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Cladodes anatomy of *Opuntia* (Cactaceae) from the province of Buenos Aires (Argentina)

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Summary: The aim of this study was to deepen the knowledge of the cladodes anatomy of nine species of *Opuntia* that grow in the province of Buenos Aires, Argentina. Fresh and herbarium samples were prepared according to usual methods for light microscope. Histochemical techniques were performed to identify starch, mucilage and oxalate salts. The main traits found were: epidermis smooth and uniseriate, covered by a thin cuticle and epicuticular waxes; large (range 36-57 µm length) stomata in low density (range 21-27/ mm^2), located at level respect to the rest of epidermal cells with a deep substomatal chamber; a multiseriate hypodermis with one crystal layer; the cortex with external chlorenchyma and internal hydrenchyma. The eustele presents different size of vascular bundles; in some species the large ones have a secretory duct adjacent to the phloem. Some species showed wide-band tracheids and a few presented fibres in their vascular bundles. Some characters (presence of fibres and ducts) may be a useful tool for plant identification. However, many open questions remain to be investigated such as the correlation of the environment factors with hypodermis, calcium oxalate crystals, and wide-band tracheids.

Key words: Buenos Aires province, *Opuntia*, segment of stems, tissues.

Resumen: Anatomía de los cladodios de *Opuntia* (Cactaceae) de la provincia de Buenos Aires (Argentina). El objetivo de este trabajo fue profundizar el conocimiento de la anatomía de los cladodios de nueve especies de *Opuntia* que crecen en la provincia de Buenos Aires, Argentina. Se hicieron preparaciones con material fresco y ejemplares de herbario siguiendo métodos habituales para microscopía óptica. Se usaron técnicas histoquímicas para identificar almidón, mucílagos y sales de oxalato. Las principales características halladas fueron: epidermis lisa y uniseriada, cutícula delgada y ceras epicuticulares; estomas grandes (entre 36-57 µm long) en baja densidad (entre 21-27/ mm^2), ubicados a nivel con respecto a las restantes células epidérmicas y con una profunda cámara subestomática; hipodermis multiseriada con capa cristalífera; la corteza con clorénquima externo e hidrénquima interno. La eustela presenta haces vasculares de diferente tamaño, en algunas especies los haces mayores presentan un conducto secretor adyacente al floema. Algunas especies presentaron traqueidas con bandas de engrosamiento secundario y muy pocas tuvieron fibras en sus haces vasculares. La presencia de fibras y conducto adyacente al floema pueden ser caracteres útiles en la identificación de especies. Sin embargo, muchos aspectos requieren mayor investigación en relación con los factores ambientales, tales como la hipodermis, los cristales de oxalato de calcio y las traqueidas con bandas de engrosamiento secundario.

Palabras clave: *Opuntia*, provincia de Buenos Aires, segmentos del tallo, tejidos.

Introduction

The family Cactaceae was assigned to order Caryophyllales, suborder Portulacineae (Ortega-Baes *et al.*, 2010). It has ample distribution in arid regions of Mexico, Brazil, Argentina and Bolivia as the principal center of diversity, but also is presents in tropical rainforests and temperate areas (Kiesling, 1975, 1988, 2003; Ortega-Baes *et al.*, 2010). In Argentina, the family is represented by 225 species of which 131 are endemic (Zuloaga *et al.*, 1999). *Opuntia* Mill. (subfamily Opuntioideae, tribe Opuntieae) contains 191 species (Nattero & Malerba, 2011), and it has a widespread distribution from northern Patagonia to southern Canada (Kiesling, 1975). In Argentina, are growing 30

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species (Kiesling, 1975, 1999, 2005), and in the province of Buenos Aires, Cabrera & Fabris (1965) described seven species of *Opuntia*. Zuloaga *et al.* (2008) cited seven species. Later, *Opuntia bonaerensis* was recognized, and Long (2012) described a new species, *Opuntia ventanensis*.

During the last years, many anatomical studies for the family, subfamilies and genera have been performed (e.g. Conde, 1975; Mauseth & Plemons-Rodríguez, 1998; Loza-Cornejo & Terrazas, 2003; Arruda *et al.*, 2005; Mauseth, 2005, 2006; Melo-de-Pinna *et al*., 2006; Pereira de Arruda & Melo-de-Pinna, 2015) introducing novelties, but only a few contains some structural data of Argentinian *Opuntia* species (Vergez Manghi *et al*., 2015). Therefore, our objective was to deepen the anatomy knowledge of segment of stems (cladodes) of the species of *Opuntia* from the province of Buenos Aires.

Materials and Methods

Plant materials studied

Accessions from the following herbaria have been studied (listed in the Appendix): BA, BAA, LP, SI (acronyms according to Thiers, http://sweetgum.nybg.org/ih). In addition, fresh samples were surveyed (included in Appendix). To identified the *Opuntia* collected, and for nomenclature were consulted the following documents and data base: Flora de la provincia de Buenos Aires (Cabrera & Fabris, 1965), Flora ilustrada de Entre Ríos (Kiesling, 2005), Instituto de Botánica Darwinion, www.darwin.edu.ar, Long (2012), Font (2014), Realini *et al.* (2014), and Las Peñas *et al.* (2017).

Fresh samples of cladodes were preserved in formalin-acetic-alcoholic solution (FAA 70%) (Johansen, 1940) in the Laboratorio de Morfología Comparada de Espermatófitas (LAMCE). A sample of the collected material was cultivated in greenhouse, Botanical Garden and Arboretum “C. Spegazzini”, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata. In Fig. 1 is showed the distribution of the studied material in the Buenos Aires province.

Anatomical and histochemical analyses

To avoid alterations on fresh samples, they were immediately fixed in FAA solution (Johansen, 1940). Dried small pieces of cladodes were reconstituted by immersion in water and placed in an oven at 30ºC for 24-72 h, then fixed in FAA. To perform anatomy study a small piece from the middle part of the cladode was used. To study characteristics in surface view transparent material was prepared by the following technique of LAMCE Laboratory: at room temperature, samples of cladodes were submerged in a solution of sodium hydroxide (5%) add sodium hypochlorite (5%), ratio 1: 1 for 2-5 days. At completion of the bleaching process, five washed in distilled water were carried out to remove the sodium hydroxide and hypochlorite. Samples were then transferred into a solution of chloral hydrate (5%) for 24-48 h. To analyze the structure, small pieces of cladodes were sectioned transversally, bleached in sodium hypochlorite (50%), washed three times with distilled water, and stained with alcoholic solution of safranin (80%). Slides were mounted on glycerin jelly (Johansen, 1940).

In surface view external characteristics of epidermal tissue, and internal traits of hypodermis and subepidermal druses were examined. The quantitative analysis was performed on four areas on both cladode surfaces. The epidermal cells size, anticlinal cell walls width; stomata guard cells length and width, and the diameter of subepidermal druses between the tips of the crystal were taken. The number of epidermal cells, stomata, and subepidermal druses per field was determined; those cells or druses which were in the border of the field were not considered in case that more than half of structure was out.

In transverse sections of the cladode, the tissues and the internal structure were analyzed. Thereby, quantitative features, such as thickness of external periclinal epidermal cell walls plus cuticle, hypodermis width and diameter of druses located in the cortex and pith were measured. All measurements were expressed in micrometer (µm). The average dimensions (e. g. cells, stomata, and druses) were established on the basis of more than 50 measurements per sample.

Histochemical localization of cell components was performed for starch with iodine-potassium iodide (IKI) (Ruzin, 1999); mucilage was determined with a color reaction with blue brilliant of cresyl (1%) (Zarlavsky, 2014), and to distinguish oxalate salts from carbonate we used acetic acid (5%) test (Yasue, 1969).

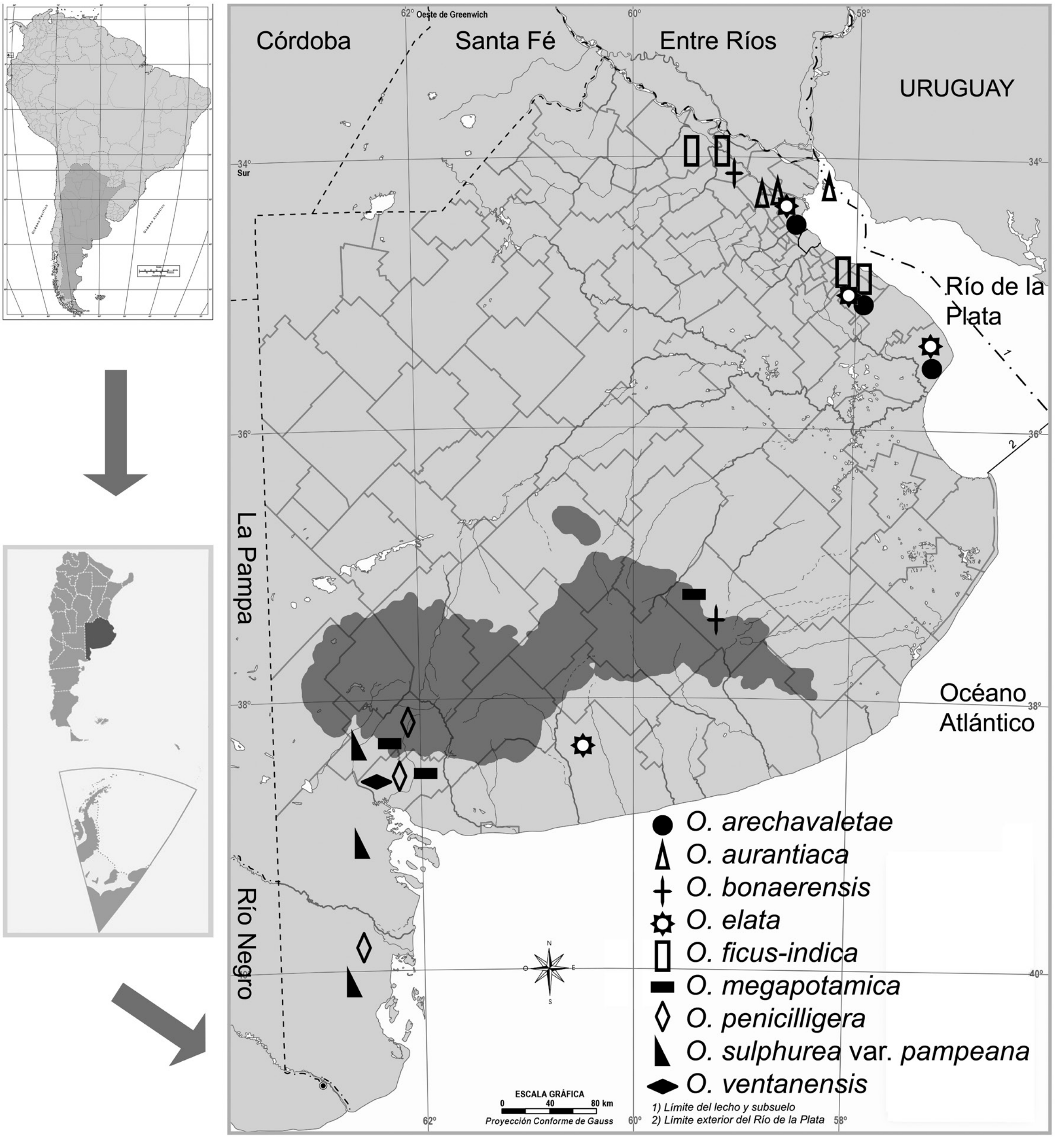

Fig. 1. Geographical points where the studied species of *Opuntia* were collected in the province of Buenos Aires (Argentina).

Microscopic studies were performed by means of a CETI T light microscope equipped with a camera lucida used to count cells, stomata and druses in surface view. The images were captured with a Moticam 1000 attached to the eyepiece of a microscope Gemalux XSZ-H, and Motic Image Plus 2.0 software. A Nikon E200 LED, equipped with digital camera and Micrometrics SE Premium software also was used.

Terminology is according to Metcalfe & Chalk (1979), and for stomata type we used the classification of Ash *et al*. (1999). Scientific names are according to the Instituto de Botánica Darwinion, www.darwin.edu.ar.

Results

Qualitative and quantitative characters are shown in Table 1 and Table 2. Quantitative values have been taken to whole numbers to facilitate reading and interpretation.

Epidermal tissue

Epidermal cells in surface view are polygonal in shape. We classified the anticlinal cell wall patterns as rectilinear to curved (e. g. *O. arechavaletae, O. aurantiaca, O. megapotamica*) (Fig. 2A); rectilinear to undulate, when some specimens exhibited linear anticlinal cell walls, and other one U undulate (*O. penicilligera* and *O. sulphurea* var. *pampeana*), and we found only U undulate in *O. ficus-indica* and *O. ventanensis* (Table 1; Fig. 2B). The epidermal cells size resulted less than 50 µm, with uniform thickness of the anticlinal cell walls; it ranged between 2-3 µm width (Table 1). The density of epidermal cells showed values lower to 1000 cells per unit of area (cells/mm^2), in *O. aurantiaca, O. bonaerensis*, and *O. elata*; whereas in the other species studied ranged from 1007 to 1696 cells/mm^2. There were not found important differences in the values of density between both cladode sides (Table 1).

In transverse section, the epidermis is smooth and uniseriate with the epidermal cells rectangular, covered by thin cuticle (2-3 µm width). The outer periclinal cell walls of the epidermis plus the cuticle ranged from 6 to 10 µm width (Table 2, Fig. 2C, D). The widest values were found in *O. penicilligera* and *O. sulphurea* var. *pampeana*. Over the cuticle there is deposited a wide layer of waxes (epicuticular waxes) that is visible at optical microscope smooth and brightly, sometimes cracked and falling-out (Fig. 2D).

Stomatal apparatus is constituted by a stoma with its guard cells and some subsidiary cells. In surface view, the stomatal aperture between the two guard cells is covered by the outer stomatal ledge or rim of cuticle, which is prolonged to both poles, frequently adopting a T-piece shape (Fig. 3A). In transverse section, the stomata were found at the same level as the ordinary epidermal cells (Fig. 3B). The deep substomatal chamber go through the collenchymatic hypodermis as a channel, and it may be extending into the cortical parenchyma (e.g. *O. arechavaletae, O. penicilligera, O. sulphurea* var. *pampeana*) (Fig. 3B).

The sizes of stomata guard cells ranged from 36 µm length x 21 µm width in *O. penicilligera* to 57 µm length x 31 µm width in *O. bonaerensis* (Table 1). The stomata density per unit of area presented low values of 21, 27, and 34 stomata/mm^2. The lowest density was found in *O. elata* and the highest in *O. arechavaletae, O. megapotamica, O. penicilligera*, and *O. sulphurea* var. *pampeana*. Coincidently, these species showed the smallest epidermal cells (Table 1).

The stomata types were variable and frequently, there was more than one type of stoma in the same epidermis (Table 1). We found the amphibrachyparacytic stomata type with two subsidiary cells flanking the sides of the guard cells but no completely enclosing them (= brachy) and surrounded by a ring of curved cells (e. g. *O. elata*), or when there are four cells flanking the sides of guard cells with or without a ring of curved cells surrounding them (e. g. *O. aurantiaca*; *O. ficus-indica*). Other type was amphibrachyparatetracytic with four subsidiary cells, disposed: two short cell lateral and parallel to the guard cells, and two wide polar subsidiary cells, and is surrounded by a ring of small curved cells (e. g. *O. megapotamica*). The brachyparahexacytic type has six subsidiary cells disposed as follows: four short cells lateral to the guard cells (two on each side), and two wide polar cells (e. g. *O. ventanensis,* Fig. 3C). It is amphibrachyparahexacytic when is surrounded by a ring of small and curved cells (e. g. *O. sulphurea* var. *pampeana,* Fig. 3D).

Cortical tissues

Hypodermis is located under the epidermis with 4-7 layers, the one under the epidermis has distinctive druses (Table 2; Figs. 2C, D; 3B, F). The hypodermis cells have primary cellulosic walls with irregular thickening of 13-32 µm width, and establish intercellular communication by primary pitted areas (Fig. 3E). This tissue is only interrupted by the stomatal chambers (Fig. 3B, E), and in transverse section, this hypodermis showed a thickness that ranged from 87 µm to 164 µm (Table 2). The druses diameter ranged from 27 µm (*O. ventanensis*) to 45 µm (*O. megapotamica*) (Table 2). The density of druses in surface view, ranged from 138 to 814 druses/mm^2. High values (469-814) were present in *O. ficus-indica, O. penicilligera, O. sulphurea* var. *pampeana,* and *O. ventanensis*.

Table 1. Epidermal features in surface view of the cladode of *Opuntia* (Cactaceae).

Taxa	Epidermal Cells				Stomata		
	Anticlinal cell walls patterns	width (µm)	Size (µm)	Density (c/mm2)	Type	Size (µm)	Density (s/mm2)
O. arechavaletae	rectilinear to curved	2	29 x 22	1041 - 1200	amphybrachyparacytic brachyparahexacytic	46 x 35	34 - 34
O. aurantiaca	rectilinear to curved	3	35 X 22	793 - 917	amphybrachyparacytic amphybrachyparahexacytic brachyparahexacytic	38 x 21	27 - 27
O. bonaerensis	rectilinear to curved	3	44 x 28	869 - 890	amphybrachyparacytic amphybrachyparahexacytic brachyparahexacytic	57 x 31	27 - 27
O. elata	rectilinear to curved	3	38 x 29	800 - 972	amphybrachyparacytic amphybrachyparahexacytic brachyparahexacytic	57 x 26	21 - 21
O. ficus-indica	U undulate	3	49 x 35	1207 - 1276	amphybrachyparacytic amphybrachyparahexacytic	45 x 27	21 - 27
O. megapotamica	rectilinear to curved	3	27 x 17	1359 - 1696	amphybrachyparacytic amphybrachyparatetracytic amphybrachyparahexacytic	48 x 27	27 - 34
O. penicilligera	rectilinear to U undulate	3	26 x 19	1524 - 1696	amphybrachyparahexacytic	36 x 21	34 - 34
O. sulphurea var. *pampeana*	rectilinear to U undulate	3	31 x 21	1593 - 1696	amphybrachyparahexacytic	46 x 28	27 - 34
O. ventanensis	U undulate	3	44 x 24	1007 - 1027	brachyparahexacytic	42 x 24	27 - 27

Ref.: c: cells; s: stomata.

The cortical parenchyma is formed by the outermost photosynthetic cortical cells, and the innermost storage cortical cells, immediately adjacent to the stele. The photosynthetic chlorenchyma tissue represents 50-70% of the cladode cortex in all studied species. In surface view, it is formed by rounded cells leaving small intercellular spaces (Fig. 4A, C), and in lateral view, rectangular to quadrangular cell outlines, disposed with its major axis perpendicular to the surface (Fig. 4B, D-F). This parenchyma has straight thin primary cell walls, but sometimes they appear undulate (Fig. 4A, B, E). However, these cells showed thicker walls with different degree of cellulose thickening in *O. penicilligera, O. sulphurea* var. *pampeana*, and *O. ventanensis* (Fig. 4C, D, F). The innermost cortical parenchyma represents 30-50% of the cortex. It is separated from the pith by a ring of vascular tissue. Both, internal parenchyma cortex and pith have isodiametric cells with thin primary cell walls, leaving small intercellular spaces. They are water-storage parenchyma (hydrenchyma), and in relation with that function they have numerous, large and globose mucilage cells (Fig. 4D). In the innermost cortical parenchyma and adjacent to the phloem of major vascular bundles may be found a reservoir (duct) of mucilage (*O. bonaerensis, O. megapotamica, O. penicilligera,* and *O. sulphurea* var. *pampeana*). Starch was identified as the principal reserve compound, and different crystal shapes of calcium oxalate were also found in the cortex and pith (Fig. 4F, f). In cross sections we measured the stellate druses with acute sharp points (Fig. 4F). The diameter of these druses ranged from 76 to 126 µm (Table 2). They were conspicuously larger than in comparison to those of subepidermal layer.

Table 2. Histological traits in transection of the cladode of *Opuntia* (Cactaceae).

Taxa	Epidermis + Cuticle width (µm)	Subepidermal druses size (µm)	Hypodermis			Parenchyma druses size (µm)	Vascular bundles		
			Density (d/mm2)	Number of layers	Width (µm)		Duct adjacent to the phloem	Fibres	Wide-band tracheids
O. arechavaletae	7	35	214-214	5 to 7	91	100	a	a	a/p
O. aurantiaca	7	32	365-386	4 to 5	87	77	a	p	a
O. bonaerensis	6	41	193-193	4 to 5	111	76	p	p	a
O. elata	7	39	138-172	4 to 5	95	83	a	a/p	a/p
O. ficus-indica	7	38	552-565	4 to 6	145	84	a	a/p	a/pm
O. megapotamica	7	45	262-276	5 to 7	164	76	p	p	pm
O. penicilligera	8	33	779-814	4 to 6	134	86	p	p	pm
O. sulphurea var. *pampeana*	10	34	690-745	5 to 7	111	126	p	a	pm
O. ventanensis	7	27	469-490	5 to 7	104	104	a	a	pm

Ref.: (a) absence; (p) presence; (pm) presence of a mass of wide-band tracheids.

Vascular tissue

The studied species of *Opuntia* have different size of collateral vascular bundles ordered in eustele. The small ones are formed by phloem, and opposite to the phloem on the inner side is the xylem, with two to ten narrow vessels, with helical secondary walls, and disposed in a row, intercalate with parenchyma of the xylem. The vascular bundle is surrounded by parenchyma differentiated from the medullary rays, it appears as a parenchymatic sheath (Fig. 5A). In the vascular bundle of medial size the xylem has a greater number of vessels with helical or pseudohelical secondary cell walls, and simple perforation plates (Fig. 5B). In general, the xylem of the studied *Opuntia* species (e. g. *O. arechavaletae*) exhibited a relatively large number of narrow vessels with helical secondary walls and parenchyma. In the xylem of *O. aurantiaca, O. bonaerensis, O. elata* (some specimens)*, O. ficus-indica, O. megapotamica,* and *O. penicilligera* were found clusters of libriform fibres (Table 2; Fig. 5C). In the xylem of *O. ficus-indica*; *O. megapotamica, O. penicilligera, O. sulphurea* var. *pampeana,* and *O. ventanensis* the wide-band tracheids (WBTs) were found. These especial cell types are located between the vessels, and around the vessels of the xylem (Figs. 5D; 6A, B). The wide-band tracheids are closed as a tracheid, and frequently also they adopt a spindle form in longitudinal view (Fig. 6A). These cells showed primary cellulosic walls, and internally two to four or six lignified annular rings or discs of secondary wall (Fig. 6A). We named ring when the secondary wall leave enough lumen, and disc when the lumen is very reduced nearly a point, after the secondary thickenings project deeply into de lumen (Fig. 6B). We found in *O. megapotamica, O. penicilligera, O. sulphurea* var. *pampeana,* and *O. ventanensis*, the WBTs forming great masses in the xylem, and extending into the pith, and they also appear connecting two to more vascular bundles (Fig. 6C, D).

Discussion

Epidermal cells in surface view showed variability of anticlinal cell wall patterns. It appears as a Cactaceae features, because it was previously mentioned by Loza-Cornejo & Terrazas (2003) for the Cactoideae of North America. The waviness of epidermal cell walls had been

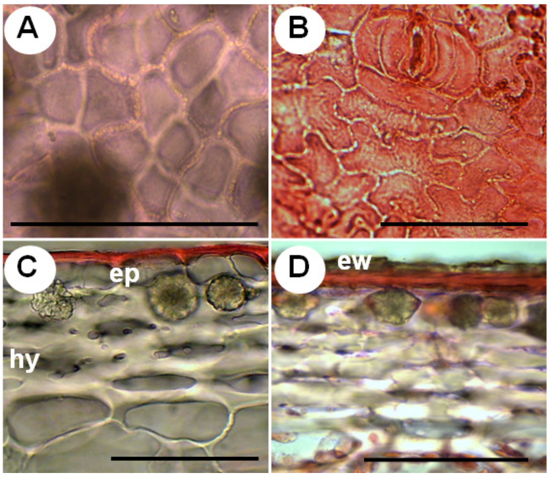

Fig. 2. Epidermis in surface view: A-B. A, rectilinear to curved anticlinal epidermal cell walls pattern (*O. megapotamica*). **B**, undulate anticlinal epidermal cell walls pattern (*O. ventanensis*). **Epidermis and hypodermis in transection of the cladode: C-D. C**, outer periclinal epidermal cell walls plus cuticle; hypodermis showing the crystal layer with druses (*O. elata*). **D**, epicuticular waxes over the cuticle (*O. ventanensis*). Ref.: **ep**, epidermis; **ew**, epicuticular waxes; **hy**, hypodermis. Scale bars = 100 µm.

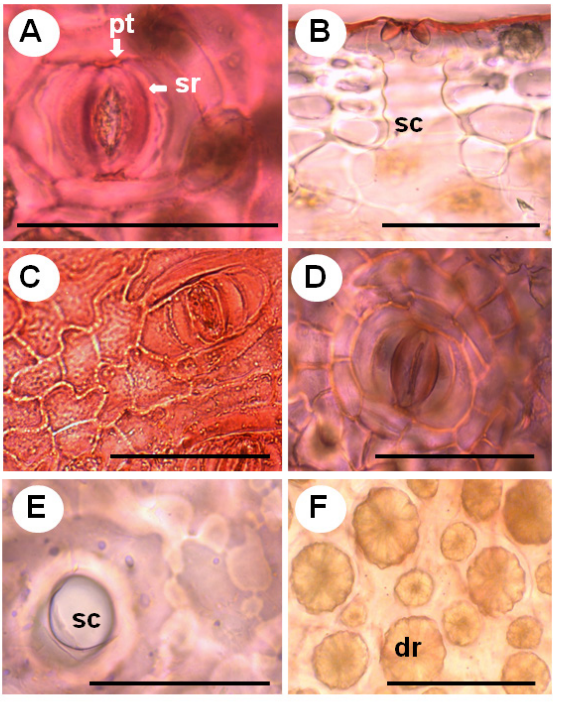

Fig. 3. Stomata and hypodermis. A, stomata in surface view, showing the outer stomatal rim of cuticle and polar thickening (*O. aurantiaca*). **B**, transection of stomata showing guard cells, subsidiaries, and substomatal chamber (*O. arechavaletae*). **C**, brachyparahexacytic stomata type (*O. ventanensis*). **D**, amphybrachyparahexacytic (*O. sulphurea* var. *pampeana*). **E**, hypodermis in surface view, showing primary pitted areas, and a substomatal chamber as a channel through the hypodermis (*O. ventanensis*). **F**, rounded druses of the subepidermal crystal layer in surface view (*O. ficus-indica*). Ref.: **dr**, druses; **sc**, substomatal chamber; **sr**, stomatal rim of cuticle; **pt**, polar thickening. Scale bars = 100 µm.

associated with environmental factors, such as latitude, altitude, and combined temperature and precipitation (Stace, 1965; Metcalfe & Chalk, 1979). In fact, it is not clear in the present research, but we are working with another organ, and we can hypothesize this characteristic may be also influenced by stem grows, flattened and rounded. In a drought-stressed condition the plants show substantial wilting, and the cell walls become to bend if they do not have enough rigidity (Schulze *et al*., 2005). Therefore, we think that those species with small epidermal cells, more or less rectilinear walls, and thicker periclinal cell walls plus cuticle and epicuticular waxes (e. g. *O. penicilligera and O. sulphurea* var. *pampeana*) are more resistant to suffer cytorrhysis. The superficial stomata position agrees with data reported by Pereira de Arruda & Melo-de-Pina (2015) in their Opuntioideae research, and it is supposed the deep stomatal chamber going through thickened multiseriate hypodermis does slow down water loss. The stomata type was found with a variable number of subsidiary cells as was noted by Eggli (1984) who named stoma “opuntioid”. These types accord well with paracytic, parallelocytic, tetracytic, and hexacytic combined with cyclocytic of Metcalfe & Chalk (1979). Results in general, agree with the stomata types indicated for the family and subfamily by previous authors (Eggli, 1984; Gibson & Nobel, 1986; Arruda *et al*., 2005; Calvente *et al*., 2008; Faigon *et al*., 2010; Pereira de Arruda & Melo-de-Pinna, 2015). We found hexacytic as the

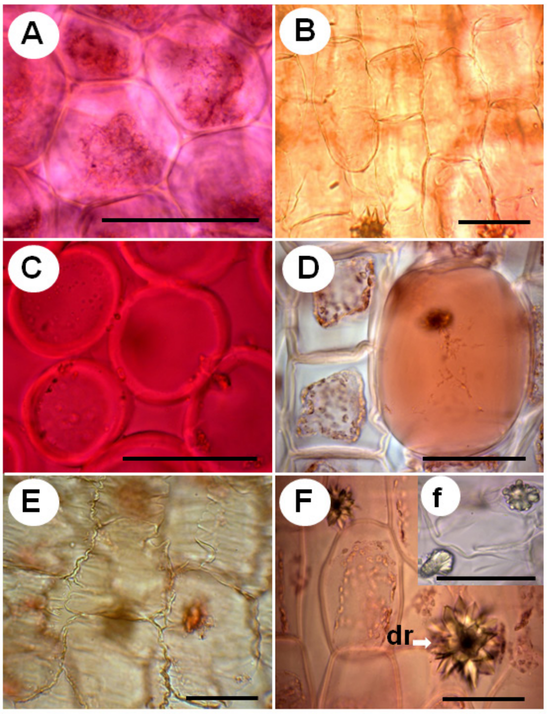

Fig. 4. **The chlorenchyma**. **A**, in surface view; **B**, in lateral view; **A, B**, both with thin cell walls (*O. arechavaletae*). **C**, in surface view; **D**, in lateral view, and one mucilage cell; **C, D**, both with thicker cell walls (*O. penicilligera*). **E**, in lateral view with thin and sinuous cell walls (*O. elata*). **F**, different calcium oxalate crystal types in the parenchyma tissue (*O. penicilligera*). Ref.: **dr**, druses; **f**, different crystal types. Scale bars = 100 µm.

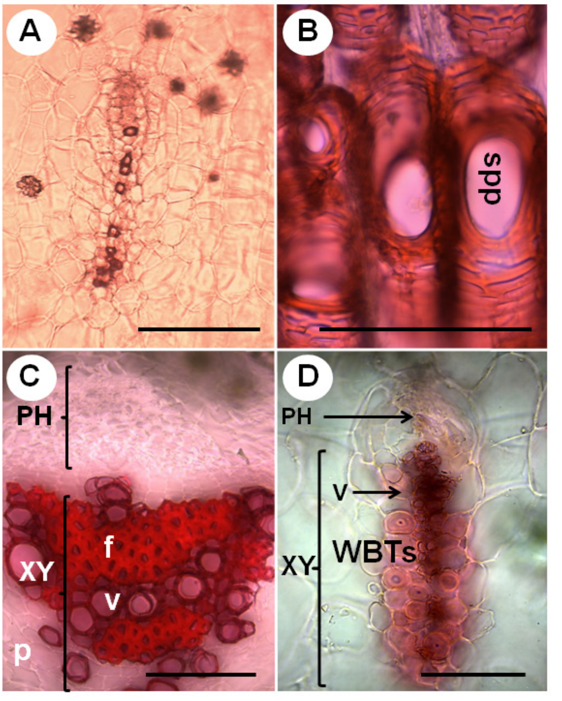

Fig. 5. **Vascular bundles**. **A**, small vascular bundle (*O. elata*). **B**, vessels with simple perforation plates (*O. elata*). **C**, vascular bundle showing phloem and xylem with vessels, fibres and parenchyma (*O. aurantiaca*). **D**, small vascular bundle showing phloem, xylem with vessels and wide-band tracheids (*O. ventanensis*). Ref.: **f**, fibres; **p**, parenchyma; **PH**, phloem; **spp**, simple perforation plates; **v**, vessels; **WBTs**, wide-band tracheids; **XY**, xylem. Scale bars = 100 µm.

most frequent type of stoma (brachyparahexacytic and amphybrachyparahexacytic). The hypodermis tissue was found 4-7 layered in the studied *Opuntia* species. Pereira de Arruda & Melo-de-Pinna (2015) found the number of layers of hypodermis variable (2-10) in different species of *Opuntia*. Whilst we doing this work observed that cladodes in specimens of *O. arechavaletae* growing under trees, in shadow conditions, had a hypodermis 2-3 layered with reduced thickening in the cell walls. It would be interesting to consider in future studies the effect of environmental factors on this tissue. In the cortex, the thickening in chlorenchyma cell walls exhibited by *O. penicilligera, O. sulphurea* var. *pampeana*, and *O. ventanensis* could be an adaptation to xerophytic environment. The thicker cell walls are more rigid doing more resistant cells (Mauseth, 1995). We can speculate that similar to what happens with epidermal cells, here the thickening of the walls support and prevent cellular collapse. The internal storage water cells are flexible, they are adapted to absorb water during wet conditions and then supply water to the chlorenchyma during drought stress as were previously described and illustrated by Mauseth (1995). All studied species of *Opuntia* exhibited a variety of crystalline forms. We found the whewellite type (Monje & Baran, 2002), and others types of crystals. We feel that is another interesting point to continuous exploring in these species of *Opuntia*. The function of oxalate crystals

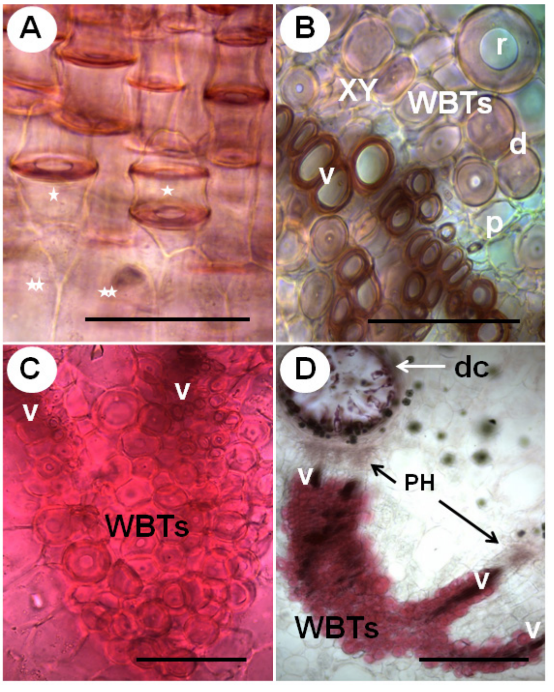

Fig. 6. **Vascular tissue**. **A**, longitudinal view of wide-band tracheids (WBTs) with spindle shapes (indicated by one asterisk). It may be seen some parenchyma cells (indicated by two asterisk) (*O. sulphurea* var. *pampeana*). **B**, xylem showing vessels, parenchyma, and WBTs, where may be distinguished between annular ring and disc thickenings (*O. ventanensis*). **C**, WBTs connecting two vascular bundles (*O. megapotamica*). **D**, one large vascular bundle with a duct adjacent to the phloem, surrounded by druses, and a mass of WBTs connecting this with other two vascular bundles forming a bunch (*O. megapotamica*). Ref.: **d**, disc; **dc**, duct; **p**, parenchyma; **PH**, phloem; **r**, ring; **v**, vessels; **WBTs**, wide-band tracheids; **XY**, xylem. Scale bars = 100 µm.

storage in plants still persists unresolved. Monje & Baran (2002) suggested that precipitation of calcium oxalate in the tissues of succulent plants may be related with preserving water. This could explain the high concentration of oxalate crystals found in specimens studies of *O. penicilligera* and *O. sulphurea* var. *pampeana* collected in semiarid region. More recently investigations (Hartl *et al.*, 2007) also referred that the subfamily Opuntioideae has predominantly monohydrated crystals. Tovar-Puente *et al.* (2007) analyzed the possibility to use crystals density as a tool to distinguish cultivars of the genera *Opuntia* and *Nopalea* and they had positive results among cultivars studied. However, they also recognize the convenience to continuous working increasing the number of samples from different edaphic conditions. In the xylem the WBTs would be involved in water storage and secondary in transference of water since the cell walls are very thin to be able to fulfill these functions, as well as to adapt to the processes of hydration and dehydration without suffering collapse they form the secondary thickenings. It is in agreement with the function concept referred by Boke (1944) and Gibson & Nobel (1986). Mauseth (1999) indicated that these cells constitute a strategy to water storage as well as minimize embolism damage. On the presence of these special cells we found some variations, for example in *O. megapotamica,* which is a species with ample area of distribution, the specimen collected in Cerro de la Ventana (a cold, dry area with stony soil) had a mass of wide-band tracheids, whereas the specimen collected in Villa María (a warm, rainy area) had no wide-band tracheids. Further studies are necessary to establish whether there is a real significant correlation between climate and wide-band tracheids. On the basis of the quantity of these special cells found in *O. penicilligera*, *O. sulphurea* var. *pampeana*, and *O. ventanensis*, we hypothesize that the development of the wide-band tracheids is favoured by extreme xeric conditions, perhaps in presence of physiological drought, when the plants are growing on the driest soils or saline soils.

Conclusions

It is the first stem anatomy description and illustration of nine species of *Opuntia*, from Buenos Aires province. The depth of the study allowed us to know superficial and internally features of this group of species. Some characters (e. g. presence of fibres and ducts) may be a useful tool for plant identification. However, many open questions remain to be investigated such as the correlation of the environment factors with hypodermis, calcium oxalate crystals, and wide-band tracheids.

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Appendix: Species of *Opuntia* from Argentina, Buenos Aires province, with representative specimens studied. References: #: cultivated, *: in preservation liquid, LAMCE.

O. arechavaletae Speg. *Prov. Buenos Aires: Pdo. San Isidro*, San Isidro, región de la costa, 15-XI-1956, *Burkart 17911* (**SI**). Det. *Kiesling* 1999. *Prov. Misiones: Dpto. Montecarlo*, Puerto Piray, 25-I-1952, *Castellanos 19411* (**BA**). Fresh material: *Prov. Buenos Aires: Pdo. Punta del Indio*, Punta Indio, X-2013, *M. P. Hernández* (#, *). *Pdo. La Plata*, La Plata, Diag. 113 esq. 116, XII-2012, *V. G. Perrotta* (*).

O. aurantiaca Lindl. *Prov. Buenos Aires: Pdo. La Plata*, Isla Martín García, 25-XI-1983, *Nuncia M. Tur et al.*, *1793* (**LP**); *Pdo. Campana*, Reserva Natural Otamendi, 25-XI-1962, *Burkart 23905* (**SI**); *Pdo. Escobar*, Belén de Escobar, barrancas cerca del Golf club, IV-1986, *Erb s. n.* (**SI** *028496*). *Prov. Entre Ríos: Dpto. Gualeguaychú*, Gualeyán, Arroyo al norte de Irazusta, 18-IV-1965, *Burkart 25761* (**SI**). Det. *Kiesling* en 1995.

O. bonaerensis Speg. *Prov. Buenos Aires: Pdo. Zárate*, Estación Las Palmas, 13/XII/1951, *Boelcke s. n.*, Det. *Leuenberger 5149* (**SI**); *Pdo. Tandil*, al oeste del Cerro San Luis, Tandilia, 1996, *B. Leuenberger 4516* (**SI**). Fresh material: *Prov. Buenos Aires: Pdo.Tandil*, Monte Calvario, 12-XI-2013, M. P. Hernández. (Identified as *O. paraguayensis* K. Schum.); *Pdo. La Plata*, Villa Elisa, XI-2013, *V. G. Perrotta* and *M. P. Hernández* (*).

O. elata Salm-Dyck. *Prov. Buenos Aires: Pdo. La Plata*, Manuel B. Gonnet, 3-XII-1942, *Cabrera 7621* (**SI**). Det. *F. Font, 2009* como *O. elata* var. *obovata* E. Whalter; *Dpto. Tres Arroyos*, Hueso Clavado, hacia ruta 228, 6-XII-1987, *Gazzaniga s. n.* Det. *Villamil 5603* (**SI**). *Pdo. Escobar*, Belén de Escobar, barrancas cerca del Golf club, IV-1986, *Erb s. n.* (**SI** *028495*). Det. *Oakley* como *Opuntia elata* aff. *elata*. *Prov. Corrientes: Dpto. San Cosme*, a 4 km al este de Paso de la Patria, 22-II-1969, *Krapovickas & Cristóbal 14939* (**LP**). Det. *F. Font* como *O. elata* var. *elata*; *Dpto. Capital*, Ruta 12 y camino Santa Ana, en quebrachal, 18-XI-1973, *Krapovickas & Cristobal 23736* (**LP**). Det. *F. Font, XI-2009*, as *O. elata* var. *elata*; *Dpto. San Martín*, Carlos Pellegrini, 30-X-1971, *Krapovickas et al. 20125* (**LP**). Det. *F. Font, dic. 2009* as *O. elata*; *Dpto. Ituzaingó*, Isla Apipé Grande, Puerto San Antonio, 10-XII-1973, *Krapovickas et al.*, *24104* (**LP**). *Det. F. Font, XI-2009* as *O. elata* (Our obs.: may be *O. elata* Salm-Dyck var. *cardiosperma* (K. Schum.) R. Kiesling, because this specimen has large oblong cladodes and vascular bundles with fibres in the xylem). *Prov. Entre Ríos*: *Dpto. Colón*, Parque Reserva Nacional “El Palmar”, 8-XII-1976, *Fernández Velazco s. n.* (**BA**). Det. *Leuenberger 2004*, as *O. cardiosperma* K. Schum. Det. *Oakley, 21-IV-2014* as *O. elata* var. *elata*. (Our obs.: may be *O. elata* var. *cardiosperma*, because this specimen has large oblong cladodes and vascular bundles with fibres in the xylem). Fresh material: *Prov. Buenos Aires: Pdo. Punta del Indio*, Punta Indio, X-2013, *M. P. Hernández* (#, *).

O. ficus-indica (L.) Mill. *Prov. Buenos Aires: Pdo. Baradero*, Baradero. sobre la barranca, 19-XII-1937, *Burkart 8500* (**LP**). Det. *F. Font, XI-2009*; *Pdo. Zárate*, Lima, sobre barranca del Paraná, 13-XI-1965, *Cabrera 17241* (**LP**). Det. *F. Font, XI-2009*. Fresh material: *Prov. Buenos Aires: Pdo. La Plata*, La Plata, Facultad de Agronomía, 27-XII-2012, *V. G. Perrotta & M. P. Hernández* (*); *Pdo. Berazategui*, Juan M. Gutiérrez, X-2017, *V. G. Perrotta* (*).

O. megapotamica Arechav. *Prov. Buenos Aires: Pdo. Tornquist*, Sierra de la Ventana, XI-1941, *Cabrera 7560* (**LP**); Proyecto Ventania 760, 26-XI-1979, Det. *F. Font*; *Pdo. Bahía Blanca*, G. Militar, 21-XII-1909 (*16416* **BAA**), Det. *Leuenberger, 19-II-2008* as *O. elata* (Our obs.: vascular bundles are coincident with *O. megapotamica*). *Prov. Córdoba: Dpto. General San Martín*, Villa María, 12-X-1964, *Cabrera & Fabris 15934* (**LP**). Det. *F. Font, XI-2009*. Fresh material: *Prov. Buenos Aires: Pdo. Tandil*, Sierra de las Ánimas, 12-XI-2013, *M. P. Hernández*. Identified as *O. vulgaris* Mill. (*).

O. penicilligera Speg. *Prov. Buenos Aires: Pdo. Tornquist*, Sierra de la Ventana, Estancia Funke, 14-XI-1943, *Cabrera 8126* (**LP**); *Pdo. Patagones*, Carmen de Patagones, XI- 1972, *Narosky & Izurieta 1* (**LP** *007081*). Det. *Kiesling*, V-1972. Fresh material: *Prov. Buenos Aires: Pdo. Bahía Blanca*. (#, *).

O. sulphurea Gillies ex Salm-Dyck var. ***pampeana*** (Speg.) Backeb. *Prov. Buenos Aires: Pdo. Patagones*, Carmen de Patagones, 5-XII-1918, *Cabrera 4793* (**SI**). Det. Kiesling; *Pdo. Tornquist*, Sierra de la Ventana, cerro cerca del hotel Ymcapolis, 6-XII-1971, *Burkart 28964* (**SI**); *Pdo. Villarino*, Salinas chicas, *s. f.*, *Fabris 5685* (**LP**); *Pdo. Villarino*, 60 km al oeste de Pedro Luro, XII-1964, *Fabris 5636* (**LP**).

O. ventanensis A. Long. *Prov. Buenos Aires: Pdo. Bahía Blanca*, cultivada en el Jardín Botánico "Lucien Hauman", Facultad de Agronomía, Universidad Nacional de Buenos Aires, IX-2017 (#, *).