

EPIDERMAL CHARACTERS OF *PTEROCACTUS* (OPUNTIOIDEAE, CACTACEAE)

FAIGÓN, A.¹; GALATI, B. G.^{2*}; ROSENFELDT, S.¹ AND KIESLING, R.³

¹ Facultad de Ciencias Exactas y Naturales. UBA

² Cátedra de Botánica. Facultad de Agronomía. UBA

³ CCT - (ex CRICyT) CONICET. C.C. 507 (5500) Mendoza, Argentina

Abstract: Stem epidermis of eight *Pterocactus* species was examined under light microscopy (LM) and scanning electron microscopy (SEM). The stomata belong to the paracytic type, with two to five subsidiary cells. The size of stomata and papillae and the density of both were measured and counted under LM. Under SEM the micromorphology of papillae was studied. These characters were used to distinguish anatomically the species of *Pterocactus*.

Resumen: Las epidermis de los tallos de 8 especies de *Pterocactus* fueron examinadas con microscopio óptico (MO) y Microscopio Electrónico de Barrido (MEB). Los estomas corresponden al tipo paracítico, con dos a cinco células subsidiarias. El tamaño de los estomas y de las papilas, como así también la densidad de ambos fueron medidos y contados con el MO. La micromorfología de las papilas fue estudiada con MEB. Estos caracteres fueron usados para distinguir anatómicamente las especies de *Pterocactus*.

Introduction

The genus *Pterocactus* contains nine species restricted to Argentina. The species range from the western edge of the Monte to the Patagonian phytogeographic provinces (Kiesling 1971, 1982). Recently *P. hickenii* and *P. australis* have been recorded in a small area in Chile (Kiesling 2002, Saldivia 2009).

Pterocactus is segregated from *Opuntia* based on its distinctive cylindrical stems, “apical flowers”[†] and winged seeds (Kiesling 1984). Recent works based on DNA analyses (Wallace and Dickie 2002) and on seed anatomy (Stuppy 2002) reaffirmed the identity of *Pterocactus* as a discrete entity of the Opuntioideae subfamily.

Although the anatomy of the vegetative structures of the Cactaceae has been studied by numerous histologists (Mauseth and Landrum 1997, Loza-Cornejo and Terrazas 1997, Nyffeler and Egli 1997, Mauseth and Plemons-Rodriguez 1998, Mauseth 1999, Terrazas and Arias 2002, Terrazas and Mauseth 2002, Loza-Cornejo and Terrazas 2003, Vázquez-Sánchez et al. 2005, Vázquez-Sánchez et al. 2007, Calvente et al. 2008), there are few recent works concerning the anatomy of genera related to *Pterocactus* (Fernandez 1995, Bullock and Martijena 1998, Fernandez 1999, Bobich and Nobel 2001).

Egli (1984) examined the stomatal types in more than 150 taxa of the Cactaceae. According to this author, the Pereskioideae and Opuntioideae show parallelcytic stomata on the leaves, but the stomata of the stem represent a different type.

According to Barthlott (1981), epidermal characters provide information that is useful in the classification of species and families in the plant kingdom. In the Cactaceae some examples among the genera of the Opuntioideae are mentioned, but not *Pterocactus*. However, his point of view can be applied here.

This paper reports the epidermal features of eight species of *Pterocactus*. The aim of this work is to provide information for a better delimitation of the species.

Materials and methods

The material was collected from wild populations in Argentina or from cultivation in Argentina.

Specimens examined:

Pterocactus australis (Weber) Backeb.

Chubut, dpto. Florentino Ameghino, ca. 185 Km Norte de Comodoro Rivadavia; Ruta 3, km 1686, 30-X-2001, Kiesling 9938 (SI).

Chubut, dpto. Sarmiento, Ruta Nac. 26; 35 km E de Sarmiento, 31-X-2001, Kiesling 9946 (SI).

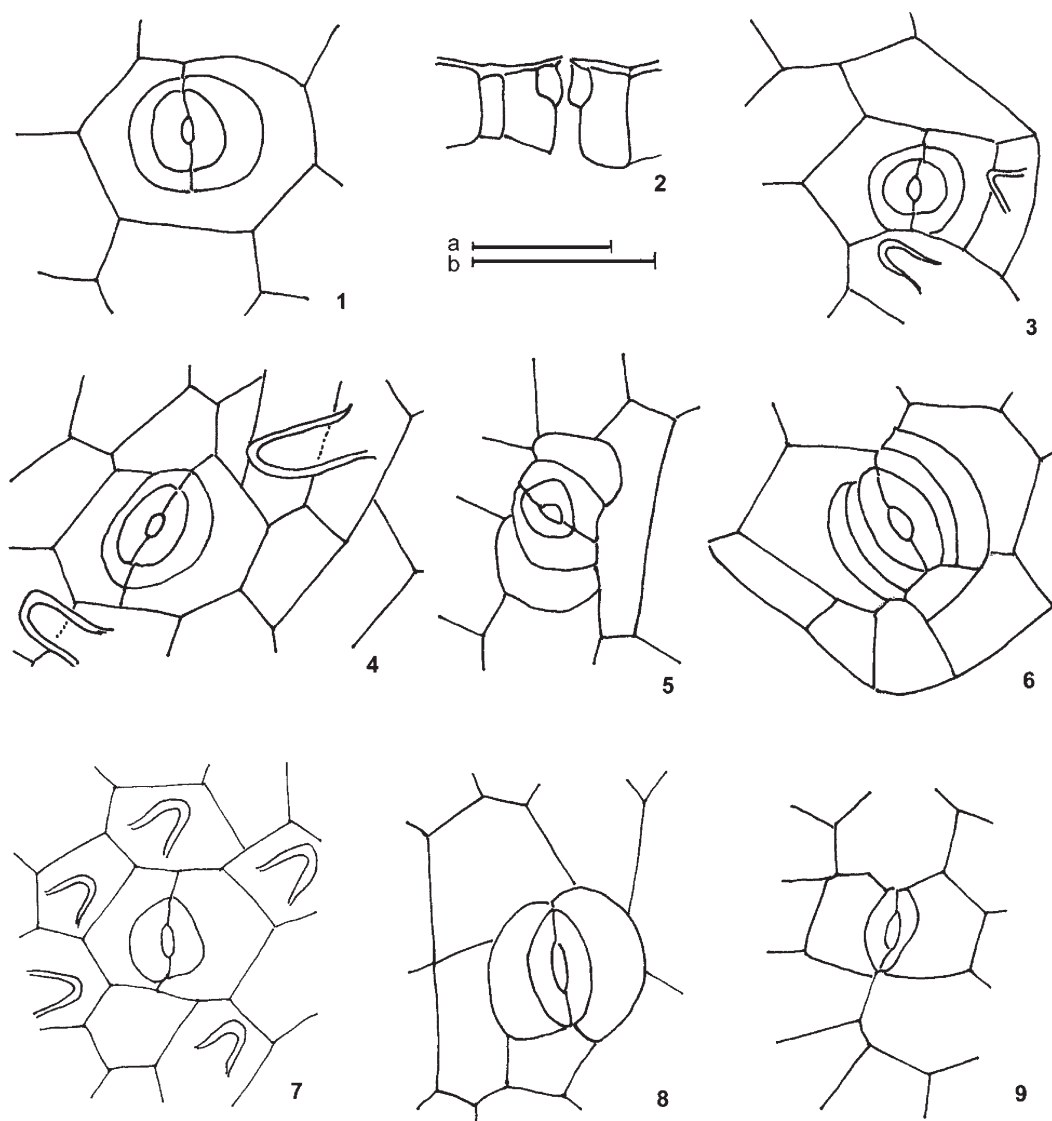
Santa Cruz, Departamento Lago Argentino, de Calafate a Tres Lagos: La Leona, 5-XI-2001, Kiesling 9966 (SI).

P. tuberosus K. Schumann

Mendoza, dpto. Luján de Cuyo, Potrerillos, cultivated.

* Corresponding author: Galati, B.G., galati@agro.uba.ar

† Morphological interpretation of the “apical flowers” in *Pterocactus* is the same as for other cacti; each flower is immersed in the receptacle or hypanthium. But in some *Pterocactus* species normal stems are absent or nearly absent, and the receptacle functionally replaces them.



Figures 1–9. Stomatal complex of *Pterocactus* spp. **1.** *P. australis*, superficial view of stomatal complex. Scale bar a = 50 μm . **2.** *P. australis*, transverse section of stomatal complex. Scale bar a = 50 μm . **3.** *P. reticulatus*, superficial view of stomatal complex. Scale bar b = 100 μm . **4.** *P. araucanus*, superficial view of stomatal complex. Scale bar b = 100 μm . **5.** *P. gonjianii*, superficial view of stomatal complex. Scale bar a = 50 μm . **6.** *P. fischeri*, superficial view of stomatal complex. Scale bar a = 50 μm . **7.** *P. megliolii*, superficial view of stomatal complex. Scale bar a = 50 μm . **8.** *P. hickenii*, superficial view of stomatal complex. Scale bar a = 50 μm . **9.** *P. tuberosus*, superficial view of stomatal complex. Scale bar a = 50 μm .

***P. megliolii* R. Kiesling**

San Juan, dpto. Zonda, camino a Ea. Maradona, cultivated.

***P. reticulatus* Kiesling**

San Juan, dpto. Iglesia, entre Rodeo y Agua Negra. Cultivated.

***P. gonjianii* Kiesling**

San Juan: dpto. Iglesia, 15 Km Oeste de Las Flores, Las Pampitas, I/ 1971. Kiesling 65 cultivated and LP.

***P. fischeri* Britton & Rose**

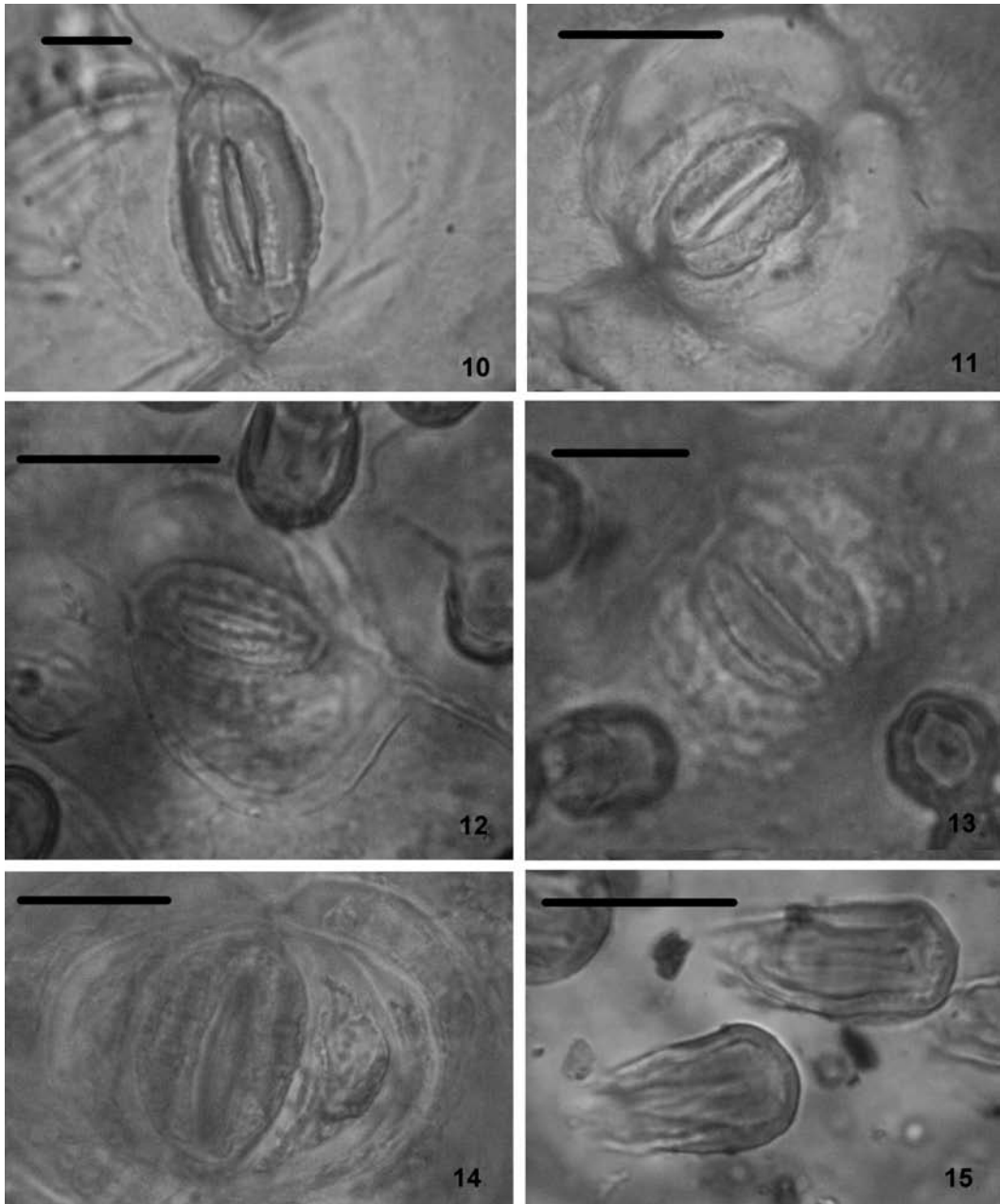
Neuquén, dpto. Chos Malal, ca. 10 Km N de Chos Malal, 12-XI-2001, Kiesling 9987 (SI).

***P. araucanus* Castellanos**

Neuquen. dpto. Zapala, Zapala, 15 km hacia Primeros Pinos; 11-XI-2001, Kiesling 9983 (SI).

***P. hickenii* Britton & Rose**

Chubut, dpto. Florentino Ameghino, ca. 185 Km Norte de Comodoro Rivadavia; Ruta 3; km 1686, 30-X-2001, Kiesling 9937 (SI).



Figures 10–15. Photomicrographs with light microscope. **10.** *P. araucanus*, detail of stomatal complex. Scale bar = 25 μ m. **11.** *P. australis*, detail of stomatal complex. Scale bar = 25 μ m. **12.** *P. meglolii*, detail of stomatal complex. Scale bar = 25 μ m. **13.** *P. reticulatus*, detail of stomatal complex. Scale bar = 25 μ m. **14.** *P. fischeri*, detail of stomatal complex. Scale bar = 25 μ m. **15.** *P. gonjianii*, detail of papillae. Scale bar = 25 μ m.

Chubut, dpto. Sarmiento, Ruta Nac. 26; 35 km E de Sarmiento, 31-X-2001, Kiesling 9945 (SI).

Chubut, dpto. Sarmiento, ladera sobre el Lago Musters, 8-XI-2001, Kiesling 9972 (SI).

The material was fixed in FAA (formaldehyde-alcohol 70%-acetic acid, 10:85:5), and small pieces of epidermis were cleared and stained with aqueous

safranin or sectioned according to the standard techniques of microtomy and stained with a safranin–fast green combination. (D’Ambrogio 1986). The following histochemical tests were carried out: Sudan IV for lipids, Cresyl Blue for cellulose, and Ruthenium Red for pectin. The preparations were observed with a Wild M 20 microscope and photographed with a Canon PowerShot A650 IS.

Measurements of length of papillae and occlusive cells were taken from cleared material. Ten papillae and stomata of each species were measured.

For scanning electron microscopy (SEM) studies, the material was dehydrated serially in acetone solutions of ascendant concentrations (70%, 80%, 90%, 100%) and subsequently freeze-dried with liquid CO₂. Sputter coating was done with gold-palladium for 3 minutes (O'Brien and McCully 1981). Scanning micrographs were taken with a Philips XL 30 microscope.

For transmission electron microscopy (TEM) studies, the material was pre-fixed in glutaraldehyde 1% in phosphate buffer (pH 7.2) at 2°C for 24 h and then post-fixed in OsO₄ 1% in the same buffer at 2°C for 3 h. It was dehydrated in a series of acetone solutions of increasing concentrations and embedded in Spurr's resin (O'Brien and McCully 1981). Fine sections were prepared using a Reichert-Jung ultramicrotome, and stained with uranyl acetate and lead citrate. They were observed and photographed using a Philips EM 301 TEM.

Results

Pterocactus stem surfaces are covered by a thick cuticle, and a dense layer of epicuticular waxes are deposited over it. These waxes are partially removed in the process of preparation of the material for SEM, using ethanol.

The epidermis of *Pterocactus* possesses three types of cells:

Type 1: Cells of the stomatal complex: kidney-shaped guard cells and subsidiary cells.

Type 2: Papillate cells: polygonal and isodiametric cells with straight anticlinal walls and a convex outer periclinal wall forming a papilla.

Type 3: Regular or ordinary epidermal cells: cells more or less elongated with sinuous or straight anticlinal walls.

Type 1: Stomatal complex

In all species studied here, the stomatal complex is of the paracytic type; each elongate stoma is accompanied on either side by one or more subsidiary cells parallel to the long axis of the pore and guard cells. The two guard cells appear enclosed by subsidiary cells that meet over the poles. In *P. australis*, *P. gonjiani*, *P. araucanus*, *P. fischeri*, and *P. reticulatus* (Figs. 1, 3, 4, 5, 10, 11, 13, 14), the stomatal complexes are formed by two guard cells and two pairs of subsidiary cells. The latter are in close contact with the guard cells and have very thin walls.

Rarely in *P. fischeri* six subsidiary cells are observed (Fig. 6), and occasionally in *P. reticulatus* only three subsidiary cells are found. In *P. hickenii*, *P. megliolii* and *P. tuberosus* (Figs. 7–9, 12) just one pair of subsidiary cells is present.

The stomatal pore is situated below the surface and the long narrow aperture visible from above is formed by cuticular ledges that largely cover the pore.

The length of the longtable axis of guard cells varies from 42–48 µm in *P. fischeri*, *P. hickenii* and *P. araucanus*, to around 28 µm in the remaining species (see Table 1).

The density of stomata (St. Den.) varies from 9 stomata per mm² in *P. fischeri* to 56 stomata per mm² in *P. gonjiani* (Table 1).

Type 2: Papillate cells

The papillate cells do not have a regular pattern of distribution (Figs. 31–35), except in *P. tuberosus*, where papillate cells form stripes which alternate with bands of non-papillate cells (Fig. 36).

Papillate cells are polygonal and isodiametric epidermal cells with straight anticlinal walls and a convex outer periclinal wall that forms a papilla (Figs. 15, 18, 19), which is a short, central, nipple-like projection. The papilla presents a thick cuticle, a thick cell wall of cutinized cellulose, and a central lumen (Figs. 18, 19). In cross section, the wall of the papilla is seen to be crossed by tiny ectodesmata arranged radially (Figs. 16, 17, 20). The length of the long axis of the papilla ranges from 22 µm in *P. megliolii* to 38 µm in *P. araucanus* (Table 1).

Table 1. Density and size of stomata and papillae in *Pterocactus* spp.

Species	Density of Stomata (number/ mm ²)	Size of Stomata (µm)	Density of Papillate Cells (number/ mm ²)	Length of Papillae (µm)
<i>P. araucanus</i>	14	42	140	38
<i>P. australis</i>	37	28	25	30
<i>P. fischeri</i>	9	48	105	26
<i>P. gonjiani</i>	50	31	610	30
<i>P. hickenii</i>	18	47	170	35
<i>P. megliolii</i>	37	28	895	22
<i>P. reticulatus</i>	29	31	510	25
<i>P. tuberosus</i>	39	31	510	27

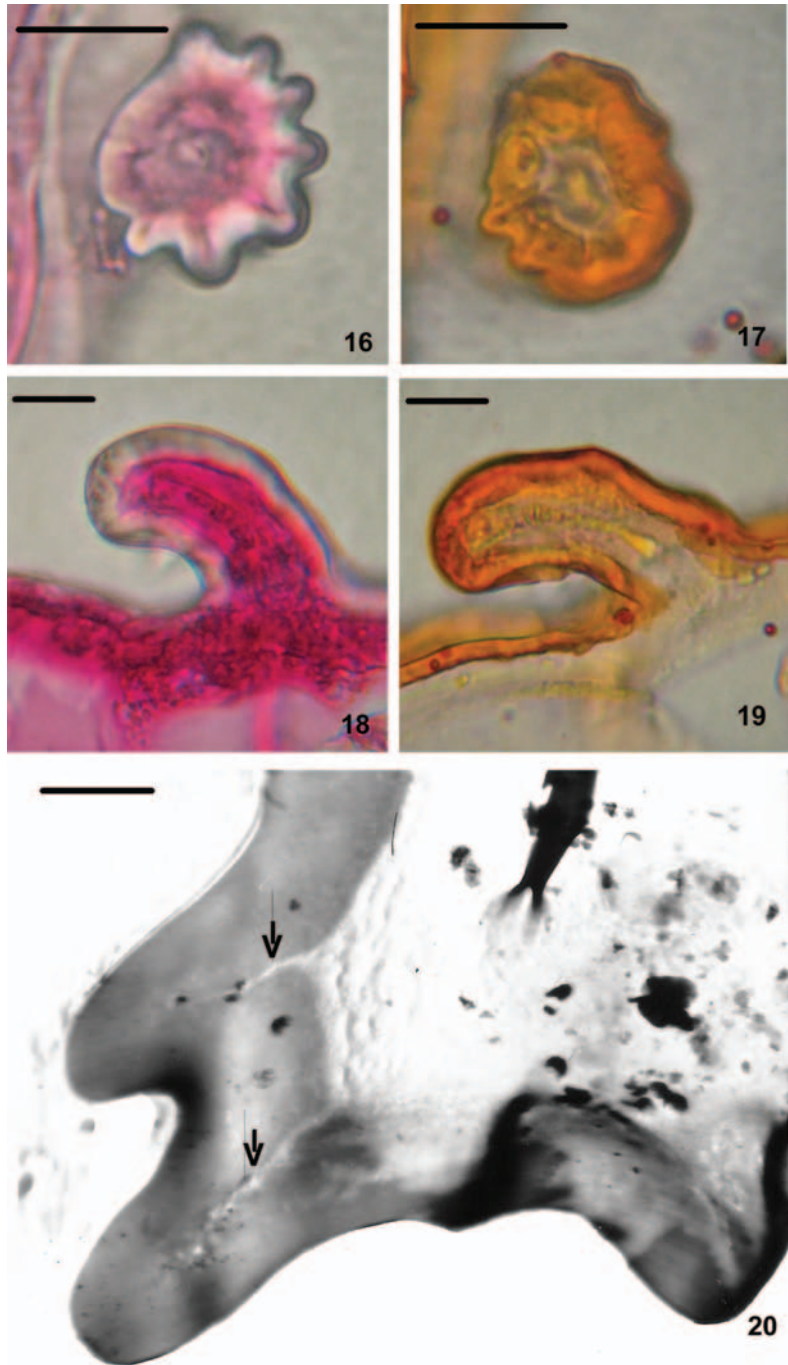
The papillae have a striate cuticle at the base and a smooth cuticle at the apex. The striations are thick and form deep channels over the surface of the papilla, and they are more numerous and conspicuous in *P. araucanus*, *P. australis*, *P. gonjianii*, *P. hickenii* and *P. reticulatus* (Figs. 21–27). In *P. araucanus* (Figs. 21, 22) the striation is extended toward the base of the papilla. In *P. tuberosus*, *P. megliolii* and *P. fischeri* (Figs. 28–30) the cuticle is mostly smooth, with scarce striation. The papilla itself is straight in *P. australis*, *P. gonjianii*, *P. hickenii* and *P. reticulatus* (Figs. 23–27), whereas it is curved in *P. araucanus*, *P. tuberosus* and *P. megliolii* (Figs. 21, 22, 28, 29). In *P. tuberosus* and *P. megliolii* (Figs. 28, 29), the base of the papilla is thinner than in the other species.

**Type 3:
Epidermal cells**

The sculpturing of the cuticle in the epidermal cells varies among *Pterocactus* species. In *P. gonjianii* the cuticle has thick, deep and parallel striations similar to those seen in papillae (Fig. 25). In *P. araucanus*, *P. tuberosus*, *P. megliolii* and *P. fischeri* the cuticle is slightly to irregularly striate (Figs. 21, 22, 28, 29, 30). *P. australis*, *P. hickenii* and *P. reticulatus* have a cell surface that exhibits practically no sculpturing (Figs. 24, 26, 27).

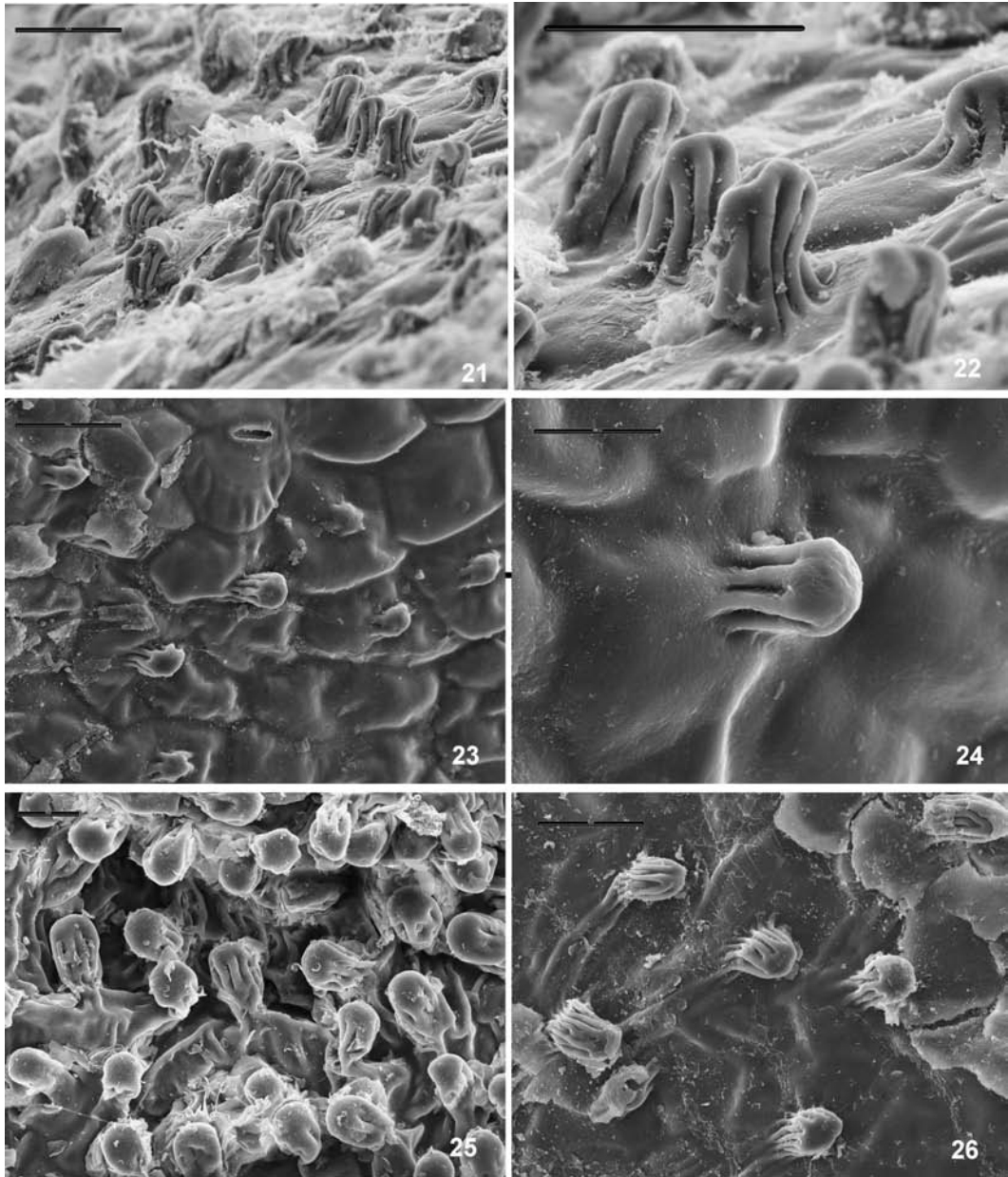
Discussion

The stomatal complex of *Pterocactus* species belongs to the paracytic type, which seems to be a common feature in the Opuntioideae. The members of the subfamilies Pereskioideae and Opuntioideae



Figures 16–20. *P. tuberosus*, detail of papillae. **16.** Transverse section of a papilla stained with ruthenium red, light microscopy. Scale bar = 25 µm. **17.** Transverse section of a papilla stained with Sudan III. Scale bar = 25 µm. **18.** Longitudinal section of a papilla stained with ruthenium red, light microscopy. Scale bar = 25 µm. **19.** Longitudinal section of a papilla stained with Sudan III. Scale bar = 25 µm. **20.** Transverse section of a papilla observed with transmission electron microscope. Detail of ectodesmata (arrows). Scale bar = 5 µm.

examined by Eggli (1984) showed parallelocytic stomata only on the leaves. This type of stomata was observed on the stems in the *Pterocactus* species studied

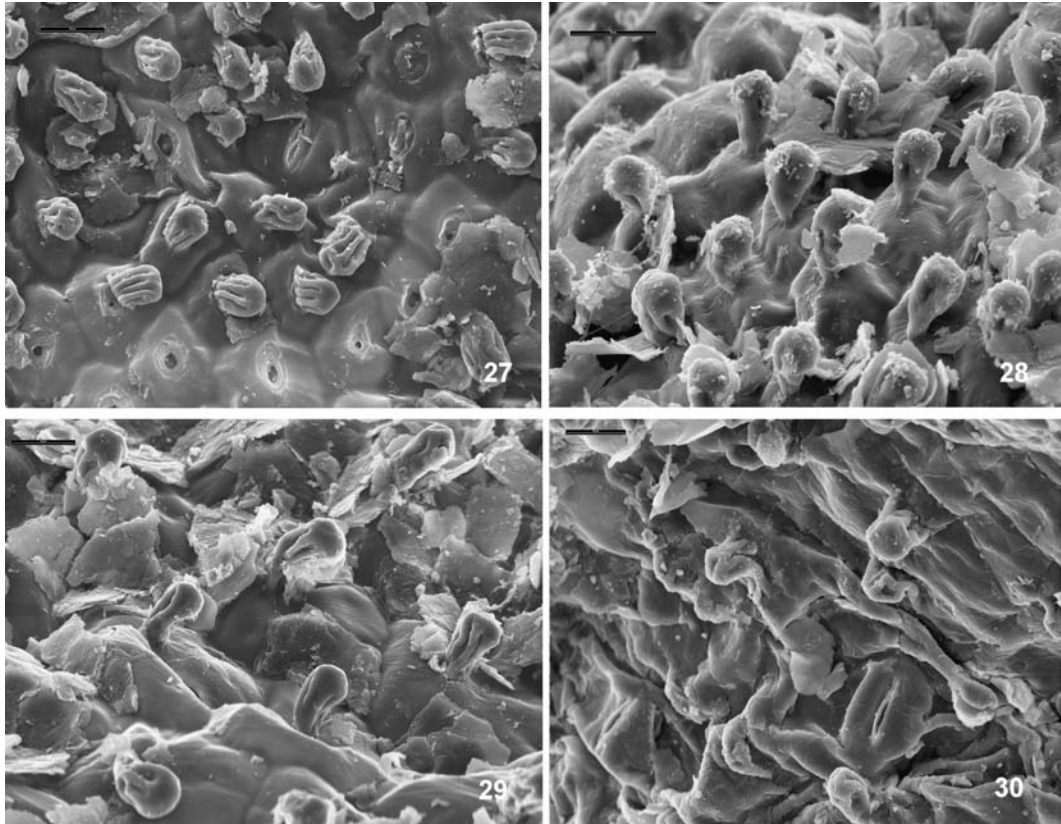


Figures 21–26. SEM observations of stem surface in *Pterocactus* spp. **21–22.** *P. araucanus*. Scale bars = 20 μm . **23.** *P. australis*. Scale bar = 50 μm . **24.** *P. australis*. Scale bar = 20 μm . **25.** *P. gonjaniii*. Scale bar = 20 μm . **26.** *P. hickenii*. Scale bar = 50 μm .

here. The asymmetrical arrangement and differences in the final number of subsidiary cells of the stomata could be determined by variation in the number of cell divisions of the subsidiary meristemoids, as was reported for five *Opuntia* species by Fernandez (1999).

Succulent xerophytes have fewer stomata per unit area, and their size is significantly larger, in comparison to non-succulent xerophytes (Fahn and Cutler 1992). According to Sunderberg (1986), stems of succulent plants have a mean density of 29.5 stomata per mm^2 , and the mean length of the stomata is

19.11 μm . Our data from the present study (Table 1) show that the mean densities of stomata in *P. australis*, *P. megliolii*, *P. reticulatus* and *P. tuberosus* are similar to the density reported by Sunderberg, but the mean sizes of the stomata we observed were somewhat larger (28–31 μm). *P. gonjaniii* is the only species in which the density of stomata is substantially higher, at 50 stomata per mm^2 . However, the size of the stomata in *P. gonjaniii* (31 μm) is similar to those of the previous species. *P. araucanus*, *P. fischeri* and *P. hickenii* have low stomatal densities, but the sizes of the stomata are considerably larger (40–47 μm).



Figures 27–30. SEM observations of stem surface in *Pterocactus* spp. **27.** *P. reticulatus*. Scale bar = 20 μm . **28.** *P. tuberosus*. Scale bar = 20 μm . **29.** *P. megliolii*. Scale bar = 20 μm . **30.** *P. fischeri*. Scale bar = 50 μm .

The difference between the densities of stomata in *P. reticulatus* (29/mm²) and *P. gonjianii* (56/mm²) is very notable. These species grow in the same localities and are similar in appearance. The second species was described some years later than the first, due to the doubts of the author about whether the two were truly different. Finally, the marked differences in fruit morphology confirmed that these were indeed separate species (Kiesling, et al. 2008), and the different stomatal densities not associated with different environments reaffirms this.

The presence of papillae is a common character of all species of *Pterocactus* studied in this work. The occurrence of this type of cells is not very frequent in the Cactaceae. Loza-Cornejo and Terrazas (2003) studied 70 species representing 21 genera of North American Cactoideae, and according to these authors papillae occur in only eight species belonging to four genera.

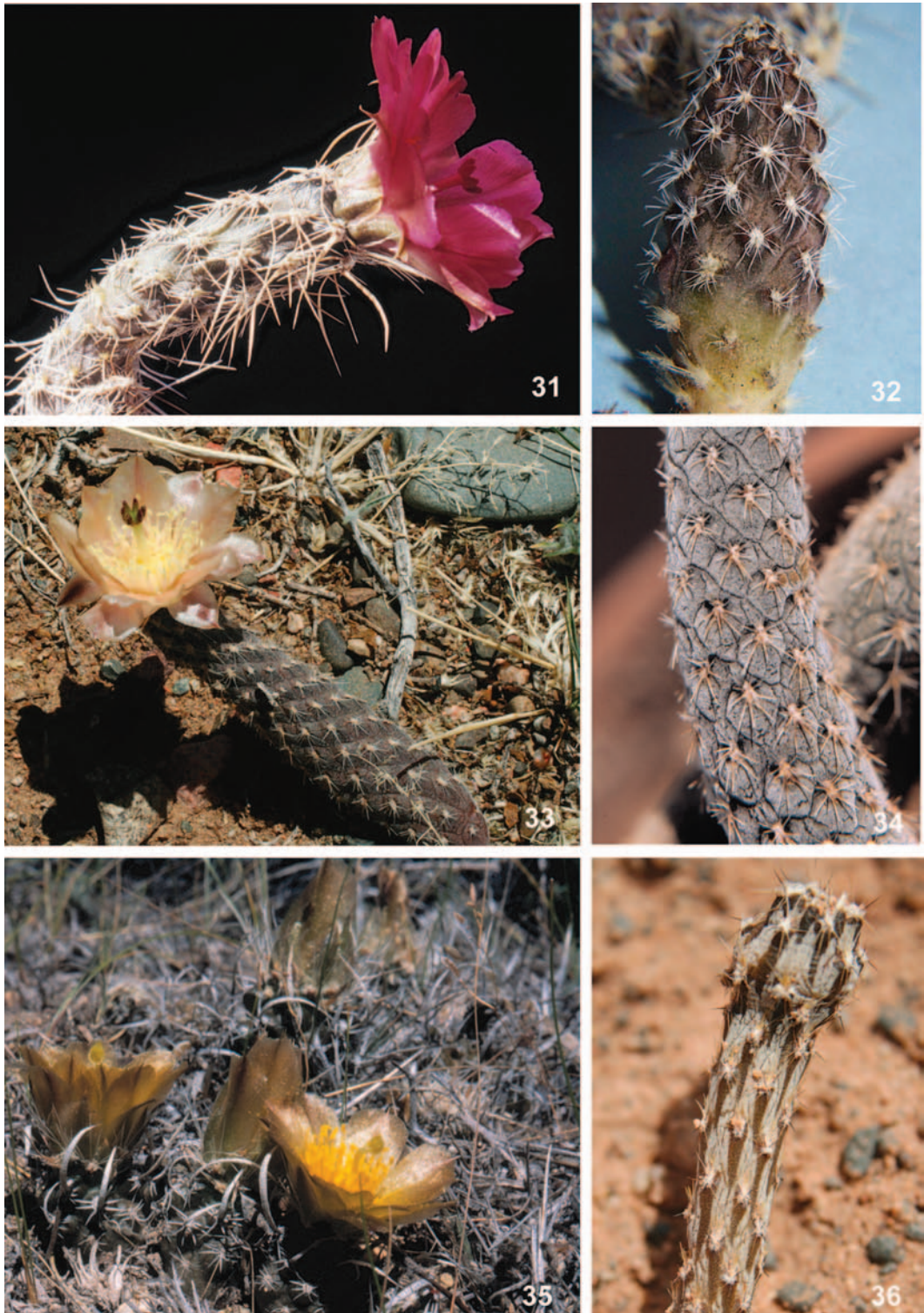
The presence of epidermal papillae is considered an adaptation to arid conditions. *Pterocactus* grows in areas where water is a restrictive factor for plant development. The dense layer of papillae increases stem reflectance and reduces radiation absorbance, resulting in a reduction of heat load and water loss by evapo-transpiration. The species that have higher densities of papillae (*P. gonjianii*, *P. megliolii*, *P. retic-*

ulatus and *P. tuberosus*) grow in the northwest of Argentina, where the UV radiation is very high, while the other species are distributed in the south of the country. A high density of papillae is observed as a silvery white epidermal surface, whereas a low density of papillae gives the appearance of a darker surface color in the living plants.

P. valentinii (Speg.) Britton & Rose is the only species of the genus not included in this study. It was excluded because in the one specimen analyzed we didn't observe any papillae. This species has small stems and areoles with many thin spines, similar to the young stems of any of the other species. Therefore, *P. valentinii* could be a neotenic form of one or more of the other species.

The ectodesmata present in the papillae of *Pterocactus* could be implicated in the secretion of the waxes which cover the epidermal surface. According to Lyshede (1982), the ectodesmata have been involved in processes of foliar absorption and excretion in xerophytes.

The nine species of *Pterocactus* were originally defined according to morphological characters of spines and stems (Kiesling 1982). A phylogenetic study based on the sequence analysis of trnL intron/trnL-trnF in chloroplast DNA did not resolve relationships within *Pterocactus*, but confirmed the



Figures 31–36. *Pterocactus* spp. 31–32. *P. fischeri*. 33–34. *P. gonjani*. 35. *P. australis*. 31–35. Papillae are distributed uniformly; the stem surface is observed as silvery white. 36. *P. tuberosus*. The distribution of the papillae is seen as white stripes that alternate with dark bands of non-papillate cells.

monophyly of it (Cota-Sanchez et al., unpublished data).

Based on cuticle sculpturing, size, density and morphology of stomata and papillae, we propose the following key to separate *Pterocactus* species:

- 1- Cuticle notably striate, density of stomata high (50/mm²)-----*P. gonjianii*
- 1'- Cuticle smooth or lightly striate, stomata density low or medium (12–40/mm²) -----2
- 2- Papillae with thinned base -----3
- 2'- Papillae without thinned base ----- 4
- 3- Papillae arranged in bands-----*P. tuberosus*
- 3'- Papillae without a regular pattern of distribution-----*P. meglolioli*
- 4- Density of papillae low (less than 30/mm²)----
---*P. australis*
- 4'- Density of papillae moderate to high (higher than 100/mm²)-----5
- 5- Density of papillae high (higher than 500/mm²)----*P. reticulatus*
- 5'- Density of papillae medium (100–200/mm²)
-----6
- 6- Stomata with one pair of subsidiary cells-----
P. hickenii
- 6'- Stomata with two pairs of subsidiary cells, or more-----7
- 7- Stomata with 5–6 subsidiary cells-----
---*P. fischeri*
- 7'- Stomata with exactly two pairs of subsidiary cells-----
-----*P. araucanus*

A paper currently in preparation, based on analyses of morphological, embryological and other anatomical characters, will further contribute to a robust delimitation of the species of *Pterocactus*.

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