Micro-morphology and anatomy of *Turbinicarpus* (Cactaceae) spines

Micromorfología y anatomía de las espinas de *Turbinicarpus* (Cactaceae)

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Abstract. Spines are a striking feature of cacti and display wide variation in size, number, shape, and texture. This study showed that *Turbinicarpus* species exhibit not only a high variability in the gross morphology of the spines, but also in their micro-morphology. Their surface can be smooth or ornamented with projections that can be low, conical, pinnate, or long trichomes. The epidermis can be continuous, broken up into single cell elements or transversely fissured, the fissures extending deeply into the underlying sclerenchyma. The mechanical properties of the spines are related to their anatomy, here documented for the first time. The woody rigid spines being made up of fibers with thick walls (> 3 μm), while papery or corky spines have a sclerenchyma made up of fibers with thin walls (< 2 μm). Alternatively, spine anatomy can be dimorphic with the outermost layers made up of thin-walled fibers and an inner core made up of thick-walled fibers. *Turbinicarpus* taxonomy mostly relies on spine features and the newly collected data can contribute to a better understanding of the interspecific relationships. The epidermal features like long trichomes or the lack of ornamentation as well as the modified anatomy of corky spines may be apomorphic characters within the genus.

Key words: spine anatomy, sclerenchyma, fibers, spine micro-morphology.

Resumen. Las espinas son una de las características más distintivas de las cactáceas y se distinguen por su variación en tamaño, número, forma y textura. Este estudio muestra que las especies de *Turbinicarpus* no sólo tienen variación en la morfología de sus espinas, sino también en su micro-morfología. Su superficie puede ser lisa u ornamentada con proyecciones bajas, cónicas, pinadas o bien con tricomas largos. En las espinas, la epidermis se mantiene continua, separada en sus células o transversalmente fisurada. Las fisuras de la epidermis pueden prolongarse hasta el esclerénquima más interno. Las propiedades mecánicas de las espinas están relacionadas con su anatomía, aquí documentada por primera vez. Las espinas rígidas están constituidas de fibras con paredes gruesas (> 3 μm), mientras que las espinas suaves o corchosas, también denominadas cerdas tienen esclerénquima de fibras con paredes delgadas (< 2 μm). Además, algunas espinas en su madurez tienen 2 tipos de fibras, las pobremente lignificadas en la parte externa y en la interna las de paredes gruesas y lignificadas. La taxonomía de *Turbinicarpus* se basa principalmente en las espinas y los datos aquí generados contribuyen a un mejor entendimiento de sus relaciones interespecíficas. El conocimiento de las características epidermales como los tricomas largos, la carencia de ornamentación o las modificaciones anatómicas de las espinas corchosas pueden interpretarse como caracteres apomórficos en el género.

Palabras clave: anatomía de la espina, esclerénquima, fibras, micro-morfología de la espina.

Introduction

Turbinicarpus (Backeb.) Buxb. et Backeb. is a small genus of Mexican cacti, which comprises about 30 species (Hunt, 2006). They are small-sized, globose, tuberculate plants with variable spination, sometimes strongly mimetic. The genus belongs to the tribe Cacteae (Cactaceae) and the most recent phylogeny of this tribe based on *rpl*16 intron sequence variation places it within a poorly supported clade together with *Ariocarpus* Scheidw., *Epithelantha* F.A.C. Weber ex Britton et Rose and *Pediocactus* Britton

et Rose (Butterworth et al., 2002). The taxonomy of *Turbinicarpus* remains the object of continuous changes that are reflected at a nomenclatural level in the large number of names which is available today to classify the known taxa (Anderson, 1986; Lüthy, 2002; Donati et Zanovello, 2004; Hunt, 2006). In this genus the spines show an incredible variation in size, number, shape, and texture and are commonly described as acicular, bristly, spongy, corky, papery or plumose, being a key feature for its taxonomy (Anderson, 1986; Lüthy, 2002).

Despite the great importance that spines have in the classification of Cactaceae, little is known about their micro-morphology, anatomy, and chemical composition.

Cactus spines are the modified bud scales of an axillary bud, originating from primordia that are morphologically indistinguishable from the leaf primordia, and at maturity they consist of only 2 cell types, both dead, libriform fibers and sclerified epidermis (Mauseth, 2006). The micro-morphology of the spine epidermal cells of Cactaceae is highly variable, as shown by Schill et al. (1973). The authors have classified the different surface features into 3 main types and 6 sub-types. Pereskioideae and Maihuenioideae have spines with a smooth surface and prosenchymatic cells, which is interpreted as the most primitive type based on phylogenetic studies (Nyffeler, 2002; Edwards et al., 2005). In the Opuntioideae the spines and glochids, which are modified spines abscised at their base, share the same surface texture, being both retrorsely barbed (Robinson, 1974). This feature, named glochidioid type by Schill et al. (1973), is characteristic of this subfamily. The Cactoideae display, instead, a much greater variation in surface characters. The epidermis can be continuous, broken up into single cell elements or crossed by deep fissures that reach the underlying sclerenchyma. Epidermal cell outer walls are smooth or, usually, bear projections that can be short or very long, giving a plumose appearance to the spines.

The anatomy of the spines is very simple. They are modified leaves without guard cells, stomata, hypodermis, chlorenchyma and vascular tissue. Instead, they consist of sclerenchyma and a sclerified epidermis (Mauseth, 2006). The structure and chemical composition of cactus spines have to date been studied only in 1 species, *Opuntia ficus-indica* (L.) P. Miller (Malainine et al., 2003). The main and most obvious function of the spines is to provide a defence against herbivores, but they also protect the plants from temperature stress (Gibson and Nobel, 1986). In many opuntias, joints and fruits are easily detached when their barbed spines easily stick to the skin, allowing animals to disperse plant parts over wide areas. The spines of *Copiapoa haseltoniana* Backeb. are finely grooved and the fog condensing on them during the night flows down the grooves to the areoles and then wets the epidermis, lowering stem temperature by evaporation (Mooney et al., 1977). In some species, it has been reported that water could be absorbed through the spines. Schill and Barthlott (1973) have found that after having applied radioactive orthophosphate to the spines of *Turbinicarpus schmiedickeanus* ssp. *klinkerianus* (Backeb. et Jacobsen) N.P. Taylor and *Discocactus horstii* Buining et Breederoo ex Buining the radioactivity could be detected in the stem close to the areole. A similar experiment was carried out by Porembski (1994) on *Corynopuntia invicta* (Brandegee) F.M.Kunth using a dye with similar results. The spines of these 3 species have an interrupted epidermis that allows a quick and easy imbibition of the underneath tissue so that water can reach the stem by capillarity. What effect this process of water absorption through the spines can have on the overall water balance of the plants has not yet been investigated.

In this study the micro-morphology and anatomy of *Turbinicarpus* spines are reported and their role in affecting the spine gross morphology as well as their use in assessing species relationships are discussed.

Materials and Methods

Spine samples were obtained from plants in the collection of the author. All the plants have been raised from documented seeds purchased from local nurseries or obtained from the Turbinicarpus Gruppe Association (Table 1). The plants, cultivated in xeric conditions, exhibited a shoot and spine morphology matching that given in the original descriptions as well as the features described in the current literature (Anderson, 1986; Lüthy, 2001; Donati and Zanovello, 2004). This suggests that no modifications occurred in spine anatomy. Five spines were collected for every studied taxon and the spine surface observed with a stereomicroscope. A single spine was prepared for SEM observations, 2 spines were observed unmounted with a light microscope to record surface characters and 2 spines were used for sectioning. For scanning electron microscopy, the spines were fixed to stubs with bi-adhesive tape, sputter-coated with gold and observed with a Stereoscan 430i Leica microscope. For light microscopy, the spines were embedded in resin (Araldite 502/EMbed 812, Electron Microscopy Sciences) and sectioned at 4 μm thickness using glass knives with a Top Ultra 150 microtome (Pabisch, Milan, Italy). The sections were stained with toluidine blue 0.5% for 30 sec, rinsed in water and immediately observed. Individual fiber cells were obtained by boiling the spines in HCl 5 N for 30 min and then the fibers separated from each other with a needle. Features evaluated were as follows: epidermis: in surface view the presence of fissures and the type of trichomes, in cross sections the presence of cell inclusions, the longest cell axis and wall thickness; for sclerenchyma: in transverse sections, cell deposits, the longest cell axis and wall thickness. In macerations fibers wall deposition, cell deposits and length. Morphometric values, mean and standard deviation, were obtained from 25 measurements with the exception of fiber length that were obtained from 4-7 measurements using ImageJ (Rasband, 2007). In a few cases, namely for *T. pseudopectinatus*, *T. valdezianus* and *T. ysabelae*, 10 to 20 measurements were done due to the difficulties encountered to obtain satisfactory sections.

Table 1. List of examined material from Mexico. Abbreviations for collectors are as follows: BZ: Battaia et Zanovello, HO: Anton Hofer, TCG: *Turbinicarpus* Gruppe

Species	Collection num.	Locality
Turbinicarpus alonsoi Gl. et Arias	TCG 21001	Xichú, Guanajuato
Turbinicarpus bonatzii Frank	TCG 20001	Los Cerritos, San Luis Potosí
Turbinicarpus dickisoniae (Gl. et F.) Gl. et Hofer	TCG 14001	El Olmo A, Nuevo León
Turbinicarpus flaviflorus Frank et Lau	TCG 13001	Santa Rita, San Luis Potosí
Turbinicarpus gielsdorfianus (Werd.) John et Riha	TCG 43001	Las Tablas, San Luis Potosí
Turbinicarpus gracilis Gl. et F.	TCG 12001	Aramberri, Nuevo León
Turbinicarpus hoferi Luethy et Lau	HO 434	Aramberri, Nuevo León
Turbinicarpus horripilus (Lem.) John et Riha	TCG 40201	San Pablo, Hidalgo
Turbinicarpus jauernigii Frank	TCG 18001	Palomas, San Luis Potosí
Turbinicarpus knuthianus (Boed.) John et Riha	TCG 45006	Pozo de Acuña, San Luis Potosí
Turbinicarpus laui Gl. et F.	TCG 11003	Mezquite Grande, San Luis Potosí
Turbinicarpus lophophoroides (Werd.) Buxb. et Backeb.	BZ 25	Las Tablas, San Luis Potosí
Turbinicarpus macrochele (Werd.) Buxb. et Backeb.	TCG 3002	San Antonio, San Luis Potosí
Turbinicarpus macrochele ssp. *frailensis* Lechner et Jantschgi	TCG 3003	El Herrero, San Luis Potosí
Turbinicarpus macrochele var. *polaski* Lechner et Jantschgi	TCG 19007	La Pastoriza, San Luis Potosí
Turbinicarpus pseudomacrochele (Backeb.) Buxb. et Backeb.	TCG 5004	Cárdonal, Querétaro
Turbinicarpus pseudomacrochele ssp. *krainzianus* (Frank) Gl.	TCG 9001	s. loc.
Turbinicarpus pseudomacrochele ssp. *lausseri* (Diers et Frank) Gl.	TCG 9201	Sierra El Doctor, Querétaro
Turbinicarpus pseudomacrochele ssp. *minimus* (Frank) Luethy et Hofer	TCG 9101	Davoxtha, Hidalgo
Turbinicarpus pseudopectinatus (Backeb.) Gl. et F.	TCG 6002	Dr Arroyo, Nuevo León
Turbinicarpus rioverdensis Frank	HO 733	Rio Verde, San Luis Potosí
Turbinicarpus saueri (Boed.) John et Riha	TCG 42001	San Vincente, Tamaulipas
Turbinicarpus schmiedickeanus ssp. *andersonii* Mosco	TCG 1101	Rancho Nuevo, Nuevo León
Turbinicarpus schmiedickeanus ssp. *rubriflorus* (Frank) Panarotto	TCG 1301	Cerros Blancos, Nuevo León
Turbinicarpus schmiedickeanus ssp. *klinkerianus* (Backeb. et Jcb) Taylor	TCG 7002	La Verdolaga, San Luis Potosí
Turbinicarpus swobodae Diers	TCG 15001	Rayones, Nuevo León
Turbinicarpus valdezianus (Moel.) Gl. et F.	TCG 2001	Saltillo, Coahuila
Turbinicarpus viereckii (Werd.) John et Riha	TCG 46001	Jaumave, Tamaulipas
Turbinicarpus viereckii ssp. *major* (Gl. et F.) Gl.	TCG 46102	Lazaro Cardenas, Nuevo León
Turbinicarpus ysabelae (Schlange ex Croizat) John et Riha	BZ 33	Tula, Tamaulipas

Results

Spines with thick-walled fibers. Thick-walled fibers compose spines that are hard, rigid to sub-rigid, mostly pungent, only the thinnest being bristly (Table 2). The epidermis is usually continuous (Fig. 1A,C,E,F), but can be broken up into single cell elements like in *T. jauernigii*, and its surface is highly variable. The epidermal cells have outer periclinal walls smooth as in *T. gielsdorfianus*, *T. jauernigii*, *T. lophophoroides*, *T. saueri* and *T. ysabelae* (Fig. 1A) or with projections at their distal endings. These projections are differently shaped: they are low domed in

T. hoferi, tubercled in *T. pseudopectinatus*, pinnate in *T. horripilus*, *T. knuthianus*, *T. laui*, *T. pseudomacrochele*, *T. swobodae* and *T. viereckii* or very long as in *T. valdezianus* (Fig. 1C-F). Epidermis is sclerified, uniseriate, with rectangular to ovoid cells with empty lumina. Underneath the epidermis there are 1 or 2 layers of sub-epidermal cells with thin walls, on average less than 1 μm thick, whose lumina can be empty or filled with tannins. These cells are replaced inwards by fibers with thick secondary walls, ranging from 2.3 μm to 5.9 μm, that form the bulk of the spine. Thick-walled cells are rounded to elliptic in cross section, with the lumina completely occluded

Table 2. Morphologycal and anatomical characters of *Turbinicarpus* spines. Cell diameter refers to the longest axis in transverse section. Morphometric values are expressed as mean ± standard deviation. na = not available, nm = not measured, no = not observed.

	Morphology		Spine surface	Epidermis		Thick-walled fibers		Thin-walled fibers		Fiber length μm	Helical pattern
	Texture	Sharpness		Surface	Projections	Cell diam. μm	Wall thickness μm	Cell diam. μm	Wall thickness μm		
Spines with thick-walled fibers											
T. gielsdorfianus radial spine	woody	pungent	even	continuous	none	10.3 ± 2	3.8 ± 0.7	9.3 ± 0.6	1 ± 0.4	nm	na
T. hoferi radial spine	woody	sub-pungent	even	continuous to slightly broken up	low tubercles	9.4 ± 2.1	3.2 ± 0.7	no	no	nm	na
T. horripilus central spine	woody	pungent	even	continuous	pinnate	8.5 ± 2	2.7 ± 0.6	13.8 ± 4.6	1.2 ± 0.3	nm	na
T. jauernigii radial spine	woody	sub-pungent	even	broken up	none	12.1 ± 3	2.3 ± 0.5	no	no	nm	na
T. knuthianus central spine	bristly or woody	sub-pungent to pungent	even	continuous	pinnate	9.9 ± 2.3	3 ± 0.7	no	no	nm	na
T. laui radial spine	bristly	non-pungent	even	continuous	pinnate	11.2 ± 2.4	1.5 ± 0.4	10.7 ± 2.8	0.8 ± 0.1	nm	na
T. lophophoroides radial spine	woody	sub-pungent to pungent	even	broken up	none	8 ± 1.9	2.3 ± 0.7	5.7 ± 1.9	0.8 ± 0.1	nm	na
T. pseudomacrochele ssp. *minimus*, radial spine	bristly	non-pungent	even	continuous	pinnate	6.8 ± 2	1.7 ± 0.5	no	no	nm	na
T. pseudopectinatus radial spine	woody	sub-pungent	even	broken up	tuberculate	9.2 ± 2.4	3.4 ± 0.8	no	no	nm	na
T. saueri central spine	woody	pungent	even	continuous to slightly broken up	none	10.8 ± 2.8	3.2 ± 0.8	no	no	nm	na
T. swobodae radial spine	bristly or woody	non pungent to sub-pungent	even	broken up	pinnate	11 ± 2.1	3.7 ± 0.8	12 ± 2.3	0.9 ± 0.3	nm	na
T. valdezianus radial spine	woody	non pungent	even	continuous	trichomes	10 ± 1.3	4.1 ± 0.9	no	no	nm	na
T. viereckii central spine	woody	pungent	even	continuous	pinnate	10.7 ± 2.1	3.2 ± 0.8	9.6 ± 1.2	0.8 ± 0.2	490 ± 90	-
T. ysabelae radial spine	woody	pungent	even	continuous	none	18.4 ± 5	5.9 ± 1.5	13.8 ± 3.5	1.6 ± 0.6	nm	na

Spines with thick- and thin-walled fibers

Table 2. Continues

T. alonsoi radial spine	corky	non-pungent	fissured	broken up	none	7.8 ± 1.4	0.9 ± 0.2	11.1 ± 5.6	0.8 ± 0.4	320 ± 120	-
T. dickisoniae central spine	bristly	non-pungent	even	broken up	none	10.1 ± 2	2.3 ± 0.5	11.2 ± 2.8	1.5 ± 0.3	530 ± 130	-
T. gracilis central spine	papery	non-pungent	even	continuous	none	13.1 ± 2.6	1.4 ± 0.3	12.5 ± 2.8	1 ± 0.2	470 ± 100	-
T. macrochele ssp. frailensis, radial spine	corky	non-pungent	fissured	broken up	none	9.4 ± 2	1.8 ± 0.5	14.3 ± 6.2	0.9 ± 0.3	540 ± 80	+
T. rioverdensis radial spine	corky	non pungent	fissured	broken up	none	9.9 ± 2.4	3.1 ± 0.9	18.9 ± 7.8	1 ± 0.2	410 ± 90	+
T. schmiedickeanus ssp. klinkerianus, radial spine	corky	non pungent	fissured	broken up	none	10.4 ± 2.3	0.7 ± 0.2	14.9 ± 5.6	0.5 ± 0.2	420 ± 70	+
Spines with thin-walled fibers											
T. bonatzii radial spine	papery	non-pungent	even	broken up	none	no	no	13.5 ± 4	1.3 ± 0.6	320 ± 70	+
T. flaviflorus radial spine	corky	non-pungent	even	continuous	none	no	no	16 ± 4.8	1.5 ± 0.3	500 ± 60	+

(Fig. 2A). In the case of the bristly spines of *T. laui* and *T. psedomacrochele*, secondary walls are not so heavily sclerified, attaining a thickness of 1.5 to 1.7 μm, and the cells have narrow lumina (Fig. 2C).

Spines with thick and/or thin-walled fibers. Papery or corky spines are sub-rigid to flexible, non-pungent (Table 2). The spine surface can be entire as in *T. bonatzii*, *T. dickisoniae*, *T. flaviflorus*, and *T. gracilis* or fissured like in *T. alonsoi* (Fig.1B), *T. macrochele*, *T. rioverdensis*, and *T. schmiedickeanus*. Epidermis can be continuous as in *T. flaviflorus*, broken up into single cell elements as in *T. alonsoi* or transversely cut by deep fissures that involve also the underneath layers in *T. alonsoi*, *T. macrochele*, *T. rioverdensis*, and *T. schmiedickeanus*. Outer periclinal walls are always devoid of any ornamentation (Fig. 1B). Epidermal cells are on average very wide, rectangular to ovoid, with the largest axis more than 20 μm in cross-section and exceptionally wide in *T. alonsoi* where were found cells with 45 μm in the largest axis (Fig. 2D). The epidermis is uniseriate. Underneath the epidermis there are 1 or 2 layers in *T. alonsoi*, *T. horripilus,* and *T. swobodae* or 2 to several layers of thin-walled fibers with wide lumina that inwards reduce gradually their diameter (Fig. 2). These thin-walled fibers usually are replaced in the center of the spine by thick-walled fibers. The inner fibers have walls 2-3 μm thick, being circular to elliptic in transverse section with occluded lumina or have walls < 2 μm, and then the cells are collapsed. In *T. bonatzii*, *T. gracilis*, and *T. flaviflorus* there are only fibers with open lumina and walls < 2 μm thick (Fig. 2E, F). In *T. bonatzii*, *T. flaviflorus*, *T. macrochele*, *T. rioverdensis*, and *T. schmiedickeanus* there are thin-walled fibers displaying cell walls with a helical pattern (Fig. 3A) as seen in macerations. Tannins were observed in the outermost sclerenchyma cells, but not in the epidermis (Fig. 2-3B).

Discussion

Spine traits. Spines are the most striking feature of cacti because of their structural characteristics and morphological variability. The spines of *Turbinicarpus* not only are highly variable in their morphology, but are also extraordinarily diverse in their surface micro-morphology. The epidermis can be continuous, broken up into single cells or fissured together with the under laying sclerenchyma layers. The epidermal cells can have the outer periclinal walls smooth or with short projections or long trichomes. At a juvenile stage, all *Turbinicarpus*, except *T. saueri*, *T. swobodae* and *T. viereckii*, have plumose spines that as development goes on undergo a reduction of trichome length that in some species, at the adult stage, are completely absent (Mosco
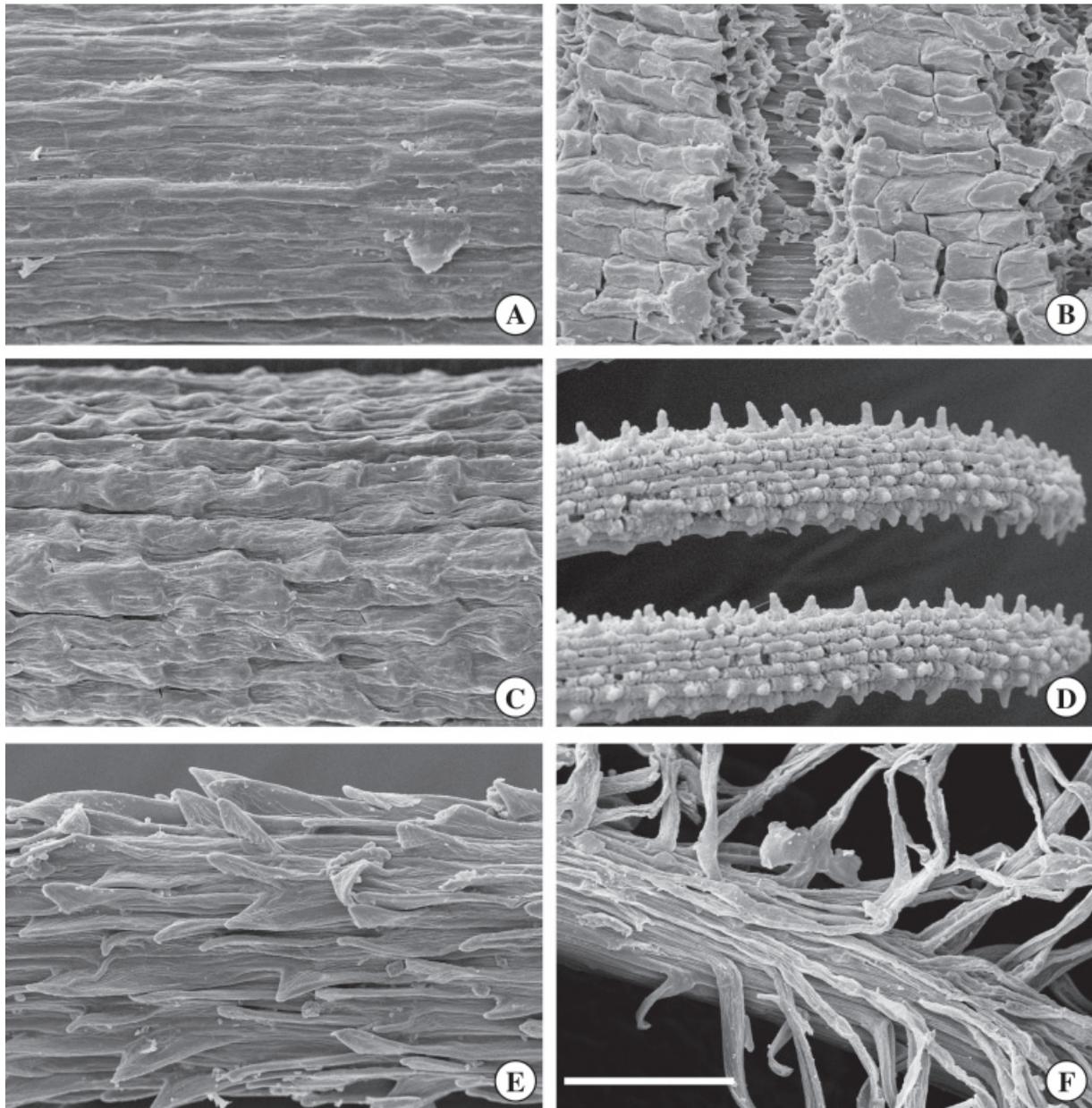
Figure 1. Scanning electron micrographs of spine epidermis in *Turbinicarpus* species. A, *T. ysabelae*, central spine with a continuous epidermis devoid of ornamentation. B, *T. alonsoi*, radial spine with the epidermis broken up into single cells devoid of projections and a deep fissure affecting epidermis and the underneath thin-walled cell layers. C, *T. hoferi*, radial spine with a continuous epidermis, epidermal cells with low domed projections. D, *T. pseudopectinatus*, radial spine with the epidermis broken up into single cells, epidermal cells with tubercled outer periclinal walls. E, *T. pseudomacrochele*, radial spine with a countinuous epidermis and epidermal cells showing pinnate projections. F, *T. valdezianus*, radial spine with a continuous epidermis and epidermal cells showing long trichomes. Scale bar = 100 μm.

and Zanovello, 2003). Unique exception is *T. valdezianus* that also at the adult stage bears feathery spines, being so the most neotenic species of the genus.

Schill et al. (1973) recognized in the Cactoideae 3 different evolutionary tendencies leading from epidermal cells with projections to epidermal cells bearing long trichomes or devoid of any projection. The third line concerns the spines with an epidermis broken up in single elements without projections. All of these character states can be found in *Turbinicarpus*. On this basis it is possible
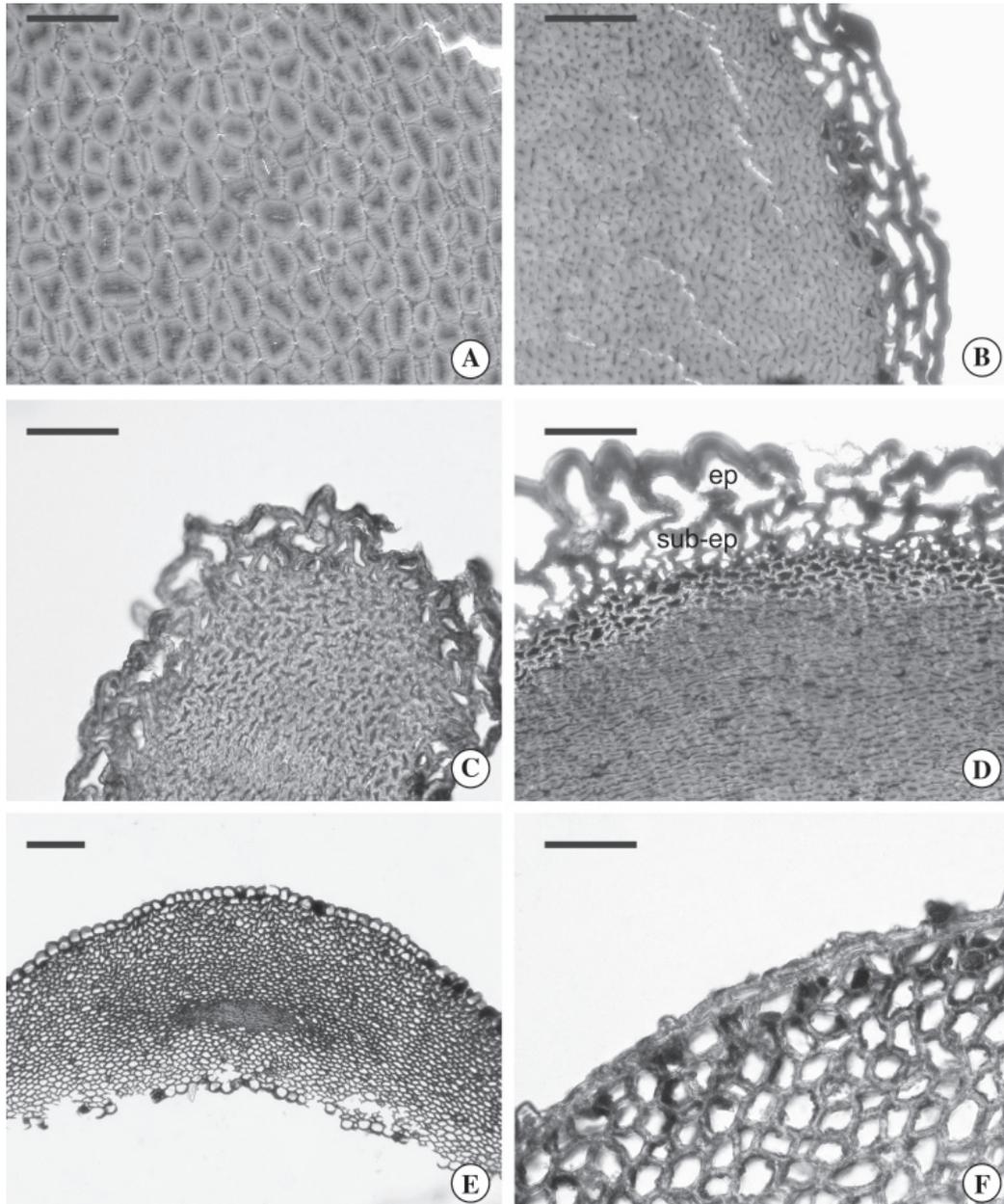
Figure 2. Spine cross sections. A, *T. ysabelae*, radial spine, fibers with very thick walls occluding lumina. B, *T. horripilus*, central spine, rectangular epidermal cells, 2 layers of sub-epidermal thin-walled cells followed by thick-walled fibers. C, *T. pseudomacrochele*, radial spine, wide epidermal cells and of the core of fibers with partially collapsed walls and narrow lumina. D, *T. alonsoi*, radial spine, wide epidermal cells with thick outer walls (ep), 1 layer of sub-epidermal cells with wide lumina (sub-ep), several outer layers of thin-walled fibers mostly filled with tannins and the inner layers of thick-walled fibers. E, *T. gracilis*, central spine, uniseriate epidermis with wide, ovoid cells and the outer layers of wide, thin-walled fibers and an inner core of fibers with collapsed walls and almost entirely occluded lumina. F, *T. flaviflorus*, radial spine, uniseriate epidermis with rectangular cells and exclusively wide, thin-walled fibers with tannins in some cells occluding lumina. Scale bars; E = 100 μm, others = 40 μm.

to consider the species with epidermal cells bearing conical or pinnate tubercles, namely *T. horripilus*, *T. laui*, *T. pseudomacrochele*, *T. pseudopectinatus*, *T. swobodae*, and *T. viereckii* as ancestral, while *T. gielsdorfianus*, *T. jauernigii*, *T. hoferi*, *T. knuthianus*, *T. lophophoroides*, *T. saueri* and *T. ysabelae* are all species with strongly reduced projections or lacking them completely and thus can be regarded as derived. The species with corky or papery spines share 2 apomorphic characters. They have an epidermis devoid of any ornamentation, which is
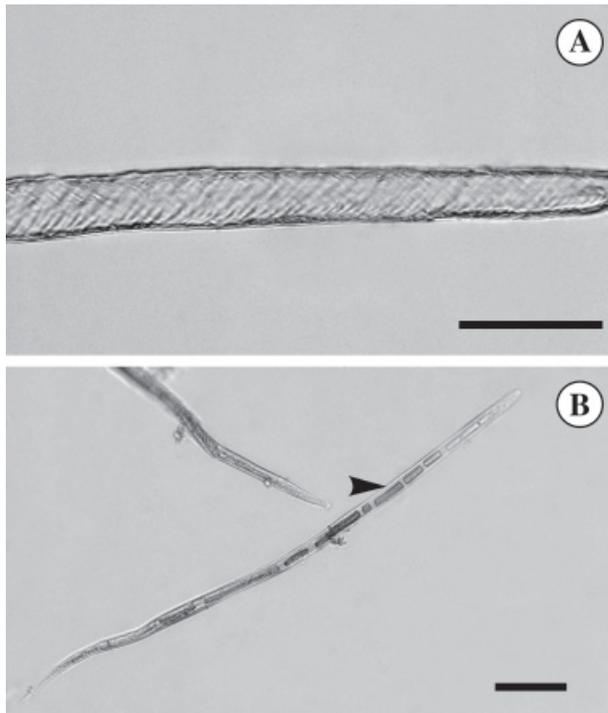
Figure 3. Spine macerated fibers. A, *T. flaviflorus*, a thin-walled fiber with the cell wall displaying a helical pattern. B, *T. dickisoniae*, a fiber filled with tannins (arrow). Scale bar = 50 μm.

usually broken up into single cell elements, and have an unusual spine anatomy. The presence of thick- and thin-walled fibers or only thin-walled fibers makes these species derived. Another highly derived species is *T. valdezianus*, which is strongly neotenic. In fact, the juvenile stages of *T. valdezianus* have feathery spines, as most turbinicarpi do, that persist for the whole life.

The survey by Schill et al. (1973) was limited to about 90 species of cacti, with 1 or 2 species per genus; therefore it did not allow evaluation of the degree of the interspecific morphological variability. An indication that the micro-morphological variability of the spines in a single genus can be rather great was given by Lüthy (1995) for *Mammillaria*. In this genus, there are species with a continuous epidermis or broken up into single cells and the epidermal cell outer walls are smooth, pinnate, or bearing long trichomes. Therefore, it was not a surprise to find that in *Turbinicarpus* too there is great variability in the micro-morphology of the epidermal cells. Nothing is known about the function that this ornamentation can have in cactus spines. Xerophytes usually have a sculptured epidermal surface that is considered an adaptation to arid environments. It has been proposed that secondary

and tertiary sculptures reduce the contamination by dust particles as well as help in temperature control. A sculptured surface may increase the thermal exchange with the surrounding air due to a wider surface and an increased turbulency in airflow (Barthlott, 1981). As spines represent an effective mean to control stem temperature (Gibson and Nobel, 1986), it may well be that trichomes improve this control. Only for *T. valdezianus* can it be speculated that the long trichomes, which give the spines a plumose appearance, serve to shade the plant greatly reducing the incidence of the strong sunlight. Nevertheless, it has to be stressed that in this species the plumose spines present at the adult stage are due to heterochrony (Mosco and Zanovello, 2003), therefore their presence is more likely due to the neoteny of this species rather than to adaptive processes to local environmental conditions.

Spines are modified leaves with a sclerified epidermis and a mesophyll made up only of a sclerenchyma. Most Cactoideae have a single-layered epidermis, while a multiple epidermis is rare. Uniseriate epidermis was observed in 2 *Turbinicarpus* species, *T. schmiedickeanus* and *T. valdezianus* (Loza-Cornejo and Terrazas, 2003). Almost all *Turbinicarpus* have spines with a single-layered epidermis, which is in agreement with the data reported for the stem epidermis, but in *T. alonsoi*, *T. horripilus* and *T. swobodae* are present 1 or 2 layers of distinctive sub-epidermal cells, whose origin can be assessed only by developmental studies. In *Turbinicarpus,* the sclerenchyma can be made up of fibers with thick walls occluding their lumina, with thin walls or with distinctive helical wall pattern deposition. The single thick walled-fibers have a length of 490 ± 90 μm, a size that is comparable to that reported for *Opuntia polyacantha* (Mauseth, 1977). The anatomical study of the corky, flexible spines of *T. alonsoi*, *T. bonatzii*, *T. dickisoniae*, *T. flaviflorus*, *T. gracilis*, *T. macrochele*, *T. rioverdensis*, and *T. schmiedikeanus* here presented has revealed the presence of thin-walled fibers together with thick-walled fibers that are restricted to an inner core. These thin-walled fibers have a length similar to that of thick-walled fibers, but have a larger diameter, thinner walls, and open lumina making a loose sclerenchyma. In *T. bonatzii*, *T. flaviflorus*, *T. macrochele*, *T. rioverdensis*, and *T. schmiedikeanus* there is evidence of a secondary helical wall pattern deposition. It is possible that the deposition of the secondary wall starts in a helical pattern; pattern which is lost as wall deposition increases. The degree of the wall thickness of the inner core fibers may be related to the plant age. In fact, younger plants of *T. dickisoniae* and *T. gracilis* showed thinner walls of centermost fibers compared to older ones.

A clear correlation exists between the morphological characters and the anatomy of the spines. Features like

flexibility, hardness, and sharpness are related to the extent of spine sclerification. Cross sections of the hard spines have shown that they are made up of fibers with thick to very thick secondary walls with almost or completely occluded lumina. It is the presence of these fibers that confers to the spines their extraordinary hardness and sharpness. Flexibility too is related to fiber wall thickness, but it depends more on the spine diameter. Indeed very thin spines like those of *T. knuthianus* are rather flexible, also if they have a compact sclerenchyma with thick-walled fibers. Papery or corky spines are instead more flexible, soft and non-pungent. They are made up of thin-walled fibers, like in *T. flaviflorus*, or the sclerenchyma can be dimorphic, like in *T. schmiedickeanus*, with the outer layers made up of thin-walled fibers and a more or less large inner core of fibers with thicker walls. Due to the presence of a loose sclerenchyma with thin-walled fibers, these spines are rather soft and their tips are never so sharply tapered. Usually they are described as flexible, and indeed they are more, but this feature depends on spine diameter, so that thicker spines are quite rigid, also because they have a larger compact inner core providing mechanical strength.

A feature unique to the corky spines are the deep fissures that extend from the epidermis to the under laying sclerenchyma layers and that are due to the thin-walled fibers of those layers that break easily. The finding of fissured spines that are made up of large fibers with wide lumina gives an anatomical-structural basis for the capacity that some cacti have to absorb water through the spines. Schill and Barthlott (1973) have shown that water can be absorbed by the spines in 2 species with fissured spines, *D. horstii* and *T. schmiedickeanus* ssp. *klinkerianus*. *C. invicta* proved to be another species that can absorb water through its spines (Porembski, 1994). It is possible to hypothesize an adaptive role for this feature in *C. invicta*, which is a species living in a fog desert, and in *D. horstii*, which has a reduced root system, but for *T. klinkerianus* and the other *Turbinicarpus* taxa with corky spines, a similar role is less likely. These species grow in arid areas, on gravely soils rich in organic matter (Sotomayor et al., 2004), and have well developed root systems so that water uptake by the spines can only be secondary. It is possible to suppose that in *Turbinicarpus* papery or corky spines have the function to provide a certain degree of mimetism, resembling tufts of dried grass, or that they contribute to an energy saving for the less organic matter needed to build the thin walls of their spine fibers.

The hollow fibers of the colored spines, ranging from straw-colored to black, contain more or less abundant tannins. Besides being responsible for the spine color, tannins probably play a protective role giving a higher protection to pathogens, a function proposed also for the tannins found in the epidermal cells of some cacti (Loza-Cornejo and Terrazas, 2003).

Spine traits value in taxonomy. *Turbinicarpus* taxonomy mostly relies on spine features and the newly collected data can contribute to a better understanding of the interspecific relationships. Epidermal surface characters that are slightly influenced by environmental conditions, can be valuable criteria for classification at species level (Barthlott, 1981). Nevertheless, very few studies dealt in cactus spines (Schill et al., 1973; Robinson, 1974). In *Turbinicarpus* some epidermal characters are strictly species-specific such as the long trichomes of *T. valdezianus*, the short conical projections of *T. pseudopectinatus* or the low tubercles of *T. hoferi*. Others, such as the pinnate tubercles or the epidermal cells devoid of ornamentation are shared by several species. A well delimited group is that made up of the species that have a sclerenchyma with thin-walled fibers, *T. alonsoi*, *T. bonatzii*, *T. dickisoniae*, *T. gracilis*, *T. macrochele*, *T. rioverdensis*, and *T. schmiedickeanus*. Papery or corky spines are very rare in Cactoideae and are restricted to a few unrelated genera such as *Leuchtembergia*, *Pediocactus*, *Sclerocactus*, and *Turbinicarpus*, and represent a highly derived feature that evolved independently several times based on the known molecular phylogeny (Butterworth et al., 2002). This character supports the decision by Lüthy (2002) to group these taxa into a series of their own (series *Turbinicarpus*) in the genus *Turbinicarpus*. Donati and Zanovello (2004) followed a different approach and in their revision of the genus grouped in the subseries *Lophophoroides* 2 species with woody spines, *T. lophophoroides* and *T. jauernigii*, together with some species bearing corky spines, *T. alonsoi*, *T. bonatzii*, *T. flaviflorus,* and *T. rioverdensis*. Zachar (2004) mostly followed the proposal by Lüthy, except that he included *T. jauernigii* together with the species bearing corky spines in the series *Turbinicarpus* and leaved out *T. alonsoi*, considering it related to *T. swobodae*. The last 2 classifications clash with the spine anatomy, underestimating the peculiarity of corky spines for which a solid anatomical background exists. More recently, Hunt (2006) considered all the taxa with corky spines as subspecies of *T. schmiedickeanus*, and included in it *T. jauernigii* too, a position for which there is no anatomical evidence as *T. jauernigii* has spines made up of a compact sclerenchyma, while the other taxa included in *T. schmiedickeanus* have a loose or dimorphic sclerenchyma. The species with woody spines share a similar spine anatomy, a sclerenchyma with thick-walled fibers, but differ in their epidermal characters. They can be divided into 2 groups based on the presence or not of epidermal projections, but these groupings do not fit any of the proposed systems. The actual classifications consider

some taxa sufficiently distinct to be grouped in different genus subdivisions. Besides series *Pseudomacrochelae* and series *Valdeziani*, the other subdivisions by Lüthy gather species with and without epidermal projections. A similar problem concerns the classification proposed by Donati and Zanovello (2004). The weight that epidermal characters, like the presence of lateral projections, can have on the building a classification of *Turbinicarpus* is linked to a wider knowledge of the infraspecific variability these characters can display. In fact there are no data that tell us how much constant they are within a population and if or how much they vary among populations. It is known that *Turbinicarpus* taxa display small morphological variations between different populations, therefore would not be surprising to find that spine micro-morphology, to some extent, can vary too.

This study has shown that anatomical and, to some extent, micro-morphological characters can be successfully used to assess species relationships and has enabled the giving an account of the great variability of spine micro-morphology in *Turbinicarpus* and, for the first time, to document the anatomy of cactus spines.

Acknowledgments

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