taxonomy

MDPI

Article

Taxonomy and Phylogeny Reveal a Cryptic New Species of *Opuntia* (Cactaceae) from Tamaulipas, Mexico †

César Ramiro Martínez-González [1], Tania Raymundo [2], Fortunato Garza-Ocañas [3], Leccinum J. García-Morales [1], Jaime Jiménez-Ramírez [4] and Jesús García Jimenéz [1,*]

[1] Instituto Tecnológico de Ciudad Victoria, Tecnológico Nacional de México, Ciudad Victoria 87010, Tamaulipas, Mexico; d22380018@cdvictoria.tecnm.mx (C.R.M.-G.); leccinum.gm@cdvictoria.tecnm.mx (L.J.G.-M.)
[2] Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Alcaldía Miguel Hidalgo, Ciudad de México 11340, Mexico; traymundoo@ipn.mx
[3] Facultad de Ciencias Forestales, Universidad Autónoma de Nuevo León, Linares 67700, Nuevo León, Mexico; fortunato.garzaoc@uanl.edu.mx
[4] Facultad de Ciencias, Universidad Nacional Autónoma de México, Alcaldía Coyoacán, Ciudad de México 04510, Mexico; jjr@ciencias.unam.mx
* Correspondence: jesus.gj@cdvictoria.tecnm.mx
† urn:lsid:ipni.org:names:77379786-1.

Abstract

Opuntia miquihuanensis (Cactaceae: Opuntioideae), a new species endemic to the locality Peña-Aserradero, part of the Sierra Madre Oriental, Tamaulipas, Mexico, is formally described and illustrated. Phylogenetic analyses used maximum parsimony, maximum likelihood and Bayesian inference, based on sequences of *trn*L-*trn*F, *psb*J-*pet*A, *matK*, *ycf1*, *ppc* and ITS. The micromorphology of spines, epidermis, stoma, and pollen was characterized with scanning electron microscopy. *Opuntia miquihuanensis* is recovered as a supported species within *Opuntia s.str*.

Keywords: cactus pear; Cactaceae; new species; *Opuntia*; phylogenetic relationships

1. Introduction

The cactus family (Cactaceae) contains charismatic ornamental horticultural plants, which belong to the order Caryophyllales, with approximately 174 genera and nearly 1850 species [1–3]. Mexico is considered the main center of diversity of the family [4].

They exhibit a range of life forms from geophytes and cushion plants to dwarf shrubs, shrubs, or small trees [5].

The tribe Opuntieae DC. (Cactaceae Juss., Opuntioideae K. Schum.) consists of the following seven genera: *Airampoa* Frič, *Brasiliopuntia* Berger, *Consolea* Lemaire, *Miqueliopuntia* Frič ex F. Ritter, *Opuntia* Miller, *Salmonopuntia* P. V. Heath *and Tacinga* Britton & Rose [3].

Opuntia (L.) Miller is one of the most iconic groups and it is recognized as the second most speciose genus within the family after *Mammillaria* Haworth [6], comprising approximately 154 species [3], 97 of which are found in Mexico and species continue to be added. The genus has undergone a rapid radiation, resulting in a broad distribution and high morphological diversity among species [7], thrives predominantly in the arid and semiarid regions of America, and is cultivated globally [7]. *Opuntia* species are stress-tolerant (e.g., salinity, drought), succulent CAM plants with highly efficient water use [8].

One of the most critical areas for Cactaceae diversity is northern Mexico, where Sthe state of Tamaulipas is located. Some studies have been carried out in Tamaulipas that

Check for updates

Academic Editor: Manuel B. Crespo

Received: 19 January 2026
Revised: 30 April 2026
Accepted: 1 May 2026
Published: 20 May 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

integrate knowledge about this family, such as the distribution analysis carried out by Martínez-Ávalos and Jurado [9]; meanwhile, García-Morales [10,11] deals with the richness and distribution of the cacti of the Sierra Madre Oriental and neighboring montane isolated regions of the Sierra de San Carlos and Sierra de Tamaulipas, and recently produced two studies at the State level, documenting 40 [12] and 36 [13] species of *Opuntia*, which suggests a lack of botanical explorations in the State of Tamaulipas.

During the scientific exploration in the Miquihuana Municipality, in the Sierra Madre Oriental, west of the State of Tamaulipas, we found a species of *Opuntia* that, due to its morphological characteristics, does not fit into known species, but shares characteristics of members of the Rhizomatosa clade. Therefore, it is proposed here as a new species.

2. Materials and Methods

2.1. Species Sampling

The specimens were widely studied in the wild at Km. 12, path Ejido La Peña-Aserradero, Miquihuana, Mexico (Figure 1). The morphological description and photographs are based on wild specimens.

Morphological observations and measurements were made with the aid of a digital caliper. The relevant literature was analyzed [13–32].

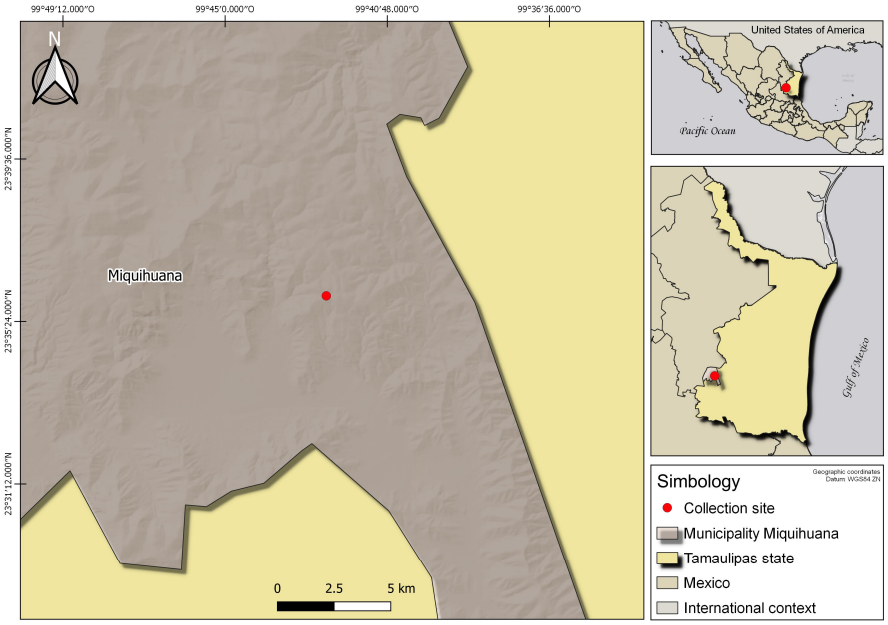


Figure 1. Map showing the type locality of *Opuntia miquihuanensis*, in the Miquihuana Municipality, State of Tamaulipas, Mexico. The map was generated using ArcMap 10 (ESRI, Redlands, CA, USA).

2.2. Surface Analysis (SEM)

Spines, epidermis and pollen were studied by SEM from material collected in situ and fixed in FAA (formol, alcohol and acetic acid) for 48 h. All materials were dissected under a stereomicroscope (Leica MZ 75, Wetzlar, Germany) (manufacturer, city, and country), dehydrated in an ethanol series, subjected to critical point drying in a Bal-Tec CPD 030, mounted on metal stubs using double-sided carbon tape, and then coated in gold in an Emitech K550X sputter coater (Kent, United Kingdom). All SEM observations were performed using a Hitachi, SU1510 model scanning electron microscope (Hitachi, Tokyo, Japan), at 10 KV at the Institute of Biology of the Universidad Nacional Autónoma de México (UNAM). The measurements were obtained using the Image Tool version 3.0 image analyzer [33].

2.3. Data from Herbaria

We reviewed the collections of the following herbaria: (ANSM, CHAP, CHAPA, CHIP, CICY, CIIDIR, ECO-TA-H, ECO-SH-H, ENCB, FCME, FMVZ, HEM, HUAA, IBUG, IEB, INEGI, INIF, ITCV, MEXU, SLPM, UAG, UAQ, UAS, UAT, UJAT, XAL and ZEA), and (virtual herbarium databases) at F, HUH, MO, NY, REMIB and US were examined (acronyms according to Thiers 2026 [34] [continuously update]). The species' identity was verified for each specimen examined.

2.4. Isolation of DNA Extraction

The DNA was obtained from 50–100 mg of the plant stem. Genomic DNA was extracted using the CTAB method [35]. The DNA was quantified with a Nanodrop 2000c (Thermo, Waltham, MA, USA). We prepared dilutions from each sample at 20 ng to amplify the genes.

2.5. PCR Amplification and Sequencing

The reaction mixture for PCR was prepared in a final volume of 15 µL. The PCR amplification reaction procedure involved pre-denaturation at 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 1 min at a specific temperature for each gene (Table 1), and extension at 72 °C for 5 min. The PCR products were analyzed using 1.2% agarose gel electrophoresis at a setting voltage of 87 V and current of 100 mA for 55~60 min. The amplified products were purified using the ExoSAP Purification kit (Affymetrix, Santa Clara, CA, USA), according to the manufacturer's instructions. These products were sequenced in both directions with an Applied Biosystems model 3730XL (Applied BioSystems, Waltham, MA, USA), at the Instituto de Biología of the Universidad Nacional Autónoma de México (UNAM).

Table 1. Primers were used in this study.

Loci/Segment	Name	Sequence 5′-3′	Tm (°C)	Reference
*mat*k	1326R	TCTAGCACACGAAAGTCGAAGT	48	[36]
	390F	CGATCTATTCATTCAATATTTC	48	[36]
ycf1	118 F	CTTATCTCTTACTTCTCCAAGCTC	52	[37]
	1330R	GCGGCTAAACTAGGTGGATGTG	52	[37]
*trn*L-*trn*F	C	CGAAATCGGTAGACGCTACG	50	[38]
	D	GGGGATAGAGGGACTTGAAC	50	[38]
	E	GGTTCAAGTCCCTCTATCCC	50	[38]
	F	ATTTGAACTGGTGACACGAG	50	[38]
psbJ-petA	psbJ	ATAGGTACTGTARCYGGTATT	50	[39]
	petA	AACARTTYGARAAGGTTCAATT	50	[39]
ITS	ITS5	GGAAGTAAAAGTCGTAACAAGG	57	[40]
	ITS4	TCCTCCGCTTATTGATATGC	57	[40]
ppc	Op 19F	GAGATGAGGGCAGGGATGAGTTACTTCC	55	[41]
	Op 569R	CTAGCCAACAAGCAAACATC	55	[41]

2.6. Sequence Assembly

The sequences of both strands of each of the genes were analyzed, edited, and assembled using BioEdit version 7.0.5 [42] to generate a consensus sequence. These consensus sequences were compared with those deposited in GenBank of the National Center for Biotechnology Information (NCBI), using the BLAST 2.2.19 tool [43].

2.7. Phylogenetic Methods

The sequences were subjected to standard BLAST searches in GenBank database to ensure taxon and gene matches.

To infer the phylogenetic relationships of the new species of *Opuntia* within the *Opuntia sensu stricto* clade an alignment was made based on the taxonomic sampling employed by Majure et al. (2012) [41], including Mexican species [44,45]. Sequences were generated from the chloroplast regions (*matK*, *ycf1*, *trn*L intron, the *trnL-trnF* spacer, and *psbj-petA*), *ppc*, and the ITS nuclear region (Table 2). Each region was independently aligned using the online version of MAFFT v7.490 with the E-INS-i algorithm, a 100PAM/k = 2 scoring matrix, a 1.3 gap open penalty, and a 0.123 offset value [46–48]. The alignments were reviewed in PhyDE v.10.0 [49], followed by minor manual adjustments to ensure character homology of every nucleotide at each position in the alignment. The collective chloroplast dataset (*matK*, *ycf1*, *psbJ-petA*, *trn*L-*trn*F), *ppc* and the ITS were analyzed separately and then concatenated to produce a total evidence dataset. Not all gene regions were available for all taxa and some taxa had incomplete datasets. Missing data were treated as a continuous series of Ns in concatenated datasets [50]. Given the dataset draws from different loci, topological incongruence between partitions (nuclear markers vs. chloroplast markers) was examined using the incongruence length difference (ILD) test implemented in PAUP* 4.0a169 [51] with 1000 heuristic after the removal of all invariable characters. The data were analyzed using maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (BI). Maximum parsimony analyses were carried out in PAUP* 4.0a169 [51] using the heuristic search mode, with 1000 random starting replicates and TBR branch swapping, with MULTREES and Collapse on. Bootstrap values were estimated using 1000 bootstrap replicates under the heuristic search mode, each with 100 random starting replicates. Bayesian inference was performed in MrBayes v3.2.6 x64 [52,53]. Two independent Markov chain Monte Carlo (MCMC) analyses were performed in parallel, each consisting of four chains run for 3.5 million generations. This run length was sufficient for the standard deviation of split frequencies to fall below 0.01, indicating convergence of the chains. Trees were sampled every 100 generations. PartitionFinder [54–56] was used to select the best-fitting mode for BI and ML analyses. Substitution models were assigned separately to each partition based on the specific evolutionary patterns of each marker as follows: a TIM1+G model was applied to *matK*, a TIM3+G model to *ycf1*, a TVM+G model to the *psbJ-petA* spacer, a TPM1uf+G model to the *trn*L intron and the *trn*L–*trn*F intergenic spacer, a HKY+G+I for ITS, and HKY+G for *ppc*. Default priors were used, and all substitution model parameters were unlinked across loci. Tracer 1.6 [57] was employed to assess stationarity and determine an appropriate burn-in period. After discarding burn-in samples, the remaining trees from both runs were combined to construct a 50% majority-rule consensus topology, with nodal support evaluated using posterior probabilities (BIPP). ML analysis was conducted using RAxML v.8.2.10, which performed 1000 rapid bootstrap replicates followed by a search for the best-scoring tree in a single run. The best-fit model GTR+G for the combined dataset for BI and ML was selected under the Akaike Information Criterion (AIC) using PartitionFinder [54,56]. The phylogenetic trees were edited using Figtree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 1 May 2026).

Table 2. GenBank accession numbers correspond to the sequences used in the phylogenetic analyses. The newly generated sequences are indicated in bold and missing sequences are indicated by –.

Species Name	GenBank Accessions					
	***trn*L-*trn*F**	***mat*K**	**ITS**	***psbJ-petA***	***ycf1***	***ppc***
Brasiliopuntia brasiliensis	JF712685	JF786712	JF786876	JF787469	JN387143	JN387207
Maihueniopsis ovata	JF712696	JF786723	JF786887	JF787479	JN387144	JN387208
Miqueliopuntia miquelii	JF712698	JF786725	JF786889	JF787480	JN387145	JN387210
Opuntia auberi	JF712699	JF786726	JF786890	JF787481	JN387146	JN387211
Opuntia chaffeyi	JF712733	JF786761	JF786922	JF787513	–	–
Opuntia cochenillifera	JF712700	JF786727	JF786891	JF787482	JN387147	JN387212
Opuntia dejecta	–	JF786729	JF786893	–	JN387148	JN387213
Opuntia gaumeri	JF712702	JF786730	JF786894	JF787484	JN387149	JN387214
Opuntia inaperta	JF712706	JF786734	JF786898	JF787488	JN387151	JN387216
Opuntia karwinskiana	JF712707	JF786735	JF786899	JF787489	JN387152	JN387217
Opuntia abjecta	JF712838	JF786865	JF787021	JF787598	JN387199	JN387264
Opuntia arechavalatae	JF712714	JF786742	JF786906	JF787496	JN387155	JN387220
Opuntia arenaria	JF712715	JF786743	JF786907	–	JN387155	JN387220
Opuntia aureispina	JF712718	JF786746	JF786910	JF787607	JN387158	JN387223
Opuntia austrina	JF712719	JF786747	JF786911	JF787499	JQ676985	JN387224
Opuntia basilaris	JF712722	JF786750	JF786913	JF787502	JN387159	JN387225
Opuntia caracassana	JF712729	JF786757	JF786918	JF787509	JN387159	JN387225,
Opuntia chisosensis	JF712734	JF786762	JF786923	JF787514	JN387159	JN387225
Opuntia chlorotica	JF712735	JF786763	JF786924	JF787608	JN387162	JN387228
Opuntia delafuentiana	KM678218	KM678215	KM678221	–	–	–
Opuntia drummondii	JF712742	JF786770	JF786930	JF787520	JN387163	JN387229
Opuntia elata	JF712746	JF786774	JF786934	–	JN387164	JN387230
Opuntia ellisiana	JF712747	JF786775	JF786935	JF787523	JN387166	JN387232
Opuntia excelsa	JF712755	JF786783	JF786942	JF787530	JN387167	JN387233
Opuntia gosseliniana	JF712761	JF786789	JF786948	JF787611	JN387169	JN387234
Opuntia humifusa	JF712762	JF786790	JF786949	JF787536	JN387169	JN387234
Opuntia hyptiacantha	MW504058	MW553200	MW475081	ON254911	–	–
Opuntia hystricina	JF712764	JF786792	JF786951	JF787538	–	–
Opuntia jamaicensis	JF712765	JF786793	JF786952	–	JN387169	JN387234
Opuntia lasiacantha	MW504050	MW545824	KM507353	–	–	–
Opuntia leiascheinvariana	KM507356	KM507350	KM507353	–	–	–
Opuntia macbridei	JF712771	JF786799	JF786957	JF787542	JN387172	JN387238
Opuntia macrocentra	JF712773	JF786801	JF786959	JF787544	JN387174	JN387240
Opuntia macrorhiza	JF712774	JF786802	JF786960	JF787545	JQ676983	JN387241
Opuntia megacantha	MW504046	MW520856	MW475069	ON254903	–	–
Opuntia megarhyza	JF712779	JF786807	JF786964	JF787549	JN387175	–
Opuntia microdasys	JF712781	JF786809	JF786966	JF787551	JN387175	JN387242
Opuntia miquihuanensis	PX965785	PX962276	PX945583		–	–
Opuntia miquihuanensis	PX965786	PX962277	PX945584		–	–
Opuntia pachyrrhyza	JF712785	JF786813	JF786970	JF787554	JN387178	JN387178
Opuntia polyacantha	JF712794	JF786822	JF786979	JF787562	JN387180	JN387245
Opuntia pusilla	JF712800	JF786827	JF786984	JF787566	JN387181	JN387246

Table 2. *Cont.*

Species Name	GenBank Accessions					
	***trn*L-*trn*F**	***mat*K**	**ITS**	***psbJ-petA***	***ycf1***	***ppc***
Opuntia pycnantha	JF712803	JF786830	JF786987	JF787565	JN387182	JN387247
Opuntia quimilo	JF712804	JF786831	JF786988	JF787569	JN387183	JN387248
Opuntia retrorsa	JF712814	JF786839	JF786995	JF787575	JN387185	JN387250
Opuntia rufida	JF712812	JF786840	JF786997	JF787577	JN387251	JF786840
Opuntia sanguinea	JF712817	–	JF787000	JF787580	JN387190	JN387255
Opuntia scheeri	JF712819	JF786847	JF787002	JF787581	JN387192	JN387257
Opuntia schickendantzii	JF712820	JF786848	JF787003	JF787582	JN387192	JN387257
Opuntia stenopetala	JF712825	JF786852	JF787008	JF787618	JN387192	JN387257
Opuntia streptacantha	MW504054	MW546909	MW745077	ON254899	–	–
Opuntia strigil	JF712829	JF786856	JF787012	JF787590	JN387195	JN387260
Opuntia tapona	JF712833	JF786860	JF787016	JF787593	JN387198	JN387263
Opuntia tehuacana	MT887611	–	MT856449	–	–	–
Opuntia triacantha	–	JN676103	JN676102	JN676105	JN387200	JN387265
Salmiopuntia salmiana	JF712815	JF786842	JF786998	JF787578	JN387188	JN387253
Tacinga funalis	–	AY042660	–	–	–	–
Tacinga inamoena	JF786870	JF786870	JF787026	JF787619	JN387201	JF787026
Tacinga lilae	JF712769	JF786797	JF786955	JF787612	JN387171	JN387237
Tacinga palmadora	JF712845	JF786872	JF787028	JF787603	JN387203	JN387267
Tacinga saxatilis	JF712846	JF786873	JF787029	JF787620	JN387204	JN387268

3. Results

3.1. Phylogenetic Results

Thef combined *matK* + *ycf1* + *psbJ-petA* + *trn*L-*trn*F + ITS + *ppc* dataset comprises 65 taxa with 5399 characters, including gaps. The three phylogenetic analyses, MP, ML, and BI, were conducted and resulted in generally congruent topologies. The best RAxML tree with a final likelihood value of –29540.258400 is presented. The matrix had 1101 distinct alignment patterns, with 9.84% undetermined characters or gaps. Estimated base frequencies were as follows: A = 0.108624, C = 0.200816, G = 0.280341, and T = 0.262750; substitution rates AC = 1.108312, AG = 2.083041, AT = 1.831201, CG = 1.030428, CT = 6.106258, and GT = 1.000000; gamma distribution shape parameter α = 0.100084. In the Bayesian analysis, the standard deviation between the chains stabilized at 0.0002 after 3.5 million generations. No significant changes in tree topology trace or cumulative split frequencies of selected nodes were observed after about 1.5 million generations, so the first 980,000 sampled trees (25%) were discarded as burn-in. To confirm that the sample size was sufficient, the parameter file was examined in Tracer 1.6 [57,58], and all parameters had an estimated sample size above 1500. Both the maximum parsimony, maximum likelihood, and Bayesian inference recovered *Opuntia miquihuanensis*, supporting the existence of a new taxon distinctive from related species of *Opuntia s. str.* (BS = 100%, BS = 100%, BI = 1) (Figure 2).

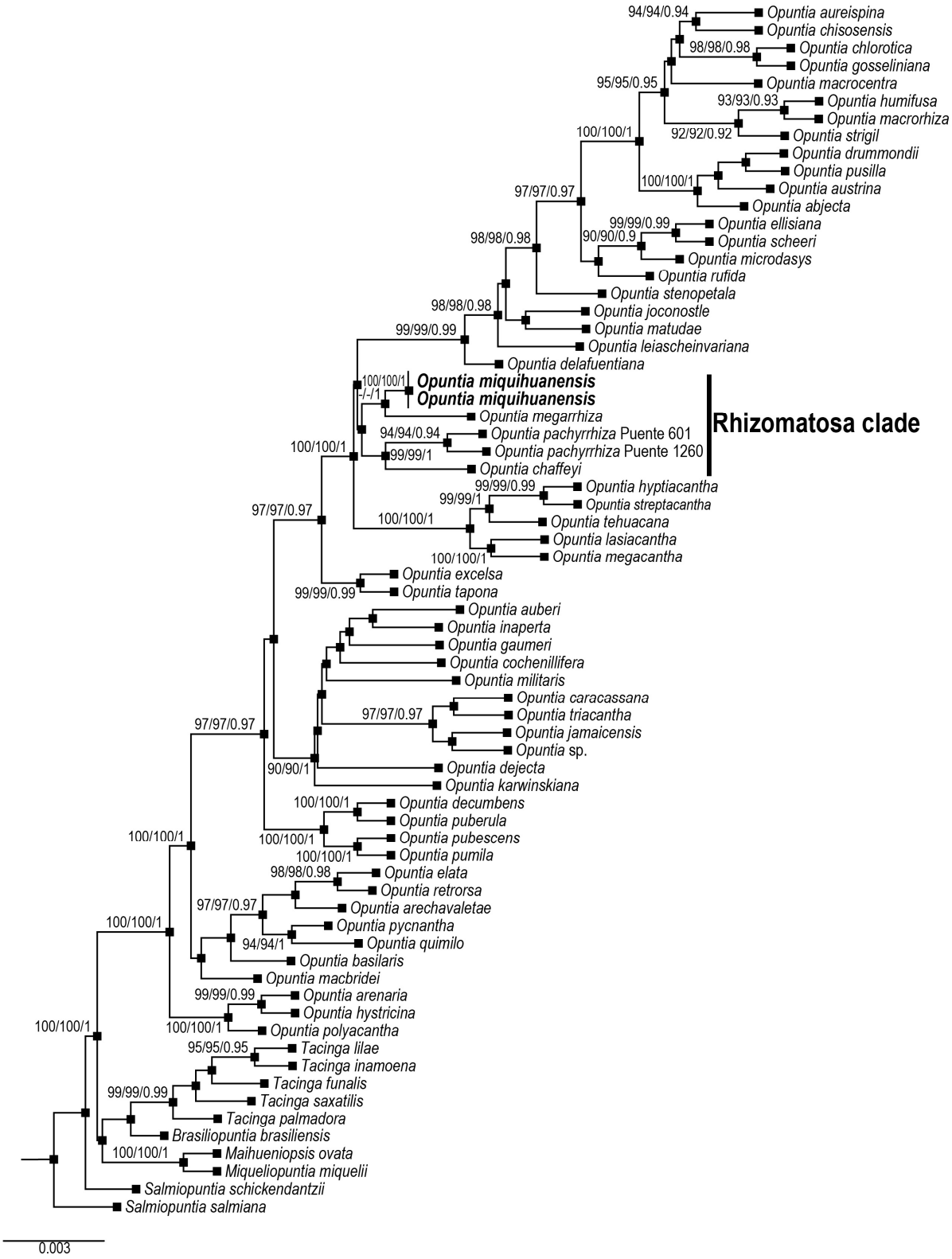


Figure 2. Phylogram of *Opuntia s.s.*, based on the concatenated *matK*, *ycf1*, *psb*J-*pet*A, *trn*L-*trn*F, ITS, and *ppc* sequence alignment. For each node, the following values are provided: maximum parsimony (MP $\geq$ 90%, left)/maximum likelihood bootstrap (ML $\geq$ 90%, middle), and Bayesian inference posterior probability (BIPP $\geq$ 0.90, right). The phylogenetic position of *Opuntia miquihuanensis* is shown in bold. The scale bar represents the expected number of nucleotide substitutions per site.

3.2. Taxonomic Treatment

Opuntia miquihuanensis Martínez-Gon., García-Mor. & García-Jim., sp. nov. (Figure 3).

Type: MEXICO, Tamaulipas, Miquihuana Municipality, 23°36′03.79″ N, 99°42′22.88″ E, elevation 2663 m, 26 March 2016, *García-Morales 5345B* (holotype: ITCV!; isotypes: CHAP! and CHAPA!).

Diagnosis: *O. miquihuanensis* shows greater similarity to *O. pachyrrhiza*, but differs in having longer cladodes, a greater number of areole series, straight (75–100°), diffuse (21–74°), and radial or adpressed (0–20°) spines, as well as longer fruits with a pedunculate-claviform shape.

Description: Low-growing habit, 30–40 cm in height (Figure 4A). Trunk not defined. Epidermis glabrous (Figure 8C). Rhizome erect, succulent, tuberiform. Cladodes elliptical, 18–21 × 12–17 cm, and approximately 1.8 cm thick, green (Figure 4C). Areoles arranged in 6–7 series, 2.3 cm apart between series, 2 cm apart between areoles, elliptical to obovate, 0.4 × 0.1–0.2 cm, with short, dark-colored trichomes. Brown, short glochids (0.2 cm). Spines 1–11 per areole, white with amber or dark-tipped apices, setose, porrect and divergent, unequal, 0.5–4.3 cm long (Figure 5B). Juvenile cladodes light green, with prominent tubercles; areoles bearing short, cream-colored trichomes; leaves subulate, erect, green with reddish and hooked apices (Figure 4B). Floral buds acute; perianth segments reddish with acuminate apices; pericarpels turbinate with elevated tubercles; areoles bearing long, cream-colored trichomes; glochids yellow (Figure 5C,D). Flowers yellow (Figure 5E), 10–11 cm long; pericarpel turbinate, ca. 4.7 × 2.3 cm; areoles arranged in three series, spaced 1.1 cm apart, with a thick basal scale; outer perianth segments obovate, emarginate at the apex, yellow with a green stripe; inner segments obovate, apices acuminate, yellow; stamens one-third the length of the perianth (Figure 6B); filaments white; anthers yellow; style cuneate, ca. 2.5 cm long, white; stigma lobes eight, papillose, ca. 0.5 cm long, green with a whitish median stripe. Pollen grains hexagonal, tectate, suprareticulate, with broad and slightly verrucose walls (Figure 8E). Fruits pedunculate (Figure 6F), 6.1–6.5 cm long, 3.1–3.6 cm wide, yellowish green; floral scar slightly depressed (0.6 cm), slightly striate (Figure 7B); areoles small, arranged in 3–4 series, circular to semicircular, with long gray trichomes (Figure 7C), spaced 1.5 cm apart and 2.2 cm between series; spines almost absent, white to reddish; walls thin, greenish-white and tasteless; pyriform 2.1–2.4 cm long; funicule white, succulent, and tasteless (Figure 7A). Seeds slightly reniform or discoidal, cream-colored, ca. 0.32 cm in diameter; aril lateral, irregular; hilum–micropylar region lateral, slightly depressed; micropyle and funicle included (Figure 7D).

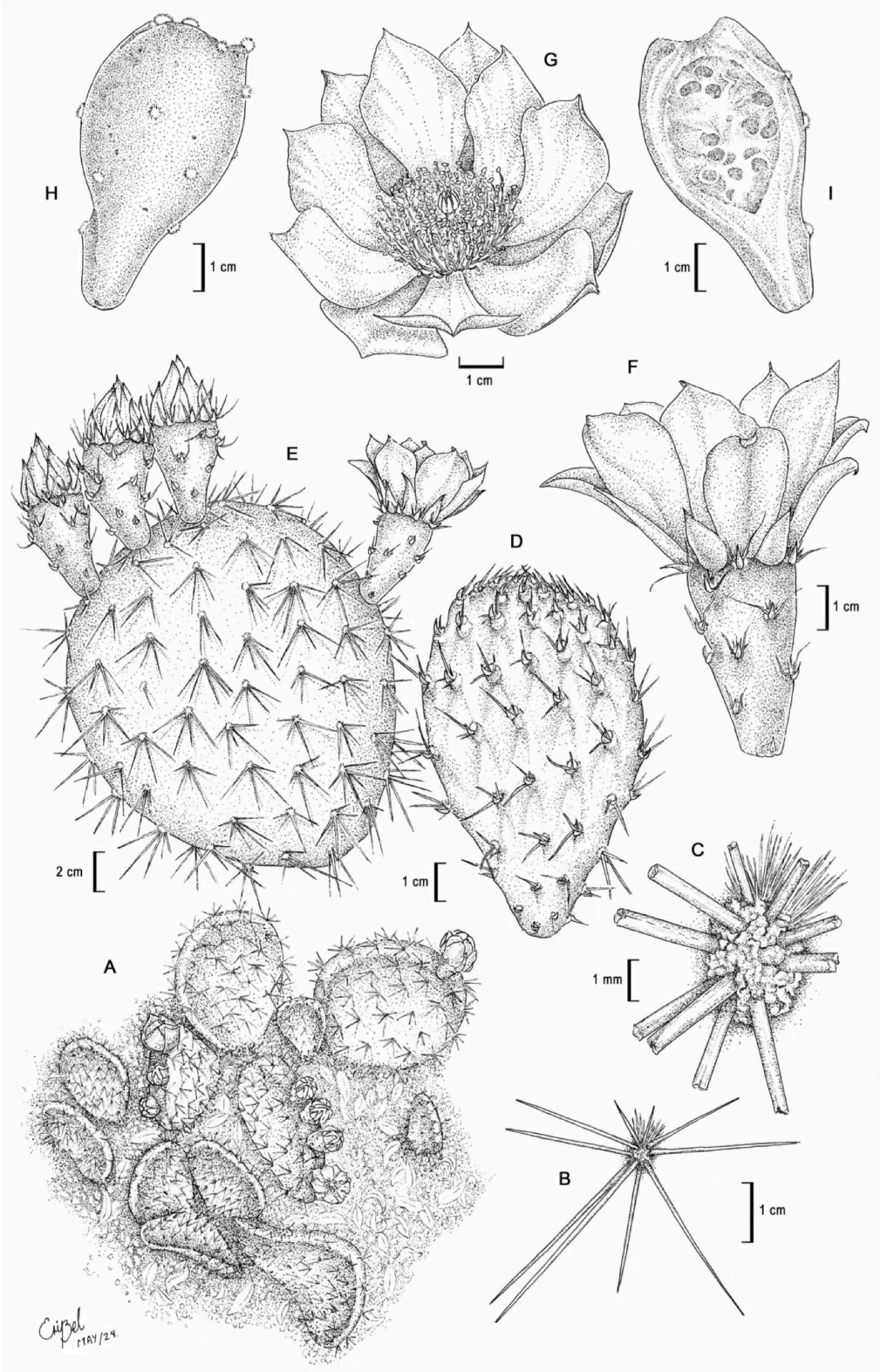


Figure 3. *Opuntia miquihuanensis.* (**A**) Low-growing habit. (**B**) Spines. (**C**) Areole. (**D**) Juvenile cladode. (**E**) Mature cladode. (**F**) Flower side view. (**G**) Flower in anthesis. (**H**) Pyriform fruits. (**I**) Inner part of the fruit. Illustration by Ericka Belén Cortez on the type specimen *García-Morales 5345B* (ITCV).

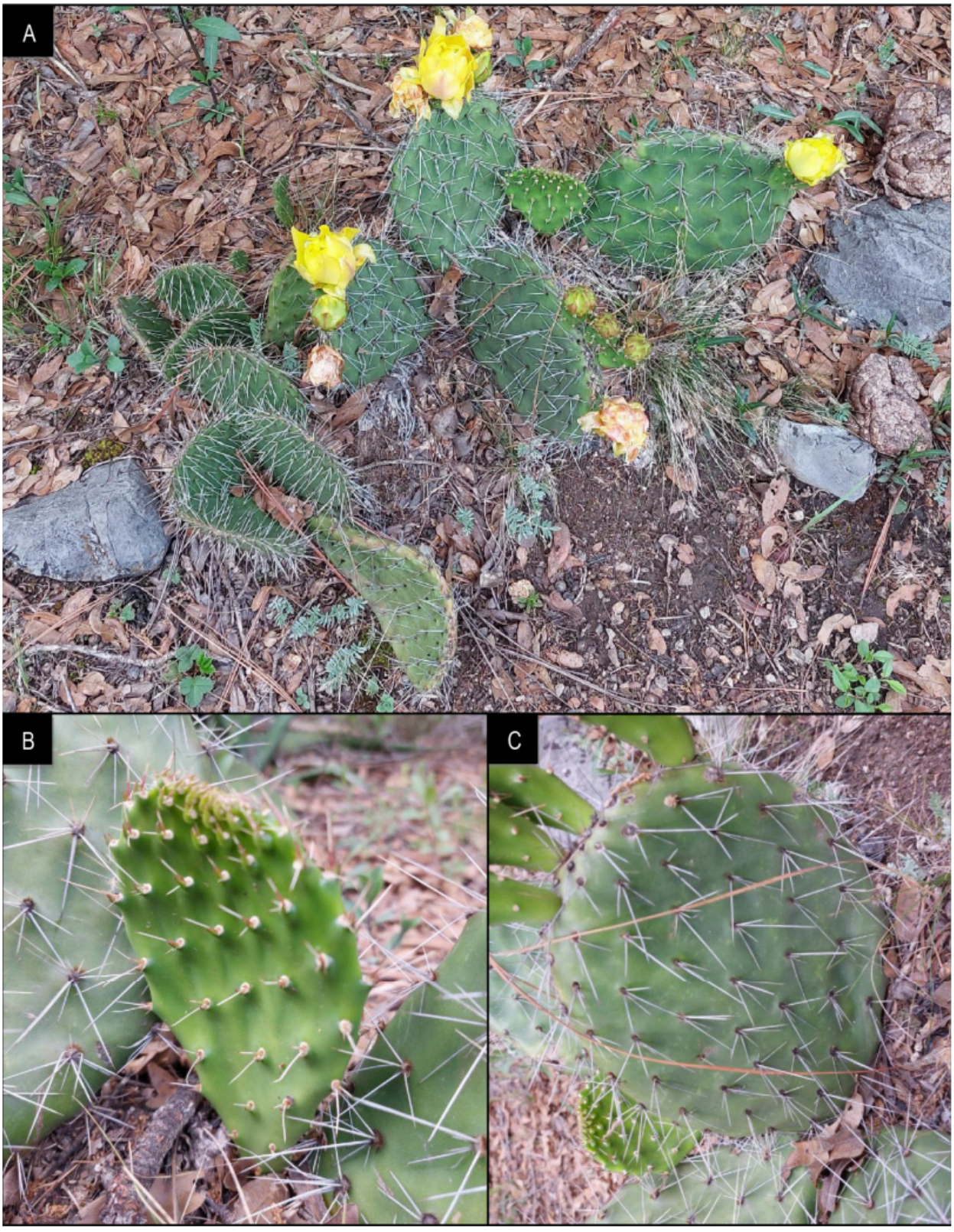


Figure 4. *Opuntia miquihuanensis*. (**A**) Plant prostate, trunk not defined. (**B**) Juvenile cladode with elevated podaria, white spines with amber tips, some with reddish bases. (**C**) Dark green elliptical cladodes covered with a wax layer.

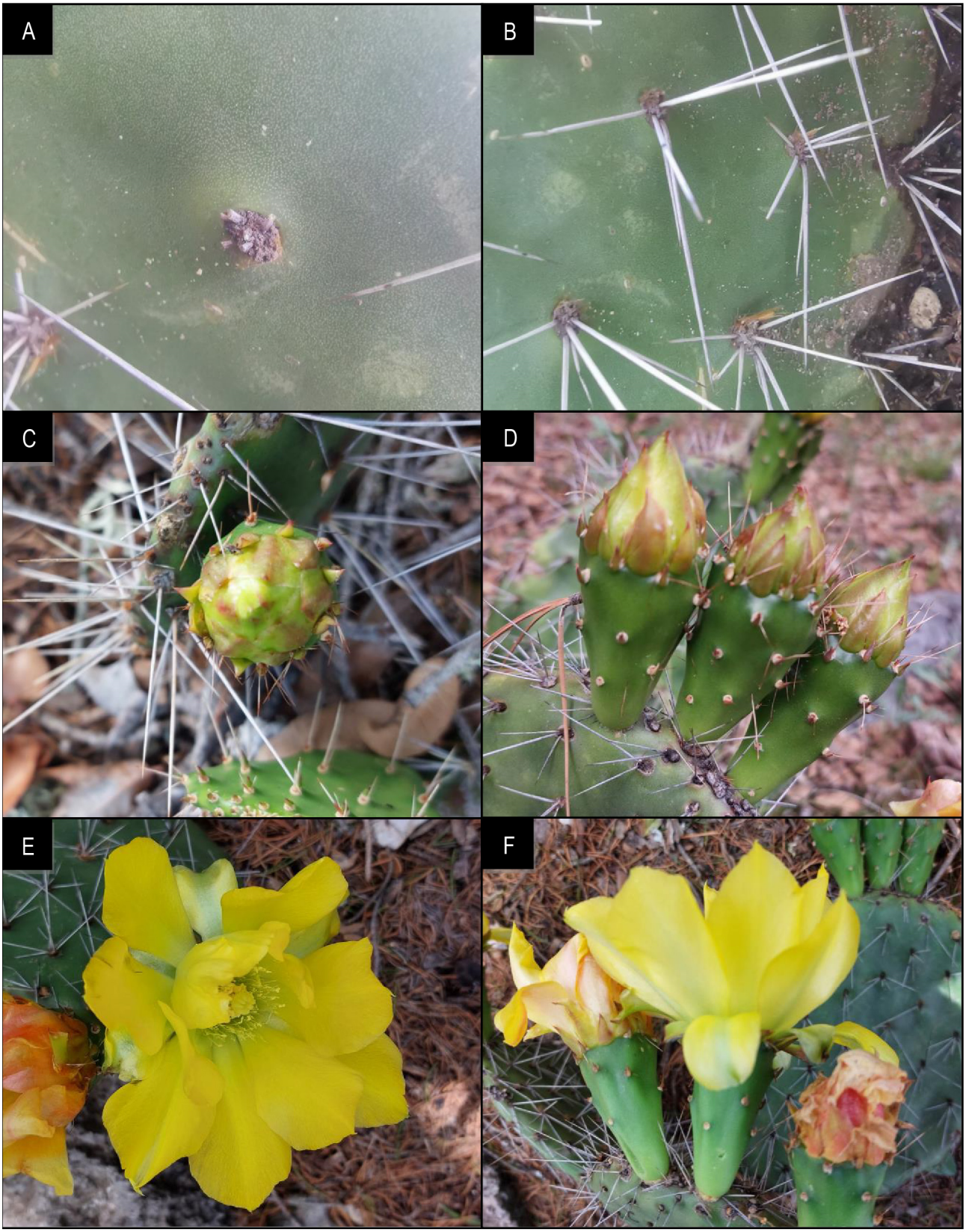


Figure 5. *Opuntia miquihuanensis*. (**A**) Elliptical black areole, and base with five spines. (**B**) White spines with amber tips. (**C**) Flower button, upper view. (**D**) Lateral view of the flower button, turbinate pericarpel with brown bristles. (**E**) Yellow flower in anthesis. (**F**) Flower side view, turbinate pericarpel with brown bristles.



Figure 6. *Opuntia miquihuanensis*. (**A**) Should be longitudinal section of flower. (**B**) Obovate yellow inner segments, acuminate apex. (**C**) Outer segments of obovate perianth, emarginate apex, yellow, with a green stripe in the middle. (**D**) Style white. (**E**) Eight green papillose stigma lobes, with a white midstripe. (**F**) Yellowish green color of the fruit.



Figure 7. *Opuntia miquihuanensis*. (**A**) Longitudinal cut of the fruit. (**B**) Floral scar depressed, slightly striate. (**C**) Areoles small, circular with long gray trichomes. (**D**) Reniform or discoidal seed with irregular lateral aril.

3.3. Micromorphology

Spines with long, wide and lignified epidermal cells in the apical part, 120.40 ± 1.2 µm and 19.17 ± 1.3 µm, respectively (Figure 8A), the middle part of the spine with epidermal cells of 88.66 ± 1.8 µm long and 16.21 ± 1.2 µm wide, non-continuous epidermal cells with fissures between cells and smooth texture (Figure 8B). Glabrous epidermis (Figure 8C), irregular epidermal cells (Figure 8D), and paracytic stomata (Figure 8D). Pollen grains, with polyhedron shape, pantoaperturate (Figure 8E), 102.90 ± 3.2 µm in diameter, 7504.45 µm^2 in area, 17.39 ± 2.9 µm, distance between each distal opening (pores), with a diameter of 25.16 ± 1.5 µm, 16–17 pores with walls of 1.77 ± 0.02 µm, width and warty (Figure 8F).

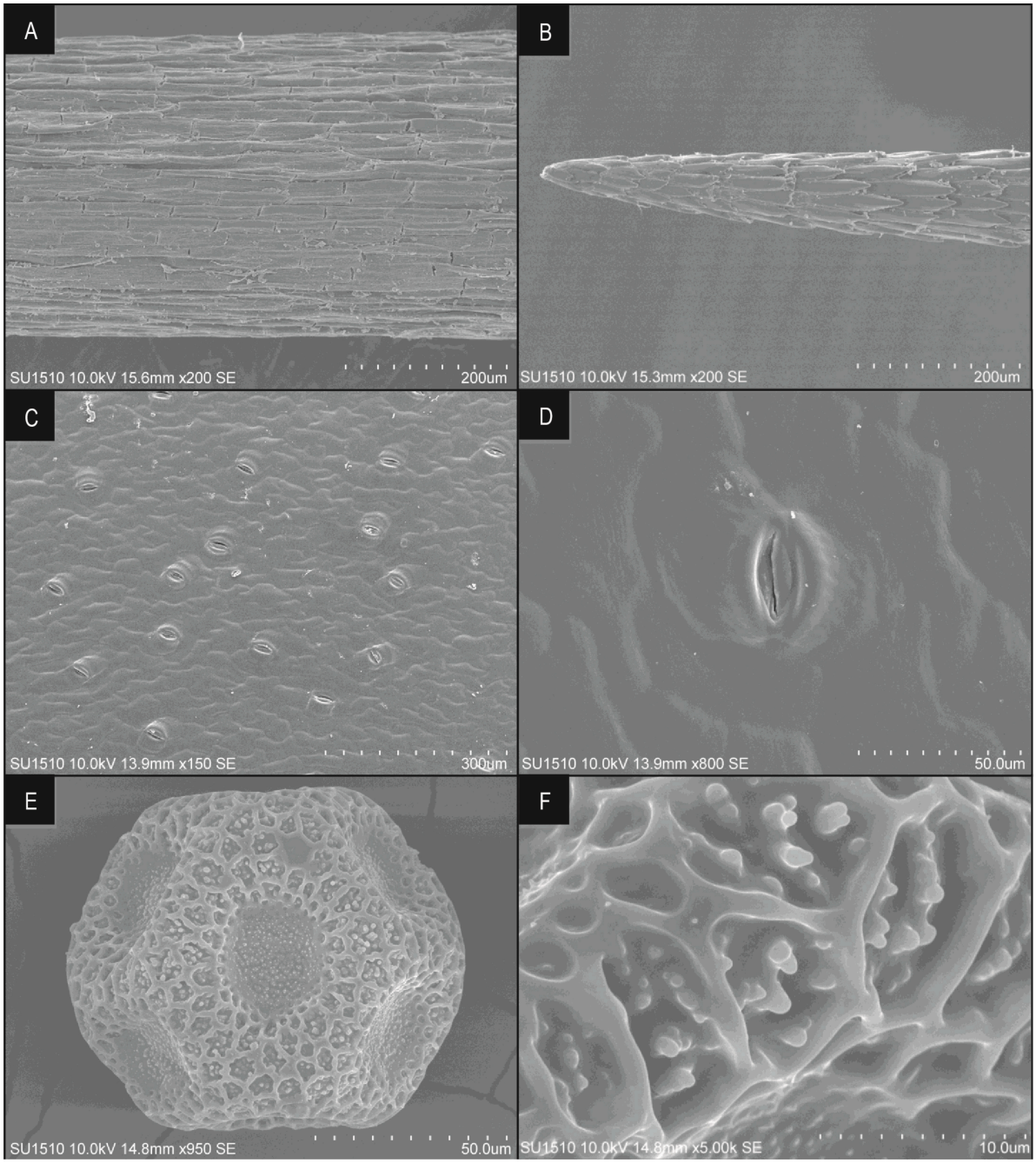


Figure 8. *Opuntia miquihuanensis*. (**A**) Epidermal cells of the middle region of the spine, with fissures between cells and rough texture. (**B**) Epidermal cells of the apical region of the spine. (**C**) Glabrous epidermis. (**D**) Irregular epidermal cells, paracytic stoma. (**E**) Pollen grains are hexagonal, tectate, and suprareticulate. (**F**) Close-up of a pore, with slightly warty walls.

3.4. Etymology

The epithet refers to the municipality of Miquihuana, Tamaulipas, where this new species is found as a microendemic taxon and the only known distribution area of this new species.

3.5. Phenology

It flowers once a year in March–April, and depending on the distribution of rainfall during the cycle, it usually reaches maturity in July–August.

3.6. Distribution and Habitat

This species is by now only found around natural open grasslands and flatlands on the top of mountains among pine and oak forests, between the 1600 and 1800 m elevation (Figure 9).



Figure 9. Habitat of *Opuntia miquihuanensis* (indicated with a white arrow) at the type locality.

3.7. Conservation Status

This species was found during visits to the type locality in 2016, and recently again in 2023 and 2024; only 10 individuals were observed in the type location, and a few dozen more were scattered along a larger area within the municipality. Considering that its natural habitat is highly altered by habitat loss and livestock grazing, it is herein proposed as "Critically Endangered" CR B1ab (i, ii, iii, v)" + 2ab (i, ii, iii, v) under the UICN criteria (IUCN Standards and Petitions Committee 2022). In addition, this species should be considered for its inclusion in a protection category (i.e., subject to special protection) within the Mexican Official Standard on threatened species (NOM-059-SEMARNAT 2010) [59].

4. Discussion

Mexico is a highly varied country with mountain ranges, plateaus, deep canyons, and coastal plains among other relief features, this complex physiography result from the interaction of five tectonic plates [60]. The Sierra Madre Oriental (SMO), located in northeastern Mexico, is home to 6981 species of vascular plants, of which 1542 are endemic [61], and constitutes one of the five mountain ranges of the Mexican Transition Zone, where the Nearctic and Neotropical biogeographic realms meet [62], and from a biological viewpoint is one of the most important mountain systems in Mexico. Temperate forests in the SMO occur in high "island mountain ranges" surrounded by contrasting vegetation types and

are characterized by high endemism, suggesting geographical isolation is an important factor enhancing allopatric speciation processes [62].

In recent years, the species documentation of *Opuntia* in Tamaulipas has increased substantially, with García-Morales et al. [12] adding 31 new records not mentioned in the Mexican Cactus Inventory [16], the checklist of Cactaceae [9] and the checklist of Mexican vascular plants [63].

A total of 5500 specimens of the genus *Opuntia* were examined from the herbaria consulted; only 12 correspond to *O. pachyrrhiza*, all from the MEXU and SLPM collections.

In the phylogenetic analysis, species belonging to the Rhizomatosa clade were included: *O. chaffeyi*, *O. megarrhiza*, *O. miquihuanensis*, and two individuals of *O. pachyrrhiza* (*Puente 601* and *1260*). These taxa formed a monophyletic clade, which is congruent with their shared possession of rhizomes. *O. miquihuanensis* is supported as an independent lineage from the other known rhizomatous species.

Morphologically, *O. miquihuanensis* shows greater similarity to *O. pachyrrhiza* (Table 3), differing in having longer cladodes, a greater number of areole series, straight (75–100°), diffuse (21–74°), and radial or adpressed (0–20°) spines, as well as longer fruits with a pedunculate–claviform shape. At the type locality of *O. miquihuanensis*, *O. pachyrrhiza* is also present, and the latter corresponds to the description provided by Hernández et al. [20]. Therefore, *O. miquihuanensis* is not a hybrid derived from *O. pachyrrhiza*, as no ambiguities were observed in the ITS region (ITS1, 5.8, ITS2).

Table 3. Morphological comparison between *Opuntia miquihuanensis* and *O. pachyrrhiza*.

Habit	***Opuntia miquihuanensis***	***Opuntia pachyrrhiza***
	Erect to Prostrate	**Erect to Prostrate**
Shape of cladodes	Elliptic	Elliptic
Long of cladodes	18–21 cm	40 cm
Diameter of cladodes	12–17 cm	12 cm
Epidermis	Glabrous	Glabrous
Number of areola series in the cladodes	6–7	8–9
Direction of the spines	Straight (75–100°) and diffuse (21–74°)	Straight (75–100°), diffuse (21–74°) and radial or adpressed (0–20°)
Flower color	Yellow	Yellow
Number of stigma lobes	8	6–10
Shape of the style	Cuneate	Subconical
Fruit shape	Pedunculate	Obovoid
Fruit length	6.1–6.5 cm long	2.7 cm long
Pedunculated fruit	Yes	-----
External color of fruits	Yellowish green	Yellow
Trichomes on the areolas	Long gray trichomes	Long yellow trichomes
Internal color of fruits	Greenish-white	-----
Color of the funiculars	White	-----
Funiculars	Tasteless	-----
Seed shape	Discoid	Discoid

Interestingly *O. miquihuanensis* lives together with *O. pachyrrhyza* in the mountains of Miquihuana, Tamaulipas, the reason it remained hidden as a cryptic species until this study.

Opuntia megarrhiza is also found nearby the known locations of this new taxon, supporting the biogeographical concept of a speciation area including three of the four known species of this group of *Opuntia*.

The diversity and phylogenetic relationships within the genus *Opuntia* remain insufficiently studied, with only a few species having been formally molecularly characterized to date.

This study establishes an integrative framework for delimiting one novel *Opuntia* species. The discovery of *O. miquihuanensis* represents a significant advancement in our understanding of the morphological and phylogenetic of *Opuntia*, a recently established genus (around 7.5 mya) of nopales [64,65].

This finding supports the original concept of the genus as a cohesive evolutionary lineage defined by both morphological and molecular characters.

Author Contributions: Conceptualization, C.R.M.-G., L.J.G.-M. and J.G.J.; methodology, T.R., J.J.-R., F.G.-O. and L.J.G.-M.; formal analysis, C.R.M.-G. and L.J.G.-M.; investigation, C.R.M.-G. and J.G.J.; resources, J.G.J.; writing—original draft preparation, C.R.M.-G.; writing—review and editing, C.R.M.-G., L.J.G.-M. and J.G.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Tecnológico Nacional de México, Instituto Tecnológico de Ciudad Victoria, throughout the Programa de Doctorado en Ciencias en Biología.

Data Availability Statement: The original contribution presented in the study is included in the article, and further inquiries can be directed to the corresponding author.

Acknowledgments: The authors thank Laura Márquez and Nelly López (LaNaBio, Instituto de Biología, Universidad Nacional Autónoma de México) for sequencing the PCR products. They also thank Berenit Mendoza (LaNaBio, Instituto de Biología, Universidad Nacional Autónoma de México) for taking the images in the scanning electron microscope and Ericka Belén Cortez for the wonderful illustration.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Novoa, A.; Le Roux, J.J.; Robertson, M.P.; Wilson, J.R.; Richardson, D.M. Introduced and invasive cactus species: A global review. *AoB PLANTS* **2014**, *7*, plu078. [CrossRef]
2. Abouseadaa, H.H.; Atia, M.A.M.; Younis, I.Y.; Issa, M.Y.; Ashour, H.A.; Saleh, I.; Osman, G.H.; Arif, I.A.; Mohsen, E. Gene-targeted molecular phylogeny, phytochemical profiling, and antioxidant activity of nine species belonging to family Cactaceae. *Saudi J. Biol. Sci.* **2020**, *27*, 1649–1658. [CrossRef] [PubMed]
3. Korotkova, N.; Aquino, D.; Arias, S.; Eggli, U.; Franck, A.; Gómez-Hinostrosa, C.; Guerrero, P.C.; Hernández, H.M.; Kohlbecker, A.; Köhler, M.; et al. Cactaceae at Caryophyllales.org—A dynamic online species-level taxonomic backbone for the family. *Willdenowia* **2021**, *51*, 251−270. [CrossRef]
4. Goettsch, B.; Hernández, H.M. Beta diversity and similarity among cactus assemblages in the Chihuahuan Desert. *J. Arid. Environ.* **2006**, *65*, 513–528. [CrossRef]
5. Ritz, C.M.; Reiker, J.; Charles, G.; Hoxey, P.; Hunt, D.; Lowry, M.; Stuppy, W.; Taylor, N. Molecular phylogeny and character evolution in terete-stemmed Andean opuntias (Cactaceae-Opuntioideae). *Mol. Phylogenet. Evol.* **2012**, *65*, 668–681. [CrossRef] [PubMed]
6. Hernández-Ledesma, P.; Berendsohn, W.G.; Borsch, T.; Mering, S.V.; Akhani, H.; Arias, S.; Castañeda-Noa, I.; Eggli, U.; Eriksson, R.; Flores-Olvera, H.; et al. A taxonomic backbone for the global synthesis of species diversity in the angiosperm order Caryophyllales. *Willdenowia* **2015**, *45*, 281–383. [CrossRef]
7. Anderson, E.F. *The Cactus Family*; Timber Press: Portland, OR, USA, 2001.
8. Vilà, M.; Burriel, J.A.; Pino, J.; Chamizo, J.; Llach, E.; Porterias, M.; Vives, M. Association between Opuntia species invasion and changes in land-cover in the Mediterranean region. *Glob. Change Biol.* **2003**, *9*, 1234–1239. [CrossRef]
9. Martínez-Avalos, J.G.; Jurado, E. Geographic distribution and conservation of Cactaceae from Tamaulipas Mexico. *Biodivers. Conserv.* **2005**, *14*, 2483–2506. [CrossRef]

10. García-Morales, L.J. Contribución al conocimiento de la diversidad taxonómica de las cactáceas (Caryophyllales, Cactaceae) en Tamaulipas, México. In *La biodiversidad en Tamaulipas*; Cruz Angón, A., López Higareda, D., Malgarejo, E.D., Rodríguez Ruiz, E.R., Eds.; Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO): Mexico City, Mexico, 2024; pp. 127–138.
11. García-Morales, L.J. Estudio Sobre la Diversidad, Distribución y Algunos Aspectos Ecológicos de las Cactáceas (Caryophyllales: Cactaceae) de la Sierra Madre Oriental, Sierra de San Carlos y Zonas Adyacentes en el Estado de Tamaulipas, México. Bachelor's Thesis, Instituto Tecnológico de Ciudad Victoria, Ciudad Victoria, Mexico, 2006.
12. García-Morales, L.J.; García-Jiménez, J.; Contreras-Medina, R.; Alcántara-Ayala, O.; Luna-Vega, I. Diversity, distribution and conservation of the Cactaceae (Caryophyllales) from Tamaulipas Mexico. *Plant Biosyst.* **2022**, *156*, 1405–1421. [CrossRef]
13. García-Morales, L.J.; Contreras-Medina, R.; Alcántara-Ayala, O.; Luna-Vega, I.; García-Jiménez, J. *Biodiversidad Tamaulipeca Vol. I*; Dirección General de Educación Superior Tecnológica (DGEST)—Instituto Tecnológico de Ciudad Victoria: Ciudad Victoria, Mexico, 2005.
14. Bravo-Hollis, H. *Las Cactáceas de México Vol. 1*; Universidad Nacional Autónoma de México: Mexico City, Mexico, 1978; 351p.
15. Britton, N.L.; Rose, J.N. *The Cactaceae: Description and illustrations of Plants of the Cactus Family, Vol. 1*; Carnegie Institution: Washington, DC, USA, 1919; 236p.
16. Guzmán, U.L.; Arias, S.; Dávila, P. *Catálogo de Cactáceas Mexicanas*; Universidad Nacional Autónoma de México: Mexico City, Mexico, 2003; 315p.
17. Hunt, D.; Taylor, N.; Charles, G. *The New Cactus Lexicon*; DH Books: Milborne Port, UK, 2006.
18. Hunt, D. Further Studies in Opuntioideae (Cactaceae). *Succ. Pl. Res.* **2014**, *8*, 185−200.
19. Hunt, D. *CITES Cactaceae Checklist*, 3rd ed.; DH Books: Milborne Port, UK, 2016.
20. Hernández, H.M.; Gómez-Hinostrosa, C.; Bárcenas, R.T. Studies on Mexican Cactaceae. I. *Opuntia pachyrrhiza*, a new species from the Chihuahuan Desert, Mexico. *Novon* **2001**, *11*, 309–314. [CrossRef]
21. Lodé, J. *Taxonomy of the Cactaceae (Supplement)*; Alphabetical INDEX of Taxa in Current Usage and Their Synonyms; Cactus Adventures International: Barcelona, Spain, 2015; 46p.
22. Martínez-González, C.R. *Opuntia* × *soumaya* (Cactaceae; Opuntioideae), a new hybrid from Mexico based on morphological, micromorphology and molecular evidence. *Phytotaxa* **2025**, *717*, 31–51. [CrossRef]
23. Parfitt, B.D.; Pinkava, D.J. Nomenclatural systematic reassessment of *Opuntia engelmanii* and *Opuntia lindheimeri* (Cactaceae). *Madroño* **1988**, *35*, 342−349.
24. Pinkava, D.J. Nomenclatural changes in *Opuntia* (Cactaceae). *Haseltonia* **1996**, *4*, 103−104.
25. Pinkava, D.J. On the evolution of the continental North American Opuntioideae. Studies in the Opuntioideae (Cactaceae). *Succ. Pl. Res.* **2002**, *6*, 59–98.
26. Scheinvar, L. *Opuntia heliabravoana, Una Especie Nueva de Cactaceae*; Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Botánica: Mexico City, Mexico, 1974; Volume 45, pp. 75–86.
27. Scheinvar, L. *Redescubrimiento de Opuntia oligacantha Forster en el Valle de México y en la Altiplanicie Mexicana*; Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Botánica: Mexico City, Mexico, 1987; Volume 57, pp. 109–122.
28. Scheinvar, L. *Opuntia zamudioi*, una nueva especie de Querétaro. *Cactáceas Suculentas Mex.* **1999**, *44*, 88−93.
29. Scheinvar, L. *Opuntia stricta* (Haw.) Haw. subsp. *esparzae*, una nueva subespecie de las dunas del río Concá, Arroyo Seco, Querétaro, México. *Cactáceas Suculentas Mex.* **2002**, *47*, 94−102.
30. Scheinvar, L.; Manzanero, G. *Opuntia chiangiana*, una nueva especie de Cactaceae de Oaxaca, México. *Novon* **2009**, *19*, 222−228. [CrossRef]
31. Scheinvar, L.; Olalde, G.; Filardo, S.; Beckler, P. *Diez Especies Mexicanas Productoras de Xoconostles: Opuntia spp. y Cylindropuntia imbricata (Cactaceae)*; Universidad Nacional Autónoma de México: Mexico City, Mexico; Universidad Autónoma del estado de Hidalgo: Mexico City, Mexico; Universidad Autónoma Metropolitana: Mexico City, Mexico, 2010; 177p.
32. Scheinvar, L.; Gallegos-Vázquez, C.; Gámez-Tamariz, N.; Olalde-Parra, G. *Atlas de los Nopales Silvestres Mexicanos*; Universidad Nacional Autónoma de México: Mexico City, Mexico, 2020; 607p.
33. Wilcox, C.D.; Dove, S.; McDavis, W.; Greer, D. *Image Tool, version 3.0*; University of Texas Health Center: San Antonio, TX, USA, 2002.
34. Thiers, B. Index Herbariorum: A Global Directory of Public Herbaria and Associated Staff. New York Botanical Garden's Virtual Herbarium. Available online: https://sweetgum.nybg.org/science/ih/ (accessed on 1 February 2025).
35. Martínez-González, C.R.; Ramírez-Mendoza, R.; Jiménez-Ramírez, J.; Gallegos-Vázquez, C.; Luna-Vega, I. Improved method for genomic DNA extraction for *Opuntia* Mill. (Cactaceae). *Plant Methods* **2017**, *13*, 82. [CrossRef] [PubMed]
36. Schmitz-Linneweber, C.; Maier, R.; Jean-Pierre, A.; Cottet, A.; Herrmann, R.; Mache, R. The plastid chromosome of spinach (*Spinacia oleracea*): Complete nucleotide sequence and gene organization. *Plant Mol. Biol.* **2001**, *45*, 307−315. [CrossRef] [PubMed]
37. Dong, W.; Xu, C.; Li, C.; Sun, J.; Zuo, Y.; Shi, S.; Cheng, T.; Guo, J.; Zhou, S. *ycf1*, the most promising plastid DNA barcode of land plants. *Sci. Rep.* **2015**, *5*, 8348. [CrossRef]

38. Taberlet, P.G.; Ludovic, P.; Guy, P.; Bouvet, J. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* **1991**, *17*, 1105−1109. [CrossRef]
39. Shaw, J.; Lickey, E.B.; Schilling, E.E.; Small, R.L. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *Am. J. Bot.* **2007**, *94*, 275−288. [CrossRef]
40. White, T.J.; Bruns, T.D.; Lee, S.B.; Taylor, J.W. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*; Innis, M.A., Gelfand, D.H., Sninsky, J.J., White, T.J., Eds.; Academic Press: New York, NY, USA, 1990; pp. 135–322.
41. Majure, L.; Puente, R.; Griffith, M.P.; Judd, W.S.; Soltis, P.S.; Soltis, D.E. Phylogeny of *Opuntia* s.s. (Cactaceae): Clade delineation, geographic origins, and reticulate evolution. *Am. J. Bot.* **2012**, *99*, 847−864. [CrossRef]
42. Hall, T.A. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp. Ser.* **1999**, *41*, 95−98.
43. Zhang, Z.; Schwartz, S.; Wagner, L.; Miller, W. A greedy algorithm for aligning DNA sequences. *J. Comput. Biol.* **2000**, *7*, 203−214. [CrossRef] [PubMed]
44. Martínez-González, C.R.; Gallegos-Vázquez, C.; Scheinvar, L.; Muñiz-Díaz de León, M.E.; Jiménez-Ramírez, J. Re-evaluation of *Opuntia matudae* (Cactaceae). *Phytotaxa* **2019**, *423*, 158−170. [CrossRef]
45. Martínez-González, C.R.; Muñiz-Díaz de León, M.E.; González-Martínez, C.A.; Jiménez-Ramírez, J.; Morales-Sandoval, J.; Gallegos-Vázquez, C. Phylogenetic placement and new data on macro- and micromorphology of *Opuntia joconostle* (Cactaceae). *Bradleya* **2020**, *38*, 158−169. [CrossRef]
46. Katoh, K.; Misawa, K.; Kuma, K.; Miyata, T. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* **2002**, *30*, 3059–3066. [CrossRef]
47. Katoh, K.; Rozewicki, J.; Yamada, K.D. MAFFT online service: Multiple sequence alignment, interactive sequence choice and visualization. *Brief. Bioinform.* **2017**, *20*, 1160–1166. [CrossRef] [PubMed]
48. Katoh, K.; Standley, D.M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **2013**, *30*, 772−780. [CrossRef]
49. Müller, K.; Quandt, D.; Müller, J.; Neinhuis, C. PhyDE®-Phylogenetic Data Editor. Program Distributed by the Authors, Versión 10.0. Available online: http://www.phyde.de/ (accessed on 16 April 2025).
50. Wiens, J.J. Missing data and the design of phylogenetic analyses. *J. Biomed. Inform.* **2006**, *39*, 34−42. [CrossRef]
51. Swofford, D.L. *PAUP*, Phylogenetic Analysis Using Parsimony (* and Other Methods); Version 4.10*; Sinauer Associates: Sunderland, MA, USA, 2002.
52. Huelsenbeck, J.P.; Ronquist, F. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* **2001**, *17*, 754−755. [CrossRef]
53. Huelsenbeck, J.P.; Larget, B.; Alfaro, M.E. Bayesian phylogenetic model selection using reversible jump Markov Chain Montecarlo. *Mol. Biol. Evol.* **2004**, *21*, 1123−1133. [CrossRef] [PubMed]
54. Frandsen, P.B.; Calcott, B.; Mayer, C.; Lanfear, R. Automatic selection of partitioning schemes for phylogenetic analyses using iterative *k*-means clustering of site rates. *BMC Evol. Biol.* **2015**, *15*, 13. [CrossRef]
55. Stamatakis, A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **2014**, *30*, 1312–1313. [CrossRef] [PubMed]
56. Lanfear, R.; Frandsen, P.B.; Wright, A.M.; Senfeld, T.; Calcott, B. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol. Biol. Evol.* **2016**, *34*, 772−773. [CrossRef] [PubMed]
57. Rambaut, A.; Drummond, A.J.; Xie, D.; Baele, G.; Suchard, M.A. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Syst. Biol.* **2018**, *67*, 901–904. [CrossRef]
58. Bouckaert, R.; Heled, J.; Kühnert, D.; Vaughan, T.; Wu, C.-H.; Xie, D.; Suchard, M.A.; Rambaut, A.; Drummond, A.J. BEAST 2: A software platform for Bayesian Evolutionary analysis. *PLoS Comput. Biol.* **2014**, *10*, E1003537. [CrossRef]
59. SEMARNAT. *Secretaría de Medio Ambiente y Recursos Naturales (2010) Norma Oficial Mexicana NOM-059-SEMARNAT*; Diario Oficial de la Federación (DOF): Mexico City, Mexico, 2010.
60. Estrada, A.E.; Arévalo, J.R.; Villarreal, J.A.; Salinas, M.M.; Encina-Domínguez, J.A.; González, H.; Cantú, C.M. Classification and ordination of main plant communities along an altitudinal gradient in the arid and temperate climates of northeastern Mexico. *Sci. Nat.* **2015**, *102*, 59. [CrossRef]
61. Salinas-Rodríguez, M.M.; Hernández-Sandoval, L.; Carrillo-Reyes, P.; Castillo-Gómez, H.A.; Castro-Castro, A.; Estrada-Castillón, E.; Figueroa-Martínez, D.S.; Gómez-Escamilla, I.N.; González-Elizondo, M.; Gutiérrez-Ortega, J.S.; et al. Diversidad de plantas vasculares de la Provincia Fisiográfica de la Sierra Madre Oriental, México. *Bot. Sci.* **2022**, *100*, 469–492. [CrossRef]
62. Halftter, G.; Morrone, J.J. An analytical review of Halffter's Mexican transition zone, and its relevance for evolutionary biogeography, ecology and biogeographical regionalization. *Zootaxa* **2017**, *4226*, 1–46. [CrossRef] [PubMed]
63. Villaseñor, J. Checklist of the native vascular plants of Mexico. *Rev. Mex. Biodiv.* **2016**, *87*, 559–902. [CrossRef]

64. Vázquez-Sánchez, M.; Terrazas, T.; Arias, S.; Ochoterena, H. Molecular phylogeny, origin and taxonomic implications of the tribe Cacteae (Cactaceae). *Syst. Biodivers.* **2013**, *11*, 103–116. [CrossRef]
65. Majure, L.C.; Achá, S.; Baker, M.A.; Puente-Martínez, R.; Köhler, M.; Fehlberg, S. Phylogenomics of One of the World's Most Intriguing Groups of CAM Plants, the Opuntioids (Opuntioideae: Cactaceae): Adaptation to Tropical Dry Forests Helped Drive Prominent Morphological Features in the Clade. *Diversity* **2023**, *15*, 570. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.