrounded by callose, undergo meiosis forming a tetrad of microspores (Fig. 1C). Once the callose degrades, the microspores are released becoming unicellular pollen grains (Fig. 1C). It is during this time that the cells of the tapetum degenerate, forming the secretory type of tapetum.

The unicellular pollen grain divides mitotically giving rise to a vegetative and a generative cell (Fig. 1D). The generative cell further divides giving rise to two spermatic cells (Figure 1E). The wall of the mature anther has a layer of epidermal cells that collapse losing their shape, an endothecium layer whose anticlinal walls have thickened a middle layer and a tapetum layer whose cells degenerate. The septa that separate the microsporangia disintegrates just before the tricellular pollen grains are released via longitudinal dehiscence.

Micromorphology of the pollen grain. Pollen grains are tricolpate, tetracolpate, and pentacolpate, tectate, and with perforations and spinules that cover all the surface (Fig. 1F).

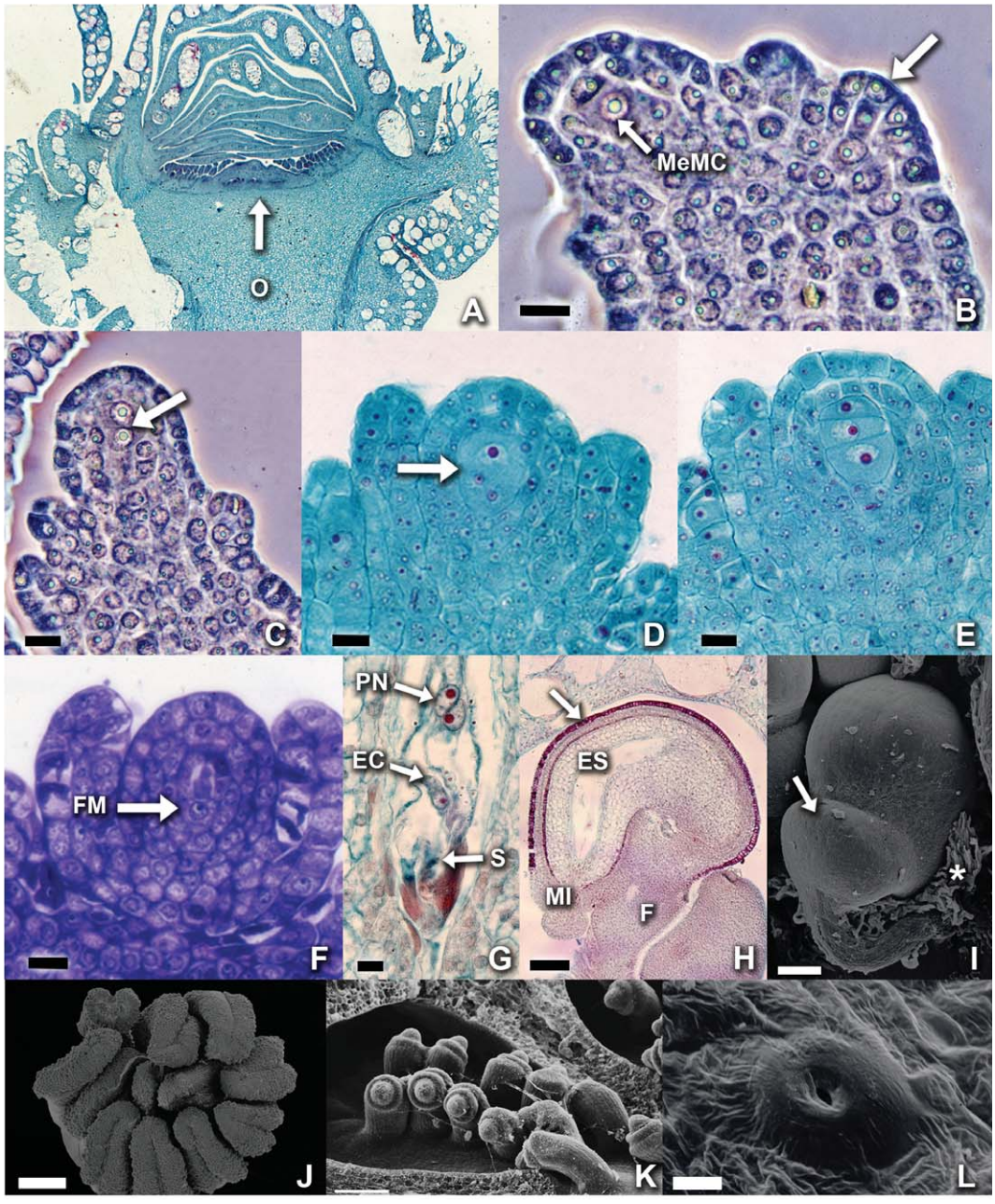

Figure 2. Longitudinal sections of flower bud showing stages of megasporogenesis and megagametogenesis in *Pereskia lychnidiflora*. **A** Flower bud showing the early stages of ovary development (O). *Scale bar* 10 µm. **B** Flower bud where the ovule primordia in which a megaspore mother cell (MeMC) can be seen. The dermal origin of the teguments can be seen (arrow). *Scale bar* 10 µm. **C** First division of the microspore mother cell produces a dyad (arrow). *Scale bar* 10 µm. **D** The chalazal cell of the dyad undergoes the second phase of meiosis (arrow). *Scale bar* 10 µm. **E** Megaspore triad. *Scale bar* 10 µm. **F** Functional megaspore in the chalazal end (FM). *Scale bar* 10 µm. **G** Embryo sac with two synergids (S), egg cell nucleus (EC), and two polar nuclei in the central cell (PN). *Scale bar* 10 µm. **H** Campilotropous ovule during pre-anthesis with a funicule (F), and embryo sac (ES), endostome micropyle (MI) with tannins in the external epidermis of the external integument (arrow). *Scale bar* 100 µm. **I–L** SEM photographs. **I** Ovule in pre-anthesis showing a funicular protuberance (arrow) and an obturator (*). *Scale bar* 100 µm. **J** Apical view of the stigmatic lobules. *Scale bar* 500 µm. **K** Basal-laminal placentation. *Scale bar* 100 µm. **L** Stomatal opening and two guard-cells along the base and central part of the ovary. *Scale bar* 5 µm.

Megasporogenesis and megagametogenesis. Gynoecium development starts when a group of meristematic cells grow conically, giving rise to the primordium of ovary and styles (Fig. 2A). Within the ovary, several nucellar primordial cells develop surrounded by a layer of protodermal cells. Within each nucellus, a hypodermal cell enlarges developing a conspicuous nucleus, thus becoming the archesporium. This archesporial cell later divides periclinally to give rise to a parietal cell and the sporogenous cell. The ovule develops two integuments of dermic origin. The ovule is crassinucellate (Fig. 2B). It is during this stage that the ovule primordia start to curve, and the sporogenous cell increases in size, becoming the megaspore mother cell. The megaspore mother cell is separated from the nucellus by a single layer of parietal cells (Fig. 2B). During this stage two cavities develop, one between the internal and external integuments and a second one between the internal integument and the nucellus close to the chalazal end. It is also during this stage that the funiculus starts forming. Once the megaspore mother cell undergoes meiosis I, it is possible to observe the presence of a dyad (Fig. 2C). From those two cells, only the one in the chalazal end follows to the second phase of meiosis (Fig. 2D) (the other degenerates as a 2C polar body), thus we only see the presence of three megaspores (Fig. 2E). Only the megaspore close to the chalazal end is functional, the other two degenerate, therefore *P. lychnidiflora* presents a monosporic embryo sac (Fig. 2F). The functional megaspore divides mitotically, giving rise to an egg apparatus, three antipodal cells and a binucleate central cell, a typical *Polygonum* embryo sac (Fig. 2G). The embryo sac is rich in starch deposits. The mature ovule is campylotropous, crassinucellated, bitegmic, funiculate, and endostomic (Fig. 2H) and shows a funicular protuberance (Fig. 2I). The vascular bundle runs from the funiculus to the chalazal zone where druses are present. The epidermal cells in the ventral part of the funiculus are differentiated into papillae that grow towards the micropyle forming an obturator (Fig. 2I).

Gynoecium. The gynoecium is formed by 10-18 connate carpels, with solid styles. During development, the carpels curve toward the center of the flower and fuse at the base to form the roof of the ovary. Commonly, the styles are branched into lobules covered by papillae (Fig. 2J). According to its position in the pre-anthesis stage, the ovary is inferior and remains at the bottom of the point of stamen insertion (Weberling 1989). A laminal-basal placentation occurs accordingly, with ovules arranged in the ovary (Fig. 2K). Stomata are present, represented by short apertures on the epidermis along the basal and central part of the ovary, composed of a couple of high, kidney-shaped guard cells (Fig. 2L).

Seed. Embryo development and storage tissue. During the globular stage, the embryo is surrounded by endosperm whose nuclei are arranged in a rosary-like disposition (Fig. 3A). The nucellar cells surrounding the embryo sac become densely packed with storage substances developing into perisperm. By the time the embryo reaches the heart-shaped stage, the endosperm starts developing cell walls in the micropylar end. When the embryo reaches the torpedo stage, the perisperm cells located in the inner curvature of the embryo contain great quantities of starch granules (Figs. 3B, C). The embryo fills most of the volume of the seeds. The cotyledons are larger than the hypocotyl, and their main storage substance are proteins. Druses are found throughout the embryo. The perisperm is persistent in mature seeds, even after the reserves from the endosperm have been depleted by the growing embryo (Figs. 3D, E). Polyembryony is uncommon. From all the seeds analyzed, only one embryo was observed in the torpedo state that coexisted with an embryo in the early torpedo state (Fig. 3F). However, in mature, seeds we never saw more than one embryo per seed.

Seed micromorphology. The outer surface of the seed coat is smooth and the cells of the exotest have flat and hexagonal periclinal walls. The region of the micropyle is found within the hilar cup. The hilum is comprised of sclereids, and, on its inner part, it is possible to see remains of the vascular bundle that runs through the funiculus (Figs. 3G, H).

Seed coat development. During pre-anthesis (Fig. 4A) and anthesis (Fig. 4B), the ovule is surrounded by an outer bi-layer integument and an inner tri-layer integument. After fertilization, and during the development of the seed, the seed coat starts to develop from the integuments. The outer integument gives rise to the testa and the inner integument forms the tegmen. Periclinal divisions of the testa cells lead to formation of an exotesta, mesotesta and endotesta, forming a multiplicative testa. The cells of the exotesta have vacuoles full of tannin deposits, and exotesta cell walls thicken in their external periclinal side. The mesotesta and endotesta are formed by only one or two strata (Figs. 4E–G). The tegmen remains tri-layered, with an endotegmen that shows high concentrations of tannins (Figs. 4C–E), and a mesotegmen and exotegmen that degenerate during a later stage (Fig. 4E). Over time, the only remaining layer of the tegmens, the endotegmen, degenerates, and only its remnants can be seen (Figs. 4F, G). Therefore, on mature seeds, the seed coat is formed by a one-layered exotesta rich in tannin deposits, a mesotesta and endotesta are comprised of only 1 or 2 strata and a degenerated tegmen (Fig. 4H).

DISCUSSION

In *P. lychnidiflora*, anther wall development follows a monocotyledonean type, the same type described in *Opuntia tomentosa* (Flores 2002), *O. stenopetala* (Orozco 2002), *O. robusta* (Silva 2007), *Pachycereus militaris* (Núñez 2004), *Mammillaria haageana* (Parada 2004), and *M. dioica* (Sánchez 2007). Therefore it seems that this type of anther wall development is an unmodified character across the different subfamilies of Cactaceae (e.g. Cactoideae, Opuntioideae, and Pereskioideae).

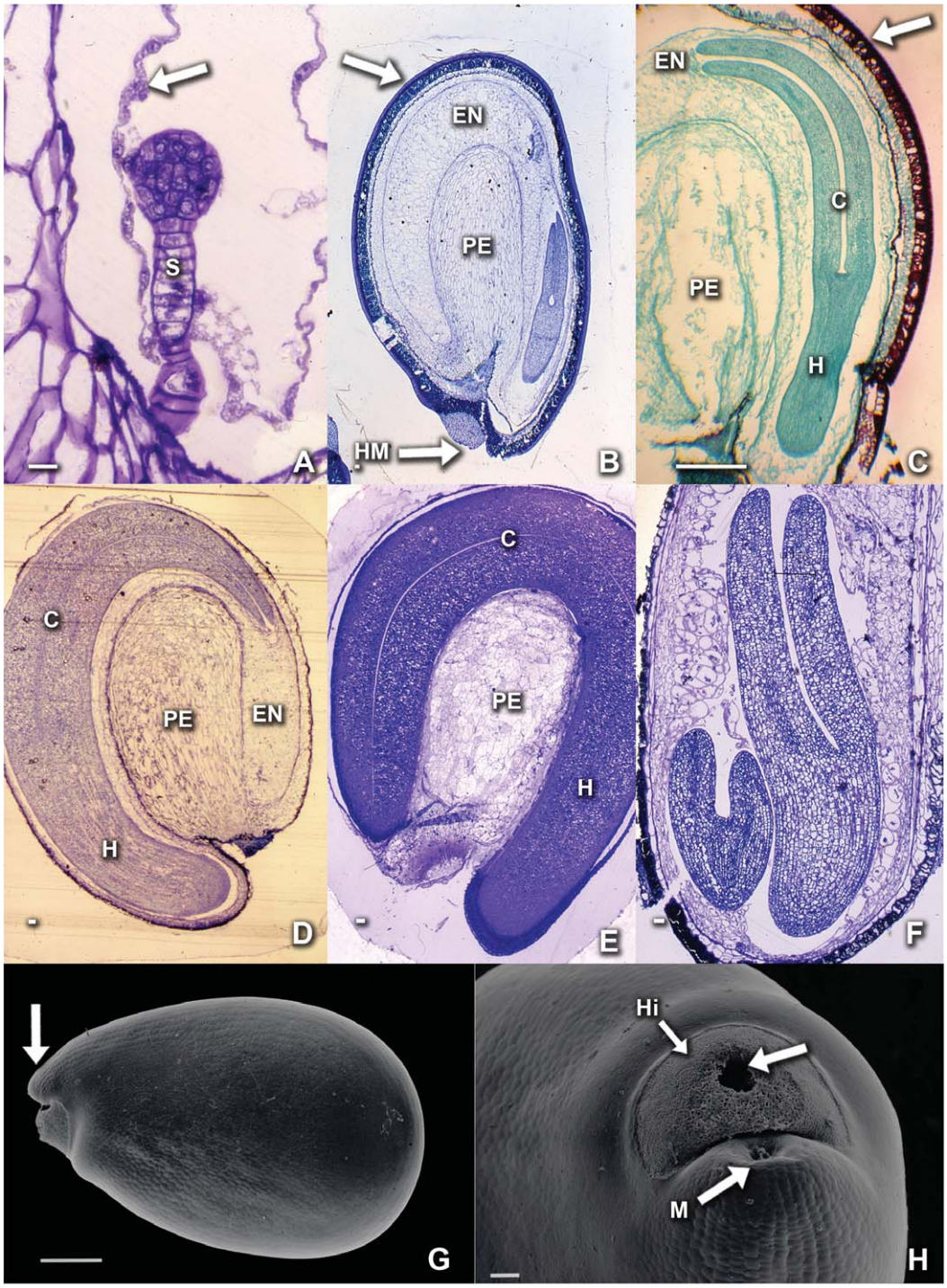

Figure 3. Embryogenesis in *Pereskia lychnidiflora*. Longitudinal sections. **A** Globular embryo with a multicellular suspensor (S) and rosary-like endosperm (arrow). *Scale bar* 10 μm. **B** Embryo during the torpedo stage. Endosperm (EN) and perisperm (PE) surrounded by a seed coat rich in tannins (arrow). The hilum-micropylar region (HM) is evident in base of the seed. *Scale bar* 10 μm. **C** Embryo during an advanced torpedo stage showing the cotyledons (C), hypocotyl (H), endosperm (EN), and perisperm (PE). *Scale bar* 10 μm. **D** Embryo in the later stages of development with cotyledons (C) that are larger than the hypocotyl (H), remnants of the endosperm (EN), and perisperm (PE). *Scale bar* 10 μm. **E** Mature embryo with perisperm (PE) surrounded by the cotyledons (C) and hypocotyl (H). *Scale bar* 10 μm. **F** Polyembryony. A rare event. *Scale bar* 10 μm. **G–H** SEM photographs. **G** Mature seed showing the hilum-micropylar region (arrow). *Scale bar* 10 μm. **H** Hilum region (Hi), micropyle (M) and the entrance of the vascular bundle (arrow). *Scale bar* 10 μm.

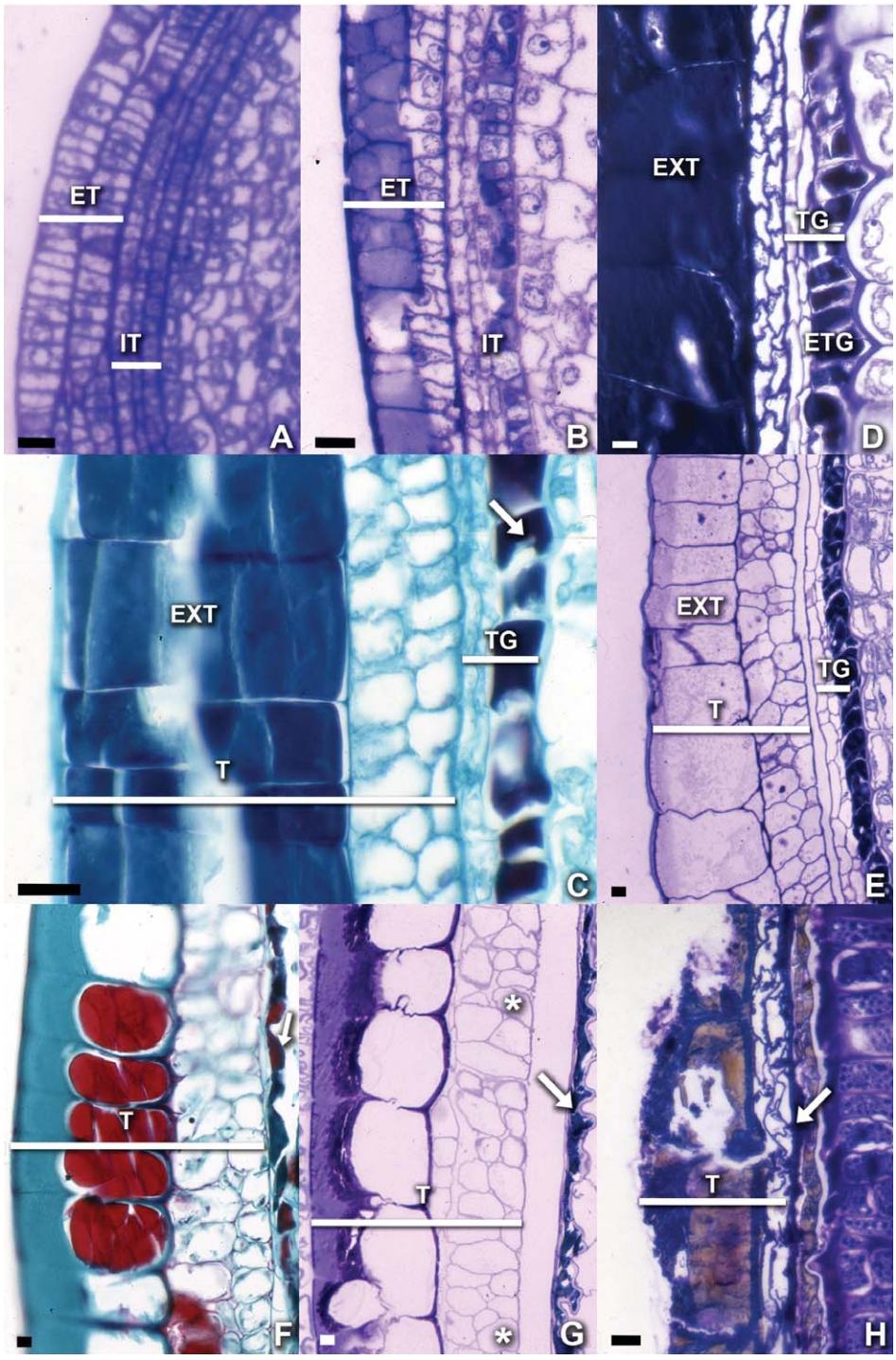

Figure 4. Seed coat development in *Pereskia lychnidiflora*. Longitudinal sections. **A** Pre-anthesis ovule, with external integument bi-stratified (ET) and internal tegument tri-stratified (IT). *Scale bar* 10 µm. **B** Anthesis ovule. External tegument (ET) with tannins, internal integuments (IT) with three or four layers. *Scale bar* 10 µm. **C** Seed coat. Testa (T) with a thick exotesta (EXT), and tegmen (TG) with tannins in the endotegmen (Arrow). *Scale bar* 200 µm **D** Seed coat. Exotesta (EXT), tegmen (TG) and endotegmen (ETG) with tannins. *Scale bar* 10 µm **E** Seed coat treated with sodium hypochlorite, showing the testa (T) exotesta (EXT), and tegmen (TG). *Scale bar* 10 µm. **F** Seed coat. Testa (T) and remnants of the endotegmen (arrow). *Scale bar* 10 µm. **G** Seed coat treated with sodium hypochlorite. The cells of the testa (T) undergo mitosis (*), remnants of the endotegmen are visible (arrow). *Scale bar* 500 µm **H** Seed coat of a mature seed. The testa (T) shows an exotesta with tannins and thickened walls. The tegmen is found in a stage of degeneration (arrow). *Scale bar* 100 µm.

Species	Boke (1963, 1966, 1968)	Leuenberger (1986)	Edwards et al. (2005)
Southern South American Clade			
P. bahiensis		Half.inferior-inferior	Half-inferior
P. grandifolia		Inferior	Half-inferior
P. nemorosa		Superior to half-inferior	Half-inferior
P. sacharosa		Half-inferior	Half-inferior
P. stenantha		Half.inferior-inferior	Half-inferior
Andean Clade			
P. aculeata		Superior to half-superior	Half-inferior
P. diaz-romeroana		Superior to half-inferior	Half-inferior
P. horrida	Superior	Superior to half-inferior	Half-inferior
P. weberiana	Superior	Half-inferior	Half-inferior
Northern Clade			
P. aureiflora		Half-inferior	Half-inferior
P. bleo		Inferior	Inferior
P. guamacho		Half-inferior	Half-inferior
P. lychnidiflora		Half-superior	Half-inferior
P. marcanoi			Inferior
P. portulacifolia	Superior	Inferior	Inferior
P. quisqueyana		Inferior	Inferior
P. zinniiflora		Inferior	Inferior

Table 1. Ovary types in the genus *Pereskia*.

The position of the ovary, on the other hand, has always proven a problematic character in *Pereskia*. Leuenberger (1986) has pointed out that the complex nature of the ovary in *Pereskia* along with its modifications during the ontogeny of the flower and fruit make this character an ambiguous one since it can lead to different interpretations. Following the argument of Weberling (1989), who determined the ovary position in the pre-anthesis phase, we identified that *P. lychnidiflora* has an inferior ovary.

Leuenberger's research (1986) refers to the type of ovary in all known species of *Pereskia*. In particular, *P. lychnidiflora* is considered to be semi-superior ovary, while Boke (1963) determined a superior ovary in this species. Phylogenetic studies carried out by Edwards et al. (2005) have shown *P. lychnidiflora* clustered together with seven other *Pereskia* species in a monophyletic group. Five of those species have been described as having an inferior ovary, while the remaining three species, including *P. lychnidiflora* were considered to have a semi-inferior or semi-superior ovary (Edwards et al. 2005; Table 1).

Placentation type is a character of taxonomic importance in the Cactaceae. Within *Pereskia,* basal and parietal placentation types have been described (Berger 1926; Leuenberger 1986; Leins and Schwitalla 1988; Judd et al. 2002). In the particular case of *P. lychnidiflora,* different placentation types have been described depending on the author, ranging from basal (Judd et al. 2002), basal-axilar (Leuenberger 1986), and basal-laminal (Leins and Schwitalla 1988), to parietal (Berger 1926). Our direct embryological observations in *P. lychnidiflora* confirm a basal-laminal placentation as described by Leins and Schwitalla (1988).

Our findings of the presence of stomata along the basal and central part of the ovary in *P. lychnidiflora* is a novelty that has never been reported before in *Pereskia*. The function of these stomata is unknown; however, it might be related to secretion (Davies et al. 2005; Wist and Davis 2008; Gudiño, pers. comm.). The modified stomata are secretory structures where nectar can be released (Nepi, 2007). The modified stomata associated with these nectaries have also been reported in other members of the Cactaceae, such as *Escontria chiotilla*, *Myrtillocactus geometrizans*, *Lophocereus* (*Pachycereus*) *schottii*, *Neobuxbaumia mezcalaensis, Pachycereus pecten-aborigium*, *Stenocereus pruinosus* (Fuentes-Pérez, 2004), *Opuntia tomentosa* (Montero, 2004), *Rhipsalis teres* (García de Almeida et al. 2010), and *Stenocereus quevedonis* (Gudiño, 2012). These modified secretory stomata could also represent a relictual trait because the flower is inserted within a modified branch forming the pericarpel. (Buxbaum 1953).

Even though a multicellular archesporium is the most common type found in the Cactaceae, a unicellular type has been described in *Mammillaria elongata*, *Opuntia ficus-indica*, and *Zygocactus truncata* (Johri et al. 1992). While within *Pereskia* a multicellular archesporium is present in *P. aculeata*, *P. grandiflora,* and *P. bleo* (Tiagi 1967), our results indicate that in the case of *P. lychnidiflora* the archesporium is unicellular.

In Cactaceae the formation of megaspores is linear or T-shaped. Our study indicates that in *P. lychnidiflora*, after the first meiotic division, only the chalazal cell undergoes the second stage of meiosis. Therefore instead of a tetrad, *P. lychnidiflora* has a linear triad, of which the chalazal cell is the functional

megaspore (monosporic embryo sac). A linear triad has been reported in other species of the family such as *Opuntia dillenii* (Maheshwari and Chopra 1955), *Astrophytum myriostigma* (Engleman 1960); *Cereus jamacaru* (Kapil and Prakash 1969), *Pachycereus militaris* (Núñez et al. 2001), *Opuntia tomentosa* (Flores 2002), *Mammillaria san-angelensis* (Parada 2004) and sometimes in *Pachycereus gaumeri* (Núñez 2004).

We were unable to find in *P. lychnidiflora* a couple of characters that have been described as diagnostic for *Pereskia*, such as the nucellar cap and the polyembryony. However, the nucellar cap has been described for another member of the genus as *P. amapola* var. *argentina* (Leuenberger 1986), and other cacti species such as *Opuntia aurantiaca* (Archibald 1939), *Rhipsalis, Thelocactus* and *Astrophytum myriostigma* (Englemann 1960). While polyembryony has been reported in various genera of Cactaceae (e.g. *Mammillaria*, *Pereskia* and *Opuntia*; Johri et al. 1992), we were only able to detect one case in 200 seeds analyzed during the torpedo zygotic embryo.

According to Leuenberger (1986), perisperm in *Pereskia* is well developed and located in the central region of the seed between the cotyledons and the hypocotyl. Within Opuntioideae (Flores 2002) and Cactoideae (Engleman 1960; Johri et al. 1992; Núñez 2004), the perisperm is greatly reduced and, in some instances, is absent. In this study *Pereskia lychnidiflora* showed abundant perisperm located in the central position of the seed, persistent in mature seeds. Our data supports the hypothesis that within Cactaceae, the evolutionary tendency is towards perisperm reduction, as suggested by Barthlott and Hunt (2000).

The seed coat has long been considered an important taxonomic characteristic for the angiosperms. Corner (1976) mentioned that the presence of a multiplicative seed coat is a basal character. We found a multiplicative seed coat in *P. lychnidiflora.* There is a lack of studies on seed coat development in Cactaceae, and the only work in the family is in *Opuntia dillenii* (Maheswari and Chopra 1955), which indicated that the seed coat is not multiplicative.

Some structures referred to as ancestral in the genus *Pereskia* have been documented by several authors (Boke 1963, 1966, 1980; Ross 1982; Gibson and Nobel 1986; Leuenberger 1986; Anderson 2001; Nyffeler 2002; Edwards et al. 2005), based on its tree habit, the presence of deciduous leaves that are not succulent, a superior or half-superior ovary, and the presence of C_3 photosynthesis. In this study, we corroborated some embryological characters for *P. lychnidiflora* described for the genus and the family. Some of these characters support the basal position of this species in the Cactaceae, such as abundant perisperm and superior ovary. We believe that it is necessary to have more embryological studies in other species of the genus *Pereskia* in order to make generalizations about the exact ovary position, the presence and function of stomata on the ovary and occurrence of multicellular layers in the seed coat, in order to corroborate the paraphyly of *Pereskia* and support the two clades in the genus.

ACKNOWLEDGEMENTS

We are grateful to Anabel Bieler-Antolin for microscopy help and to Ricardo Wong for his assistance in the technical implementation and reviewing the manuscript. Thanks to Patricia Soffiatti for many helpful comments.

REFERENCES

Anderson EF. 2001. *The cactus family*. Timber Press. Portland, Oregon.

Archibald EE. 1939. The development of the ovule and seed of jointed cactus (*Opuntia aurantiaca*. Lindley). *South African Journal of Science* 36: 195–211.

Bailey I. 1963. The xylem of pereskias from southern Mexico and Central America. *Journal of the Arnold Arboretum* 44: 211–221.

Barthlott W, Hunt DR. 2000. Seed diversity in the Cactaceae subfamily Cactoideae. *Succulent Plant Research* 5: 1–173.

Berger A. 1926. *Die entwicklungslinien der Kakteen*. G. Fisher. Jena, Germany.

Boke NH. 1963. Anatomy and development of the flower and fruits of *Pereskia pititache*. *American Journal of Botany* 50: 843–858.

Boke NH. 1966. Ontogeny and structure of the flower and fruit of *Pereskia aculeata. American Journal of Botany* 53: 534–542.

Boke NH. 1968. Structure and development of the flower and fruit of *Pereskia diaz-romeroana*. *American Journal of Botany* 55: 1254–1260.

Boke NH. 1980. Development morphology and anatomy in Cactaceae. *BioScience* 30: 605–610.

Butterworth CA, Wallace RS. 2005. Molecular phylogenetics of the leafy cactus genus *Pereskia* (Cactaceae). *Systematic Botany* 3: 800–808.

Buxbaum F. 1953. *Morphology of cacti. Section II: Flower*. Abbey Garden Press. Pasadena, California.

Corner E. 1976. *The seed of dicotyledons*. Volume. I. Cambridge University Press. Cambridge, Massachusetts.

Davies KL, Stpiczynska M, Gregg A. 2005. Nectar secreting floral stomata in *Maxillaria anceps* Ames & C. Schweinf (Orchidaceae). *Annals of Botany* 96: 217–227.

Edwards EJ, Nyffeler R, Donoghue MJ. 2005. Basal cactus phylogeny: Implications of *Pereskia* (Cactaceae) paraphyly for the transition to the cactus life form. *American Journal of Botany* 92: 1177–1188.

Edwards ER, Donoghue MJ. 2006. *Pereskia* and the origin of the cactus life-form. *American Naturalist* 167: 777–793.

Edwards EJ. 2006. Correlated evolution of stem and leaf hydraulic traits in *Pereskia* (Cactaceae). *New Phytologist* 172: 479–489.

Engleman ME. 1960. Ovule and seed development in certain cacti. *American Journal of Botany* 47: 460–467.

Flores RL. 2002. Estudio embriológico de *Opuntia tomentosa* Salm-Dyck var. *tomentosa* Salm-Dyck (Cactaceae). Undergraduate Thesis. Universidad Nacional Autónoma de México. México.

Fuentes-Pérez M. 2004. Anatomía floral de siete especies de la Tribu Pachycereeae (Cactaceae). MS Thesis. Universidad Nacional Autónoma de México. México.

García de Almeida O, Santori-Paoli A, De Souza L. 2010. Flower morpho-anatomy in *Epiphyllum phyllanthus* (Cactaceae). *Revista Mexicana de Biodiversidad* 81: 65–80.

Gibson C, Nobel PS. 1986. *The cactus primer*. Harvard University Press. Cambridge, Massachusetts.

Gudiño W. 2012. Ecofisiología reproductiva de algunas cactáceas columnares de la Tribu Pachycereereae. D. Phil. Thesis. Universidad Nacional Autónoma de México. México.

Hunt DR. 2006. *The new cactus lexicon*. DH Books. Milborne Port, England.

Johri BM, Ambergaskar KB, Srivastava S. 1992. Comparative embryology of angiosperms. Volume I. Springer-Verlag. New York.

Judd WS, Campbell CS, Kellogg EA, Stevens PF, Donoghue MJ. 2002. *Plant systematics. A phylogenetic approach.* Sinauer Associates. Sunderland.

Kapil N, Prakash N. 1969. Embryology of *Cereus jamacaru* and *Ferocactus wislizeni* and comments on the systematic position of the Cactaceae. *Botaniska Notiser* 122: 409–426.

Leins P, Schwitalla S. 1988. Placentation in Cactaceae. In: Leins P, Tucker SC, Endress PK (eds) *Aspects of floral development*. Berlin-Stuttgart, Germany.

Leuenberger B. 1986. *Pereskia* (Cactaceae). *Memories of New York Botanical Garden* 41: 1–141.

Maheshwari P, Chopra RN. 1955. The structure and development of the ovule and seed of *Opuntia dillenii* Haw. *Phytomorphology* 5: 112–122.

Mauseth JD, Landrum JV. 1997. Relictual vegetative anatomical characters in Cactaceae: the genus *Pereskia*. *Journal of Plant Research* 112: 307–315.

Montero O. 2004. Localización, estructura e histología de nectarios florales en Cactaceae. Undergraduate Thesis. Universidad Nacional Autónoma de México. México.

Nepi M. 2007. Nectary structure and ultrastructure. In: Nicolson S, Nepi M, Pacini E (eds). *Nectaries and nectar*. Springer. The Netherlands.

Núñez MC. 2004. Cactáceas columnares de México: Estudios embriológico comparativo de cinco especies de la Tribu Pachycereeae. D. Phil. Thesis. Universidad Nacional Autónoma de México. México.

Núñez M, Engleman EM, Márquez J. 2001. Embriología de *Pachycereus militaris* (Audut) Hunt (Cactaceae). *Boletín de la Sociedad Botánica de México* 68: 5–13.

Nyffeler R. 2002. Phylogenetic relationships in the cactus family (Cactaceae) based on evidence from *trnK/matK* and *trnL-trnF* sequences. *American Journal of Botany* 89: 312–326.

Orozco AG. 2002. Sistemas reproductivos de *Opuntia stenopetala* Engelmann (Cactaceae). Undergraduate Thesis. Universidad Nacional Autónoma de México. México.

Parada PD. 2004. Estudio comparativo de las estructuras reproductoras de *Mammillaria san-angelensis* Sánchez Mejorada y *Mammillaria haageana* Pfeiffer (Cactaceae). Undergraduate Thesis. Universidad Nacional Autónoma de México. México.

Ross R. 1982. Initiation of stamens, carpels, and receptacle in the Cactaceae. *American Journal of Botany* 69: 369–376.

Sánchez CD. 2007. Embriología de *Mammillaria dioica* K. Brandegee (Cactaceae). Thesis. Universidad Nacional Autónoma de México. México.

Silva MJ. 2007. Embriología de *Opuntia robusta* Wendland (Cactaceae). Thesis. Universidad Nacional Autónoma de México. México.

Tiagi YD. 1967. Contribution to the embryology of the genus *Pereskia*. *Proceedings of the 54th Indian Science Congress. (Hyderabad)* 324–325.

Wallace R, Gibson A. 2002. Evolution and systematic. pp: 1-21. In: Nobel P (ed) *Cacti: biology and uses.* University of California Press. Berkeley.

Weberling F. 1989. *Morphology of flowers and inflorescences*. Cambridge University Press. Cambridge.

Wist TJ, Davis AR. 2008. Floral structure and dynamics of nectar production in *Echinacea pallid* var. *angustifolia* (Asteraceae). *International Journal of Plant Sciences* 169: 708–722.