

## A NEW SPECIES AND SPECIES RICHNESS DISTRIBUTION OF THE GYPSOPHYTE BUTTERWORTS (*PINGUICULA*, LENTIBULARIACEAE) IN MEXICO

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### Abstract

**Background:** Mexico harbours the highest species richness of *Pinguicula*, with 54 species. Plants collected on gypsum soils in Oaxaca do not coincide with any known species.

**Questions:** Do plants collected represent a new species? What is its conservation status? How many *Pinguicula* gypsophytes are there in Mexico? How are they different? Where are they distributed?

**Studied species:** *Pinguicula* gypsophytes.

**Study site and dates:** Santo Domingo Tonalá, Oaxaca, Mexico, 2023-2024.

**Methods:** A morphological analysis and description were made based on herbarium specimens and living plants. We employed Bayesian Inference and Maximum Likelihood to analyse the regions *rpl32-trnL*, *trnQ-rps16*, and ITS to infer their phylogenetic position concerning similar species. The conservation status of the species was assessed following the IUCN Red List Criteria and Categories. Gypsophyte specimens of *Pinguicula* in Mexico were examined to construct a key. The species richness distribution of gypsophyte *Pinguicula* was analysed by state, physiographic provinces, and  $1 \times 1^\circ$  cells.

**Results:** *Pinguicula tonalaensis* is proposed as new species distinguished by linear-lanceolate summer leaves, geniculate corolla tube, and without propagules in the apex of summer leaves. *Pinguicula tonalaensis* is sister of the clade *P. heterophylla*-*P. medusina* and together form a monophyletic group. *Pinguicula tonalaensis* is Critically Endangered. There are 10 gypsophytes of *Pinguicula* in Mexico. Nuevo León and the Sierra Madre Oriental are the richest. The location of the cell richest coincide with these.

**Conclusions:** The morphology and phylogenetic hypothesis support *Pinguicula tonalaensis* as new species. This increasing the taxonomic richness of *Pinguicula* in Mexico to 55.

**Keywords:** carnivorous plants, gypsum soils, Mexican Transition Zone, Oaxaca, *Pinguicula* diversification.

### Resumen

**Antecedentes:** México concentra la mayor riqueza de *Pinguicula*, con 54 especies. Plantas gipsófilas colectadas en Oaxaca no corresponden con alguna especie descrita.

**Preguntas:** ¿Los especímenes representan una especie nueva? ¿Cuál es su estado de conservación? ¿Cuántas especies gipsófilas de *Pinguicula* crecen en México? ¿Cómo se diferencian? ¿Dónde están distribuidas?

**Especies estudiadas:** *Pinguicula* gipsófilas.

**Sitio de estudio y fecha:** Santo Domingo Tonalá, Oaxaca, México, 2023-2024.

**Métodos:** Realizamos un análisis morfológico y una descripción con base en especímenes de herbario y plantas vivas. Empleamos Inferencia Bayesiana y Máxima Verosimilitud para analizar las regiones *rpl32-trnL*, *trnQ-rps16* e ITS y estimar su posición filogenética con respecto a especies similares. Su categoría de riesgo fue evaluada con las Categorías y Criterios de la Lista Roja de la UICN. Examinamos especímenes de herbario para generar una clave de pinguículas gipsófilas mexicanas. La distribución de su riqueza fue analizada por estado, provincia fisiográfica y celdas de  $1 \times 1^\circ$ .

**Resultados:** *Pinguicula tonalaensis* es una especie nueva, distinguida por el tubo de la corola geniculada y las hojas de verano linear-lanceoladas sin propágulos. *Pinguicula tonalaensis* es hermana del clado *P. heterophylla*-*P. medusina* y juntas forman un grupo monofilético. *Pinguicula tonalaensis* está en Peligro Crítico. En México crecen 10 especies gipsófilas de *Pinguicula*. Nuevo León y la Sierra Madre Oriental son los más ricos. La localización de la celda más rica coincide con estos.

**Conclusiones:** La morfología e hipótesis filogenética sustentan a *Pinguicula tonalaensis* como especie nueva. Esto incrementa la riqueza taxonómica de *Pinguicula* en México a 55.

**Palabras clave:** Diversificación de *Pinguicula*, Oaxaca, plantas carnívoras, suelos yesosos, Zona de Transición Mexicana.

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**L**entibulariaceae is the richest family of carnivorous plants, including the genera *Genlisea* A.St.-Hil. with 30 species, *Pinguicula* L. with 110 species, and *Utricularia* L. with 271 species. *Pinguicula* is distinguished by the presence of roots, leaves arranged in a basal rosette, and ascapes with a single flower (Casper 1966). Based mainly on floral characters, Casper (1966) divided *Pinguicula* into *P.* subg. *Pinguicula*, *P.* subg. *Isoloba* Barnhart, and *P.* subg. *Temnoceras* Barnhart. However, the estimated phylogenies of Cieslak *et al.* (2005), Degtjareva *et al.* (2006), and Shimai *et al.* (2021) suggest that the subgenera are polyphyletic.

Mexico harbours 54 species of *Pinguicula* and 46 (85 %) of them are endemic (López-Pérez *et al.* 2024a,b). Based on this, Mexico represents a centre of diversity (Cheek 1994, Zamudio 1995, Burelo-Ramos *et al.* 2018) and their geographical distribution is almost exclusive to the main Mexican mountain ranges (López-Pérez *et al.* 2024b). Therefore, Shimai *et al.* (2021) suggested that geodiversity and the Quaternary climatic oscillations promoted the diversification of the genus.

The main factor correlated to plant diversity in Mexico is the heterogeneity of the physical space, results from its tectonic, geological, and climatic history (Espinosa *et al.* 2008). According of Hulshof & Spasojevic (2020), the soil is one of the main factors that determinate the species distribution. Gypsum soils occur in many areas of the world and are especially common in arid or semiarid regions. Nevertheless, they can also arise as small outcrops in wetter areas (Herrero & Porta 2000). Gypsum is a stressful environment that imposes severe physiological restrictions for the plants (Escudero *et al.* 2015). Plant taxa restricted to this type of substrate tend to be endemic or even microendemic (Pérez-García *et al.* 2017).

During a systematic study of *Pinguicula* in Mexico, we collected plants growing on gypsum soils in the municipality of Santo Domingo Tonalá, Oaxaca. The morphology of the plants did not correspond with any previously known species. Here, we propose and describe it as a new gypsophyte *Pinguicula* species based on morphological and phylogenetic evidence. Additionally, we analysed the species richness distribution of gypsophytes *Pinguicula* in Mexico by state, physiographic province, and 1° × 1° cells.

## Materials and methods

*Taxonomic treatment.* Specimens were legally collected according to the scientific permit SPARN/DGVS/08149/23. We examined the putative new species and compared with all morphologically similar *Pinguicula* specimens from the ENCB, FCME, IBUG, IEB, MEXU, OAX, SERO, UAMIZ, and XAL herbaria (acronyms according to Thiers 2024). The morphological description was elaborated based on herbarium specimens and living plants. The terminology followed Casper (1966), Moreno (1984), Zamudio (2001), and López-Pérez (2017). The colour of the structures was designated in accordance with the RHS Colour Chart (Royal Horticultural Society 2015).

*Conservation status.* The conservation status of the new species was evaluated based on the IUCN Red List Criteria and Categories (IUCN 2022). The Extent of Occurrence (EOO) and Area of Occupancy (AOO) were estimated with the GeoCAT tool (Bachman *et al.* 2011).

*Plant material, DNA extraction, amplification, and sequencing.* The sampling comprised eight *Pinguicula* species from the clade VII of the nuclear phylogenetic hypothesis of Shimai *et al.* (2021). This clade includes morphologically similar *Pinguicula* species to the putative new species. Taxa were selected to represent the subclades within the clade. The study included 16 accessions available in GenBank. Further, fresh material of *P. acuminata* Benth., *P. emarginata* Zamudio & Rzed., *P. heterophylla* Benth., *P. medusina* Zamudio & Studnička, *P. parvifolia* B.L.Rob., and the putative new taxon were collected. The samples were processed to generate herbarium specimens and leaves were dried in silica gel for DNA extraction according to Funk *et al.* (2017). *Genlisea lobata* Fromm, *G. violacea* A.St.-Hil., *Utricularia foliosa* L., *U. floridana* Nash, and *U. gibba* L. were employed as outgroups. The accession and collection numbers of these are listed in [Appendix 1](#). The specimens were deposited in the IBUG herbarium.

DNA extraction was carried out with the protocol of Doyle & Doyle (1987). The chloroplast intergenic spacers *rpL32-trnL*, *trnQ-rps16* (Wicke *et al.* 2014, Shaw *et al.* 2014), and the Internal Transcribed Spacer (ITS, Cheng *et al.* 2016) were amplified and sequenced (Table 1). PCRs were performed in a reaction mix of 8.5  $\mu$ L, including 0.5  $\mu$ L DNA (~ 50 ng/  $\mu$ L), 0.5  $\mu$ L of each primer [10 mM], 3.0  $\mu$ L of DreamTaq™ Green PCR Master Mix (2x), and 4.0  $\mu$ L of RNase-Free Water. The amplification parameters for the *rpL32-trnL* and *trnQ-rps16* included: 1) 80 °C for 5 min for an initial denaturation, 2) 30 cycles of denaturation at 95 °C for 1 min, first annealing at 50 °C, followed by a 0.3 °C ramp to 65 °C for 1 min and extension at 65 °C for 4 min, 3) a final extension at 65 °C for 5 min. For ITS, the PCR parameters were: 1) 80 °C for 5 min for an initial denaturation, 2) 30 cycles of denaturation at 95 °C for 1 min, an alignment at 52 °C for 1 min, an extension of 65 °C for 4 min, 3) a final extension at 65 °C for 4 min. DNA extraction, amplification, and sequencing were carried out in the Laboratorio Nacional de Identificación y Caracterización Vegetal (LaniVeg) of the Universidad de Guadalajara.

**Table 1.** Primers employed for the DNA amplification.

Primer	Sequence (5' to 3')	Reference
<i>rpL32</i>	CAG TTC CAA AAA AAC GTA CTT C	Shaw <i>et al.</i>
<i>trnL</i>	CTG CTT CCT AAG AGC GT	(2007)
<i>trnQ</i>	GCG TGG CCA AGY GGT AAG GC	
<i>rps16</i>	GTT GCT TTY TAC CAC ATC GTT T	
<i>ITS-u1</i>	GGA AGK ARA AGT CGT AAC AAG G	Cheng <i>et al.</i>
<i>ITS-u4</i>	RGT TTC TTT TCC TCC GCT TA	(2016)

*Phylogenetic analyses.* Phylogenetic relationships were estimated with three data sets: *rpL32-trnL* + *trnQ-rps16*, ITS, and *rpL32-trnL* + *trnQ-rps16* + ITS. We used Bayesian Inference (BI) and Maximum Likelihood (ML) methods. The *Utricularia-Genlisea* clade was defined as the outgroup (Jobson & Albert 2002, Müller *et al.* 2004, Shimai *et al.* 2021). The best molecular evolution models were estimated for each partition with jModelTest v. 2.1.10 (Posada 2008). Thus, the best evolutionary model was inferred for each intergenic spacer, and the best-of-fit DNA models were selected based on the corrected Akaike Information Criterion (AICc, Akaike 1998, Burnham & Anderson 2002). BI analyses was performed using MrBayes v. 3.2.2 (Ronquist *et al.* 2012) with  $50 \times 10^6$  generations and a sampling frequency of 1,000. The first 25 % of the trees were discarded as burn-in to ensure that the chains reached stationarity. Posterior probabilities (PP) were obtained by a majority-rule consensus tree (> 50 %). For the ML analyses, we employed RAXML HPC BlackBox (Stamatakis 2014) tool in CIPRES Science Gateway v. 3 portal (Miller *et al.* 2010). We used the option Let RAXML halt bootstrapping automatically. The likelihood of the final tree was estimated under the GTR + G + I model (Stamatakis 2006, Yang 1993). Cladograms were drawn with FigTree v. 1.4.4 347 software (Rambaut 2018).

*Database and species richness distribution of Pinguicula gypsophytes in Mexico.* We included all the gypsophytes species of *Pinguicula* in Mexico considering the definition of Pérez-García *et al.* (2017), who mention that a gypsophyte is a plant species growing exclusively (or almost) on gypsum soils. The taxa were selected based on the information attached to herbarium specimens, digital herbaria, digital databases and original descriptions. The *Pinguicula* records from CAS (digital), CHAP, CHAPA, CIIDIR, ENCB, IBUG, IEB, INEGI, MEXU, MO (digital), SLPM, UAMIZ, XAL, and ZEA herbaria (acronyms according to Thiers 2024), the information available in the Global Biodiversity Information Facility (GBIF 2023), and the Southwest Environmental Information Network (Gilbert *et al.* 2019) were compiled and curated to construct the database and species distribution maps. To assure the correct taxonomic identity of the digital records, only specimens with images were examined. Specimens without geographical data were georeferenced using Google Earth Pro v. 9.191.0.0 (Google 2023) and the Mapa Digital de México v. 6.1 (INEGI 2023) following the recommendations of Garcia-Milagros & Funk (2010). Specimens with ambiguous information of location were excluded.

The species richness distribution of *Pinguicula* gypsophytes in Mexico was estimated by 1) state, 2) physiographic province, and 3)  $1 \times 1^\circ$  cells. The species richness distribution analysis by state was carried out through a direct count of species documented in each polygon according to INEGI (2018) limits. We used the limits established by Ferrusquía-Villafranca (1993) for the species richness analysis by physiographic provinces, while for the grid cell richness analysis we employed a cell size of  $1 \times 1^\circ$ .

## Results

***Pinguicula tonalaensis*** López-Pérez & Zamudio sp. nov. (Figures 1 and 2, Table 2).

*Type.* Mexico, Oaxaca, municipio Santo Domingo Tonalá,  $\pm 3.5$  km al suroeste de Tonalá (Santo Domingo), 1,416 m asl, 01 August 2023, J. López-Pérez & G. Munguía-Lino 680 (Holotype: IBUG; Isotypes: MEXU, OAX).

*Diagnosis.* *Pinguicula tonalaensis* shares winter and summer leaf forms and size with *P. medusina*. The geniculate corolla tube and the lack of apical propagules on the summer leaves set *P. tonalaensis* apart.

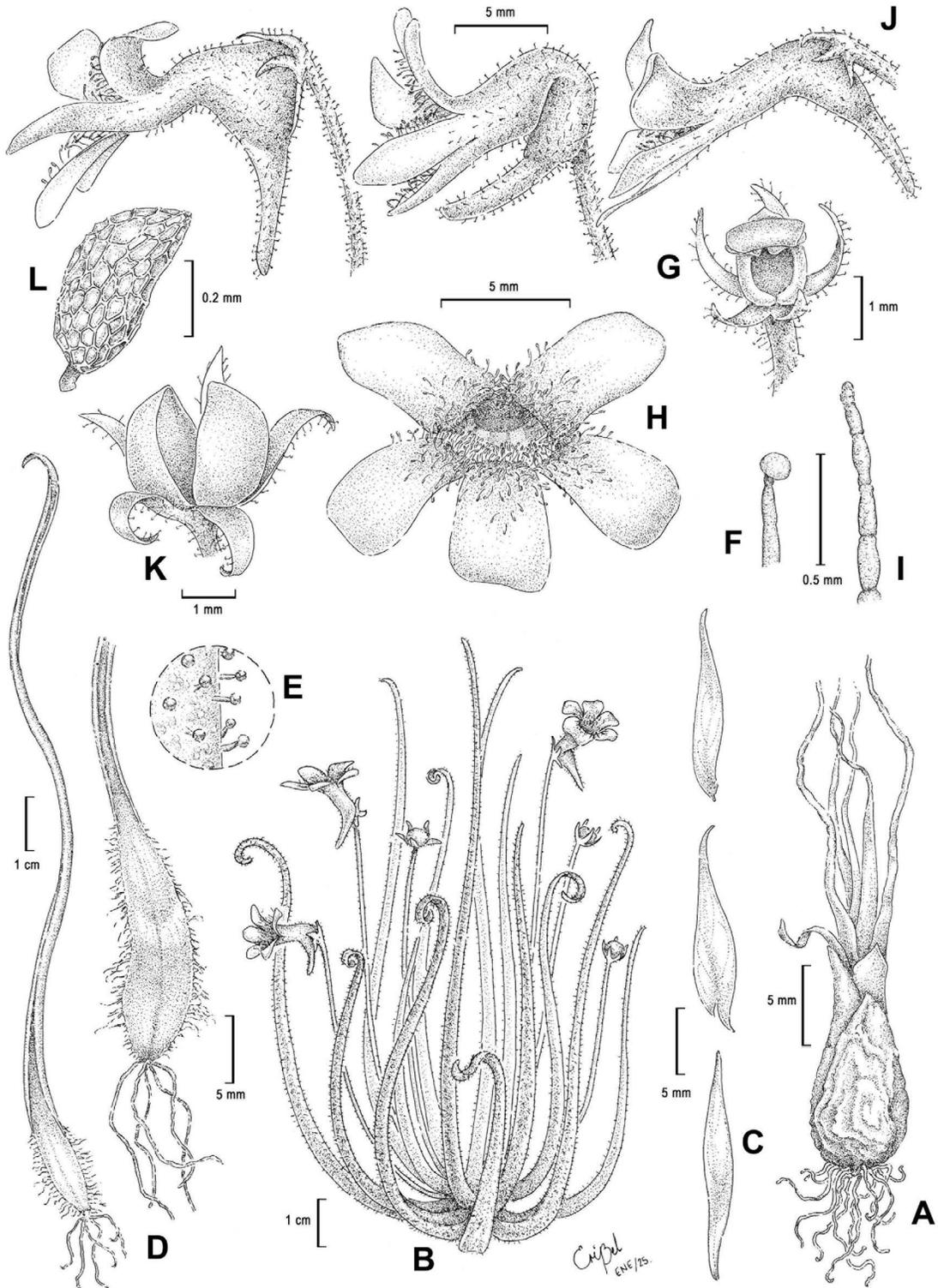
*Description.* Perennial herbs. Leaves dimorphic, arranged into basal rosettes; winter rosette compact, hypogeous, 7.5-12.0 mm in diameter, leaves 55-70, succulents, sessile,  $8.0-18.0 \times 1.2-3.0$  mm, elliptic-lanceolate, acuminate, glabrous; summer rosette lax, leaves 7-12, sessile, erect,  $3.5-15.5$  cm  $\times$   $1.6-4.5$  mm, linear, acuminate, margin revolute, pubescent on the upper surface with sessile and stipitate glandular trichomes, ciliate at the base, cilium ca. 4.5 mm, without apical propagules. Scapes 3-5 per plant, 5.5-12.0 cm long, pilosulous with glandular trichomes. Flowers (14.0)16.5-22.5 mm long including the spur. Calyx bilabiate, outer surface pilosulous with stalked glandular trichomes; upper lip trilobate, triangular lobes,  $1.5-2.5 \times 0.5-1.0$  mm; lower lip bilobate, lobes triangular to lanceolate,  $2.0 \times 0.5$  mm. Corolla subisolobate, white, with a Strong Yellow-Green (Yellow-Green Group N144-C) macula at the base of the lips that extends to the throat, lobes obovate to obovate-oblong, apex rounded to truncate, pilosulose; upper lip bilobate (3.0)4.5-6.5  $\times$  2.5-4.0(5.5) mm; lower lip trilobate, lateral lobes 5.0-7.7  $\times$  2.0-4.2 mm, the medium larger than the laterals ones, 5.3-9.0  $\times$  2.5-5.0 mm. Corolla tube geniculate, Strong Yellow-Green to Deep Purple (Yellow-Green Group N144 B, Violet Group 83 A), 5.0-8.5  $\times$  2.5-4.0 mm, pilosulose with stalked glandular trichomes. Spur tubular, 2.6-6.0 mm long, Strong Yellow-Green to Brownish Orange (Yellow-Green Group N144 C, Greyed-Orange Group 165 B), acute, occasionally emarginated. Capsule globose,  $\pm 2.0$  mm in diameter, pilosulose. Seeds elliptic,  $0.5 \times 0.2$  mm, reticulate, apiculate.

*Distribution and ecology.* *Pinguicula tonalaensis* grows in the municipality of Santo Domingo Tonalá, Oaxaca (Figure 3). The area is part of the Sierra Madre del Sur physiographic province (Ferrusquía-Villafranca 1993). It inhabits northeastern facing gypsum ravines and hills at 1,416 m asl, covered by xeric scrubland. The plants grow in sympatry with *Begonia tlapensis* Burt-Utley & Utley, *Calochortus multicolor* García-Mend., D.Sandoval & C.Chávez, *Fouquieria ochoteranae* Miranda, *Physodium corymbosum* C.Presl, *Polystemma calcicola* (Greenm.) Morillo, *Dahlia* sp., and *Sellaginella* sp.

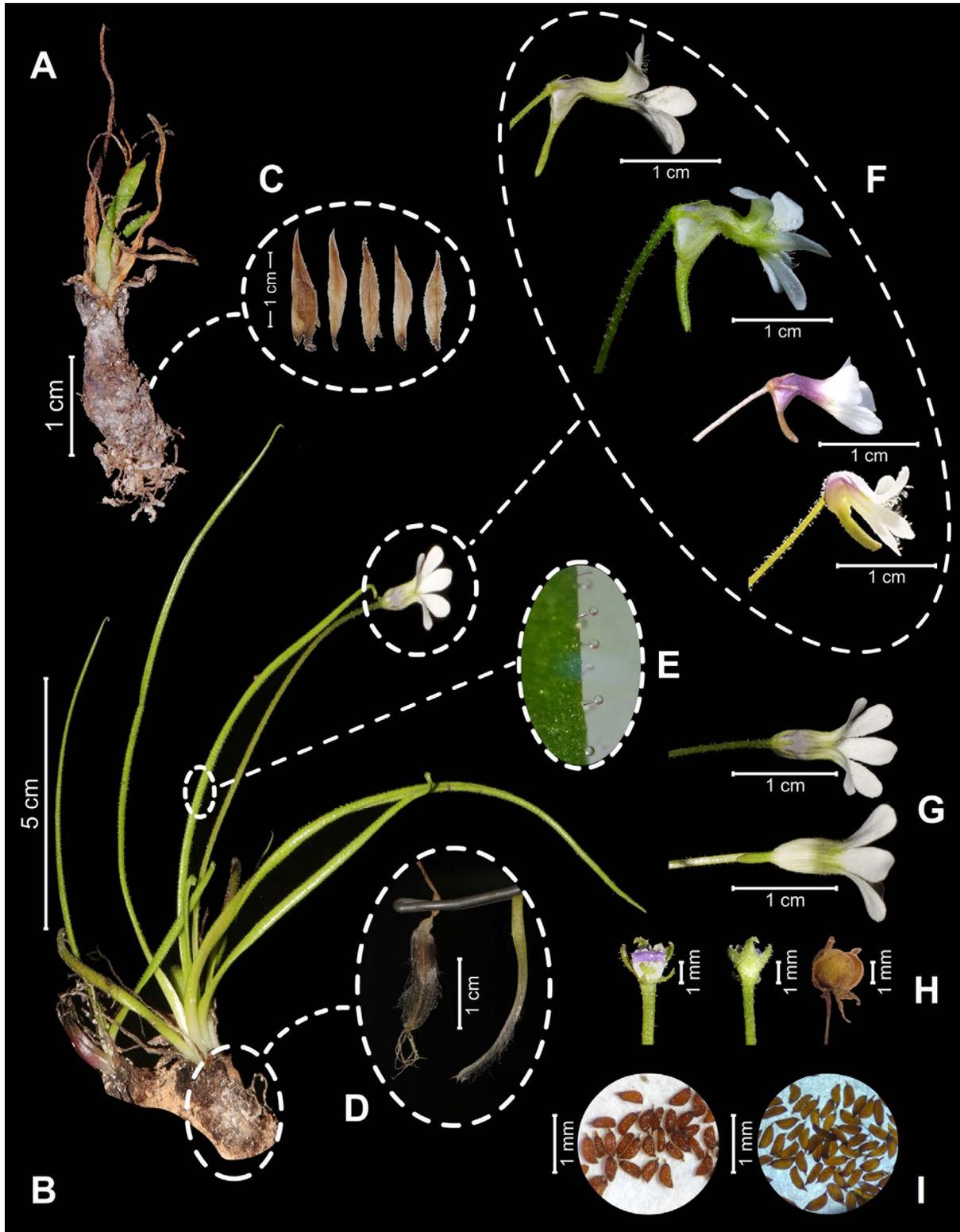
*Conservation status.* *Pinguicula tonalaensis* is known from three localities in municipality of Santo Domingo Tonalá, Oaxaca. GeoCAT calculated an EOO of 0.045 km<sup>2</sup> and an AOO of 8.0 km<sup>2</sup> (based on a cell width of 2 km). According to the IUCN Red List guidelines and criteria B1 and B2, we preliminary recommend the category of Critically Endangered (CR), criterion B1ab(iii)+2ab(iii).

*Phenology.* The plants bloom from July to September. Flowering occurs when the summer leaves are completely developed. The summer leaves survive until November, then they shrivel to the winter rosette. The winter rosette remains underground during the dry season and the summer rosette emerges at the beginning of the rainy season of the following year.

Mexican gypsophyte butterworts



**Figure 1.** *Pinguicula tonalaensis*. A) Winter rosette; B) summer rosette; C) winter leaves; D) summer leaves; E) surface of summer leaves; F) trichomes of leaves, peduncle, calyx and corolla tube surface; G) calyx; H) frontal corolla view; I) trichomes of the surface corolla lobes; J) lateral view of corolla tube variation; K) fruit mature; L) seeds. Illustrated by Ericka Belén Cortez Castro based on type (*J. López-Pérez & G. Munguía-Lino 680*) and fresh material.



**Figure 2.** *Pinguicula tonalaensis*. A) Winter rosette; B) summer rosette; C) winter leaves; D) base of the summer leaves; E) surface of summer leaves; F) variation in the corolla tube; G) dorsal and ventral view of the corolla tube; H) ventral and dorsal view of the calyx, and mature fruit; I) seeds. A-I) Photographs of Jorge López-Pérez.

Mexican gypsophyte butterworts

**Table 2.** Morphological comparison of *Pinguicula tonalaensis*, *P. medusina*, and *P. heterophylla*.

Character	<i>P. tonalaensis</i>	<i>P. medusina</i>	<i>P. heterophylla</i>
Winter rosette			
Rosette diameter (mm)	7.5-12.0	9.0-20.0	11.0-24.0
Leaf number	55-70	70-90	~100
Leaf length (mm) × width (mm)	8.0-18.0 × 1.2-3.0	8.0-23.0 × 1.5-3.5	0.9-25.0 × 2.5-7.0
Leaf shape	Elliptic-lanceolate	Lanceolate	Lanceolate
Summer rosette			
Leaf number	7-12	6-12	3-15
Leaf length (cm) × width (mm)	3.5-15.5 × 1.6-4.5	7.0-19.0 × 1.5-3.5	6.0-18.0 × 2.5-7.0
Apical propagules	Absent	Present	Present
Flower length (mm)	14.0-22.5	16.0-23.0	16.0-24.0
Upper lobes length × width (mm)	3.0-6.5 × 2.5-5.5	5.0-8.0 × 2.5-5.0	7.5-9.0 × 3.5-5.0
Lateral lower lobes length × width (mm)	5.0-7.7 × 2.0-4.2	6.0-9.0 × 2.5-4.0	5.5-10.0 × 3.5-6.5
Medium lower lobe length × width (mm)	5.3-9.0 × 2.5-5.0	6.0-9.0 × 3.0-5.5	8.0-11.0 × 4.5-6.0
Corolla tube	Geniculate	Straight	Straight
Corolla tube length × width (mm)	5.0-8.5 × 2.5-4.0	5.0-8.0 × 2.5-4.0	6.0-11.0 × 3.0-5.0
Spur length (mm)	2.6-6.0	3.0-5.0	3.0-6.0
Flowering time	July to September	May to September	April to August
Habitat	Gypsum soils in xeric scrubland	Gypsum soils in xeric scrubland and tropical deciduous forest	Limestone soils in oak, pine, pine-oak and tropical deciduous forests

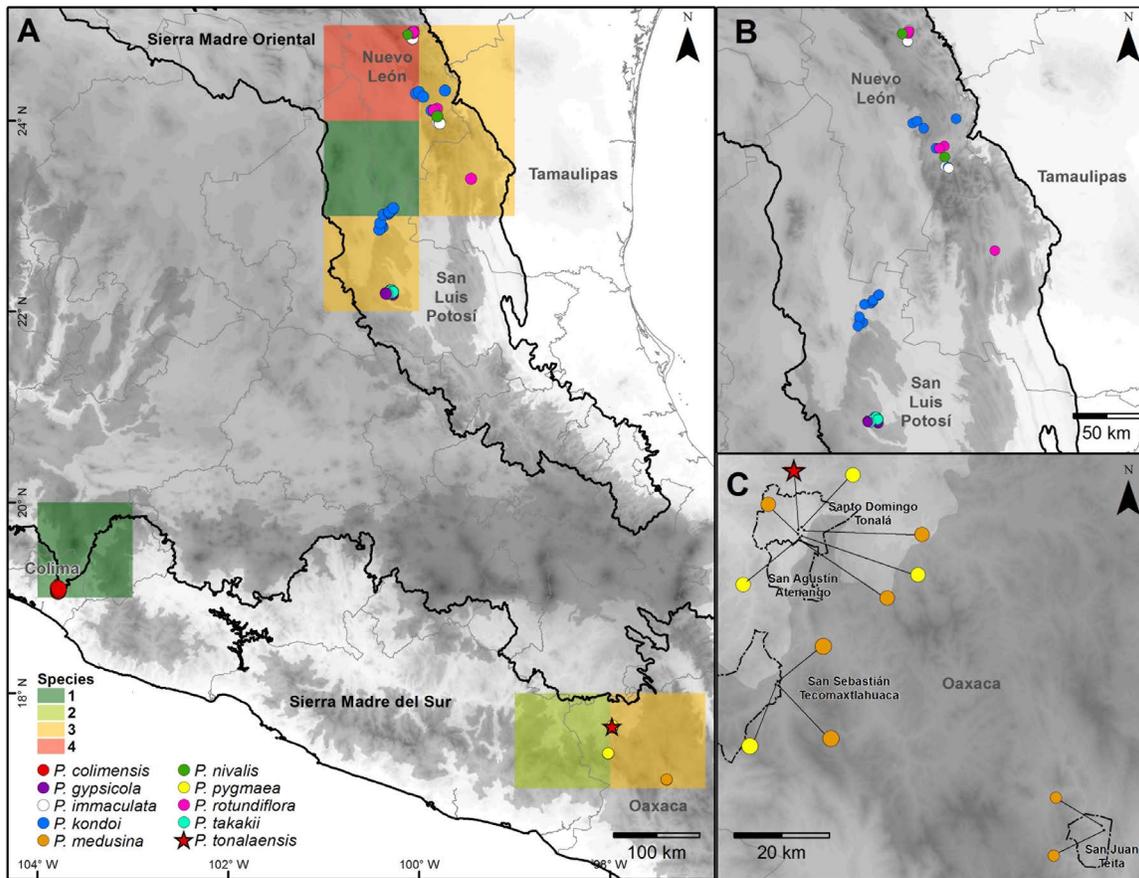
*Etymology.* The specific epithet refers to Santo Domingo Tonalá municipality, where the plants grow.

*Additional specimens examined.* Mexico, Oaxaca, municipio Santo Domingo Tonalá, carretera 15 (tramo Río Santiago Copala a Juxtlahuaca), ± 2 km al suroeste de Yetla de Juárez, 1,449 m asl, 23 September 2024, *J. López-Pérez & G. Munguía-Lino* 767 (IBUG, IEB, MEXU); Carretera 15 (tramo Santo Domingo Tonalá a San Agustín Atenango), 1,437 m asl, 23 September 2024, *J. López-Pérez & G. Munguía-Lino* 768 (IBUG, IEB, MEXU).

*Phylogenetic relationships.* Three genera, 15 species, and 37 sequences were employed in the phylogenetic analyses ([Appendix 1](#)). This included 26 *Pinguicula* samples. The data set had a total length of 1,637 bp for *rpL32-trnL + trnQ-rps16*, 901 bp for ITS, and 2,537 bp for *rpL32-trnL + trnQ-rps16 + ITS*. The corrected AIC criterion estimated the model TPM1uf + G for *rpL32-trnL*, TPM3uf for *rps16-trnQ*, and GTR + I + G for ITS. The results of the analyses of the three data sets showed *P. tonalaensis* as the sister group of the clade formed by *P. heterophylla* and *P. medusina* (PP ≥ 0.84, BS ≥ 79 %; [Figure 4](#)).

*Pinguicula gypsophytes in Mexico.* There are 10 gypsophyte *Pinguicula* species in Mexico ([Figure 5](#), [Table 3](#)), and all of them are endemic to the country. These species can be identified as follows:

1. Corolla isolobate to subisolobate, lobes equal or almost equal between the superior and inferior lips ..... 2
2. Annual plants; palate minute, pubescent ..... *P. takakii*
2. Perennial plants; palate absent ..... 3
3. Winter rosette hypogaeal, compact; summer leaves linear-lanceolate ..... 4

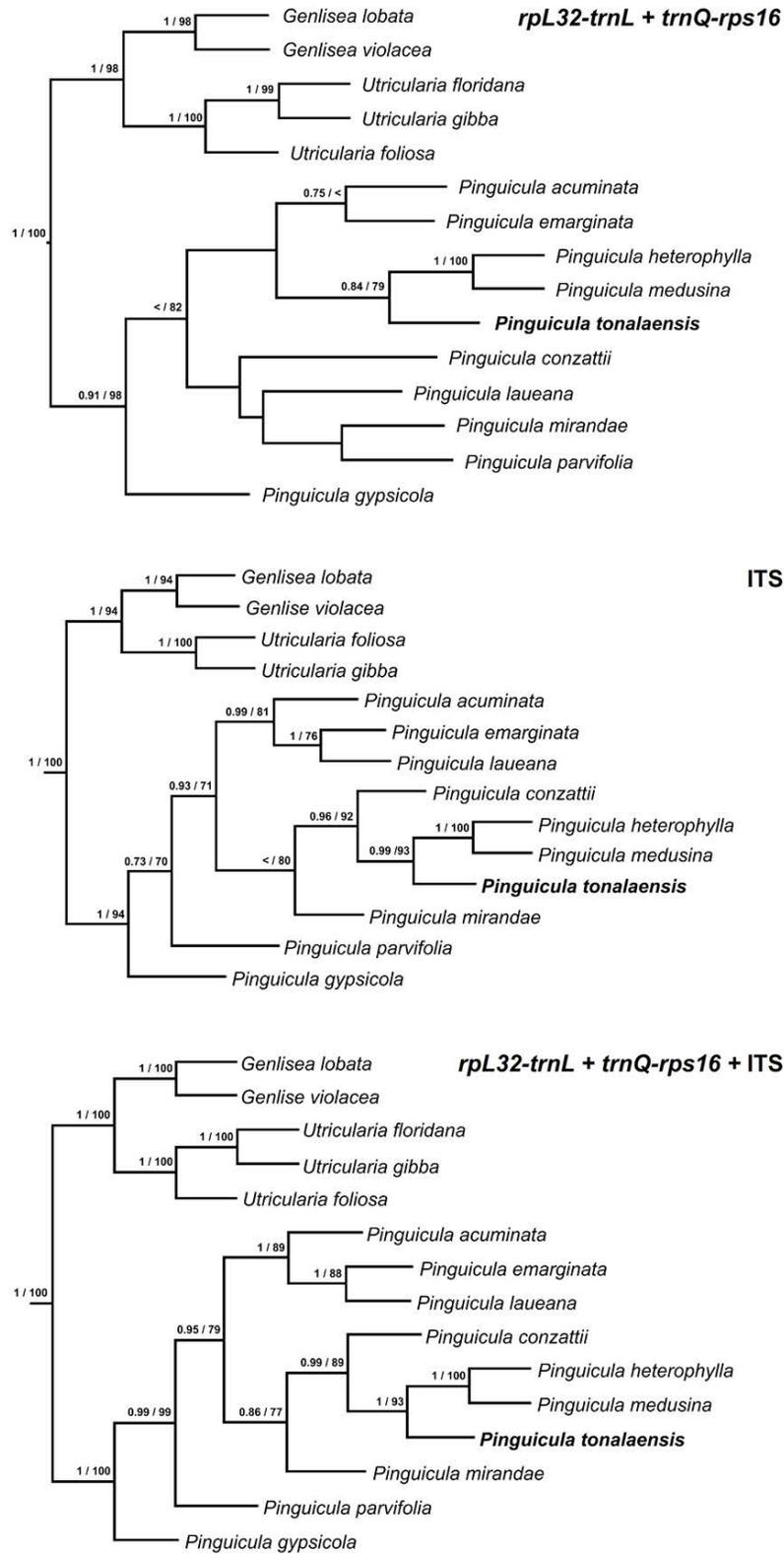


**Figure 3.** Distribution of *Pinguicula gypsophytes* in Mexico. A) Species richness distribution; B) species in the Sierra Madre Oriental; C) species in the Oaxaca State. Solid black line: physiographic limit. Grey line: state limit. Solid dot line: municipal limit in Oaxaca state.

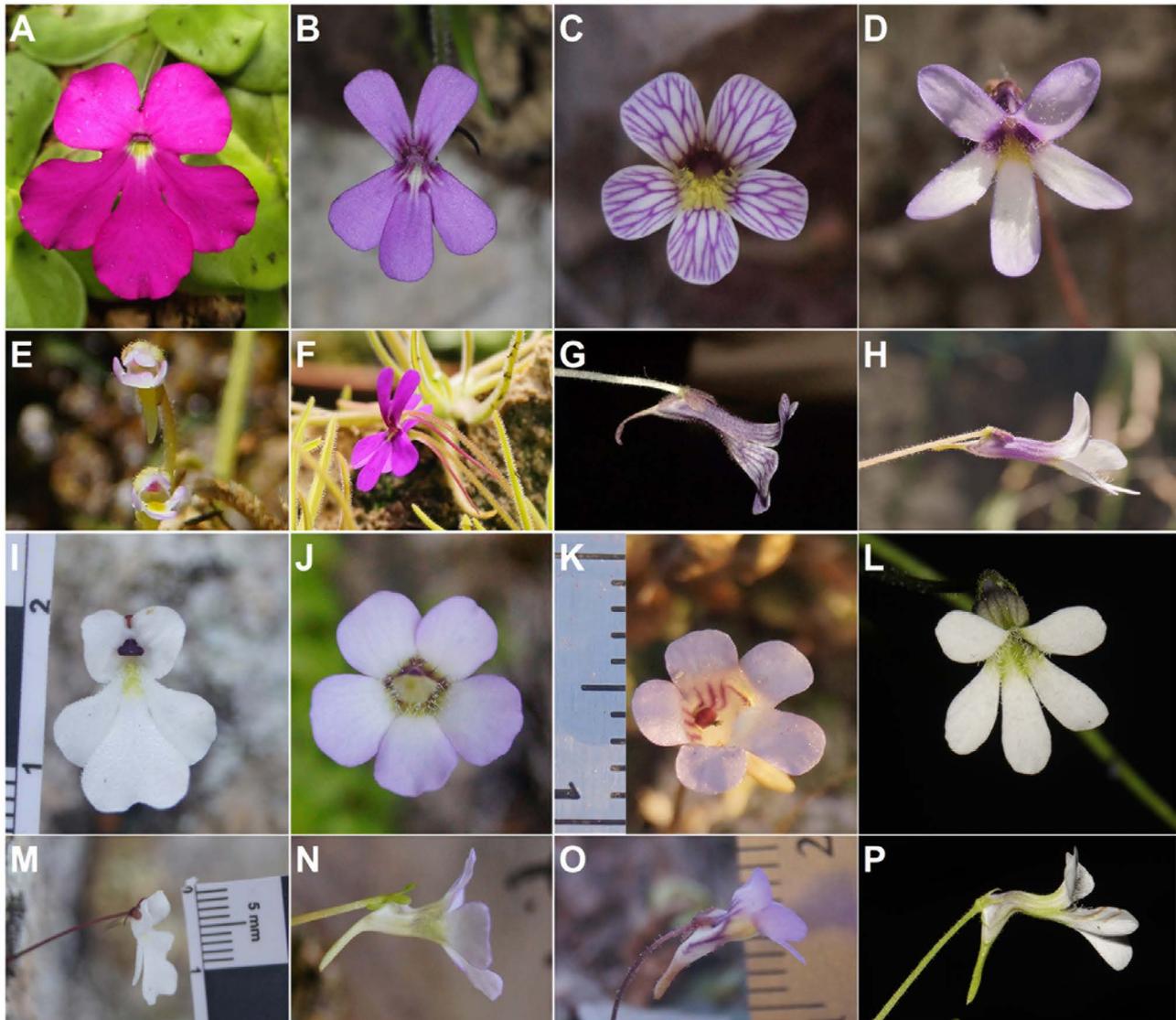
- 4. Corolla tube straight; with propagules in the apex of the summer leaves ..... *P. medusina*
- 4. Corolla tube geniculate; without propagules in the apex of the summer leaves ..... *P. tonalaensis*
- 3. Winter rosette epigeal, lax; summer leaves cuneate or spatulate ..... 5
- 5. Corolla lobes with evident purple veins; base of the lower corolla lip covered with yellow trichomes ..... *P. kondoi*
- 5. Corolla lobes with inconspicuous veins or the same colour as the lobes; base of both corolla lips covered with white trichomes ..... *P. rotundiflora*
- 1. Corolla lobes with a clear distinction between the superior and inferior lips ..... 6
- 6. Annual plants ..... *P. pygmaea*
- 6. Perennial plants ..... 7
- 7. Corolla white; spur shorter than the corolla ..... 8
- 8. Upper corolla lobes obovate, undulate, 3-7 × 3-6 mm ..... *P. nivalis*
- 8. Upper corolla lobes oblong, entire, 1-3 × 1.0-1.5 mm ..... *P. immaculata*
- 7. Corolla pink or purple; spur larger than the corolla ..... 9
- 9. Summer leaves linear-lanceolate; corolla lobes oblong ..... *P. gypsicola*
- 9. Summer leaves elliptic to suborbicular; corolla lobes suborbicular to obovate ..... *P. colimensis*

*Species richness distribution of Pinguicula gypsophytes in Mexico.* The analyses showed that gypsophyte *Pinguicula* species are present in the states of Colima, Nuevo León, Oaxaca, San Luis Potosí, and Tamaulipas (Figure 3, Table 3). Nuevo León had the most species, where *P. immaculata*, *P. kondoi*, *P. nivalis*, and *P. rotundiflora* were

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**Figure 4.** Phylogenetic relationships of *Pinguicula tonalaensis*. The numbers in the nodes show the posterior probabilities (PP) and the bootstrap support (BS %). Nodes with support  $\geq 70\%$  are indicated.



**Figure 5.** Gypsophyte species of *Pinguicula* in Mexico. A) *P. colimensis*; B, F) *P. gypsicola*; C, G) *P. kondoii*; D, H) *P. medusina*; E) *P. pygmaea*; I, M) *P. nivalis*; J, N) *P. rotundiflora*; K, O) *P. takakii*; L, P) *P. tonalaensis*. A-P) Photographs by Jorge López-Pérez.

**Table 3.** Species list and distribution of *Pinguicula* gypsophytes in Mexico. SMS: Sierra Madre del Sur, SMOr: Sierra Madre Oriental.

Species	Subgenus	State	Physiographic province
<i>P. colimensis</i> McVaugh & Mickel	<i>Pinguicula</i>	Colima	SMS
<i>P. gypsicola</i> Brandegee	<i>Pinguicula</i>	San Luis Potosí	SMOr
<i>P. immaculata</i> Zamudio & Lux	<i>Temnoceras</i>	Nuevo León	SMOr
<i>P. kondoii</i> Casper	<i>Isoloba</i>	Nuevo León, San Luis Potosí	SMOr
<i>P. medusina</i> Zamudio & Studnicka	<i>Isoloba</i>	Oaxaca	SMS
<i>P. nivalis</i> Luhrs & Lampard	<i>Temnoceras</i>	Nuevo León	SMOr

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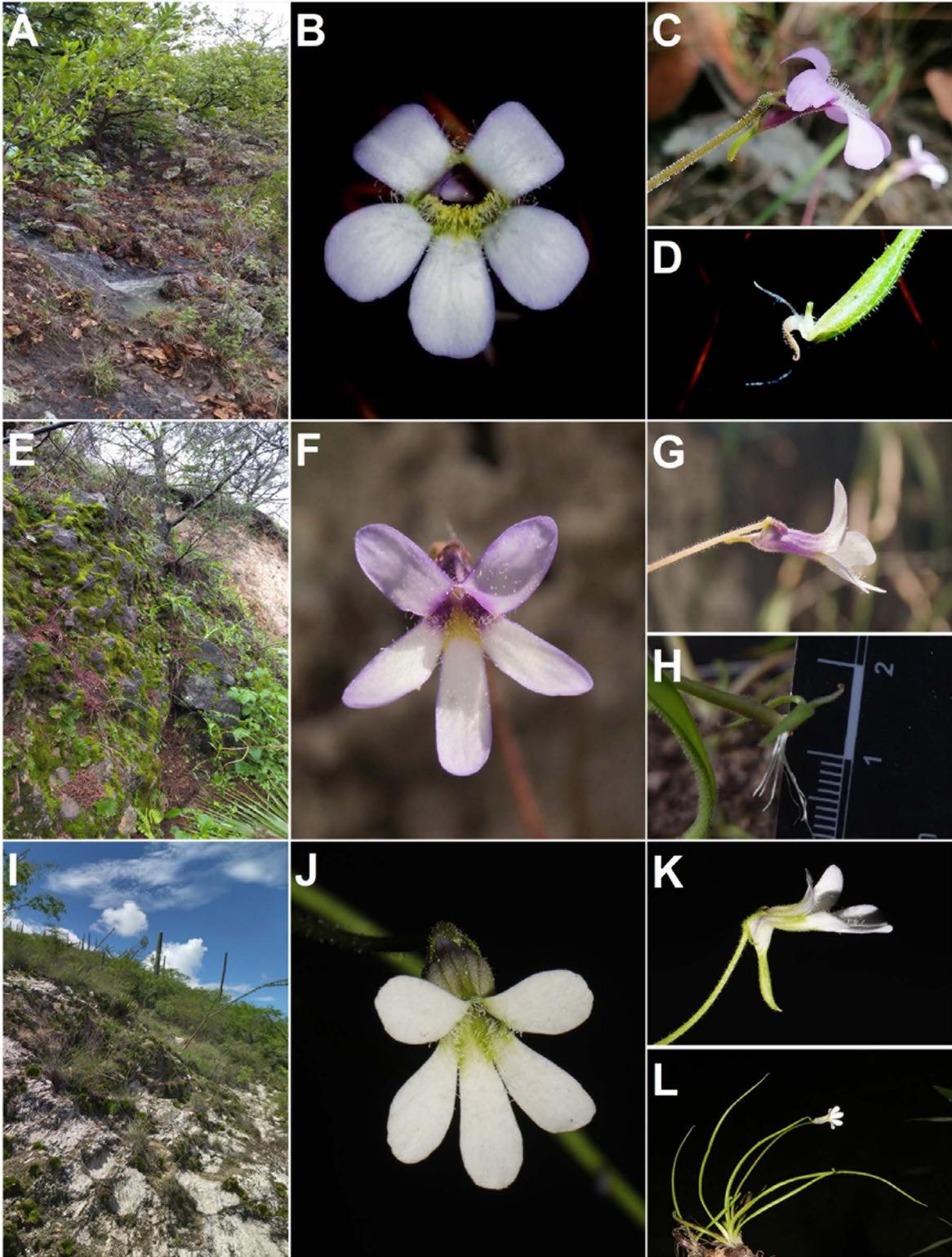
Species	Subgenus	State	Physiographic province
<i>P. pygmaea</i> Rivadavia, E.L.Read & A.Fleischm.	<i>Isoloba</i>	Oaxaca	SMS
<i>P. rotundiflora</i> Studnicka	<i>Isoloba</i>	Nuevo León, Tamaulipas	SMOr
<i>P. takakii</i> S.Z.Ruiz & Rzed.	<i>Isoloba</i>	San Luis Potosí	SMOr
<i>P. tonalaensis</i> López-Pérez & Zamudio	<i>Isoloba</i>	Oaxaca	SMS

registered (Figure 3B). Colima and Tamaulipas had one species each, *P. colimensis* and *P. rotundiflora*. The gypsophyte species are restricted to the physiographic provinces of Sierra Madre Oriental (SMOr) and Sierra Madre del Sur (SMS) (Figure 3). The SMOr included six species: *P. gypsicola*, *P. immaculata*, *P. kondoi*, *P. nivalis*, *P. rotundiflora*, and *P. takakii* (Figure 3A, B, Table 3). The SMS had four species: *P. colimensis*, *P. medusina*, *P. pygmaea*, and *P. tonalaensis* (Figure 3A, C). The grid cell analysis placed the cell with the highest species values on the SMOr (Figure 3A, B).

## Discussion

*Taxonomic treatment.* According to the classification of Casper (1966), the new taxon pertains to *Pinguicula* subg. *Isoloba*. The presence of winter and summer rosettes, the isolobate corollas, the subcylindric corolla tube, and the spur shorter than the corolla tube characterize the subgenus. *Pinguicula tonalaensis* is morphologically similar to *P. heterophylla* and *P. medusina* (Figure 6, Table 2). The morphology of the winter rosette in *P. heterophylla*, *P. medusina*, and *P. tonalaensis* is similar. It is composed of thick underground leaves strongly grouped together. These are covered by the dry ciliate base of the summer leaves from the previous growing season. This structure resembles a tunicate bulb (Figures 1A, 2A). However, *P. tonalaensis* differs from *P. heterophylla* by the absence of propagules in the apex of summer leaves and by growing up on gypsum soils covered with xeric scrubland. In contrast, *P. heterophylla* grows in pine-oak forest. The new species is more similar to *P. medusina*, but differs by the absence of propagules at the apex of summer leaves. In addition, the presence of a geniculate corolla tube in *P. tonalaensis* distinguished it from both species (Figure 6C, G, K). Zamudio & Studnicka (2000) considered that *P. medusina* develops propagules in the apex of summer leaves, whereas *P. heterophylla* lacks them. The authors highlight the absence of propagules in *P. heterophylla* as a taxonomic character. However, observations in the field and cultivated plants show that this taxon does develop apical foliar propagules in the summer leaves too (Figure 6D).

*Species richness distribution of gypsophyte Pinguicula in Mexico.* The Mexican Transition Zone (MTZ) represents the boundary between the Nearctic and Neotropical regions (Villaseñor *et al.* 2020). It is a set of morphotectonic and physiographic provinces with different ages and origins (Ferrusquía-Villafranca 1993, Mastretta-Yanes *et al.* 2015). Within the MTZ, the SMOr represents an area of high species richness and endemism for *Pinguicula* (Zamudio 2005, Salinas-Rodríguez *et al.* 2017, 2022, Domínguez *et al.* 2024, López-Pérez *et al.* 2024b). A similar pattern for other groups was observed by Contreras-Medina & Luna-Vega (2007), Torres-Miranda *et al.* (2011), Luna-Vega *et al.* (2013), Sanginés-Franco *et al.* (2015), and Tellez *et al.* (2020), who emphasized that the taxonomic richness of gymnosperms, oaks, vascular plants, and native trees of Mexico and Central America is centred in this province. Concerning species richness and endemism of gypsophytes in Mexico, Ortiz-Brunel *et al.* (2023) also highlighted this province as the richest. Based on grid cell analyses, they identified the Cuatro Ciénegas Basin in Coahuila as the richest area and as an Endemism Centre. Also they highlight the relevance of the surroundings of Santo Domingo Tonalá in Oaxaca, for its elevated number of restricted species. Finally, the description of this new gypsophyte species from Oaxaca increases to 55 the number of *Pinguicula* species in Mexico. This work also emphasizes the importance of gypsum soils for the formation of endemic species of *Pinguicula*.



**Figure 6.** Morphologically comparison of *Pinguicula tonalaensis* and similar species. A-D) *P. heterophylla*; E-H) *P. medusina*; I-L) *P. tonalaensis*; A, E, I) habitat; B, F, J) frontal corolla view; C, G, K) lateral corolla view; D, H) propagules in *P. heterophylla* and *P. medusina* respectively; L) summer leaves without propagules in *P. tonalaensis*. A-B, D-L) Photographs by Jorge López-Pérez; C) photograph by Erick Vélez-Sánchez.

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## Literature cited

- Akaike H. 1998. Information Theory and an Extension of the Maximum Likelihood Principle. *In*: Parzen E, Tanabe K, Kitagawa G, eds. *Selected Papers of Hirotugu Akaike*. New York: Springer Series in Statistics. pp. 199-213. ISBN: 978-1-4612-1694-0
- Bachman S, Moat J, Hill AW, de la Torre J, Scott B. 2011. Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool. *ZooKeys* **150**: 117-126. DOI: <https://doi.org/10.3897/zookeys.150.2109>
- Burelo-Ramos CM, Zamudio-Ruiz S, González-Aguilar MA. 2018. *Pinguicula olmeca* (Lentibulariaceae) una nueva especie del sur de México. *Botanical Sciences* **96**: 359-365. DOI: <https://doi.org/10.17129/botsci.1927>
- Burnham KP, Anderson DR. 2002. *Model Selection and Multimodel Inference*. New York, United States of America: Springer-Verlag. ISBN: 0-387-95364-7
- Casper SJ. 1966. Monographie der gattün *Pinguicula* L. *Bibliotheca Botanica* **127/128**: 1-209.
- Cheek M. 1994. *Pinguicula greenwoodii* (Lentibulariaceae), a new butterwort from Mexico. *Kew Bulletin* **49**: 813-815. DOI: <https://doi.org/10.2307/4118077>
- Cheng T, Xu C, Lei L, Li C, Zhang Y, Zhou S. 2016. Barcoding the kingdom Plantae: new PCR primers for *ITS* regions of plants with improved universality and specificity. *Molecular Ecology Resources* **16**: 138-149. DOI: <https://doi.org/10.1111/1755-0998.12438>
- Cieslak T, Polepalli JS, White A, Müller K, Borsch T, Barthlott W, Steiger J, Marchant A, Legendre L. 2005. Phylogenetic analysis of *Pinguicula* (Lentibulariaceae): chloroplast DNA sequences and morphology support several geographically distinct radiations. *American Journal of Botany* **92**: 1723-1736. DOI: <https://doi.org/10.3732/ajb.92.10.1723>
- Contreras-Medina R, Luna-Vega I. 2007. Species richness, endemism and conservation of Mexican gymnosperms. *Biodiversity and Conservation* **16**: 1803-1821. DOI: <https://doi.org/10.1007/s10531-006-9072-3>
- Degtjareva GV, Casper SJ, Hellwig FH, Schmidt AR, Steiger J, Sokoloff DD. 2006. Morphology and nrITS Phylogeny of the Genus *Pinguicula* L. (Lentibulariaceae), with Special Attention to Embryo Evolution. *Plant Biology* **8**: 778-790. DOI: <https://doi.org/10.1055/s-2006-924560>
- Domínguez Y, Temple P, Pančo I, Miranda VF. 2024. Biogeographical patterns of *Pinguicula* L. (Lentibulariaceae) in the Americas revealed by endemism and habitat suitability analyses. *Flora* **313**: 152484. DOI: <https://doi.org/10.1016/j.flora.2024.152484>
- Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11-15.
- Escudero A, Palacio S, Maestre FT, Luzuriaga AL. 2015. Plant life on gypsum: a review of its multiple facets. *Biological Reviews* **90**: 1-18. DOI: <https://doi.org/10.1111/brv.12092>
- Espinosa DS, Ocegueda S, Aguilar C, Flores O, Llorente-Bousquets J, Vázquez B. 2008. El conocimiento biogeográfico de las especies y su regionalización natural. *In*: Capital natural de México. Soberón J, Halfter G, Llorente-Bousquets J, eds. *Capital Natural de México. Vol. I*. México: Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, pp. 31-35. ISBN: 978-607-7607-03-8
- Ferrusquía-Villafranca I. 1993. Geología de México: una sinopsis. *In*: Ramamoorthy TP, Bye R, Lot A, Fa M, eds. *Biological Diversity of Mexico: Origins and Distribution*. New York: Oxford University. pp. 3-107. ISBN: 0-19-506674-X
- Funk VA, Gastel M, Devine A, Kelloff CL, Wurdack K, Tuccinardi C, Radosavljevic A, Peters M, Coddington J.

2017. Guidelines for collecting vouchers and tissues intended for genomic work (Smithsonian Institution): Botany Best Practices. *Biodiversity Data Journal* **5**: e11625. DOI: <https://doi.org/10.3897/bdj.5.e11625>
- García-Milagros E, Funk V. 2010. Improving the use of the information from museum specimens: Using Google Earth® to georeference Guiana Shield specimens in the US National Herbarium. *Frontiers of Biogeography* **2**: 71-77. DOI: <https://doi.org/10.21425/F5FBG12348>
- GBIF [Global Biodiversity Information Facility]. 2023. GBIF Occurrence. DOI: <https://doi.org/10.15468/dl.pujhuj> (accessed November 10, 2023).
- Gilbert EM, Gries C, Franz NM, Leslie RL, Nash TH. 2019. SEINet: a centralized specimen resource managed by a distributed network of researchers. *Biodiversity Information Science and Standards* **3**: e37424. DOI: <https://doi.org/10.3897/biss.3.37424>
- Google. 2023. Google Earth Pro, version 9.191.0.0. <https://earth.google.com/> (accessed August 1, 2023).
- Herrero J, Porta J. 2000. The terminology and the concepts of gypsum-rich soils. *Geoderma* **96**: 47-61. DOI: [https://doi.org/10.1016/S0016-7061\(00\)00003-3](https://doi.org/10.1016/S0016-7061(00)00003-3)
- Hulshof C, Spasojevic MK. 2020. The edaphic control of plant diversity. *Global Ecology and Biogeography* **29**: 1634-1650. DOI: <https://doi.org/10.1111/geb.13151>
- INEGI [Instituto Nacional de Estadística y Geografía]. 2018. División política municipal, 1: 250,000. Instituto de Estadística y Geografía. [http://geoportal.conabio.gob.mx/descargas/mapas/imagen/96/muni\\_2018gw](http://geoportal.conabio.gob.mx/descargas/mapas/imagen/96/muni_2018gw) (accessed September 15, 2023).
- INEGI [Instituto Nacional de Estadística y Geografía]. 2023. Mapa Digital de México, v. 6.1. <https://gaia.inegi.org.mx/> (accessed July 1, 2023).
- IUCN [International Union for Conservation of Nature]. 2022. Standards and Petitions Committee. Guidelines for Using the IUCN Red List Categories and Criteria, version 15.1. Prepared by the Standards and Petitions Committee. <https://www.iucnredlist.org/documents/RedListGuidelines.pdf> (accessed October 1, 2024).
- Jobson RW, Albert VA. 2002. Molecular Rates Parallel Diversification Contrasts between Carnivorous Plant Sister Lineages. *Cladistics* **18**: 127-136. DOI: <https://doi.org/10.1006/clad.2001.0187>
- López-Pérez JD. 2017. Revisión taxonómica de la familia Lentibulariaceae Rich. del estado de Oaxaca. BSc Thesis. Universidad de la Sierra Juárez.
- López-Pérez JD, Zamudio S, Munguía-Lino G, Rodríguez A. 2024a. A new endemic species and species richness distribution of the genus *Pinguicula* (Lentibulariaceae) in the Trans-Mexican Volcanic Belt, Mexico. *Botanical Sciences* **102**: 995-1008. DOI: <https://doi.org/10.17129/botsci.3485>
- López-Pérez JD, Zamudio S, Munguía-Lino G, Rodríguez A. 2024b. Species richness, geographic distribution and endemism of *Pinguicula* (Lentibulariaceae) in the Mexican Transition Zone. *Phytotaxa* **641**: 031-046. DOI: <https://doi.org/10.11646/phytotaxa.641.1.3>
- Luna-Vega I, Espinosa D, Rivas G, Contreras-Medina R. 2013. Geographical patterns and determinants of species richness in Mexico across selected families of vascular plants: implications for conservation. *Systematics and Biodiversity* **11**: 237-256. DOI: <https://doi.org/10.1080/14772000.2013.797517>
- Mastretta-Yanes A, Moreno-Letelier A, Piñero D, Jorgensen TH, Emerson BC. 2015. Biodiversity in the Mexican highlands and the interaction of geology, geography and climate within the Trans-Mexican Volcanic Belt. *Journal of Biogeography* **42**: 1586-1600. DOI: <https://doi.org/10.1111/jbi.12546>
- Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: 2010 Gateway Computing Environments Workshop, GCE 2010. DOI: <http://dx.doi.org/10.1109/GCE.2010.5676129>
- Moreno NP. 1984. *Glosario botánico ilustrado*. Veracruz, México: Instituto Nacional de Investigaciones sobre Recursos Bióticos. ISBN: 968-26-0434-6.
- Müller K, Borsch T, Legendre L, Porembski S, Theisen I, Barthlott W. 2004. Evolution of Carnivory in Lentibulariaceae and the Lamiales. *Plant Biology* **6**: 477-490. DOI: <https://doi.org/10.1055/s-2004-817909>
- Ortiz-Brunel JP, Ochoterena H, Moore MJ, Aragón-Parada J, Flores J, Munguía-Lino G, Rodríguez A, Salinas

- Rodríguez MM, Flores-Olvera H. 2023. Patterns of richness and endemism in the Gypsiculous Flora of Mexico. *Diversity* **15**: 522. DOI: <https://doi.org/10.3390/d15040522>
- Pérez-García FJ, Martínez-Hernández F, Mendoza-Fernández AJ, Merlo ME, Sola F, Salmerón-Sánchez E, Garrido-Becerra JA, Mota JF. 2017. Towards a global checklist of the world gypsophytes: a qualitative approach. *Plant Sociology* **54**: 61-76. DOI: <http://dx.doi.org/10.7338/pls2017542S1/06>
- Posada D. 2008. jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* **25**: 1253-1256. DOI: <https://doi.org/10.1093/molbev/msn083>
- Rambaut A. 2018. *FigTree: Tree Figure Drawing Tool, Version 1.4.4*. Institute of Evolutionary Biology. <http://tree.bio.ed.ac.uk/software/figtree> (accessed September 2, 2024).
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology* **61**: 539-542. DOI: <https://doi.org/10.1093/sysbio/sys029>
- Royal Horticultural Society. 2015. *R.H.S colour chart, Sixth Edition*. London, UK: RHS Media.
- Salinas-Rodríguez MM, Estrada-Castillón E, Villareal-Quintanilla JA. 2017. Endemic vascular plants of the Sierra Madre Oriental, Mexico. *Phytotaxa* **328**: 1-52. DOI: <https://doi.org/10.11646/phytotaxa.328.1.1>
- Salinas-Rodríguez MM, Hernández-Sandoval L, Carrillo-Reyes P, Castillo-Gómez HA, Castro-Castro A, Estrada-Castillón E, Figueroa-Martínez DS, Gómez-Escamilla IN, González-Elizondo M, Gutiérrez-Ortega JS, Hernández-Rendón J, Munguía-Lino G, De-Nova JA, Ortiz-Brunel JP, Rubio-Méndez G, Ruíz-Sánchez E, Sánchez-Sánchez C, Sandoval-Mata TN, Soltero-Quintana R, Steinmann V, Valencia-A S, Zamudio-Ruiz S. 2022. Diversidad de plantas vasculares de la Provincia Fisiográfica de la Sierra Madre Oriental, México. *Botanical Sciences* **100**: 469-492. DOI: <https://doi.org/10.17129/botsci.2864>
- Sanginés-Franco C, Luna-Vega I, Contreras-Medina R, Espinosa D, Tejero-Díez JD, Rivas G. 2015. Diversity, endemism and conservation of ferns (Polypodiales) in the Mexican mountain component. *Journal of Mountain Science* **12**: 891-904. DOI: <https://doi.org/10.1007/s11629-014-3070-9>
- Shaw J, Lickey EB, Schilling EE, Small RL. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* **94**: 275-288. DOI: <https://doi.org/10.3732/ajb.94.3.275>
- Shaw J, Shafer HL, Leonard OR, Kovach MJ, Schorr M, Morris AB. 2014. Chloroplast DNA sequence utility for the lowest phylogenetic and phylogeographic inferences in angiosperms: the tortoise and the hare IV. *American Journal of Botany* **101**: 1987-2004. DOI: <https://doi.org/10.3732/ajb.1400398>
- Shimai H, Setoguchi H, Roberts DL, Sun M. 2021. Biogeographical patterns and speciation of the genus *Pinguicula* (Lentibulariaceae) inferred by phylogenetic analyses. *Plos One* **16**: e0252581. DOI: <https://doi.org/10.1371/journal.pone.0252581>
- Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688-2690. DOI: <https://doi.org/10.1093/bioinformatics/btl446>
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312-1313. DOI: <https://doi.org/10.1093/bioinformatics/btu033>
- Tellez O, Mattana E, Diazgranados M, Kühn N, Castillo-Lorenzo E, Lira R, Montes-Leyva L, Rodriguez I, Flores C, Way M, Dávila P, Ulian T. 2020. Native trees of Mexico: diversity, distribution, uses and conservation. *PeerJ* **8**: e9898. DOI: <https://doi.org/10.7717/peerj.9898>
- Thiers B. 2024. *Index herbariorum: a global directory of public herbaria and associated staff*. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/science/ih/> (accessed September 2, 2024).
- Torres-Miranda A, Luna-Vega I, Oyama K. 2011. Conservation biogeography of red oaks (*Quercus*, section *Lobatae*) in Mexico and Central America. *American Journal of Botany* **98**: 290-305. DOI: <https://doi.org/10.3732/ajb.1000218>
- Villaseñor JL, Ortiz E, Delgadillo-Moya C, Juárez D. 2020. The breadth of the Mexican Transition Zone as defined by its flowering plant generic flora. *Plos One* **15**: e0235267. DOI: <https://doi.org/10.1371/journal.pone.0235267>

- Wicke S, Schäferhoff B, Depamphilis CW, Müller KF. 2014. Disproportional plastome-wide increase of substitution rates and relaxed purifying selection in genes of carnivorous Lentibulariaceae. *Molecular Biology and Evolution* **31**: 529-545. DOI: <https://doi.org/10.1093/molbev/mst261>
- Yang Z. 1993. Maximum-likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. *Molecular Biology and Evolution* **10**: 1396-1401. DOI: <https://doi.org/10.1093/oxfordjournals.molbev.a040082>
- Zamudio S. 1995. Las plantas mexicanas del género *Pinguicula*, un grupo de interés hortícola. *Revista Chapingo. Serie: Horticultura* **1**: 63-70. DOI: <https://doi.org/10.5154/r.rchsh.1994.03.025>
- Zamudio S. 2001. Revisión de la sección *Orcheosanthus*, del género *Pinguicula* (Lentibulariaceae). PhD Thesis. Universidad Nacional Autónoma de México.
- Zamudio S. 2005. Dos especies nuevas de *Pinguicula* (Lentibulariaceae) de la Sierra Madre Oriental, México. *Acta Botanica Mexicana* **70**: 69-83. DOI: <https://doi.org/10.21829/abm70.2005.988>
- Zamudio S, Studnicka M. 2000. Nueva especie gipsícola de *Pinguicula* (Lentibulariaceae) del estado de Oaxaca, México. *Acta Botanica Mexicana* **53**: 67-74. DOI: <https://doi.org/10.21829/abm53.2000.863>

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**Author contributions:** JDLP designed the project, conducted the field and laboratory work, and analysed the data; GM did field work and analysed the data; JDLP, GM, SZ, and AR prepared the manuscript.

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**Appendix 1.** Sampled taxa, GenBank accession numbers, and voucher specimen of samples employed in the phylogenetic analyses. GenBank accession numbers in bold are sequences generated in this study.

<b>Taxon</b>	<i>rpL32-trnL</i>	<i>rps16-trnQ</i>	<b>ITS</b>
<i>Pinguicula acuminata</i>	LC348618	<b>PV424175</b>	AB199751
<i>Pinguicula conzattii</i>	LC348630	N/A	AB199754
<i>Pinguicula emarginata</i>	LC348640	<b>PV424178</b>	AB199759
<i>Pinguicula gypsicola</i>	<b>PV424179</b>	N/A	AB199763
<i>Pinguicula heterophylla</i>	LC348648	<b>PV424177</b>	AB199765
<i>Pinguicula laeana</i>	LC348654	N/A	AB199768
<i>Pinguicula medusina</i>	LC348664	<b>PV424176</b>	AB199771
<i>Pinguicula mirandae</i>	LC348665	N/A	AB251605
<i>Pinguicula parvifolia</i>	<b>PV424180</b>	<b>PV434152</b>	AB199777
<i>Pinguicula tonalaensis</i>	<b>PV424181</b>	<b>PV424182</b>	<b>PV357563</b>
<i>Genlisea violacea</i>	NC 37083	FN641794.1	AB212116
<i>Genlisea lobata</i>	LC348688.1	FN641793.1	AB212113.1
<i>Utricularia foliosa</i>	KY025562	N/A	MG027754
<i>Utricularia floridana</i>	LC348693.1	N/A	N/A
<i>Utricularia gibba</i>	NC_021449.1	N/A	LC682655.1