

## Pollen development and morphology in four species of *Pterocactus* (Cactaceae)

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Received 10 June 2008, revised version received 15 Aug. 2008, accepted 15 Aug. 2008

Gotelli, M. M., Scambato, A., Galati, B. & Kiesling, R. 2009: Pollen development and morphology in four species of *Pterocactus* (Cactaceae). — *Ann. Bot. Fennici* 46: 409–415.

We report a detailed study of the development and morphology of pollen in four species of the genus *Pterocactus* (Cactaceae), carried out by using LM and SEM. The anther is tetrasporangiate, its wall consists of epidermis, endothecium, one middle layer and a binucleate secretory tapetum. Microspore tetrads are tetrahedral and pollen grains are shed at bicellular stage. Pollen grains are pantoporate, with a perforate tectum and supratectate spinules.

Key words: microgametogenesis, microsporogenesis, pollen morphology, *Pterocactus*

### Introduction

The genus *Pterocactus* (Cactaceae) comprises nine species of small plants, with cylindrical or globose segments, apical flowers, winged seeds and tuberous roots (Kiesling 1982). It belongs to the subfamily Opuntioideae and is endemic to the south and west of Argentina.

Embryological studies on the Cactaceae are scarce (Johri *et al.* 1992) and restricted to *Consolea spinosissima* (Strittmatter *et al.* 2002), various species of *Opuntia* (Tiagi 1954, Maheshwari & Chopra 1955, García Aguilar & Pimienta-Barrios 1996) and some species of *Rhipsalis*, *Mammillaria* and *Pereskia* (Mauritzon 1934, Chopra 1957). Kurtz (1948, 1963) describes the general morphology of pollen grains of the Cactaceae, while Leuenberger (1976b) compares palynological characters between the subfamilies Pereskioideae, Opuntioideae and Cactoideae. Pollen

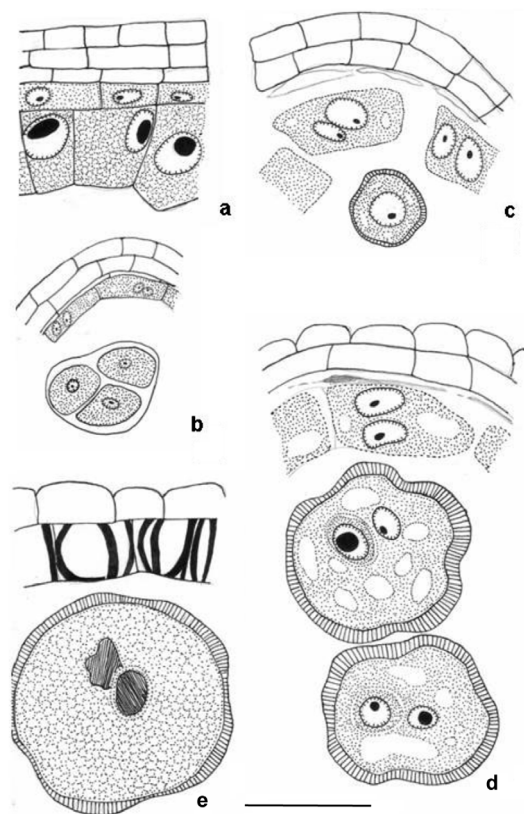
morphology has also been studied for *Trichocereus atacamensis*, *Denmoza rodacantha* and *Trichomoxa roseiflora* (Font & Picca 2001).

Historically, embryological studies have been used to assess taxonomic relationships among the families and genera (Johri *et al.* 1992). Resurrection of several genera in the Cactaceae based on their seed characters has been recently proposed (Stuppy 2002).

So far the pollen grain ontogeny in the genus *Pterocactus* has not been examined. The aim of the present study is to provide detailed pollen developmental and morphological descriptions in order to shed light on the species' taxonomic position.

### Material and methods

Flowers of *P. australis*, *P. araucanus*, *P. hickenii*



**Fig. 1.** Microsporogenesis and microgametogenesis in *Pterocactus*. — **a:** Archesporic tissue and young microsporangium wall. — **b:** Microspore tetrad with tetrahedral disposition. — **c:** Free microspore, two-nuclei tapetal cells, middle layer partly consumed. — **d:** Young bicellular pollen grain, middle layer mostly consumed. — **e:** Mature pollen grain, endothecium with fibrous thickenings, epidermal cells enlarged. Scale bar: 40  $\mu\text{m}$ .

and *P. valentinii* in different stages of development were fixed in FAA and embedded in paraffin. Sections (5–10  $\mu\text{m}$ ) were cut and stained with safranin combined with fast green (D'Ambrogio 1986) and observed with a Wild M20 microscope. Photographs were taken with a Zeiss microscope. For SEM studies critical point was made to pollen grains, which were then sputter-coated with gold-palladium for three minutes (O'Brien & McCully 1981) and observed with a SEM Philips Series XL, Model 30.

## Results

The descriptions apply to all of the four species.

The distinctive features are specifically elaborated.

### Microsporangium

The anther is tetrasporangiate and dehisces longitudinally. The anther wall consists of an epidermis, endothecium, one middle layer and a binucleate secretory tapetum. The anther wall development coincides with the dicotyledonous type (Davis 1966) since the middle layer and the endothecium share the same origin (Fig. 1a).

In floral primordia, cells that form the four wall layers have a similar size and shape (Fig. 2a). During anther development they differentiate and acquire special features. Tapetum cells are the first to enlarge and become binucleate by normal mitosis at late microspore mother cell stage (Figs. 1b, 2b). These cells are densely cytoplasmic and surround the sporogenous cells completely. At early bicellular pollen grain stage the tapetal cells reach their maximum size, breaking down once the anther has reached maturity (Fig. 1c–e).

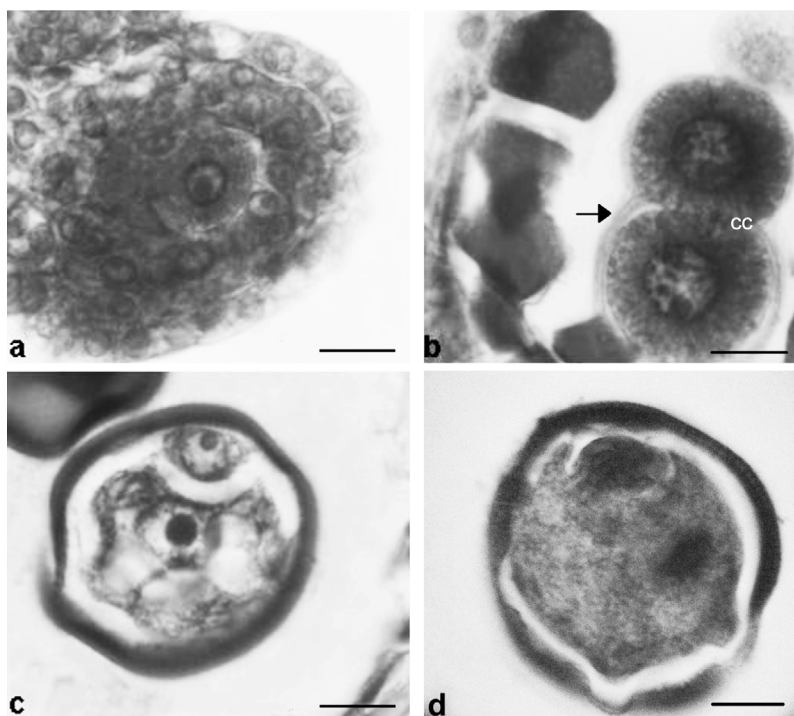
Epidermal and endothecial cells as well as the middle layer cells grow radially and tangentially as the anther matures. After the first meiotic division, the middle layer cells start a slow degeneration process. At the early bicellular pollen grain stage, most of the middle layer cells have been consumed, while a few persist and are restricted to the zone of the locule that contacts with the connective tissue until the pollen grains reach maturity (Fig. 1d).

During microgametogenesis fibrous thickenings develop from the inner tangential and radial walls of the endothecium cells. These ribs are interrupted in the outer tangential face (Fig. 1e). Therefore the endothecium acts mechanically in the dehiscence of the anther. Epidermal cells enlarge considerably throughout the development of the microspores. Epidermis remains as a well-defined layer in the mature anther (Fig. 1e).

### Microsporogenesis and microgametogenesis

The sporogenous tissue differentiates once the four layers of the anther wall have been formed.

**Fig. 2.** Light micrographs of *Pterocactus australis*. Microsporogenesis and microgametogenesis. — **a**: Archeporic tissue and young microsporangium wall. — **b**: microspore mother cells with callose wall (arrow), cytoplasmic connections (cc). — **c**: Young bicellular pollen grain. — **d**: Mature bicellular pollen grain. Scale bars: **a–c**: 15  $\mu$ m, **d**: 20  $\mu$ m.



This tissue is distinguishable by cells of large size, dense cytoplasm and prominent nuclei (Fig. 2a). Microspore mother cells walls become thicker due to deposition of callose wall between the plasmalemma and the primary wall. Subsequently, they come apart by the dissolution of the middle lamella and primary walls that kept the sporogenous tissue together. Each microspore mother cell undergoes simultaneous reductive divisions and gives rise to microspore tetrads with tetrahedral arrangement (Fig. 1b).

Each individual microspore separates out from the tetrad by a sudden dissolution of the callose wall. The deposition of sporopollenin begins immediately after the release of microspores into the anther locule. Consequently, a thick exine wall is developed. Young microspores have a vacuolated cytoplasm and increase in size (Fig. 1c).

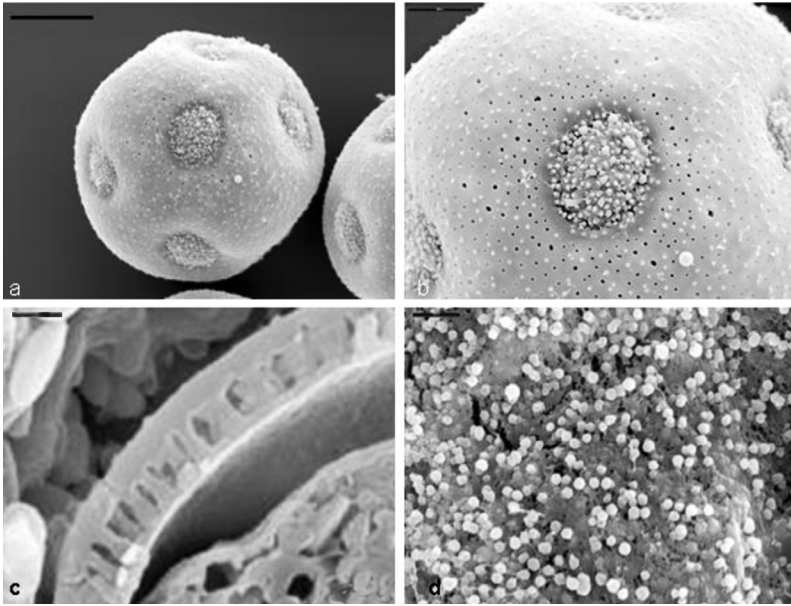
The first division of the microspore gives rise to a small generative cell and a large vegetative cell with a vacuolated cytoplasm (Figs. 1d, 2c). Soon after pollen grains are formed they increase their volume, which generates a stretching and slimming of the exine. After microspore mitosis the vegetative cell continues to grow, the vacuoles gradually disappear and the cytoplasm

becomes filled with starch grains (Figs. 1e, 2d). At this stage the pollen grains are shed. The division of the generative cell into gametes was not observed.

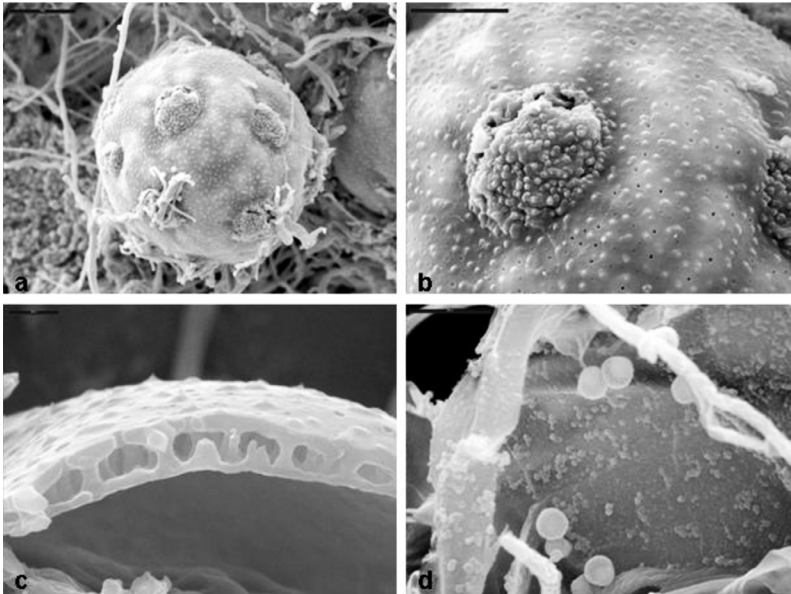
### Pollen grain morphology

Pollen grains are pantoporate with a perforate tectum and supratectate spinules (Figs. 3a, 4a, 5a, 6a). The pore membrane is either straight (Figs. 3b, 5b, 6b) or convex (Fig. 4b) and presents a higher density of spinules, some of which fuse to each other. The outline of the pollen grains seen in polar view, or amb (Punt *et al.* 1994), is spheroidal in *P. australis*, *P. araucanus* and *P. hickenii* (Figs. 3a, 4a, 6a) and suboblate in *P. valentinii* (Fig. 5a).

Pollen grain size and pore size are similar in the four species studied. However, *P. hickenii* presents pores in which the diameter varies (Fig. 6a). *Pterocactus australis* has fewer pores than the other species studied, while *P. hickenii* presents the most. Puncta, defined as a rounded or elongated tectal perforations, less than 1  $\mu$ m in length or diameter, are constant in *P. arauca-*



**Fig. 3.** *Pterocactus australis*. Pollen grains and orbicules observed with SEM. — **a**: General aspect. — **b**: Detail of the pore. — **c**: Detail of a sectioned pollen grain wall. — **d**: Detail of the orbicules. Scale bars: **a**: 20  $\mu\text{m}$ , **b**: 8  $\mu\text{m}$ , **c** and **d**: 2  $\mu\text{m}$ .



**Fig. 4.** *Pterocactus araucanus*. Pollen grains and orbicules observed with SEM. — **a**: General aspect. — **b**: Detail of the pore. — **c**: Detail of a sectioned pollen grain wall. — **d**: Detail of the orbicules. Scale bars: **a**: 20  $\mu\text{m}$ , **b**: 10  $\mu\text{m}$ , **c**: 2  $\mu\text{m}$ , **d**: 5  $\mu\text{m}$ .

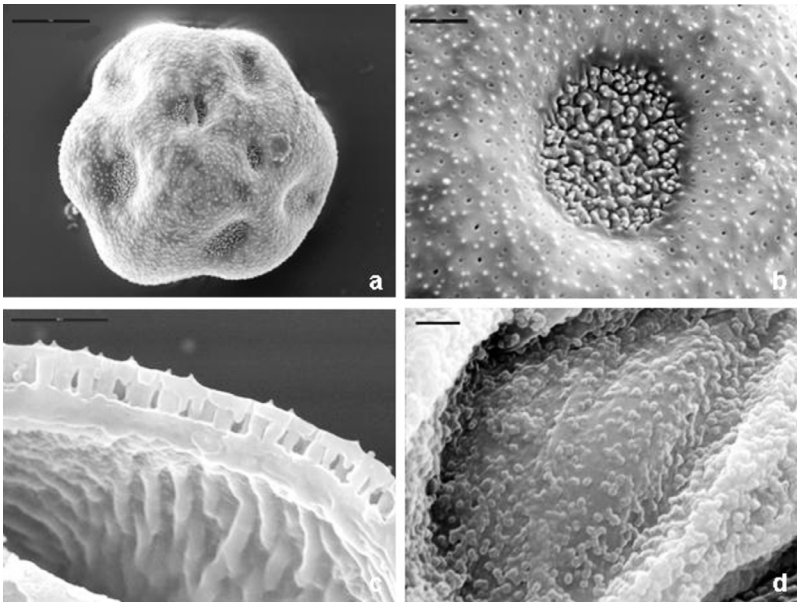
*nus* and *P. hickenii*, whereas *P. australis* and *P. valentinii* have smaller and larger puncta. The former shows the greatest differences, having puncta that range from 200  $\mu\text{m}$  up to 800  $\mu\text{m}$ .

Most differences between species are found in the pollen grain wall sections. The tectum is thicker than the wall sections group formed by the foot layer, endexine and intine in *P. australis* (Fig. 3c) and thinner in *P. valentinii* (Fig. 5c and Table 1), while in *P. araucanus* and in *P. hickenii*

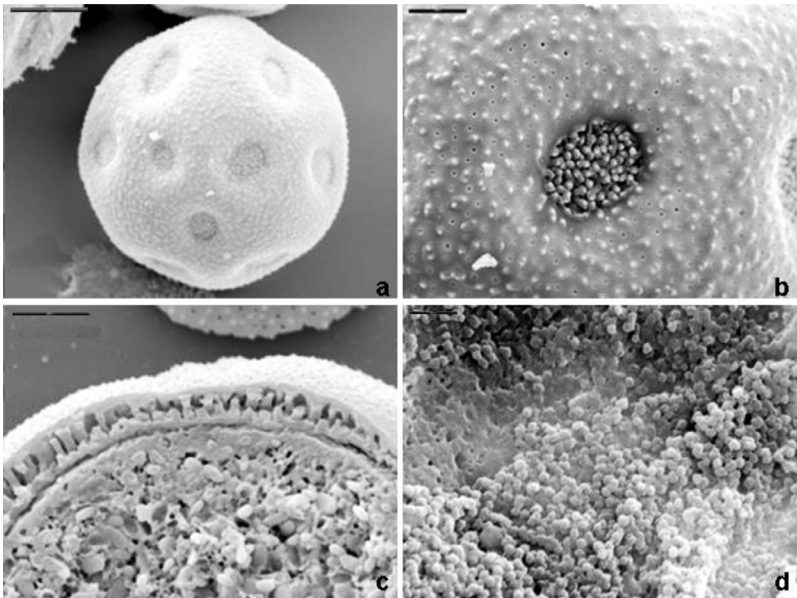
such structures are of the same thickness (Figs. 4c, 5c and Table 1). *Pterocactus valentinii* has the largest columellae, which are straight with occasional bifurcations in the four species studied (Figs. 3c, 4c, 5c, 6c and Table 1). The exine is considerably thicker in *P. valentinii* than in *P. australis*, *P. araucanus* and *P. hickenii*. The latter three species have exine of similar size.

The inner surface of the anther locule is covered by orbicules, or Ubisch bodies, which





**Fig. 5.** *Pterocactus valentinii*. Pollen grains and orbicules observed with SEM. — **a**: General aspect. — **b**: Detail of the pore. — **c**: Detail of a sectioned pollen grain wall. — **d**: Detail of the orbicules. Scale bar: **a**: 20 μm, **b** and **c**: 5 μm, **d**: 2 μm.



**Fig. 6.** *Pterocactus hickonii*. Pollen grains and orbicules observed with SEM. — **a**: General aspect. — **b**: Detail of the pore. — **c**: Detail of a sectioned pollen grain wall. — **d**: Detail of the orbicules. Scale bars: **a**: 20 μm, **b** and **c**: 5 μm, **d**: 2 μm .

**Table 1.** Comparison of pollen morphology among the four species of *Pterocactus*. T = tectum, C = columellae, L = length, W = width, FL = foot layer, E = endexine, I = intine, TT = total thickness.

Taxa	N	Pollen size (μm)	Pore size (μm)	Pore number	Punctum size (μm)	Exine (nm)					Orbicules (μm)
						T	C		FL + E + I	TT	
							L	W			
<i>P. australis</i>	20	70.8	12	10 ± 2	200–800	1143	857	571	286	2286	0.600
<i>P. araucanus</i>	20	66.7	13	12 ± 2	340	667	1000	667	667	2334	0.454
<i>P. hickonii</i>	20	78	9	18 ± 2	263	500	1000	500	500	2000	0.500
<i>P. valentinii</i>	20	73	12	14 ± 2	263–447	1250	1875	1250	3125	6250	0.300

are spherical to subspherical and with a smooth surface pattern (Figs. 3d, 4d, 5d, 6d).

## Discussion

This report contributes to the embryological knowledge of the genus *Pterocactus* since little was known about the pollen morphology and nothing about its development.

Anther wall development, microsporogenesis and microgametogenesis coincide with the descriptions made for the functionally staminate and superficially perfect flowers of *Consolea spinosissima* (Strittmatter et al. 2002). However, the pollen grains in that species are shed at tricellular stage, while in the four species of *Pterocactus* studied they are released at bicellular stage. It is inferred that the generative cell divides in the pollen tube. Therefore, we cannot say if the sperm cells are monomorphic or dimorphic.

Several authors have described the pollen morphology of different species of Opuntioideae, whose characteristics are similar to *Pterocactus*. Pollen grains of Argentine species of the genera *Austrocylindropuntia*, *Maihueniopsis*, *Opuntia* and *Tephrocactus* are spheroid apolar, radio-symmetric, pantoaperturate and large, between 50 and 100  $\mu\text{m}$  (Garralla & Cuadrado 2007). Kiesling (1984) described similarly the pollen of that subfamily. *Opuntia schickendantzii* is the only species of *Opuntia* with perforated tectate pollen grains (Kiesling 1984). Leuenberger (1976a) also analyzed *O. schickendantzii* and *O. aurantiaca*, and indicated the predominance of 12-porate pollen in these two species, while, according to Kiesling's (1984) descriptions, the former has approximately 24 pores. Pollen grains of *Puna bonnieae* are very similar to those of *Tephrocactus halophilus* and *T. weberi*, with tectate spinulose, perforated, and each perforation surrounded by a ring (Ferguson & Kiesling 1997).

Garrallo and Cuadrado (2007) divide the analyzed species into two large groups: (1) species with tectate, imperforate/perforate, spinulose/nanospinulose grains; and (2) species with semitectate, reticulate grains. Pollen grains of *Pterocactus* species are large, pantoporate, tectate, perforate with spinules that fuse to each other.

The pore number of the species of *Pterocactus* here studied varies from 10 to 18. According to these characteristics, *Pterocactus* falls into the first category.

Pollen morphology of *Pterocactus* differs from that observed by Font and Picca (2001) in *Trichocereus atacamensis*, *Denmoza rodacantha* and  $\times$  *Trichomoza roseiflora*. Therefore, it could be induced that pollen characteristics differ between the subfamilies Opuntioideae and Cactoideae.

Although the differences in pollen morphology between species are few, to separate the four species studied based on the descriptions of the pores and wall sections the following key can be used:

1. Tectum and wall section's group formed of foot layer, endexine and intine of equal thickness ..... 2
1. Tectum and wall section's group formed of foot layer, endexine and intine of different thickness ..... 3
2. Pores of variable diameter with straight membrane ..... *P. hickenii*
2. Pores of similar diameter with convex membrane ..... *P. araucanus*
3. Tectum thicker than wall section's group formed of foot layer, endexine and intine ..... *P. australis*
3. Tectum thinner than wall section's group formed of foot layer, endexine and intine ..... *P. valentinii*

Our results support the current taxonomic position within the genus of the four species of *Pterocactus* studied. It is beyond the scope of this paper whether this data supports the taxonomic position of *Pterocactus* within the Opuntioideae.

## References

- Chopra, R. N. 1957: The mode of embryo sac development in *Opuntia aurantiaca* Lindl.: a reinvestigation. — *Phytomorphology* 7: 403–406.
- D'Ambrogio, A. 1986: *Manual de técnicas en histología vegetal*. — Hemisferio Sur, Buenos Aires.
- Davis, G. L. 1966: *Systematic embryology of the angiosperms*. — John Wiley, New York.
- Ferguson, D. J. & Kiesling, R. 1997: *Puna bonnieae* (Cactaceae), a new species from Argentina. — *Cactus Succul. J. (U.S.)* 69: 283–287.
- Font, F. & Picca, P. 2001: Natural hybridization between *Trichocereus atacamensis* (Philippi) Marshall and *Denmoza rodacantha* (Salm-Dyck) Britton & Rose (Cactaceae). — *Bradleya* 19: 59–66.

- García-Aguilar, M. & Pimienta-Barrios, E. 1996: Cytological evidences of agamospermy in *Opuntia* (Cactaceae). — *Haseltonia* 4: 39–42.
- Garralla, S. & Cuadrado, G. A. 2007: Pollen morphology of *Austrocylindropuntia* Backeb., *Maihueniopsis* Speg., *Opuntia* Mill. and *Tephrocactus* Lem. (Cactaceae, Opuntioideae) of Argentina. — *Rev. Palaeobot. Palynol.* 146: 1–17.
- Johri, B. M., Ambegaokar, K. B. & Srivastava, P. S. 1992: *Comparative embryology of angiosperms*, vols. 1 & 2. — Springer-Verlag, Berlin.
- Kiesling, R. 1982: The genus *Pterocactus*. — *Cactus Succul. J. (Great Britain)* 44: 51–56.
- Kiesling, R. 1984: Estudios en Cactaceae de Argentina: *Maihueniopsis*, *Tephrocactus* y géneros fines (Opuntioideae). — *Darwiniana* 25: 171–215.
- Kurtz, E. B. 1948: Pollen grain characters of certain Cactaceae. — *Bull. Torrey Bot. Club* 75: 516–522.
- Kurtz, E. B. 1963: Pollen morphology of the Cactaceae. — *Grana Palynol.* 4: 367–372.
- Leuenberger, B. 1976a: Die Pollen Morphologie der Cactaceae. — *Diss. Bot.* 31: 1–321.
- Leuenberger, B. E. 1976b: Pollen morphology of Cactaceae. — *Cactus Succul. J. (Great Britain)* 38: 79–94.
- Maheshwari, P. & Chopra, R. N. 1955: The structure and development of the ovule and seed of *Opuntia dillenii* Haw. — *Phytomorphology* 5: 112–122.
- Mauritzon, J. 1934: Ein Beitrag zur Embryologie der Phytolaccaceen und Cactaceen. — *Botaniska Notiser* (Lund, 1934): 111–135.
- O'Brien, T. P. & McCully, M. E. 1981: *The study of plant structure. Principles and selected methods*. — Termarcaphi, Melbourne.
- Strittmatter, L. I., Negrón-Ortiz, V. & Hickey, J. R. 2002: Subdioecy in *Consolea spinosissima* (Cactaceae): breeding system and embryological studies. — *Am. J. Bot.* 89: 1373–1387.
- Stuppy, W. 2002: Seed characters and classification of the Opuntioideae. — *Succul. Plant Res.* 6: 25–58.
- Tiagi, Y. D. 1954: Studies in the floral morphology of *Opuntia dillenii* Haworth. 1. Development of the ovule and gametophytes. — *Bot. Notiser* 107: 343–356.