UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE BIOLOGÍA
SISTEMÁTICA

# DIVERSIFICACIÓN DE *ANTIPHYTUM* DC. EX MEISN. (BORAGINACEAE): IMPLICACIONES EN LA SISTEMÁTICA DEL GÉNERO

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

**NIDIA MENDOZA DÍAZ**

TUTORA PRINCIPAL DE TESIS: DRA. HILDA FLORES OLVERA
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SCIENCE CENTER, OBERLIN COLLEGE, OHIO

CIUDAD UNIVERSITARIA, CD. MX., SEPTIEMBRE, 2022.

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**CIUDAD UNIVERSITARIA, CD. MX., 2022**

**COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS**

**INSTITUTO DE BIOLOGÍA**

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**ASUNTO: Oficio de Jurado**

**M. en C. Ivonne Ramírez Wence**
**Directora General de Administración Escolar, UNAM**
**Presente**

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **20 de junio de 2022** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **MENDOZA DÍAZ NIDIA** con número de cuenta **407043381** con la tesis titulada **“DIVERSIFICACIÓN DE ANTIPHYTUM DC. EX MEISN. (BORAGINACEAE): IMPLICACIONES EN LA SISTEMÁTICA DEL GÉNERO”,** realizada bajo la dirección de la **DRA. MARÍA HILDA FLORES OLVERA,** quedando integrado de la siguiente manera:

| | |
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| Vocal: | DR. ANDRÉS ERNESTO ORTÍZ RODRÍGUEZ |
| Secretario: | DRA. HELGA OCHOTERENA BOOTH |

Sin otro particular, me es grato enviarle un cordial saludo.

**ATENTAMENTE**
**“POR MI RAZA HABLARÁ EL ESPÍRITU”**
Ciudad Universitaria, Cd. Mx., a 16 de agosto de 2022

**COORDINADOR DEL PROGRAMA**

**DR. ADOLFO GERARDO NAVARRO SIGÜENZA**

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# DEDICATORIA

A Dino
A Lori
A Bru y a todos mis niños

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# RESUMEN

El género americano *Antiphytum* DC. ex Meisner (Boraginaceae, subfamilia Echiochiloideae) es mayormente diverso en México. La lectotipificación con *A. cruciatum* preservó el nombre sobre taxones sudamericanos disyuntos a Norteamérica, pero actualmente éstos se encuentran mal entendidos en cuanto a circunscripción taxonómica, número de especies, distribución geográfica y su relación con las especies norteamericanas. En un estudio previo con dos marcadores moleculares (ITS y *ndh*F–*rpl*32), *Antiphytum cruciatum* se recuperó como hermana a algunos representantes norteamericanos. No obstante, ningún análisis filogenético ha incluido un muestreo a nivel de subfamilia, ni ha representado satisfactoriamente a las especies de *Antiphytum*, particularmente las sudamericanas. Dado este escenario, mi proyecto de tesis se propone circunscribir a *Antiphytum* y a sus especies, obtener una hipótesis filogenética sobre las relaciones del género dentro de la subfamilia Echiochiloideae y entre las especies de *Antiphytum* y esclarecer la relación entre los linajes norteamericano y sudamericano. El alcance de estos objetivos se planteó como la base para ubicar el género en las zonas ecológicas de las regiones anfitropicales, postular una direccionalidad en la colonización de América y plantear los posibles escenarios que dieron lugar a la diversificación de *Antiphytum*. La primera parte de la tesis presenta la reevaluación taxonómica del género con el hallazgo de dos nuevas especies de Norteamérica, que añade una nueva forma a las ginobases de *Antiphytum*. La clarificación de la diversidad de Sudamérica incluye la publicación de una nueva especie uruguaya de corolas blancas, que rompe la disrupción morfológica en el color de las corolas que se asociaba fuertemente con la geografía; la transferencia y lectotipificación de una especie distribuida en Brasil y Uruguay, y una clave para todas las especies sudamericanas. Todo este estudio taxonómico apoyado en morfología, datos moleculares, trabajo de campo, los ejemplares tipo y la nomenclatura, permitió circunscribir 13 especies en el género, de las cuales nueve se distribuyen en Norteamérica y cuatro en Sudamérica. Este resultado incrementa al doble la diversidad conocida en la zona austral, actualiza el conocimiento del género en Norteamérica y delimita el género en términos morfológicos dentro de Echiochiloideae, principalmente con la combinación de los siguientes caracteres: sufrútices perennes como forma de crecimiento; corola con simetría radial y sobrepasando el cáliz; presencia de apéndices faucales; fruto no encerrado por el cáliz, sino el cáliz extendido y subtendiendo al fruto; forma de la ginobase de plana a triangular; cicatriz en la mitad proximal del eremocarpo, no rebasando la zona media de la cara ventral de éste. La segunda parte de esta tesis presenta una propuesta filogenética de *Antiphytum* con un muestreo de 12 de las 13 especies reconocidas en la primera parte y la inclusión de terminales de los otros géneros de Echiochiloideae. El árbol consenso obtenido mediante cinco regiones moleculares (ITS, *ndh*F–*rpl*32, *trn*L–F, *mat*K y *rps*16) recupera a *Antiphytum* como monofilético y formado por tres

clados en una politomia: clado norteamericano (NA) de especies sin roseta basal, clado NA de especies con roseta basal y clado sudamericano (SA), lo que, aunado al hecho de que el grupo hermano, *Ogastemma*, es africano, genera ambigüedad en la interpretación de direccionalidad de colonización. Sin embargo, dos de los tres posibles escenarios para resolver esta politomia sugieren que las especies norteamericanas no comparten un ancestro común inmediato e implican un origen único y más probable en Norteamérica, en contraste a la necesidad de invocar dos colonizaciones independientes de esta región. Todos los escenarios implican que el subclado con especies con corolas azules es derivado. Según el análisis de los cinco marcadores, hay una diferencia en el número de cambios hacia *A. charruasorum* (29) con respecto a las otras especies en SA (18), lo que apuntaría a que las especies con flores azules son más recientes. En conjunto esta evidencia sugiere que el patrón anfitropical ocurrió en un único evento de Norteamérica a Sudamérica, probablemente de dispersión a larga distancia, como ha sido el caso para otras Boraginaceae. En cuanto a diversificación, se sugiere que la mayor diversidad en NA se debe a la distribución geográfica de *Antiphytum* en las zonas áridas de siete diferentes regiones biogeográficas de México. Además, hay una preferencia edáfica a diversos suelos restrictivos, como los yesosos (restringidas o no), calcáreos y de origen volcánico. En cambio, el linaje sudamericano se encuentra espacialmente más restringido y sólo asociado a suelos de origen volcánico o calcáreos. El clado NA sin rosetas presenta el mayor número de especies y es en el que se encuentran todas las preferencias edáficas, pero las modificaciones en la corola y la quilla ventral de los eremocarpos están asociadas a especies que crecen sobre suelos de origen volcánico, tanto en SA como NA. El género *Ogastemma* se recuperó como monofilético y hermano de *Antiphytum* en los análisis combinados, como fuera sugerido por estudios previos, aunque las características palinológicas son muy similares entre ambos. Finalmente, *Antiphytum* se suma a los taxa de Boraginaceae con distribución anfitropical y se provee de una clave taxonómica para todas las especies del género.

## ABSTRACT

The American genus *Antiphytum* DC. ex Meisner (Boraginaceae, subfamily Echiochiloideae) is mostly diverse in Mexico, but the name is preserved over the South American species after the lectotypification with *A. cruciatum.* The South American species are disjunct to North American ones, but they are currently misunderstood in terms of taxonomic circumscription, number, geographic distribution and their relationships with the North American taxa. In a previous study with two molecular markers (ITS and *ndh*F–*rpl*32), *Antiphytum cruciatum* was recovered as sister to some North American taxa. However, all the phylogenetic analyses lack a representative subfamily level sampling and do not include the whole of the known *Antiphytum* species, particularly from South America. Therefore, this study aims to circumscribe *Antiphytum* and its species; to obtain a phylogenetic hypothesis on the relationship of the genus within Echiochiloideae and among *Antiphytum* species; and to clarify the relationship between the North American and South American lineages. The attainment of these objectives was proposed as the basis for locating the genus in the ecological zones of amphitropical regions, postulating directionality in the colonization of America, and positing possible scenarios that led to the diversification of *Antiphytum*. The first part of this thesis presents the taxonomic reevaluation with the finding of two new species from North America that adds a new gynobase shape. Clarification of South American diversity includes the description of a new Uruguayan species with white corollas. This feature breaks the morphological disruption in corolla color that was strongly associated with geography. This part of the study also derived in the transfer and lectotypification of one species from Brazil and Uruguay, and provided a published taxonomic key to all species of this region. All this taxonomic work supported by morphology, molecular data, field work, type specimens and nomenclature, allowed the circumscription of 13 species, of which nine are distributed in North America and four in South America. This result doubles the known diversity in South America, updates the genus knowledge in North America and delimits *Antiphytum* in morphological terms within Echiochiloideae, mainly by the combination of the following characters: suffruticose perennial as growth-form; corolla radially symmetrical surpassing the calyx; presence of faucal appendages; fruit not enclosed into the calyx, rather an extended calyx subtending the fruit; gynobase shape from flat to triangular; cicatrix in proximal half of eremocarp, not exceeding the middle zone of the ventral face. The second part of this thesis presents a phylogenetic hypothesis of *Antiphytum* sampling 12 of the 13 recognized species and terminals of the other genera of Echiochiloideae. The consensus tree based on five markers (ITS, *ndh*F–*rpl*32, *trn*L–F, *mat*K and *rps*16) recovers *Antiphytum* as monophyletic and formed by three main clades in a polytomy: the North American (NA) clade of species without basal rosette, the NA clade of species with basal

rosette, and the South American (SA) clade, which along with the fact that *Ogastemma*, the sister group, is African, generates ambiguity in directionality interpretation of colonization. However, two of the three possible scenarios for resolving this polytomy suggest that North American species do not share an immediate common ancestor and entail a single, more likely origin in North America, in contrast to the need to invoke two independent colonizations of this region. All scenarios imply that the subclade with blue-corolla species is derived. According to the analysis of the five markers, there is a difference in the number of changes toward *A. charruasorum* (29) relative to the other species in SA (18), which would point to the blue-corolla species being more recent. Taken together this evidence suggests that the amphitropical pattern occurred in a single event from North to South America, probably by long-distance dispersal, as has been the case for other Boraginaceae. In terms of diversification, it is suggested that NA presents a greater diversity because *Antiphytum* distributes in the arid zones of seven different biogeographic regions of Mexico. In addition, there is an edaphic preference to diverse restrictive soils such as gypsiferous (restricted or not), calcareous and volcanic soils. In contrast, the South American lineage is spatially more restricted and associated only with soils of volcanic or calcareous origin. The NA clade without rosettes presents the largest number of species and is the one in which all edaphic preferences are found, but modifications in the corolla and ventral keel of eremocarps are associated with species growing on soils of volcanic origin, both in SA and NA. *Ogastemma* was recovered as monophyletic and sister to *Antiphytum* in the combined analyses, as suggested by previous studies, although the palynological characteristics are very similar between the two. Finally, *Antiphytum* joins the Boraginaceae taxa with amphitropical distribution and a taxonomic key is provided for all species of the genus.

# INTRODUCCIÓN GENERAL

## I. LA FAMILIA BORAGINACEAE JUSS.

El clado Boraginales Juss. ex Bercht. & J.Presl comprende alrededor de 2750 especies de distribución cosmopolita (Luebert et al. 2016), que a su vez se resuelve en dos clados (Refulio-Rodríguez y Olmstead 2014; Weigend et al. 2014; Luebert et al. 2016): Boraginales I, compuesto de las familias Codonaceae Weigend & Hilger, Wellstediaceae Novák y **Boraginaceae s. str**.; y Boraginales II, con las familias Hydrophyllaceae R.Br., Namaceae Molinari, Heliotropiaceae Schrad., Cordiaceae R.Br. ex Dumort. s.l. y Ehretiaceae Mart. s.l. Boraginales I tiene a África como área ancentral, mientras que para Boraginales II es Sudamérica (Luebert et al. 2017).

Dentro del clado Boraginales I, la familia Codonaceae es tempranamente divergente y, al igual que Wellstediaceae, está restringida al continente africano (Luebert et al. 2016, 2017, Weigend et al. 2016). Boraginaceae s. str. (a partir de aquí, citada como Boraginaceae), que antes fue referida como subfamilia Boraginoideae Arn. por De Candolle (1846), Bentham y Hooker (1976), Gürke (1897), Takhtajan (1980, 1997), Cronquist (1981), Johnston (todos sus estudios sobre Boraginaceae, indexados por Hilger y Zippel 2001) y Al-Shehbaz (1991), es subcosmopolita y hermana de Wellstediaceae (Cohen 2014, Weigend et al. 2014; Luebert et al. 2016). Las plantas de Boraginaceae se distinguen por ser, en su mayoría, hierbas con un indumento hirsuto a híspido, tener estilo ginobásico, ovario dos-partido que se divide en cuatro clusas e inflorescencia tipo cima escorpioidea; otras características vegetativas, florales, palinológicas y de los frutos varían entre sus integrantes (Cohen 2014).

En las últimas décadas, la clasificación intrafamiliar de Boraginaceae se ha modificado con el descubrimiento, reducción y recircunscripción de sus tribus (Långstrom y Chase 2002; Långstrom y Oxelman 2003; Weigend et al. 2010, Nazaire y Hufford 2012; Weigend et al. 2013; Cohen 2014; Chacón et al. 2016). Chacón et al. (2016) redefinieron la clasificación intrafamiliar de Boraginaceae en tres subfamilias, Echiochiloideae Weigend (≡tribu Echiochileae Långstrom y Chase), Boraginoideae Arn. (integrada por las tribus Lithospermeae Dumort. y Boragineae Rchb.) y Cynoglossoideae Weigend (la más grande y problemática con ocho tribus: Asperugeae Zakirov ex Ovczinnikova; Craniospermeae DC. ex Meisn.; Cynoglosseae W.D.J.Koch; Lasiocaryeae Weigend; Myosotideae Rchb.f.; Omphalodeae Weigend; Rochelieae A.DC.; y Trichodesmeae Zakirov ex Riedl). Mientras las jerarquías más inclusivas continúan siendo ratificadas por los diferentes estudios (Långstrom y Chase 2002; Långstrom y Oxelman 2003; Weigend et al. 2010; Nazaire y

Hufford 2012; Weigend et al. 2013, Cohen 2014; Chacón et al. 2016), las relaciones al interior de las tribus y géneros de Boraginaceae no se han resuelto de forma definitiva.

Por ejemplo, se han recuperado géneros no monofiléticos, como *Cryptantha* Lehmann ex G.Don. (Hasenstab-Lehman y Simpson 2012; Mabry y Simpson 2018), *Cynoglossum* L. (Cohen 2011; Selvi et al. 2011; Weigend et al. 2013; Cohen 2014, 2015; Chacón et al. 2016; Pourghorban et al. 2020), *Lithospermum* (Cohen y Davis 2009; Weigend et al. 2009), *Omphalodes* Moench (Weigend et al. 2013, Otero et al. 2014), *Plagiobothrys* (Hasenstab-Lehman y Simpson, 2012; Guilliams 2015; Simpson et al. 2017a); cuyas complejas relaciones han derivado en cambios de circunscripción y en la descripción de nuevos géneros y especies (Cohen y Davis 2009; Hansestab-Lehman y Simpson 2012; Otero et al. 2014; Cohen 2015; Mabry y Simpson 2018; Simpson et al. 2019; Simpson y Rebman 2021; Rebman y Simpson 2022). En algunos casos, las decisiones taxonómicas son consideradas prematuras por algunos autores [p. ej. la descripción de géneros segregados de *Cynoglossum* s.l., como *Adelinia* J.I.Cohen y *Andersonglossum* J.I.Cohen descritos por Cohen (2015), y *Oncaglossum* Sutorý descrito por Sutorý (2010); véase Weigend et al. (2016)].

También se han observado contradicciones en la resolución de clados a nivel de las tribus y subtribus de Boraginaceae, dependiendo del muestreo y marcadores empleados (p. ej. Weigend et al. 2013; Cohen, 2014, 2015; Chacón et al. 2016); o la falta de resolución en la base de la filogenia (p. ej. Pourghorban et al. 2020). Por ello, para continuar con el esclarecimiento de los grupos de Boraginaceae, es necesario solucionar los problemas taxonómicos a nivel de los géneros.

## II. DISTRIBUCIÓN GEOGRÁFICA DE LA FAMILIA BORAGINACEAE EN AMÉRICA

La mayoría de los representantes de la familia Boraginaceae se encuentra principalmente en Asia y Europa. A excepción de las tribus Craniospermeae, Lasiocaryeae y Trichodesmeae (Al-Shehbaz 1991; Chacón et al. 2016; Weigend et al. 2016), en América se encuentran unos cuantos taxones de cada una de las tribus principales.

La presencia de los géneros de Boraginaceae en América no es igual entre los dos más grandes subcontinentes americanos. Por ejemplo, de los géneros compartidos con el Viejo Mundo, *Eritrichium* Schrad. ex Gaudin (Rochelieae), *Hackelia* Opiz (Rochelieae), *Lappula* Moench (Rochelieae) y *Mertensia* Roth (Asperugeae) sólo se encuentran en Norteamérica (Nazaire et al. 2014; Weigend et al. 2016); *Plagiobothrys* s. l. (Cynoglosseae) tiene mayor diversidad en Norteamérica (Amsinckiinae Working Group 2022) y *Lithospermum* (el único género de la tribu Lithospermeae en América) tiene su centro de diversificación en el suroeste de EUA y México (Cohen y Davis 2012; Cohen 2022).

Los géneros endémicos americanos *Amsinckia* Lehm. (Cynoglosseae), *Antiphytum* (Echiochiloideae), *Cryptantha* s.l. (Cynoglosseae), y *Pectocarya* DC. ex Meisn. (Cynoglosseae) tienen su mayor diversidad en el hemisferio norte del continente (Johnston 1927; Hasenstab-Lehman y Simpson 2012; Guilliams et al. 2013; Mendoza-Díaz 2015). En contraste, otros géneros son endémicos de Norteamérica, como *Dasynotus* I.M.Johnst. (Cynoglosseae), *Mimophytum* Greenm. (Omphalodeae) y *Oncaglossum* (Cynoglosseae) (Johnston 1948; Sutorý 2010; Holstein et al. 2016a); o de Sudamérica, como *Moritzia* DC. ex Meisn. (Boragineae), *Selkirkia* Hemsl. (Omphalodeae) y *Thaumatocaryon* Baill. (Boragineae) (Weigend et al. 2010, 2016; Chacón et al. 2016; Holstein et al. 2016b). La subtribu Moritziinae Weigend (integrada por *Moritzia* y *Thaumatocaryon*) es la única representante de la tribu Boragineae en el continente americano (Weigend et al. 2010); sin embargo, el registro fósil indica que durante el Mioceno Tardío y el Plioceno Temprano taxones de Boragineae también se distribuyeron en Norteamérica, y ahí la tribu alcanzó una gran diversidad, abundancia y extensión (Weigend et al. 2010).

La distribución en Sudamérica tampoco es homogénea, pues los cerca de 20 géneros (Jørgensen et al. 2014; Zuloaga et al. 2021) y aproximadamente 90 especies (Johnston 1927) de Boraginaceae se distribuyen principalmente en la zona occidental del subcontinente (Argentina, Chile, Colombia, Perú y Venezuela). Únicamente tres géneros (*Antiphytum*, *Moritzia* y *Thaumatocaryon*) se distribuyen en el área comprendida entre Uruguay y el sur de Brasil.

Respecto a los géneros compartidos entre Norte y Sudamérica, Boraginaceae presenta 19 ejemplos de distribución anfitropical en América ("*American amphitropical disjunct*", AAD por sus siglas en inglés, sin incluir a *Antiphytum*) y es la segunda familia con mayor número de AAD después de Poaceae, con 51 ejemplos (Simpson et al. 2017b). La relación filogenética dentro de Boraginaceae explica un patrón biogeográfico en el cual Sudamérica fue colonizada a partir de elementos de origen norteamericano (p. ej. Weigend et al. 2010; Cohen y Davis 2012; Hasenstab-Lehman y Simpson 2012; Mabry y Simpson 2018; Otero et al. 2019), es decir que, a nivel de géneros, la dispersión y el establecimiento en el continente fue exclusivamente de norte a sur (Chacón et al. 2017).

La presencia de taxones con disyunciones entre Norteamérica y Sudamérica tiene dos principales explicaciones potenciales de acuerdo a Raven (1963): 1) individuos de un linaje ancestral norteamericano o sudamericano atravesaron los Trópicos en uno o más pasos por medio de dispersión a larga distancia, ya sea por aves o grandes mamíferos. Si fue por mamíferos, la dispersión debió ocurrir durante el Gran Intercambio Biótico Americano (GABI por sus siglas en inglés) (Guilliams et al. 2017). 2) Congéneres o coespecíficos de Norteamérica y Sudamérica llegaron a estar aislados tras la fragmentación histórica de un intervalo continuo de distribución que abarcó el área ocupada actualmente por los Trópicos Americanos (llamada hipótesis de la "fragmentación del intervalo" por Guilliams et al. 2017).

La evidencia publicada en Boraginaceae sugiere que no hay datos que respalden una distribución ancestral amplia que abarcara toda la zona tropical de América (Chacón et al. 2017; Mabry y Simpson 2018; Otero et al. 2019). Asimismo, los datos publicados hasta ahora foverecen la explicación de dispersión a larga distancia vía aves y no por mamíferos (Chacón et al. 2017; Guilliams et al. 2017).

## III. LA SUBFAMILIA ECHIOCHILOIDEAE

Desde que fuera propuesto como tribu (Långstrom y Chase 2002), el clado de la subfamilia Echiochioideae ha sido recuperado con alto soporte en las filogenias moleculares, con independencia del método aplicado por los autores (Weigend et al. 2010; Nazaire y Hufford 2012; Weigend et al. 2013; Cohen, 2014; Weigend et al. 2014; Mendoza-Díaz 2015; Chacón et al. 2016).

La subfamilia Echiochiloideae es hermana del resto de Boraginaceae (Weigend et al. 2010, 2014; Nazaire y Hufford 2012; Weigend et al. 2013; Cohen 2014; Weigend et al. 2014; Chacón et al. 2016) y presenta la menor diversidad dentro de la familia con tres géneros, *Antiphytum*, *Ogastemma* Brummitt y *Echiochilon* Desf., y aproximadamente 30 spp. (Lönn 1999; Långström y Oxelman 2003; Mendoza-Díaz 2015). La relación filogenética al interior de Echiochiloideae se ha recuperado como (*Echiochilon*, (*Ogastemma*, *Antiphytum*)) aunque sin un muestreo significativo del género *Antiphytum*, pues en muchos trabajos se omite (p.ej. Weigend et al. 2010 y Otero et al. 2014), o sólo se incluyen hasta tres especies, ninguna de Sudamérica (Långström y Chase 2002; Långström y Oxelman 2003; Nazaire y Hufford 2012; Weigend et al. 2013; Cohen 2014; Chacón et al. 2016, 2017; Luebert et al. 2017).

También la diversidad de formas de crecimiento en Echiochiloideae es menor en comparación con las otras subfamilias, que incluyen formas desde efímeras a arborescentes (Weigend et al. 2014). La subfamilia Echiochiloideae, al igual que las familias Codonaceae y Wellstediaceae, está compuesta en su mayoría por plantas desérticas sufruticosas/fruticosas (Weigend et al. 2014) que habitan en condiciones adversas o suelos empobrecidos (Lönn 1999; Setubal 2010; Mendoza-Díaz 2015). Los taxones de la subfamilia se distribuyen en zonas áridas y no tienen representantes asiáticos. *Antiphytum* es el único elemento americano; *Ogastemma*, el género hermano, se distribuye en las Islas Canarias, el norte de África y la Península Arábiga; a su vez, *Echiochilon* se encuentra desde el Norte de África hasta Kenia, en la Península Arábiga y en Paquistán e India (Lönn 1999). Echiochiloideae tiene, pues, una disyunción anfiatlántica.

Anteriormente, un taxón con distribución anfiatlántica se explicaba por un origen Gondwánico y la ruptura posterior de este continente (Raven y Axelrod 1974), de modo que la extensión del taxón en el Hemisferio Norte era producto de las migraciones desde el sur. Sin embargo, las edades de muchos grupos han resultado más recientes que la separación total de África y Sudamérica (Wilkström et al. 2001; Givnish y Renner 2004) fechada hace ca. 105 Ma. (McLoughlin 2001).

En su lugar, el registro fósil y la afinidad de las floras del Hemisferio Norte se ha explicado a través de una flora ancestral denominada boreotropical (Wolfe 1975), es decir, durante el Paleogeno existió un intercambio preferencial entre Norteamérica y Eurasia de plantas tropicales facilitada por la proximidad entre ambas placas y el máximo térmico del Eoceno (Lavin y Luckow 1993). La hipótesis de la flora boreotropical (Lavin y Luckow 1993) predice que si existió un intercambio preferencial durante el Terciario Temprano a través del Atlántico Norte, los grupos de plantas y animales, con Norteamérica como su lugar de diversificación y con registro fósil en la región desde el Terciario, tienen una alta probabilidad de tener a sus grupos hermanos en los Paleotrópicos y grupos derivados en Sudamérica. Varias explicaciones biogeográficas recientes sobre grupos disyuntos han invocado la hipótesis de la flora boreotropical (Lavin y Luckow 1993; Givnish y Renner 2004; Calvillo-Canadell y Cevallos-Ferriz 2005; Smerdmark y Anderberg 2007; Erkens et al. 2009), de tal forma que muchos taxones sudamericanos tuvieron sus ancestros en Norteamérica (Cevallos-Ferriz y Ramírez 2004; Pérez-García et al. 2012).

Parte de la hipótesis de la flora boreotropical implica una conexión por medio de puentes intercontinentales transatlánticos que existieron durante el Paleogeno hasta probablemente el Mioceno-Medio (Wolfe 1975; Grønlie 1979; Tiffney 1985; Sanmartín et al. 2001; Brikiatis 2014). Tiffney y Manchester (2001) consideraban a las conexiones directas de tierra como una mejor explicación que invocar varios eventos paralelos y repetidos de dispersión a larga distancia de taxones no relacionados y con una amplia variedad de mecanismos de dispersión. Sin embargo, la conexiones físicas entre América, Groenlandia y Europa llegaron a ser inusuales durante el Oligoceno y el Mioceno, y el puente terrestre noratlántico (NALB, por sus siglas en inglés) ya estaba roto por el Eoceno Tardío (Tiffney y Manchester 2001). Por ello, en los grupos que tienen tiempos de diversificación más recientes que las edades de estos puentes se explica la afinidad anfiatlántica por dispersión a larga distancia (Kadereit y Baldwin 2012).

La distribución de los géneros que integran a la subfamilia Echiochiloideae podría encajar dentro de la hipótesis de la flora Boreotropical. Långström y Chase (2002) plantearon como posible explicación de esta distribución un origen en Eurasia, con *Antiphytum* diseminándose a Norteamérica vía el Puente Thulean. Este puente conectó el oeste de Europa con el este de Norteamérica a través de Groenlandia desde el Paleoceno Tardío hasta el Eoceno Tardío –o incluso más– (Grønlie 1979; Tiffney 1985; Sanmartín et al. 2001). Para *Echiochilon* y *Ogastemma*, los mismos autores postularon una diseminación hacia el sur desde Eurasia, debido al enfriamiento que inició en el Eoceno Temprano. La publicación de Långström y Oxelman (2003) mantiene la idea del paso por el puente Thulean, pero explica el patrón actual de distribución de *Echiochilon* y *Ogastemma* por medio de una distribución amplia del ancestro probable de estos géneros, seguida de eventos de vicarianza y especiación.

La edad de los nodos Echiochiloideae y *Antiphytum* fechados por Chacón et al. (2017) en 45 Ma y en 9.4 Ma, respectivamente, indican que el arribo de la subfamilia al continente americano fue por dispersión a larga distancia desde el oeste de Europa a Norteamérica cuando las conexiones por el NALB ya se habían interrumpido (Chacón et al. 2017). El evento de dispersión implicó cladogénesis y un cambio en el modo de dispersión de epizoocoria a endozoocoria, de acuerdo a estos mismos autores.

Después de los trabajos de Lönn (1999); Långstrom y Chase (2002) y Långstrom y Oxelman (2003), la subfamilia Echiochiloideae no ha sido objeto de investigación reciente a excepción de Mendoza-Díaz (2015). En consecuencia, aunque ha habido esfuerzos de compilar la información taxonómica, morfológica, cromosómica y palinológica que describe a la familia Boraginaceae, en muchos de estos campos la subfamilia Echiochiloideae carece de información (véase Weigend et al. 2016) y en algunos aspectos morfológicos y evolutivos se puede considerar imprecisa (obs. pers.).

Este proyecto propone completar la información de la subfamilia, enfocado al único y poco estudiado género americano *Antiphytum*, a la vez que busca responder a preguntas evolutivas y biogeográficas partiendo de una filogenia del género con marcadores moleculares.

## IV. EL GÉNERO *ANTIPHYTUM*

*Antiphytum* es un pequeño género de plantas sufruticosas perennes cuyas especies fueron descritas mayormente en el siglo XIX (Chamisso 1829; Martens y Galeotti 1844; De Candolle 1846; Torrey 1859; Watson 1883; Rose 1890), muchas de ellas bajo distintos géneros como *Anchusa* L., *Eritrichium*, *Krynitzkia* Fisch. & C.A.Mey. y *Lithospermum*.

Hasta que Långström y Chase (2002) propusieron la otrora tribu Echiochileae, el género *Antiphytum* presentó por mucho tiempo dificultades para ser ubicado dentro de la clasificación tribal de Boraginaceae, al igual que otros géneros "problemáticos" de la familia. *Antiphytum* figuró dentro de la tribu Cynoglosseae (De Candolle 1846), Lithospermeae (Bentham y Hooker 1876; Baillon 1890; Gürke 1897; Johnston 1923), Eritrichieae Gürke (Johnston 1954) y finalmente, fue incluido dentro de la tribu artificial Trigonotidae Riedl subtribu Antiphytinae Riedl (Riedl 1967, 1968).

La historia de *Antiphytum* inicia con su publicación (Meisner 1840) con cuatro especies, tres sudamericanas y una mexicana correspondiente a una iconografía de la Real Expedición Botánica a la Nueva España. El género había sido propuesto por De Candolle a partir del material iconográfico de Sessé y Mociño, responsables de la Real Expedición. No obstante, la iconografía que formalmente dio origen a *Antiphytum* no fue la correcta, ya que Meisner (1840) citó a "*A. Mexicana*, DC. ap. Moc. ic. Fl. Mex. ined.". En la descripción póstuma de Sessé y Mociño (1887), *"Anchusa mexicana* Sessé & Moc." (McVaugh 2000) tiene filotaxia alterna, lo cual no es congruente con la filotaxia opuesta de la diagnosis original de *Antiphytum* [*"Genus jam foliis oppositis (unde nomen) insigne"*, Meisner 1840, ii, p. 188; y "folia opposite (unde nomen)", De Candolle 1846, p. 121]. Contrariamente, las especies sudamericanas sí presentaban filotaxia opuesta. De ellas, sólo *"Anchusa cruciata* Cham." y "*Anchusa stoechadifolia* Cham." permanecerían subsecuentemente en el género, pues el tercer elemento sudamericano enlistado ("*Anchusa oppositifolia* Kunth") es en realidad *Plagiobothrys linifolius* (Willd. ex Lehm.) I.M.Johnst. (Holstein y Weigend 2016).

En el primer tratamiento formal del género, De Candolle (1846) realizó la combinación nomenclatural para las especies sudamericanas enlistadas por Meisner (1840), *Antiphytum cruciatum* (Cham.) DC., *A. stoechadifolium* (Cham.) DC. y *A. linifolium* (HBK) DC., y aclaró que la iconografía correcta de Sessé y Mociño era la correspondiente a la especie *Symphytum fruticosum* Sessé & Moc. (Sessé y Mociño 1887). McVaugh (2000) la refiere portando el número 288 y en ella se puede observar que De Candolle escribió el nombre "*Antiphytum oppositifolium*" (McVaugh 2000; Mociño y Sessé 2010), aunque la combinación final fue "*Antiphytum Mexicanum* DC." (De Candolle 1846). Actualmente se sabe que la especie mexicana que dio origen al género en realidad corresponde a *Euploca mexicana* (DC.) M.W.Frohl. & M.W.Chase, Frohlich et al. 2020). De Candolle (1846) también incorporó una nueva especie norteamericana (*Antiphytum heliotropioides* A. DC.) y otras dos especies de Sudamérica: *A. walpersii* A.DC., en realidad *Plagiobothrys kunthii* (Walp.) I.M.Johnst., Johnston (1923), y *A. tetraquetum* (Cham.) DC., en realidad *Thaumatocaryon* *tetraquetrum* (Cham.) I.M. Johnst. (Johnston 1924) en calidad de dudosa, la cual permanecería por mucho tiempo dentro de *Antiphytum*.

Bentham y Hooker (1876) consideraron que el género *Antiphytum* era heterogéneo y que la iconografía que lo originaba (de acuerdo a Meisner 1840) era inconsistente con la descripción morfológica del mismo. Por ello, restringieron *Antiphytum* a las especies sudamericanas *A. cruciatum*, *A. stoechadifolium* y *A. tetraquetum* con las que Fresenius (1857) trató el género en su trabajo regional *Flora brasiliensis*. Con esta decisión, el género por primera vez era dividido de acuerdo a un patrón geográfico.

Como consecuencia de esta circunscripción, Gray (1885) incluyó a las especies norteamericanas segregadas de *Antiphytum* en el subgénero *Amblynotus* de *Krynitzkia* junto con otras dos especies no relacionadas y Macbride (1916) creó a su vez el género *Amblynotopsis* J.F.Macbride para reunir en un grupo natural a las otrora especies norteamericanas de *Antiphytum*. Al crear este género, Macbride (1916) no las comparó con las especies sudamericanas.

Johnston (1923), tras comparar *Amblynotopsis* con *Antiphytum*, encontró insuficientes las diferencias morfológicas entre ambos géneros y los unificó bajo *Antiphytum*, reconociéndolo como un género con especies norteamericanas y sudamericanas de distribución disyunta, como fuera inicialmente. El trabajo de Johnston (1923) es el tratamiento más reciente publicado del género y el único trabajo del autor que trata con la totalidad del mismo. En 1927, Johnston hizo un cambio de circunscripción al excluir finalmente a *Thaumatocaryon tetraquetrum* (Cham.) I.M. Johnst., la cual había sido heredada desde De Candolle (1846), y reconocer a *A. cruciatum* y *A. stoechadifolium* como las únicas especies sudamericanas de *Antiphytum* (Johnston 1927). La suma de los trabajos en los que se incluye *Antiphytum* (Johnston 1923, 1924, 1925, 1927, 1957, 1966) dan cuenta de nueve especies, con las especies sudamericanas como las menos entendidas en cuanto a número y circunscripción. Más recientemente, bajo la circunscripción genérica de Johnston, Higgins y Turner (1983) describieron *A. hintoniorum*, especie norteamericana restringida a suelos yesosos y endémica del municipio de Galeana, en Nuevo León.

La historia taxonómica de *Antiphytum* plantea por igual un problema nomenclatural y uno de circunscripción. Dado el origen errado del género, Johnston (1923) lectotipificó *Antiphytum* con la especie sudamericana *A. cruciatum,* pues era parte de la publicación original y correspondía con la diagnosis genérica de Meisner (1840). Johnston (1923) había considerado *A. stoechadifolium* un sinónimo taxonómico de *A. cruciatum,* por lo que esta decisión no sólo preservaba el nombre sobre las especies sudamericanas, sino que planteaba el reconocimiento de una única especie en esta región. Aunque posteriormente Johnston (1927) reconocería que no eran coespecíficas, *A. steochadifolium* es un taxón prácticamente sin colectas fuera del material tipo. Por otro lado, el nombre *Amblynotopsis* es necesario para las especies norteamericanas si éstas no quedan circunscritas con las especies sudamericanas a las que está asociado el nombre *Antiphytum*.

En algunos trabajos, Johnston (1927, 1957) se cuestionó la naturalidad de *Antiphytum*, pues consideró que el género aún requería ser revisado, especialmente las especies sudamericanas que él conocía principalmente por descripción (Johnston 1927). Incluso llegó a llamar verdadero *Antiphytum* a las especies sudamericanas (Johnston 1927) y *Amblynotopsis* a las especies de México (Johnston 1957), aunque en el último trabajo en que trató con el género, Johnston (1966) lo siguió considerando disyunto. Por el contrario, Brand (1929, 1931) consideró no natural la unión de las especies sudamericanas y norteamericanas de *Antiphytum* e inválida la lectotipificación de Johnston, por lo que no se justificaba el uso del nombre. El nombre *Antiphytum* empero es válido y está correctamente aplicado.

La circunscripción de *Antiphytum* ha cambiado a lo largo de su historia taxonómica, con especies *entrando* y *saliendo* de acuerdo al concepto del autor que tratara con el género. Circunscrito tal como lo planteara Johnston (1923, 1925, 1927) al excluir a *Thaumatocaryon tetraquetrum*, el género es un grupo monofilético (Mendoza-Díaz 2015), de distribución disyunta, que según criterios de Simpson et al. (2017b) podría considerarse un grupo “trans-Norteamérica oriental-Sudamérica AAD”, es decir, que aunque presenta un patrón de distribución anfitropical en América, tiene taxones distribuidos en los sistemas montañosos tropicales de Norteamérica y taxones en la zona oriente de Sudamérica. Sin embargo, tal consideración geográfica requiere primero una evaluación taxonómica, tanto a nivel del género como de las especies, valorando tanto la morfología como otras fuentes de evidencia. Particularmente, la circunscripción de las especies sudamericanas requiere un estudio pormenorizado con colectas y observaciones de campo, dada la problemática de acceso al material herborizado y a la pobreza de la información bibliográfica. En el pasado, caracteres presentes en las especies sudamericanas, como la filotaxia opuesta, la inserción totalmente basal de las clusas y el color azul de la corola frente al blanco de las norteamericanas, fueron empleados para proponer la división de *Antiphytum*. Sin embargo, todos estos caracteres no son disruptivos porque a lo largo del género, los mismos se presentan como una gradación (Mendoza-Díaz 2015). Por otra parte, todas las colectas sudamericanas son determinadas como *A. cruciatum*, lo cual subestima el número de especies y oscurece la distribución del género en Sudamérica.

Para explicar los patrones de distribución y diversificación de *Antiphytum* se requiere de una filogenia bien sustentada que permita plantear hipótesis biogeográficas y esclarecer las relaciones entre sus especies y grupos hermanos, la cual tenga implicaciones directas en la sistemática del género, como la circunscripción del mismo, la delimitación de especies y el reconocimiento de caracteres morfológicos y moleculares diagnósticos o de agrupación. En el caso particular de *Antiphytum*, su distribución disyunta en el continente americano, aunada a su distribución amplia y diferencial en México, requiere un enfoque a nivel de subfamilia para explicar la biogeografía histórica del mismo.

## OBJETIVOS

**-Circunscribir** el género *Antiphytum* y **las especies** que lo integran por medio de datos morfológicos, geográficos y moleculares.

**-Obtener una hipótesis filogenética** del género que aborde la relación con los géneros de la subfamilia Echiochiloideae y las relaciones entre las especies de *Antiphytum*.

**-Esclarecer** la relación entre los linajes norteamericano y sudamericano.

-**Evaluar la evolución de caracteres morfológicos** clave en el género por medio de la filogenia.

**-Plantear posibles escenarios** que han dado lugar a la diversificación de *Antiphytum* y la direccionalidad en la colonización de América a partir de los datos disponibles.

## METODOLOGÍA GENERAL

-La revisión taxonómica preliminar de Mendoza- Díaz (2015) constituye el antecedente más reciente y directo para esta investigación que se robusteció para la ulterior circunscripción genérica y de especies de *Antiphytum*. Se llevó a cabo trabajo de herbario con la revisión de ejemplares de ARIZ, ASU, B, BM, CAS, CHAPA, CIIDIR, ENCB, F, FCME, G, HCIB, HUH, IBUG; IEB, K, MEXU, MO, NMC, NY, P, R, RSA-POM, TEX, UAT, UC, y US a partir de préstamos, visita a herbario o imágenes disponibles en línea (AAH, BR, G, G-DC, GH, K, UC, US, y W).

-Se realizó trabajo de campo en localidades esenciales para completar el muestreo pertinente para la reconstrucción filogenética, procesamiento de polen y observación de eremocarpos, y para conocer en su medio natural a las poblaciones de taxones poco entendidos o desconocidos del género. El trabajo de campo también se diseñó para corroborar localidades que eran registros únicos o atípicos para algunas especies. Recolectas en Brasil y Uruguay complementaron el trabajo de campo realizado en México.

-Se hizo una revisión de caracteres morfológicos y palinológicos diagnósticos del género y de importancia taxonómica en la delimitación de especies de *Antiphytum*, pero también de *Ogastemma*: forma de crecimiento; filotaxia; presencia o ausencia de roseta basal o de tricomas glandulares; tirsoides homocládicos o heterocládicos, surgiendo desde la base de la rama o más distalmente; número de ferófilos en cada ramificación dicasial; paraclados opuestos o alternos, formando o no cimas escorpioideas; largo de las cimas escorpioideas; presencia o ausencia de metaxifilos; flores sésiles, subsésiles o pediceladas, bracteadas o ebracteadas; corola rebasando o no el cáliz; tubo de la corola de igual o menor tamaño que los lóbulos de cáliz; presencia o ausencia de fornículos en la garganta de la corola, superficie y color de los fornículos; color de la corola; número de eremocarpos por fruto; fruto envuelto o no por el cáliz acrescente; extensión del cáliz cuando sustenta a los eremocarpos; forma de la ginobase; forma, ornamentación y número de quillas del eremocarpo; quilla ventral formando o no un estípite o una especie de giba; posición de la cicatriz del eremocarpo; tejido de la cicatriz proyectado o no, probablemente un elaiosoma; forma y tamaño de la mónada de polen; ornamentación y características de la exina; número de aberturas en la mónada. Se tomaron medidas de estructuras morfológicas para la descripción taxonómica y se fotografiaron muestras de los eremocarpos de la subfamilia en el Laboratorio de Microscopía y Fotografía de la Biodiversidad (II) del IBUNAM. Fotografías de microscopía electrónica de barrido de algunas muestras de polen procesadas durante el trabajo de laboratorio y algunos eremocarpos fueron tomadas en el Laboratorio de Microscopía Electrónica y Fotografía de la Biodiversidad (I).

-Se obtuvieron hipótesis filogenéticas de *Antiphytum* y géneros hermanos con análisis independientes y concatenados de cinco marcadores moleculares (la región nuclear ITS y cuatro de cloroplasto: *ndh*F–*rpl*32, *trn*L–F, *rps*16 y *mat*K). La elección de los marcadores se basó en la bibliografía que contiene análisis filogenéticos para el orden Boraginales que buscaban resolver la relación desde niveles de género, tribu, subfamilia y orden (Moore y Jansen 2006; Cohen 2014; Gottschling et al. 2014; Nazaire et al. 2014; Weigend et al. 2014; Chacón et al. 2016). Se dio especial atención a buscar marcadores de evolución adecuada al nivel de género, así como a complementar los datos con secuencias depositadas en GenBank**.** El muestreoincluyó al menos un terminal para el género monotípico *Ogastemma* y algunas de las especies de *Echiochilon* (*E. collenettei* I.M.Johnst., *E. fruticosum* Desf., *E. johnstonii* Cufod., *E. kotschyi* (Boiss. & Hohen.) I.M.Johnst., *E. longiflorum* Benth. o *E. persicum* (Burm.f.) I.M.Johnst., dependiendo del marcador y la preservación del terminal en la matriz combinada), y dos o más individuos por especie de *Antiphytum.* De las 13 especies circunscritas, la sudamericana *A. stoechadifolium* no fue incluida en el análisis filogenético, ya que no se pudo extraer ADN del escaso material herborizado disponible. El muestreo por especie se redujo a uno enlos casos donde sólo se pudo amplificar un individuo para el marcador (*mat*K: *A. peninsulare*; *ndh*F–*rpl*32*: A. charruasorum, A. berroi, A. cruciatum, A. geoffreyi* y *A. hintoniorum*). La extracción de ADN y los protocolos de amplificación de las regiones genómicas se realizaron en el Laboratorio de Biología Molecular del Instituto de Biología, UNAM. Los productos de PCR fueron limpiados y secuenciados en LaNABio (IBUNAM).

Dada la interdependencia de los métodos mencionados, el trabajo fue recursivo y se retroalimentó en todo momento. Por ello, los datos moleculares fueron empleados en dos contextos: en la primera parte de reevaluación taxonómica, como otra línea de evidencia con un muestreo dirigido para el reconocimiento de una nueva especie y en la segunda parte, para obtener una hipótesis filogenética de las especies de *Antiphytum* en su contexto dentro de la subfamilia Echiochiloideae. La morfología y datos geográficos y ambientales permitieron la toma de decisiones taxonómicas en la primera parte de la tesis y la discusión de los patrones obtenidos en la segunda. Mayor detalle de los métodos se da en los capítulos correspondientes de la tesis.

# PARTE I: REEVALUACIÓN TAXONÓMICA DE LAS ESPECIES DE *ANTIPHYTUM*

## PARTE A. ESPECIES DE NORTEAMÉRICA

### CAPÍTULO I: Morphological and molecular evidence reveals a new species of *Antiphytum* (Echiochiloideae, Boraginaceae) from Guerrero, Mexico.

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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, gypsovag, 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. hintoniorum* 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 eremocarps (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. hintoniorum* and Mendoza-Díaz et al. (2018) described *A. geoffreyi* N. Mend. & Flores Olv., 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 (GAAGTACGYTTTTTTGGAACTGC; ACATAGGAACTGTWAGTGGAAG), 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 (Epicentre, 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 Olv. & 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-oblanceolate to oblanceolate, 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

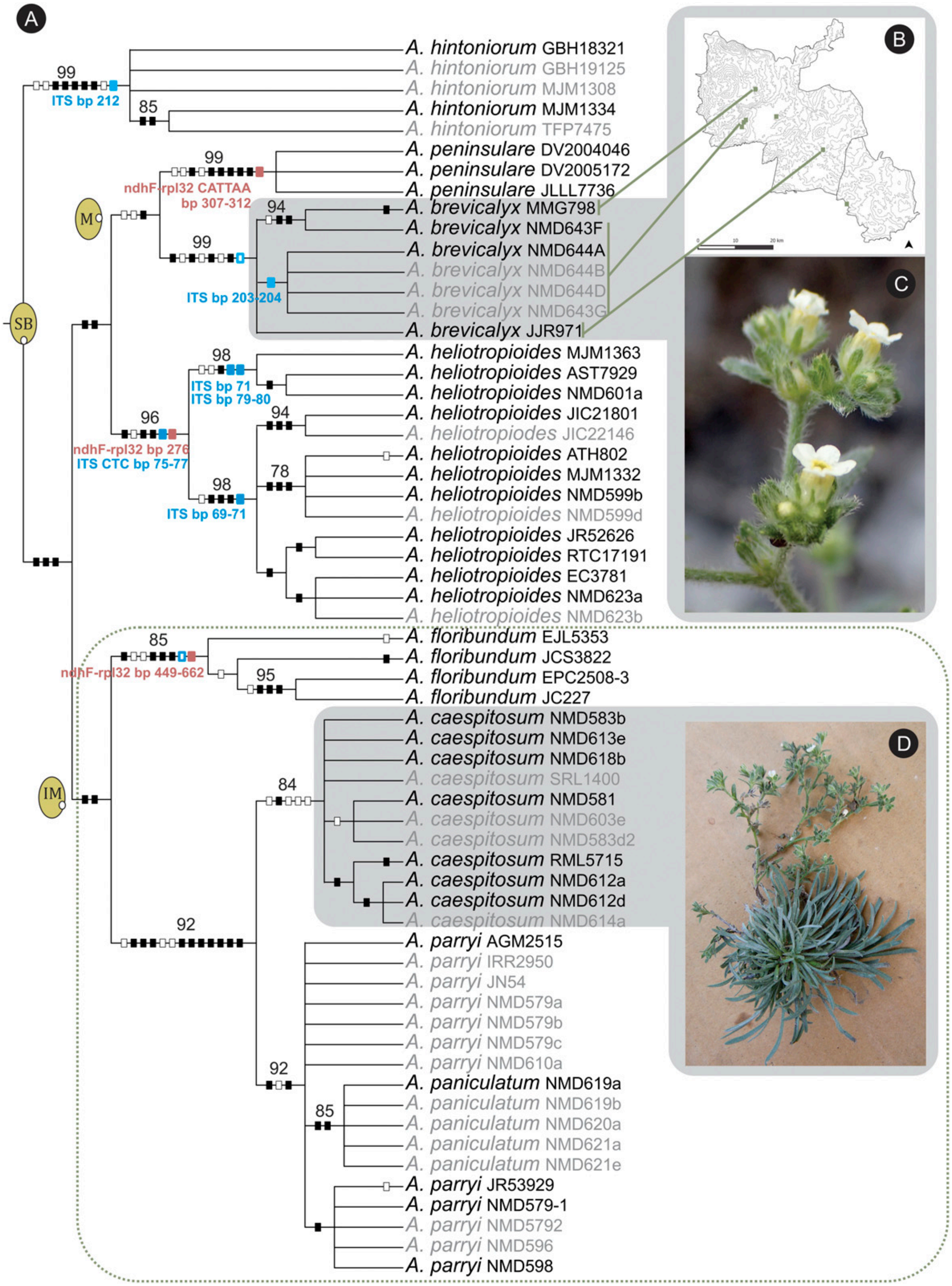

FIG. 1. Combined parsimony tree (L = 172) using ITS and *ndhF-rpl32* sequences, including microstructural DNA characters. A. Single most parsimonious tree highlighting *Antiphytum brevicalyx.* Sequences in gray are identical to the sequence of the first black-colored accession name above. Numbers above 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).

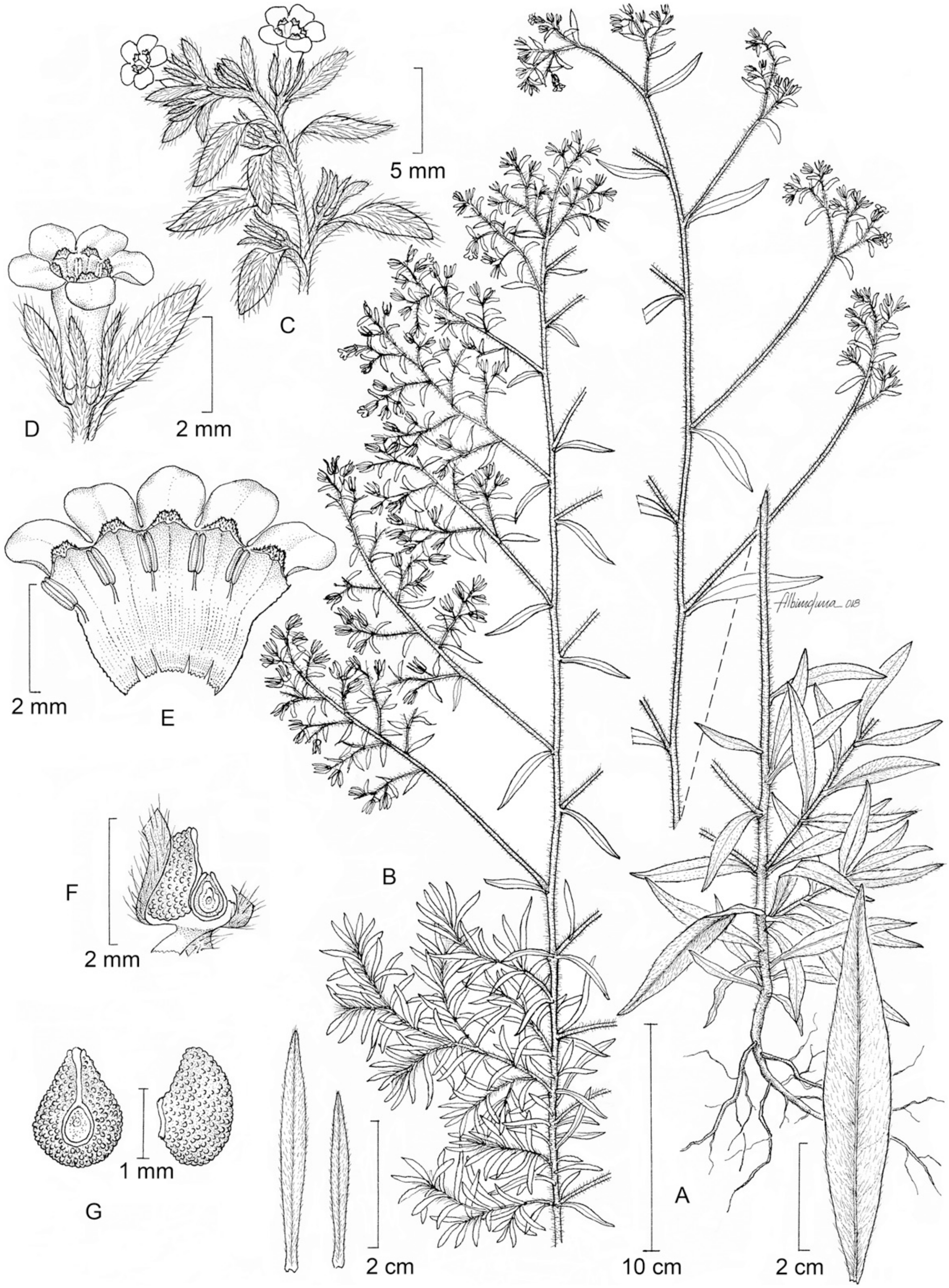

FIG. 2. *Antiphytum brevicalyx*. A. General appearance of a young plant showing detail of the abaxial surface of a basal leaf (from *Mendoza-Díaz et al. 644C*). B. General appearance of an older individual showing detail of the adaxial surface of the cauline leaves (from *Mendoza-Díaz et al. 643A*). 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 7.0–11.0 × 3.2–4.0 mm, gradually smaller upward along the cyme. **Flowers** perfect, sessile or subsessile (pedicel 0.2–0.5 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. Thyrsoid 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. 2.0–2.2 × 0.3–0.4 mm, the bigger one obovate and resembling a floral bract, 2.5 × 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 × (1.0–) 1.2–1.4 mm, abaxially strigose at the middle; stamens

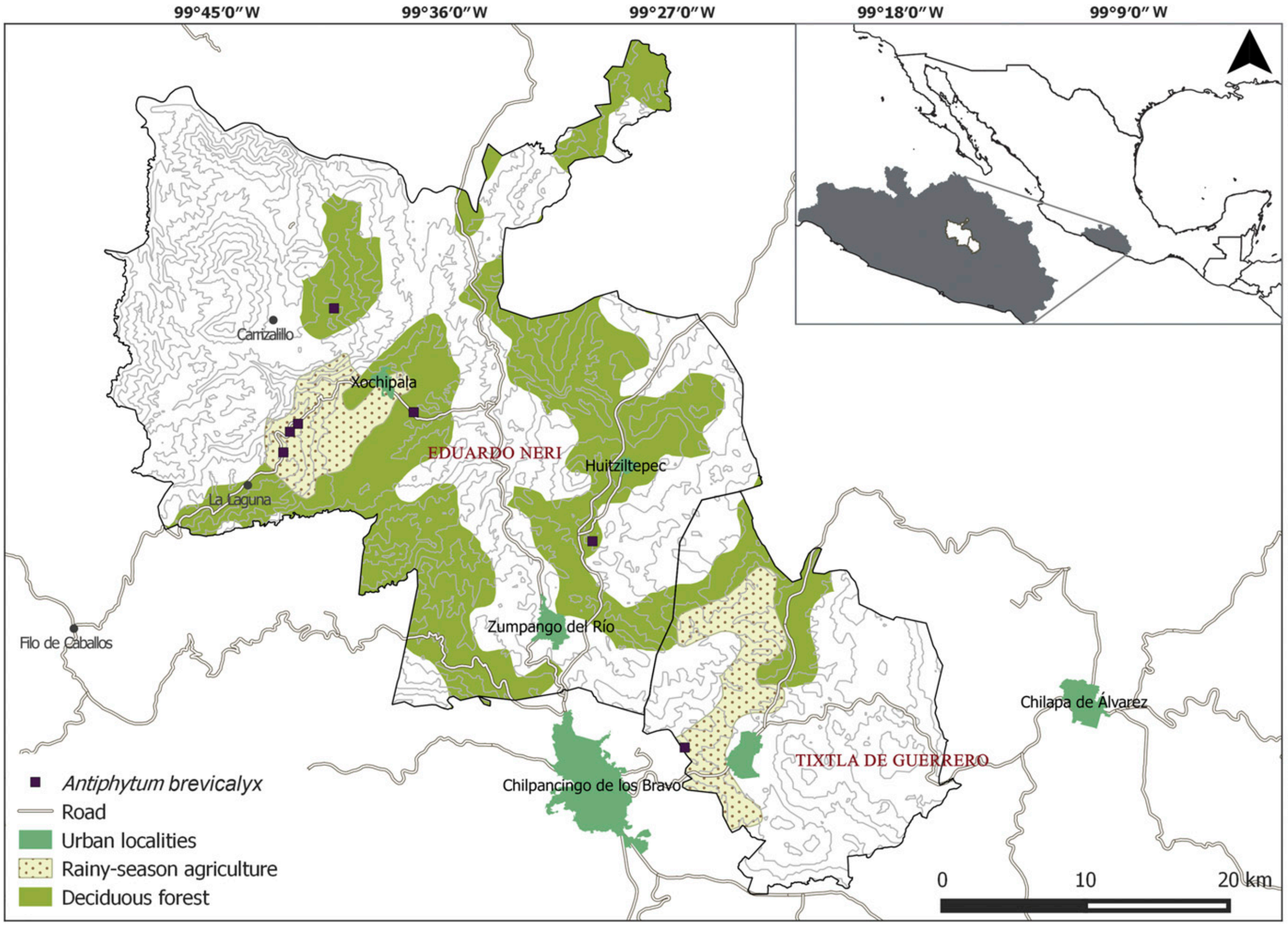

FIG. 4. Distribution map of *A. brevicalyx* within Guerrero, showing the two municipios (Eduardo Neri and Tixtla de Guerrero) in which the species has been recorded, and vegetation types. The inset map shows the locations of the two municipios within Guererro and the location of Guerrero in Mexico.

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.

| | *A. brevicalyx* | *A. floribundum* | *A. heliotropioides* | *A. peninsulare* |
|---|---|---|---|---|
| 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 | **All calyx lobes shorter** | 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 |

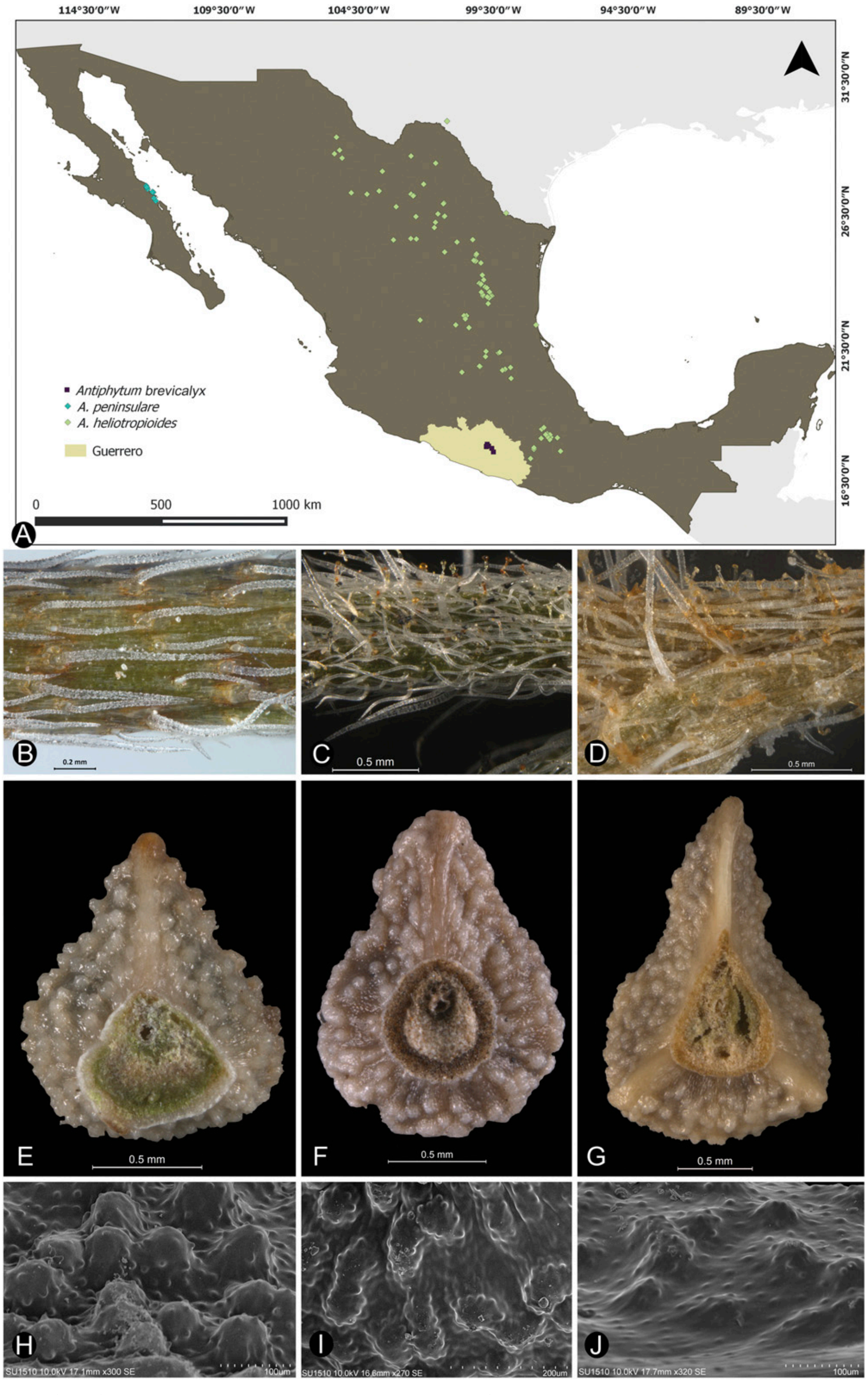

FIG. 5. Comparison of *A. brevicalyx* to the morphologically most similar species of *Antiphytum*. A. Distribution. B–D. Comparison among trichomes; note the capitate-glandular trichomes in C and D. E–G. Eremocarps; note the medial position of the cicatrix in F and G. H–J. Pericarp ornamentation of tubercles 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 municipios 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$^2$ 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. hintoniorum*, 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 eremocarp 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. brevicayx* 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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We thank the directors and curators of the herbaria cited in this paper. We recognize the facilities provided by Martha Juana Martínez at FCME; she and several other colleagues of the Facultad de Ciencias, UNAM, have collected invaluable specimens for the Flora de Guerrero project, which are also important to the knowledge of the Mexican flora in general. The authors thank the following colleagues at IBUNAM: Berenit Mendoza-Garfias for assistance with the scanning microscope, Susana Guzmán-Gómez for help with the stereoscopic microscope, Lidia Cabrera for use of the facilities at Laboratorio de Biología Molecular, IBUNAM, and Laura Márquez and Nelly López at the sequencing service LaNaBio. We especially thank Alejandro Torres-Montúfar and Cecilia Rojas-Martínez for fieldwork help and company, María Aguilar-Morales for support to get one stereoscopic photograph, and Albino Luna for the botanical illustration. The first author is a recipient of a doctoral fellowship (CONACYT grant 288698). We also thank the US National Science Foundation for support (DEB 1054539 to MJM).

## 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 Olv. & 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.**—MICHOACÁN: *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), MN265444, MN313540; *Mendoza-Díaz et al. 599d* (MEXU), MN265445, MN313541; *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.

# PARTE I:
# REEVALUACIÓN TAXONÓMICA DE LAS ESPECIES DE *ANTIPHYTUM*

## PARTE A. ESPECIES DE NORTEAMÉRICA

## CAPÍTULO II: A new and unusual endemic species from the Chihuahuan Desert, Mexico: *Antiphytum geoffreyi* (Boraginaceae, Echiochiloideae).

Nidia Mendoza-Díaz, Hilda Flores-Olvera, Michael G. Simpson & Michael J. Moore

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# A new and unusual endemic species from the Chihuahuan Desert, Mexico: *Antiphytum geoffreyi* (Boraginaceae, Echiochiloideae)

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## Abstract

A new and unusual species of *Antiphytum,* here named *A. geoffreyi*, is described and illustrated. This new species is assigned to the genus *Antiphytum* because it possesses eremocarps directly attached to a pyramidal gynobase, which forms a ventral cicatrix on the eremocarp. However, it is unique within *Antiphytum* in having multiple inflorescence branches arising nearly at the base of the plant, a triangular-pyramidal gynobase, and the proportionally longest eremocarp cicatrix of the genus. Some of these characteristics are reminiscent of *Ogastemma*, the sister genus of *Antiphytum*, making *A. geoffreyi* of potential great significance in the systematics of Boraginaceae subfam. Echiochiloideae. Among species of *Antiphytum, A. geoffreyi* is most similar to *A. peninsulare*, with which it shares a triangular-shaped eremocarp, but it differs from this species in possessing an infra-medial cicatrix and a smaller corolla limb diameter. The new species is known from only two collections in Coahuila, Mexico, both made by the Hinton family, who have discovered many new species in their extensive explorations of the country and provided important specimens to the country's flora.

**Keywords:** endangered species, fruit, gynobase, North America

## Introduction

*Antiphytum* DC. ex Meisner (1840: 280) (Boraginaceae, Echiochiloideae Weigend; see Chacon *et al.* 2016) is an American genus with nine recognised species, following Johnston (1923, 1927) who last revised the genus. *Antiphytum* is characterised by having eremocarps (terminology sensu Hilger 2014; also termed mericarps or nutlets) directly attached to a flat or depressed-pyramidal gynobase, forming a ventral or basal attachment scar on the eremocarp, the scar termed cicatrix by Hilger (2014). Most species are North American, with seven species ranging from the southwestern USA to southern Mexico, but two species are endemic to South America, in Brazil and Uruguay (Johnston 1927).

The most recent species described in the genus is *Antiphytum hintoniorum* Higgins & Turner (1983: 457), which is endemic to gypsum soils in Nuevo Leon, Mexico. This species was discovered by James Hinton and named in honor of the Hinton family, who for three generations have explored the botanical richness of Mexico, leading to the discovery and description of several hundred new species (Hinton & Rzedowski 1972). George B. Hinton, who was the first botanist of the family, concentrated his fieldwork in the states of Guerrero, Michoacan, and Mexico (Rico *et al.* 2008). His son James Hinton collected in the mountains of Nuevo Leon, Coahuila, Michoacan, Guerrero, and Oaxaca (Hinton 2008), and his grandson George S. Hinton has continued the family tradition through his extensive exploration of northern Mexico. Their collections are available at the G. B. Hinton herbarium (GBH).

In this manuscript we describe yet another important taxonomic novelty, *Antiphytum geoffreyi*, collected by George S. Hinton in Coahuila, Mexico. This species represents an important new addition to the flora of the Chihuahuan Desert and, due to its unusual characters, is important for understanding the systematics of the genus and the subfamily. This new species is assigned to the genus *Anthiphytum* by having eremocarps directly attached to a pyramidal gynobase, forming a ventral cicatrix on the eremocarp.

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## Material and Methods

Herbarium specimens (acronyms according to Thiers continuously updated):—The revision of Boraginaceae at GBH revealed two collections of an undescribed species of *Antiphytum* of the state of Coahuila, Mexico. These specimens were collected by George S. Hinton, who provided duplicates as donation to MEXU. Images provided by Hinton from GBH specimens were also studied. To properly describe the characteristic inflorescence of the species, we follow Weberling (1989) and Weigend *et al.* (2016). This terminology is defined as part of the description when necessary.

Fruit observations:—Mature eremocarps and gynobases from specimens of the new species, as well as from the morphologically similar *Antiphytum peninsulare* (Rose) Johnston (1923: 51), and *Ogastemma pusillum* (Coss. & Durieu ex Bonnet & Barrate) Brummit (1982: 680), were observed 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 Laboratorio de Microscopía y Fotografía de la Biodiversidad (II) of Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). Also, SEM photographs were taken (Hitachi SU1510, Hitachi Ltd., Tokyo, Japan) at Laboratorio de Microscopía Electrónica y Fotografía de la Biodiversidad (I). The image files were cleaned in Adobe Photoshop CS5 12.0 (Adobe Systems Incorporated, San Jose, California).

Distribution map:—A distribution map was produced using QGIS Brighton (2.6.1) employing layers from Instituto Nacional de Estadística y Geografía (2017).

## Taxonomy

***Antiphytum geoffreyi*** N. Mend. & Flores Olv., *sp. nov.* (Figs. 1–2).

**Type:**—MEXICO. Coahuila: Municipality Francisco I. Madero, West side of Sierra de Tlahualilo, 1275 m, 26° 36′ 46.8″ N, 103° 21′ 13″ W, 21 April 2015. *Hinton et al. 29613* (Holotype: GBH!, Isotypes: ANSM, CIIDIR, GBH, MEXU!, TEX).

**Diagnosis:**—Differs from all other species of the genus by possessing a terminal inflorescence arising at the bottom of each branch rather than from the distal part, by possessing a triangular-pyramidal rather than a deltate-pyramidal or flat gynobase, and by possessing the proportionally longest eremocarp cicatrix in the genus.

**Suffruticose perennial**, erect (or decumbent?), up to 20 cm tall, covered by strigose and hispid indumenta with a multicellular lithocystic structure, and hyaline glandular-capitate trichomes on branches, leaves, and other leafy organs. **Stems** of numerous branches arising from almost the base of a short main stem, branches alternate or opposite, non-fistulose, slender, with white exfoliating bark on the older parts. **Leaves** cauline and pseudobasal, without a basal rosette, sessile, blades linear-oblanceolate to linear-oblong, margins flat, entire, apices acute, bases truncate connate, surfaces concolorous, veins not visible; cauline leaves opposite, alternate (?) when subtending inflorescences, 8.0-10.0 × 1.0 mm, gradually smaller upward along the stem; pseudobasal leaves at the base of some branches, the newest ones 3.0 × 0.5 mm. **Inflorescence** arising at the bottom of each branch**,** ending in a flower (monotelic) with alternate paraclades, forming a terminal homocladic determinate thyrse (thyrsoid) with dichasial branching, one side a scorpioid cyme and the other side branching many times; first internode of a lateral axis (hypopodium) 4.3–8.2 mm in length; mature scorpioid cymes up to 133 mm in length, many-flowered, bracteate; floral bracts oblanceolate to linear-oblong, sessile, strigose, with glandular capitate-trichomes at margins, veins not visible, blades 6.0–6.5 × 1.7 mm, gradually smaller upward along the cyme. **Flowers** perfect, sessile or subsessile (pedicel ca. 0.7 mm in length); calyx strigose on both surfaces, glandular-capitate and hispid trichomes at margins, accrescent in fruit, divided almost to the base, lobes asymmetric, acute, scarious at the base, three linear-oblong and two oblanceolate, ca. 2.2–3.3 × 0.4–1.2 mm; corolla hypocrateriform (rotate), white, with five papillose faucal appendages of color unknown on the throat opposite to corolla lobes, tube not surpassing the calyx, 1.4–1.5 mm in length, 0.8 mm in diameter at the base, limb 4.7(–5.0) mm in diameter, lobes oriented nearly perpendicular to the tube, imbricate, rounded, sinuate at apex, ca. (2.0–) 2.1–2.4 × (1.9–) 2.0–2.2 mm, abaxially strigose at the middle; stamens inserted, alternate to corolla lobes, adnate at ca. 0.6 mm from the base of the corolla tube; filaments up to 0.4 mm in length, slender; anthers oblong, slightly lanceolate, dorsifixed, ca. 0.6–0.7 × 0.3–0.4(–0.5) mm; ovary with four ovules, style gynobasic, 0.7–0.8 mm in length at anthesis, stigma bilobed to near the apex of the style (subterminal). **Fruit** dry, covered by a persistent calyx, usually with 3–4 developed eremocarps; eremocarps lustrous, triangular and apically acute in ventral view, 1.6–1.7 × 1.3–1.4 mm, dorsally convex, tuberculate and papillate except to the tubercles apex, cicatrix at infra-medial position on the ventral face, triangular, ca. 0.9 mm in length, three keels straight, a ventral (apical) not forming a stipe, and two lateral (basal)

**HERBARIUM of G. B. HINTON** **29613**

Boraginaceae
*Cryptantha mexicana* (Brandegee) I.M. Johnst.

Determined by G.S. Hinton

**HERBARIUM of G. B. HINTON** **No. 29613**

Collected by Hinton et al. 2015.04.21

West side of Sierra de Tlahualilo 1275 m.
Francisco I. Madero, Coahuila, México.
Rocky outcrop on gypsum hill.
Fl. white. 20 cm. Colony.

**FIGURE 1**. Holotype of *Antiphytum geoffreyi sp. nov*. Photo provided by George S. Hinton from the GBH specimen.

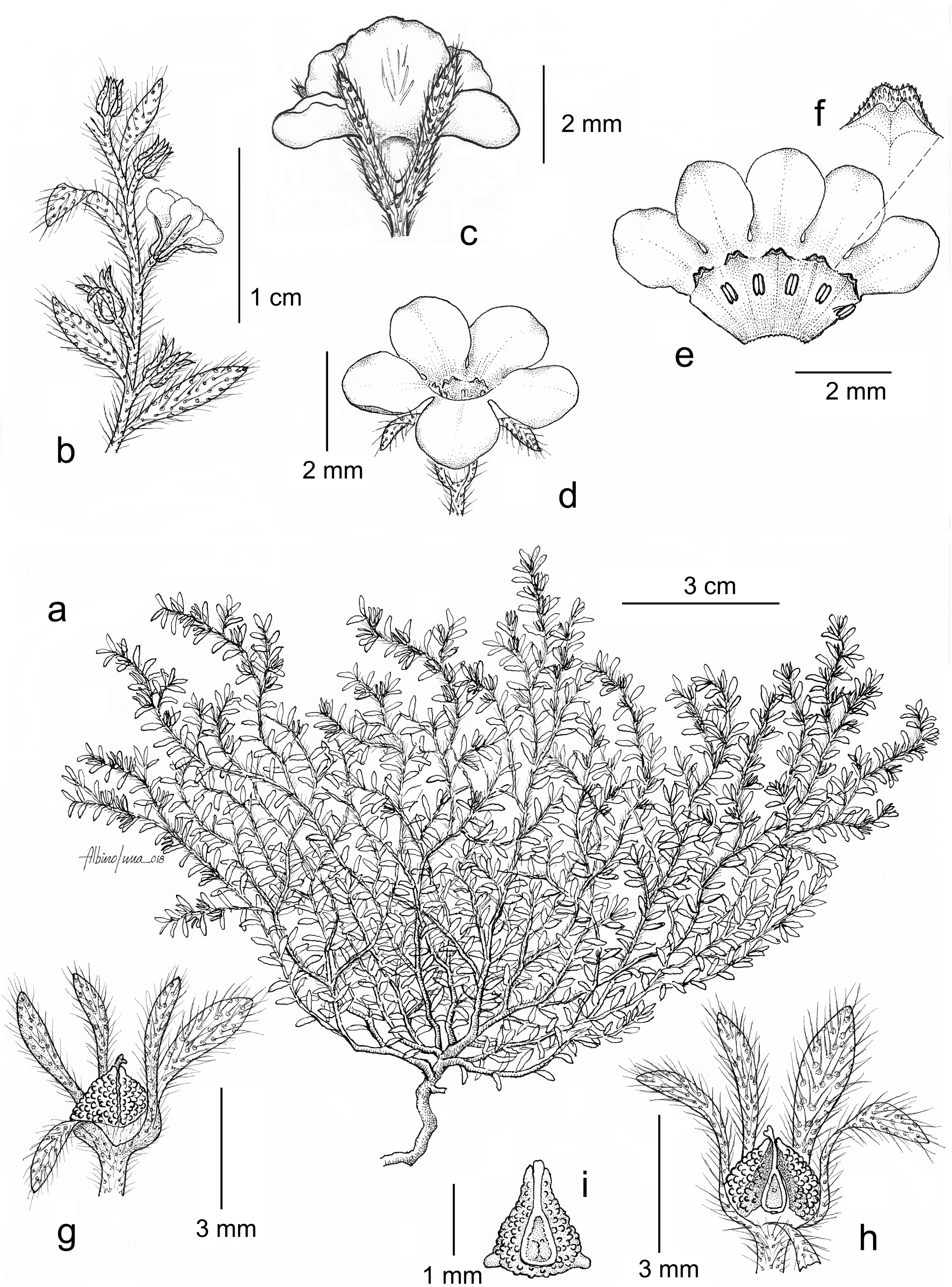

**FIGURE 2**. *Antiphytum geoffreyi*: a, general appearance, b, fragment of the scorpioid cyme, c, flower in lateral view, d, flower showing the corolla limb, e, open flower, f, detailed of faucal appendage, g, fruit, h, fruit showing the gynobase and eremocarp insertion, i, eremocarp. (Illustration by Albino Luna from *Hinton et al. 29613 & 29132* in MEXU and GBH).

from the vertices of the eremocarp to the corresponding cicatrix vertices; gynobase triangular-pyramidal, with four areoles corresponding to the cicatrix of each eremocarp, style plus stigmas 1.8 (–1.9) mm, surpassing the eremocarps.

**Additional specimens examined (paratypes):—**MEXICO. Coahuila: Municipality Parras, South of Parras, 1326 m, 25° 18′15.4″ N, 102° 13′ 42.5″ W, 30 April 2010. *Hinton et al. 29132* (ANSM, CIIDIR, GBH!, MEXU!, TEX).

**Additional specimens examined (other species):—*Antiphytum cruciatum*** (Chamisso) DC. in Candolle & Candolle (1846: 121). BRASIL. Rio Grande do Sul, Porto Alegre: Belem novo (Hindenburgshöhe) near Porto Alegre, November 1926. *Jüngers 254* (B!, GH!). Itapoan, Granja Neugebauer p. Itapoan, 11 October 1950. *Rambo 48956* (B!, US!). Sao Sepe, 02 October 1988. *Wasum et al. 4538* (MO!, NY!). URUGUAY. Cerro Largo: Río Negro y Palleros, December 1937. *Rosengurtt 2346* (GH!). ***Antiphytum floribundum*** (Torrey) Gray (1874:55)**.** MEXICO. Coahuila: Municipality Ocampo, Sierra de Hechiceros, vicinity of Rancho El Tule, near Chihuahuan boundary, 17–19 September 1940. *Johnston & Muller 1330* (GH!). ***Antiphytum heliotropioides*** A. DC. in Candolle & Candolle (1846: 122). MEXICO. Coahuila: Municipality Muzquiz, ca. 130 road km NW of Muzquiz on Coa Hwy 2A (Muzquiz-Boquillas), then SW of 2A on tunnel road to La Encantada basin, Sa. Buenavista of Sa. La Encantada, 1650 m, 28° 34′ N, 102° 30′ W, 03 June 1992. *Nesom & Mayfield 7421* (MEXU!). Municipality General Cepeda, in upper reaches of Canada el Aguirreno, from 0–1 km W of old Barita mine, ca. 4–5 air km from mouth of canyon, ca. 11 air km SE of Las Coloradas, on NW side of Sierra La Paila; Las Coloradas 1:50 000, 26° 06′15″ N, 101° 35′ 30″ W, 05 April 1990. *Bridges et al. 13074* (MEXU!). Municipality Arteaga, El Chorro, 1800 m, July–August 1942. *Lyonnet 3681* (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!). Municipality Mulege, N side of river, botton of sandy/gravelly arroyo, 5 m, 26.8998 N, 111.9762 W, 18 January 2005. *Valov 2005017* (MEXU!). Municipality Mulege, N side of river; bottom of sandy/gravelly arroyo, 5 m, 26° 59.990′ N, 111° 58.570′ W, 04 February 2004. *Valov 2004046* (HCIB!). Municipality Santa Rosalia, along the wash emptying at Punta El Aterrizaje, just N of airstrip, ca. 5.6 km N of Santa Rosalia, along gravel road turning N from Mex Hwy 1, 5 m, 27° 23.6′ N, 112° 18.2′ W, 28 February 1995. *Porter & Machen 11056* (HCIB!). Municipality Santa Rosalia, Isla San Marcos, 15 m, 27° 14′ N, 112° 06′ W, 27 October 1995. *León de la Luz 7736* (HCIB!). ***Ogastemma pusillum***. KUWAIT. Wadi Al-Batin, 12 km N Al-Salmi border station with Saudi Arabia, 280 m, 17 April 1990. *Boulos & Cope 17614* (K!). Shu´aib Al-Batin, 25 km E Al-Shagayah, 280 m, 17 April 1990. *Boulos & Cope 17685* (BM!, K!). Medairah halophytes project north of city in National Park, 05 March 1990. *Hepper 8000* (K!). 35 km north-east of Jahra along Jahra-Al Subbiyah road, 29° 36′ N, 47° 56′ E, 30 March 1995. *Mathew 2633* (K!). SAUDI ARABIA. 27-48 N 47-18 E, 27 February 1970. *Mandaville Jr. 2720* (BM!).

**Additional specimens examined (type specimens):—*Antiphytum***. Type: *Anchusa cruciata* Chamisso (1829:438) (designated by Johnston 1923):—BRASIL. Brasilia Meridionalis, *Sellow s/n* (UC https://plants.jstor.org/stable/10.5555/al.ap.specimen.uc493522). ***Antiphytum peninsulare***. Type:—MEXICO. Baja California Sur, Santa Rosalia, 23 February to 3 March 1889. *Palmer 203* (GH!). ***Ogastemma pusillum***. Type:—ALGERIA. Biskra, sur les collines incultes, 10 May 1853, *Balansa 1035* (E https://plants.jstor.org/stable/10.5555/al.ap.specimen.e00115613).

**Etymology:—**The epithet honours Geoffrey Hinton, who accompanied his father George S. Hinton in the discovery of the new species in Sierra Tlahualilo, Coahuila.

**Phenology:—**Flowers appear at the beginning of April, perhaps earlier, with fruits appearing at the end of April. Whether the species can also flower in the late summer during the rainy season is unknown.

**Distribution and habitat:—***Antiphytum geoffreyi* is known from only two collections by George S. Hinton, in disjunct localities in the municipalities of Francisco I. Madero in the Sierra Tlahualilo, and Parras, Coahuila (Fig. 3). It occurs at elevations of 1,275 m and 1,326 m, in a steep rockslide and a rocky outcrop on a gypsum hill. This new species is a gypsovag (signifying that it grows on and off gypsum), as are many other North American species of the genus, such as *A. caespitosum* Johnston (1923: 52), *A. heliotropioides*, *A. parryi* Watson (1883: 122), and *A. peninsulare*. So far as is known, *A. geoffreyi* is endemic to Coahuila and hence represents the third known *Antiphytum* in the state, along with *A. heliotropioides* and *A. floribundum*, the latter only known in Coahuila from one collection (*Johnston & Muller 1330*, GH!) close to the border with Chihuahua.

**Conservation status—**The new species is known only from two disjunct subpopulations in the state of Coahuila, Mexico (Fig. 3) and the estimated area of occupancy is geographically limited to less than 500 km$^2$. According to the IUCN (2012), it meets the criteria under B2ab(iii) for the category of Endangered (EN).

**Discussion:—**A number of unusual traits characterize *Antiphytum geoffreyi,* most prominently involving the fruit. Fruit characters are useful for the classification and taxonomy of Boraginaceae (Weigend *et al.* 2016). When the eremocarps are released, the part of the fruit that remains on the plant is termed a gynobase (Hilger 2014), which in *Antiphytum* is either flat or depressed-pyramidal (Johnston 1927). Within the genus, the length/width ratio of the gynobase can be classified as deltate (1:1) to shallowly triangular (1:2) to totally flat (*pers. obs.*). The shape of the gynobase is linked with the eremocarp orientation (Weigend *et al.* 2016), and is therefore also associated with the position and shape of the eremocarp cicatrix. *Antiphytum geoffreyi* possesses a triangular-pyramidal gynobase (2:1; see Table 1), which represents a new gynobase shape for the genus (Fig. 4). The new species also possesses the longest cicatrix, relative to the eremocarp (cicatrix length/ventral eremocarp face length) in the genus (Table 1). The unusual gynobase shape in *A. geoffreyi* is closer to the almost narrowly triangular shape present in the sister taxon of *Antiphytum,* the monotypic *Ogastemma pusillum* (Längstrom & Oxelman 2003, Cohen 2014), which has a relatively long triangular cicatrix (Fig. 4) that extends from the middle to the infrabasal part of the eremocarp (Table 1).

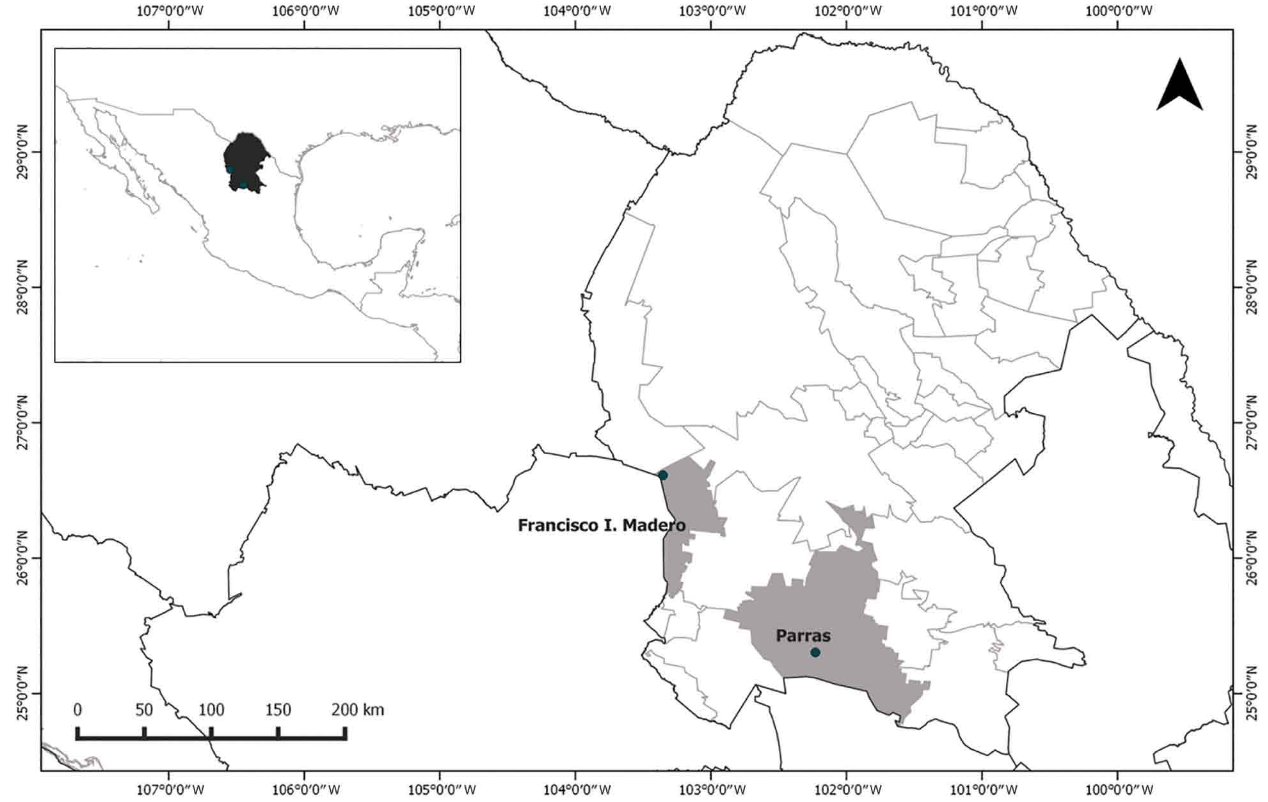

**FIGURE 3.** Map of the known distribution of *Antiphytum geoffreyi*. The areas shaded in grey indicate the municipalities where specimens have been collected, dots the location of the two individual collections.

The inflorescences of *Antiphytum geoffreyi* are also similar to those of *Ogastemma.* Specifically, *A. geoffreyi* is the only species in the genus with multiple branches bearing inflorescences that arise nearly at the base of the plant, similar to *Ogastemma pusillum.* Despite the similarities of *A. geoffreyi* with *Ogastemma pusillum*, we consider the new species to be part of *Antiphytum* due to shared floral characters, such as the calyx exceeding the corolla in *Ogastemma* compared to the reverse in *Antiphytum*. Other species of *Antiphytum*, such as *A. heliotropioides*, *A. hintoniorum,* and *A. peninsulare*, have principal stems branching multiple times with inflorescences distal from the base. As with *A. geoffreyi,* all of these species lack leaves arranged in basal rosettes. Those species with basal rosettes, such as *A. caespitosum,* have distal inflorescences bearing well-defined unbranching flowering stems.

Within *Antiphytum*, *A. geoffreyi* is most similar to *A. peninsulare*. Both species share white colouration on old branches, the presence of capitate glandular trichomes, a triangular-shaped eremocarp and similar pericarp ornamentation (Fig. 5). Nevertheless, *Antiphytum geoffreyi* differs in several key characters from *A. peninsulare*, including the basal (vs. distal) position of inflorescences, a corolla limb diameter of 4.7–5.0 mm (vs. 10.0–12.0 mm), and an infra-medial (vs. medial) and relatively long eremocarp cicatrix.

The unusual characters of *Antiphytum geoffreyi* and the similarity of some of its characters to *Ogastemma* make it a potentially important species for the systematics of *Antiphytum* and the subfamily Echiochiloideae as a whole. Future molecular phylogenetic studies may elucidate its position in the complex. This distinctive new species is also an important addition to the Chihuahuan Desert flora, a region still in need of exploration as shown by the collections of the Hinton family.

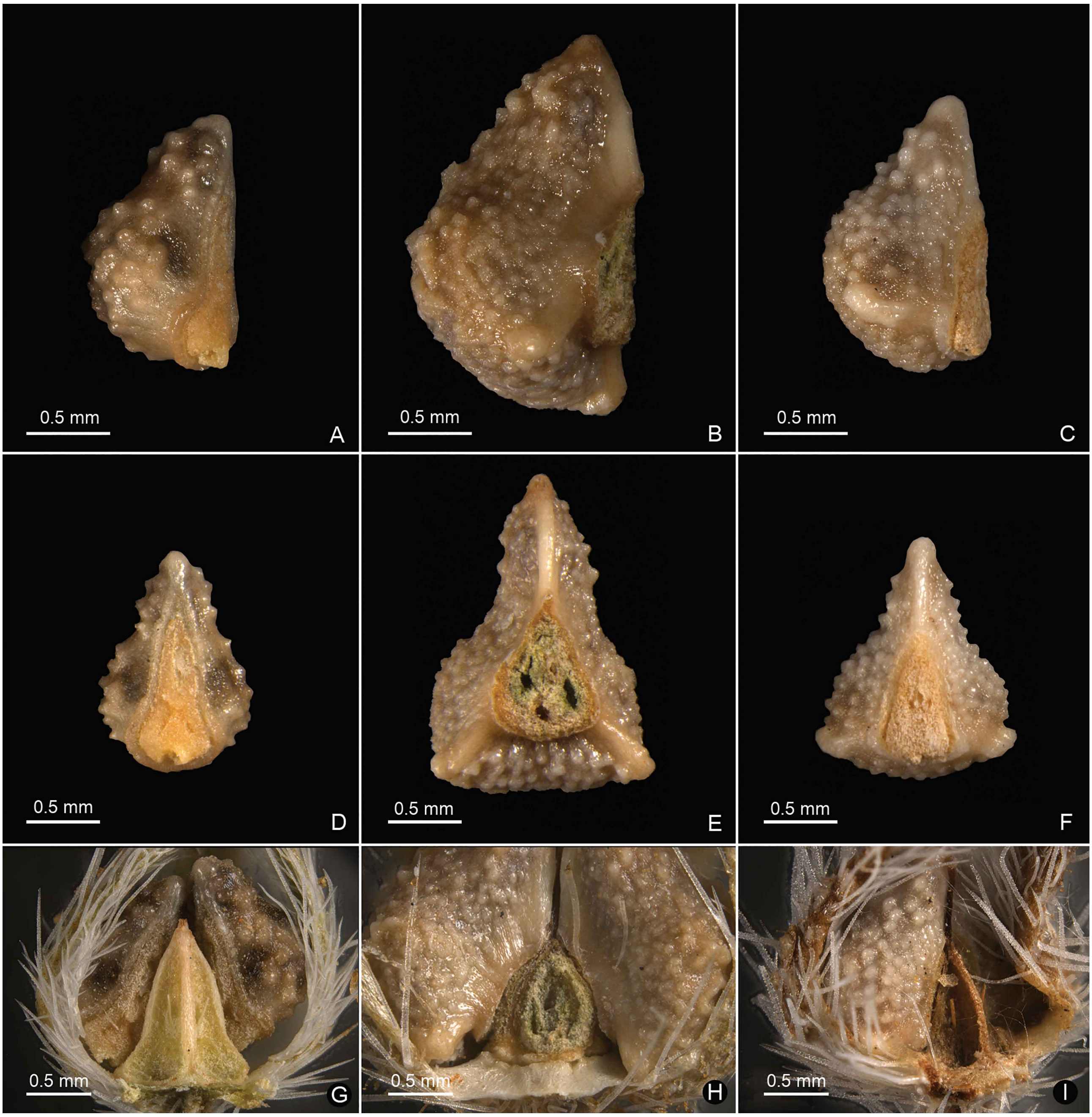

**FIGURE 4.** Comparison of eremocarps in oblique (A–C) and ventral views (D–F), with their respective gynobases (G–I). A, D & G, *Ogastemma pusillum* (*Boulos &. Cope 17614*, K), B, E & H, *Antiphytum peninsulare* (*Wiggins 17328*, MEXU), C, F & I, *A. geoffreyi* (*Hinton et al. 29613*, MEXU).

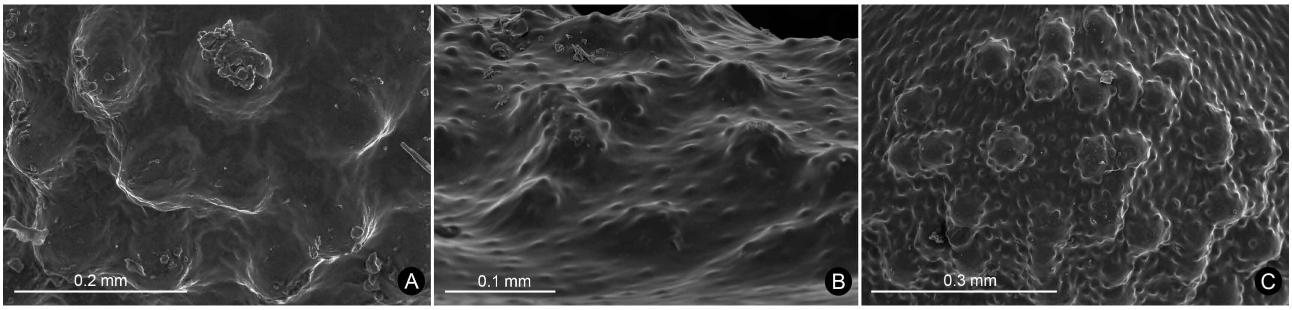

**FIGURE 5.** Pericarp ornamentation. A, *Ogastemma pusillum* (*Boulos &. Cope 17614*, K), B, *Antiphytum peninsulare* (*Wiggins 17328*, MEXU), C, *A. geoffreyi* (*Hinton et al. 29613*, MEXU). Note larger tubercles and smaller papillae in B and C.

**TABLE 1.** Differences among *Antiphytum geoffreyi* and morphologically similar taxa.

| | *Antiphytum geoffreyi* | *A. peninsulare* | *Ogastemma pusillum* |
|---|---|---|---|
| Corolla diameter (mm) | 4.7–5.0 | 10.0–12.0 | 2.0 |
| Eremocarp cicatrix position | infra-medial | medial | extending from the middle to the infrabasal part |
| Gynobase (length: width) | triangular (2:1) | deltate (1:1) | almost narrowly triangular (5:2) |
| Ratio of cicatrix length/eremocarp ventral face length | 50% | 30–40% | 70% |

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https://doi.org/10.1007/978-3-319-28534-4_5

# PARTE I: REEVALUACIÓN TAXONÓMICA DE LAS ESPECIES DE *ANTIPHYTUM*

## PARTE B. ESPECIES DE SUDAMÉRICA

## CAPÍTULO III: White flowers in South America: a new species of *Antiphytum* (Echiochiloideae, Boraginaceae)

Nidia Mendoza-Díaz, Marina Díaz, Patricia Brussa, Fabián Muñoz, José M. Bonifacino & Hilda Flores-Olvera

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# White Flowers in South America: A New Species of *Antiphytum* (Echiochiloideae, Boraginaceae)

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***Abstract*—*Antiphytum charruasorum***, a new endemic species from western Uruguay, is described. This is the only South American species in the genus with white corolla and an infra-medial cicatrix on the ventral face of the eremocarp, both features shared with the North American species of *Antiphytum*, in sharp contrast with the blue corolla and the basal cicatrix at the end of a stipe-like prolongation that characterizes all South American species in the genus. *Antiphytum charruasorum* increases the diversity of the genus in South America, as well as the endemism and distribution range into Uruguay. Moreover, this new species adds novel features in *Antiphytum* such as the irregularly dichasial inflorescences, the hirsute faucal appendages, the eremocarps with a tissue plug from the gynobase, and the habitat where the species has been reported.

***Keywords*—**Endemism, grasslands, North America, Uruguay.

*Antiphytum* DC. ex Meisn. (Boraginaceae, Echiochiloideae Weigend; see Chacón et al. 2016) is an American disjunct genus with 11 species, nine in North America, two of them recently described to Mexico (Mendoza-Díaz et al. 2018, 2020), and two endemic to South America (Johnston 1927). The known South American species of *Antiphytum* are subshrubs restricted to Uruguay and adjacent Brazil (Johnston 1927; Mendoza-Díaz 2015), which differ from their North American relatives by having blue corollas, opposite leaves throughout, and flat gynobases forming a basal cicatrix (attachment scar) on the nutlets (eremocarps sensu Hilger 2014) that are basally attached by a short stipe-like prolongation to the gynobase (Johnston 1923). In contrast, the North American species have white corollas, mostly alternate leaves, and eremocarps directly attached to a short-pyramidal gynobase that leaves a medial to suprabasal cicatrix on the eremocarp ventral face (Johnston 1923; Mendoza-Díaz 2015).

During fieldwork carried out in Paysandú Department in western Uruguay we discovered a hitherto unknown species of *Antiphytum.* Our objective is to describe this as a new species for which we propose *Antiphytum charruasorum*; we present illustrations and offer comments on its morphology and on the geographically and morphologically comparable species within the genus.

## Materials and Methods

***Plant Material and Fruit Observation*—**We discovered the new species in December 2016 in El Pucará plantation forest farm, in Uruguay´s Paysandú Department, as part of the diversity evaluation ran by the UPM Forestal Oriental company. Additional collections were later obtained in 2017 and 2018 in the same known locality in order to have a broader sampling of the morphological variation. During 2019 we discovered a new population in Los Cobres farm, Río Negro Department, Uruguay. We obtained herbarium specimens and silica gel dried material for phylogenetic studies (Mendoza-Díaz in preparation).

Mature and immature fruits of the new species and of morphologically related species were studied with a confocal stereo microscope (Leica Z16 APO A, Leica Microsystems GmbH, Wetzlar, Germany), and photographed with an attached camera (Leica DFC490, Leica Microsystems GmbH) at the Laboratorio de Microscopía y Fotografía de la Biodiversidad (II) of the Instituto de Biología of Universidad Nacional Autónoma de México (IBUNAM). Fruit samples were also mounted on stubs with common double stick carbon tape, and coated with gold to 20 μÅ for two minutes in a sputter coater (Quorum Q150OR ES, Quorum Technologies Ltd, East Sussex, UK) to be studied with a scanning electron microscope (Hitachi SU1510, Hitachi Ltd., Tokyo, Japan) at the Laboratorio de Microscopía Electrónica y Fotografía de la Biodiversidad (I) of IBUNAM.

The new species was compared to the known species of the genus. We are using morphological operational criteria for species delimitation. The taxonomic hypothesis is corroborated using geographical, morphological, and ecological data that "break out" of the taxonomic circle (DeSalle et al. 2005).

***Distribution Map*—**A distribution map was produced using QGIS Brighton (2.6.1) employing layers from Agencia de Gobierno Electrónico y Sociedad de la Información y del Conocimiento (AGESIC 2019), Dirección Nacional de Medio Ambiente (DINAMA 2019), Dirección General de Recursos Naturales (DGRN 2019), Infraestructura de Datos Espaciales del Ministerio de Transporte y Obras Públicas (IDE MTOP 2019), Instituto Nacional de Estadísticas (INE 2011), and the collections coordinates.

## Taxonomic Treatment

***Antiphytum charruasorum*** N.Mend. & Marina Díaz, sp. nov. Type: Uruguay. Paysandú: Municipio Quebracho, UPM Forestal Oriental "El Pucará" plantation forest farm, 50 m, 31°57′02.08″S, 57°54′48.06″W, 15 November 2017, *F. Muñoz* with *P. Brussa, C. Brussa* and *M. Díaz* (Holotype: MVJB! MVJB30748; Isotype: MVJB! MVJB30749).

*Antiphytum charruasorum* is unique in the genus by irregularly dichasial inflorescences, by possessing hirsute-pubescent corolla faucal appendages, and the eremocarp is detached with a tissue plug from the gynobase, leaving a hole in the areola after detachment.

**Suffrutex** erect, 15–18(–20) cm tall, stems (mainly 3–4) arising from a subterraneous stem, branches opposite, solid (non-fistulose), slender (ca. 1.5 mm diameter), later exfoliating,

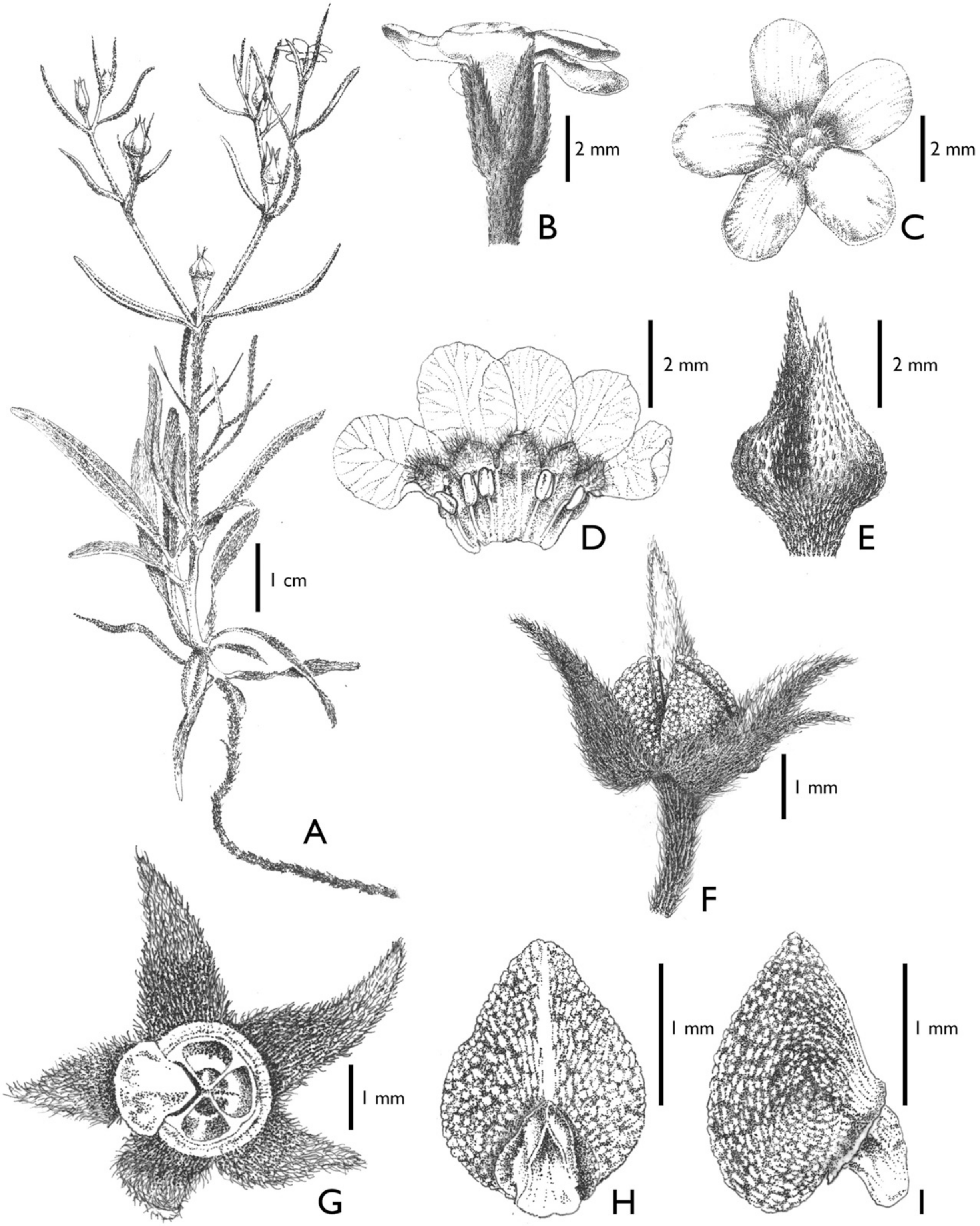

FIG. 1. *Antiphytum charruasorum*. A. General appearance. B. Lateral view of flower. C. Superior view of flower. D. Open corolla. E. Lateral view of immature fruit. F. Lateral view of fruit. G. Mature fruit with several eremocarps removed. H. Eremocarp in ventral view. I. Eremocarp in lateral view. Drawing by Javier Lage.

densely strigose, each trichome with several mineralized foot cells (lithocystic structure), eglandular. **Leaves** basal, pseudobasal, and cauline, decussate, sessile, blades linear to narrowly elliptic, bases truncate, connate-sheathing, apex acute, mucronate, margins flat, entire, surfaces strigose concolorous, midvein not visible on the adaxial side, visible and raised on the abaxial side, secondary veins not visible; basal leaves marcescent, not forming a rosette, up to 40.0 × 2.0–2.8 mm; cauline leaves 18.0–38.0 × 1.0–2.0 mm, gradually smaller towards the apex; pseudobasal leaves at the beginning of each

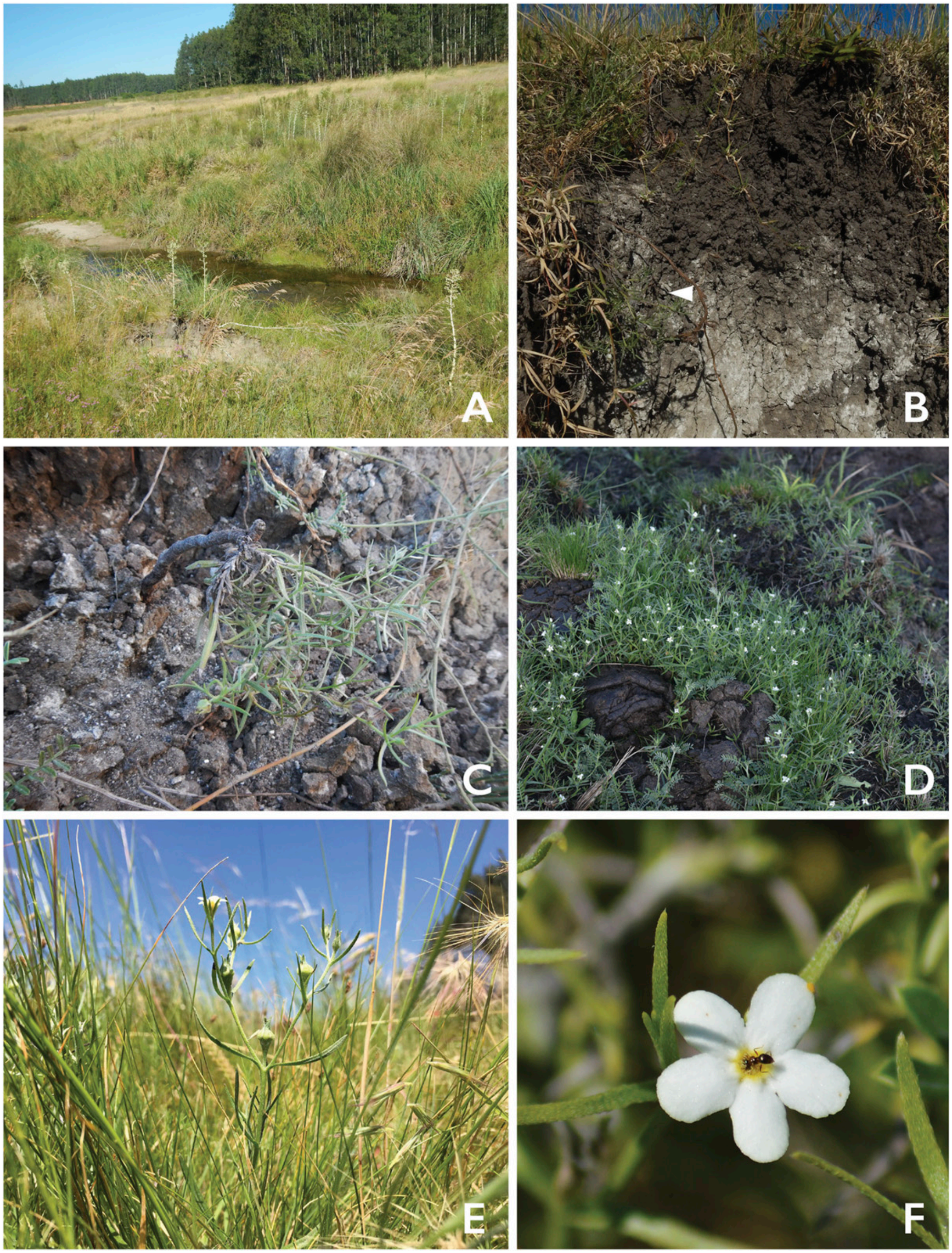

FIG. 2. *Antiphytum charruasorum* in the field (*Brussa et al. MVJB30747*; *Muñoz et al. MVJB30748*; *Muñoz and Díaz MVJB31567, MVJB31669*; *Mendoza-Díaz 649 et al.*). A. Habitat. B. Soil detail; individual of *A. charruasorum* near a water body marked with a white arrow. C. Individual showing the subterranean stem. D. View from above of the general appearance. E. Portion of the inflorescence bearing flowers and immature fruits. F. Flower. Photos A, B, C by Nidia Mendoza-Díaz; E by Patricia Brussa; D, F by Fabián Muñoz.

branch, the youngest ones ca. 5.0 × 0.5 mm. **Inflorescences** ending in a flower (monotelic) subtending by opposite paraclades, forming a terminal heterocladic determinate thyrse (thyrsoid) with dichasial branching, in some cases twice dichasial, and sometimes one side as a new branch with dichasial branching at the distal nodes, first internode of a lateral axis 14.0–15.0 mm in length; bracts subtending each terminal dichasial flower 2, 23.5–33.0 × 1.7–2.0 mm, gradually smaller upward along the cyme, linear to narrowly elliptical, sessile, apex acute, nearly mucronate, strigose. **Flowers** perfect, pedicel (–1.5)2.0–3.5 mm in length; calyx strigose on both sides, with scattered hispid trichomes on abaxial surface and numerous hispid trichomes adaxially, accrescent, divided almost to the base, lobes 3.0–4.0 × 1.2–1.4 mm, triangular, narrowly asymmetric, acute, all longer or equal than the corolla tube at anthesis, up to 5.6 mm in length in fruit; corolla hypocrateriform (rotate), white, with five yellow hirsute-pubescent faucal appendages on the throat in the sinuses of the corolla lobes, tube 2.7–3.0 mm in length, 1.0 mm diameter at the base, not surpassing the calyx, yellow, limb 7.5–8.0 mm in diameter, lobes 2.5–3.0 × 2.5 mm oriented perpendicular to the tube, imbricate, oblong, rounded sinuate at apex, abaxially strigose at the middle; stamens included, alternate to the corolla lobes, adnate at ca. 1.8 mm from the base of the corolla tube; filaments up to 0.5 mm in length, slender; anthers oblong, sub-lanceolate, dorsifixed, ca. 0.8 mm long; style gynobasic, 1.5–2.0 mm in length at anthesis, stigma bilobed at the apex of the style (terminal), included, ovules 4. **Fruit** dry, subtending by a spreading calyx, 4 mature eremocarps; eremocarps 2.2–2.5 × 1.8–2.0 mm, ovate and apically acute-acuminate in ventral view, dorsally convex, lustrous, striate-tuberculate and papillate under the external layer; cicatrix ca. 1.0 mm long, at infra-medial position on the ventral face, ovate to triangular, with a tissue projection without pericarp of ca. 1.0 mm in length resembling a stipe; one ventral keel (apical) straight from the apex of the eremocarp to the cicatrix apex, not forming a stipe, lateral (basal) keels absent; gynobase very shallowly triangular (ca. 1:3), with four big and sunken areoles corresponding to the cicatrix projection of each eremocarp, style plus stigmas 1.8–2.0 mm long, not surpassing the eremocarps. Figures 1, 2.

***Etymology***—The specific epithet honors the Charrúas, an indigenous community of Uruguay that inhabited the land before Spanish colonization. Nowadays, *Charrúas* is used as an adjective that defines Uruguayans' spirit of courage, fight, and persistence.

***Phenology***—Flowers and fruits were observed in October, November, and December, although probably the flowering period starts earlier.

***Distribution and Habitat***—*Antiphytum charruasorum* is only known from two disjunct localities from the West Sedimentary Basin ecoregion (Brazeiro et al. 2015) in Uruguay, in the Paysandú and Río Negro Departments at less than 50 km east of Río Uruguay (Fig. 3). Populations are very small and individuals occur in sparse grasslands in well drained calcareous soils (José F. Ramos, UPM senior soil specialist, pers. comm.; División de Suelos y Aguas 2002). Both locations have soils developing over sedimentary parent rock deposited during a desertic continental environment from Mercedes Formation (Bossi 1966; Bossi et al. 1975).

The first population was found in El Pucará plantation forest farm, Paysandú Department, municipio Quebracho, in grasslands over flood plains together with *Eragrostis lugens* Nees, *Bouteloua megapotamica* (Spreng.) Kuntze, and *Adesmia incana* var. *grisea* (Hook. f.) Burkart (Fig. 2). The new species occurs at elevations of 50 m amid grasses, forming clusters of scattered individuals, in a narrow strip of land of 10 m wide, along small slopes. The second population occurs in the Río Negro Department, Los Cobres farm, near El Ombú, 100 km south from the first locality, on rocky hillsides with steep slopes (> 6%) over calcareous sandstone (División de Suelos y Aguas 2002). This second population extends towards a

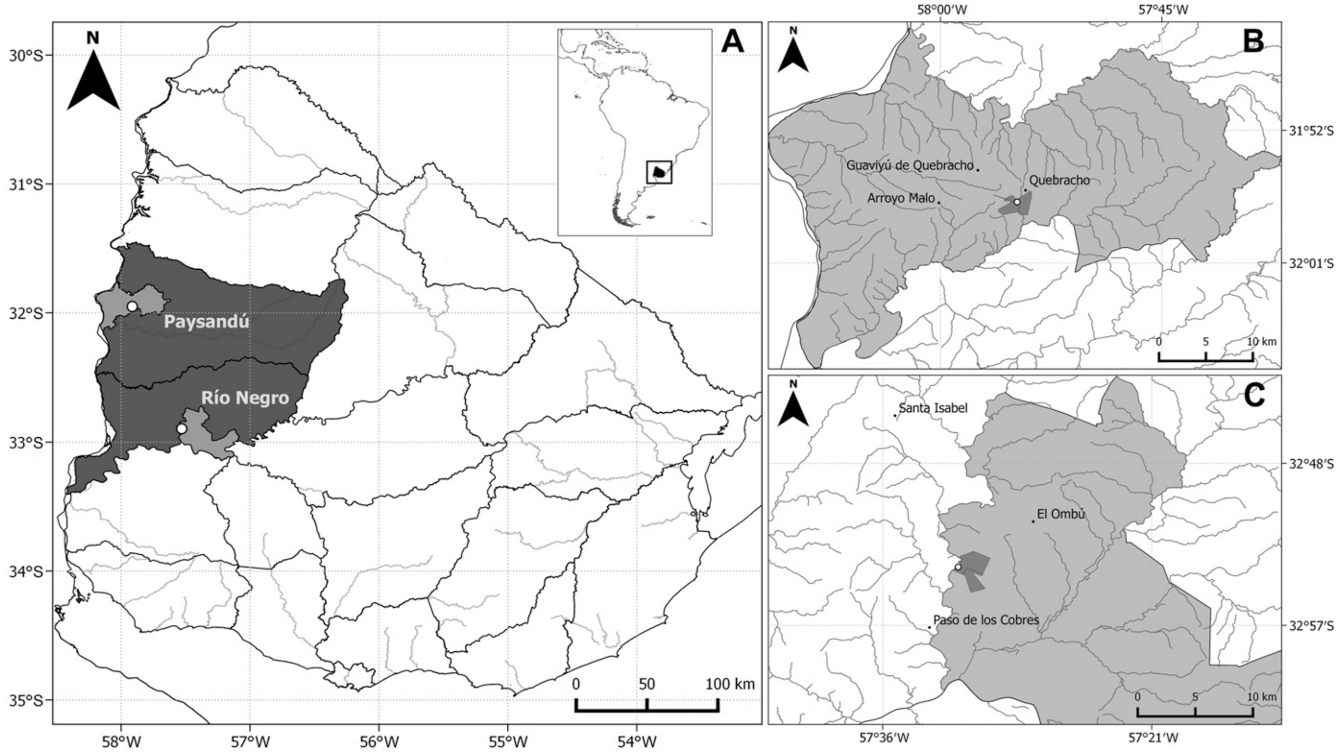

FIG. 3. Distribution map of *A. charruasorum* (white dots) in Uruguay. A. Paysandú and Río Negro Departments where the species occurs; note in light gray the respective municipality and land section of the collections. B. Municipio Quebracho in Paysandú Department; in darker gray the plantation forest farm El Pucará is highlighted. C. Part of the 11va Seccional Policial El Águila in Río Negro Department in light gray; in darker gray the farm Los Cobres where the collection was done is highlighted.

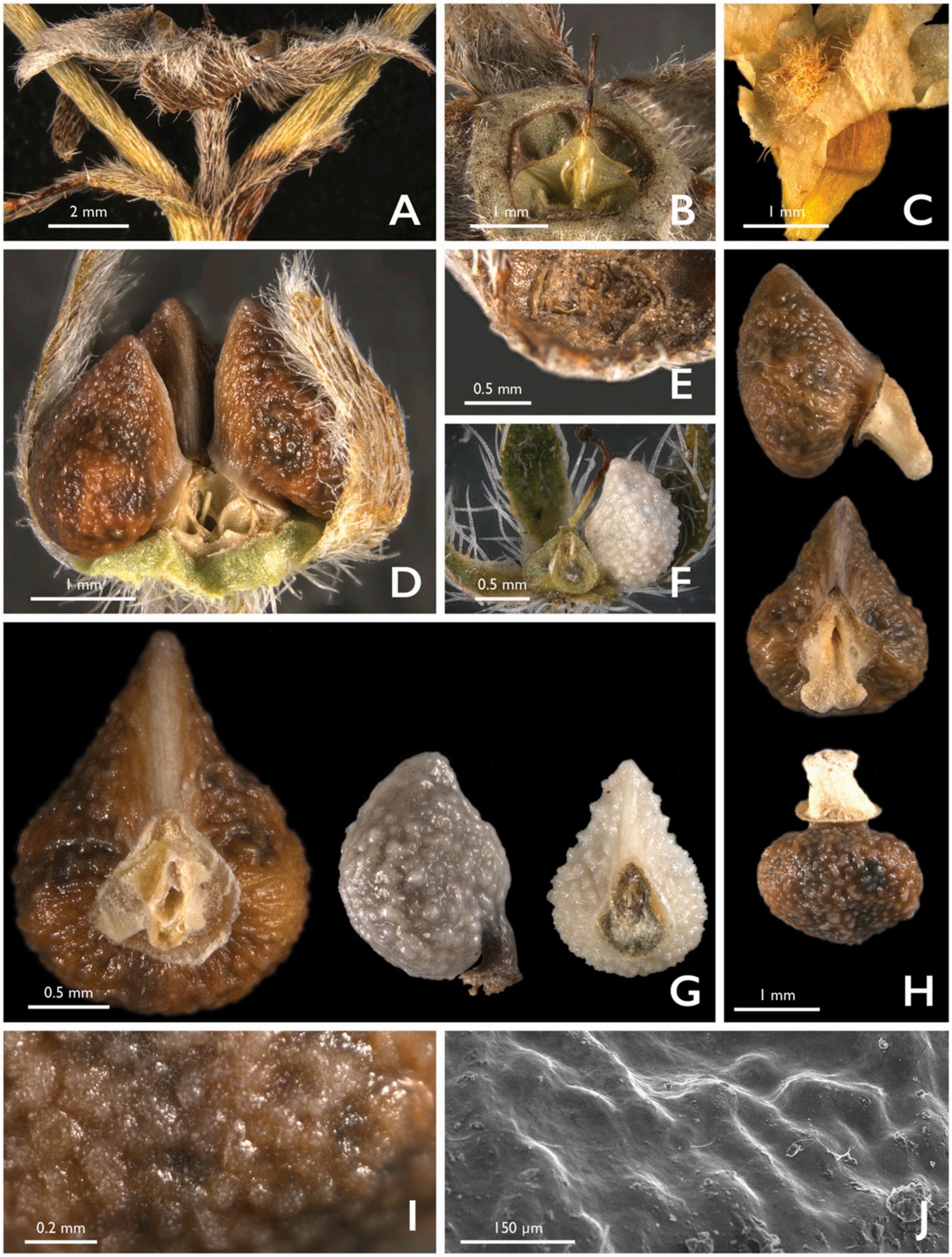

FIG. 4. Characteristic fertile structures of *A. charruasorum*. A. Mature gynobase of *A. charruasorum* in lateral view. B. Oblique view from above of the mature gynobase of *A. charruasorum*; note the sunken areolas. C. Detail of a faucal appendage showing the dense hirsute indumenta. D. Immature fruit of *A. charruasorum*, note the very shallowly triangular gynobase with the full areola. E. Mature flat gynobase of *A. cruciatum*. F. Mature deltate gynobase of *A. heliotropioides*. G. Comparison of *A. charruasorum* immature eremocarp with broken projection (left; *Mendoza-Díaz 649 et al.*), with respect to its morphologically and geographically comparable species *A. cruciatum* (in the middle, note the clear stipe in lateral view; *Mendoza-Díaz 651 and Santos de Mello*) and *A. heliotropioides* (right; *Mendoza-Díaz 623 et al.*). H. Mature eremocarp of *A. charruasorum* (*Mendoza-Díaz 648 et al.*) in lateral, ventral, and basal views. Note the length of the scar projection. I. Pericarp ornamentation seen in stereoscopic microscopy; note the papillas. J. Pericarp ornamentation seen in SEM.

Table 1. Main morphological characters to distinguish *Antiphytum charruasorum* from the geographically[1] and morphologically[2] comparable species of the genus.

| | *A. charruasorum* | *A. cruciatum*[1] | *A. geoffreyi*[2] | *A. heliotropioides*[2] | *A. stoechadifolium*[1] |
|---|---|---|---|---|---|
| Cauline leaf blades (mm) | Linear to narrowly elliptic (18.0–38.0 × 1.0–2.0) | Oblanceolate (15.0–30.0 × 3.0–6.0 mm) | Linear-oblanceolate to linear-oblong (8.0–10.0 × 1.0) | Linear-oblanceolate to oblanceolate (10.0–60.0 × 1.5–8.0) | Linear (25.0–50.0 × 0.15–1.0) |
| Flower arrangement | Only the terminal flower of each dichasial branching | Scorpioid cymes | Scorpioid cymes | Scorpioid cymes | Scorpioid cymes |
| Corolla diameter (mm) | 7.5–8.0 | 4.0–5.0 | 4.7–5.0 | 4.0–6.0 | 5.0–7.0 |
| Corolla color | White | Blue | White | White | Blue |
| Faucal appendage surface | Densely hirsute | Papillose | Papillose | Papillose | Papillose |
| Eremocarp ventral keel | Not forming a stipe | Forming a stipe | Not forming a stipe | Not forming a stipe | Forming a stipe |
| Eremocarp cicatrix position | Infra-ventral | Basal | Infra-ventral | Infra-ventral | Basal |
| Cicatrix tissue | Forming a long projection | Not forming a projection | Not forming a projection | Not forming a projection | Not forming a projection |
| Eremocarp size (mm) | 2.2–2.5 × 1.8–2.0 | 1.3–1.4 × 1.0–1.1 | 1.6–1.7 × 1.3–1.4 | (–1.3)1.5–1.7 × (–0.9)1.1–1.2 | 1.8–2.0 × 1.4–1.6 |
| Gynobase shape (length:width) | Very shallowly triangular (ca. 1:3) | Flat | Triangular (2:1) | Deltate (ca. 1:1) | Flat |
| Areola tissue | Forming part of the eremocarp projection | Remaining in the gynobase | Remaining in the gynobase | Remaining in the gynobase | Remaining in the gynobase |
| Areolas | Sunken in mature fruits | Solid in mature fruits | Solid in mature fruits | Solid in mature fruits | Solid in mature fruits |

watercourse in a grassland characterized by *Bouteloua megapotamica*. No other *Antiphytum* populations are reported from Paysandú nor Río Negro Departments. Adding to the morphological singularity of this discovery, the habitat of *A. charruasorum* is unusual for the entire genus, since *Antiphytum* species occur mainly in xeric habitats.

***Conservation Status***—*Antiphytum charruasorum* is only known from two localities, both in farms near *Eucalyptus* plantations. According to the IUCN (2012), this species meets the criteria B2ab (iii, v) and we recommend that if a formal assessment were performed that it would be categorized as Critically Endangered (CR).

## Identification Key to the South American Species of *Antiphytum*

1. Inflorescences with only the terminal flower of each dichasial branching; corollas white; eremocarp cicatrix infra-ventral. . . . . . *A. charruasorum*
1. Inflorescences with flowers in scorpioid cymes; corollas blue; eremocarp cicatrix basal. . . . . . 2
    2. Leaves 15.0–30.0 × 3.0–6.0 mm, oblanceolate; eremocarps 1.3–1.4 × 1.0–1.1 mm. . . . . . *A. cruciatum*
    2. Leaves 25.0–50.0 × 0.15–1.0 mm, linear; eremocarps 1.8–2.0 × 1.4–1.6 mm. . . . . . *A. stoechadifolium*

## Discussion

The discovery of *Antiphytum charruasorum* breaks down the taxonomic knowledge of the genus in South America due to its very shallowly triangular gynobase, the absence of a stipe, the cicatrix on the ventral face of the eremocarp in infra-medial position, and the white corollas. Prior to the discovery of *A. charruasorum*, there was a clear morphological pattern for the North and South American species of *Antiphytum* (Johnston 1923; Mendoza-Díaz 2015). The South American species were distinguishable by the combination of opposite leaves, flat gynobases, basal eremocarp cicatrix at the end of a stipe-like projection of the ventral keel, and blue corollas. The North American species were characterized by leaves mostly alternate, short-pyramidal gynobases, ventral cicatrix without a stipe-like projection, and white corollas. Since the gynobase and the eremocarp shape are basic in the taxonomy of Boraginaceae (Weigend et al. 2016), the differences between the North American and South American species of *Antiphytum* were used to hint (Johnston 1927) or even propose their recognition as two separate genera (Brand 1929, 1931), but without "fundamental characters which set off the Mexican species from the true *Antiphyta* of South America" (Johnston 1927, p. 12). Johnston (1923) already knew that opposite leaves occur in North American species at least basally on the stem, but opposite leaves along the stem were described in the North American species *A. hintoniorum* L.C.Higgins and B.L.Turner (Higgins and Turner 1983). Moreover, flat gynobases sometimes are observed in *A. floribundum* (Torr.) A.Gray (Brand 1929; NMD pers. obs.) from western Mexico and southern US.

The new species is morphologically closer to the North American species *A. heliotropioides* A.DC. and *A. geoffreyi* N.Mend. and Flores Olv. with which it shares infra-medial position of the cicatrix and the absence of a basal leaf rosette. Nevertheless, both species and the known South American species, *A. cruciatum* (Cham.) DC. and *A. stoechadifolium* (Cham.) DC., have the flowers arranged in scorpioid cymes as the basic unit of the inflorescences. In sharp contrast, *A. charruasorum* only bears the terminal flower of each dichasial branching. The presence of trichomes on the faucal appendages and the tissue projection of the cicatrix, with the corresponding sunken areolas in the gynobase, are features not previously reported from *Antiphytum*. Moreover, the entire fruit and individual eremocarps reach the largest size in the genus (Fig. 4; Table 1). Eremocarps have a peg-shaped projection of gynobase tissue that is common in almost all Boragineae and in some *Lithodora*

Griseb. and *Myosotis* L. (Hilger 2014; Weigend et al. 2016). Detachment of the mature eremocarp occurs when an abscission or separation zone forms between this structure and the gynobase (Roth 1977), while removal from the immature fruit breaks off this "plug tissue" and makes evident the very shallowly triangular gynobase shape as well as the area and position of the cicatrix (Fig. 4). At least in Boragineae and *Myosotis*, the peg-shaped plug is a food body serving in myrmecochory (elaiosome) (Hilger 2014; Weigend et al. 2016). Further studies are needed to better understand the role and development of this structure in *A. charruasorum* where ants' visits have been documented (Fig. 2F).

The habitat and distribution of *Antiphytum charruasorum* are novelties for the genus since the new species grows near to a water body on wet soils with a high content of calcium carbonate. The remaining species grow in dry places and some on calcareous soils in North America. No other localities of *Antiphytum* in Paysandú nor Río Negro Departments have been reported, which are nearer to the Argentina border than to southern Brazil and northern Uruguay. *A. charruasorum* thus represents an isolated outpost of the genus (Fig. 3).

In short, the new species represents an addition to *Antiphytum* that adds novel characters to the genus and removes the morphological distinction of fertile structures (corolla color and eremocarp cicatrix position) between the South and North American species, expands the range of ecological conditions under which *Antiphytum* has been reported, as well as its distribution area and diversity in South America, and adds a new endemic species to Uruguay.

***Paratypes*—Uruguay.** —PAYSANDÚ: Municipio Quebracho, Forestal Oriental "El Pucará" plantation forest farm, 50 m, 31°57′03.5″S, 57°54′48.4″W, 14 December 2016, *Brussa et al.* (MVJB! 30747); 48 m, 31°57′03.7″S, 57°54′48.3″W, 07 December 2017, *Mendoza-Díaz 648 et al.* (MEXU!, MVFA!); 50 m, 31°57′02.9″S, 57°54′46.2″W, 07 December 2017, *Mendoza-Díaz 649 et al.* (MEXU!, MVFA!); 50 m, 31°57′03.33″S, 57°54′47.88″W, 18 October 2018, *Muñoz and Díaz* (MVJB! MVJB 31567). RÍO NEGRO: 11va Seccional Policial, "Los Cobres" farm, "El Ombú" place, 31 m, 32°53′45.14″S, 57°31′47.73″W, 19 October 2019, *Muñoz and Díaz* (MVJB! 31669).

***Additional Specimens Examined (Other Species)—Antiphytum cruciatum.* Brazil.** —RIO GRANDE DO SUL: Porto Alegre, Morro São Pedro, 173 m, 30°10′30.7″S, 51°06′42.9″W, 14 December 2017, *Mendoza-Díaz 651 and Santos de Mello* (MEXU!, MVFA!). ***Antiphytum floribundum.* Mexico.** —MICHOACÁN: Municipio Morelia, La Alberca de Teremendo de los Reyes, 2194 m, 19°48′11.1″N, 101°26′58.8″W, 09 Oct 2013, *Contreras J. 52* (MEXU!). ***Antiphytum geoffreyi.* Mexico.** —COAHUILA: Municipio Francisco I. Madero, west side of Sierra de Tlahualilo, 1275 m, 26°36′46.8″N, 103°21′13″W, 21 April 2015, *Hinton 29613 et al.* (MEXU!). ***Antiphytum heliotropioides.* Mexico.** —QUERÉTARO: Municipio Landa de Matamoros, 43 km (on road) east of Landa de Matamoros, on the road Jalpan de Serra-Xilitla, 1570 m, 21°16′22.7″N, 99°12′19.4″W, 13 September 2014, *Mendoza-Díaz 623 et al.* (MEXU!). —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 629 et al.* (MEXU!). ***Antiphytum hintoniorum.* Mexico.** —NUEVO LEÓN: Municipio Rayones, road Galeana-Rayones, 6 km N of Los Nogales and 17 km N of Galeana, 1348–1360 m, 24°55′38.7″N, 100°03′52.2″W, 24 September 2016, *Mendoza-Díaz 630 et al.* (MEXU!).

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## AUTHOR CONTRIBUTIONS

Nidia Mendoza-Díaz led manuscript preparation. Marina Díaz and Fabián Muñoz contributed to the manuscript writing. Patricia Brussa helped with fieldwork, map elaboration and description. José M. Bonifacino and Hilda Flores-Olvera contributed to manuscript preparation.

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# PARTE I: REEVALUACIÓN TAXONÓMICA DE LAS ESPECIES DE *ANTIPHYTUM*

## PARTE A. ESPECIES DE SUDAMÉRICA

## CAPÍTULO IV: A taxonomic reevaluation of the South American *Myosotis* species described by José Arechavaleta

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# A Taxonomic Reevaluation of the South American *Myosotis* Species Described by José Arechavaleta

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ABSTRACT. In *Flora Uruguaya*, José Arechavaleta described two Uruguayan species of *Myosotis* L. (Boraginaceae) that I. M. Johnston later placed within *Antiphytum* DC. ex Meisn. Johnston considered *M. uruguayensis* Arechav. to be a doubtful species that was probably in *Antiphytum*, apparently nearest to *A. cruciatum* (Cham.) DC., whereas he treated *M. berroi* Arechav. as a synonym of *A. stoechadifolium* (Cham.) DC. We studied the type specimens and protologues of these two names in *Myosotis*. We conclude that *M. berroi* belongs to *Antiphytum* but is not conspecific with *A. stoechadifolium* based on eremocarp features, such as pericarp ornamentation and size, together with shorter leaves. We provide an updated circumscription and distribution, publish the new combination *A. berroi* (Arechav.) N. Mend. & Flores Olv., and clarify the type. A taxonomic key for the South American species of *Antiphytum* is provided. *Myosotis uruguayensis* does not belong to *Antiphytum* due to its shallowly divided calyx entirely covering the immature eremocarps; it retains its uncertain taxonomic status due to the scanty and immature original material and inadequate protologue.

*Key words:* *Antiphytum*, Boraginaceae, Brazil, *Myosotis*, new combination, Uruguay.

The genus *Antiphytum* DC. ex Meisn. (Boraginaceae subfamily Echiochiloideae; see Chacón et al., 2016) was published by Meisner (1840), containing two South American species previously described by Chamisso (1829) as *Anchusa cruciata* Cham. and *Anchusa stoechadifolia* Cham. Both species were transferred to *Antiphytum* by de Candolle (1846), i.e., *Antiphytum cruciatum* (Cham.) DC. and *Antiphytum stoechadifolium* (Cham.) DC. These species were later treated as heterotypic synonyms by Johnston (1923), who lectotypified the genus with *Antiphytum cruciatum*. In a subsequent work, Johnston (1927) accepted both species as the only two well-supported South American species of *Antiphytum*. At the same time, Johnston (1927) also cited for the first time in the literature the two species described by José Arechavaleta, a Spanish botanist based in Uruguay, under *Myosotis* L. in the *Flora Uruguaya* (Arechavaleta, 1898–1911): *M. berroi* Arechav. and *M. uruguayensis* Arechav. Johnston (1927) treated the former as a heterotypic synonym of *Antiphytum stoechadifolium* and the latter as probably a species of *Antiphytum*, apparently nearest to *Antiphytum cruciatum*. However, he did not transfer the name to *Antiphytum*. In the meantime, between Arechavaleta's and Johnston's publications, no work dealing with Boraginaceae referred to *Flora Uruguaya* nor the *Myosotis* species there described.

As part of the taxonomic revision of the genus *Antiphytum* currently underway by the lead author, we reviewed the treatment of the Boraginaceae in the *Flora Uruguaya* (Arechavaleta, 1898–1911) with a focus on the two species of *Myosotis* as possible species of *Antiphytum*. In parallel, we revisited Johnston's hypothesis on Arechavaleta's species. We searched for the types in Uruguayan and international herbaria where Arechavaleta's specimens may be, according to Stafleu and Cowan (1976–1988) in the second edition of *Taxonomic Literature* (TL-2), checked the protologues, and examined specimens to clarify the taxonomic affinities of these names.

## TAXONOMIC HISTORY

*Flora Uruguaya* (Arechavaleta, 1898–1911) is one of the first botanical works published in South America together with *Flora de Chile* (Reiche, 1896–1911). In *Flora Uruguaya*, Boraginaceae s. str. is only represented by *Myosotis*, for which Arechavaleta described

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*M. berroi* and *M. uruguayensis* (Arechavaleta, 1911a, 1911b).

*Myosotis berroi* was named to honor Mariano B. Berro, a botanist who collected plants of the species in the department of Rivera in northern Uruguay. The collection information on *Berro 4975* (MVFA and MVM; acronyms according to Thiers, 2022) perfectly matches the information in the protologue: "Vive en campos de Rivera en los llamados Cerros del Gobierno, pedregosos áridos. Florece en Diciembre. Hallado por el Sr. Berro a quien se lo dedico" (Arechavaleta, 1911a: 70). As annotated on the specimen at MVFA (Fig. 1), this final epithet was chosen after Arechavaleta initially named the specimen in sched. as "*Myosotis rupestris* n. sp. non Pall. nec Willd." *Myosotis rupestris* had been used already by Pallas (published in Georgi, 1775) and even by Willdenow (Ledebour, 1847) as a later homonym. Arechavaleta later wrote a second label with the name that would eventually be validly published, along with the publication information (Fig. 1).

*Myosotis uruguayensis* was also collected in northern Uruguay in the department of Tacuarembó in sand-clay soils (Arechavaleta, 1911a). It seems that the collection was not made by Arechavaleta himself, although the protologue lacks a collector's name and number. The name Pantaleón Pintos appears on the label of the only located original material (MVM; Fig. 2). Pantaleón Pintos likely is the collector of this specimen, as in the case of *Bambusa tacuara* Arechav. as recognized in the protologue (Arechavaleta, 1897). No information regarding a specific locality is given either in *Flora Uruguaya* or on the label of the specimen; "Campos de Tacuarembó" is reported, but Tacuarembó covers a very large area. As in the case of *M. berroi*, the final epithet "*uruguayensis*" was chosen after two unavailable epithets were considered, as the label shows.

Before the publication of *Flora Uruguaya*, Meisner (1840) had published the genus *Antiphytum*, and de Candolle (1846) had formalized it. However, neither *Antiphytum* nor the two species originally described in *Anchusa* by Chamisso (1829) were mentioned in the *Flora Uruguaya*. Arechavaleta may not have been aware of the presence of *Antiphytum* in Uruguay, or he may have considered that the morphological features defining the genus did not match the Uruguayan specimens known to him.

Johnston (1927) had access to Arechavaleta's *Flora Uruguaya* when he published the revision of the South American "Boraginoideae." However, Johnston did not have access to specimens, so his understanding of the two *Myosotis* species came from Arechavaleta's descriptions alone. Based on the description and the type locality, Johnston (1927) considered *M. berroi* a heterotypic synonym of *Antiphytum stoechadifolium*. According to Johnston (1927), the German botanist Friedrich Sellow explored northern Uruguay during the early part of 1823. Because Sellow's collections were used by Chamisso to describe *Anchusa stoechadifolia* (the basionym of *Antiphytum stoechadifolium*), Johnston wrote that, "it is, hence, not impossible that the types of *Anchusa stoechadifolia* and *Myosotis Berroi* were both obtained in northern Uruguay and perhaps in the same region" (Johnston, 1927: 13–14). On the other hand, Johnston (1927) considered *M. uruguayensis* a doubtful species of *Antiphytum*. He wrote that it is "a fruticose plant probably of this genus [*Antiphytum*] and apparently nearest to *A. cruciatum* but differing in its alternate leaves and tubular calyx. I have seen no material of this species" (Johnston, 1927: 14). Johnston's description of *Antiphytum cruciatum* was thus adapted from the original description only (Johnston, 1927: 12).

None of Johnston's subsequent publications include South American species of *Antiphytum* or Uruguayan species of *Myosotis*. In an unpublished letter to Uruguayan botanist Bernardo Rosengurtt dated 1938 (held at MVFA), Johnston considered *M. berroi* to be a third species of the genus *Antiphytum* based on observation of the specimen *Rosengurtt 2346*. We studied this specimen on loan from GH (see Additional specimens examined). Although this specimen actually corresponds to *A. cruciatum*, Johnston annotated it as "*Antiphytum Berroi* (Arech.) Johnston" (Fig. 3B), this being the first time that this combination was used, although he never formally published it. No mention of *M. uruguayensis* was made in the above-mentioned letter.

The taxonomic status of *Myosotis* species described by Arechavaleta therefore remains yet unresolved. Although both species were validly published, and the names are cited in current floristic works such as *Catálogo de las Plantas Vasculares del Cono Sur* (Zuloaga et al., 2021), their circumscriptions have not been updated and the type specimens have not yet been examined.

## MATERIALS AND METHODS

Literature and herbarium specimens of South American species of *Antiphytum*, including protologues and types, were located and examined. To locate original material of the species of *Myosotis* described by Arechavaleta (1898–1911) in *Flora Uruguaya*, we first searched Uruguayan herbaria MVFA and MVM for historic collections of Mariano Berro and José Arechavaleta, who were both based in Uruguay and collected throughout the country in the late 1800s and early 1900s. We found one specimen of *M. berroi* each at MVFA and MVM, and one specimen of *M. uruguayensis* at MVM. Based on information in TL-2 (Stafleu & Cowan, 1976–1988), we then sought information from the following herbaria to determine whether they housed

Figure 1. Lectotype of *Antiphytum berroi* (Arechav.) N. Mend. & Flores Olv. at MVFA collected by Mariano Berro. Note the label with the basionym *Myosotis berroi* Arechav. and the data of publication annotated by José Arechavaleta.

Figure 2. Original material of *Myosotis uruguayensis* Arechav. at MVM collected by Pantaleón Pintos.

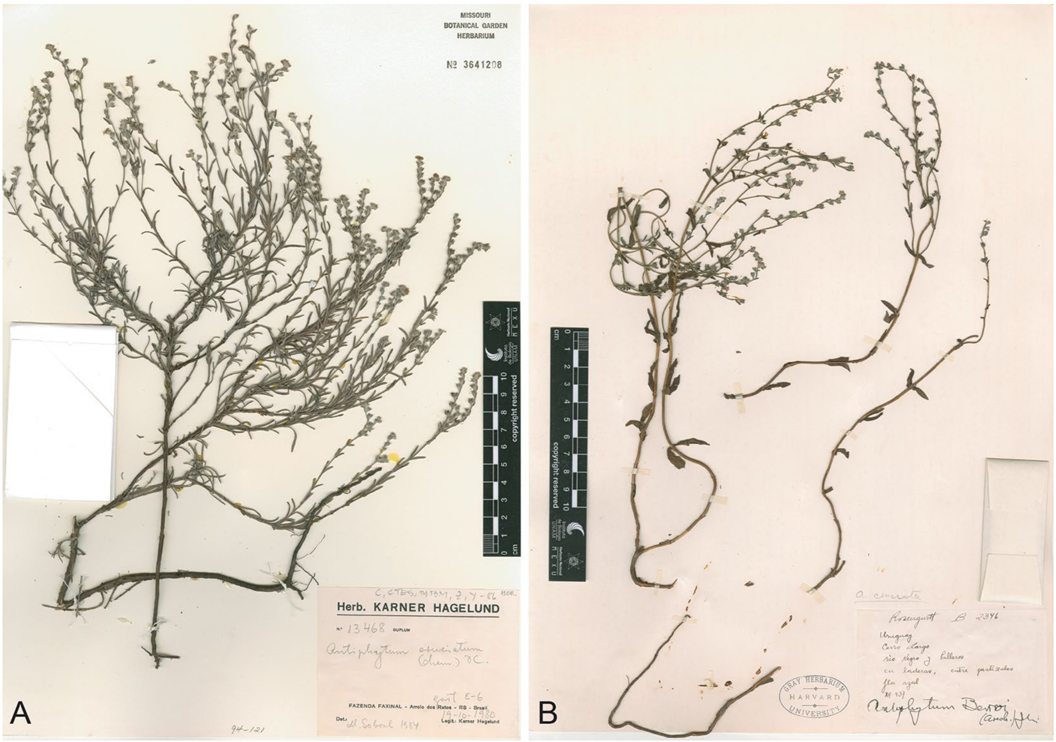

Figure 3. Reference specimens. —A. Specimen of *Antiphytum berroi* (Arechav.) N. Mend. & Flores Olv. collected by Karner Hagelund in Rio Grande do Sul, Brazil (MO). Note the mention of his own herbarium. —B. Specimen of *Antiphytum cruciatum* (Cham.) DC. collected in Uruguay and annotated as "*Antiphytum Berroi*" by I. M. Johnston (GH).

original material from these species: B, BAF, CORD, F, G, K, L, LD, M, MIN, NY, PH, R, WRSL, and ZT. Answers from most of the herbaria indicate that there is no more additional material out of Uruguay, but GH holds a phototype of the MVM specimen of *M. berroi*. As we found collections referable to *M. berroi* that were made in southern Brazil, the ICN herbarium was also checked for additional collections to upgrade taxonomic and geographic information of the species. Fieldwork was conducted in the departments of Tacuarembó and Rivera in Uruguay.

Morphological characters, particularly from the fruit and the inflorescence (following Weberling, 1989) were used to differentiate and to describe the species: eremocarp number and size, pericarp ornamentation, cleft of the calyx lobes, kind of diaspora. Material from *Myosotis berroi* was available from type specimens and recent collections, including our own collections (see Additional specimens examined). Since there are no duplicates or additional collections of *M. uruguayensis*, it was not possible to make direct observation of the fruits. Mature eremocarps (nutlets or mericarps, terminology sensu Hilger, 2014) from our collected specimens of *M. berroi* and one specimen of *Antiphytum stoechadifolium* (*Marchesi 1420*) were observed 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 Laboratorio de Microscopía y Fotografía de la Biodiversidad II of Instituto de Biología, Universidad Nacional Autónoma de México (UNAM). SEM photographs were also taken (Hitachi SU1510, Hitachi Ltd., Tokyo, Japan) at Laboratorio de Microscopía Electrónica y Fotografía de la Biodiversidad I.

A distribution map was made using QGIS (2.6.1-Brighton) (QGIS Development Team, 2014) and the GIS layers from official websites of Argentina (Instituto Geográfico Nacional de la República Argentina, 2020), Brazil (Fundação Estadual de Proteção Ambiental Henrique Luiz Roessler, 2020; Núcleo de Economía Regional e Urbana da Universidade de São Paulo, 2020; Serviço Geológico do Brasil, 2020), and Uruguay (Infraestructura de Datos Espaciales del Ministerio de Transporte y Obras Públicas de Uruguay, 2020; Instituto Nacional de Estadística, 2020; Ministerio de Industria, Energía y Minería de Uruguay, 2020). For the delimitation of Serra Geral Formation, Brazil, we followed Wildner et al. (2004).

The designation of types follows McNeill (2014) and Article 9 of the *International Code of Nomenclature for algae, fungi, and plants* (Turland et al., 2018).

## RESULTS

We propose transferring *Myosotis berroi* to the genus *Antiphytum*, which would make it the fourth species native to South America, and the third with blue corollas (see Mendoza Díaz et al., 2020). Features from the eremocarp, along with the cleft of the calyx lobes almost to the base in the flower, and the extended calyx in the fruit that does not enclose the four eremocarps, are characteristic of *Antiphytum* and exclude *A. berroi* (Arechav.) N. Mend. & Flores Olv. from genera of Moritziinae. *Antiphytum berroi* comprises a well-defined species that differs from *A. stoechadifolium* by the eremocarp ornamentation and leaf length. From the two syntypes found, the specimen at MVFA (*M. B. Berro 4975*) is designated as the lectotype.

By contrast, based on the short protologue description and the type found in MVM, we conclude that *Myosotis uruguayensis* does not belong in *Antiphytum* due to its shallowly divided calyx and the eremocarps that remain enclosed in the accrescent calyx. *Myosotis uruguayensis*, although valid, remains with uncertain taxonomic status since there are not enough features to reliably assign it to any genus of Boraginaceae. After our search to find duplicates, only the specimen at MVM is known. In the face of the uncertainty of the original gathering, it seems unnecessary to designate a lectotype according to McNeill (2014).

## TAXONOMIC TREATMENT

**Antiphytum berroi** (Arechav.) N. Mend. & Flores Olv., comb. nov. Basionym: *Myosotis berroi* Arechav., Anales Mus. Nac. Montevideo ser. 2, 1(3): 69. 1911. TYPE: Uruguay. Dpto. Rivera: “Vive en campos de Rivera en los llamados Cerros del Gobierno, pedregosos áridos,” *M. B. Berro 4975* (lectotype, designated here, MVFA!; isolectotype, MVM!, MVM photo at GH!). Figure 4C–H.

Suffrutex, erect, 25–35(–40) cm tall; stems (usually 3) broadly branching, arising from a main slender stem (ca. 2–3 mm diam.); branches opposite, solid (nonfistulose), slender (ca. 1 mm diam.), later exfoliating, densely strigose, each trichome with several mineralized foot cells (lithocystic structures). Leaves pseudobasal and cauline, the cauline opposite, without a basal rosette, sessile; lamina linear-lanceolate or linear-oblong to linear-oblanceolate, in cauline leaves (10–)14.6–20.9(–30) × 1–1.6(–2.3) mm, becoming gradually smaller on the branch distally, in youngest pseudobasal leaves ca. 5 × 0.4 mm; base truncate, connate-sheathing; apex acute, mucronate; margins thickened, entire; surfaces strigose, with trichomes more densely and finely distributed on abaxial surface, concolorous; midvein not visible on adaxial surface, but groovelike at the very base, visible and raised on abaxial surface; secondary veins not visible. Inflorescences ending in a flower (monotelic) forming a terminal homocladic determinate thyrse (thyrsoid); most distal node with paired scorpioid cymes (boragoids) up to 170 mm when mature, loosely many-flowered, bracteate, with well-developed terminal flower arising from a dichasial division; previous nodes (until 3) often with opposite boragoids as paraclades of the thyrsoid, but sometimes one side on the distal nodes as a new flower branch; most proximal internode of a lateral axis 13–26 mm; bracts subtending the inflorescence and inflorescence nodes opposite, sessile, triangular-lanceolate, the largest 11.5 × 2.5–3 mm, becoming gradually smaller distally, base truncate, apex acute, mucronate, margins hispid, indumenta and midvein as on leaves; flower-subtending bracts sessile, triangular-lanceolate, the largest 4.5 × 1.5–2 mm, becoming gradually smaller toward the apex of the cyme, apex mucronate, margins hispid, indumenta and midvein as on the leaves. Flowers monoclinous, pedicellate; pedicel 1.2–1.5 mm; calyx accrescent, divided ± to the base, strigose on both surfaces, scarious at the very base, calyx lobes ± symmetric, lanceolate, 2.2–3.5 × 0.5–1.5 mm, longer than or equal to corolla tube at anthesis, up to 4 mm in fruit, apex slightly acuminate, mucronate in fruit, trichomes more densely distributed on abaxial surface, few amber-colored and hyaline glandular-capitate at margins, more at base; corolla hypocrateriform (rotate), blue, with 5 white trapezoid papillose faucal appendages on throat in sinuses of corolla lobes, tube light blue, 2.2–2.7 mm, 1 mm wide at base, limb purple-blue, 6 mm diam., lobes oriented perpendicular to tube, imbricate, oblong, 2.1–2.3 × 1.6–2.2 mm, apex rounded-sinuate, abaxial surface densely pilose at middle; stamens included, alternate to corolla lobes, adnate to corolla tube ca. 1.2 mm from base of tube; filaments up to 0.5 mm, slender; anthers dorsifixed, oblong, sublanceolate, ca. 1.3 mm; style gynobasic, 1.7–2.2 mm at anthesis; stigma bilobed, branches short (subterminal), included; ovules 4. Fruit dry, subtended by a persistent extended calyx with acuminate lobes apically recurved; eremocarps usually 4, rarely 3 by abortion, 1.6–1.9 × 1.3–1.5 mm, ovate and apically acute in ventral view, dorsally convex, lustrous, striate; cicatrix at basal position under a stipelike projection, ovate to elliptic, ca. 1 mm; 1 ventral keel (apical) straight from apex of eremocarp to base, forming a stipe; lateral (basal) keels absent; gynobase flat to slightly raised (length:width ratio ca. 1:6), with 4 areoles corresponding to the cicatrix of each eremocarp; style plus stigmas 2.4 mm, surpassing the eremocarps.

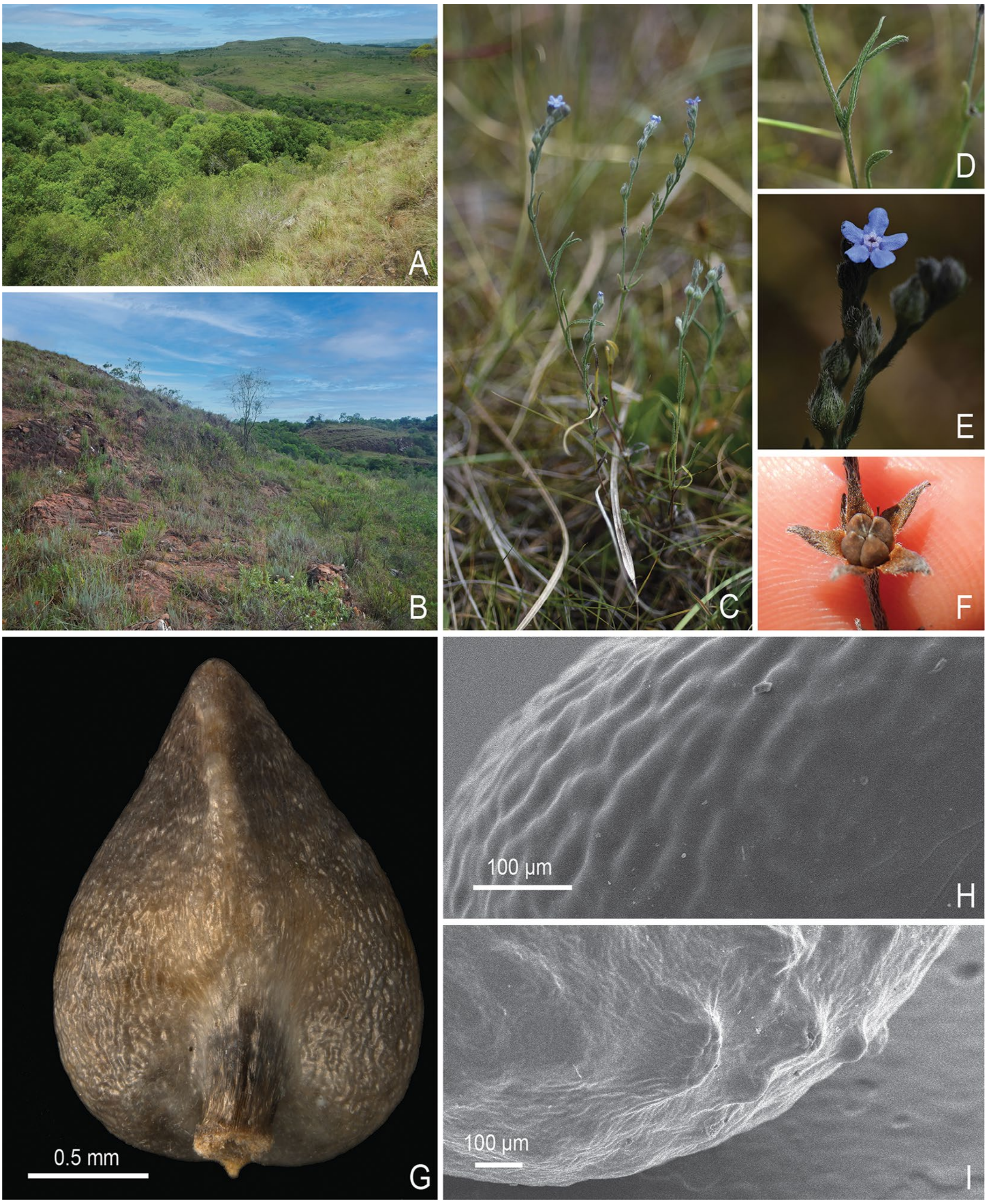

Figure 4. *Antiphytum berroi* (Arechav.) N. Mend. & Flores Olv. —A. Habitat; note the grassland near to deciduous forest. —B. Habitat close up, with sedimentary rocks in foreground. —C. Habit. —D. Linear leaves. —E. Portion of scorpioid cyme and flower. —F. Fruit. —G. Front view of one eremocarp (*Mendoza-Díaz et al. 650A*). —H. Eremocarp ornamentation of *A. berroi* (*Mendoza-Díaz et al. 650B*). I. Eremocarp ornamentation of *A. stoechadifolium* (Cham.) DC. (*Marchesi 1420*). Photographs: A, B, F, G by Nidia Mendoza-Díaz; C–E by Camilo Pérez; H, I by Berenit Mendoza-Garfias.

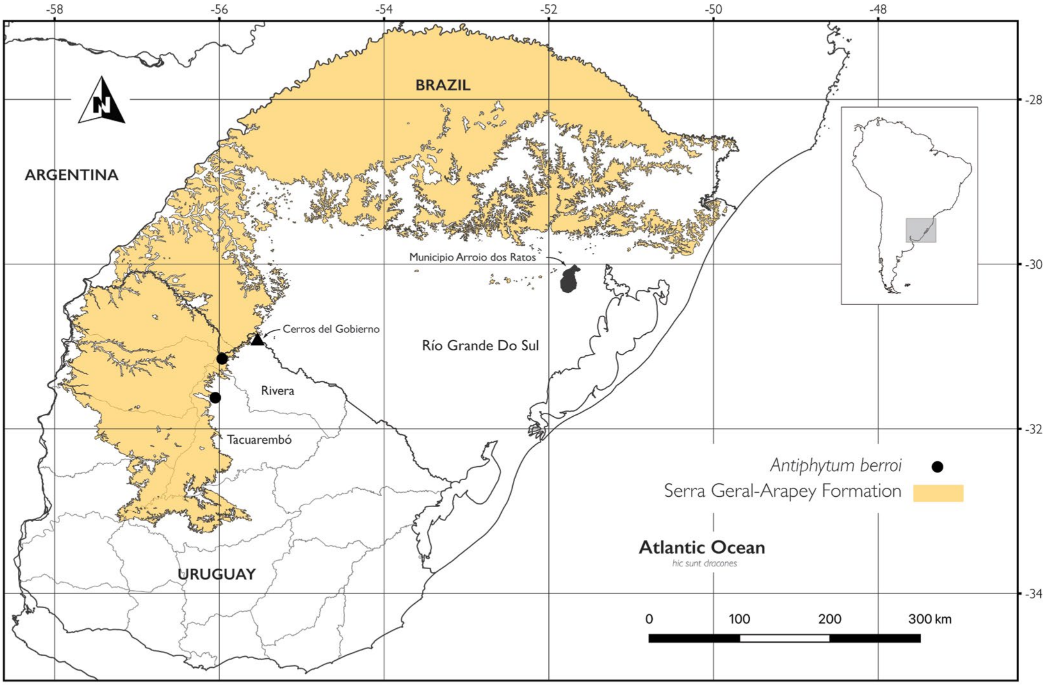

Figure 5. Distribution map of *Antiphytum berroi* (Arechav.) N. Mend. & Flores Olv. in both Uruguay (Tacuarembó and Rivera Departments) and Brazil (Rio Grande do Sul State, not indicated). ▲ "Cerros del Gobierno" locality [now destroyed]; ● other localities. Note the distribution of the Uruguayan localities over the Serra Geral–Arapey Formation and the distant Municipio Arroio dos Ratos.

*Distribution.* *Antiphytum berroi* is native to South America, occurring in southern Brazil (Rio Grande do Sul only) and northern Uruguay (adjacent departments Tacuarembó and Rivera) in disjunct localities (Fig. 5). A third locality in Rivera Department, i.e., the rocky habitat of the type locality of "Cerros del Gobierno" (*Berro 4975*; MVFA, MVM), no longer exists as the hill has been transformed into a quarry (Díaz, pers. obs.; Fig. 5, labeled triangle). The distribution of the species in southern Brazil is supported by Hagelund's collections from Rio Grande do Sul, mainly from the ICN herbarium, but the precise localities remain unknown (see Notes).

*Habitat and underlying geology.* According to Uruguayan records from the departments of Rivera and Tacuarembó, *Antiphytum berroi* is found in grasslands near a riverside subtropical forest formed in the canyons ("quebradas") with herbaceous species such as *Baccharis trimera* (Less.) DC., *Crocanthemum brasiliensis* Spach., *Plantago myosuros* Lam., *Schlechtendalia luzulifolia* Less., and species of *Andropogon* L., *Aristida* L., *Borreria* G. Mey., and *Chascolytrum* Desv. The area falls into what Lezama et al. (2019) classified as *Eryngium horridum* [Malme]–*Juncus capillaceus* [Lam.] community, a densely vegetated grassland, dominated by mesophytic species, encompassing stands with high plant cover values (near 100%) that occupy medium and deep soils of the Eastern Hills, North Eastern Sedimentary Basin, and the South Central regions. We found and collected scattered individuals from one small population (*Mendoza-Díaz et al. 650*, to be sheltered in MEXU and MVFA) at ca. 200 m.s.m., mainly on a sedimentary rock wall in one locality in the department of Tacuarembó whose substrate comprised 2%–10% rocky outcrops and 1%–10% stones (according to GIS layers provided by Dirección General de Recursos Naturales Renovables, 2002). The underlying geology of these areas comprises basic lavas of tholeiitic basalt type with flow structure and intercalation of aeolian sandstones (Preciozzi et al., 1985). The localities of occurrence in Uruguay are part of the Arapey Formation (Upper Jurassic–Lower Cretaceous) of igneous volcanic origin that alternates on the east side with sandstones of sedimentary origin from Tacuarembó Formation. The Arapey Formation is an extension of the Serra Geral Formation in Brazil (Walther, 1911).

*Phenology.* The main flowering and fruiting period is from September to December.

*Notes.* After examining the type material and reviewing the protologue, we confirmed that the species *Myosotis berroi* belongs to the genus *Antiphytum*, a conclusion supported by the nature of the calyx almost entirely divided to the base, the flat gynobase, the eremocarp with its stipelike projection and basal cicatrix (scar), the bracteate flowers arranged in scorpioid cymes, the blue corolla, the open extended calyx subtending the four eremocarps, and the opposite leaves throughout the length of the stem. The calyx lobe features and the kind of diaspora exclude this species from any genera of Moritziinae, which include *Thaumatocaryon* Baill. and *Moritzia* DC. ex Meisn., whose species are rosette herbs with fruits that consist of a single, ventrally and dorsally keeled nutlet enclosed into and dispersed with the calyx (Chacón et al., 2016). *Antiphytum berroi* is morphologically most similar to the South American species *A. cruciatum* and *A. stoechadifolium*. The blue corolla alone sets it apart from the North American species and the recently described Uruguayan species *A. charruasorum* N. Mend & Mar. Díaz (Mendoza-Díaz et al., 2020), all of which have white corollas.

*Antiphytum berroi* is easily distinguishable from *A. cruciatum* by its linear leaves that are 0.4–1.6 mm wide (vs. oblanceolate leaves ca. 3–4 mm wide), but also by the eremocarp size and ornamentation (1.3–1.4 × 1–1.1 mm and rugose in *A. cruciatum*). The general vegetative appearance is similar to that of *A. stoechadifolium*, which also bears linear leaves, but the leaves in *A. stoechadifolium* are considerably longer (ca. 5 cm long) than those of *A. berroi* (1–2[–3] cm long). Therefore, the length of the leaves gives a lax appearance to the internodes in *A. berroi*, in contrast to *A. stoechadifolium*, whose internodes are covered with leaves. Berro's specimens (*Berro 4975*, MVFA and MVM) have a pericarp ornamentation of the eremocarp that was described as "somewhat rugose" (Arechavaleta, 1911a: 69, "un tanto rugosa") but is rather more or less striate as shown in the original illustration (Arechavaleta, 1911a: fig. 6F), and also in the SEM and stereoscope photographs (Fig. 4H, I). In marked contrast with this, Chamisso (1829: 440) described the pericarp ornamentation of *A. stoechadifolium* as "tuberculato-granulatae," a description later ratified by Johnston (1927) and by our own observations (Fig. 4H, I). Although the descriptions of vegetative characters of both species by Chamisso (1829) and Arechavaleta (1911a, 1911b) are apparently similar, we found enough evidence in leaves and eremocarps to recognize *A. berroi* as a different species within *Antiphytum*, not conspecific with *A. stoechadifolium*, which seems to be allopatric.

Regarding the known distribution, specimens from Rio Grande do Sul are geographically unreliable. The collector of these specimens, Karner Hagelund (1913–1988), was a researcher and collector who had his own herbarium in "Fazenda Faxinal," in the municipality of Arroio dos Ratos, Rio Grande do Sul (Eisinger, 1987), a farm with a local traditional agrosilvopastoral system (*faxinal*) that is performed in some areas of southern Brazil (Antoneli et al., 2020). In 1988, Hagelund donated to the ICN herbarium his collections from areas of Rio Grande do Sul currently used for farming, especially from the northern region Alto Uruguai e das Missões, whose flora prior to their devastation is well represented in Hagelund's collection (Catálogo REMAM, 2013). Jesuit missions were located in western areas of Rio Grande do Sul, following the Uruguay River, along the Serra Geral Formation. This basaltic formation extends southward into Uruguay, where it is named Arapey Formation (Walther, 1911) and in which the Uruguayan populations of *Antiphytum berroi* are located. Hagelund's collections of *A. berroi* are labeled using the herbarium locality as the only geographic reference, "Fazenda Faxinal, Arroio dos Ratos, RGS [Rio Grande do Sul]" (Fig. 3A), which is far away from the known Uruguayan localities (Fig. 5); hence, there is doubt whether Arroio dos Ratos is actually the locality of distribution of *A. berroi* in Brazil. Therefore, new surveys in the Brazilian state of Rio Grande do Sul are warranted to relocate historical populations and find new populations of *A. berroi*. Such surveys should be conducted in areas where the Jesuit missions were based, as well as at additional localities in the Serra Geral and Arroio dos Ratos, to establish the current distribution and status of *A. berroi* in Brazil.

*Additional specimens examined.* BRAZIL. **Rio Grande do Sul:** Arroio dos Ratos, Fazenda Faxinal, 5 Sep. 1974, *Hagelund 8161* (ICN-162311); 18 Oct. 1976, *Hagelund 10541* (ICN-153403); 19 Oct. 1980, *Hagelund 13468* (ICN-153402, MO-3641208); 10 Oct. 1982, *Hagelund 14046* (ICN-162310). URUGUAY. **Dpto. Rivera:** 2 Nov. 1926, *Felippone 5224* (K-H2002/02368); Arroyo Gajo del Lunarejo, 17 Sep. 1995, *Bonifacino s.n.* (MVFA-25051). **Dpto. Tacuarembó:** Cd. Tacuarembó, Cerros Chatos, 31°37′16.4″S, 56°02′33.3″W, 215 m, 9 Dec. 2017, *Mendoza-Díaz et al. 650* (MEXU, MVFA).

*Additional specimens examined (other species). Antiphytum cruciatum* (Cham.) DC.: BRAZIL. **Rio Grande do Sul:** Arroio dos Ratos, Fazenda Faxinal, 5 Oct. 1979, *Hagelund 12846* (ICN); Canguçú, estrada Amaral Ferrador-Canguçú, 8 Oct. 1977, *Miotto 558* (ICN), entre Amaral Ferrador e Canguçú, 8 Dec. 1977, *Fleig 751* (ICN); Camaquã, Boa Vista, Sep. 1985, *Sobral et al. 4165* (ICN); Caçapava do Sul, Pedra do Segredo, 29 Nov. 2002, *Kinupp & Irgang 2504* (ICN), BR 290, 31 Oct. 2010, *Silva Filho 1603* (ICN); Guaíba, Centro Agronômico de Guaíba, 10 Oct. 1974, *Schultz s.n.* (ICN); Lavras do Sul, Mina Volta Grande, 5 Oct. 1984, *Sobral 3074* (ICN); Piratini, Ponte Piratini, 15 Nov. 1975, *Arzivenco s.n.* (ICN); Porto Alegre, morro São Pedro, 30 Sep. 1956 (ICN), morro São Pedro, Econsciência Espaço de Conservação, 20 Oct. 2005, *Setubal 492* (ICN); Rio Pardo, na beira da RS-7, Km. 32, 4 Oct. 1972, *Lindeman et al. s.n.* (ICN); Santana da

Boa Vista, 2 Nov. 1995, *Jarenkow & Sobral 2777* (ICN); Toca do Tigre, perto de Itapoan, 27 Sep. 1950, *Rambo 48861* (ICN); Torres, Butiazal, 26 Feb. 1985, *Hagelund 15492* (ICN), Butiazal, 2 Oct. 1975, *Irgang s.n.* (ICN), Butiazal, 12 Oct. 1970 (ICN); Vacaria, BR 116, Km. 50, 6 Oct. 1985, *Miotto 1072* (ICN); Viamão, Itapuã, Sep. 1983, *Sobral 2237* (ICN); Viamópolis, 14 Nov. 1969, *Korner s.n.* (ICN). URUGUAY. **Dpto. Cerro Largo:** Río Negro y Palleros, Dec. 1937, *Rosengurtt 2346* (GH).

*Antiphytum stoechadifolium* (Cham.) DC.: BRAZIL (?). Brasilia meridionali, *Sellow s.n.* (holotype, GH [barcode] 00032786 fragment! and image!; isotype GH [barcode] 00032785!). URUGUAY. **Dpto. Maldonado:** Sierra de las Ánimas, 26 Dec. 1965, *Marchesi 1420* (MVFA).

## KEY TO THE SOUTH AMERICAN SPECIES OF *ANTIPHYTUM*

1. Inflorescences with flowers not arranged in scorpioid cymes, but only the terminal flower in each dichasial branch; corollas white; eremocarp cicatrix infra-ventral . . . . . . . . . . . . . . . . . . . . . . . . . . *A. charruasorum* N. Mend. & Mar. Díaz
1′. Inflorescences with flowers arranged in scorpioid cymes, therefore more than the terminal flower in each dichasial branch; corollas blue; eremocarp cicatrix basal.
    2. Leaves 3–4(–6) mm wide, oblanceolate; eremocarps 1.3–1.4 × 1–1.1 mm . . . . . . . . . . . . *A. cruciatum* (Cham.) DC.
    2′. Leaves < 3 mm wide, linear-lanceolate or linear-oblong to linear-oblanceolate; eremocarps 1.6–2 × 1.3–1.6 mm.
        3. Pericarp ornamentation ± striate; leaves up to 3 cm long. . . . . . . . *A. berroi* (Arechav.) N. Mend. & Flores Olv.
        3′. Pericarp ornamentation tuberculate-granulate; leaves ca. 5 cm long . . . . . . . . . *A. stoechadifolium* (Cham.) DC.

## UNCERTAIN TAXONOMIC STATUS

**Myosotis uruguayensis** Arechav., Anales Mus. Nac. Montevideo ser. 2, 1(3): 68. 1911. TYPE: Uruguay. Dpto. Tacuarembó: “Vive en Tacuarembó, en terrenos arcillo-arenosos. Florece por noviembre y diciembre,” *P. Pintos s.n.* (type, MVM!).

*Distribution and habitat.* *Myosotis uruguayensis* is known from only the type collection and reportedly occurs in Tacuarembó, Uruguay, on sandy-clay soils.

*Phenology.* Flowering is in November and December (Arechavaleta, 1911a).

*Notes.* According to the original description and illustrations of the calyx and corolla (Arechavaleta, 1911a: 68 and fig. 4), *Myosotis uruguayensis* bears alternate linear leaves (ca. 4–5 × 0.3 cm), violet corollas, pubescent corolla faucal appendages (“escamillas trapezoides circundadas de pelos gruesos dactiloides”), and a shallowly divided calyx (“lacinias breves”). We were not able to fully observe all these features on the type specimen because of the immature and scanty nature of the original material, which prevented direct examination of the necessary fruit features to identify this specimen, but what we observed allowed us to associate the material with the protologue.

The short description of the fruit allows placement of the taxon under Boraginaceae, but it lacks characters to place it confidently within any genus since it only mentions three to four ovoid eremocarps (“aquenios”) with an acute apex, without an accompanying illustration. Even with the poor original material and short description, we can exclude *Myosotis uruguayensis* from *Antiphytum* because the calyx is not divided almost to the base and the eremocarps are not free from the calyx, as is characteristic of that genus. *Myosotis uruguayensis* seems closer to the Moritziinae subtribe (see Weigend et al., 2010: fig. 1), whose genera *Moritzia* and *Thaumatocaryon* have no records from Uruguay. Species in Moritziinae may have alternate leaves, a shallowly divided calyx, and pubescent corolla faucal appendages. However, *Thaumatocaryon* and *Moritzia* produce only one eremocarp due to abortion of the other three (Weigend et al., 2010, 2016), a key feature that may exclude *Myosotis uruguayensis* from these genera. In addition, *Moritzia* species have ebracteate inflorescences and uncinate trichomes in the calyx, unlike the original material of *Myosotis uruguayensis*; hence, we strongly doubt its affiliation to this genus. Furthermore, in both genera the species have conspicuous basal leaves forming a rosette. Since Arechavaleta was not the collector of the specimen, the absence of the basal rosette in both the description and the collection can be expected. *Myosotis uruguayensis* could also be part of *Myosotis*, as diagnosed by the length of the calyx cleft and the number of eremocarps. *Myosotis* has exotic representatives in Uruguay, such as *Myosotis latifolia* Poir. and *Myosotis verna* Nutt. (Zuloaga et al., 2021). Unfortunately, an exact identification is not possible, even at the generic level. Except for *A. berroi*, which occurs in Tacuarembó, and recent records of *A. cruciatum* (Patricia Brussa, pers. comm.), no other native Boraginaceae have been reported from the type locality of *Myosotis uruguayensis* nor from neighboring areas. Therefore, *Myosotis uruguayensis*, which is a validly published name, remains as a name with uncertain status. Finding new populations of this taxon will be the only reliable way to know its identity by examining key fruit characteristics, such as pericarp ornamentation and cicatrix position. However, the lack of information on the locality of collection will be a challenge due to the large area of Tacuarembó.

*Typification notes.* The MVM specimen is currently the only known original material, but following the recommendation in McNeill (2014), lectotypification is not required.

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# PARTE II: FILOGENIA Y PATRONES DE DIVERSIFICACIÓN DE *ANTIPHYTUM*

## CAPÍTULO V: *Antiphytum*: initial insights about its phylogeny, diversification and biogeographic patterns

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**Borrador**

Prepared to Plant Systematics and Evolution

Original research article

Mendoza-Díaz et al.: Initial insights to *Antiphytum*

# ***Antiphytum*** **(Boraginaceae, Echiochiloideae): initial insights on its phylogeny, diversification, and biogeographic patterns**

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## Abstract

*Antiphytum* (Boraginaceae) has been an elusive genus whose disjunct endemic distribution in North and South America and apparently unlike morphology has suggested a non-natural group. Recent works on Boraginales included no more than two or even no species of *Antiphytum*, lacking South American samples. In this study, we aimed to get a phylogeny of the entire genus with a broad sampling that includes all the known species from both North and South America derived from taxonomic revision. We used five molecular markers (nuclear ITS and cpDNA regions *ndh*F–*rpl*32, *trn*L–F, *mat*K, and *rps*16) to carried out parsimony, maximum likelihood and Bayesian analyses. *Antiphytum* was recovered as a monophyletic member of Echiochiloideae with *Ogastemma* closer related. The backbone of the phylogeny had no resolution, but we recovered three clades within *Antiphytum*: a South American (SA) clade; a North-American (NA) clade with species bearing basal-rosettes, and a North American one with species lacking basal-rosettes. Each clade has morphological and geographical patterns that could be related to edaphic factors, tectonic events or changes associated to dispersal that we discuss as possible explanations for the similar diversity in each. The direction of the colonization of America is proposed north to south, with a disjunction by a single event and probably long-distance dispersal. According to this proposal and additional results, corolla color changed from white to blue after the colonization of South America. We discussed our data in light of possible process that explained the patterns found, following the recent knowledge in Boraginaceae and groups with similar patterns.

**Keywords**: American amphitropical disjunction, Boraginales, circumscription, phylogeny, morphology, taxonomy

## Introduction

*Antiphytum* DC. ex Meisn. (subfamily Echiochiloideae, see Chacón et al., 2016, Boraginaceae) is an American genus with a disjunct distribution, whose messy taxonomic history and apparently unlike morphology between the North and South American species, has suggested a non-natural group.

*Antiphytum* has a complicated taxonomic history, which begins with its publication in Meisner's Genera (1840) without the corresponding nomenclatural changes over the four species that were included in the new genus: "***Anchusa oppositifolia*** **HBK** [correct name *Plagiobothrys linifolius* (Willd. ex Lehm.) I.M.Johnst., according to Holstein and Weigend 2016]; ***A. cruciata*** **et** ***stoechadifolia*** **Cham.** [correct name *Antiphytum cruciatum* (Cham.) DC., lectotype of the genus (Johnston 1923) and *Antiphytum stoechadifolium* (Cham.) DC., respectively]; and ***A. Mexicana,*** **DC. ap. Moc. ic. Fl. Mex. ined.**". This first publication of the genus comprised two parts, a brief description of the new genus in a diagnostic table (Meisner 1840, i, p. 280), and the "Commentarius", with the previously listed four species (here transcribed in the same order and typing of the original work) and its bibliography (Meisner 1840, ii, p. 188).

Except for the Sessé and Mociño species from Mexico, the new genus *Antiphytum* was composed mainly by South American species, but only *Anchusa cruciata* and *A. stoechadifolia* further kept into *Antiphytum* through the time. On the other hand, it is very likely that the species that originated the new genus was erroneously cited by Meisner, because according to the posthumous publication of Sessé and Mociño (1887), *Anchusa mexicana* Sessé and Moc. bears alternate phyllotaxy, which does not coincide with the intention expressed in the name *Antiphytum* (*"Genus jam foliis oppositis (unde nomen) insigne"*, Meisner 1840, ii, p. 188; and "folia opposite (unde nomen)", De Candolle 1846, p. 121). Rather the Sessé and Mociño's iconography no. 288 (Mociño and Sessé 2010), corresponding to *Symphytum fruticosum* Sessé and Moc. (Sessé and Mociño 1887; see De Candolle 1846, p. 121), must be the correct one and whose new combination *Antiphytum Mexicanum* DC. [correct name *Euploca mexicana* (DC.) M.W.Frohl. and M.W.Chase, according to Frohlich et al. 2020] was given by De Candolle (1846) in the first formal synthesis of the genus.

De Candolle (1846) made the nomenclatural combinations of the South American Meisner's *Antiphytum* species, added an additional North American species (*A. heliotropioides* A.DC.), and two South American ones, from which "*A. tetraquetum* (Cham.) DC." [actually *Thaumatocaryon* *tetraquetum* I.M.Johnst., Johnston 1924] was considered as a doubtful species, but later remained in *Antiphytum* during a large time.

Fresenius' Flora of Brazil (Fresenius 1857) treated *Antiphytum* comprised by *A. cruciatum*, *A. tetraquetum*, and *A. stoechadifolium*, which would be the circumscription proposed by Bentham and Hooker (1876) for a segregated genus *Antiphytum*. The North American species published, either as *Antiphytum* or within other genera, such as *Eritrichium* Schrad. ex Gaudin, *Krynitzkia* Fisch. and C.A.Mey. or *Lithospermum* L. (see Martens and Galeotti 1844; Torrey 1859; Watson 1883; Rose 1890) were included later within the genus *Amblynotopsis* J.F.Macbride (Macbride 1916).

Johnston (1923) rejected the segregation of *Antiphytum* and added three additional North American species. He suggested that corolla color (blue for the South American taxa vs white for the North American ones) and phyllotaxy (through opposite vs mainly alternate, respectively) were scarcely generic differences to validate the segregation. Johnston's contribution (1923) is a cornerstone on *Antiphytum* taxonomy, from which derives our nowadays concept of the genus and the lectotypification that validates the name and join it to the South American species, although currently, North America is the richest area. In posterior works, Johnston changed the genus circumscription, getting out *A. tetraquetum* for the first time since De Candolle's work (1846) and recognized only two South American species, *A. cruciatum* and *A. stoechadifolium* (Johnston 1927). He even questioned himself the genus concept (Johnston 1927, 1957), considering that it was still subject to revision, naming “true *Antiphytum*” to the South American species and applicable the generic name *Amblynotopsis* to the North American ones. In the last work dealing with the genus Johnston (1966) kept *Antiphytum* as a disjunct genus, opposing to Brand (1929, 1931) that proposed that *Antiphytum* must not even to exist, mainly due to its mess origin.

No molecular work has tested the monophyly of *Antiphytum*. The efforts focused from order to subfamily level have included only one or two (e.g., Längstrom and Chase 2002; Längstrom and Oxelman 2003; Nazaire and Hufford 2012; Weigend et al. 2013, 2014; Cohen 2014; Chacón et al. 2016; Luebert et al. 2016) or even no *Antiphytum* species (e.g., Weigend et al. 2010), and no studies have included species from South America. Since Längstrom and Chase (2002) proposed tribe Echiochileae, currently subfamily Echiochiloideae (Chacón et al. 2016), this clade has been subsequently retrieved with high support and includes *Antiphytum*, *Ogastemma* Brummitt and *Echiochilon* Desf., being *Antiphytum* and *Ogastemma* sister groups (e.g., Längstrom and Oxelman 2003; Weigend et al. 2013; Cohen 2014; Chacón et al. 2016). Whereas the position of *Antiphytum* within the subfamily seems robust, the relationships among the species of the unique American genus of Echiochiloideae are poorly known. *Ogastemma* is monotypic and its unique species *O.*

*pusillum* (Coss. & Durieu ex Bonnet & Barratte) Brummitt is distributed in North Africa and Arabian Peninsula and recent records had been done in Iraq (Mousa and Shahatha 2021). *Echiochilon*, mainly also from North Africa and Arabian Peninsula, was the subject of study in Längstrom and Oxelman᾽s (2003) work, although the phylogeny was built only with ITS as molecular marker and morphology. Figure 1 shows distribution of subfamily Echiochiloideae based in Lönn (1999) and our observations.

Overall, Echiochiloideae requires a taxonomic and phylogenetic update, with a sampling that incorporates *Antiphytum*, the genus more poorly sampled until now and that it is the focus of the present work. With the recent discovery of new species from North and South America (Higgins and Turner 1983; Mendoza-Díaz et al. 2018, 2020a,b), questions involving monophyly, circumscription and biogeographic history of *Antiphytum* need to be encompassed. In order to cover these objectives, we sampled the currently known species according to the taxonomic updated work carried on by the first author for the molecular phylogenetic reconstruction, and morphological features were observed from herbaria collections and material collected during fieldwork. We presented the first insights over the phylogeny, diversification and geographic patterns of *Antiphytum* based on a broad morphological and geographic revision plus the retrieved topologies from molecular phylogenetic analyses.

## Material and methods

### Morphological study, field work and terminal selection

As a first step, taxa that once were included under the genus, but recent studies indicated them out of *Antiphytum* were excluded. The morphological study was based on the revision of protologues, bibliography and herbarium specimens. Herbarium specimens (including types) were examined directly or through loans from ARIZ, ASU, B, BM, CAS, CHAPA, CIIDIR, ENCB, F, FCME, G, HCIB, HUH, IBUG; IEB, K, MEXU, MO, NMC, NY, P, R, RSA-POM, TEX, UAT, UC, and US (Thiers continuously updated). Online specimens from AAH, BR, G, G-DC, GH, K, UC, US, and W were also revised. In order to broaden the sampling, especially for taxa with old records, few specimens, or major problems with its geographic distribution and taxonomic circumscription, field work was carried on different localities of Mexico, Uruguay, and Brazil.

A morphological concept of the genus was achieved through this taxonomic revision, which allowed the recognition of *Antiphytum* among Boraginaceae by the combination of the following characters: suffruticose perennial as growth-form; corolla radially symmetrical surpassing the calyx;

presence of faucal appendages; fruit not enclosed into the calyx, rather an extended calyx subtending the fruit; gynobase shape from flat to triangular; cicatrix in proximal half of eremocarp, not exceeding the middle zone of the ventral face. Subsequently, every species was defined by diagnostic traits.

As a consequence of these preliminary steps, three new species were described (Mendoza-Díaz et al. 2018, 2020a,b) and one species resurrected that derived in a new combination (Mendoza-Díaz et al. 2022). Updating Johnston's works (1923, 1927, 1966), that recognize nine *Antiphytum* species with only two of South America (including as heterotypic synonyms two Brand's species described as *Amblynotopsis* (Brand 1926): *A. ehrenbergii* Brand and *A. humilis* Brand), a total of 13 species, four from South America, were achieved (Table 1). Based on this taxonomic revision, we recruited samples for palynological observation and molecular analysis for almost all the resulting 13 species to test with phylogenetic reconstruction, in an illumination reciprocal process.

For the remaining genera of Echiochiloideae subfamily, herbaria specimens were revised for *Ogastemma pusillum* and some *Echiochilon* species, such as *E. collenettei* I.M.Johnst., *E. fruticosum* Desf., *E. johnstonii* Cufod., *E. longiflorum* Benth., and *E. persicum* (Burm.f.) I.M.Johnst.

### Palynological study and fruits observation

Whole flowers, sampled from fresh and herbarium collections of *Ogastemma pusillum* and eight *Antiphytum* species (*A. berroi*, *A. brevicalyx, A.caespitosum*, *A. cruciatum*, *A. heliotropioides*, *A.hintoniorum*, *A. floribundum*, and *A. peninsulare*) were macerated while hydrating in a water bath. Macerated material was dehydrated with acetic acid to concentrate it through centrifugation, and acetolyzed by Erdtman (1960) method with modifications from Laboratorio de Paleopalinología of Instituto de Geología, UNAM (Martínez-Hernández personal comm.). Pollen was mounted in glycerinated gelatin and some samples deposited in the palynological collection of Instituto de Geología, UNAM. Samples were analyzed in an optic microscope and photographed with a Zeiss AxioCam.

Mature eremocarps (nutlets o mericarps, terminology *sensu* Hilger 2014) from *Ogastemma pusillum*, some *Echiochilon* species, and all the *Antiphytum* species (Table 1) samples were observed 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 Laboratorio de Microscopía y Fotografía de la Biodiversidad (II) of Instituto de Biología, UNAM. SEM photographs were also taken (Hitachi SU1510, Hitachi Ltd., Tokyo, Japan) at Laboratorio de Microscropía Electrónica y Fotografía de la Biodiversidad (I) after fruits samples were critical-point dried and mounted on stubs with common double stick carbonated tape, and coated with gold to 20 µÅ during two minutes in a sputter coater (Quorum Q150OR ES).

### DNA extraction, PCR amplification and sequencing

Total DNA was isolated from herbarium specimens or field-collected leaves dried in silica gel using the CTAB extraction method of Doyle and Doyle (1987) modified by the addition of 2% of polyvinylpyrrolidone (PVP) to the extraction buffer. Several individuals from 19 taxa of Echiochiloideae (the sequences will be uploaded to GenBank) were newly sequenced for five molecular markers: nuclear internal transcribed spacer (ITS) using the primer pairs ITS4 and ITS5 (White et al. 1990), and the plastid markers for chloroplast *ndh*F–*rpl*32 intergenic spacer (newly designated, repl32.4R GAAGTACGYTTTTTTGGAACTGC; ndhF.40R.Bor. ACATAGGAACTGTWAGTGGAAG); *trn*L–F intergenic spacer (pairs C and F, Taberlet 1991); *rps*16 intron (pairs F and R2, Oxelman et al. 1997), and *mat*K gene (partial maturase gene, pairs 2F and 3R, Sang et al. 1997). Final marker selection was based on the bibliography on Boraginales for genus (e.g., Moore and Jansen 2006; Cohen and Davis 2012; Nazaire et al. 2014), order (e.g., Weigend et al. 2014; Luebert et al. 2016, Chacón et al. 2016), or subtribe level (e.g., Pourghorban et al. 2020), and amplification success. Previously ITS and *ndh*F–*rpl*32 matrix from Mendoza-Díaz et al. (2020a) was expanded to include newly generated sequences (60 for ITS, 28 for *ndh*F–*rpl*32, 111 for *trn*L–F, 112 for *rps*16, and 65 for *mat*K). Also, to the data matrix of every separate marker we added available GenBank sequences of both Echiochiloideae and other Boraginaceae subfamilies to function as outgroup (68 for ITS, 41 for *ndh*F–*rpl*32, 211 for *trn*L–F, 174 for *rps*16, and 128 for *mat*K).

PCR conditions were the same as Mendoza-Díaz et al. (2020a), as well the amplification programs for ITS and *ndh*F–*rpl*32. Both *trn*L–F region and *rps*16 intron were amplified following Moore and Jansen (2006) program for *rps*16. Initially, *mat*K partial gene was difficult to amplify for North American species of *Antiphytum*, but it was successful when it was followed the program of Nazaire and Hufford (2014) for the marker.

PCR products were sent unpurified to LaNABio (IBUNAM) for sequencing with an ABI 3730xL sequencer (Applied Biosystems, Waltham, Massachusetts).

### Sequence assembly, alignment, coding of microstructural characters and phylogenetic reconstruction

All sequence chromatograms were edited, assembled, and manually aligned and annotated with PhyDE 0.9971 (Müller et al. 2010). Regions of mutational hot spots were removed prior to phylogenetic analyses. Only for the parsimony analyses (MP), microstructural characters (gaps, simple sequence repeats or SSRs, and inversions) were added at the end of every marker matrix. Each microstructural character was named including the locus, type of character, and the corresponding aligned positions. Gaps were coded using simple indel coding according to Simmons and Ochoterena (2000) and microstructural characters were coded according to Ochoterena (2009).

Resulting matrices did not match each other either in the number or identity of the terminals, even for the Echiochiloideae taxa, because the sampling was not equalled successfully amplified for all the markers. Concatenated matrices of microstructural character and nucleotide for every individual marker were analysed under MP 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 (not shown). Ambiguously supported nodes were collapsed and identical trees removed. 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 on to the consensus tree using WinClada. We considered support to be lacking when the values were below 64% (Farris et al. 1996).

Separate analyses were performed to evaluate the position and monophyly of the putative species of Echiochiloideae in the recovered topologies, in order to strengthen the hypothesis of the concatenated five markers matrix, in which individual sampling by species was reduced, both ingroup and outgroup.

Concatenated five markers matrix (hereafter 5-loci matrix) included 43 terminals, 12 *Antiphytum* taxa (five taxa with only one individual), one *Echiochilon* taxa (one individual), and *Ogastemma pusillum* (one individual). As outgroup we used concatened GenBank sequences from *Lithospermum californicum* A.Gray and *Echium vulgare* L. Although sequences of these species from every marker came from different vouchers, we can rule out mixing of information, as these species were recovered in the same phylogenetic position in the separate analysis and suffice the outgroup function.

MP analyses were performed using the 5-loci matrix with nucleotides and microstructural characters with the described procedure. For Bayesian inference (BI) and maximum likelihood (ML) analyses we used only the nucleotide 5-loci matrix. Substitution models for each DNA region were selected based on the Akaike information criterion (AIC) implemented in jModelTest 2 (Darriba et al. 2012) using the following parameters: 203 substitution schemes, +F base frequencies, +I and +G rate variation, nCat=4 and SPR tree search. Every marker obtained a different model of molecular evolution: GTR+G for ITS; GTR for *ndh*F–*rpl*32; GTR+I for *trn*L–F; HKY for *rps*16, and GTR+G+I for *mat*K. ML analysis was performed with RaXML (Stamatakis 2014) using raxmlGUI 2.0 (Edler et al. 2021) implementing the "ML+thorough bootstrap" option and 1000 ML bootstrap replicates for estimating branch supports. We considered nodes with weak (50–70%), moderate (71–80%) and strong support (81–100%) following to Ortíz-Rodríguez et al. (2018).

BI analyses were conducted using MrBayes v.3.2.7 (Ronquist et al. 2012) with the following parameters: number of Markov Chain Monte Carlo generations=1,000,000, sampling frequency=1000, burn-in=100,000 generation. The burn-in was determined using Tracer 1.7.1 (Rambaut et al. 2018). We considered a clade to be well supported if posterior probabilities (PP) were $\geq 0.95$ (according to Alfaro and Holder 2006), and not supported when PP $< 0.85$.

Phylogenetic trees were visualized using FigTree 1.4.4 (Rambaut 2018). MP and BI consensus as well as the ML tree were graphically summarized, keeping only the supporting nodes by at least one inference method.

### Distribution Maps

Maps were produced using QGIS Brighton (2.6.1). The distribution of the species was obtained from specimen labels or from data obtained directly in the fieldwork, using geographic coordinates when available. Specimens without coordinates were georeferenced using Google Earth 7.1.8.3036 (32 bit).

## Results

### Morphology

At the level of macromorphology, *Antiphytum* exhibits two well-defined groups: species bearing a non-marcescent basal rosette and species that lack it. While the first group is exclusively North American, species without basal rosettes are found both in North and South America. The rosette group includes *A. floribundum*, which is morphologically and geographically distant from the remaining three Mexican rosette-forming species *A. caespitosum*, *A. paniculatum* and *A. parryi* (from here, "eastern rosette species").

Cauline leaves are totally opposite in the South American species and in *A. hintoniorum* from North America; the remaining North American species have alternate phyllotaxy *throughout* the stem or branch, but sometimes are opposite at the base. Midvein is visible mainly on the adaxial side of the leaves, but secondary venation is besides only visible in two species, *A. brevicalyx* and *A. floribundum*.

Glandular-capitate trichomes were solely found in *A. berroi*, *A. cruciatum*, and *A. stoechadifolium* from South America; and *A. brevicalyx*, *A. geoffreyi*, and *A. peninsulare* from North America. They are wider distributed on young branches, leaves and leafy organs such as bracts (pherophylls), flower-subtending bracts, and calyx in *A. brevicalyx*, *A. geoffreyi*, *A. peninsulare*, and *A. stoechadifolium*. This kind of trichomes were more difficult to observe in *A. berroi* (only on the calyx) and *A. cruciatum* (on the branchlets), probably because they are deciduous in the latter species, since not in all the specimens were possible to find. *Ogastemma pusillum* also has this kind of indumentum on the calyx and branchlets.

The inflorescence in *Antiphytum* ends in a flower (monotelic) forming a terminal homocladic or heterocladic determinate thyrse (thyrsoid) with dichasial division. Except for *A. charruasorum* and *A. hintoniorum*, the basic unit of the inflorescence is the bracteate boragoid. Boragoids ranges from less of 10 mm long in *A. parryi* to 200 mm in *A. heliotropioides*. In *A. charruasorum* the inflorescence has only the terminal flower (without flower-subtending bracts) and no branch becomes a boragoid in every dichasial division subtending by two opposite pherophylls. In *A. hintoniorum* there is a reduced terminal dichasial cymoid to a one-flowered cymoid (only the terminal flower of each unique dichasial division). It could be interesting to highlight the arrangement of the boragoids in several species. For instance, in the blue-corolla species from South America there are ca. 3–4 paired boragoids at the previous nodes before the terminal dichasial division of the last node, which bears the terminal flower and the last paired boragoids. Every paired boragoids arise from the leaf axils of a pair of opposite pherophylls. In the North American species, boragoids have alternate arrangement and arise from the leaf axil of one pherophyll. *Antiphytum geoffreyi* has the boragoids more proximal to the base of the plant, similar to *Ogastemma pusillum*, in contrast with the rather distal position common in the genus. The species with basal-rosettes have distal inflorescences bearing well-defined unbranching flowering stems, but in *A. floribundum* the thyrsoid is heterocladic, such as in the non-rosetted species *A. brevicalyx*. Both *A. heliotropioides* and *A. peninsulare* has foliose metaxyphylls (or intercalated sterile bracts) preceding the terminal flower (which apparently is concaulescent in *A. heliotropioides*) of every dichasial division; therefore, boragoids may appear to be isolated and axillary.

Regarding corolla color, except for three exclusively South American species with blue corollas (*A. berroi*, *A. cruciatum*, and *A. stoechadifolium*), the genus exhibits mainly white corollas in all the North American species and in one of Uruguay (*A. charruasorum*). The presence of faucal appendages (or fornices) is usual, but not the rule, since *A. floribundum* and *A. paniculatum* lack of them. Fornices surfaces are often papillose, but hirsute trichomes are found in *A. charruasorum*. Calyx lobes are divided almost to the base, but except in *A. brevicalyx*, they always are equal or longer than the corolla tube. The corolla is hypocrateriform (rotate) when fornices are present, but *A. paniculatum* has also a rotate corolla, while rather it is infundibuliform-campanulate in *A. floribundum*. Corolla diameter ranges from 2–4 mm of diameter in *A. parryi* to 10–12 mm in *A. peninsulare*; other species with large corolla diameter are *A. hintoniorum* (8–9 mm) and *A. charruasorum* (7.5–8 mm). Flowers are sessile or subsessile (pedicel 0.2–2.7 mm in length), but in *A. heliotropiodes* are pedicelate (up to 12 mm in length).

Pollen traits are common in *Antiphytum* and shared with *Ogastemma*: isopolar subprolate (in *Antiphytum* vs. oblate spheroidal in *Ogastemma*) tricolporate monads, ectoapertures with colpal membrane verrucate and endoapertures with converging transverse costae, meridional edges lacking or indistinct; large polar area, polar outline circular (*Antiphytum* and *Ogastemma*) to slightly concave (*Antiphytum*); tectate exin with perforations and columellar structure and microreticulate pattern (Fig. 2a). Although *Ogastemma pusillum* along with *A. berroi* and *A. cruciatum* have the largest polar area of all the sampling, the two South American blue corolla species have overall the monads with both the largest equatorial diameter and polar axis. The monads in the North American species varied in size from the smallest *A. heliotropioides* to the larger *A. brevicalyx* (Fig. 2a).

In contrast, eremocarps discriminates very well among species of *Antiphytum* (although in the eastern rosette species group, eremocarps are very similar) and with respect to *Ogastemma pusillum*

(Fig. 2b). Diagnostic features are: 1) form and size; 2) number; 3) ornamentation of the pericarp; 4) modification of the ventral keel such as projection-like stipites or a kind of bump; 5) position and area of the cicatrix; 6) projection of the cicatrix tissue. Although the predominant eremocarp form in the genus is ovate (also in *Ogastemma pusillum*), rather it is clearly triangular in *A. geoffreyi* and *A. peninsulare*; the size ranges from the biggest eremocarps in *A. charruasorum* (up to 2.5 × 2.0 mm) to the more reduced in size such as in *A. floribundum* (up to 1.2 × 1.0 mm); *A. cruciatum* (up to 1.3 × 1.0 mm); *A. parryi* (1.35 × 1.0 mm); *A. paniculatum* (average 1.3–1.5× 1.0 mm, but the smallest measured eremocarp 1.17 × 1.0 mm) or *A. caespitosum* (1.5-1.7 × 1.2-1.4 mm). While from South America *A. charruasorum* has the biggest eremocarp, from North America *A. peninsulare* and *A. hintoniorum* have the biggest sizes with 2.0–2.3 × 1.2–1.6 mm and 1.8–2.0× 1.2–1.4 mm, respectively, close to those in the South American species *A. stoechadifolium* (1.8–2.0 × 1.4–1.6 mm).

Usually there are four eremocarps per fruit, but reduction by abortion diminishes the number mainly in *A. caespitosum* (until two), *A. brevicalyx* (generally 2–3), to the extreme reduction to one (rarely two, but one almost abortive) in *A. hintoniorum*.

Pericarp ornamentation varies in *Antiphytum* from tuberculate (or verrucate), papillate (or scabrate)-echinate, tuberculate-papillate (or verrucate-scabrate), to striate. Tubercules (or verrucas) could be aligned and form a striate pattern in some cases. Verrucate surfaces are characteristic to all the basal rosette-species and to the South American *A. cruciatum*; scabrate-echinate surfaces are found only in *A. hintoniorum*, while striate surfaces are privative of *A. berroi*. The remaining species of the genus, both from North and South American, have verrucate-scabrate ornamentation. *Ogastemma pusillum* has verrucas and a scale-like pattern in the surface.

Three straight keels on the ventral side of the eremocarp could be present or not: two lateral (basal) and one ventral (apical), which is always present. This apical keel could be modified forming a projection-like stipites in all the blue corolla species from South America, or a kind of bump only in *A. floribundum* (Fig. 2b). While the cicatrix of the eremocarp varies in position and form in correspondence with the shape of the gynobase, the cicatrix area keeps correspondence with the presence of the projection of the ventral keel, since the smallest cicatrixes are found in the aforementioned species. Position of the cicatrix is necessary basal in the species with projection-like stipites, suprabasal in *A. heliotropioides* and *A. hintoniorum*; inframedial in *A. charruasorum*, *A. geoffreyi* and all the basal-rosette species; and medial in *A. brevicalyx* and *A. peninsulare*. Only in *A. charruasorum* there is a projection of the cicatrix tissue resembling a stipe, which could be an elaiosome (Fig. 2b). In *Ogastemma pusillum*, the cicatrix extends from the middle to the infrabasal part of the eremocarp.

Finally, gynobase columella ranges in *Antiphytum* from flat to triangular, whereas is almost narrowly triangular (length/width relation 5:2) in *Ogastemma*. Blue corolla species from South America have flat to slightly raised (1:6) gynobases, but also they are found in *A. floribundum* and *A. hintoniorum* from North America. Very shallowly triangular (1:3) are found in *A. charruasorum*, and *A. stoechadifolium* from South America and *A. floribundum*, which besides could has shallowly triangular gynobases (1:2). Broadly triangular gynobases (6:5) are found in *A. caespitosum* and *A. heliotropioides*; but also very broadly triangular or deltate (1:1 and 5:6), such as in *A. paniculatum*, *A. parryi* and *A. peninsulare*. Finally, *A. geoffreyi* has a triangular columella (2:1).

## Distribution

*Antiphytum* is an American genus with species mainly with restricted distributions (Figs. 3,4). In some cases, known occurrences are from several adjacent localities with no more of 100 km of distance in total (e.g., *A. hintoniorum* and *A. peninsulare*); two or three disjunct localities in a determinate political state (e.g., *A. brevicalyx*, *A. geoffreyi*) or narrow region (i.e. no more of 100 km long (e.g., *A. berroi*¸ *A. charruasorum*); or only currently known of one reliable locality (e.g., *A. paniculatum* and *A. stoechadifolium*). *Antiphytum caespitosum* occurs mainly in Oaxaca state, Mexico and adjacent regions from the surrounding states; its distribution seems rather discrete, except for a southern locality disjunct to the main concentration of populations (Fig. 3). This species is sympatric in several localities with *A. parryi*, whose distribution range is the widest for the eastern species with basal-rosettes, and for many species of the genus. *Antiphytum parryi* distribution is only surpassed by the distribution ranges of *A. floribundum* and *A. heliotropioides*, which run from southern USA to central Mexico by the Sierra Madre Occidental or to southern Mexico by the Sierra Madre Oriental, respectively; both also in the Altiplano Mexicano. Except for these species, seven North American species of *Antiphytum* are endemic from Mexico (Fig. 3).

In South America, nearly all the genus is either endemic or restricted to Uruguay, according to the updated geographical information (Fig. 4). *Antiphytum cruciatum* has the widest distribution, covering northern Uruguay and the entire longitudinal extension of Rio Grande do Sul State, in Brazil. Although *A. berroi* has historical records from the same Brazilian state, no current records have been made since then.

The genus exhibits in North America a marked edaphic affinity to calcareous and gypsum soils, being *A. hintoniorum* a gypsophile, *A. peninsulare* a gypsocline and, except *A. floribundum*, that never has been collected in gypsum, all are gypsovags (Meyer, 1986). Species gypsovags occurs out of gypsum only in calcareous soils, while *A. peninsulare* also grow in soils of volcanic origin, from which *A. floribundum* is restricted. The South American species also has preference for impoverished soils, and for *A. charruasorum* is known its preference to the calcareous ones.

## Phylogenetic Datasets

Among the commonly used markers in Boraginaceae the spacer *trn*S–G could not be used in this study because of failure to amplifying it. Basic data set characteristics of the five loci used are provided in Table 2. Among the five amplified loci, *mat*K and *ndh*F–*rpl*32 had the fewest available GenBank sequences for Echiochiloideae. Among the markers that successfully amplified, ITS was the easier to amplify, but most difficult to align. The intron *rps*16 was easy to amplify and relatively easy to align, but required a careful assessment of ambiguous alignment due to the relatively high number of SSRs, hotspots and complex gaps. The intergenic spacers *trn*L–F and *ndh*F–*rpl*32 behave quite different; *trn*L–F behaved similar to *rps*16, while *ndh*F–*rpl*32 was problematic to amplify requiring many trials, but it was one of the easier to align, including the inversion in one of the outgroups. The coding region of *mat*K in general was easy to amplify for the South American species, but for the North American species it required an adjustment of the PCR program; as expected, it was easy to align.

Because the terminals were combined only avoiding missing regions, the matrix with most terminals was ITS+*trn*L–F+*rps*16 with 113 terminals (102 from Echiochiloideae). In contrast, the combined matrix with the four chloroplast markers (*mat*K+*ndh*F–*rpl*32 +*trn*L–F+*rps*16) had only 43 terminals (41 from Echiochiloideae); all of those terminals had sequences for ITS. Except for terminals of *A. stoechadifolium*, all *Antiphytum* species were available in each dataset, with good geographic representation for the more widely distributed species in North America. The combined matrix with all the regions had 594 DNA microstructural characters (Table 2; Appendix 1), of which only 29 were potentially parsimony informative, mainly from gaps, plus seven that were informative in the original matrices (Table 3).

## Tree topologies

In all the analyses the earlier divergent clades are well resolved and supported while the tips of the trees are less resolved in greater or less degree depending on the marker. The combination of

ITS+*ndh*F–*rpl*32 has more terminals and retrieved the same topology obtained with the 5-loci dataset, although the support values may vary among them. Similarly, *mat*K+*ndh*F–*rpl*32 also recovered the 5-loci topology with differences in some of the tip branches. As expected, the chloroplast markers contribute proportionally more to the resolution of the deeper branches, while ITS proportionally resolves better the grouping within a species (Fig. 5). All methods retrieve congruent trees with the consensus of the MP trees for the 5-loci trees (with more or less resolution) and therefore the consensus of these trees was used to map the support values obtained in the PP, MP-JK, ML-BS analyses (Fig. 6).

The Echiochiloideae subfamily was retrieved as monophyletic in all analyses except for *rps*16. Both genera sampled with more than one species within Echiochiloideae, *Antiphytum* and *Echiochilon*, were recovered as monophyletic. The genus *Echiochilon* was the earliest divergent within the subfamily, although some of the sampled species were not recovered as monophyletic. Consistent with its phylogenetic position, *E. persicum* stands out by its branch lengths (number of changes), with 111 changes (in the 5-loci tree; Fig. 5).

The phylogenetic position of *Ogastemma* is not consistent among the molecular markers, but all sampled individuals of this monotypic genus formed a clade in all cases. The genus is retrieved as sister to *Antiphytum* with the intergenic spacers *ndh*F–*rpl*32 and *trn*L–F, with the chloroplast combined matrix and with the 5-loci combined reduced matrix. With the other markers (ITS, *mat*K, and *rps*16), as well as with ITS+*trn*L–F+*rps*16, *Ogastemma* appears nested in different positions within *Antiphytum*. In the 5-loci consensus, *O. pusillum* has a similar branch length (17 changes) as in any given species of *Antiphytum* (12.5 in average; Fig. 5).

All loci were congruent supporting the monophyly of *Antiphytum*, including both the North and South American species. Three clades appear in at least one marker analysis –i.e. some locus or combined loci datasets retrieved at least one or none of these clades–: 1) a North American clade with all the basal-rosetted species (NA-rosette clade); 2) a North American clade without-rosette species (NA-clade), and 3) a clade of the three sampled South American species (SA-clade). None clade is retrieved with ITS, *rps*16 and the combination *trn*L–F+*rps*16; in contrast, the three clades appear at one time only with some combined datasets. In none of the consensus, the North American species form a monophyletic group. The resulting polytomy among these three clades can be caused by conflict or by lack of information, depending on the set of loci. These three clades appear in the 5-loci hypothesis (Fig. 6), no matter the method of reconstruction. However, the ML suggests a sister relationship between the SA-clade and the NA-rosette clade, but this relationship lacks statistical support. Whereas the NA-rosette clade and the SA-clade are supported in all the 5-

loci trees (PP, MP-JK, ML-BS), the NA-clade was supported only by PP in the Bayesian analysis and weakly supported by ML-BS in the ML tree (Fig. 6). This clade has weak or lacks statistical support (ML-BS or MP-JK, respectively) in the relationships among species (Fig. 6).

In the 5-loci analysis (Fig. 6), all species in *Antiphytum* are supported as monophyletic, except for *A. parryi* that is paraphyletic with respect to *A. paniculatum*. Nevertheless, this is not the case for all the individual chloroplast marker analyses or their partial concatenations, where some species are not resolved (a polytomy includes more than one species) or they are retrieved as paraphyletic. In most chloroplast analyses *A. berroi* and *A. cruciatum* are unresolved, as well as *A. caespitosum*, *A. paniculatum*, and *A. parryi*. Two examples of paraphyletic species can be found in the *ndh*F–*rpl*32 analysis, with *A. peninsulare* paraphyletic to *A. geoffreyi* and *A. brevicalyx* paraphyletic to *A. heliotropioides*. The most problematic marker in this respect is *rps*16, that only retrieves two species as monophyletic. ITS provides an important contribution for supporting the monophyly of the species.

For the 5-loci trees (Fig. 6), in the NA-rosette clade, *A. floribundum* is the earlier divergent species, followed by *A. caespitosum*, and *A. parryi*+*A. paniculatum*. In the NA-clade *A. hintoniorum* (gypsum edaphic endemic) is sister to two clades with the remaining species, one including *A. heliotropioides* and *A. brevicalyx*, the other including *A. peninsulare* and *A. geoffreyi*. In the SA-clade the white-flowered species, *A. charruasorum*, is retrieved as sister to the subclade with the two blue-corolla species, *A. berroi* and *A. cruciatum*. In general, relationships among *Antiphytum* species retrieved from chloroplast loci are not incongruent with those from the nuclear locus (from ITS), despite the lack of resolution (polytomies) for several subclades in the individual plastid loci trees. The main discrepancy depicted by both sources of evidence is the relationship between *A. peninsulare* and *A. brevicalyx*, which are sister in the ITS analysis, but appear in different clades in all the chloroplast analyses: *A. peninsulare* is sister to *A. geoffreyi*, while *A. brevicalyx* to *A. heliotropioides*. When ITS is combined with *trn*L–F+*rps*16 (the dataset with more terminals) the ITS phylogenetic signal is recovered while in the 5-loci analysis the chloroplast signal is recovered.

In the 5-loci consensus there is a clear asymmetry in the different species branch lengths (Fig. 5): *A. paniculatum* (2), *A. parryi* (3), *A. caespitosum* (5), *A. berroi* (6) vs *A. floribundum* (16), *A. geoffreyi* (18), *A. hintoniorum* (24), *A. charruasorum* (29). The latest two are the earliest divergent species within their clades. The species with the broadest geographic distribution, *A. heliotropioides*, has the average number of changes (12), but notwithstanding provided mostly by chloroplast loci; within it, the branches are mostly supported by ITS.

### Microstructural characters

Microstructural characters were identified in all loci. For the 5-loci matrix, *mat*K only had one informative SSR while ITS had 14, 13 of them gaps (Table 3; Fig. 7).

Most of the microstructural characters are concentrated at the Echiochiloideae branch, mainly one-site or multiple-sites gaps in all regions but in *mat*K (Fig. 7; Table 3). A few others are distributed along the 5-loci consensus without a clear pattern (Fig. 7). Two multiple-sites gaps in the *ndh*F–*rpl*32 locus characterize *Echiochilon* (Table 3). In the same region, a SSR (GAAAGA) in the aligned position 90–95 characterizes to *Ogastemma*, but the 5-loci dataset keept only one terminal. The sister relationship between *Antiphytum* and *Ogastemma* is characterized by two microstructural characters in the *trn*L–F: a synapomorphic 16-site gap (aligned positions 550–566) and one diagnostic but not synapomorphic SSR (CCTTG in the aligned positions 181–185), i.e., the insertion of the SSR occurred once within Echiochiloideae, but it is also present in the outgroup. A 10-sites gap in the same region (aligned positions 1115–1124) is diagnostic but not synapomorphic of *Antiphytum* (Table 3).

Within *Antiphytum* no main clade is corroborated by microstructural characters, but some subclades and species are. One-site gaps in ITS supported both blue-corolla subclade and eastern rosette species subclade. On the other hand, the sister relationship between *A. geoffreyi* and *A. peninsulare* is characterizes by an SSR (CATTAA) in the *ndh*F–*rpl*32 region (aligned positions 343–348). From the same region, an SSR (AG in the aligned positions 688–690) is diagnostic for the eastern rosette species subclade, but it could be synapomorphic for the entire NA-rosette clade.

Although several one-site gaps contribute to support species, all but one individual in *A. floribundum* have a notoriously big gap (263 sites) in the *ndh*F–*rpl*32 region (aligned positions 500–762). Likewise, at the interior of the species, gaps provide evidence of divergence among individuals or populations. A prominent case is the 55-sites gap in the *trn*L–F region (aligned positions 977–1031) share by two individuals of *A. brevicalyx*.

SSRs are less frequent than gaps and in general were not detected within species, except for one SSR (AAAT) in the *trn*L–F region (aligned positions 1573–1577) characteristic of a subclade within *A. hintoniorum*; this SSR is nevertheless not unique to the subclade occurring also outside Echiochiloideae. This species is by the way the only one corroborated by a microstructural character in the *mat*K region, one SSR (CATTAA, aligned positions 343–348). In general, SSRs are less prone to homoplasy than gaps, but every rule has an exception: in the 5-loci matrix only one SSR (TTCA in the *rps*16 aligned positions 157-160) is highly homoplastic (Fig. 7).

## Discussion

### Morphology of *Antiphytum*

At first glance, morphological variation in *Antiphytum* might appear to be considerable, but it is rather a continuum or, better said, a set of modifications around a common morphological pattern. For instance, the inflorescence morphology varies around the number of pherophylls in each node, or the number of dichasial divisions, or the presence of foliose metaxyphylls, or the development or not of the boragoid in the dichasial division, but the inflorescence is in all the cases a terminal homocladic or heterocladic determinate thyrse. It would be bad enough that "the inflorescences from members of the same genus have to be associated with different inflorescence types" (Weberling 1989: 222).

Although some features of *Antiphytum* such as corolla color, projection-like stipites, or presence a basal-rosette seem like a gap among the variation distribution, these features are associated to clearly delimited ecological o geographic groups in the genus, and hence, they could be explained in this context. Moreover, in contrast with the other genera of the subfamily, the variation is low, with the exception of the monotypic *Ogastemma.* In *Echiochilon* extreme variation is observed (Lönn 1999): from perennial to, even rare, annual plants with alternate or opposite phyllotaxy; glandular or eglandular trichomes; corollas actinomorphics to zygomorphics, with white, yellow, pink, blue, reddish, bluish or yellowish color; and projection-like stipites in the eremocarp of *E. paciflorum* (Stocks ex Wight) Långström & M.W.Chase. This genus is however monophyletic (Långström and Oxelman 2003; our results), although some species deserve more work.

Within *Antiphytum*, we found that eremocarps are very useful for discriminating among species (Fig. 2b), but may be insufficient for discriminating some species of the eastern rosette species group, which could be related with recent divergence times. A curious pattern was found in the gynobase columella form, which southwards in the continent tends to be flat, but rather triangular northwards.

Pollen morphology is not informative to separate *Ogastemma* from *Antiphytum,* but other morphological features related with the flower, such as presence of faucal appendages on the corolla throat, the corolla surpassing the calyx, and the height at which the stamens are inserted, for instance, clearly differentiates the genera.

At a morphological level, we therefore agree with Johnston (1923) that corolla color and phyllotaxy were scarcely generic differences to validate the segregation of *Antiphytum*. The monophyly of the genus as recovered in this study supports this agreement.

## Phylogeny

The subfamily Echiochiloideae is monophyletic (Fig. 6), but some internal relationships and the corresponding taxonomic decisions are not free of conflict.

Even though several ITS sequences for *Echiochilon* were taken from Långstrom and Oxelman (2003) through GenBank, the addition of other samples inside and outside the genus resulted in the possibility that *E. johnstonii*, *E. longiflorum*, and *E. persicum* are not monophyletic. Otherwise, our results are comparable with those of Långstrom and Oxelman (2003).

This study raises awareness that the monotypic genus *Ogastemma* (*O. pusillum*) could be transferred to *Antiphytum* as not all the analyses support their sister relationship and contrary some place it well nested inside *Antiphytum*. Furthermore, the sister relationship would not prevent this taxonomic decision to be taken: the branch length of its species is like that in the many species of *Antiphytum*; fruit and inflorescence features have been compared to *A. geoffreyi* (Mendoza-Díaz et al. 2018); pollen morphology is almost identical to that in *Antiphytum* (see above). The fact that previous studies (Längstrom and Oxelman 2003; Weigend et al. 2013; Cohen 2014; Chacón et al. 2016) also recovered *Ogastemma* as sister to *Antiphytum* does not pose a strong argument because all those studies included very limited number of species of *Antiphytum*. Despite all this apparently overwhelming evidence, we consider that the 5-loci analyses are very robust and that the sister relation of *Ogastemma* and *Antiphytum* reflects the actual cladogenesis within the subfamily. Although the taxonomic concept of *Antiphytum* could be expanded to include the sister genus, there are several morphological differences among them. Within Echiochiloideae only *Antiphytum* has faucal appendages (see above); according to Lönn (1999) *O. pusillum* is annual, whereas all the species in *Antiphytum* are perennial; the otherwise diagnostic corolla surpassing the calyx, position of the stamen insertion within the corolla, and eremocarp cicatrix relative size and position in *Antiphytum* (see above) would have to become variable. At this time, we see no reason to destroy the clear diagnosis of *Antiphytum* and to disturb the nomenclatural stability of both genera: transferring the paleotropical *Ogastemma* to the neotropical *Antiphytum* would imply changing the current concepts that have been accepted in several regional floras.

Our study has the most comprehensive sampling in *Antiphytum* including 12 of the 13 recognized species as well as several representative individuals per species covering most of its distribution range and morphological variation. It corroborate the recognition of the three species recently described (Mendoza-Díaz et al. 2018, 2020a,b). More important, this is the first time that the South American taxa are included in a phylogeny. The effect of broadening the taxon sampling can be observed by comparing the results of Mendoza-Díaz et al. (2020a) with a sampling focused on the recognition of a new Mexican species using ITS+*ndhF*–rpl32. In that study, only the NA-rosette clade was retrieved, while the same loci combination retrieves three clades in our study. In the same way, the increasing of molecular markers with a subset of taxon sampling implemented with the 5-loci analyses, also retrieved those three clades. Notwithstanding, the reduced taxon sampling did not compromise the distribution range or morphological diversity representation for the genus. It is worth saying that it would have been ideal to have a more comprehensive loci coverage per sample, but even the fresh silica dried material posed amplification failures. The convergent results obtained when taxon representations or marker representation are privileged give us confidence that the backbone of the phylogenetic hypothesis, for Echiochiloideae in general and for *Antiphytum* in particular, has achieved stability. Nevertheless, it remains pending to further test the presence of the NA-clade and to solve its internal conflict depending on the marker; to confidently solve the relationships among the three major clades; to include *A. stochaedifolium* in phylogenetic analyses; and to further test the potential paraphyly of *A. parryi*.

The potential paraphyly of *A. parryi* raises the possibility of merging it with *A. paniculatum*. Nevertheless, as currently circumscribed, both can be differentiated by the flower size and the absence or presence of faucal appendages (see above). Although both species are vegetatively very similar, as is the case in the entire subclade, these two floral features are strongly associated to the potential pollinators and thus can represent important traits driving divergence (Stebbins 1970; Bolnick and Fitzpatrick 2007); even though both species have not reached reciprocal monophyly at the molecular level. The subclade, which also includes *A. caespitosum* is supported by chloroplast and ITS changes, but the species inside are only supported by ITS (Fig. 5). This is an indication that the species within this subclade have diverged recently (Baldwin et al. 1995; Drouin et al. 2008; Poczai and Hyvönen, 2010; Leliaert et al. 2014). The geographic distribution of *A. parryi* overlaps with that of the other species, but *A. paniculatum* is only known from a single precise locality within the Tehuacan valley, where it is sympatric with *A. parryi*. It is possible that a genetic reproductive barrier has not yet be fully formed between both species, as *A. paniculatum* is only supported by two ITS synapomorphies while *A. caespitosum* is supported by five ITS changes, including three synapomorphies.

In contrast to the limited phylogenetic information supporting species within the *A. caespitosum-A. paniculatum-A. parryi* subclade, also in North America, *A. hintoniorum* outstands for the long branch supporting it (24 changes) and the several changes (4 and 5) distinguishing its individuals (Fig. 5). These long branches can be explained by being a relative ancient lineage, by an accelerated evolution due to edaphic factors, by other species in the lineage becoming extinct, or by a combination of all or some of them (as discussed by Phillipe et al. 2011; Swenson 2011; Bromham et al. 2015; but also see Sauquet 2013). Because the molecular support for *A. hintoniorum* is coupled with morphological uniqueness and given that it is the only species in the genus restritect to gypsum outcrops, which are naturally exposed as fragmented habitats and are believed to accelerate mutations (Moore and Jansen 2007; Aguirre-Liguori et al. 2014; Moore et al. 2014), and because the species is geographically restricted it would not be striking that all mentioned factors influenced this branch length pattern (see below for further discussion).

With also a notoriously long branch (29 changes), but growing in South America, the recently described *A. charruasorum* (Mendoza-Díaz et al. 2020b) broke the paradigms for the genus because it has more in common with the NA species than with its sister group, unique with blue flowers. In addition to the flower color, there are many changes in the phenotypic traits among these species and the North American ones (see above). The long branch of *A. charruasorum* suggests that this species is more ancient than those with blue flowers (Swenson 2011), corroborating the polarity implied by the character optimization. Among the South American species, the phylogenetic position of *A. stoechadifolium* remains to be tested. Despite the relevance of this species for its historical role in the description of the genus, it is in general poorly known, with only two records from one reliable locality in Uruguay. Nevertheless, the morphological features in common with *A. berroi* (Mendoza-Díaz et al. 2022) suggest that they are probably sister.

### Microstructural characters

Few sequence-based studies analyze the evolution of microstructural characters, (e.g., Graham et al. 2000; Kelchner 2000; Borsch et al. 2003, 2005; Drábková et al. 2004; Wanke et al. 2007; Borsch and Quandt 2009; Tian et al. 2019; Song et al. 2022) using them to support and diagnose clades in the phylogenetic hypothesis (e.g., Torres-Montúfar et al. 2017; Torres-Montúfar et al. 2020). For Boraginaceae this is very seldom (Mendoza-Díaz et al. 2020a).

Despite the dramatic reduction in the number of potentially parsimony informative microstructural characters (from 594 to 29+7) in the 5-loci analyses, all markers contributed with at least some information and provided phylogenetic signal at several hierarchical levels, from the subfamily to the individuals (Fig. 7). Contrary to the general pattern in the phylogenetic signal coming from the nts (transitions/tranversions), where the chloroplast signal is proportionally more concentrated towards the deepest branches and ITS towards the tips (Fig. 5), both the nuclear and chloroplast microstructural characters are more or less evenly distributed along the cladogram (Fig. 7). While short gaps coming from chloroplast are more concentrated in the deepest branches of the cladogram, chloroplast SSRs are more concentrated in the shallow branches. ITS in contrast only has one SSR that is autapomorphic for *A. geoffreyi*. Most gaps in both ITS and chloroplast are of one or two sites, but chloroplast also has longer gaps, all synapomorphic except the *A. floribundum* autapomorphic 263 positions deletion in *ndh*F–*rpl*32 (positions 500–762; character 21 in Table 3; Fig. 7), which is not present only in one of the sampled individuals of the species. In this molecular region there is an SSR that characterizes *Ogastemma* (GAAAGA; character 15 in Table 3, Fig. 7).

Neither of the three main clades within *Antiphytum* is supported by microstructural characters. Within these clades, almost all SSRs are concentrated in the NA-clade, but only one of them is a synapomorphy supporting the conflicting relationship between *A. peninsulare* and *A. geoffreyi*. The broadening of this study sampling revealed that this SSR actually is a synapomorphy of the latter both species and not an autapomorphy of *A. peninsulare*, as was previously retrieved lacking *A. geoffreyi* samples (Mendoza-Díaz et al. 2020a). The other three SSRs are autapomorphic, and one more is even present at the level of individuals in *A. hintoniorum*. Worth mentioning is the *mat*K SSR (CTAGAA) in *A. hintoniorum* (character 25 in Table 3, Fig. 7) because, as in any coding region, indels in multiples of three result in a differential number of amino acids (Metzgar et al. 2000), in this case adding two. This is the only *mat*K microstructural change for the 5-loci matrix and coincides with the species inhabiting the most specialized habitat (it is a gypsophile) and that has one of the longest branches among *Antiphytum*. The type of character and hence its quality adds evidence to the already discussed scenarios (see above) that this species may represent an ancient lineage within *Antiphytum*, or it could reflect the distant gypsum outcrops where the species distributes. The single SSR outside the NA-clade is diagnostic of the eastern rosette species subclade, but this short insertion in *ndh*F–*rpl*32 (AG; character 22 in Table 3; Fig. 7) could have appeared earlier than the 263 positions deletion in the same marker in *A. floribundum*, thus being a synapomorphy of the entire NA-rosette clade. This clade hence is prone to microstructural mutations in this region of the marker.

We have not found information on microstructural characters in Boraginaceae apart from Mendoza-Díaz et al. (2020a). Therefore, this study also provides the first insights into molecular evolution in the selected markers and sampling of Boraginaceae (Appendix 1). We found that the *mat*K region appeared in Boraginales as a functional gene by its trimeric structural mutations (Wanke et al. 2007). Also, within Echiochiloideae no inversions were found in any of the marker matrices, but outside the subfamily. Describing microstructural characters is important to generate a more complete understanding of molecular evolution in *Antiphytum*, but also in Boraginaceae.

## Marker selection

In this study, we used some commonly used markers for assessing phylogenetic relationships at generic and higher levels in Boraginales. For example, to assess a phylogeny of *Tiquilia* Pers., Moore and Jansen (2006) used nuclear ITS; *rps16* intron and *mat*K and *ndh*F genes; in turn, Cohen and Davis (2012) a chloroplast set of five markers, including *mat*K, for *Lithospermum* L.; Nazaire et al. (2014) used ITS and a set of 12 regions of chloroplast, including *mat*K, *ndh*F–*rpl*32, *trn*L and *trn*L–*trn*F in their study on *Mertensia* Roth. For *Heliotropium* L., Luebert et al. (2011) used *rps*16, *psb*A–*trn*H, *trn*L–F, *trn*S–G, and ITS, almost the same set that Chacón et al. (2016) used in a broader sampling of Boraginaceae. In general, the combination of ITS with one of two chloroplast intergenic spacers, *trn*L–F or *trn*S–G, are commonly employed in phylogenetic analyses of genera of Boraginaceae (Cohen and Davis, 2012).

Pourghorban et al. (2020) recently published a phylogenetic study of subtribe Cynoglossinae with ITS+*rps*16+*trn*L–F, which was the combined matrix with most terminals in our analyses. As in our case, they obtained a relatively poor topological resolution in the backbone at subtribe level. As it was previously shown (Mendoza-Díaz et al. 2020a) the combination of ITS+*ndh*F–rpl32 did not introduce significant conflict and provided support at different hierarchical levels, as the chloroplast genes evolved more slowly than the nuclear genes (Drouin et al. 2008). In our study, this combination retrieved the three described clades with internal resolution, but lacked support (MP-JK) for the *Antiphytum* entire clade, the three main clades and some internal relationships. Adding *rps*16+*trn*L–F corroborated the topology from and generally improved the support values. In this case, the sum of the five markers allowed "secondary signals" that maximized congruence among different sources of data in the phylogenetic inference (Nixon and Carpenter 1996). This includes the combination of chloroplast and nuclear markers because there is no evidence to suspect reticulation among species and, hence, the phylogenetic signal from both types of markers is congruent (Bull et al. 1993; Johnson and Soltis 1998).

Despite the fact that *rps*16 is commonly used for phylogenetic analysis in Boraginaceae (e.g., Chacón et al. 2016; Chacón et al. 2019; Otero et al. 2019; Pourghorban et al. 2020) our results agree with those of Shaw et al. (2005 and references therein), who indicated that in general *rps*16 is not variable enough to resolve infrageneric relationships. In our analyses, *rps*16 showed the lowest resolution even at subfamily level, not yet recovering Boraginaceae subfamilies, although it did support some tribes. After all, this marker added, at the most, little contribution to branch support in the combined matrix and probably could have been avoidable. In contrast, *ndh*F–rpl32 was a very useful marker with a good variation level, in agreement with the findings of Song et al. (2022), although is seldom used.

To get a fully resolved phylogeny of *Antiphytum*, additional molecular information, including markers with an intermediate rate of evolution, is needed. Overall, we have achieved significant progress in our understanding of the group and the generation of much larger data sets, such as complete plastome data sets and/or hyb-seq data sets, can be guided by our results.

### Biogeography

The retrieved monophyly of *Antiphytum* has not only implications for its systematics, but also for biogeography. First, our results confirm *Antiphytum* as another example of an American genus with amphitropical disjunct distribution (AAD), in this case trans-NA eastern-SA AAD (Simpson et al. 2017), since it occurred from southern USA to southern Mexico in North America and southern Brazil and mainly northern Uruguay in South America (Figs. 3,4). Species distribution agrees with the ecological zones characteristics of the amphitropical regions, SM, SBSh, SBWh in NA, and SCf in SA, but extends into southern Mexico in the TM zone (abbreviations and classification according to Davis and Holmgren 2001). Although Boraginaceae (sensu Chacón et al. 2016) has the second highest number of AAD representatives among all plant families (19 examples according to Simpson et al. 2017, without count *Antiphytum*), the south American distribution of the genus *Antiphytum* is unusual in that most Boraginaceae taxa occur mainly in western South America (Argentina, Chile, Colombia, Peru, and Venezuela), while *Antiphytum* is the only native Boraginaceae in Uruguay (according to records on Flora del Conosur, Zuloaga et al. 2018 and personal observation). In southern Brazil, *Antiphytum* occurs along with *Moritzia* DC. ex Meisn. and *Thaumatocaryon* Baill., unique genera from Boraginae tribe, subfamily Boraginoideae, and exclusively South American (Weigend et al. 2010).

Second, with our data, we can propose an interpretation of the directionality of colonization of America from north to south. We argue that our evidence suggests a feasibly more recent blue-corolla subclade, in which occurred a corolla-pigment gain. Besides, two of the three possible scenarios for resolving the polytomy in the backbone of *Antiphytum* suggest that North American species do not share an immediate common ancestor. These hypotheses entail a single, more likely origin in North America and subsequent dispersal into South America, rather than the need for two independent North America colonization events. In addition with our results, the extinct taxa from late Tertiary assignable to Echiochiloideae in North America (not published, but the fossils were erroneously described as *Cryptantha* by Thomasson 1979) along with the node age of the crown *Antiphytum* estimate in 9.4 Ma (Chacón et al. 2017), support a posterior colonization of Uruguay and Brazil from North America, very likely by long-distance dispersal (LDD), such as many examples in Boraginaceae (Chacón et al. 2017 and references therein; Guilliams et al. 2017; Mabry and Simpson 2018; Otero et al. 2019). This interpretation of the direction in the colonization may be associated with the biggest size of eremocarp in *A. charruasorum*, which may be disadvantageous for dispersal, but more advantageous at early stages of colonization after a dispersal event (Schenk and Saunders 2017; Otero et al. 2019). Therefore, we discuss further based on this hypothesis.

Since *Antiphytum* is a small genus of suffruticose plants with all the South American species retrieved in one clade, no more than one LDD event is necessary to explain the colonization of South America. As in many cases, arises the question on how these plants reached this region, especially for *Antiphytum*, whose South American taxa are distributed outside of the general Pacific pattern of the others Boraginaceae genera, except for the endemics *Moritzia* and *Thaumatocaryon* (Weigend et al. 2010).

Diaspore morphology usually is associated with some dispersal syndrome (van der Pijl 1982), but some LDD could be caused by both standard and nonstandard mechanisms of dispersal (i.e. not linked with the vector associated to the diaspore morphology) (Higgins et al. 2003). Schenk and Saunders (2017) argued, though, that the distance magnitude (7000–13,000 km) in AAD species implies that diaspores are more likely to disperse through their adaptive structures than in a nonstandard dispersal. Based on their results, they also argued that despite the effectiveness of endozoochory at shorter distances, epizoochory is a more likely dispersal mode, and that anemochory should not be discounted a priori as a dispersal syndrome in AAD plants (Schenk and Saunders 2017). For long distances, adhesive diaspores (epizoochory) could travel further than diaspores carried internally (endozoochory) or dispersed by the wind (anemochory), since it depend over the detection and removal by the vector (Sorensen 1986).

Endozoochory has been postulated for *Moritzia* (Weigend et al. 2016; Chacón et al. 2017) and *Cryptantha* fruits (Mabry and Simpson 2018), with the latter example surmises the fruits (including the hispid calyces) being transported on the feathers of migratory birds flying south, perhaps in a single uninterrupted flight, as the best hypothesis (Mabry and Simpson 2018). Epizoochorous traits also were found in Omphalodeae and related with the LDD events in the tribe (Otero et al. 2019).

Lönn (1999) did not describe the diaspore in *Ogastemma*, but Chacón et al. (2017) considered that it presents two dispersal modes: epizoochory and anemochory. Lönn (1999) postulated the latter possibility of dispersal because sand storms should also be considered. Nevertheless, it is not clear how Chacón et al. (2017) classified the diaspora of Echiochiloideae members, since for both *Antiphytum* and *Echiochilon* sampled species are nutlets, but for *Ogastemma* is "fruit", while there is also the "entire fruit" classification in other species (Chacón et al. 2017 in Supplementary Material). Calyx lobes in Echiochiloideae are divided almost to the base, and although the mature nutlets in many cases are kept within the acrescent calyces, they are easily visible and removable from. In *Antiphytum*, except for *A. hintoniorum* with only one eremocarp firmly appressed into the calyx, the calyx lobes extend horizontally and exhibit the eremocarps. Since features in *Ogastemma pusillum* fruit and individual eremocarps are pretty similar to those in *Antiphytum*, we suggest that the nutlet is mainly the diaspore in both cases, but it is possible that the entire fruit and/or a piece of plant , for example, a fruiting stem, could be the diaspore in epizoochory. Therefore, epizoocory may be applicable also for *Antiphytum* (and not only endozoocory, as postulated Chacón et al. 2017), but not anemocory, due that *Antiphytum* does not distribute in deserts where sand storms occur. Schenk and Saunders (2017) in their study used the term achory (without a dispersal syndrome) for some species that have fruits and seed that lack obvious structures associated with dispersal; they found that this was a common syndrome in AAD species (Schenk and Saunders 2017). These authors included in this category diaspores that were too large to be considered dust diaspores (>1 mm) and lacked structures to enhance dispersal, such as *Antiphytum* nutlets. If nutlet is the diaspore in *Antiphytum*, then achory implies that we know that the nutlet somehow dispersed, but we cannot hypothesize how (Schenk and Saunders 2017). Epizoochory by chance or endozoochory could be explanations of a concept that might be better characterized as polychorous (dispersal through multiple syndromes), according to Schenk and Saunders (2017). In any case, an in-depth study is needed to understand how these diaspores dispersed to successfully generate the AAD pattern.

About the vectors, for the "long-distance dispersal" hypothesis concept, Guilliams et al. (2017) included both direct dispersal and stepwise dispersal and two nonmutually exclusive subhypothesis: dispersal by birds and dispersal by large mammals during the Great American Biotic Interchange (GABI). Biro and Whittal (2015) discussed over the plausibility of GABI as an alternative hypothesis to avian dispersal to explain amphitropic plant disjunctions for lineages whose dispersal events date coincide with the timing of the GABI migration (2.6-0.125 Ma) and without obvious characteristics that would facilitate bird dispersals. In general, american amphitropical disjunct studies often conclude that birds are the most likely animal vector, but due the long distance between the North and South American localities, the challenge for the successful dispersal is related to the birds making such a travel without defecating (in endozoochory) or preening (in epizoochory) (Schenk and Saunders 2017 and references therein). Mammals, on the other hand, have dispersal limitations typical of terrestrial vertebrates (Guilliams et al. 2017), given their propensity to have small, intracontinental ranges and relatively show travel times (Schenk and Saunders 2017 and references therein).

In the study of Guilliams et al. (2017), the GABI hypothesis was rejected for Amsinckiinae due to the asynchronous pattern inferred and the broad range of dates among the AAD events in the subtribe. Conversely, the estimated node age for crown *Antiphytum* (9.4 Ma by Chacón et al. 2017) does not preclude the possibility of dispersal affected by terrestrial vertebrates during the GABI, which joints with avian dispersal as scenarios to be tested in further studies.

## Patterns of diversification in *Antiphytum*

The three clades retrieved in *Antiphytum* each have almost the same number of species. The NA-clade is the most diverse with five species and the NA-rosette and SA-clades has four species each (see above). Following the "taxonomic category" of Simpson et al. (2017), diversification in *Antiphytum* after the LDD could be assigned to a clade-clade category (diversification results in a clade that is sister to another clade). Simpson et al. (2017) found a correlation between this category and oldest divergence times, since it is expected that more time would be required for diversification of a single taxon into multiple species within a clade. Conversely, we argue that this pattern is not fulfilled in *Antiphytum*, due to our phylogenetic findings along with the expected recent divergence dates of the clades that comprise the genus.

There are several interesting geographic, ecological and morphological aspects associated with each clade of *Antiphytum* that may be explaining its species richness. However, linking these patterns to process could be risky until further methods don't be applied to test those (Ng and Smith 2014). Despite this fact, we consider relevant and necessary to expound some hypothesis that could explain these patterns.

NA-clade has species with all the edaphic preferences known in *Antiphytum* and includes the unique gypsophile, *A. hintoniorum*, a narrow edaphic endemic (Ferreira and Boldrini 2011) retrieved as sister of the remaining species, and the gypsocline *A. peninsulare*. This is the unique clade in *Antiphytum* in which its entire species have some degree of fidelity to gypsum (Meyer 1986). Distribution of this clade is equally broad, as in Mexico the species occur in the arid zones of five biogeographical regions, that slightly varies according to the different definitions (Morrone 2019). These are the provinces of Baja Californian, the Chihuahuan Desert, the Sierra Madre del Sur, the Sierra Madre Oriental, the Transmexican Volcanic Belt and the Balsas Basin (Morrone 2019, Fig. 3). Verrucate-scabrate pericarp ornamentation of the eremocarp prevails in almost all the species of the clade (scabrate-echinate is only present in the gypsophile), that besides possesses the most different presentations of the inflorescence. Three species have glandular-capitate trichomes on young branches, leaves and leafy organs such as bracts (pherophylls), flower-subtending bracts, and calyx.

Most of the NA-rosette clade species are gypsovags, but *A. floribundum*, which is the sister taxon of the of eastern rosette species subclade, is the only NA gypsophobe of the genus. The main morphological feature of this clade is the presence of a basal-rosette, which originates one time in *Antiphytum*. The clade species distributes in arid zones of six biogeographical regions in Mexico, which coincides with the aforementioned ones, except for the Baja Californian province, but additionally along both the Sierra Madre Occidental and the Transmexican Volcanic Belt provinces (Morrone 2019). This clade possesses the only two species without fornices in the corolla throat associated to two corolla shapes and, according to the branching pattern of the phylogeny, to two independently origins: the absent of fornices in *A. floribundum* is associated with a campanulate corolla, but in *A. paniculatum* the corolla keeps the infundibuliform rotate shape common to all the species, which suggests a different and perhaps more recent process to that occurred in *A. floribundum*, maybe for being an incipient species (see above). The inflorescences are thyrsoids heterocladics in *A. floribundum*, but rather homocladics in the eastern rosette species subclade. Ornamentation of the pericarp is homogeneus in this clade (verrucate). Western distribution of *A. floribundum* correlates with the presence of basaltic parental rock, product of the uplift of the Sierra Madre Occidental and the Transmexican Volcanic Belt, and the volcanic derived soils as islands in the Chihuahuan Desert (Aide and Auken 1985), because this species does not occur outside soils derived from this substrate. None species has glandular-capitate trichomes.

None of the SA-clade species has affinity to gypsum soils, but the unique white-flowered species is distributed over calcareous soil, such as the majority of species of *Antiphytum*. The SA-subclade of blue corollas distributes over soils which could be associated to basaltic parental rock. Then, this is the unique clade in *Antiphytum* with none fidelity degree to gypsum. The inflorescence in the blue corolla subclade is homogeneous, but in the SA clade overall is characterized by the presence of two pherophylls subtending each node of the inflorescence. Three pericarp ornamentations exist in this clade: verrucate, verrucate-scabrate, and striate. Glandular-capitate trichomes are found only in the subclade of blue corollas.

As diversification factors, we could surmise the following: 1) in the NA-clade could be invoked as diversity causal the edaphic component plus tectonic events that leaded to the formation of the regions where the species distribute; 2) in the NA-rosette clade could be the rosette basal along with the edaphic and geographic factors; 3) in the SA-clade, we could argue for the dispersal event plus the gain of the corolla pigment.

In order to discuss some of these scenarios, diversification in North America, the edaphic factor and the morphological traits that could lead diversification in the genus are discussed below.

### Diversification in North America

Many results relate the diversification of many xeric plants from North America with the Neogene uplift and the subsequent formation and differentiation of the various deserts in the Miocene (e. g. Moore and Jansen 2007; Loera et al. 2012; De Nova et al. 2018; Sosa et al. 2018). These regions, among super-arid, arid and semi-arid, occupy in Mexico more than fifty percent of territory (Sousa et al. 2018). Wilson and Pitts (2010) have already suggested that "evolutionary events [in North America] dating anywhere between 15 and 2 Ma could be considered associated with mountain building or desert formation activity" (p. 434).

We know that *Antiphytum* probably originates in America during the Late Miocene (the node age of the crown *Antiphytum* is 9.4 Ma, according to Chacón et al. 2017), and that the subfamily Echiochiloideae overall has features to deal with arid environments: the species of *Echiochilon* and *Ogastemma* are shrublets or perennial herbs with more or less obvious adaptations to dry conditions (Lönn 1999) and according to this study *Antiphytum* inhabiting arid or at least dry regions both in North and South America. Hence, we could surmise niche conservatism to dry conditions in *Antiphytum* that along with the expansion of available habitat in North America may drive the diversification of the genus in this area (Loera et al. 2012; Gándara et al. 2014). For instance, Chacón et al. (2019) suggested the aridity as promoting habitat openness, which in turn acts as precursor to soil specialization; thus the increasing availability of semiarid open habitats in the Mid-Miocene may have likely allowed the diversification of Lithospermeae through its adaptations to arid habitats (Chacón et al. 2019). Sosa et al. (2018) also argued that arid regions formed in the Miocene offered suitable areas for colonization by arid-adapted plants that later diversified there.

Distribution of *Antiphytum* in North America (Fig. 3) coincides with most of the complex geological history of Mexico: the mountain systems known as Sierra Madre Oriental and Sierra Madre Occidental that run from north to south; the Trans-Mexican Volcanic Belt that rather runs west to east; the Sierra Madre del Sur mountain system; the Chihuahuan Desert, the Balsas Basin; and the Baja California Peninsula, which in the past was connected to the Mexican mainland, with an generally accepted split around 6 Ma (Wilson and Pitts 2010). In this regard, Sosa et al. (2018) found that the most important areas of endemism and phylogenetic endemism for the Mexican flora are associated with zones of topographical complexity, such as the main mountain systems and desert areas. Also, De Nova et al. (2018) found that vicariance by tectonic events during the Neogene drove the rapid diversification of *Fouqueria*.

According to *Antiphytum* distribution in the aforementioned regions, it is more feasible to suppose that the Mexican Plateau was the ancestral area for *Antiphytum* and that events in more than one step, such as vicariance by tectonic events, isolating by abiotic or biotic factors, and dispersal, played a substantial role in the diversification of *Antiphytum* in North America. For instance, rifting of the Baja California Peninsula from Mexican mainland that produced speciation with a subsequent narrowly distribution (Dolby et al. 2015) is the highly likely case for *A. peninsulare* origin, which has its sister species (*A. brevicalyx*) mainland. Likewise, dispersal could have occurred from the Mexican Plateau to the Sierra Madre Oriental and southwards, to Sierra Madre del Sur, including the Balsas Basin, in both the two NA clades. This latter pattern of dispersal, from Mexican Plateau to Sierra Madre del Sur, was found in the dry land *Milla* clade (Gándara et al. 2014).

The diversification of a number of plant lineages from arid lands in North America has been correlated with events from different geological periods (Gándara et al. 2014); Sosa et al. (2018) found that the majority of clade divergences in vascular plants of Mexico occurred during the Miocene (5–20 Ma). The complex geological and climatic history of Mexico that has given rise to the country's present appearance is a mosaic of distinct geological provinces with different ages and origins (Mastretta-Yanes et al. 2015; Sosa et al. 2018). Therefore, it is not striking that *Antiphytum* found favorable conditions to diversify into the nine species known so far in North America once the genus originated in America, either by vicariance and/or by dispersal in the Mexican territory. Testing is needed to tackle this question.

## Edaphic factors

Along with the traits to dealing with arid habitats, plants of the Echiochiloideae subfamily have to cope with the soils of these environments, since they have been reported to grow over limestone, gypsum, granite, or volcanic ground (Lönn 1999; personal observation).

*Antiphytum* shows a marked edaphic preference for a particular kind of impoverished or challenging soils. Most species of this genus have affinity to calcareous soils, but six species are facultative in gypsum (*A. brevicalyx, A. caespitosum, A. geoffreyi*, *A. heliotropiodes*, *A. paniculatum*, and *A. parryi*); one is an edaphic endemic to gypsum (*A. hintoniorum*) and four species only seems to grow in soils with basaltic parental material (*A. floribundum* and SA-subclade of blue corollas); *A. peninsulare* (a gypsocline) distributes both in gypsum and basaltic parental material, but not in calcareous soil; and *A. charruasorum* is only known from this kind of soil. The *Antiphytum* clades with gypsum affinities species distribute only in Mexico, where gypsum outcrops are most highly concentrated in the Chihuahuan Desert region, but outcrops also occur in Baja California, Campeche, Chiapas, Guerrero, Oaxaca, Puebla, and Tamaulipas (Ochoterena et al. 2020). Insofar is known, within Echiochiloideae lineage only *A. hintoniorum* is gypsophile, and examples of gygsovagues also are *Echiochilon longiflorum* and *E. persicum*, but *Ogastemma* lacks of gypsovagues (Lönn 1999). Preference to limestone is high in the subfamily; nevertheless, gypsum can also be misidentified when occurring with calcium carbonate or more soluble salts (Casby-Horton et al. 2015) and therefore records in gypsum may be underestimated.

Calcareous soils and gypseous soils are found naturally in arid and semiarid regions, where the annual low rainfall is insufficient for the leaching of the calcium carbonate or calcium sulfate (gypsum) accumulated in the soil (Parsons 1976; Casby-Horton et al. 2015; Weil and Brady 2017; Jha and Sivapullaiah 2017). This kind of soils has high pH level, low organic matter and unavailability or reduced micronutrient availability (Weil and Brady 2017), but rather physical constraints to plant growth, including surface crusts and root-restrictive layers in the subsoil (Casby-Horton et al. 2015) explain the limited flora: seedlings are unable to penetrate the hard crust typical of these soils in semiarid and arid climates (Douglas and Manos 2007; Moore et al. 2014; Casby-Horton et al. 2015; Escudero et al. 2015, and references therein). However, at chemical level, when carbonate concentrations may be found at or near the soil surface, serious micronutrient and phosphorus deficiencies can be induced in plants that are not adapted to calcareous conditions (Weil and Brady 2017).

Lineages specialized on gypsum are unlikely to have arisen from lineages with no latent or expressed gypsum tolerance (Douglas and Manos 2007). Some taxonomic lineages may have ‘preadaptations’ to coping with restrictions in gypsum soils (Escudero et al. 2015), as we propose that is the case for Echiochiloideae. Although multiple origins of gypsophyly are typical within plant lineages that appear to be ancestrally tolerant of gypsum (Moore et al. 2014), this is not the case in this subfamily. Despite this, the clade containing *Antiphytum hintoniorum* (NA-clade) possesses one gypsocline and numerous other taxa that are gypsovags, meeting the pattern reviewed by Moore et al. (2014). To explain the origin of the gypsophyle lineage of *Antiphytum*, two possible scenarios could be referred. One is that gypsum promoted the differentiation and origin of *A. hintoniorum* with or without specific adaptive responses to these environments (Escudero et al. 2015); the other one is that the lineage originated in other stressful environments from which it colonized gypsum soils (Escudero et al. 2015). We ignore the leaf chemistry of *A. hintoniorum* to know if there are or not specialized chemical adaptations to cope with the chemical restrictions of gypsum soils and hence, if *A. hintoniorum* follows a refuge model, since it is narrowly distributed (Palacio et al. 2007; Escudero et al. 2015). Then, it remains pending to test if its restriction to gypsum soils is related with special adaptation to gypsum soils or with a low competitive ability linked to its nature as stress-tolerant plant (Palacio et al. 2007).

*Antiphytum hintoniorum* is precisely the most morphological divergent species in the genus, and its branch has many changes (Fig. 5). Accumulation of molecular changes can be expected by time or by environmental pressures in a relative short time span (Phillipe et al. 2011; Bromham et al. 2015). Coupled with the latter, this species shows reduction of vegetative and fertile structures (the inflorescence is a terminal dichasial cymoid reduced to one flower and each fruit bears only one eremocarp) that could be a response to the impoverished soil or a characteristic of endemism. For instance, reduction in flowers and seeds as a common trait among most of endemic species of the French Mediterranean region was reported by Lavergne et al. (2004).

That *A. hintoniorum* is the only gypsophyle species in *Antiphytum* contrasts with the gypsophile flora of the Chihuahuan Desert region reviewed by Moore et al. (2014). This lack of diversity could be explained by the fact that gypsophily may be a relatively recent phenomenon in *Antiphytum*, of less than 9 Ma (conversely to that hypothesized for gypsophile species in general; see Moore and Jansen 2007; Aguirre-Liguori et al. 2014 and references therein), or an evolutionary dead end (Ng and Smith 2014), leading to the extinction of other species in the lineage, if there ever were any. The few and separate localities where *A. hintoniorum* occurs could be the result of posterior habitat fragmentation from a wider distribution due to climatic reasons or have been independently colonized by long-distance dispersal, a process that several authors relate to evolutionarily old taxa due to the time they need to colonize isolated gypsum outcrops by chance (Moore and Jansen 2007; Escudero et al. 2015). The position of *A. hintoniorum* being the earliest divergent species of the NA-clade perhaps corresponds with being relative ancient, although long branches do not necessarily imply long times (Sauquet 2013). It could also be the unique survivor species of its lineage. Morphological changes with respect to the other taxa of the genus, and at molecular level among the individuals of different gypsum outcrops, shows how gypsum soil restriction has promoted morphological change and has drove genetic differentiation in *A. hintoniorum* (Bromham et al. 2015) by the island-like pattern of the gypsum deposits that are found in North American deserts (Moore and Jansen 2007; Aguirre-Liguori et al. 2014; Moore et al. 2014).

Edaphic factors could also be involved in the origin of *A. floribundum*. We could suppose a parapatric speciation explanation for this species, since the soils of the Chihuahuan Desert are predominantly calcium carbonate, whereas volcanic derived soils distributed more locally throughout the desert as islands (Aide and Auken 1985). Calcareous soils are primarily preferred by *Antiphytum*, thus individuals of the ancestral *Antiphytum* rosetted lineage populations colonizing volcanic outcrops on the Mexican Plateau may have acquired traits related to this different soil as a new niche. Colonization of *Antiphytum floribundum* from the Mexican Plateau to the Sierra Madre Occidental and the Trans-Mexican Volcanic Belt, where volcanic soils are common, could be posterior and reached by dispersal.

Lastly, we would like to point out a couple of correlation patterns involving soils of volcanic origin. It is interesting that only *A. floribundum* and SA-subclade of blue corollas species have a modification of the ventral keel of the eremocarp, either as a stipe (SA-subclade) or as a hump (*A. floribundum*). Moreover, the loss of the fornices with the consequent modification of the corolla occurred in *A. floribundum*, while the gain of pigment in the corolla occurred in the SA species with this kind of parental material in the soils. This raises the question if the soil of basaltic origin has been a factor driven or influenced these modifications in the eremocarps and corolla.

### Basal rosette

It is far known that arid regions of North America have a wide range of rosette plants, from annuals (Mulroy and Rundel 1977) to evergreen (Kemp and Gardetto 1982; Martorell and Ezcurra 2002). Martorell and Ezcurra (2002) postulated that succulent evergreen rosette plants in Mexico not only are good water storing plants, but also that the succulent rosette morphologies could be an adaptive response to environmental conditions prevailing on the mid-slopes because of their high capacity to harvest fog and low-intensity rains.

In the case of *Antiphytum* species inhabit arid regions, leaves arranged in rosettes are basal, non-succulent and in contact with the soil. In fact, we were able to observe in some localities individuals from the subclade of eastern rosette species growth as prostrates in often openness areas. The fact that four species of *Antiphytum* (*A. caespitosum*, *A. floribundum*, *A. paniculatum*, *A. parryi*) bearing such conspicuous morphological feature, were retrieved in a clade, arises the question over whether the apparition of this trait leads to the diversification in the genus.

In general, it has been found that grazing increases the abundance of rosette plants (Díaz et al. 2007), maybe by protecting the apical meristems. In another way, rosette growth-form was found to have a positive correlation with aridity (Nunes et al. 2017). Further, Cramer and Morrow (2007) found that prostrate leaves allow a small proportion of the total photosynthetic $CO_2$ to be derived from soil $CO_2$ with consequently reduced $H_2O$ loss. They concluded that probably other prostrate-leaved plants (e.g., geophytes, rosette-form herbs) also derive advantage from $CO_2$ fluxing from the soil (Cramer and Morrow 2007). Maybe this trait has some advantages under drought (Nunes et al. 2017) and to conquer bare new habitats.

### Corolla color

Flower color is evolutionarily labile, with examples of evolutionary divergence between closely related species from diverse genera and families (Wessinger and Rausher 2012). In *Antiphytum*, flower color transition occurred one time within the SA-clade by the gain of anthocyanin pigmentation from white; therefore, the blue color of the corollas in the subclade (*A. berroi*, *A. cruciatum*) is a derived trait, posterior to the long-distance dispersal event and, although differing in flower color does not by itself implicate a change at speciation, as other characters could have caused the initial divergence with flower color evolving anagenetically (Smith and Goldberg 2015), probably product of cladogenesis. In contrast, white flowers (lacking floral anthocyanins) is the condition for all the North American species of *Antiphytum* and the Uruguayan *A. charruasorum*, the sister taxa to the SA-subclade with blue corollas.

Anthocyanin pigments are a flavonoid subclass that are a major contributor to the diversity of flower colors found across angiosperms (Ng et al. 2018) and are synthetized by the anthocyanin biosynthetic pathway (ABP), which is conserved across angiosperms and is well-characterized genetically (Wessinger and Rausher 2012). The ABP and its regulation are conserved through large spans of evolutionary time, regardless of whether pigment is produced in flowers (Smith and Goldberg 2015), because nearly all higher plants produce anthocyanin in vegetative tissue (Sobel and Streisfeld 2013), with exception of the Caryophyllales (anthocyanins are entirely replaced by betalain pigments [Winkel 2006]). Anthocyanin pigments are also expressed in sepals, stems, leaves and even roots where they can be crucial to a plant's abiotic and biotic stress response (Dick et al. 2011), since they are involved in a variety of physiological functions (Winkel-Shirley 2001): in addition to their role in pigmentation in flowers, fruits, seeds, and leaves, which is useful for attracting pollinators and fruit dispersal agents (Rausher 2006), flavonoids have key roles for the defense and survival of the plants, and protection against UV radiation and drought (Winkel-Shirley 2001; Rausher 2006). According to Smith and Goldberg (2015), this range of functions may explain the deep conservation of the pathway across flowering plants. While almost all the functions of flavonoids are ancient (Rausher 2006), since the “early” steps in the pathway are found in the bryophytes (Winkel-Shirley 2001), the role of anthocyanin pigments as visual signals in angiosperms was acquired late in the evolutionary diversification of flavonoids (Rausher 2006).

Although gains of novel traits are expected to occur less frequently than losses because there are typically more ways to break a pathway than to restore it (Sobel and Streisfeld 2013), this is not the case for the ABP, and therefore, gains of floral anthocyanin pigmentation may occur more readily than gains in other phenotypic traits (Sobel and Streisfeld 2013). A pattern common to all floral color transitions is that a mutation at a single locus is sufficient to cause a shift in flower color (Ellis and Field 2016), hence, transitions between pigmented and unpigmented flowers are frequent and reversible (e.g., Smith and Goldberg 2015; Ellis and Field 2016; Roguz et al. 2020). According to Ng et al. (2018), this macroevolutionary lability appears to arise from regulatory and coding mutations in rapidly evolving MYB transcription factors, which control anthocyanin expression and whose changes at these loci underlie polymorphisms within species and differences between closely related taxa, such as in this case between *A. charruasorum* and the SA-subclade of blue corollas. The gaining floral anthocyanin in this *Antiphytum* lineage likely did not require the restoration of a non-functioning pathway or the re-evolution of the entire pathway de novo, but instead a simple shift in regulation of the ABP genes into this expression domain through activation of this existing pathway in petals (Smith and Goldberg, 2015).

Although flower color divergence may occur through genetic neutral drift or linkage to pleiotropic traits experiencing selection, in many cases such change is likely adaptive (Wessinger and Rausher 2012). Therefore, this morphological novelty in *Antiphytum* raises the question of whether the change was adaptative (or not), and if so, whether it was to biotic or abiotic factors, i.e. new pollinators, herbivores or nectar robbers or dispersal agents, or by the environment and the geography, respectively. It is worthy not underrate the role of non-pollinator agents of selection in color traits mediated by flavonoids, because due to the numerous non-pollinator functions, correlations in anthocyanin expression in different plant tissues may lead to indirect selection on flower color or even predispose plants to a particular evolutionary trajectory (Strauss and Whittall 2006). Thus, in some systems, flower color may not be the primary target of natural selection, nor pollinators the primary agent (Vaidya et al. 2018). Since we don't know the pollinators of *Antiphytum* in both disjunct regions, we are not able to compare and conclude changes in pollinators' preferences.

On the other side, although some studies have shown that in species with flower color polymorphism (FCP) –i.e. individuals produce white or pigmented mediated by anthocyanins flowers- white phenotypes have disadvantages relating to pollinator visits, sensibility to drought and water stress, greater inbreeding depression under stressful conditions, foliar herbivory, or reduction in the fitness (reviewed by Narbona et al. 2018), North American species of *Antiphytum* are distributed in arid environments and exposed to stressful drought and water conditions, but they are successful and diverse. Therefore, stress-related functions of anthocyanine are active in the vegetative tissues of these species, and probably the genus *Antiphytum* can independently regulate pigment expression in petals and vegetative tissues avoiding the deleterious effects of pigment loss in vegetative organs (Dick et al. 2011). At least the arid component in the environments that inhabits *Antiphytum* is shared among the species in North and South America.

Other abiotic factors have regulatory effects on anthocyanin expression (Stimpson et al. 2021). In species with FCP, for example, changes to floral color can occur either through regulation of the amount of anthocyanin produced, through modulation to the pH of the vacuole, or through complexation with metal ions accumulated from soils (Stimpson et al. 2021). Anthocyanins are stored in the vacuole and vacuolar pH plays an important function in coloring anthocyanin pigments (Grotewold 2006). In petunia flowers and flowers of *Ipomoea*, blue flowers are produced when the vacuolar pH increases (Grotewold 2006), but in *Hydrangea macrophylla* (Thunb.) Ser. (Hydrangaceae), more acidic vacuoles by accumulation of aluminum due to acidic soils (at lower pH, aluminum bioavailability increases) produce a color change of the floral sepals to blue by the formation of an stable blue complex from the otherwise red anthocyanin (Stimpson et al. 2021). Then, edaphic conditions like soil pH and mineral nutrient concentration can also induce variation in flower color and flower color variation could be maintained as a phenotypically plastic trait driven by environmentally variable conditions (Vaidya et al. 2018). Nevertheless, it is mostly unknown how soil properties influence flower color in nature (Vaidya et al. 2018). For *Antiphytum* with blue corollas, it is known that *A. cruciatum* grows in some arid localities of Rio Grande do Sul with marked acidic soils (Setubal 2010).

Whether the edaphic factor triggered color change in the genus requires that the ancestral population in which the gain of pigmentation mutant appeared was already settled on acid soils and the floral ABP was functional. In contrast, a new floral color different from the ancestral white flowered lineage maybe not only leaded to the formation of a new lineage (SA-subclade of blue-corollas), but also was able to promote dispersal to a new region, altering patterns of gene flow and therefore, affecting the diversification in the genus (Ng & Smith 2014), since the species with blue-corolla are spatially disjunct with respect to *A. charruasorum*, which distributes only over calcareous soils in Uruguay, far away from the localities where are distributed the blue-corolla species. At this point it could be a circular argument, because we don't know what factor actually triggered the expression of floral anthocyanins in South America: if the mutant in the ancestral population appeared first and afterwards the colonization of environments with acid soils ocurred, or the mutant appeared over acid soils that promoted the blue color and sympatric speciation. We can suspect due to the above said, that the ABP likely is decoupled and therefore expressed in the vegetative tissues in white flowered lineage, as well as in the ancestral lineage of blue-corolla flowers and therefore, prone to become active in the petals.

Nevertheless, even if we ignore all these factors, we can conclude that the pigment gain allowed the formation of the SA-subclade of blue-corollas, whose known diversity currently are represented by three species, and therefore it is a trait that affected the diversification in *Antiphytum*. Whether the origin of this novel trait was adaptive or not, and to what extent was adaptive, remains an open question worthy of further biological investigation.

## Conclusions

The results of this work improve our understanding of the circumscription and phylogenetic relationships of *Antiphytum*, although some interspecific relationships are not resolved. *Antiphytum*, like many Boraginaceae genera, was long an elusive genus, but it is nowadays clear that it belongs to Echiochiloideae subfamily and that is a sister clade of *Ogastemma*. The genus is the second richest in the subfamily with 13 species (compared to 15 in *Echiochilon*) disjunct in North and South America and retrieved in three clades: NA-with basal-rosette plants; NA-without basal-rosette plants and SA-without basal rosette plants; the relationships among them remain to be determined. México is the most diverse country for the genus, since it hosts nine species, while

Uruguay has four species. USA and Brazil are the northernmost distribution for two species each in its corresponding region, respectively. The direction of the colonization of America is proposed from north to south and the disjunction is explained by a single event, probably long-distance dispersal, as in almost all the Boraginaceae groups with American amphitropical disjunct distribution. One event of corolla color (white to blue) and cicatrix position (basal in SA) change occurred likely after the dispersal event. Edaphic preferences, patterns of distribution and morphological traits associated to each clade of the genus open several questions about the process behind the diversification in *Antiphytum*. We suggest that tectonic events together with specialization to harsh soils were the main drivers of diversification in NA, while blue corolla probably promoted the colonization of new habitats in SA likely by the recruitment of novel pollinator or dispersal agents. The discoverer of *A. charruasorum* was fundamental to place relevant morphological, geographical and phylogenetic patterns, but overall the prior species recognition through taxonomic revision was the most important step before the phylogenetic reconstruction. We realized the dramatic change in the phylogenetic hypothesis as a function of sampling, even with the same markers. Therefore, we encourage continuing field exploration, mainly in South America, whose species were long behind a veil, in order to improve the field knowledge and sampling, to discover more populations or to include lacking species, such as *A. stoechadifolium*. Moreover, our results with ITS nuclear marker reveal some problems within *Echiochilon*, hence the whole subfamily Echiochiloideae requires additional study. Future studies should test other markers or methods in addition to a more comprehensive sampling to get a better phylogenetic resolution. Since the success in PCR amplification of all the markers and the informative degree of these are not guaranteed, next-generation sequencing methods might be a better option. Nevertheless, despite the lacking of resolution on the backbone of the phylogeny of *Antiphytum*, this work has the most comprehensive sampling of the genus, with almost all the known species from North and South America. With this study, we contribute with initial insights about its phylogeny, diversification and biogeographic patterns, and posit several questions to be dealt in-depth in the future.

## Identification key to the species of *Antiphytum*

1a. Suffruticose plants with white corolla flowers arranged or not in scorpioid cymes; eremocarp cicatrix at suprabasal, inframedial or medial position (known from North and South America)…..…………………………………………...……………………………………..………2

    2a. Cauline leaves opposite *throughout* the plant, basal-rosette absent; flowers not arranged in scorpioid cymes (known from North and South America)……………………..…………….3

3a. Inflorescences with only one dichasial branching, therefore, branches with only one terminal flower (one-flowered cymoid); faucal appendages papillose; one whitish eremocarp per fruit generally; eremocarp cicatrix tissue not forming a projection; cicatrix at suprabasal position (known from North America)…………………………..…....***A. hintoniorum*** **L.C.Higgins & B.L.Turner**

3b. Inflorescences with more than one dichasial branching, therefore, branches with more than a terminal flower; faucal appendages hirsute-pubescent; four brown eremocarp per fruit generally; eremocarp cicatrix tissue forming a projection; cicatrix at inframedial position (known from South America)………………………………….***A. charruasorum*** **N.Mend. & Mar.Díaz**

2b. Cauline leaves alternate *throughout* the plant, but sometimes opposite at the base, basal-rosette present or absent; flowers arranged in scorpioid cymes (known from North America)..………………………………………………………………………...….……...4

4a. Plants with a basal-rosette; eremocarp cicatrix at inframedial position…………………………………………………………………………….....5

5a. Flowers with five faucal appendages on the throat opposite to the corolla lobes…………………………………..………………………….6

6a. Corolla limb 5–8 mm diameter; scorpioid cymes up to 40 mm in length…...………………………..……***A. caespitosum*** **I.M.Johnst.**

6b. Corolla limb (1–)2–4 mm diameter; scorpioid cymes up to 10 mm in length.......................................………***A. parryi*** **S.Watson**

5b. Flowers without five faucal appendages on the throat opposite to the corolla lobes…………..……………………………………………….....7

7a. Corolla hypocrateriform (rotate); homocladic thyrsoids; scorpioid cymes up to 20 mm in length; eremocarp apical keel straight and flat ……………...……….***A. paniculatum*** **I.M.Johnst.**

7b. Corolla infundibuliform-campanulate; heterocladic thyrsoids; scorpioid cymes up to 40 mm in length; eremocarp apical keel forming a bump…………………...***A. floribundum*** **(Torr.) A.Gray**

4b. Plants without a basal-rosette; eremocarp cicatrix at suprabasal, inframedial or medial position ……………………………………………………...…………....8

8a. Calyx lobes shorter than the corolla tube at anthesis; cicatrix at medial position……………...….***A. brevicalyx* N.Mend., Flores Olv. & H.Ochot.**

8b. Calyx lobes equal or longer than the corolla tube at anthesis; cicatrix at suprabasal, inframedial or medial position…………………...…………9

9a. Scorpioid cymes proximal to the base of the plant; inflorescences without foliose metaxyphylls; gynobase columella triangular (2:1); cicatrix at medial position........***A geoffreyi* N.Mend. & Flores Olv.**

9b. Scorpioid cymes distal to the base of the plant; inflorescences with foliose metaxyphylls; gynobase columella broadly triangular (6:5) to very broadly triangular or deltate (1:1 and 5:6); cicatrix at suprabasal or medial position……………………………….…10

10a. Flowers subsessile, pedicels 1.2 mm in length; corolla limb 10–12 mm diameter; gynobase columella very broadly triangular or deltate (1:1 and 5:6); cicatrix at medial position……..................***A. peninsulare* (Rose) I.M.Johnst.**

10b. Flowers pedicellate, pedicels up to 14 mm in length; corolla limb 4–6 mm diameter; gynobase columella broadly triangular (6:5); cicatrix at suprabasal position…………………..…..….***A. heliotropioides* A.DC.**

1b. Suffruticose plants with blue corolla flowers arranged in scorpioid cymes; eremocarp cicatrix at basal position (known from South America)……………..……………………………...……….11

11a. Leaves 3–4(–6) mm wide, oblanceolate; eremocarps 1.3–1.4 × 1.0–1.1 mm…………………………………….………….….……….***A. cruciatum* (Cham.) DC.**

11b. Leaves less than 3 mm wide, linear-lanceolate or linear-oblong to linear-oblanceolate; eremocarps 1.6–2.0 × 1.3–1.6 mm……………………………...…………………………12

12a. Pericarp ornamentation more or less striate, leaves up to 3 cm long ……………………………...…….***A. berroi* (Arechav.) N.Mend. & Flores Olv.**

12b. Pericarp ornamentation tuberculate-granulate; leaves ca. 5 cm long…………………...…………………………..***A. stoechadifolium* (Cham.) DC.**

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Table 1. *Antiphytum* species achieved through taxonomic revision of the genus

| **Species** | **Publication date** | **Nomenclatural synonyms** | **Taxonomic synonyms** | **Geographic distribution** |
|---|---|---|---|---|
| *A. berroi* (Arechav.) N.Mend. & Flores Olv. **comb. nov.** | 2022 | *Myosotis berroi* Arechav. (basionym, 1911) | – | SA |
| *A. brevicalyx* N.Mend., Flores Olv. & H.Ochot. | 2020 | – | – | NA |
| *A. caespitosum* I.M.Johnst. | 1923 | *Amblynotopsis caespitosa* (I.M.Johnst.) Brand (1929) | *Antiphytum nudicalces* I.M.Johnst. (1923)<br>*Amblynotopsis nudicalces* (I.M.Johnst.) Brand (1929) | NA |
| *A. charruasorum* N.Mend. & Mar.Díaz | 2020 | – | – | SA |
| *A. cruciatum* (Cham.) DC. | 1846 | *Anchusa cruciata* Cham. (basionym, 1829)<br>*Chamissoniophila cruciata* (Cham.) Brand (nom. superfl., 1929) | – | SA |
| *A. floribundum* (Torr.) A.Gray | 1874 | *Eritrichium floribundum* Torr. (basionym, 1859)<br>*Krynitzkia floribunda* (Torr.) A. Gray (1885)<br>*Amblynotopsis floribunda* (Torr.) J.F.Macbr. (1916) | *Amsinckia mexicana* M.Martens and Galeotti (1844)<br>*Amphibologyne mexicana* (M.Martens and Galeotti) Brand (nom. superfl., 1931)<br>*Amblynotopsis durangensis* J.F.Macbr. (1916) | NA |
| *A geoffreyi* N.Mend. & Flores Olv. | 2018 | – | – | NA |
| *A. heliotropioides* A.DC. | 1846 | *Eritrichium heliotropioides* (A.DC.) Torr. (1859)<br>*Krynitzkia heliotropioides* (A.DC.) A.Gray (1885) | *Amblynotopsis ehrenbergii* Brand (1926) | NA |

| | | | | |
|---|---|---|---|---|
| | | *Cryptantha heliotropioides* (A.DC.) Loes. (1913)<br>*Amblynotopsis heliotropioides* (A.DC.) J.F.Macbr. (1916) | | |
| *A. hintoniorum* L.C.Higgins & B.L.Turner | 1983 | - | – | NA |
| *A. paniculatum* I.M.Johnst. | 1923 | *Lithospermum linifolium* M.Martens and Galeotti (replaced synonym, 1844)<br>*Amblynotopsis paniculata* (I.M.Johnst.) Brand (1929)<br>*Amblynotopsis linifolia* (M.Martens and Galeotti) Brand (nov. comb., 1931) | – | NA |
| *A. parryi* S.Watson | 1883 | *Krynitzkia parryi* (Watson) A.Gray (1885)<br>*Amblynotopsis parryi* (Watson) J.F.Macbr. (1916) | *Amblynotopsis humilis* Brand (1926) | NA |
| *A. peninsulare* (Rose) I.M.Johnst. | 1923 | *Krynitzkia peninsularis* Rose (basionym, 1890)<br>*Amblynotopsis peninsularis* (Rose) J.F.Macbr. (1916) | – | NA |
| *A. stoechadifolium* (Cham.) DC. | 1846 | *Anchusa stoechadifolia* Cham. (basionym, 1829)<br>*Chamissoniophila stoechadifolia* (Cham.) Brand (nom. superfl., 1929) | – | SA |

Table 2. Individual and combined datasets characteristics. nts= nucleotides; mch=non-gap microstructural characters

| | ITS | *ndh*F–rpl32 | *trn*L–F | *rps*16 | *mat*K | ITS+*trn*L–F+*rps*16 | Nuclear-plastid combined (5-loci dataset) |
|---|---|---|---|---|---|---|---|
| Number of sequences (Echiochiloideae/Outgroup) | 253 (207/46) | 134 (95/39) | 322 (123/199) | 286 (115/171) | 193 (65/128) | 113 (102/11) | 43 (41/02) |
| Sequence length (nts) variation (minimum–) most common (–maximum) | (267–)551—663 | (305–)494—698(–749) | (534–)848—909(–922) | (560–)720—862(–897) | (584–)725—1017 | NA | NA |
| Aligned sequence length (with hotspots/without hotspots) | 721/712 | 849/836 | 1609/1463 | 1350/893 | 1053 | NA/2213 | NA/3907 |
| Number of mch | 13 | 19 | 90 | 50 | 9 | 153 | 181 |
| Number of coded gaps | 73 | 36 | 220 | 69 | 15 | 362 | 413 |
| Potentially parsimony informative characters (nts/mch/gaps) | 330/11/58 | 94/04/20 | 342/59/124 | 192/29/43 | 336/06/07 | 343/15/49 | 263/06/23 |

Table 3. Description of the parsimony informative microstructural characters in the 5-loci reduced matrix (43 terminals). The interpretation of the informativeness of the event is based on both, the consensus of the reduced matrix and the optimization of each character onto the full corresponding matrices

| Number | Locus | Microstructural character | Aligned positions | Derived state | Supported group | Interpretation |
|---|---|---|---|---|---|---|
| 1 | ITS | Gap | 139–141 | – | – | Sensitive to actran or deltran optimization |
| 2 | ITS | Gap | 237 | Deletion | *Antiphytum brevicalyx* | Autapomorphic but not diagnostic |
| 3 | ITS | Gap | 241 | Deletion | Echiochiloideae | Synapomorphic and diagnostic |
| 4 | ITS | Gap | 245 | Deletion | *A. hintoniorum* | Autapomorphic within *Antiphytum*, but also present in one species outside the genus |
| 5 | ITS | Gap | 512–513 | Insertion | Echiochiloideae | Ambiguous mapping of the insertion: either synapomorphic for the subfamily or for the second branch within it |
| 6 | ITS | Gap | 513 | Insertion | Blue-corolla subclade | Diagnostic, but not synapomorphic within *Antiphytum* |
| 7 | ITS | Gap | 518–519 | Insertion | – | Homoplastic for *A. geoffreyi* and the OG |
| 8 | ITS | Gap | 518 | Deletion | Eastern rosette species subclade | Diagnostic, but not synapomorphic |
| 9 | ITS | Gap | 519 | Insertion | Eastern rosette species subclade | Diagnostic, but not synapomorphic |
| 10 | ITS | SSR (TG) | 522–523 | Insertion | *A. geoffreyi* | Autapomorphic and diagnostic |
| 11 | ITS | Gap | 610 | Insertion | *A. floribundum* | Autapomorphic and diagnostic |
| 12 | ITS | Gap | 617 | Insertion | Echiochiloideae | Diagnostic but also present in Cynoglossoideae |
| 13 | ITS | Gap | 651 | Insertion | *A. brevicalyx* | Autapomorphic and diagnostic |
| 14 | ITS | Gap | 674 | Deletion | Echiochiloideae | Synapomorphic and diagnostic |
| 15 | *ndh*F–rpl32 | SSR ( GAAAGA) | 90–95 | Insertion | *Ogastemma* | Autapomorphic and diagnostic |
| 16 | *ndh*F–rpl32 | Gap | 308 | Insertion | *A. heliotropioides* | Autapomorphic and diagnostic |
| 17 | *ndh*F–rpl32 | Gap | 334–338 | Deletion | *Echiochilon* | Synapomorphic and diagnostic |
| 18 | *ndh*F–rpl32 | SSR (CATTAA) | 343–348 | Insertion | *A. geoffreyi* +*A. peninsulare* | Synapomorphic and diagnostic |
| 19 | *ndh*F–rpl32 | Gap | 412 | Deletion | Echiochiloideae | Synapomorphic and diagnostic |
| 20 | *ndh*F–rpl32 | Gap | 472–691 | Deletion | *Echiochilon* | Synapomorphic and diagnostic |
| 21 | *ndh*F–rpl32 | Gap | 500–762 | Deletion | *A. floribundum* | Autapomorphic but not diagnostic because it is absent in one indivudual |

| | | | | | | |
|---|---|---|---|---|---|---|
| 22 | *ndh*F–rpl32 | SSR (AG) | 688–690 | Insertion | Eastern rosette species subclade | Diagnostic, but maybe synapomorphic for all the NA-rosette clade |
| 23 | *ndh*F–rpl32 | Gap | 835–345 | Deletion | *A. brevicalyx* | Potentially autapomorphic and diagnostic, but with ambiguous mapping because it is close to the end of the reading |
| 24 | *ndh*F–rpl32 | Gap | 835–837 | Insertion | *A. floribundum* | Potentially autapomorphic and diagnostic, but with ambiguous mapping because it is close to the end of the reading |
| 25 | *mat*K | SSR (CTAGAA) | 161–166 | Insertion | *A. hintoniorum* | Diagnostic, but also present outside Echiochiloideae |
| 26 | *rps*16 | SSR (TTCA) with mutations | 157–160 | Deletion | – | Highly homoplastic |
| 27 | *rps*16 | Gap | 361–365 | Deletion | Echiochiloideae | Synapomorphic and diagnostic |
| 28 | *trn*L–F | SSR (CCTTG) | 181–185 | Insertion | *Antiphytum+Ogastemma* | Diagnostic, but also present outside Echiochiloideae |
| 29 | *trn*L–F | Gap | 349 | Insertion | Echiochiloideae | Diagnostic, but also present outside Echiochiloideae |
| 30 | *trn*L–F | Gap | 550–566 | Deletion | *Antiphytum+Ogastemma* | Synapomorphic and diagnostic |
| 31 | *trn*L–F | Gap | 977–1031 | Deletion | *A. brevicalyx* | Shared by two individuals of the species |
| 32 | *trn*L–F | Gap | 1115–1124 | Deletion | *Antiphytum* | Diagnostic, but also present outside Echiochiloideae |
| 33 | *trn*L–F | SSR with mutation (GGAA/TGAA) | 1209–1216 | Deletion | Echiochiloideae | Synapomorphic and diagnostic in the reduced matrix, plesiomorphic in the complete *trn*L–F matrix |
| 34 | *trn*L–F | Gap | 1420–1425 | Deletion | Echiochiloideae | Synapomorphic and diagnostic in the reduced matrix, plesiomorphic in the complete *trn*L–F matrix |
| 35 | *trn*L–F | Gap | 1460–1465 | Deletion | *Echiochilon* | Synapomorphic and diagnostic |
| 36 | *trn*L–F | SSR (AAAT) with mutations | 1573–1577 | Insertion | Subclade of *A. hintoniorum* | Shared by several individuals of the species and also present out of Echiochiloideae |

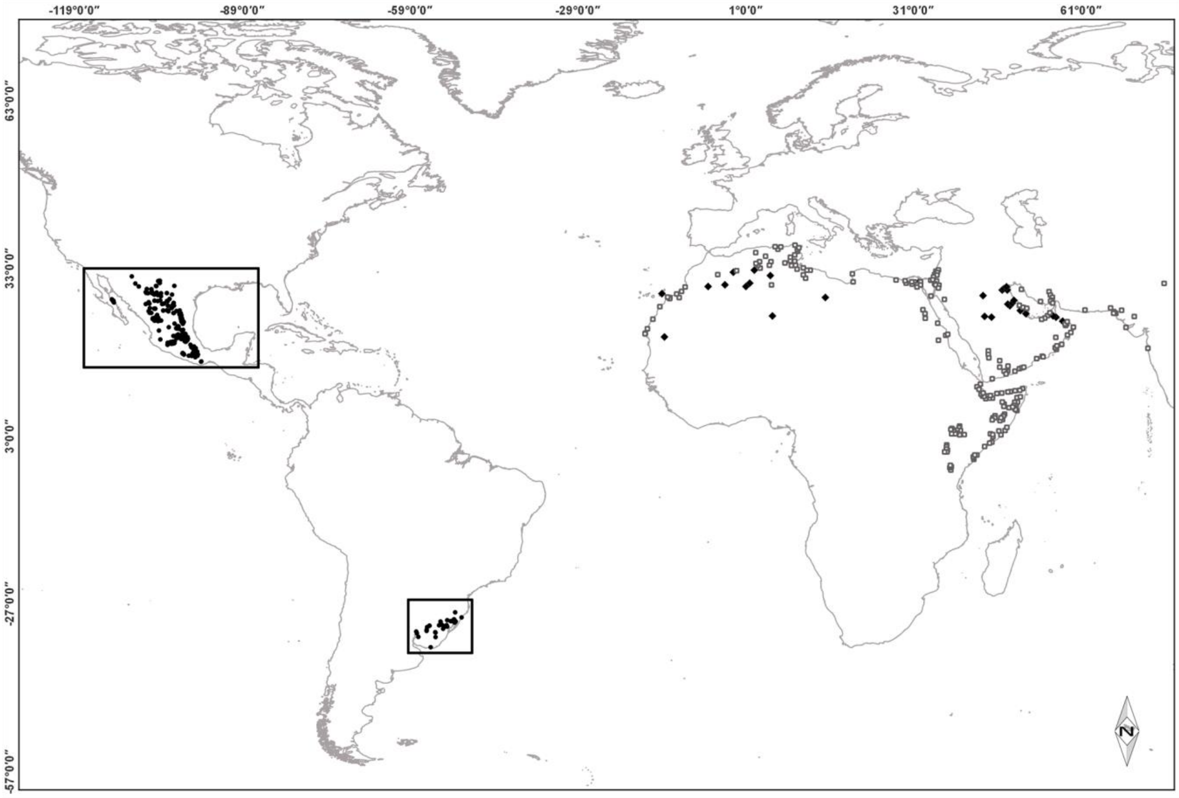

**Fig. 1** Distribution of subfamily Echiochiloideae: *Antiphytum* is American (black dots); *Ogastemma* (black diamonds) and *Echiochilon* (empty gray squares) are found in Africa and the Arabian Peninsula. The boxes in America show the regions where *Antiphytum* is found

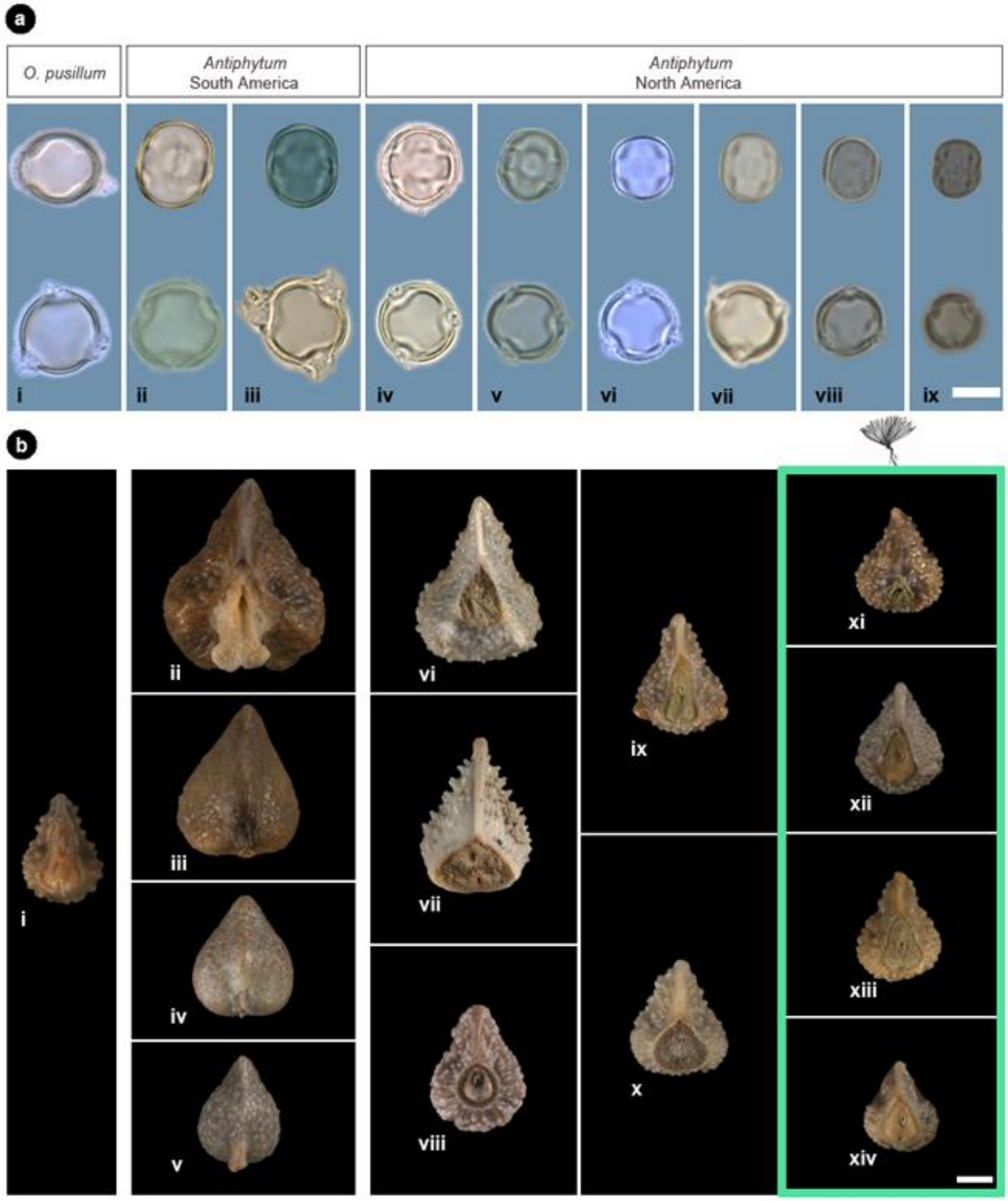

**Fig. 2** Pollen and eremocarps of *Antiphytum* in comparison to *Ogastemma pusillum*. The species are divided by genus and, within of *Antiphytum*, they are sorting according to geographical distribution and size. **a** Pollen in equatorial or oblique (above) and polar (below) view: i *O. pusillum*; ii *A. berroi*; iii *A. cruciatum*, iv *A. brevicalyx*, v *A. caespitosum*; vi *A. floribundum*; vii *A. hintoniorum*; viii *A. peninsulare*; ix *A. heliotropioides*. Scale bar equals to 10 µm. **b** Eremocarps in ventral view. The basal-rosette bearing species are highlighted with a bright green box: i *O. pusillum*; ii *A. charruasorum*; iii *A. stoechadifolium*; iv *A. berroi*; v *A. cruciatum*; vi *A. peninsulare*; vii *A. hintoniorum*; viii *A. brevicalyx*; ix *A. geoffreyi*; x *A. heliotropioides*, xi *A. floribundum*; xii *A. caespitosum*; xiii *A. parryi*; xiv *A. paniculatum.* Scale bar equals to 0.5 mm

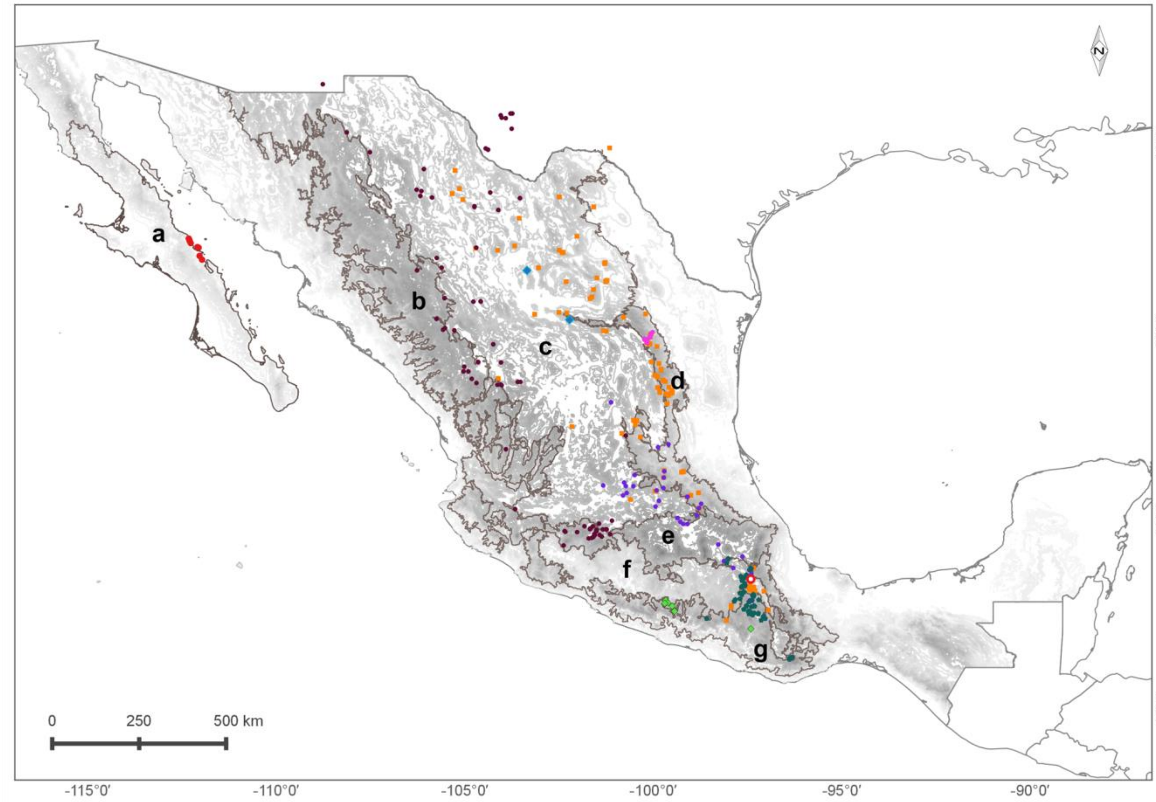

**Fig. 3** Distribution of the nine North American species of *Antiphytum*: *A. brevicalyx* (bright green diamonds); *A. caespitosum* (green pentagons); *A. floribundum* (wine-colored dots); *A. geoffreyi* (blue diamonds); *A. heliotropioides* (bright orange dots); *A. hintoniorum* (bright pink dots); *A. paniculatum* (red-lined empty circle); *A. parryi* (purple dots); *A. peninsulare* (bright red dots). Biogeographical regions in Mexico (according to Morrone 2019): **a** Baja Californian; **b** Sierra Madre Occidental; **c** Chihuahuan Desert; **d** Sierra Madre Oriental; **e** Transmexican Volcanic Belt; **f** Balsas Basin; **g** Sierra Madre del Sur provinces

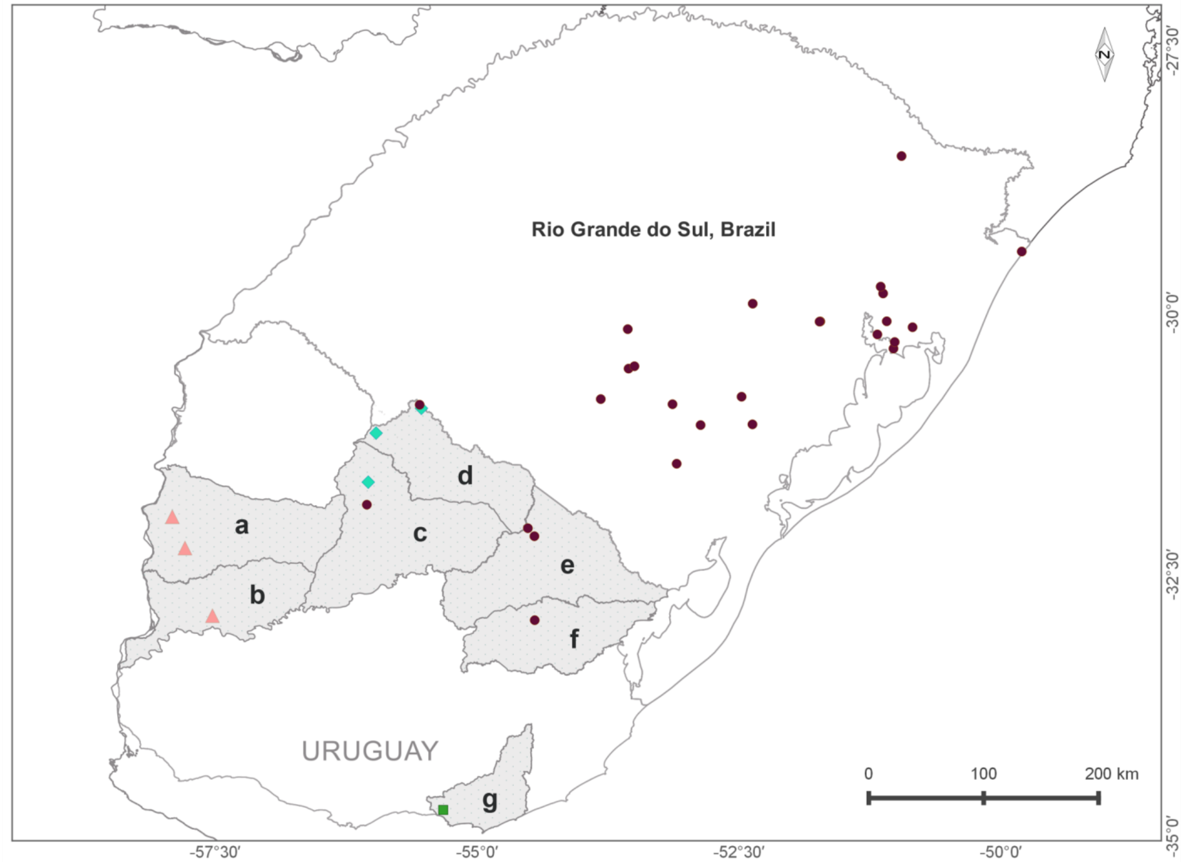

**Fig. 4** Distribution of the four South American species of *Antiphytum*: *A. berroi* (turquoise diamonds); *A. charruasorum* (pink triangles); *A. cruciatum* (wine-colored dots); *A. stoechadifolium* (green square). Distribution of *A. berroi* in Brazil is not shown (see Mendoza-Díaz et al. 2022). Departments of Uruguay: **a** Paysandú; **b** Río Negro; **c** Tacuarembó; **d** Rivera; **e** Cerro Largo; **f** Treinta y Tres; **g** Maldonado

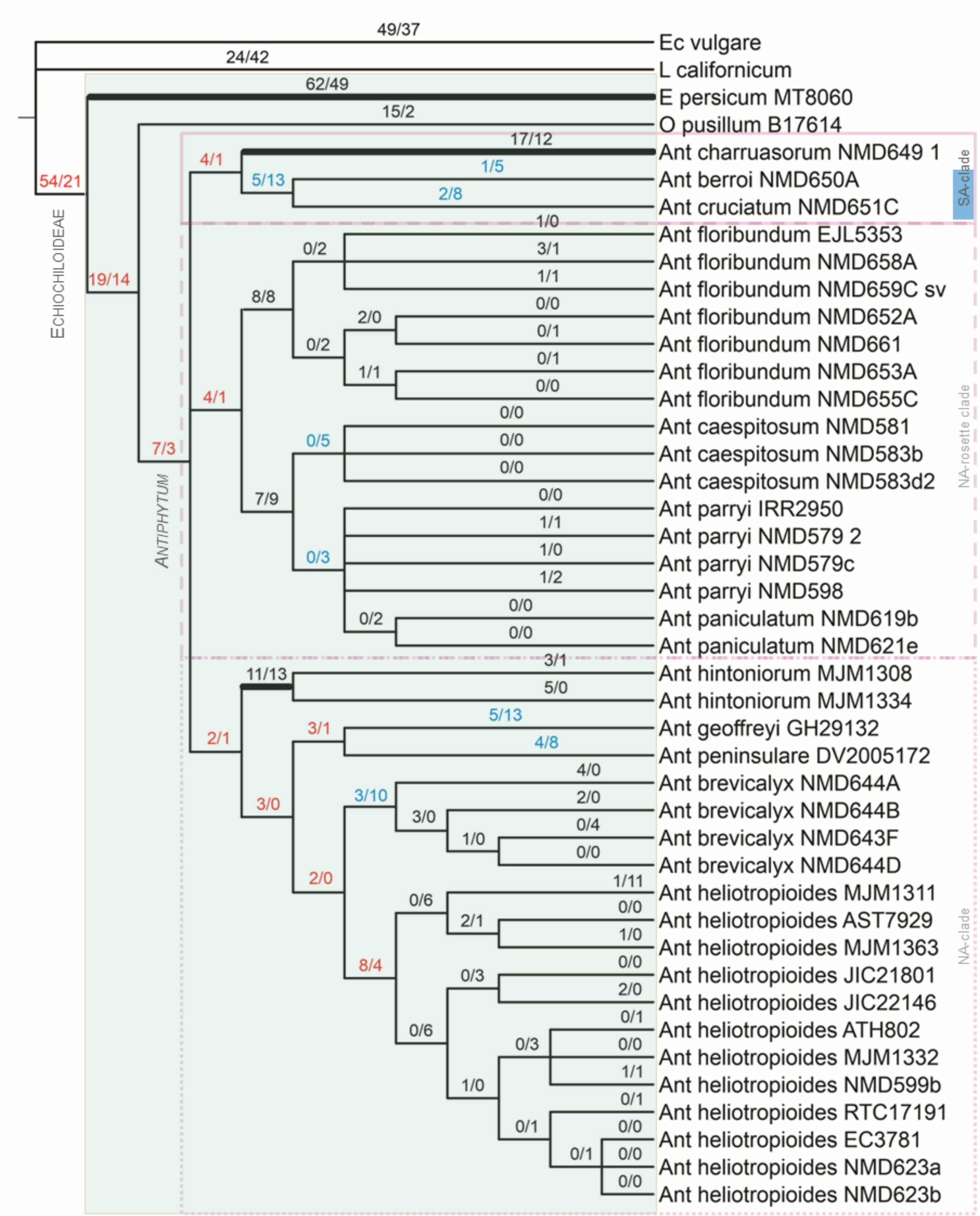

**Fig. 5** Strict consensus tree of the MP trees from the 5-loci matrix showing branch lengths (number of changes) and the contribution of either the chloroplast markers (on the left of the forward slash) or the nuclear ITS region (on the right). The label is red when most of the changes are from chloroplast markers; blue when due to ITS. The thicker branches are relevant for its number of changes and their length was corroborated against all MP trees. The explanation for other figure notations can be found in Fig. 6

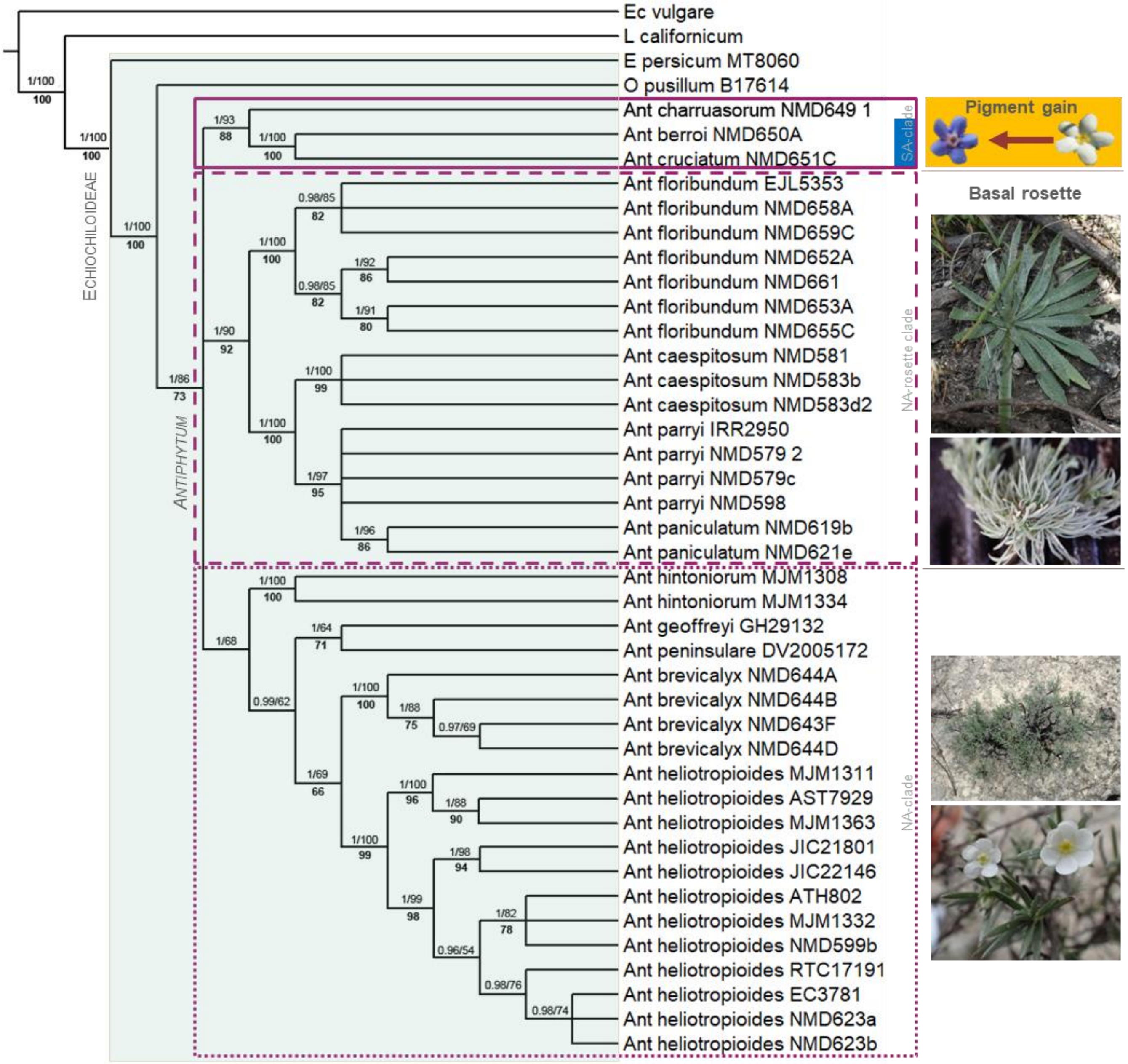

**Fig. 6** Consensus tree based on the MP analysis of the 5-loci dataset. In shaded green is highlighted the Echiochiloideae subfamily. The boxes show the three clades retrieved in *Antiphytum*: SA-clade (solid line box); NA-clade of species bearing basal-rosette (broken line box), and NA-clade of species lacking basal-rosette (dotted line box). The SA-subclade of blue corolla species is shown with a blue square, and in front, the pigment gain representation. In front of NA clade with basal-rosette images from this feature are shown. The NA clade lacking basal-rosette has species with some degree of fidelity to gypsum (soil shown in the figure) and the unique gypsophile species of the whole subfamily, *A. hintoniorum* (shown in the figure). Support values are shown on each branch (PP/ML-BS above; MP-JK below); MP-JK values below 64 are not shown

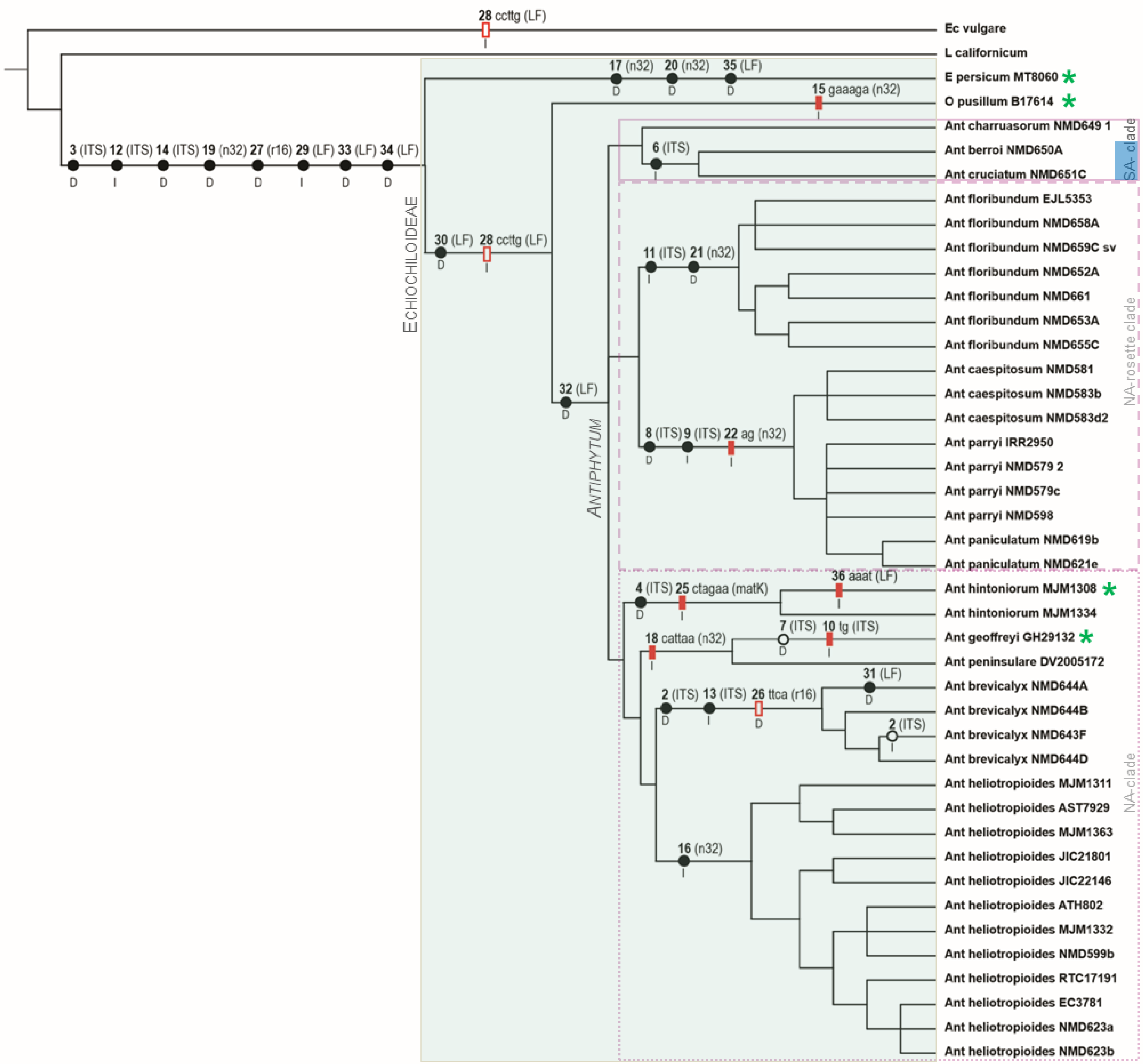

**Fig. 7** Informative microstructural characters in any given matrix mapped onto the consensus of the MP trees of 5-loci dataset. Red hashmarks represent SSRs and black hashmarks gaps; solid hashmarks when the character state shows no homoplasy. Numbers above the hashmark correspond to the character number of the 43-terminal matrix after deletion of the non-potentially informative characters. Letters below hashmarks indicate if the character state corresponding to a deletion (D) or an insertion (I). Marker codes are as follows: n32=*ndh*F–*rpl*32; LF=*trn*L–F; r16=*rps*16. Green asterisks in front of terminals indicate that the apparently autapomorphic microstructural character(s) is synapomorphic in the full corresponding matrix. Table 3 describes the microstructural characters that group the main clades in Echiochiloideae. The explanation for other figure notations can be found in Fig. 6

## Appendix 1. Lists of microstructural characters

### A. List of microstructural characters and their aligned positions found in the ITS region coded as absent (0) or present (1)

#### Gaps

1. 4
2. 6
3. 24
4. 35–38
5. 35
6. 50–52
7. 51–52
8. 52–53
9. 52
10. 59
11. 62
12. 65
13. 68–85
14. 69
15. 81–82
16. 81
17. 84–87
18. 84–85
19. 93
20. 103–104
21. 104
22. 107
23. 125
24. 139–141
25. 140–141
26. 143
27. 148–151
28. 150
29. 161–163
30. 162–163
31. 171
32. 188
33. 201
34. 219
35. 237
36. 239
37. 241
38. 245
39. 251
40. 253
41. 264–266
42. 276–381
43. 448
44. 452–453
45. 453–456
46. 453
47. 479
48. 507–519
49. 507
50. 512–513
51. 513
52. 518–519
53. 518
54. 519
55. 537
56. 566–569
57. 584
58. 610
59. 617
60. 618
61. 627
62. 651
63. 654–672
64. 656
65. 660
66. 662–663
67. 663
68. 673–674
69. 673
70. 674
71. 677
72. 683
73. 694

#### Inversions

74. GAGTTAAAAAA to TTTTTTAACTC 36–43; 47–49
75. AA to TT 636–637

#### SSRs

76. AAC 44–46
77. GC 72–73
78. TTA 114–116
79. GGC 130–132
80. AT 133–134
81. GT 148–149
82. AT 199–200
83. CA 459–460
84. TGTG double with some mutations in the first (CGTG) and inversion of the second 499–506
85. TG 522–523
86. TT 638–639

### B. List of microstructural characters and their aligned positions found in the *ndh*F–*rpl*32 region coded as absent (0) or present (1) unless otherwise stated

#### Gaps

1. 18–19
2. 58–61
3. 114–125
4. 167–173
5. 174–179
6. 181
7. 249–254
8. 308
9. 316–320
10. 326
11. 334–338
12. 406–414

13. 406–407
14. 412
15. 414
16. 419–420
17. 431–716
18. 436–677
19. 436–668
20. 449–458
21. 451–458
22. 463–466
23. 472–691
24. 482–490
25. 500–762
26. 502–505
27. 571–578
28. 594–600
29. 613–615
30. 634–637
31. 662–666
32. 686–689
33. 688–749
34. 724
35. 835–845
36. 835–837

**Inversions**

37. PolyA to polyT 669–672

**SSRs**

38. CTTTTTTAAT with change of G for C 36–46
39. GAAAGA 90–95
40. AAAAAAGA 116–123
41. AATATT 154–159
42. TCA 242–244
43. AAGATAACA with change of T for G 298–307
44. CATTAA 343–348
45. CACTT 377–381
46. CACTTTATAAA 388–398
47. ATT 488–490
48. TTA 494–499 without SSR=0; simple SSR=1; double SSR=2
49. AGGTTTA with extra G 512–518
50. ATCGATT with shorter T strings 538–544
51. ACACGGC 552–558
52. TTT 649–651
53. AG 688–690
54. TTTTCATCC 707–715
55. ATT 756–758

### C. List of microstructural characters and their aligned positions found in the *mat*K region coded as absent (0) or present (1) unless otherwise stated

**Gaps**

1. 7–12
2. 13–27
3. 16–215
4. 52–75
5. 150–152
6. 157–171
7. 157–159
8. 382–384
9. 394–399
10. 484–906
11. 694–696
12. 706–711
13. 706–708
14. 802–825
15. 820–825

**Inversions**

16. AAA to TTT 598–600

**SSRs**

17. TCA 421–423
18. TTC 439–450 without SSR=0; simple SSR=1; double SSR=2; quadruple SSR=3.
19. ATTTCT 16–21
20. AATGAT with intermediate triplet 31–49
21. CTAGAA 160–165
22. GCTAAA 601–606
23. AAA 721–723
24. ATAAACCAA 802–810

## D. List of microstructural characters and their aligned positions found in the *trn*L–F region coded as absent (0) or present (1) unless otherwise stated

### Gaps

1. 7
2. 24
3. 31
4. 35–37
5. 36
6. 56
7. 64–71
8. 70–72
9. 74–76
10. 75–76
11. 76
12. 121
13. 175–191
14. 175–188
15. 180–192
16. 197–418
17. 201–210
18. 217–231
19. 217–219
20. 233–259
21. 236–349
22. 236–259
23. 243
24. 253–259
25. 256–259
26. 263–270
27. 265–270
28. 262–264
29. 269–303
30. 269–272
31. 309–346
32. 309–326
33. 323–325
34. 323–324
35. 348–349
36. 349
37. 351
38. 361–500
39. 361–418
40. 361–414
41. 361–406
42. 374–375
43. 410–420
44. 414–443
45. 424–443
46. 424–434
47. 435–443
48. 436–437
49. 439–443
50. 439
51. 437
52. 442–443
53. 445–448
54. 445
55. 446–490
56. 446–448
57. 448–490
58. 447–448
59. 449–492
60. 449–490
61. 451–492
62. 451–512
63. 451–500
64. 451–491
65. 451–493
66. 454–456
67. 489–490
68. 494–512
69. 509–640
70. 512
71. 550–761
72. 550–566
73. 550–554
74. 550
75. 554
76. 557
77. 559
78. 562–573
79. 562–570
80. 583–611
81. 596–611
82. 615–618
83. 634–642
84. 639–640
85. 676–684
86. 688–696
87. 692–696
88. 731–747
89. 745
90. 771–807
91. 778
92. 787
93. 795–796
94. 796
95. 833–837
96. 833
97. 835–839
98. 837–859
99. 838–856
100. 839
101. 859
102. 860–862
103. 861–867
104. 866–869
105. 872–884
106. 872–874
107. 876–898
108. 901–924
109. 901–902
110. 901
111. 928–952
112. 929–956
113. 929–952
114. 933–1059
115. 952
116. 960–962
117. 967–969
118. 971–1006
119. 977–1031
120. 1020–1077
121. 1021–1071
122. 1028–1060
123. 1028–1031
124. 1034
125. 1038
126. 1041–1061
127. 1041–1052
128. 1082–1221
129. 1087–1101
130. 1087–1089
131. 1089
132. 1089–1100
133. 1089–1092
134. 1091–1094
135. 1091–1092
136. 1093–1094
137. 1094
138. 1097–1223
139. 1097–1123
140. 1097–1111
141. 1097–1101
142. 1101
143. 1105–1124
144. 1115–1126
145. 1115–1124
146. 1123
147. 1126–1131
148. 1127–1158
149. 1134–1138
150. 1140–1158
151. 1141–1158
152. 1147–1161
153. 1149–1191
154. 1161–1163
155. 1163–1164
156. 1178–1180

157. 1190–1191
158. 1194–1206
159. 1194–1203
160. 1195–1199
161. 1202–1301
162. 1204–1365
163. 1207–1223
164. 1209–1301
165. 1210–1216
166. 1211–1365
167. 1211–1217
168. 1219–1223
169. 1220–1223
170. 1223–1465
171. 1223
172. 1223–1243
173. 1223–1241
174. 1220–1221
175. 1236–1311
176. 1241–1257
177. 1242–1311
178. 1239–1241
179. 1242–1244
180. 1250–1263
181. 1250–1261
182. 1261
183. 1299–1301
184. 1308–1314
185. 1309–1314
186. 1338–1347
187. 1337–1352
188. 1338–1365
189. 1338–1350
190. 1372–1393
191. 1373–1404
192. 1373–1395
193. 1380–1391
194. 1401–1417
195. 1398–1404
196. 1400–1404
197. 1409–1425
198. 1420–1448
199. 1420–1425
200. 1425–1448
201. 1428–1429
202. 1432
203. 1435–1460
204. 1435–1459
205. 1435–1455
206. 1460–1465
207. 1461–1464
208. 1464
209. 1466–1507
210. 1466–1469
211. 1468
212. 1475–1515
213. 1498–1500
214. 1550
215. 1554
216. 1555–1556
217. 1573–1597
218. 1578–1603

**Inversions**

219. TTTGAATGA to TCSTTCAAA 1236–1244

**SSRs**

220. AAGG 112–118 without SSR=0; simple SSR=1; double SSR with deletion of one A=2
221. SRR ATAGG 125–129
222. CCTTG 181–185
223. TC 213–214
224. TCATAAA 220–226
225. TAAA 227–230
226. TG 236–237
227. GTG 238–240
228. GAAA 244–247
229. TGAAA 248–252
230. CA 269–270
231. AT 276–279 without SSR=0; simple SSR=1; double SSR=2
232. ATTTGT 280–285
233. GAAAGTATATACATTTTT 286–303
234. TGTATTGA 309–316
235. TATTGA 317–322
236. ACTTTATCTTACTTT triple with flipflop to TC in the second repetition 332–346
237. ATCAAA 355–360
238. TGA 410–412
239. TAAT 424–427
240. AAT double 428–433
241. CTAATATATT 451–463
242. CAAAATATATT 464–474
243. TTAATATATT with TT instead of AA 475–484
244. TATT 485–488
245. CTGTT 496–500
246. TTAA 563–566
247. ATAGAA583–588
248. AAGAA 591–600 without motif=0; motif without SSR=1; motif and SSR=2
249. GAAGAAAGAA 601–610
250. TAA 629–631

251. GATCAAA 634–640
252. AATCA 644–648
253. TTTT 676–679
254. TTA 693–695
255. TC 699–700
256. GTC 720–722
257. CAA 751–753
258. TTG 843–851 without SSR=0; simple SSR=1; double SSR=2; triple SSR=3
259. TTGA 853–856
260. TA triple 879–884 without motif= 0; motif without SSR=1; motif and triple SSR=2
261. TATCT 888–894: with repetition of the last CT
262. ATCT 895–898
263. TTATCTATTT 904–915: with changes of the first nt
264. TATCTATTT 916–924
265. GT 929–930
266. TTA 933–935
267. TAGT 938–941
268. TTTAGTTAGT 942–951
269. CTT 973–975
270. TTCCAAATTCCTCATCTTT 977–995
271. CTCATCTTT 996–1004
272. ATCATT 1011–1016
273. ACTCT 1023–1027
274. ATT 1035–1037
275. GTT 1041–1043
276. GTTTT 1046–1050
277. ACAA 1056–1059
278. TAGAAATG 1064–1071
279. GAT 1075–1077
280. CTGG with mutations in some terminals 1082–1085
281. CTTT 1097–1100
282. CATC 1105–1108
283. ATC 1109–1111
284. TCATCTTA 1115–1122
285. TCTT 1130–1137 without motif=0; motif without SSR=1; motif and SSR=2
286. TGAGAAACGT 1149–1158
287. ATGATATAC with mutation C to T 1168–1176
288. TACAAA 1184–1189
289. GGAA/TGAA with mutations 1209–1216 without motif=0; motif without SSR=1; motif and SSR=2
290. TTATTTAC 1250–1257
291. TTC 1309–1311
292. TTAT 1355–1358
293. TCTTAT with insertion of one T 1359–1365
294. TTG 1368–1370
295. TGAA 1373–1376
296. GAA 1377–1379
297. ACAGTA 1412–1417
298. GATAAAAGT with flip flop 1435–1443
299. TAAAA 1444–1448
300. GTT 1452–1454
301. AAAGTTGGTAATCTTCTTTAATA 1475–1497
302. TTTAATA 1501–1507
303. CTTT 1512–1515
304. TTTAATACTTTTAATTGACATA 1527–1548
305. AAAAT 1573–1577
306. ATAAAT 1578–1584

307. AAT 1586–1591 without SSR= 0; simple SSR=1; double SSR=2
308. AAG 1595–1597

### E. List of microstructural characters and their aligned positions found in the *rps*16 region coded as absent (0) or present (1) unless otherwise stated

#### Gaps

1. 6
2. 30–50
3. 37–52
4. 43–54
5. 70–73
6. 79–84
7. 123–126
8. 138–141
9. 138
10. 164
11. 188–191
12. 239–274
13. 242–247
14. 244–248
15. 287–295
16. 302–312
17. 304–312
18. 350–362
19. 350
20. 353–359
21. 354–365
22. 354–360
23. 361–365
24. 364–365
25. 400–421
26. 418–419
27. 425–426
28. 433–435
29. 434–437
30. 441–461
31. 455–471
32. 521–552
33. 549
34. 558–564
35. 559
36. 565–578
37. 565–567
38. 572–584
39. 632–635
40. 657–668
41. 659–722
42. 660–672
43. 669–678
44. 670–678
45. 670–676
46. 675–677
47. 694–700
48. 698–710
49. 707–722
50. 707–711
51. 714–731
52. 716–728
53. 725–726
54. 731–732
55. 738–740
56. 792
57. 793–796
58. 820–832
59. 822
60. 824–826
61. 827–830
62. 850–860
63. 874
64. 1202–1278
65. 1212–1270
66. 1216–1225
67. 1282–1284
68. 1309
69. 1347

#### SSRs

70. ATT 27–32 without motif=0; motif without SSR=1; motif with SSR=2
71. TGTTC 38–42
72. TCCAC 46–50
73. GGG 79–81
74. TGT 91–93
75. AAATA with C insertion 99–104
76. GTA 130–132
77. GTA 150–152
78. TTCA with mutations 157–160
79. AATCAAT with T deletion 199–204
80. AAATT with change of T to A 205–209
81. AAATAT with change of A to G 227–232
82. GATATAGAAA 255–264
83. ATAGAAA 265–271
84. CCAATT with insertion of A 306–312
85. TGAT 354–357
86. TGTA 392–395
87. TCGGAA double 406–417
88. ATAAA 448–452
89. ATAAAAG 455–461
90. GGA with mutation 469–471
91. CAAAA 482–486
92. TAAAA 560–564

93. AAA 572–574
94. CAAA with change of C to G 575–578
95. AAGGAT 589–594
96. CAGAA 602–606
97. TTTTTAATTGTCTCAA 639–654
98. ATAACT 660–665
99. AAT 670–672

# DISCUSIÓN GENERAL

## I. Reevaluación taxonómica de las especies de *Antiphytum*

El género *Antiphytum* nació con problemas taxonómicos que requirieron la lectotipificación del mismo por Johnston (1923), uniendo el nombre a las especies sudamericanas, que desde entonces eran de difícil acceso y poco conocidas. Un estudio del género debía, por fuerza, incluir la diversidad austral para poder plantear escenarios de distribución y diversificación. Empero, las especies sudamericanas representaban un problema logístico, pues no había disponibilidad de colectas para estudios filogenéticos moleculares. Los herbarios brasileños no permiten ni el envío ni el muestreo de ejemplares de sus colecciones biológicas, mientras que el herbario uruguayo MVFA no cuenta con recursos para envío de material. Por otra parte, además de los pocos ejemplares de herbarios internacionales (B, G, GH, K, MO, NY, P, US), trabajos como el de De Melo y de Stapf (2014) rechazaban la existencia de *Antiphytum* en Brasil, contradiciendo las colectas de herbario y trabajos recientes en que se ha documentado el género, como el de Setubal (2010) y las bases de datos de los herbarios brasileños.

La disponibilidad de ejemplares para una adecuada revisión taxonómica afecta la circunscripción genérica y de especies. Por ejemplo, en el tratamiento del género, Johnston (1923) consideró *A. stoechadifolium* la misma especie que *A. cruciatum*; posteriormente y con reserva, el mismo autor reconoció a las dos especies sudamericanas, aunque el material que pudo consultar fue más bien pobre (Johnston 1927). Mendoza-Díaz (2015) concordó con el reconocimiento de estas dos especies principalmente por la ornamentación de las clusas y coincidió en lo exiguo del material para concluir la delimitación de especies en Sudamérica: la información sobre la distribución de los especímenes sudamericanos es escasa en concordancia con la antigüedad de los mismos, precisando si acaso el país o la región donde fueron colectados. Empero, las conclusiones taxonómicas preliminares de Mendoza-Díaz (2015) para estas especies son diferentes con respecto a lo obtenido en este trabajo.

La posibilidad de muestrear en Sudamérica y consultar los herbarios de la región fue la piedra angular para los resultados de este proyecto, evidenciando que el trabajo de campo y de herbario siguen siendo pasos vitales en Sistemática. El estudio completo de material perteneciente a *Antiphytum* permitió delimitarlo dentro de Echiochiloideae en términos morfológicos y reconocer nuevas especies como parte del género. Resultado de ello fue la publicación de una nueva especie sudamericana, *Antiphytum charruasorum*, que coincide morfológicamente con las especies distribuidas en Norteamérica y que estableció importantes patrones morfológicos, biogeográficos y filogenéticos para entender el género; la transferencia a *Antiphytum* y lectotipificación de una especie sudamericana, *A. berroi*, que no sólo aumentó la riqueza de especies en Sudamérica, sino que clarificó que esta especie, además de *A. cruciatum*, se distribuye en el sur de Brasil. Finalmente, de las dos especies incluidas en la publicación original de *Antiphytum* (Meisner 1840), *A. stoechadifolium* era la menos entendida, pero gracias a la revisión de los herbario MVFA e ICN, pudimos concluir que no se distribuye en el actual Brasil, sino sólo en Uruguay, en concreto al sur de este país. Las etiquetas de las colectas de Sellow (ejemplares tipo), que sólo especifican como localidad "Brasilia meridionalis", aluden a un territorio en el que no se hacía distinción de la entonces inexistente República de Uruguay. Sin embargo, esta especie sigue siendo objeto de estudio, pues aparte de las colectas tipo del siglo XIX, dos ejemplares del MVFA (MVFA-25860, *Bonifacino* s.n.; MVFA-s.n, *Marchesi 1420*) son los únicos registros recientes (siglo XX) que aclaran la distribución de la especie en el Departamento de Maldonado, disyunta al resto de las especies uruguayas distribuidas en la zona norte del país. La preservación de los herborizados con alcohol-mercurio impidió la extracción de ADN para incluir a la especie en la reconstrucción filogenética molecular. No obstante, la morfología permite anticipar que se recuperará dentro del subclado sudamericano de especies con corolas azules e hipotetizar una relación hermana con *A. berroi.*

En Norteamérica, donde pareciera que el número de especies estaba claro, material herborizado permitió el reconocimiento de dos nuevas especies que cambiaron el muestreo disponible del género, y por ende, la hipótesis filogenética de Mendoza-Díaz (2015), aun empleando los mismos marcadores de ese trabajo; ampliando, además, la distribución geográfica conocida del mismo.

Por todo lo anterior, se considera que el más valioso resultado de este trabajo fue la actualización taxonómica del género (primera parte de la tesis) que deriva en 13 especies distribuidas de forma disyunta en América, y que fue el paso preliminar para la segunda parte de esta tesis. En términos morfológicos, *Antiphytum* se puede diferenciar principalmente por la combinación de los siguientes caracteres: sufrutices perennes como forma de crecimiento; corola con simetría radial y sobrepasando el cáliz; presencia de apéndices faucales; fruto no encerrado por el cáliz, sino el cáliz extendido y subtendiendo al fruto; forma de la ginobase de plana a triangular; cicatriz en la mitad proximal del eremocarpo, no rebasando la zona media de la cara ventral de éste.

La circunscripción actualizada de *Antiphytum* incrementa los 19 ejemplos de distribución anfitropical en América (AAD por sus siglas en inglés) de Boraginaceae, la segunda familia con mayor número de AAD (Simpson et al. 2017b), sumándose a los patrones previamente estudiados en esta familia, pero añadiendo la particularidad geográfica y morfológica de *Antiphytum*.

*Antiphytum* presenta una distribución que, de acuerdo a Simpson et al. (2017b), puede considerarse como trans-NA oriental-SA anfitropical, porque tiene representantes en zonas que se encuentran en el Neotrópico de Norteamérica. Por otro lado, la distribución en Sudamérica es atípica para la familia Boraginaceae, que principalmente se distribuye en la franja occidental del subcontinente. Los únicos géneros en el oriente de Sudamérica, entre el sur de Brasil y norte de Uruguay, son *Moritzia* y *Thaumatocaryon* (subfamilia Boraginoideae, tribu Boragineae, sólo en Brasil) y *Antiphytum* (subfamilia Echiochiloideae, en ambos países), los tres endémicos del continente americano.

México alberga la mayor riqueza de especies de *Antiphytum*, con *A. heliotropioides* y *A. floribundum* alcanzando el sur de EUA, y las demás especies norteamericanas distribuidas principalmente sobre las cadenas montañosas de las Sierras Madres, el Altiplano Mexicano y una en la Península de Baja California, teniendo a Oaxaca como su distribución más sureña en Norteamérica. Asociado a esta distribución, se encuentra la preferencia edáfica del género, pues a excepción de *A. floribundum*, que sólo crece sobre suelos volcánicos, las especies norteamericanas se encuentran en suelos calcáreos y son facultativas para suelos yesosos, siendo *A. hintoniorum* la única especie gipsófila del género y *A. peninsulare* una gipsoclina. Reapareciendo hasta Uruguay y el sur de Brasil –en el estado de Río Grande do Sul–, las especies sudamericanas de corola azul se distribuyen sobre suelos de origen volcánico, algunos de baja fertilidad, de acidez acentuada, con deficiencia de P, Ca y Mg y con granulometría de limos, arcillas y arenas (Setubal 2010), mientras que *A. charruasorum*, de corola blanca, se distribuye sobre suelos calcáreos como sus contrapartes norteamericanas a las que se parece más morfológicamente.

## II. Filogenia y patrones de diversificación de *Antiphytum*.

El primer resultado que deriva de los análisis filogenéticos de este estudio es la monofilia de *Antiphytum* en concordancia con la delimitación morfológica del género. Las tres posibles topologías para resolver la politomia por conflicto de los cinco marcadores moleculares son ambiguas respecto a la interpretación de la direccionalidad de colonización, pues el grupo hermano, *Ogastemma*, es africano. Sin embargo, dos de ellas sugieren que las especies norteamericanas no comparten un ancestro común inmediate e implican un origen único y más probable en Norteamérica, en contraste a la necesidad de invocar dos colonizaciones independientes de esta región. Además, el clado de las especies sudamericanas de corola azul y de clusas estipitadas es derivado, y de acuerdo al número de cambios del árbol consenso de cinco marcadores, más reciente con respecto a *A. charruasorum*. En conjunción con lo anterior, la distribución geográfica de los otros géneros de Echiochiloideae, la mayor diversidad en Norteamérica, que incluye taxones extintos asignables a la subfamilia (no publicado, descripción como *Cryptantha* por Thomasson 1979) y la edad del nodo *Antiphytum* (Chacón et al. 2017), apoyan una colonización de norte a sur del continente, que es la que se propone en este trabajo. Esto coincide con los patrones hallados en la familia y, especialmente por distribución geográfica, a los de *Moritzia* y *Thaumatocaryon* (Weigend et al. 2010). Estos últimos autores postularon una direccionalidad de colonización del linaje americano de Boragineae de norte a sur, apoyado en la actual presencia de *Moritzia* y *Thaumatocaryon* y la presencia de fósiles del grupo en Norteamérica. Además, la revisión de Simpson et al. (2017b) infirió que cerca de 53.8% de taxa desérticos de diferentes linajes (sin incluir helechos) con eventos AAD tuvieron direccionalidad de Norte a Sudamérica.

El clado sudamericano presenta dos cambios morfológicos, ganancia de pigmento en las corolas y presencia de un estípite, los cuales se plantean posteriores al evento de colonización. El primero se propone como promotor de diversificación del linaje sudamericano, mientras que el segundo se correlaciona con el tipo de suelo de origen volcánico. Ninguna especie sudamericana presenta rosetas basales ni registros en yeso.

En Norteamérica, se definen dos clados que morfológicamente se pueden separar por la presencia o ausencia de roseta basal. El clado sin rosetas presenta un mayor número de especies, todas con algún registro en suelos yesosos y con la especie gipsófila *A. hintoniorum* como hermana al resto de las especies de este clado. En contraposición, el clado de arrosetadas, aunque presenta especies gipsovagas, también incluye a *A. floribundum*, la única especie norteamericana que no se distribuye en yesos, sino sólo en suelos de origen volcánico.

Algunas de las áreas de distribución de *Echiochilon* y *Ogastemma* (subfamilia Echiochiloideae) en África y la Península Arábiga presentan yeso. En el género *Echiochilon* también hay especies gipsovagas, por lo que la subfamilia puede considerarse pre-adaptada a zonas áridas de suelos pobres con yeso o calizas, y por ello, estas pre-adaptaciones pudieron permitir la colonización y posterior endemismo edáfico de *A. hintoniorum*. En México los afloramientos yesosos se concentran principalmente en la región del Desierto Chihuahuense, aunque también hay en Baja California, Campeche, Chiapas, Guerrero, Oaxaca, Puebla y Tamaulipas (Ochoterena et al. 2020). En contraste, afloramientos de yeso en Uruguay son menos comunes. De acuerdo al Ministerio de Industria y Energía de Uruguay (1987), el yeso se encuentra en los materiales Devónicos en la localidad de Blanquillo, en el Departamento de Durazno, y en materiales Cuaternarios. En ninguna de estas zonas hay algún registro conocido de *Antiphytum*. La explotación de yeso sólo ocurre en el Departamento de Río Negro, en la localidad de Bellaco, aproximadamente a 35 km al NO de la población de *A. charruasorum* en Río Negro.

La disyunción biogeográfica de *Antiphytum* es un patrón clado-clado, de acuerdo a la terminología de Simpson et al. (2017b) e implica una diversificación de especies de ambos linajes tras un evento de AAD, y por ende, tiempos mayores de divergencia. Esto no coincide con lo observado en el género, pues más bien se infiere una diversificación rápida y reciente de sus especies, en concordancia con los datos de Chacón et al. (2017), que fechan el nodo de *Antiphytum* en 9.4 Ma.

Aunque en Norteamérica se presentan nueve especies del género, cada clado tiene aproximadamente el mismo número de especies (clado sudamericano 4; clado norteamericano arrosetado 4, clado norteamericano sin rosetas 5). La preferencia edáfica es más amplia en Norteamérica sólo por la presencia de especies gipsovagas y una gipsófila. El clado sin rosetas presenta individuos en tres tipos de suelo: calcáreo, yesoso y volcánico (*A. peninsulare* se distribuye en yeso y suelos volcánicos). El clado de arrosetadas se distribuye en suelos de origen volcánico (sólo *A. floribundum*), calcáreos y yesosos (como gipsovagas). La roseta basal se originó en un único evento dentro del género y se plantea su importancia como promotor de diversificación. En el clado de especies con rosetas se presenta la única especie no recuperada como monofilética, el grado de *A. parryi* dentro del subclado de especies arrosetadas del este de México. La especie, sin embargo, reúne otras características morfológicas y de distribución para considerarse un linaje independiente de *A. paniculatum*, del que es parafilética. Conjuntamente, la relación entre la distribución de las especies norteamericanas y los eventos geológicos que han configurado el relieve de México parecen sugerir eventos relacionados con la tectónica, preferencia edáfica y dispersiones posteriores en la especiación de *Antiphytum* en esta zona. El clado sudamericano sólo presenta distribución en suelos calcáreos y de origen volcánico.

Ya que, tanto el subclado de especies con roseta del este de México, como el subclado sudamericano de corolas azules, no se resuelven en los análisis individuales y concatenados con marcadores de cloroplasto, se pueden especular como recientes. Por otra parte, aunque las especies del clado norteamericano sin rosetas se recuperaron en la mayoría de los análisis concatenados e individuales como monofiléticos, este clado no presentó soporte en los análisis de parsimonia, pero sí en el bayesiano y débil en el de máxima verosimilitud, lo que podría asociarse con una rápida diversificación del clado.

Ante la falta de resolución entre los tres clados previamente descritos en la filogenia recuperada de *Antiphytum* con cinco marcadores moleculares, la morfología tampoco es concluyente. El subclado de corolas azules presenta tricomas glandulares capitados, también presentes en tres especies del clado norteamericano sin rosetas (ausentes en el resto de las especies); ambos sin roseta basal. La ginobase plana característica del clado de corolas azules y la modificación de la quilla ventral se presenta en *A. floribundum* (especie arrosetada sin fornículos), con la que, además, comparte la preferencia a suelos volcánicos, por lo que estos cambios morfológicos en corola y eremocarpos podrían estar correlacionados al tipo de suelo. Con el clado arrosetado de Norteamérica, el clado sudamericano también comparte la ornamentación de tubérculos (o verrugas) en la mayoría de las especies. También, la poca variación molecular en los marcadores de cloroplasto en los subclados de especies arrosetadas del este de México y de corolas azules de Sudamérica en los marcadores de cloroplasto puede apoyar un escenario donde ambos clados sean hermanos.

Aun sin resolución del esqueleto en la filogenia propuesta de *Antiphytum*, este estudio permite concluir un género natural con circunscripción taxonómica y geográfica actualizada, lo que descarta el color de la corola como característica de segregación taxonómica. Los resultados sugieren que la distribución disyunta ocurrió en un único evento de norte a sur del continente, con la ganancia de pigmento en la corola como posterior al evento de colonización y como un posible promotor de diversificación. Por ello, los resultados, aunque no resuelven la totalidad de preguntas que derivan de *Antiphytum*, plantean escenarios de futura investigación sobre bases sustentadas, aunque perfectibles, pues más muestreo en campo y trabajo de herbario siempre aportarán nuevas y valiosas evidencias. También, se proporciona una herramienta para la identificación de las especies del género.

## CONCLUSIONES GENERALES

-La Sistemática es una disciplina que se nutre de diferentes líneas de evidencia para hacer propuestas respecto a la circunscripción y explicación de la biodiversidad. Sus primeros pasos de descubrir, describir y nombrar la diversidad son claves para atender preguntas sobre diversificación y distribución de los taxa bajo estudio.

-Bajo la aseveración anterior, la publicación de tres nuevas especies del género *Antiphytum* (*A. brevicalyx*, *A. charruasorum* y *A. geoffreyi*) es un resultado de gran envergadura, porque cubre con datos necesarios los espacios en blanco de nuestro conocimiento sobre el género, no sólo en cuanto a diversidad y distribución, sino a caracteres morfológicos y moleculares que se añaden al concepto genérico y de especies. Además, propicia el interés de la comunidad por explorar zonas potenciales de distribución y aumentar los registros y conocimiento del hábitat de las especies. Como ejemplo de ello, tras la publicación de la especie uruguaya *A. charruasorum* se sumó una localidad de distribución para la especie, gracias al ingeniero agrónomo uruguayo Andrés González, quien pudo asociar su hallazgo con la nueva especie (com. pers.).

-La aclaración de las especies sudamericanas de *Antiphytum* por medio de la transferencia genérica de *Myosotis berroi* a *Antiphytum* y su delimitación geográfica y morfológica con respecto a *A. stoechadifolium*, que a su vez se ha delimitado con respecto a *A. cruciatum*, han sido fundamentales para conocer la diversidad del género en Sudamérica y desenmarañar la oscura situación taxonómica que se había heredado desde los tiempos de publicación del género. Prueba de ello han sido las constantes muestras de interés por el reconocimiento de *A. stoechadifolium* como especie independiente de *A. cruciatum*, bajo la cual se etiquetaba todo el material sudamericano (p. ej. Dr. José Iranildo Miranda de Melo, com. pers.).

-México es el país en el que se encuentra la mayor riqueza y diversidad de *Antiphytum*, con dos nuevas especies publicadas como producto de este trabajo, además de las conclusiones taxonómicas que reducen algunos nombres a la sinonimia taxonómica, p. ej. *Antiphytum nudicalces* (≡*Amblynotopsis nudicalces*) =*Antiphytum caespitosum*; *Amblynotopsis ehrenbergii* = *Antiphytum heliotropioides*; *Amblynotopsis humillis* =*Antiphytum parryi*; *Amphibologyne mexicana* = *Antiphytum floribundum*, y *Amblynotopsis durangensis* = *Antiphytum floribundum*; o que se denotan como superfluos (*Chamissoniophila cruciata*, *C. stoechadifolia*). Cambios taxonómicos y nomenclaturales derivados de este estudio requieren la integración en una síntesis monográfica de *Antiphytum*.

-La circunscripción de *Antiphytum* resultado de este trabajo apoya a un género endémico del continente americano, monofilético y de distribución disyunta (trans-AAD) integrado por 13 especies conocidas, nueve en Norteamérica (NA) y cuatro en Sudamérica (SA). En Norteamérica se encuentran *A. brevicalyx*, *A. caespitosum*, *A. floribundum*, *A. geoffreyi*, *A.heliotropiodes*, *A. hintoniorum*, *A. paniculatum*, *A. parryi* y *A. peninsulare*. De Sudamérica se conocen *A. berroi*, *A. charruasorum*, *A. cruciatum* y *A. stoechadifolium*. Con excepción de *A. parryi*, que se recuperó como parafilética a *A. paniculatum*, todas las especies en el género se recuperaron como monofiléticas en el análisis combinado. Bajo el concepto general de especie, *A. parryi* cuenta con suficientes características morfológicas y geográficas para ser delimitada de *A paniculatum*. El color azul de las flores y la filotaxia totalmente opuesta no son características morfológicas que segreguen al género por distribución geográfica, dados los patrones geográficos y filogenéticos obtenidos.

-*Antiphytum* se recuperó como integrante de la subfamilia Echiochiloideae, ratificando la relación hermana con *Ogastemma* obtenida en trabajos anteriores. Aunque algunos marcadores individuales (ITS, matK, *rps16*) recuperaron a *Ogastemma* al interior de un clado más grande con todos los terminales de *Antiphytum*, se concluye que este último es un género diferente por la evidencia filogenética de los marcadores concatenados y características diagnósticas propias del mismo.

-El análisis de parsimonia con todos los marcadores de cloroplasto y los tres métodos de reconstrucción filogenética con los cinco marcadores moleculares recuperaron tres clados al interior de *Antiphytum*: clado NA con rosetas basales; clado NA sin rosetas basales; y clado SA (sin rosetas basales). El clado NA sin rosetas basales tiene la particularidad de estar integrado por especies que tienen algún grado de afinidad al yeso y contener a la única especie gipsofila del género (*A. hintoniorum*), la cual se recupera como la primeramente divergente del clado, con una rama con muchos cambios moleculares. El clado NA con rosetas presenta elementos gipsovagos y la única especie norteamericana gipsofoba (*A. floribundum*), hermana al resto. El clado sudamericano tiene especies sin presencia en yeso, pero con afinidad a suelos calcícolas (*A. charruasorum*, corolas blancas) como en la mayoría de gipsovagas de NA; o a suelos de origen volcánico (especies sudamericanas de corolas azules), como en las norteamericanas *A. floribundum* y *A. peninsulare*.

-Las filogenias consenso de los cinco marcadores obtenidas por parsimonia y reconstrucción bayesiana no resuelven las relaciones entre los tres clados, ya que se recuperan dos posibles escenarios: 1) las especies de NA son monofiléticas y hermanas al clado SA, o 2) las especies NA no comparten un ancestro común inmediato y, por ende, el clado de SA es hermano al clado NA con rosetas o al clado NA sin rosetas, respectivamente. El árbol de máxima verosimilitud presenta una relación hermana entre el clado SA y el clado NA de rosetas, sin soporte estadístico y con una rama muy corta.

-Aun con la incertidumbre filogenética, el escenario del árbol de máxima verosimilitud se presenta factible por las características morfológicas en común entre las especies del clado arrosetado y las especies sudamericanas, la distribución hacia el sur en Norteamérica del primero y al hecho de que ambos clados posiblemente son linajes más recientes por la poca variación molecular presente en los marcadores de cloroplasto.

-Morfológicamente es posible identificar a *Antiphytum* por ser sufrútices perennes con corolas con simetría radial sobrepasando el cáliz y con apéndices faucales; por poseer lóbulos del cáliz divididos casi hasta la base y acrescentes durante la fructificación, que se extienden y subyacen a los cuatro eremocarpos expuestos (o menos por aborto, según la especie). Las ginobases van de planas a triangulares, con las correspondientes cicatrices en la mitad proximal del eremocarpo, no rebasando la zona media de la cara ventral, ovadas o triangulares en posición basal (asociada a un estípite formado por la quilla ventral), suprabasal, inframedial o medial. Las especies son fácilmente reconocibles por los eremocarpos, a excepción del grupo de especies con rosetas basales integrado por *A. caespitosum*, *A. paniculatum* y *A. parryi*. La morfología del polen es común para las especies de *Antiphytum* muestreadas de Sudamérica y Norteamérica, y es muy similar en *Ogastemma*.

-Características morfológicas como presencia o ausencia de roseta basal, presencia o ausencia de fornículos en la garganta de la corola, filotaxia opuesta o alterna, presencia o no de un estípite en el eremocarpo, ornamentación de los eremocarpos, o el color blanco o azul de las corolas, definen morfológicamente no sólo a las especies, sino a grupos al interior del género.

-La filotaxia totalmente opuesta no es privativa de las especies sudamericanas, sino que está también presente en la especie norteamericana *A. hintoniorum*. Las ginobases planas también se encuentran tanto en tres especies sudamericanas (las de corola azul) como en dos norteamericanas. El carácter de rosetas basales tuvo un solo origen en el género *Antiphytum* y deberá investigarse su papel como promotor de diversificación.

-El color blanco de las flores no es particular de las especies norteamericanas, ya que también se encuentra en un representante del linaje sudamericano. El color azul de las flores se propone como una adquisición posterior al evento de colonización de Sudamérica, al ser *A. charruasurom*, de corola blanca, el elemento tempranamente divergente en el clado recuperado con todas las especies sudamericanas. La ganancia de pigmento característico del subclado sudamericano se sugiere como un carácter que afectó la diversificación en *Antiphytum*, probablemente por facilitar la especiación o la colonización de nuevos ambientes al incorporar nuevos polinizadores o agentes de dispersión.

-El género *Antiphytum* presenta una marcada preferencia edáfica a suelos empobrecidos, principalmente a los calcáreos, pero casi la mitad de las especies, seis, son facultativas a yeso, una es gipsófila y una es una gipsoclina. Las especies con afinidad a yeso se distribuyen sólo en México. La preferencia a caliza es alta en la subfamilia, y ejemplos de gipsovagas también existen en el género *Echiochilon*. Suelos de origen volcánico son también colonizados por *Antiphytum* y algunas modificaciones morfológicas se presentan en las especies que habitan este tipo de suelos. Por ende, se plantea la preferencia edáfica como un factor involucrado en la diversificación del género.

-Los antecedentes bibliográficos respecto a la biogeografía de la familia Boraginaceae y la probable no monofilia de las especies norteamericanas, con el clado sin rosetas probablemente más antiguo, permiten suponer que en *Antiphytum* también ocurrió una colonización de norte a sur del continente por un evento de dispersión a larga distancia. Probablemente el linaje sudamericano comparta un ancestro común con el linaje norteamericano de rosetas basales.

-La mayor riqueza del género en México parece asociada a eventos geológicos más complejos del territorio mexicano, así como a la diversidad en el tipo de suelos restrictivos donde se establecieron las especies (yesosos, calcáreos y de origen volcánico).

-La subfamilia Echiochiloideae en conjunto requiere de mayor trabajo, pues se hallaron conflictos al interior de las especies de *Echiochilon* con el marcador ITS y queda pendiente obtener una filogenia resuelta de *Antiphytum*. El escenario provisional biogeográfico sugiere una dispersión desde África hacia Norteamérica (donde se originó *Antiphytum*), a través de Europa y como último punto de colonización el subcontinente de América del Sur. Fósiles de Echiochiloideae en el centro de EUA respaldan esta direccionalidad y son prueba de la presencia durante el Neogeno de representantes en latitudes mayores del continente, antes de restringirse a la distribución actual de *Antiphytum* en la zona sur de EUA, en lo que se considera Megaméxico 1.

-El presente estudio es un claro ejemplo de la necesidad del trabajo de campo y herbario para generar datos fiables previos al abordaje de preguntas sobre biogeografía y diversificación. En particular, la recolección de material sudamericano y la visita a los herbarios MVFA e ICN fue vital para completar la circunscripción del género *Antiphytum* y ubicar el momento del cambio de color de la corola en el género.

-La gran diferenciación de los eremocarpos a nivel del reconocimiento de las especies hace deseable conocer el desarrollo ontogenético de los mismos, para entender cómo se acomodan espacialmente con respecto a la ginobase y dan esa posición característica de la cicatriz. Asimismo, lo es entender el elongamiento de la quilla ventral para formar un estípite o una giba.

-Es necesario el uso de marcadores moleculares con una tasa de sustitución más rápida, o el uso de una mayor cantidad de genes usando técnicas de secuenciación masiva, pues el género tiene elementos muy recientes, y no sólo la relación entre los clados no se resuelve, sino que la separación entre los linajes internos del grupo sin rosetas en NA no presenta una resolución concluyente. Por ello, tanto el género como la subfamilia siguen siendo candidatos a estudios a diferentes niveles: genéricos, específicos, poblacionales, o de individuos.

-A pesar de las limitaciones impuestas por los resultados, este trabajo recopila la mayor información del género *Antiphytum* y aclara la situación de las especies del género en Sudamérica, proveyendo las primeras hipótesis de relación entre las especies y los primeros patrones de diversificación y biogeografía del mismo.

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