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Opuntia delafuentiana (Cactaceae: Opuntioideae), a new *xoconostle* from central Mexico

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Abstract

We describe a new species of a wild Mexican *Opuntia* producer of edible acidic prickly pears (*xoconostles*), locally known as “*xoconostle de cerro blanco*” (white hillside *xoconostle*), based on external morphology and micromorphology attributes (mainly characters of the spines). This species is morphologically compared with another four species of wild *xoconostles* distributed in the same region: *O. joconostle*, *O. leiascheinvariana*, *O. oligacantha* and *O. matudae*. A taxonomic key for the *xoconostles* of the area is provided. Based on the phylogenetic analysis of one chloroplast gene, one plastid intergenic spacer and one nuclear molecular marker, the new species was recognized as a monophyletic group within *Opuntia s.s. sensu* Majure *et al.*

Key words: endemism, ITS, *matK*, micromorphology, phylogenetic relationships, *trnL-trnF*

Introduction

Mexico is considered the most important center of diversity for the American cacti (Bravo-Hollis 1978; Bravo-Hollis & Sánchez-Mejorada 1991, Hunt 1999, Anderson 2001). In Mexico the Querétaro-Hidalgo zone is an arid and small discontinuous area in the south of the Chihuahuan Desert, which flora includes several endemic cactus species, many of them categorized as threatened or endangered (Hernández *et al.* 2004, Sánchez-Martínez *et al.* 2006, Hernández-Oria *et al.* 2007).

Opuntia Miller (1754: without pagination) is one of the seven genera in the tribe Opuntieae DC. and several species of this genus have distribution in the country. The group has been recovered as monophyletic in recent phylogenetic analyzes using morphology and DNA sequences (Majure *et al.* 2012, Majure & Puente 2014).

There is no agreement regarding the number of species of *Opuntia* occurring in Mexico [58 species according with Britton & Rose (1919), 66 by Bravo-Hollis (1937), 76 by Guzmán *et al.* (2003), 93 by Hunt (1999)]. The species delimitation is particularly difficult in this genera because of: 1) the high amounts of hybridization documented (Segura *et al.* 2007), 2) the lack of chromosomic counts, and 3) the lack of phylogenetic studies, among many other reasons (Majure & Puente 2014). Species belonging to *Opuntia* may have a particularly high level of hybridization, and subsequent speciation via polyploidy, but further studies are needed.

The *Opuntia* species are characterized by having fruits with little pulp and thick and acid peel, known in Mexico as *xoconostles* (Nahuatl: *xoco* = sour; *noxtle* = prickly pear) (see Bravo-Hollis 1978, Reyes-Agüero *et al.* 2006). The fleshy and acidic mesocarp of *xoconostles* is used in Mexico as food and in folk medicine (cholesterol, obesity and hypoglycemic control). Species producing *xoconostles* are not a monophyletic group, but most of them are included in *Opuntia* (Reyes-Agüero *et al.* 2006).

Wild *xoconostles* are frequent in the semiarid highlands of Central Mexico, tolerating scanty rainfall and poor soils (Gallegos-Vázquez *et al.* 2012). As well as in *Opuntia*, there is no agreement regarding the number of species that produce *xoconostles* [e.g. Olivares-Orozco *et al.* (2005) recognized 9 species, García *et al.* (2005) 8 species, Scheinvar

et al. (2010) recognized 9 species plus one further taxon in *Cylindropuntia*]. Other authors such as Arias *et al.* (1997) reported species of *Opuntia* producing *xoconostles* in the Tehuacán-Cuicatlán Reserve [*Opuntia tehuacana* Arias, Gama & Guzmán (1997: 131)], and Pérez *et al.* (2005) reported a new *xoconostle* species also from Oaxaca, known as *Opuntia olmeca* Pérez, Reyes & Brachet (2005: 90).

The present study has the following different goals: 1) to formally describe the new species *Opuntia delafuentiana*, which can be recognized by distinctive characters in areoles, spines, glochides, epidermis and seeds, 2) to compare *O. delafuentiana* with four other species producing *xoconostles* that inhabit the same geographic area; for this purpose a taxonomic key will be provided to help in the proper identification of specimens, 3) to characterize the new species from the phylogenetic point of view.

Materials and methods

We detected five populations of this new species as part of a larger study of wild cactus producing *xoconostles* in the state of Hidalgo (Martínez 2010). This cactus has a distribution restricted to the municipality and it is locally known as “*xoconostle de cerro blanco*”. A considerable part of the range of this species has been altered by human activities, mainly agriculture (maize) and animal husbandry (mainly cattle). For the present study only wild populations were considered. This study compares some morphological, micromorphological and molecular attributes of this new species with other species of *Opuntia* that produce *xoconostles* distributed in the same region: *O. joconostle* F.A.C. Weber (1928: fig. 28), *O. leiascheinvariana* Martínez-González, Gallegos & Luna-Vega (2015: 1), *O. oligacantha* Förster (1846: 945), and *O. matudae* Scheinvar (1981: 324).

The study area is located in Villa de Tezontepec, Municipality of Tezontepec, Villa Hidalgo (geographical coordinates: 19°52'55"–19°53'11" N, 98°49'16"–98°48'53" W), at elevation of 2329 meters. The area has a BS1kw (w) (i) g climate, which according to the Köppen classification (modified by García 1973) corresponds to a semi-dry climate, temperate, with summer rainfall, with a percentage of winter rain 5% lower, isothermal and the hottest month of the year occurs before June. The predominant vegetation type in the area is desert scrub (*sensu* Rzedowski 1978), with sedimentary soils.

Extensive literature about *xoconostles* of *Opuntia* was reviewed, mainly concerning the following studies: Bravo-Hollis (1978), Britton & Rose (1919), Parfitt & Pinkava (1988), Pinkava (1996, 2002), Guzmán (2003), Hunt (2006), Scheinvar (1974, 1981, 1982, 1985, 1987, 1999, 2002, 2004), Scheinvar & Rodríguez (2003), Scheinvar & Olalde (2008), Scheinvar & Manzanero (2009) and Scheinvar *et al.* (2001, 2010).

Herbarium specimens of *xoconostles* were examined in the following Herbaria: Nacional de la Universidad Nacional Autónoma de México (MEXU), Instituto de Botánica Universidad de Guadalajara (IBUG), Instituto de Investigaciones de Zonas Desérticas (SLPM), Instituto Nacional de Estadística y Geografía (INEGI), Instituto de Ecología en Michoacán (IEB), Universidad Juárez Autónoma de Tabasco (UJAT), National Museum of Natural History, Smithsonian Institute (US) and New York Botanical Garden (NY) (acronyms according to Thiers 2011).

The morphological description is based on personal observations of fresh specimens made in the field by the first author. The micromorphological description was performed on specimens previously treated with FAA solution (formol, alcohol and acetic acid) for 48–60 hours. Samples of areoles, spines, glochids, epidermis and seeds were set. The material was washed with distilled water to remove the excess FAA. Then, it was dehydrated with a series of dilutions of ethyl alcohol (ETOH) of 50%, 70%, 95% and 100%, in periods of 10 hours for each dilution. Subsequently, the material was submerged in acetone for 100 hours to remove waxes. At that point, the acetone was exchanged for absolute ethyl alcohol. When the structures are clean, we dried them to critical point and set them on slides to be bathed with gold for their further observation in a scanning electron microscope (Hitachi, S-2460N model). Pollen grains were cleaned and then set on slides directly for further description under the scanning electron microscope.

The micromorphometric values of the spines were obtained from 50 measurements of 15 individuals per species using the image analyzer Image Tool version 3.0 (Wilcox *et al.* 2002). Based on the spine measures, we built a basic data matrix, where the micromorphometric values are expressed through the standard deviation and mean.

Molecular methods. Genomic DNA was extracted from fresh tissue from a sample of 20–50 mg of the plant stem following the CTAB method (Weising *et al.* 1995), modified by Martínez-González *et al.* (2014) for reducing the amount of phenols and polysaccharides in the sample. DNA was quantified in a Nanodrop (Thermo, USA). To amplify the ITS region we used the ITS5 and ITS4 primers (White *et al.* 1990). For the *trnL-trnF* region we used the *trnL-c* and *trnL-f* primers (Taberlet *et al.* 1991), and for the *matK* loci we used the 390F and 1326R primers (Schmitz-

Linneweber *et al.* 2001). PCR amplification reactions were performed on samples of 25 µL of final volume, using an initial denaturalization step at 96°C for 2 minutes, followed by 35 cycles at 94°C for 1 minute, annealing temperature differing according to the primer for 1 minute (Table 1), 72°C elongation temperature for different time durations, depending on the length of the product. All the reactions were carried in a Peltier Thermal Cycler PTC-200 (Biorad, Mexico). All the products were observed under electrophoresis, ensuring that only single bands were present. PCR products were cleaned with the Wizard SV gel PCR clean up System (Promega USA), then sequenced at Colegio de Posgraduados (www.colpos.mx). Contigs were assembled on BioEdit 7.0.5 (Hall 1999), then compared with Genbank sequences using BLAST (blast.ncbi.nlm.nih.gov) in order to identify possible contaminations.

TABLE 1. Primers used in the amplification and sequencing of the DNA fragments.

Locus/segment	Name	Sequence 5'–3'	Tm (°C)	Reference
cp <i>matK</i>	1326R	TCTAGCACACGAAAGTCGAAGT	48	Schmitz-Linneweber <i>et al.</i> (2001)
	390F	CGATCTATTCAATCAATATTTTC	48	Schmitz-Linneweber <i>et al.</i> (2001)
<i>trnL-trnF</i>	C	CGAAATCGGTAGACGCTACG	50	Taberlet <i>et al.</i> (1991)
	D	GGGGATAGAGGGACTTGAAC	50	Taberlet <i>et al.</i> (1991)
	E	GGTTCAAGTCCCTCTATCCC	50	Taberlet <i>et al.</i> (1991)
	F	ATTTGAACTGGTGACACGAG	50	Taberlet <i>et al.</i> (1991)
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	57	White <i>et al.</i> (1990)
	ITS4	TCCTCCGCTTATTGATATGC	57	White <i>et al.</i> (1990)

Phylogenetic methods. Phylogenetic affinities for the new species were investigated in two steps: affinities in the context of Cactaceae and phylogenetic placement in *Opuntia* s.s. a) Relationships in Cactaceae. For assessing the correct placement of *Opuntia delafuentiana* in the context of Cactaceae, we selected one of the type specimens for the new species (Martínez-González 4), and manually aligned the sequence of the plastid region *matK* obtained from the specimen (Genbank accession number KM678215) into the alignment for the plastid regions *trnL-F* and *matK* published by Hernández-Hernández *et al.* (2011; treebase.org S11087). The resulting data matrix was analyzed with maximum parsimony using PAUP* 4.0 (Swofford 2003) with a heuristic search of 500 replicates, MAXTREE set to auto increase and CHUCK=2 and CHUCKSCORE=100 to optimize the use of memory. Character support was estimated with a non-parametric bootstrap (Felsenstein 1985) of 500 replicates, each with heuristic searches of 50 replicates. The alignment also was analyzed under the criteria of maximum likelihood with RAxML 8.0.2 (Stamatakis 2014) on the CIPRES platform (Miller *et al.* 2010, www.phylo.org) using the GTRCAT model and estimating character support with a non-parametric bootstrap of 500 replicates. b) Relationships in *Opuntia* s.s. Once confirmed that the new species belongs to the Opuntioideae *sensu* Hernández-Hernández *et al.* (2011), we investigated its placement within the group. Sequences for three loci were generated, one protein-coding (*matK*), and two non-coding regions (ITS and *trnL-F*) were sequenced for the three specimens designated as nomenclatural types for the new species, for a total of nine new sequences (Genbank accession numbers KM678215–KM678223). Using the taxonomic sampling of Majure *et al.* (2012) for *Opuntia* s.s. as a reference, sequences of the selected markers were retrieved from Genbank for those species known to be diploids, in order to avoid the potential artifacts resulting from the inclusion of polyploids in the phylogenetic analysis. Each loci was independently aligned with MAFFT 7.182 (Katoh & Standley 2013) using the automatic strategy; alignments were inspected and corrected by eye. Alignments were concatenated with Mesquite 3 (Maddison & Maddison 2014, mesquiteproject.org) and analyzed under three different optimality criteria. The resulting data matrix was analyzed with maximum parsimony using PAUP* 4.0 with a heuristic search of 500 replicates, MAXTREE set to auto increase and CHUCK=2 and CHUCKSCORE=100 to optimize the use of memory. Character support was estimated with a non-parametric bootstrap (Felsenstein 1985) of 500 replicates, each with heuristic searches of 50 replicates. Maximum likelihood analysis was conducted using RAxML 8.0.2 with a strategy for locating the best-known likelihood tree, with 250 replicates, declaring partitions for each loci and using the GTR model with the CAT approximation for modelling rate heterogeneity. Character support was estimated with a thorough bootstrap approach: with 500 replicates under the same conditions of partitions and substitution model, the bootstrap frequencies were drawn over the best tree found in the previous analysis. A Bayesian inference was conducted using MrBayes 3.2 (Ronquist *et al.* 2012); two independent sets of MC3 chains were run along seven-hundred thousand generations; three partitions were declared corresponding with the loci; the strategy of reversible-jump (Huelsenbeck *et al.* 2004) was

used for model selection for each partition; rate heterogeneity was modeled with reference to the gamma distribution using four categories; and a percentage of the sites were considered invariants. All the parameters were un-linked from each partition. Once the standard deviation between the chains was stabilized at a value of 0.01, the sample size for the parameters were examined with Tracer 1.6 (Rambaut *et al.* 2014, beast.bio.ed.ac.uk/Tracer). The results were summarized in a majority rule consensus computed with MrBayes 3.2, and posterior probabilities and character support values were drawn over the maximum clade credibility tree recovered with TreeAnnotator 2.1.2 (Bouckaert *et al.* 2014, beast2.org), as recommended by García-Sandoval (2014).

Taxonomic treatment

Opuntia delafuentiana Martínez-González, Luna-Vega, Gallegos-Vázquez & García-Sandoval, *sp. nov.*

Type:—MEXICO. Hidalgo: Villa Tezontepec Municipality, Villa de Tezontepec, 19 ° 53 '0.7" N, 98 ° 48' 46" W, 2329 m, *Martínez-González 4* (Holotype, MEXU!; Isotypes, ARIZ, MO and CHAPA). Figs. 1 to 4.

Diagnosis:—The new species is similar to the sympatric ones *Opuntia joconostle*, *O. matudae*, *O. leiascheinvariana* and *O. oligacantha*, but differs in having the following characteristics: length and diameter of the pericarpel and fruit (that is smaller than in the other species), reniform to lenticular small seeds and the maturation stage of the fruit that occurs in January of the year following the blooming. The color of the styles is always white and the walls of the fruit are always whitish. The mature cladodes are obovate, the areoles are arranged in 10 to 12 series, epidermis pubescent, spines arranged around the cladode, setoses, porrect and reflexes, white with yellowish apices; pericarpel obovate; style always white, stigma lobes 8 generally papillose, green; small fruits ellipsoid to obovate, yellowish with green-whitish tones, with thick walls, acidic, edible; funicles semi-dry, tasteless; mature fruit persistent, observed on cladodes for 5–6 months or more.

Etymology:—The species epithet is dedicated to the former chancellor of the Universidad Nacional Autónoma de México, Dr. Juan Ramón de la Fuente Ramírez for his commitment and devotion to the UNAM: “we will give way to a new, brighter cycle in the life of the University”.

Vernacular name:—Known locally as “*xoconostle de cerro blanco*” for the whitish color of the interior walls of the fruit.

Description (macromorphology):—Bushy habit, 1.13 m in height (Fig. 1A). Trunk undefined (inconspicuous, little apparent), 25 cm in diameter. Epidermis pubescent, unicellular and conical (Fig. 3E). Cladodes obovate, 30 × 18 cm, about 2.1 cm thick, grayish-green, covered with a thin layer of wax (Fig. 1B). Areoles arranged in 10–12 series, 2.5 cm separation between series, 2.2 cm separation between areoles, semicircular (0.3–0.4 × 0.3 to 0.4 cm), trichomes short, blackish. Glochids yellow, short, arranged on the top of the areole (Fig. 3D). 1–6 spines arranged around the cladode, setoses, porrect and reflexes, the central ones erect, unequal, 0.5–3 cm long, white with yellowish apices. Young cladodes medium green, with prominent tubercles, areoles with short trichomes, pink, setose hairs white, subulate leaves, erect, reddish with uncinat apex (Fig. 1C). Flower buds acute, perianth segments brown, apex acuminate, pericarpels obovate, elevated tubercles, areoles with short trichomes, pink, brown glochids, some bordered with brown bristles (Fig. 2A and 2B). Yellow flowers (Fig. 2C), 5–7 cm long, pericarpel obovate of ca. 2.9 × 2.1 cm, areoles arranged in 7 series, 0.3 cm distant from each other, basal scale thick, outer perianth segments deltoid, apex mucronate, reddish, with the medium groove green, inner segments obovate, apex emarginate, yellow, stamens 1/3 the length of the perianth, filaments yellowish, anthers white, style cuneiform, about 1.9 cm long, white, stigma lobes 8 generally papillose, about 0.3 cm long, green, with medium groove grayish (Fig. 2D). Fruits ellipsoids and obovate (Fig. 2E), 5.8 cm long, yellowish-with green whitish zones, floral scar slightly sunken (0.7 cm), striate, small areolas, semicircular, arranged in 4–6 series, 0.8 cm distant from each other and 0.9 cm between series, spines absent, glochids yellowish-brown, walls thick, whitish, acidic, funicles whitish, semi-dry and tasteless (Fig. 2F). Seeds reniform to lenticular, white with gray tones, about 0.3 cm diameter, irregular lateral aryl, lateral micropylar region, deep, inclusive funicle and micropyle, doted testa (Fig. 4C).

Description (micromorphology):—Spines with lignified epidermal cells, long and wide in the apical part, 109.08 µm and 29.54 µm respectively (Fig. 3B), middle part of the spine with epidermal cells of 92.02 µm long and 10.76 µm wide, continuous epidermal cells without fissures between the cells and rough texture (Fig. 3C). Glochides with barbellate epidermal cells with elevated apices (Fig. 3D). Epidermis pubescent, unicellular and conical papillae, isodiametric epidermal cells with smooth texture anticlinal walls (Fig. 3E) and paracytic stomata of 16.96 µm wide

and 37.13 μm long (Fig. 3F). Pollen grain polyhedral, 103.86 μm diameter, 7353.64 μm^2 of area, 15.91 μm of distance between each distal opening (pores), with a diameter of 17.86 μm (Fig. 4A), 14–16 pores with width walls of 1.61 μm and glabrous. Seed lenticular to reniform with irregular lateral aril, micropylar-hilum region lateral, inclusive funicle and micropyle and dotted testa (Fig. 4E).

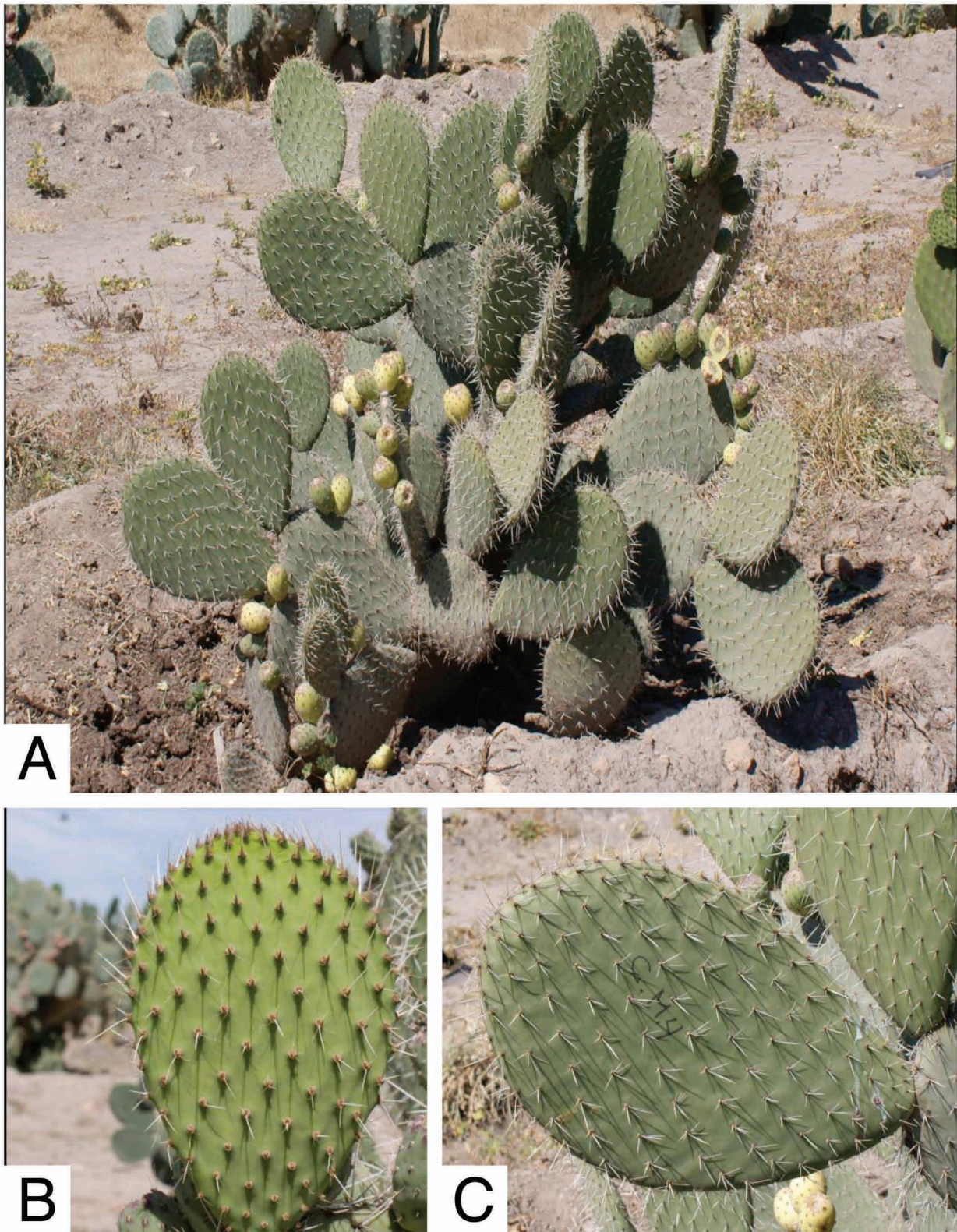


FIGURE 1. —A. Bushy habit, 1.13 m height. Native plant from Villa de Tezontepec Municipality, Hidalgo, Mexico. —B. Obovate cladodes, spines present on the areoles. —C. Young cladode, prominent tubers, areoles with short trichomes, pinkish, setose hairs, white, leaves subulate, erect, reddish and with an uncinat apex. Reference specimen: *C. Martínez-González 4* (MEXU).

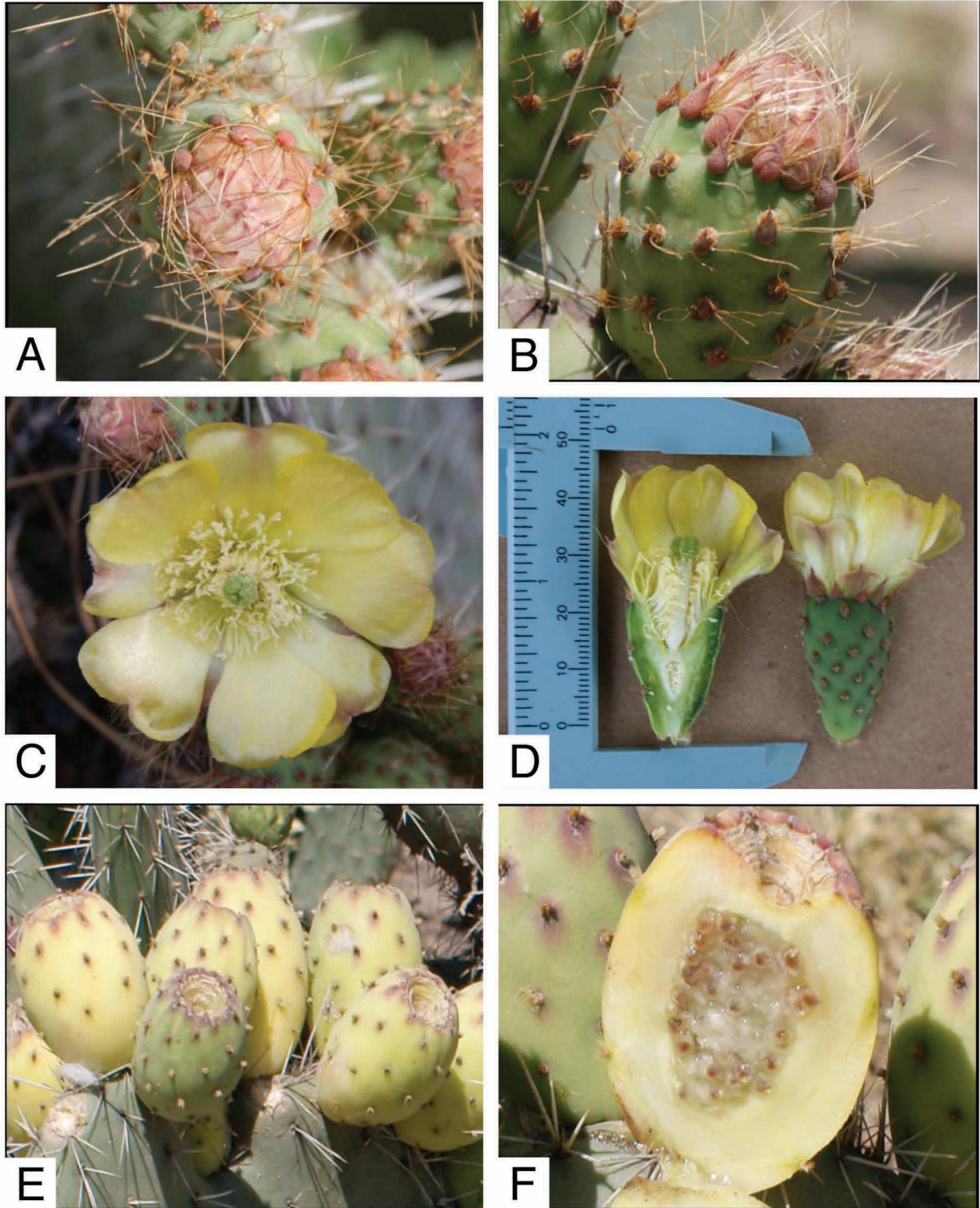


FIGURE 2. —**A y B.** Acute floral bud, segments of the perianth brown, acuminate apex, reddish, obovate pericarpel, prominent tubers, areoles with short trichomes, pinkish, brown glochides and some areoles with brown bristles. —**C.** Flower in anthesis, superior view, interior segments yellow, obovate with an emarginate apex. —**D.** Longitudinal cut of the flower, yellowish filaments and white anthers, white style, green lobes of the stigma. —**E.** Elliptic to obovate fruits, yellowish with green-whitish tones, 4–6 series of areoles, spines absent, glochides brown-yellowish. —**F.** Longitudinal cut of the fruit, 5.8 cm long, wide walls, green whitish and acids, funicles whitish, semidry and insipid. Reference specimen: *C. Martínez-González 4* (MEXU).

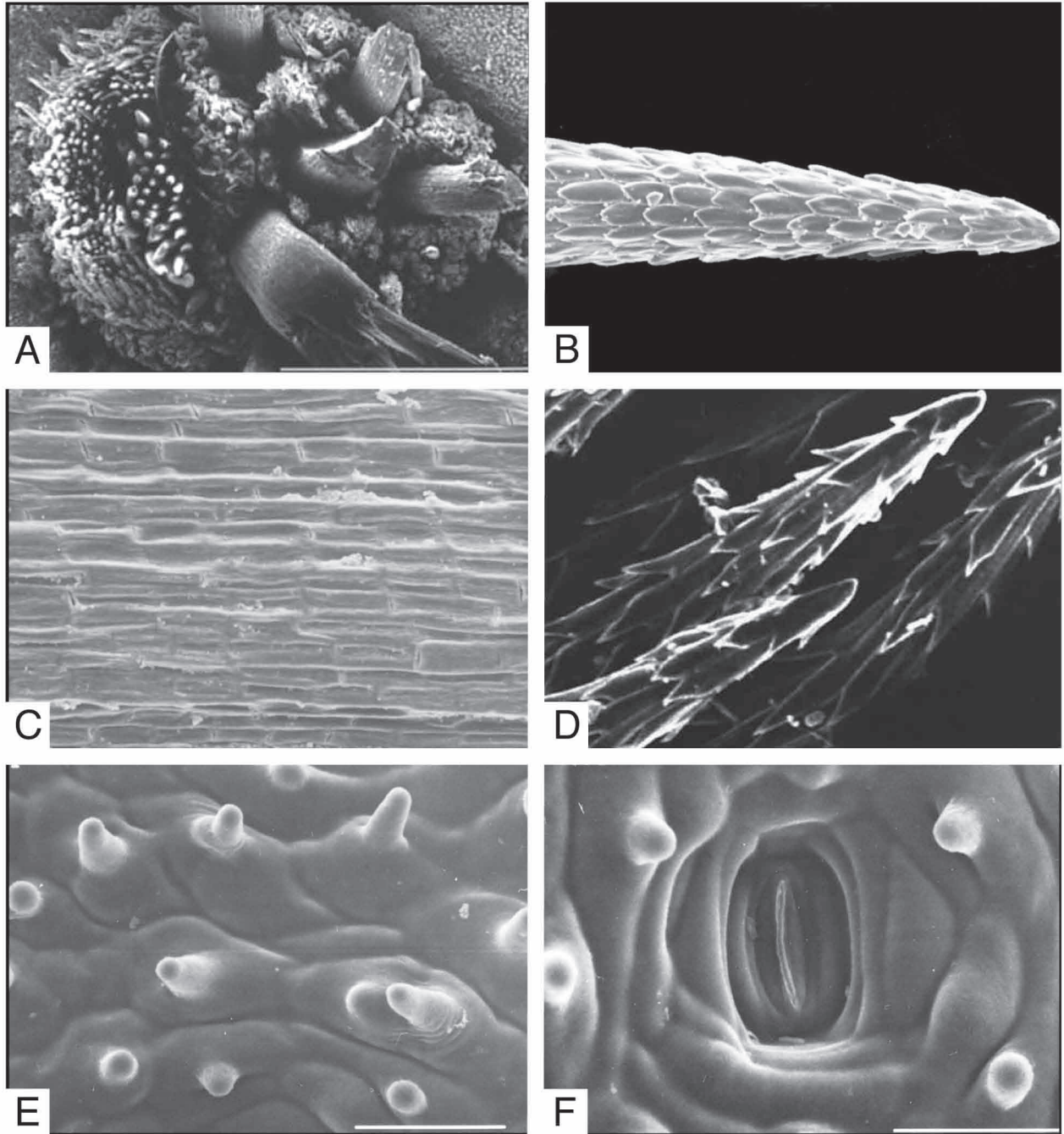


FIGURE 3.—**A.** Areole with short trichomes, glochides, base of a triangular central spine and four radial spines (x25). —**B.** Apical part of a spine, short epidermic cells (x125). —**C.** Middle part of a spine, continuous epidermic cells without fissures between the cells and with rugose texture (x110). —**D.** Glochides with prominent apices of the epidermic cells (x250). —**E.** Pubescent epidermis, epidermic cells with smooth anticlinal walls (x400). —**F.** Paracytic stomata (x500). Reference specimen: *C. Martínez-González 4* (MEXU).

Morphological comparison (Table 2):—The larger values in the length of the lignified epidermal cells of the apical part of the spine were recorded in *O. delafuentiana* (109.08 μm), *O. oligacantha* (101.90 μm), *O. matudae* (97.60 μm), while the smallest value in *O. joconostle* (94.62 μm). Concerning the width of the lignified epidermal cells of the apical part of the spine, larger values were recorded in *O. delafuentiana* (29.54 μm), which comes from wild populations, followed by *O. joconostle* (24.00 μm), *O. matudae* (22.00 μm) and *oligacantha* (21.04 μm), found in the wild and sunny orchards in the state of Hidalgo. The correlation of the two previous characters shows that the area of the apical portion of the spine of *O. delafuentiana* is significantly different to the other species, having the largest value (2544.5 μm^2), because they are setose long spines with a size up to 3 cm.

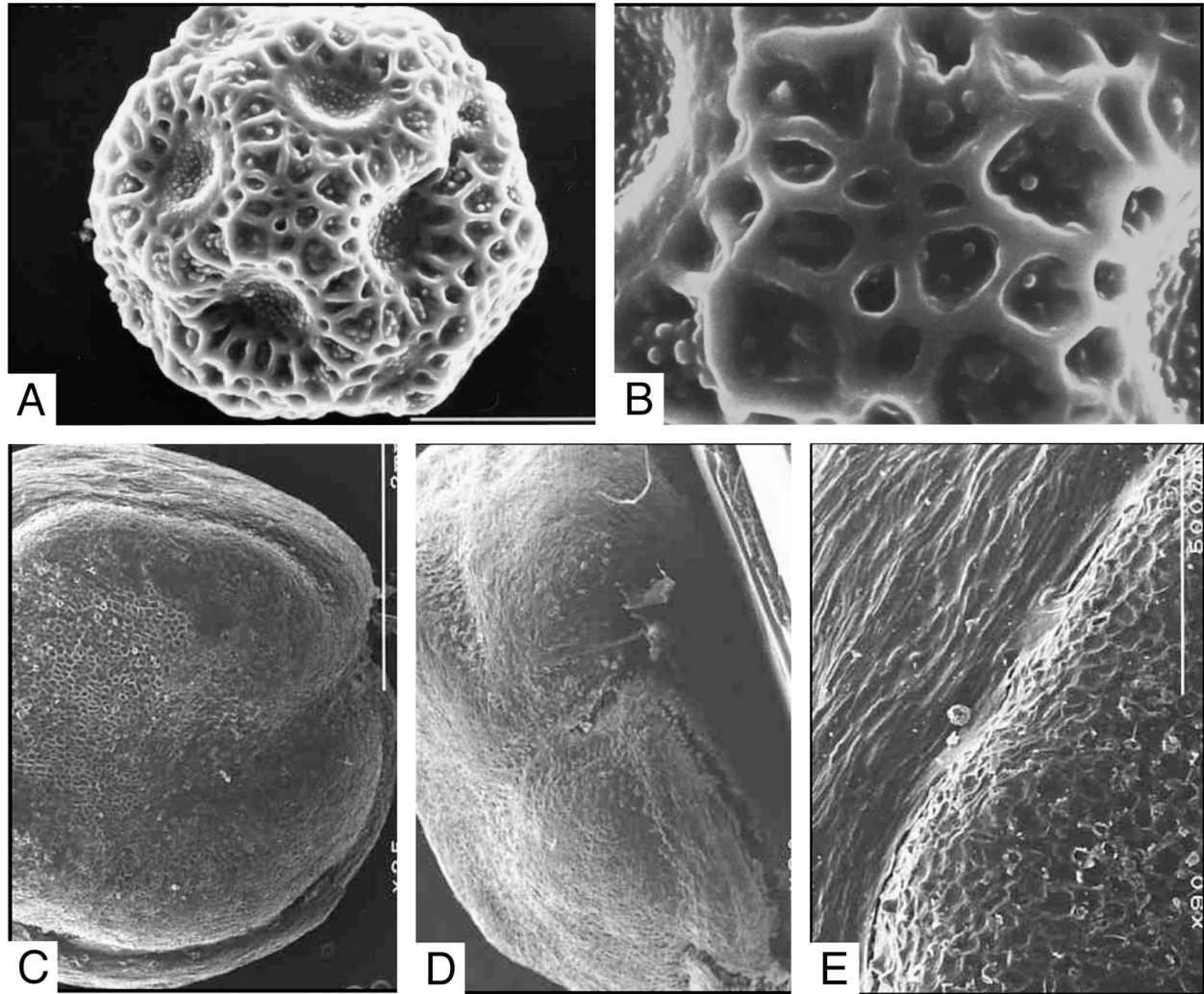


FIGURE 4. —A. Polyhedric pollen grain, 14–16 pores (x800). —B. Zoom of a pollen grain: reticle, forams and part of two pores, with wide and glabrate walls (x2500). —C. Lenticular seed, irregular lateral aril, wider in the inferior left side (x25). —D. Basal view of the seed, lateral hilo-micropilar region, deep, micropile and funicle included, testa punctate (x30). —E. Lateral aril cells (x90). Reference specimen: *C. Martínez-González 4* (MEXU).

TABLE 2. Comparison of micromorphological spine characters of four species of *Opuntia* producing *xoconostles*. The average micromorphometric values of the spines were obtained from 50 measurements of 15 individuals per species. The micromorphological values (μm or μm^2) are expressed through the standard deviation and mean.

Species	Lignified epidermic cells, apical part			Lignified epidermic cells, medium part		
	Longitude	Width	Area	Longitude	Width	Area
<i>O. joconostle</i>	94.62 ± 2.2	24.00 ± 1.8	1867.1 ± 1.5	88.58 ± 1.5	16.98 ± 2.1	1448.5 ± 1.4
<i>O. leiascheinvariana</i>	121.1 ± 1.7	27.97 ± 1.3	2492 ± 1.6	105.7 ± 1.6	23 ± 1.3	1778 ± 1.4
<i>O. matudae</i>	97.60 ± 1.1	22.00 ± 1.3	1515.4 ± 1.4	105.01 ± 1.5	21.20 ± 1.4	2064.4 ± 1.5
<i>O. oligacantha</i>	101.90 ± 1	21.04 ± 1.3	1546.0 ± 1.1	87.19 ± 1.1	10.10 ± 1.2	1074.2 ± 1.1
<i>O. delafuentiana</i>	109.08 ± 1.4	29.54 ± 1.7	2544.5 ± 1.6	92.02 ± 1.8	10.76 ± 1.1	840.0 ± 1.7

Concerning the interspecific variability of the size of the lignified epidermal cells (medium part of the spine), the length of the middle part in *O. delafuentiana* shows significant differences with respect to the other species studied, registering a value of 92.02 μm , a greater value than that observed in *O. oligacantha* and *O. joconostle* (87.19 and

88.58 μm , respectively), lower than the one presented by *O. matudae* (105.01 μm). We found the largest value of the width of the middle part of the spine in *O. matudae*. This variability was also highlighted by Martínez-González *et al.* (2014).

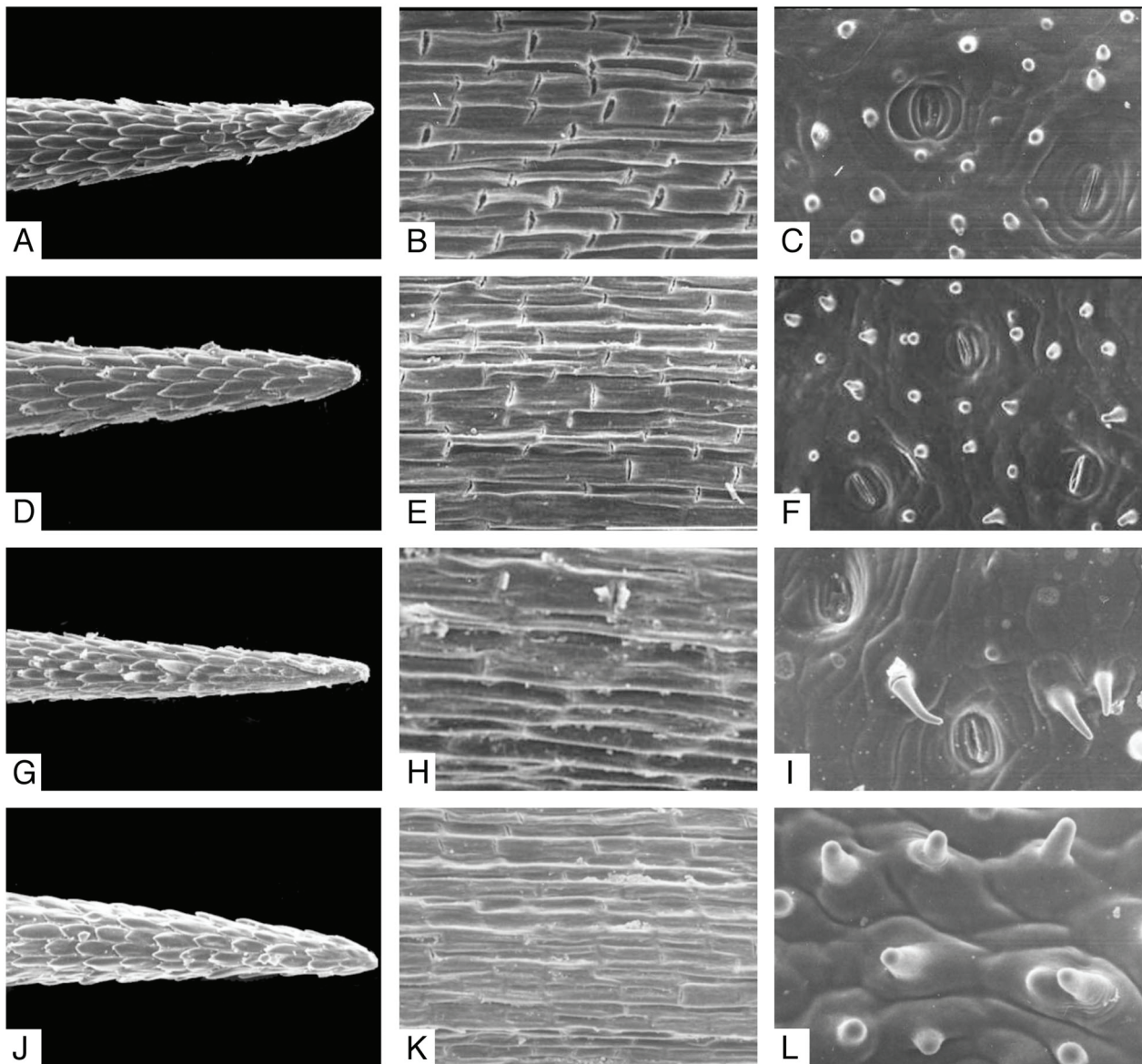


FIGURE 5. Micromorphologic differences of four species of *Opuntia* producing *xoconostles*. Images from the scanning electron microscope of the epidermic cells in spines of the apical and middle part. **A.** *O. joconostle*, apical part of the spine with epidermic cells very short and wide. **B.** *O. joconostle*, middle part of the spine with epidermic cells of 88.58 μm long and 16.98 μm wide, epidermic cells with fissures between each other and smooth texture. **C.** *O. joconostle*, glabrous epidermis, anticlinal walls with rugose texture and paracytic stomata. **D.** *O. matudae*, apical part of the spine with short and narrow epidermic cells. **E.** *O. matudae*, middle part of the spine with epidermic cells of 105.01 μm long and 21.20 μm wide, epidermic cells with fissures between the cells and smooth texture. **F.** *O. matudae*, glabrous epidermis, anticlinal walls with rugose texture and paracytic stomata. **G.** *O. oligacantha*, apical part of the spine with long and narrow epidermic cells. **H.** *O. oligacantha*, middle part of the spine with epidermic cells of 87.19 μm long and 10.10 μm wide, continuous epidermic cells without fissures between the cells and rugose texture. **I.** *O. oligacantha*, pubescent epidermis, anticlinal walls with rugose texture and paracytic stomata. **J.** *O. delafuentiana*, apical part of the spine with epidermic cells long and wide. **K.** *O. delafuentiana*, middle part of the spine with epidermic cells of 92.02 μm long and 10.76 μm wide, continuous epidermic cells without fissures between the cells and rugose texture. **L.** *O. delafuentiana*, pubescent epidermis, anticlinal walls with smooth texture and paracytic stomata.

Phylogenetic results:—The alignment for Cactaceae (modified from Hernández-Hernández *et al.* 2011) included 203 taxa and 2659 characters, 564 characters are parsimony informative. The parsimony analysis of the matrix found 42

trees of 2123 steps (CI=0.5066, HI=0.4934, RI=0.8615, RC=0.5446). The strict consensus recovers *O. delafuentiana* within the Opuntioideae clade, with a bootstrap frequency of 98%; maximum likelihood analysis of the same matrix recover the new species in the Opuntioideae clade as well, with bootstrap frequency of 94% (results not shown).

The alignment for *Opuntia* s.s. included fifty-two species (*O. delafuentiana* was sampled with three specimens) and 1955 characters, 108 characters are parsimony informative. The parsimony analysis of the alignment found 374 trees of 205 steps (CI=0.7033, HI=0.2967, RI=0.9075, RC=0.7880). The strict consensus recovers specimens of *O. delafuentiana* as a monophyletic group with a bootstrap frequency of 87%, but no clear affinities can be recognized because of the lack of resolution in the consensus, probably resulting from the lack of parsimony informative characters. Maximum likelihood analysis of the same alignment recovers the same monophyletic clade with the specimens of *O. delafuentiana* with 98% of bootstrap frequency (Fig. 6), as a sister group with *O. leiaschenvariana*, another Mexican *xoconostle* (Martínez-González *et al.* 2014), in the *Rhizomatosa* clade *sensu* Majure *et al.* (2012). Bayesian MC3 chain ran for 750 thousand generations, until standard deviation between the chains was stabilized at 0.01. Estimated sample size for the parameters was 332 or above for all the parameters. After discarding 25% of the trees, the majority rule consensus recovered specimens of the new species in a monophyletic group with a posterior probability of 1.0, and in the *Rhizomatosa* clade next with *O. leiaschenvariana* (Fig. 6).

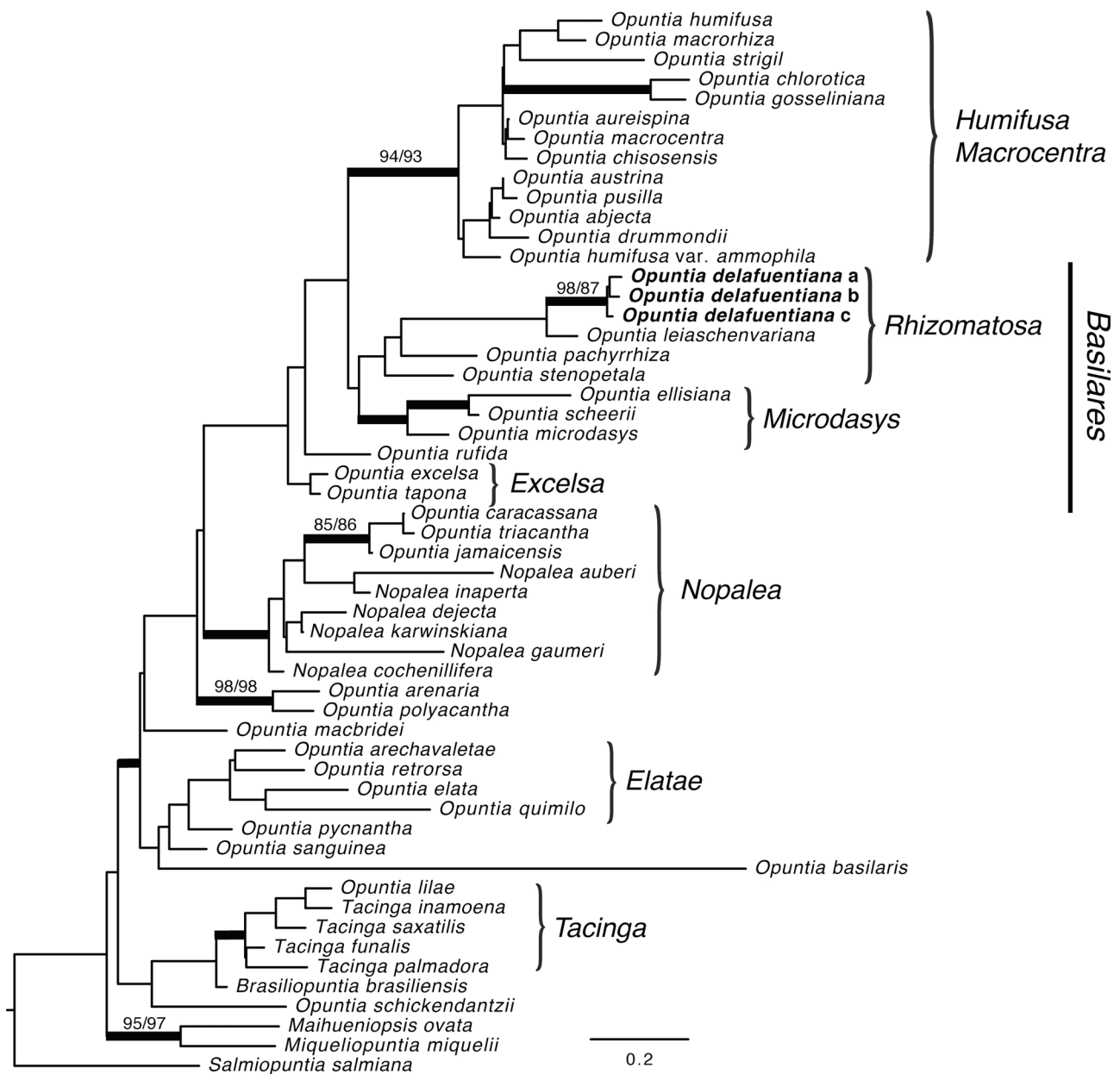


FIGURE 6. Maximum clade credibility tree for the *Opuntia* s.s., including *O. delafuentiana*. Character support values along branches for maximum likelihood and parsimony non-parametric bootstrap respectively. Bold branches correspond with Bayesian posterior probabilities of 0.95 or above. Clade names follows Majure *et al.* (2012).

Distribution and phenology:—The existence of *Opuntia* species in Mexico producing *xoconostles* is more widespread than previously thought. Nowadays we can recognize 11 species. There is only one reference locality for *O. delafuentiana*, wild species and distributed in the municipality of Villa Tezontepec, Hidalgo, Mexico. It blooms in March–April and fruits occur from January to March the year following their blooming. The fruits persist for 5–6 months in the cladodes, where they can be consumed by cattle or by wild animals when they fall off of the cladodes. The wild populations of *O. delafuentiana* help prevent soil erosion and are used as a refuge for several species of birds, reptiles and small mammals.

Conservation status:—According to the IUCN Red List of Threatened Species criteria, *O. delafuentiana* may be assessed in some of the categories for threatened species, mainly because of its very restricted distribution range, similar to the narrow endemic distributional pattern described by Hernández *et al.* (2010) for the cacti in the Chihuahuan Desert, and its extensive use in human activities observed in the field by the first author. The species is cultivated in the Botanical Garden of the Instituto de Biología, Universidad Nacional Autónoma de México, in the “Colección Nacional de Especies Silvestres de Nopales Mexicanos: National Collection of wild Mexican Nopales” in walkway OP-7 with the registration number CM4.

The Mexican list of threatened and endangered species (NOM 059; SEMARNAT 2010) includes only three *Opuntia* species, i.e. *O. arenaria* Engelm (1856: 301), *O. bravoana* Baxter (1933: 149) and *O. excelsa* Sánchez-Mejorada (1972: 68), all of them *tunas*. It is necessary to include many other species of *Opuntia*, mainly those that are endemic to the central part of Mexico, including many species of *xoconostles*.

Taxonomic key for selected species bearing *xoconostles* from central Mexico:

- 1 Spines with papery sheaths completely deciduous, cylindric stem segments *Cylindropuntia imbricata*
- Spines sheathless, stem segments flattened into cladodes 2
- 2 Plant with glabrous epidermal surface 3
- Plant with pubescent epidermal surface 5
- 3 Plant decumbent or scarcely erect, up to 30–65 cm high, stem not forming a trunk, yellow fruits.....
- *Opuntia heliabravoana* Scheinvar (1974: 75)
- 3 Plant forming shrubs with clearly differentiate trunk 4
- 4 Rigid spines; cladodes subcircular; 11.5–18.5 cm long, 18–28 cm wide, bright green, 7–9 rows of areolae per cladode
- *Opuntia joconostle*
- Flexible spines; cladodes up to 25 cm long, 20–25 cm wide, bluish-green with shades of grey; 13–14 rows of areoles per cladode, with red spots under areole
- *Opuntia matudae*
- 2 Plant with pubescent epidermal surface 5
- 5 Fruits yellowish with greenish-glaucous shades *Opuntia delafuentiana*
- Fruits reddish-pink with some shades of green *Opuntia leiascheinvariana*

Discussion

The new species shares the distribution area with other four species of *Opuntia* producing *xoconostles*: *O. joconostle*, *O. leiascheinvariana*, *O. oligacantha*, and *O. matudae*. However, the morphological features are clearly distinct (Fig. 5, Table 3). Diagnostic characters refer to the number of series of areoles, color and direction of the spines, flower and fruit size and fruit-ripening season. Common characteristics are: bushy habit, presence of *xoconostles* and the fruit is not dropped off the cladode when ripe. It has been observed that birds do not eat the walls of the fruit, avoiding acidity and drilling in search of the funicles with insipid flavor. Regarding micromorphological characters, significant characters are the epidermis type, the length and the width of the lignified epidermal cells of the apical half of the spine (Fig. 5).

The three specimens from the new species were recovered as a monophyletic group under three different optimality criteria, with moderate (87% with parsimony) to strong (98 with maximum likelihood) character support and 1.0 of posterior probability (Fig. 6). The strict consensus of the trees found with maximum parsimony has low resolution, probably resulting from the reduced number of parsimony informative characters, as reported previously by Majure *et al.* (2012). We recommend that additional loci should be explored for the *Opuntia* clade.

Phylogenetic placement for the new species is consistent with its morphological characters like the presence of glochids borne on areoles and a bony aril surrounding a campylotropous ovule, which are diagnostic for the group

(Griffitt & Porter 2009). *Opuntia delafuentiana* was consistently recovered as the sister species of *O. leiascheinvariana*, another *xoconostle* from Mexico, in the *Rhizomatosa* clade—which also includes the Mexican endemic *tunas* *O. pachyrrhiza* Hernández, Gómez & Bárcenas (2001: 309) and *O. stenopetala* Engelman (1856: 289) but with low or absent character support (Fig. 6). The presence of a clade including Mexican endemic species is congruent with a northern-central distribution in xeric habitats, but without any morphological character supporting this relationship.

Besides *O. delafuentiana* and *O. leiascheinvariana*, some other species of Mexican *xoconostles* are endemic to the area and that distribution may be indicative of a phenomenon of rapid radiation in the group, as it was suggested by Hernández-Hernández *et al.* (2014), whom documented a significant increase in the diversification rate for the *Nopalea+Opuntia* clade. Also considering that this clade may date from the early Pliocene (Hernández-Hernández *et al.* 2014), it may represent a relatively young clade, still in its period of relatively diversification (Morlon 2014). This pattern may be related with an ongoing expansion of the distribution range for these two genera after the aridification of the region and subsequent expansion of the Chihuahuan Desert.

TABLE 3. Distinctive morphological characteristics of four species of *Opuntia* producing *xoconostles*

	<i>O. joconostle</i>	<i>O. leiascheinvariana</i>	<i>O. matudae</i>	<i>O. oligacantha</i>	<i>O. delafuentiana</i>
Form of the mature cladodes	Obovate	Obovate	Narrowly obovate	Obovate with a narrow base	Obovate
Number of series of the areoles in the cladodes	7–9	11–13	9–10	11–12	10–12
Epidermis	Glabrous	Pubescent	Glabrous	Pubescent	Pubescent
Color of the spines in the mature cladodes	White with an ambarine apex	White with a yellowish apex	White-greyish with a translucent apex	White with a yellowish apex	White with a yellowish apex
Form of the pericarpel	Obovate	Oblong	Obovate	Elliptic to obovate	Obovate
Length of the pericarpel	3.6–4.1 cm	3.7–4.7 cm	3.7–4.5 cm	3.8–4.7 cm	2.9–3.1 cm
Diameter of the pericarpel	2.3–2.5 cm	2.0–2.7 cm	2.4–2.7 cm	2.3–2.8 cm	1.8–2.1 cm
Color of the filaments	White	White with a greenish base	Yellow	White-yellowish	Yellowish

Processes leading to polyploidy have been argued as the main speciation mechanism for this rapid diversification, mainly because many polyploids have been reported in the group (Majure *et al.* 2012), but the precise role of these processes remains to be assessed, and is part of current research by the lead author.

Majure *et al.* (2012) and Majure & Puente (2014) noted that many species included in Basilares have pubescent cladodes. This is the case of the species described in this study, resulting congruent with its location in the phylogenetic tree. These authors argued that the Basilares is the ancient group of species of the North American clade of *Opuntia*. The members of this clade have been involved in the production of many hybrids and polyploid species. The first author of this study is now conducting research to deepen in the genetic characterization of many Mexican species of *xoconostles*, in order to elucidate their precise phylogenetic history where reticulate evolution has been repeatedly documented (i.e. Segura *et al.* 2007, Majure *et al.* 2012, Majure y Puente 2014).

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