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## Molecular and Morphological Evidence Reveals a New Species of *Antiphytum* (Echiochiloideae, Boraginaceae) from Guerrero, Mexico

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**Abstract**—Molecular and morphological evidence supports a new species in the genus *Antiphytum* from the Sierra Madre del Sur, in the state of Guerrero, Mexico, here described as *Antiphytum brevicalyx*. This species is unique in the genus by possessing a calyx shorter than the corolla tube at anthesis; it is similar to *A. floribundum* in inflorescence arrangement, but differs from that species in lacking a basal leaf rosette and having appendages on the corolla throat. According to phylogenetic analysis of nuclear ITS and plastid *ndhF-rpl32* sequences representing seven of the eight North American species of the genus besides the proposed new species, accessions of *A. brevicalyx* form a well-defined clade within *Antiphytum*, corroborating its distinctive morphology. However, the analyses do not resolve the phylogenetic position of *A. brevicalyx* within the genus.

**Keywords**—Mexican flora, endemism, gypsosag, vulnerable.

*Antiphytum* DC. ex Meisn. (Boraginaceae) is the only American genus of the subfamily Echiochiloideae Weigend (Chacón et al. 2016). According to Johnston (1923, 1927) and Mendoza-Díaz (2015), the genus includes 10–12 species of erect or prostrate subshrubs with sessile leaves, the cauline ones opposite throughout or only at the base. Some species of *Antiphytum* possess a true basal leaf rosette whereas others have no basal rosette, but each new branch possesses a pseudobasal rosette (terminology according to Cohen 2011). The inflorescence unit is a scorpioid cyme (but solitary flowers occur in *A. hintoniurum* L.C.Higgins & B.L.Turner) arranged as paraclades of heterocladic or homocladic thyrsoids (terminology according to Weigend et al. 2016). The flowers are perfect, pentamerous, actinomorphic, and gamopetalous, and the corolla is infundibuliform or hypocrateriform, white or blue, and may or may not possess faucal appendages. The gynobase is flat to short-pyramidal bearing lustrous, ovoid or triangular eremocarp (sensu Hilger 2014, also known as mericarps or nutlets), with the cicatrix (attachment scar) in the medial to supra-basal position in the North American species, but in a basal position in the South American taxa. Almost all of the species in the genus were described in the 19th century (Chamisso de Boncourt 1829; Martens and Galeotti 1844; de Candolle and de Candolle 1846; Torrey 1859; Watson 1883; Rose 1890), although two unusual species from northern Mexico were described more recently. Higgins and Turner (1983) described the atypical gypsum endemic *A. hintoniurum* and Mendoza-Díaz et al. (2018) described *A. geoffreyi* N. Mend. & Flores Oliv., which has an unusual gynobase shape.

During ongoing phylogenetic study of the genus, a new species from the state of Guerrero, Mexico, was found, which is here described.

### MATERIALS AND METHODS

**Plant Material**—As part of an ongoing revision of the genus *Antiphytum*, specimens were borrowed and/or examined from AAH, ARIZ, ASU, B, BM, BR, CAS, CHAPA, CIIDIR, ENCB, FCME, G, G-DC, GH, HCIB, HUH, IEB, K, MEXU, MO, NMC, NY, P, R, RSA-POM, TEX, UAT, UC, US, and W (acronyms according to Thiers 2019). Several specimens were encountered that were assigned to *Antiphytum* based on the position of the eremocarp cicatrix and the shape of the gynobase, but do not

correspond to any known species of the genus. These specimens, all from the Mexican state of Guerrero, are unique within the genus by possessing a calyx shorter than the corolla tube at anthesis, a medial position of the cicatrix on the ventral face of the eremocarp, and heterocladic thyrsoid inflorescences with alternate hypopodia (following Weberling 1989).

Based on these unusual specimens, field work was carried out near Xochipala, on the road to Filo de Caballos, Guerrero, where specimens were collected and leaf samples dried in silica gel for DNA studies. The hypothesized new species was tested with cladistic analyses using molecular data.

**Fruit Observation**—Mature fruits were studied with a confocal stereo microscope (Leica Z16 APO A, Leica Microsystems GmbH, Wetzlar, Germany), and photographed with a camera (Leica DFC490, Leica Microsystems GmbH) at the Laboratorio de Microscopía y Fotografía de la Biodiversidad (II) of the Instituto de Biología, UNAM (IBUNAM). Eremocarps were also studied using a scanning electron microscope (Hitachi SU1510, Hitachi Ltd., Tokyo, Japan) in the Laboratorio de Microscopía Electrónica y Fotografía de la Biodiversidad (I) of IBUNAM. To prepare samples for electron microscopy, eremocarps were mounted on stubs with common double stick carbonated tape, and coated with gold to 20 μÅ for two minutes in a sputter coater (Quorum Q150OR ES, Quorum Technologies Ltd, East Sussex, UK).

**DNA Extraction, PCR Amplification, and Sequencing**—Total DNA was isolated from herbarium specimens or fresh leaves dried in silica gel for 53 accessions representing seven North American species of *Antiphytum* and seven accessions of the proposed new species. DNA was isolated using the GE Healthcare Nucleon PhytoPure kit (#RPN8511, Sigma-Aldrich, St. Louis, Missouri) or using the CTAB extraction method of Doyle and Doyle (1987) modified by the addition of 2% of polyvinylpyrrolidone (PVP) to the extraction buffer.

Sequences of the nuclear internal transcribed spacer (ITS) and the plastid *ndhF-rpl32* spacer were amplified using the primer pairs ITS4 and ITS5 (White et al. 1990) and the newly designed primers rpl32.4R and ndhF.40R.Bor (GAAGTACGYTTTTTGGAACTGC; ACATAGGAACT-GTWAGTGAAG), respectively. PCR conditions were as follows: DNA was amplified using GoTaq® Flexi DNA Polymerase (#9PIM829, Promega, Madison, Wisconsin) combined with FailSafe™ PCR 2X PreMix E (Epi-centre, Illumina, Madison, Wisconsin), or GoTaq® Colorless MasterMix (#9PIM713, Promega), adapting the manufacturer's protocol for 12.5 μl reaction volumes (0.1 μl GoTaq plus 6.25 μl PreMix and 5.15 μl double distilled water, or 6.25 μl GoTaq and 5.25 μl nuclease free water), adding 0.25 μl of each primer (10 μM concentration) and 0.5 μl DNA template of varying concentrations. PCRs were run in a PTC-200 DNA Engine® thermocycler (Bio-Rad, Hercules, California) under the following conditions for both loci: initial denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 50 sec, 50°C for 50 sec, and 72°C for 1 min 20 sec, with a final extension at 72°C for 7 min. PCR products were purified using ExoSAP-IT (USB Corp., Cleveland, Ohio) prior to sequencing at Neogenomics (Houston, Texas) or were sent unpurified to LaNABio (IBUNAM) for sequencing. At

both locations, sequences were generated using an ABI 3730xL sequencer (Applied Biosystems, Waltham, Massachusetts).

Sequence chromatograms were edited, assembled, and manually aligned with PhyDE 0.9971 (Müller et al. 2010); SSRs and polynucleotide regions were annotated in both individual marker matrices and the corresponding aligned positions were used to label the base pair number(s) in the motif codifications (see below). *Echium vulgare* L. was included as an outgroup, using GenBank sequences for both markers from Långström and Oxelman (2003) and Cohen and Davis (2009) (Appendix 1); no other close outgroup was available, but the inclusion of this single species suffices to test phylogenetic evidence for the proposed new species. Matrices for both loci were imported to WinClada 1.00.24 (Nixon 1999–2002) and merged to create a concatenated matrix.

For descriptive comparative purposes, distances among the concatenated sequences of the *Antiphytum* species were calculated as absolute nucleotide differences using PAUP 4.0a165 (Swofford 2003) (Table S1 in Supplementary Material 1; Mendoza-Díaz et al. 2019).

**Phylogenetic Analysis**—Gaps were coded using simple indel coding according to Simmons and Ochoterena (2000) and microstructural characters were coded according to Ochoterena (2009); both were added to the nucleotide concatenated matrix to build the final matrix for the analysis. The concatenated matrix was analyzed in WinClada to remove non-informative characters prior to analysis.

Phylogenetic analyses were performed with the parsimony criterion using TNT 1.1 (Goloboff et al. 2000) alternating the four new technology search (NTS) strategies: sectorial searches (Goloboff 1999), ratchet (Nixon 1999), tree drifting, and tree fusing (Goloboff 1999) until the consensus was stabilized three times by a factor of 100. The most parsimonious trees were saved and analyzed in WinClada. Ambiguously supported nodes were collapsed and identical trees removed. Unambiguous character transformations were plotted onto the tree using the Fitch criterion only for the potentially informative characters.

Jackknife (JK) support values were calculated with TNT using NTS (excepting sectorial searches) using a 36% of character removal probability and 10,000 replicates, until the minimum length was found four times. The frequency of appearance for each node was calculated among these trees and mapped onto the consensus tree using WinClada. We considered support to be lacking when the values were below 64% (Farris et al. 1996), moderate support 65–80%, and strong support above 80%. The resulting tree was saved as an EMF file and edited with Corel Draw X5, in which microstructural characters were manually colored and labeled according to the optimization in WinClada.

The same procedure and analyses were also conducted for each locus separately (Figs. S1, S2 in Supplementary Material 1, Mendoza-Díaz et al. 2019). Single-locus aligned matrices with labeled microstructural characters (PhyDE format) and the final concatenated matrix (nucleotide and microstructural characters in nexus format) are available in the Dryad Digital Repository (Mendoza-Díaz et al. 2019). The list of microstructural characters is also available as Supplementary Material 2 (Mendoza-Díaz et al. 2019).

**Distribution Map**—Maps were produced using QGIS Brighton (2.6.1) employing layers from INEGI (2017) and CONABIO (1997, 1998). The distribution of the species was obtained from specimen labels, using geographic coordinates when available. Specimens without coordinates were georeferenced using Google Earth 7.1.8.3036 (32 bit) (see paratypes).

## RESULTS

Sequence variation among individuals of the putative new species *Antiphytum brevicalyx* (average nucleotide differences between sequences = 1.3; Table S1) was similar to that in other species with restricted distributions (average = 0.99) vs. species with broad distributions (average = 7.6). The average sequence difference of *A. brevicalyx* compared to other species of *Antiphytum* was similar to the average sequence differences among all species pairs in the genus (28.24 vs. 28.17). Average sequence divergence between *A. brevicalyx* and other species of *Antiphytum* was highest for *A. caespitosum* I.M. Johnston and *A. paniculatum* I.M. Johnston (36.01 and 35.06, respectively) and lowest (19.1) for *A. peninsulare* (Rose) I.M. Johnston (Table S1).

The analyses of the concatenated matrix resulted in a single most parsimonious tree (two before collapsing ambiguously supported nodes) of 172 steps (Fig. 1A, where length = L). The

non-rosette forming species *A. hintoniorum* was sister to all remaining *Antiphytum*, which were recovered in two main clades, one of which was composed of species bearing a basal leaf rosette (Fig. 1A). All accessions corresponding to *A. brevicalyx* were retrieved in a clade with strong support (JK 99%) within the clade of species without a basal leaf rosette. *Antiphytum peninsulare* of Baja California was sister to the new species clade although without jackknife support (Fig. 1A). This was also the case for the ITS analysis (Fig. S1), but not for the chloroplast analysis, in which all the accessions of *A. brevicalyx* were paraphyletic to a clade including all the accessions of *A. heliotropioides* A. DC. (Fig. S2). Little or no geographic structure was found within the new species (Fig. 1B).

## TAXONOMIC TREATMENT

*Antiphytum brevicalyx* N. Mend., Flores Oliv. & H. Ochoterena, sp. nov. TYPE: MEXICO. Guerrero: Municipio Eduardo Neri, 9 km (on the road to Filo de Caballos) west of Xochipala, 1635 m, 17°46'41.4"N, 99°46'41.4"W, 18 November 2016, N. Mendoza-Díaz 643 with H. Flores Olvera, H. Ochoterena, A. Torres-Montúfar & C. Martínez-Rojas (Holotype: MEXU! Isotypes: ENCB!, FCME!, K!, MEXU!, MO!).

*Antiphytum brevicalyx* is the only species in the genus with the calyx shorter than the corolla tube at anthesis. It is similar to *A. floribundum* (Torr.) A. Gray in the inflorescence arrangement, but differs by the absence of a basal rosette (vs. present in *A. floribundum*) and the presence of appendages on the corolla throat (vs. absent in *A. floribundum*). Compared to *A. peninsulare*, the eremocarp of *A. brevicalyx* shares a medial position of the cicatrix as well as tuberculate and papillate ornamentation, but differs in shape (ovate in *A. brevicalyx* vs. triangular in *A. peninsulare*).

**Suffruticose perennial**, erect, up to 1.20 m tall, covered by strigose and hispid indumenta, each trichome with several mineralized foot cells (lithocystic structure), with amber-colored and non-persistent glandular-capitate trichomes mainly on the younger parts of branches, leaves, bracts, bracteoles, and calyx. **Stems** numerous and broadly branched, arising from a main stem, branches alternate or opposite, non-fistulose (solid), slender (ca. 2.0 mm diam), with the most external layer exfoliating. **Leaves** basal, pseudobasal and cauline, sessile, blades linear-oblongate to oblongate, and lanceolate to narrowly elliptical-acuminate, margins flat or slightly thickened, entire, apices acute, nearly mucronate, bases truncate and non-connate, glandular trichomes only on the margins at the very base, surfaces concolorous, midvein visible as a groove in the adaxial face and as raised-strigose on the abaxial, secondary veins often not visible; basal leaves marcescent, not forming a rosette, up to 70.0(–80.0) × 10.0(–15.0) mm; cauline leaves mostly alternate and only opposite at the base of the stem or the branches, 40.0–50.0(–60.0) × 2.0–4.0(–5.0) mm, gradually smaller upward along the stem; pseudobasal leaves at the beginning of each branch, the newest ones 10.0 × 2.0 mm. **Inflorescence** ending in a flower (monotelic) with alternate paraclades, forming a terminal heterocladic determinate thyrse (thyrsoid) with dichasial branching, one side ending in a scorpioid cyme and the other side branching up to several times, first internode of a lateral axis (hypopodium) 40.0–60.0(–70.0) mm in length; mature scorpioid cymes up to 90 mm in length, loosely

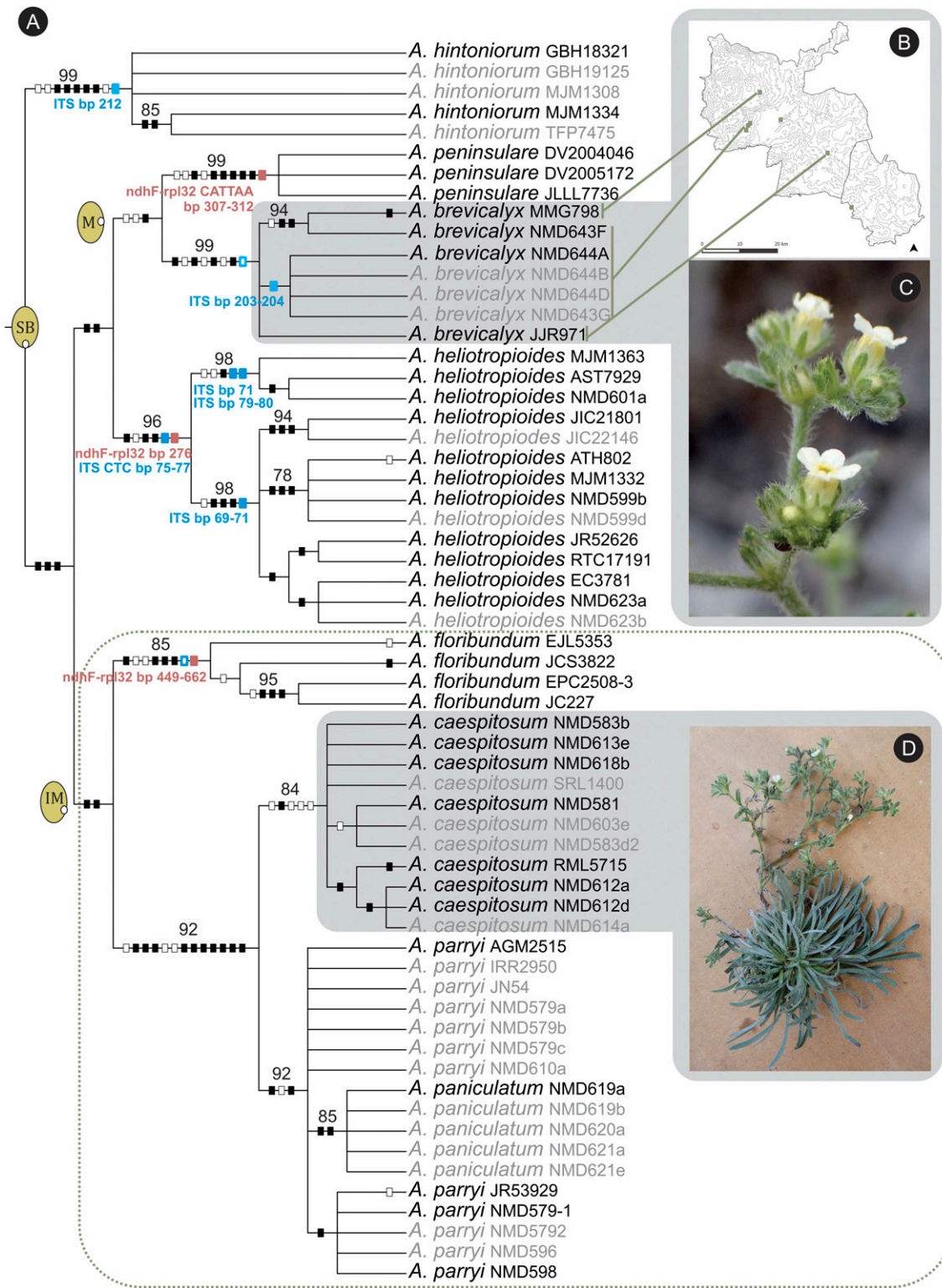


FIG. 1. Combined parsimony tree ( $L = 172$ ) using ITS and *ndhF-rpl32* sequences, including microstructural DNA characters. A. Single most parsimonious tree highlighting *Antiphytum brevicalyx*. Sequences in gray are identical to the sequence of the first black-colored accession name above. Numbers above branches correspond to jackknife support above 64%. Solid boxes on branches indicate synapomorphic changes, open boxes indicate homoplasy, and colored boxes correspond to microstructural characters, as follows: blue boxes represent gap characters, and pink boxes represent simple sequence repeats (SSRs). Numbers next to the microstructural synapomorphies indicate their base pair position(s) in the aligned matrix of the respective locus. A schematic representation of the eremocarp cicatrix position is shown next to the hypothetical ancestor of each condition: SB = supra-basal; M = medial; IM = infra-medial. B. Geographic origin of *A. brevicalyx* accessions from Guerrero, Mexico. Mapped collections that are not connected to the phylogeny lack sequences. C. Flowers of *A. brevicalyx* showing the characteristic calyx: shorter than the corolla tube. D. *A. caespitosum*, the other species distributed in Guerrero, showing the basal leaf rosette characteristic of the entire clade (dotted box).

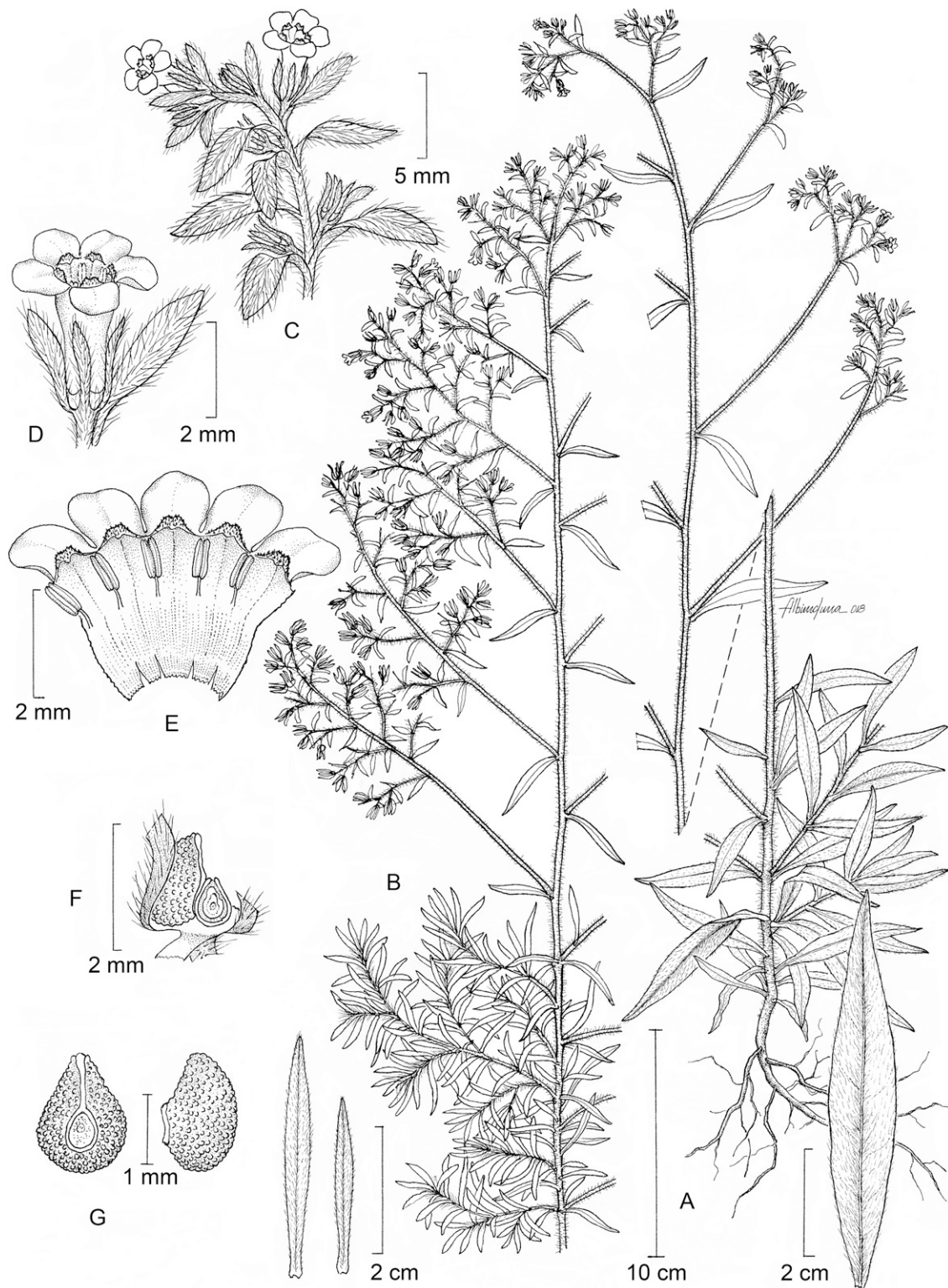


FIG. 2. *Antiphytum brevicalyx*. A. General appearance of a young plant showing detail of the abaxial surface of a basal leaf (from Mendoza-Díaz et al. 644C). B. General appearance of an older individual showing detail of the adaxial surface of the cauline leaves (from Mendoza-Díaz et al. 643A). C. Portion of cyme inflorescence (from Mendoza-Díaz et al. 644A). D. Flower (from Mendoza-Díaz et al. 644A). E. Open corolla (from Mendoza-Díaz et al. 644A). F. Fruit with three eremocarps removed showing the attachment point at the gynobase (from Mendoza-Díaz et al. 643A). G. Eremocarp in ventral and lateral view (from Mendoza-Díaz et al. 643A).

many-flowered, bracteate; floral bracts obovate (2:1) to oblong, sessile, mucronate, indumenta and midvein as on the leaves, blades  $7.0\text{--}11.0 \times 3.2\text{--}4.0$  mm, gradually smaller upward along the cyme. **Flowers** perfect, sessile or subsessile (pedicel

$0.2\text{--}0.5$  mm in length); calyx strigose, glandular-capitate and scattered hispid trichomes on abaxial surface, accrescent in fruit, divided almost to the base, lobes asymmetric, mucronate to acuminate, scarious at the very base, two of them lanceolate



FIG. 3. *Antiphytum brevicalyx* in the field (Mendoza-Díaz *et al.* 643, 644). A. Young individual with flowers and associated vegetation. B. Mature individual. C. Basal leaves in a young individual. D. Individual showing the habitat and soil. E. Thyrroid view from above. F. Partial inflorescence. G. Portion of inflorescence. H. Flower at anthesis showing the faucal appendages. Photos by Helga Ochoterena.

and three obovate, ca.  $2.0\text{--}2.2 \times 0.3\text{--}0.4$  mm, the bigger one obovate and resembling a floral bract,  $2.5 \times 1.0$  mm, all shorter than the corolla tube at anthesis, up to 4.3 mm in length in fruit; corolla hypocrateriform (rotate), white, with five yellow papillose faucal appendages on the throat opposite to the corolla

lobes, tube widely surpassing the calyx, 2.1–3.3 mm in length, 1.3 mm diam at the base, limb (4.0–) 4.25–5.25 mm diam, lobes oriented nearly perpendicular to the tube, imbricate, rounded, sinuate at apex, ca. (1.0–) 1.2–1.4  $\times$  (1.0–) 1.2–1.4 mm, abaxially strigose at the middle; stamens

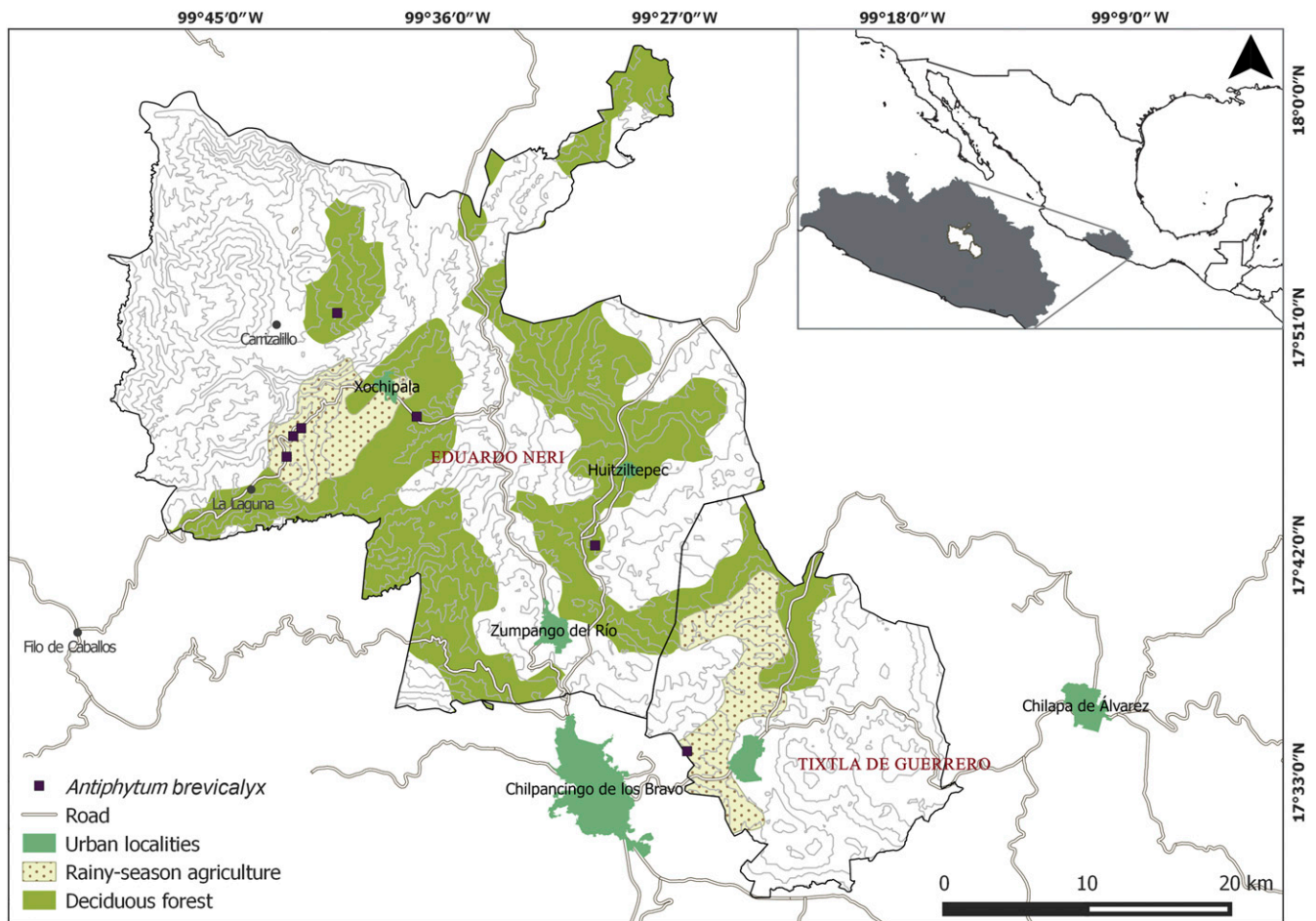


FIG. 4. Distribution map of *A. brevicalyx* within Guerrero, showing the two municipios (Eduardo Neri and Tixtla de Guerrero) in which the species has been recorded, and vegetation types. The inset map shows the locations of the two municipios within Guerrero and the location of Guerrero in Mexico.

inserted, alternate to the corolla lobes, adnate at ca. 1.7 mm from the base of the corolla tube; filaments up to 0.5 mm in length, slender; anthers oblong, slightly lanceolate, dorsifixed, ca. 0.8–0.9 × 0.2–0.3 mm; ovary with four ovules, style gynobasic, 0.7–0.8 mm in length at anthesis, stigma bilobed at the apex of the style (terminal). **Fruit** dry, covered by a persistent calyx, usually with 2–3 mature eremocarps; eremocarps lustrous, ovate and apically acuminate in ventral

view, 1.8–1.9 × 1.3 mm, dorsally convex, tuberculate and papillate except at the apex of the tubercles; cicatrix at medial position on the ventral face, ovate, ca. 0.65 mm in length; one ventral keel (apical) straight from the apex of the eremocarp to the cicatrix apex not forming a stipe, without lateral (basal) keels; gynobase deltate-pyramidal, with four areoles corresponding to the cicatrix of each eremocarp, style plus stigmas 1.7(–2.0) mm, surpassing the eremocarps. Figures 2, 3.

TABLE 1. Main morphological characters that distinguish *Antiphytum brevicalyx* from morphologically similar species of the genus. The diagnostic feature of the new species is highlighted in bold.

	<i>A. brevicalyx</i>	<i>A. floribundum</i>	<i>A. heliotropioides</i>	<i>A. peninsulare</i>
Glandular-capitate trichomes	Present on vegetative parts	Absent	Absent	Present on vegetative parts
Basal leaf rosette	Absent	Present	Absent	Absent
Main axis of the inflorescence with alternate hypopodia	Present	Present	Absent	Absent
Corolla diameter (mm)	4.0–5.25	5.0–7.0	4.0–6.0	10.0–12.0
Relationship of calyx/corolla tube length at anthesis	<b>All calyx lobes shorter</b>	Calyx lobes equal to longer	Calyx lobes equal to longer	Calyx lobes equal to longer
Faucal appendages	Present	Absent	Present	Present
Eremocarp shape	Ovate	Ovate	Ovate	Triangular
Eremocarp ornamentation	Tuberculate and papillate	Tuberculate	Tuberculate and papillate	Tuberculate and papillate
Eremocarp cicatrix position	Medial	Supra-basal	Infra-medial	Medial

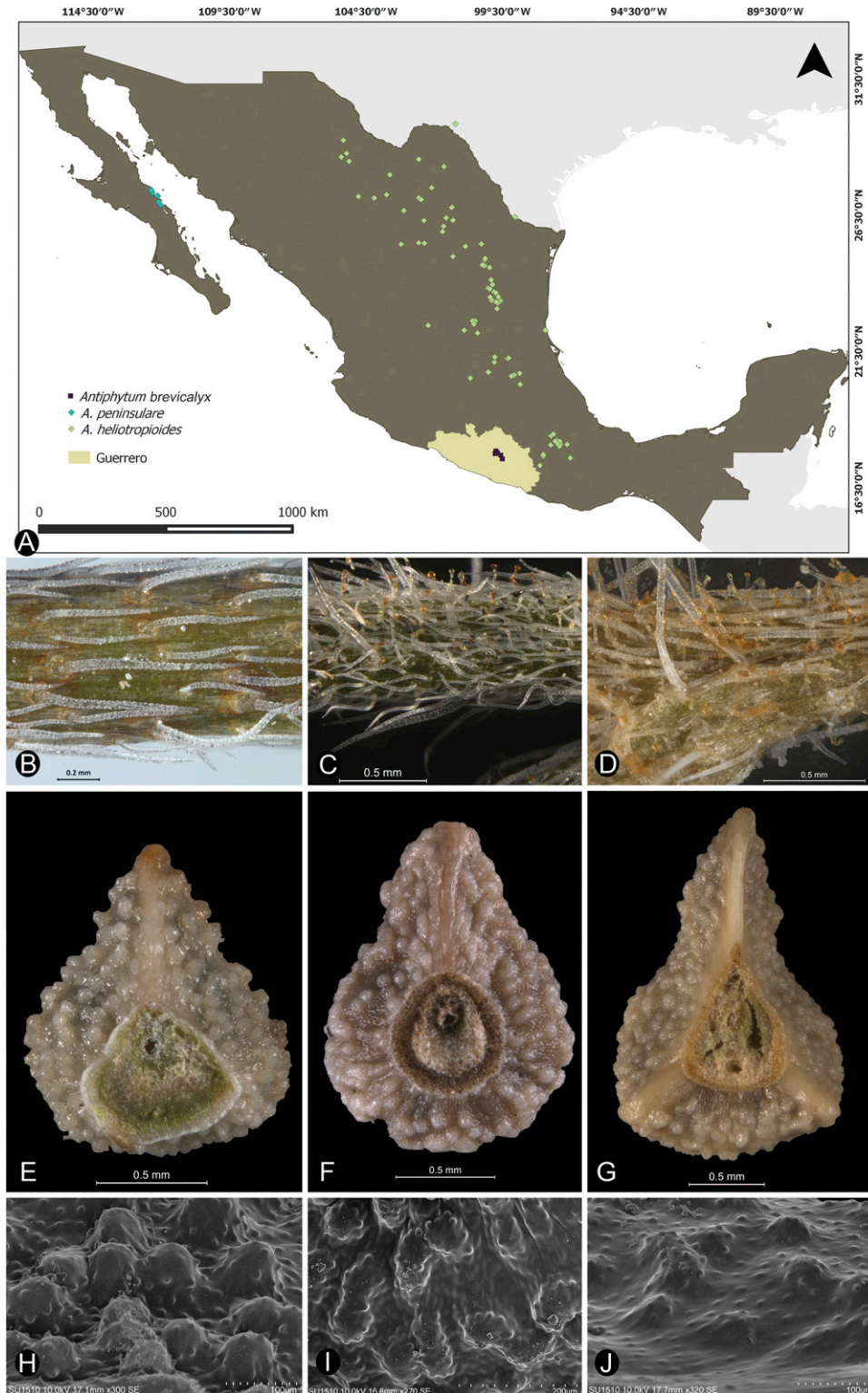


FIG. 5. Comparison of *A. brevicalyx* to the morphologically most similar species of *Antiphytum*. A. Distribution. B–D. Comparison among trichomes; note the capitate-glandular trichomes in C and D. E–G. Eremocarps; note the medial position of the cicatrix in F and G. H–J. Pericarp ornamentation of tubercles and papillae. B, E, H: *A. heliotropioides* (B, E, Mendoza-Díaz et al. 629, H, Torres-Hernández & Hernández 802). C, F, I: *A. brevicalyx* (Mendoza-Díaz et al. 643A). D, G, J: *A. peninsulare* (Wiggins 17328).

**Etymology**—The specific epithet refers to the main morphological character that distinguishes *A. brevicalyx* from other *Antiphytum*; it is the only taxon known in the genus in which the calyx is shorter than the corolla tube at anthesis (Figs. 1C, 2C–D, 3F–G).

**Phenology**—Flowering and fruiting from May to November.

**Distribution and Habitat**—*Antiphytum brevicalyx* is only known from the central region of Guerrero, in the municipalities of Eduardo Neri and Tixtla de Guerrero (Fig. 4). It occurs at



elevations ranging from 1080 to 1900 m, on limestone soils and probably also on gypsum, in deciduous forest with *Brahea*, *Bursera*, *Juniperus*, and *Quercus*.

**Conservation Status**—*Antiphytum brevicalyx* is known only from seven subpopulations in the state of Guerrero, Mexico (Fig. 4). As a whole, the extent of occurrence is less than 261 km<sup>2</sup> and some subpopulations occur among areas of rainy season agriculture (Fig. 4). Hence, habitat loss is the principal threat for the subpopulations. According to the IUCN (2012), this species meets the criteria B1ab(iii) for the category of Vulnerable (VU).

**Paratypes**—Mexico.—GUERRERO: Municipio Eduardo Neri, 9 km (on the road to Filo de Caballos) west of Xochipala, 1635 m, 17°46'41.4"N, 99°46'41.4"W, 18 November 2016, *Mendoza-Díaz et al. 644* (MEXU!, FCME!, ENCB!, K!); La Yesera, 12.3 km N de Zumpango del Río, 1420 m, 17°42'23"N, 99°30'04"W, 25 October 1994, *Jiménez-Ramírez & Luna-Flores 971* (FCME!); cerca de carretera, desv. a Xochipala, 1080 m, [17°47'27.7"N, 99°37'08"W, ±2 km], 26 October 1990, *Peralta & Villegas 127* (FCME!); 14 km SW de Xochipala, carretera a Filo de Caballos, 1900 m, [17°45'52.6"N, 99°42'17.2"W, ±2 km], 17 October 1983, *Soto-Núñez 5626* (MEXU!); along the road to Filo de Caballo, just above Xochipala at km 22, below La Laguna, 1600 m, 17°48'N, 99°36'W, 21 May 1987, *Miller & Goreti 2826* (MEXU!, MO!); 7 km O Carrizalillo, 1600 m, 17°51'34"N, 99°40'17"W, 09 September 1993, *Martínez-Gordillo 798* (FCME!, MEXU!). Municipio Tixtla de Guerrero, Summit of mountains northeast of Chilpancingo on road to Chilapa, 6000 ft, [17°34'14"N, 99°26'25.1"W, ±2 km], 19 August 1948, *Moore Jr. & Wood, Jr. 4672* (GH!).

**Additional Specimens Examined for the Morphological Study**—*Antiphytum caespitosum*. Mexico.—GUERRERO: Municipio Tlapa, on El Salado bridge, 8 km N of Tlapa, road to Huamuxtitlan, 990 m, 16 November 1982, *Martínez et al. 2680* (MEXU!). *Antiphytum heliotropioides*. Mexico.—NUEVO LEÓN: Municipio Rayones, road Galeana-Rayones, 6 km N of Los Nogales and 17 km N of Galeana, 1360 m, 24°55'43.7"N, 100°03'51.1"W, 24 September 2016, *Mendoza-Díaz et al. 629* (MEXU!). *Antiphytum peninsulare*. Mexico.—BAJA CALIFORNIA SUR: Gulf of California, volcanic hillside near north west end of Isla San Marcos, 27°15'N, 112°07'W, 28 March 1962, *Wiggins 17328* (MEXU!).

## DISCUSSION

*Antiphytum* has a preference for impoverished soils, such as limestone and gypsum in North America. Except for *A. hintoniolum*, the only gypsum endemic species of the genus, and *A. floribundum*, which is not known to occur on gypsum (Mendoza-Díaz 2015), all remaining species grow on and off gypsum (such species are called gypsovags; Moore et al. 2014). Although we only observed populations growing in calcareous soils, *A. brevicalyx* also seems to be a gypsovag, because the collection *Jiménez-Ramírez & Luna-Flores 971* (FCME) grew on gypsum soils at “La Yesera” (Spanish for “gypsum place”).

Before the description of *A. brevicalyx*, only *A. caespitosum* was known from Guerrero, near the boundary with the state of Oaxaca. The discovery of this new species was possible due to the systematic floristic exploration of the state of Guerrero, which began in 1979 with the Flora of Guerrero project at Facultad de Ciencias, UNAM (Flores-Olvera et al. 2010). Earlier collections in the state, mainly gathered by foreign botanists, are also important for the knowledge of the flora of Guerrero, and such collections are also known for the new species (see paratypes).

Although *A. brevicalyx* and *A. caespitosum* are both distributed in Guerrero, they are allopatric, morphologically distinct, and not phylogenetically sister. Moreover, *A. brevicalyx* lacks a basal leaf rosette and reaches 1.2 m in height, whereas *A. caespitosum* reaches a height of less than 1 m.

Morphological features of the inflorescence of *A. brevicalyx* are most similar to *A. floribundum*, from which it differs by the presence of appendages on the corolla throat and the lack of

leaves arranged in a basal rosette (Table 1). In addition, *A. floribundum* is distributed in western Mexico, principally along the Sierra Madre Occidental. A sister relationship between *A. brevicalyx* and *A. peninsulare* was also supported by morphological characters (Table 1; Figs. 1, 5). Detailed observations show similarities in the presence of capitate glandular trichomes, the medial position of the eremocarpaceous cicatrix and the ornamentation of the fruit, which is also shared with *A. heliotropioides*, a species without capitate glandular trichomes and with a cicatrix in an infra-medial position (Fig. 5B–J; Table 1). Despite these similarities, *A. brevicalyx* is disjunct geographically from both species (Fig. 5A).

In short, both morphological and molecular data strongly support the recognition of *A. brevicalyx* as a new species, which is unique in the genus by possessing a calyx shorter than the corolla tube at anthesis, emphasized by the specific epithet of the name.

In spite of the lack of jackknife support and the conflicting results among the nuclear and chloroplast loci, the combined molecular analysis recovered all the accessions of *A. brevicalyx* in a clade. The phylogenetic relationships among *Antiphytum* species and the exact position of *A. brevicalyx* inside the genus require further study with a broader sampling of loci and taxa, including additional outgroups and all species of *Antiphytum*, including the South American ones (Mendoza-Díaz et al. in prep.).

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## AUTHOR CONTRIBUTIONS

Nidia Mendoza-Díaz conducted the research as part of the D.Sc. thesis guided by Hilda Flores-Olvera. Helga Ochoterena guided the phylogenetic study. All coauthors conducted field work. Michael J. Moore provided lab space and support for parts of the molecular study. The manuscript was written with the collaboration of all the authors.

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APPENDIX 1. List of accessions used in the morphological and molecular phylogenetic analyses. Asterisks (\*) indicate lack of morphological observations. The following information is included for each accession: species, geographic origin, collector(s) and collection number, herbarium, and GenBank accession numbers for ITS and *ndhF-rpl32*, respectively. An em-dash indicates missing data while boldface indicates sequences obtained from outside sources, which are correspondingly cited.

**Outgroup:** *Echium vulgare* L. Great Britain, cult., \*Chase 6061 (K), **AJ555896**, Långström and Oxelman (2003), —; Cornell Plantations, \*Cohen 212, —, **FJ827364**, Cohen and Davis (2009).

***Antiphytum brevicalyx* N. Mend., Flores Oliv. & H. Ochoterena. Mexico.**—GUERRERO: Jiménez-Ramírez & Luna-Flores 971 (FCME), MN265414, MN313511; Martínez-Gordillo 798 (MEXU), MN265415, MN313571; Mendoza-Díaz et al. 643F (MEXU), MN265416, MN313512; Mendoza-Díaz et al. 643G (MEXU), MN265417, MN313513; Mendoza-Díaz et al. 644A (MEXU), MN265418, MN313514; Mendoza-Díaz et al. 644B (MEXU), MN265419, MN313515; Mendoza-Díaz et al. 644D (MEXU), MN265420, MN313516. ***Antiphytum caespitosum* I.M. Johnston. Mexico.**—OAXACA: Mendoza-Díaz et al. 581 (MEXU), MN265421, MN313517; Mendoza-Díaz et al. 583b (MEXU), MN265422, MN313518; Mendoza-Díaz et al. 583d2 (MEXU), MN265423, MN313519; Mendoza-Díaz et al. 603e (MEXU), MN265424, MN313520; Mendoza-Díaz et al. 612a (MEXU), MN265425, MN313521; Mendoza-Díaz et al. 612d (MEXU), MN265426, MN313522; Mendoza-Díaz et al. 613e (MEXU), MN265427, MN313523; Mendoza-Díaz et al. 614a (MEXU), MN265428, MN313524; Mendoza-Díaz et al. 618b (MEXU), MN265429, MN313525; Rangel-Landa 1400, MEXU, MN265431, MN313527.—PUEBLA: Medina-Lemos & Martínez-Salas 5715 (MEXU), MN265430, MN313526. ***Antiphytum floribundum* (Torr.) A.Gray. Mexico.**—MICHUACÁN: Pérez-Cálix 2508 (MEXU), MN265433, MN313529; \*Cohen 227 (—), **KF287948**, Cohen (2014), **JF488879**, Cohen and Davis (2009).—JALISCO: Calónico & Flores 3822, MEXU, MN265434, MN313530 USA.—TEXAS: Lott et al. 5353 (MEXU), MN265432, MN313528. ***Antiphytum heliotropioides* A.DC. Mexico.**—GUANAJUATO: Rzedowski 52626 (MEXU), MN265440, MN313536.—HIDALGO: Mendoza-Díaz et al. 599b (MEXU), MN265441, MN313540; Mendoza-Díaz et al. 599d (MEXU), MN265445, MN313544; Mendoza-Díaz et al. 601a (MEXU), MN265446, MN313542.—NUEVO LEÓN: Moore et al. 1332 (MEXU), MN265442, MN313538; Moore et al. 1363 (MEXU), MN265443, MN313539.—OAXACA: Calzada 21801 (MEXU), MN265438, MN313534; Calzada 22146 (MEXU), MN265439, MN313535; Salinas & Martínez-Correa 7929 (MEXU), MN265435, MN313531; Torres-Hernández & Hernández 802 (MEXU), MN265436, MN313532.—QUERÉTARO: Carranza 3781 (IEB), MN265437, MN313533; Mendoza-Díaz et al. 623a (MEXU), MN265447, MN313543; Mendoza-Díaz et al. 623b (MEXU), MN265448, MN313544.—SAN

LUIS POTOSÍ: *Torres-Colín & Terrazas 17191* (MEXU), MN265449, MN313545. ***Antiphytum hintoniorum* L.C.Higgins & B.L.Turner. Mexico.** —NUEVO LEÓN: *Hinton et al. 18321* (MEXU), MN265450, MN313546; *Hinton et al. 19125* (MEXU), MN265451, MN313547; *Moore et al. 1308* (MEXU), MN265452, MN313548; *Moore et al. 1334* (MEXU), MN265453, MN313549; *Patterson et al. 7475* (MEXU), MN265454, MN313550; ***Antiphytum paniculatum* I.M.Johnston. Mexico.** —PUEBLA: *Mendoza-Díaz et al. 619a* (MEXU), MN265455, MN313551; *Mendoza-Díaz et al. 619b* (MEXU), MN265456, MN313552; *Mendoza-Díaz et al. 620a* (MEXU), MN265457, MN313553; *Mendoza-Díaz et al. 621a* (MEXU), MN265458, MN313554; *Mendoza-Díaz et al. 621e* (MEXU), MN265459, MN313555. ***Antiphytum parryi* S.Watson. Mexico.** —OAXACA: *García-Mendoza et al. 2515* (MEXU), MN265460, MN313556; *Mendoza-Díaz et al. 579* (MEXU), MN265464, MN313560; *Mendoza-Díaz et al. 579\_2* (MEXU), MN265465, MN313561; *Mendoza-Díaz et al. 579a* (MEXU), MN265466, MN313562; *Mendoza-Díaz et al. 579b* (MEXU), MN265467, MN313563; *Mendoza-Díaz et al. 579c* (MEXU), MN265468, MN313564; *Mendoza-Díaz et al. 610a* (MEXU), MN265471, MN313567. —HIDALGO: *Mendoza-Díaz et al. 596* (MEXU), MN265469, MN313565; *Mendoza-Díaz et al. 598* (MEXU), MN265470, MN313566. —PUEBLA: *Rosas et al. 2950* (MEXU), MN265461, MN313557; *Náder et al. 54* (MEXU), MN265462, MN313558. —QUERÉTARO: *Rzedowski 53929* (MEXU), MN265463, MN313559. ***Antiphytum peninsulare* (Rose) I.-M.Johnston. Mexico.** —BAJA CALIFORNIA SUR: *Valov 2004046* (HCIB), MN265472, MN313568; *Valov 200517* (MEXU), MN265473, MN313569; *León 7736* (HCIB), MN265474, MN313570.