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Molecular Phylogeny and Taxonomic Revision of the Genus *Diastatea* (Campanulaceae: Lobelioideae)

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Abstract—*Diastatea* (Campanulaceae-Lobelioideae) is a genus of small, herbaceous annuals found mostly in Mexico. Differences in the number of recognized species in the historical treatments, and the lack of a phylogeny, suggested that a revision of the genus was necessary. Species boundaries and species level relationships were tested based on a sampling of nearly 50 individuals. Sequences of the chloroplast spacer regions, *atpB-rbcL* and *ndhF-rpl32*, as well as the ITS, were generated and then analyzed using maximum likelihood and Bayesian inference methods. The analyses of the separate datasets produced congruent topologies. Two strongly supported clades of *Diastatea* are newly recognized as subgenera in the taxonomic treatment. *Diastatea* Scheidw. subg. *Diastatea* and *Diastatea* subg. *Angustifolia* E.P.Johnson & T.J.Ayers can be distinguished morphologically by leaf shape and pubescence, hypanthium shape, and the presence or absence of a gibbous projection on the ventral side of the corolla. A morphological treatment of the genus corroborated by the molecular analysis revealed one new species, *Diastatea aptera* E.P.Johnson & T.J.Ayers, one new subspecies, *Diastatea tenera* subsp. *durangensis* E.P.Johnson & T.J.Ayers, and elevated an existing subspecies, *Diastatea ciliata* (McVaugh) E.P.Johnson & T.J.Ayers, to specific rank.

Keywords—Mexico, *atpB-rbcL*, *ndhF-rpl32*, nrITS.

The genus *Diastatea* Scheidw. (Campanulaceae) is composed of herbaceous annuals found primarily in the pine-oak forests of central and southern Mexico. *Diastatea* is delimited from the larger cosmopolitan genus *Lobelia* L. by the combination of three main features: a superior ovary, a persistent corolla lacking a dorsal fissure, and an annual habit (Otto and Dietrich 1841; McVaugh 1940a).

The most recent treatments of *Diastatea* were completed over 60 years ago by Rogers McVaugh (1940b) and Franz E. Wimmer (1953). Prior to McVaugh's work, *Diastatea* had been included in the cosmopolitan genus *Laurentia* P.Micheli ex Adans. sensu lato, a catchall genus for any herbaceous lobelioid with blue flowers and a corolla tube lacking a dorsal slit. In his revision of *Laurentia*, McVaugh (1940b) decided that a lack of a dorsal slit was not a sufficient indicator of relatedness and dismantled the genus. He grouped the Australian genus *Isotoma* Lindl. and most of the broadly distributed *Laurentia* into *Lobelia* section *Isotoma* R.Br., but made an exception for a few "small and homogeneous genera ... mostly recognized by some striking combination of characters." These included genera such as *Hippobroma* G.Don, *Porterella* Torrey, and *Diastatea*, which he described as forming a "natural genus" (McVaugh 1940b). Since McVaugh's treatment of *Laurentia* the recognition of small genera with narrow geographic distributions has continued with publication of *Wimmerella* (Serra and Crespo 1999) and *Solenopsis* (Crespo et al. 1998). None of these segregates of *Laurentia* sensu lato are considered possible close relatives of *Diastatea* because they lack both the characteristic superior ovary and fruit enclosed in a persistent hyaline corolla.

McVaugh resurrected Scheidweiler's *Diastatea*, into which he grouped three existing species and three newly described taxa. All possessed a superior ovary, a persistent corolla lacking a dorsal fissure, and an annual habit. The three existing species were *D. virgata* Scheidw., *D. micrantha* (Kunth) McVaugh, and *D. tenera* (A.Gray) McVaugh. He also described three new taxa: *D. costaricensis* McVaugh, *D. expansa* McVaugh, and a new subspecific taxon, *D. virgata* var. *ciliata* McVaugh. These five taxa are all currently recognized in the World Checklist and Bibliography of Campanulaceae (Lammers 2007).

Franz E. Wimmer's (1953) treatment of *Diastatea* recognized all of the taxa treated by McVaugh (1940b), as well as four described earlier under *Laurentia* (Wimmer 1935, 1937) and later transferred to *Diastatea* (Wimmer 1948). These included *D. maximiliana* E.Wimm., *D. lemairei* E.Wimm., and two varieties of *D. micrantha*: var. *longibracteata* (E.Wimm.) E.Wimm. and var. *ovatifolia* (B.L.Rob.) E.Wimm. *Diastatea ghiesbreghtii* (Lem.) E.Wimm., a synonym of *D. lemairei*, is the only taxon of Wimmer's that is currently recognized in the World Checklist of Campanulaceae (Lammers 2007).

To date, there has been no revised taxonomic treatment of *Diastatea*. Partial treatments and keys to *Diastatea* have been published and included in many floras representing the Americas (McVaugh 1943; Matuda 1950; Nash and Williams 1976; Wilbur 1976, 2001; Jeppesen 1981; Rzedowski and Rzedowski 1997; Slanis et al. 2009; Rzedowski 2019), but many of these include only the geographically widespread species, *D. micrantha*, and none include all of the species.

Other publications about the Campanulaceae and Lobelioideae briefly mention *Diastatea* (Ayers 1990; Serra and Crespo 1997; Lammers 2007; Antonelli 2008; Knox et al. 2008; Senterre and Castillo-Campos 2008; Rzedowski 2018), but a thorough revision of the genus including a molecular phylogeny has not been completed until now. The overlap in morphological characters between *Diastatea* and some species of Mexican *Lobelia* originally noted by Rogers McVaugh (1940a, 1940b, 1965) remains an important issue that must be resolved. Lammers' (2011) revision of the infrageneric classification of the genus *Lobelia* serves to highlight this overlap in his description of *Lobelia* section *Stenotium* (C.Presl) Lammers. This section includes two species that possess a partially inferior to superior ovary (*L. diastateoides* McVaugh, *L. xalapensis* Kunth). In addition, *Lobelia* section *Cryptostemon* (E.Wimm.) J.Murata, likely closely related to section *Stenotium* (Antonelli 2008; Lammers 2011), includes the species *L. sinaloae* Sprague, which lacks a dorsal cleft. Finally, both *L. xalapensis* and *L. diastateoides* are often confused with *D. micrantha* in herbarium collections, further highlighting similarities worthy of examination.

A more recent publication draws attention to the character overlap in the newly described genus *Wimmeranthus* Rzed. (Rzedowski 2018). Rzedowski concludes a close relationship between *Wimmeranthus* and *Diastatea* based on its annual habit and lack of a dorsal cleft, but delimits the new genus based on its white flower color, leaf arrangement, and semi-inferior ovary.

We undertook a revision of the genus *Diastatea* to provide a current species level taxonomic treatment and to assess the character overlap between *Diastatea* and some species of *Lobelia*. The main goals of this study were to: 1) test the monophyly of *Diastatea* to determine whether the generic circumscription is sound; 2) improve our understanding of the number of species and the species level relationships within *Diastatea*; and 3) provide a taxonomic revision of the genus which accurately reflects both the molecular phylogeny and the morphological diversity present in the group. A revision of *Diastatea* will serve to inform a future re-circumscription of *Lobelia*, a notoriously paraphyletic genus (Murata 1995; Antonelli 2008; Knox et al. 2008; Lammers 2011).

MATERIALS AND METHODS

Taxon Sampling—Leaf material was taken from herbarium sheets or from silica-dried plants collected in the field. All vouchers collected by T. J. Ayers are deposited at ASC and MEXU (see Appendix 1 for locality information). For the more widely distributed species, samples were taken from collections representing a broad geographical range to ensure that genetic diversity was represented in the phylogeny. Sampling included five of the six currently accepted species of *Diastatea*, excluding only *D. ghesbreghtii* (Lem.) E.Wimm. (see Doubtful and Excluded Taxa section). In addition, we sampled both subspecies of *D. virgata*, and an entity separated by Wimmer (1948) as *D. maximiliana*, but which is often treated as a synonym of *D. micrantha* (World Flora Online 2021). Together these taxa comprise the ingroup and are represented by 43 specimens.

The subfamily Lobelioideae is notorious for its problematic classification (McVaugh 1949; Wimmer 1953; Lammers 1992, 1993, 2011; Murata 1995). To explore the relationships between *Diastatea* and closely related genera, we chose to include taxa shown by Antonelli (2008) to be related to *Diastatea* including several Mexican species from his clade of North American, Mexican, and Caribbean *Lobelia*. In addition, we included *Palmerella* A.Gray, a perennial monotypic genus with an entire corolla tube that occurs from southern California to northern Baja California (Lammers 2007).

The genera noted above are represented by the following taxa: 18 species of *Lobelia* representing three sections: *Lobelia* sect. *Lobelia* is represented by *L. cardinalis* L., *L. siphilitica* L., and *L. dortmanna* L.; *Lobelia* sect. *Cryptostemon* is represented by *L. fenestralis* Cav., *L. hartwegii* Benth. ex A.DC., *L. irasuensis* Planch. & Oerst., *L. jaliscensis* McVaugh, and *L. sinaloae* Sprague; *Lobelia* sect. *Stenotium* is represented by *L. anatina* E.Wimm., *L. aurita* (Brandege) T.J.Ayers, *L. cordifolia* Hook. & Arn., *L. divaricata* Hook. & Arn., *L. flexuosa* (C.Presl) A.DC., *L. knoblochii* T.J.Ayers, *L. mcvaughii* T.J.Ayers, *L. nana* Kunth, *L. volcanica* T.J.Ayers, and *L. xalapensis* Kunth. Two collections of *Palmerella debilis* A.Gray comprise the representation for this genus. *Trachelium caeruleum* L. and *Campanula medium* L., two species from the subfamily Campanuloideae, were chosen to represent a more distantly related outgroup.

Voucher information for the taxa used in the molecular component of this study are listed in Appendix 1. *Trachelium caeruleum* sequences for the ITS (Accession DQ304570) and the cpDNA loci (Accession EU090187) were obtained from GenBank.

DNA Extraction—Genomic DNA was extracted using a Qiagen DNeasy 96 Plant Mini Kit (Qiagen, Valencia, California) with the following modification: due to the age of some of the sampled specimens, the AP1 buffer was applied to the material and left in a 65°C water bath for 1 hr to ensure adequate lysing of the cells. One specimen (*D. virgata* subsp. *ciliata* (McVaugh) Lammers, F. M. Ruiz 308, ASC) was extracted using a slightly modified CTAB protocol (Doyle and Doyle 1987). The quality of the extracted DNA was visualized on a 1% agarose gel and quantified using a NanoDrop1000 Spectrophotometer v3.3.1 (NanoDrop Technologies, Wilmington, Delaware).

Amplification and Sequencing—The genomic DNA was used for amplification of two non-coding regions of the chloroplast genome (*atpB-rbcL* and *ndhF-rpl32* intergenic spacer regions) and the internal transcribed spacer (ITS) region of nuclear ribosomal DNA, including ITS1, ITS2, and the 5.8S gene. By sequencing highly variable non-coding cpDNA intergenic spacer regions such as *ndhF-rpl32*, as well as regions with far less variation, such as *atpB-rbcL* spacer region (Shaw and Lickey 2005, 2007), we hoped to resolve species relationships as well as generic boundaries.

All loci required varying components and conditions to achieve quality sequences and all reactions were performed on a PTC-100 Thermal Cycler (MJ Research, Waltham, Massachusetts) or a Bio-Rad iCycler (Bio-Rad Laboratories, Hercules, California). The *atpB-rbcL* spacer region was amplified using Platinum Taq DNA polymerase (Invitrogen Life Technologies, Grand, New York), and 5% DMSO. Primers and PCR reaction conditions follow Chiang et al. (1998). The *ndhF-rpl32* spacer region was amplified via PCR using the primers and protocols developed by Shaw and Lickey (2007) with minor variations. PCR was carried out using KAPA Taq Hot Start DNA polymerase (Kapa Biosystems, Wilmington, Massachusetts). The ITS region was amplified using the universal primers designed by White et al. (1990). PCR reactions were carried out with KAPA2G Fast Hot-Start Polymerase (Kapa Biosystems Inc., Wilmington, Massachusetts) using the amplification conditions outlined in Koopman and Ayers (2005).

All PCR products were visualized on a 1% agarose gel and cleaned using a Qiagen QIAquick PCR purification protocol (Qiagen, Valencia, California). Product sequencing was done on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Foster City, California) at the University of Arizona Genetics Core.

Alignment—Sequences were assembled and edited using Lasergene-SeqMan Pro (DNASTAR, Madison, Wisconsin). They were aligned in MAFFT (Katoh and Standley 2013) using the L-INS-i refinement method for the *atpB-rbcL* and *ndhF-rpl32* regions, and the Q-INS-i method for the ITS region. Q-INS-i was chosen to align the ITS region due to the presence of secondary structures in that region (Katoh and Toh 2008). The datasets were concatenated using SeaView (Gouy et al. 2010). All sequences have been submitted to GenBank (Appendix 1). Alignments are available in the Dryad Digital Repository (Johnson and Ayers 2022).

Phylogenetic Analysis—IQ-Tree version 1.6.8 (Nguyen et al. 2015; Trifinopoulos et al. 2016) was used to conduct the phylogenetic analysis on a concatenated dataset that included *atpB-rbcL*, *ndhF-rpl32*, and the ITS. Analyses were also run on the nuclear and chloroplast loci separately, using the same parameters as the combined dataset. The total concatenated alignment was partitioned as follows: ITS1: 1–397; 5.8S: 398–559; ITS2: 560–903; *ndhF-rpl32*: 904–2236; *atpB-rbcL*: 2237–3010. BIC scores were used to determine that IQ-Tree's -spp partition model was the best fit for our data, allowing each partition its own evolutionary rate (Chernomor et al. 2016). ModelFinder (Kalyaanamoorthy et al. 2017) was used to find the best fit substitution model for each of the five partitions and are as follows: ITS1 and ITS2: GTR + F + I + G4; 5.8S: JC + R2; *ndhF-rpl32*: TVM + F + G4; *atpB-rbcL*: TVM + F. Maximum likelihood (ML) analyses were run using ultrafast bootstrapping (UFBoot) (Hoang et al. 2017) for 1000 replicates. Phylogenetic trees were visualized using FigTree (v. 1.4.4).

A Bayesian inference analysis was performed using MrBayes 3.2.2 (Ronquist et al. 2012). Two independent runs of 30,000,000 MCMC generations with four chains (one hot, three cold) using a chain temperature of 0.2 was run on the combined dataset. The trees were sampled every 100 generations and the first 25% of the trees were discarded. Resulting posterior probabilities were appended to the ML tree.

Taxonomy—A comparative morphological study was conducted on 375 specimens from the herbaria ASC, GH, LL, MEXU, MO, NY, TEX, and WIS (Appendix 1); abbreviations in accordance with Index Herbariorum (Thiers 2019). All quantitative measurements were taken from pressed, dried specimens, often using stereomicroscopy and a micrometer. For each recognized taxon, representative specimens are listed in the taxonomic treatment. Type specimens not on loan were viewed in JSTOR Global Plants (plants.jstor.org). Species distribution maps were produced using ArcGIS Desktop v. 10.8.1 (ESRI, Redlands, California) using locality information found on specimen labels. If no latitude/longitude information was available, coordinates were estimated from place names.

Micromorphological characters were assessed for taxonomic importance in a SEM study. Anther trichomes and seeds were removed from dried specimens, mounted on stubs and sputter-coated for 30 s with gold palladium. The images were taken at the NAU Imaging and Histology Core Facility on a Zeiss Supra 40 VP Scanning Electron Microscope.

Chromosome counts were made from germinated seeds with 2–3 mm long root tips treated with 0.05% colchicine for 90 min followed by fixation

in formalin-acetic acid-alcohol (1:1:3) at room temperature for 2 hrs. The root tips were then stored at 4°C for 1–4 hrs before processing. After fixation the tips were treated with 60°C 1M HCl for 2–3 min to loosen tissue, rinsed in distilled water, and then stained for 1 hr in 1% aceto-orcein before the seed coat was removed and the root tip was mounted on a slide in 45% acetic acid-glycerol (9:1) and flattened. Photos were taken at 1000× using MagnaFire 2.0 imaging software on a Leica DMLD compound microscope.

RESULTS

Alignment and Phylogenetic Analyses—The final concatenated alignment of 3010 positions, representing 43 *Diastatea* samples and 22 outgroup samples, contained 857 parsimony-informative sites, 353 unique sites, and 1800 constant sites. Table S1 (Johnson and Ayers 2022) shows the number of samples representing each individual locus, before concatenation of the dataset. Of the 43 *Diastatea* samples in the analysis we were unable to sequence 15% (Appendix 1) (Johnson and Ayers 2022).

Reconstruction of the relationships within *Diastatea* using the combined analysis resulted in a resolved tree with 100% bootstrap support for most nodes and 98% bootstrap support (1 PP) for the monophyly of the genus (Figs. 1, S1 Johnson and Ayers 2022). Individual trees representing the ITS and combined chloroplast regions are included as supplemental figures (Figs. S2, S3, Johnson and Ayers 2022).

Taxon Delimitation—Our phylogenetic data and morphological analysis combine to support recognition of a new species, *Diastatea aptera* E.P.Johnson & T.J.Ayers (100 BS, 1 PP) and a new subspecies of *D. tenera*, *D. tenera* subsp. *durangensis* E.P.Johnson & T.J.Ayers (100 BS, 1 PP), both described below. In addition, we have elevated *D. ciliata* (McVaugh) E.P.Johnson & T.J.Ayers (100 BS, 1 PP) from subspecific rank (formerly *D. virgata* subsp. *ciliata*). Further, two clades within *Diastatea* were recovered with high support (100 BS, 1 PP) in our analyses and are the basis for recognition of two subgenera: *Diastatea* subg. *Diastatea* and *Diastatea* subg. *Angustifolia* E.P.Johnson & T.J.Ayers.

Diastatea subgenus *Diastatea* includes *D. micrantha*, *D. virgata*, *D. maximiliana*, *D. costaricensis*, and newly elevated from subspecific rank, *D. ciliata*. *Diastatea* subgenus *Angustifolia* includes *D. tenera* subsp. *tenera*, *D. tenera* subsp. *durangensis*, *D. expansa*, and the newly recognized species, *D. aptera*. Within *D. subg. Diastatea* there are two highly supported clades: one supports *D. micrantha* as sister to *D. virgata* (100 BS, 1 PP) and the other supports *D. ciliata* as sister to a clade containing *D. maximiliana* and *D. costaricensis* (99 BS, 1 PP). Within *D. subg. Angustifolia* there are also two highly supported clades: one supports *D. tenera* as sister to *D. expansa* (100 BS, 1 PP) and the other supports the new species, *D. aptera*, as sister to the *D. tenera/D. expansa* clade (100 BS, 1 PP).

All North American species of *Lobelia* included in the molecular study, even those sharing one or two morphological traits with *Diastatea*, formed a monophyletic group sister to the *Palmerella/Diastatea* clade (64 BS). *Palmerella* was strongly supported as sister to *Diastatea* (100 BS, 1 PP) (Figs. 1, S1).

Morphology—**ROOTS**—The roots of this annual genus are branching and fibrous.

STEMS—The stems of both subgenera are minutely winged due to decurrent leaf bases. The notable exception is the newly described *D. aptera*, the only species in the genus to lack these winged projections on the stem (Fig. 2F, G).

LEAVES—The width and shape of the leaves help to distinguish between the subgenera. The pubescent, ovate to lanceolate, and less often narrowly elliptic leaves can always distinguish species in *Diastatea* subg. *Diastatea* from the species belonging to *D. subg. Angustifolia*, which have glabrous, narrowly elliptic to linear and sometimes needle-like leaves.

FLOWERS—The flowers resemble many others in the subfamily Lobelioideae in that they are resupinate (Fig. 3A–H). The pedicels are ebracteolate, and many are curved upward in fruit so that the capsule is vertical or slightly bent toward the stem (Fig. 3H).

COROLLA—The absence of a dorsal fissure in the corolla tube is one of the main characters that distinguish *Diastatea* from *Lobelia*. Figure 3A, C, and E show this clearly. In fruit, the corolla tube persists and becomes shiny and hyaline (Fig. 3H).

HYPANTHIUM—Two types of hypanthia are seen in *Diastatea*. In *D. subg. Angustifolia* the pedicel merges with a short obconic hypanthium causing the pedicel to become slightly thickened distally, while in *D. subg. Diastatea* the hypanthium is almost non-existent, and is more like a flattened disc in shape. In *D. subg. Diastatea* the hypanthium never swells in fruit, while in *D. subg. Angustifolia* the hypanthium may swell to envelop the basal portion of the capsule (Fig. 2C, D).

ANDROECIUM—The anthers and filaments in this group, as in other Lobelioideae, are connate and surround the style (Fig. 3A–H). In outcrossing taxa, the unreceptive style acts like a pump and pushes the pollen out of the anther tube. In self-pollinating individuals, the style remains within the anther tube and becomes receptive. At their apex, the two lower anthers each have a larger central triangular trichome flanked by many secondary shorter trichomes (Figs. 3B, 4A–H). When viewed via SEM it appears that the large central trichomes seen in *D. subg. Diastatea* are broader than those in *D. subg. Angustifolia*. In addition, the secondary trichomes in *D. subg. Angustifolia* (Fig. 4A–D) are larger and shaped more similarly to the central trichome than those in *D. subg. Diastatea* (Fig. 4E–H), which are much smaller in both length and width than the central trichome. *Diastatea virgata* (Fig. 4H) is an outlier within *D. subg. Diastatea* by sometimes possessing secondary trichomes that are more similar in size and shape to those in *D. subg. Angustifolia*.

SEEDS—The seed coats of all *Diastatea* have faint striations that can be observed through a microscope and via SEM (Fig. S4A–D, Johnson and Ayers 2022). No taxonomically useful variation was seen in the seed coats.

Cytology—We were able to complete chromosome counts on three species: *D. aptera*, *D. micrantha*, and *D. virgata*. In all cases the count was $2n = 14$ (Fig. S5A–C, Johnson and Ayers 2022).

Distribution—*Diastatea* is found in pine-oak forests from Sonora, Mexico to Argentina at elevations ranging from 600–3850 m. The genus occurs in 16 Mexican states, as well as Honduras, Guatemala, Costa Rica, and Panama, with *D. micrantha* found further south through the Andean highlands to Argentina (McVaugh 1943; Lammers 2007; Knox et al. 2008; Slanis et al. 2009). Both subgenera are distributed along the Trans-Mexican Volcanic Belt (Figs. 5, 6, 7), which is known for high levels of diversity and endemism and is part of the larger transitional zone between Nearctic and Neotropical flora (Marshall and Liebherr 2000; Devitt 2006). The

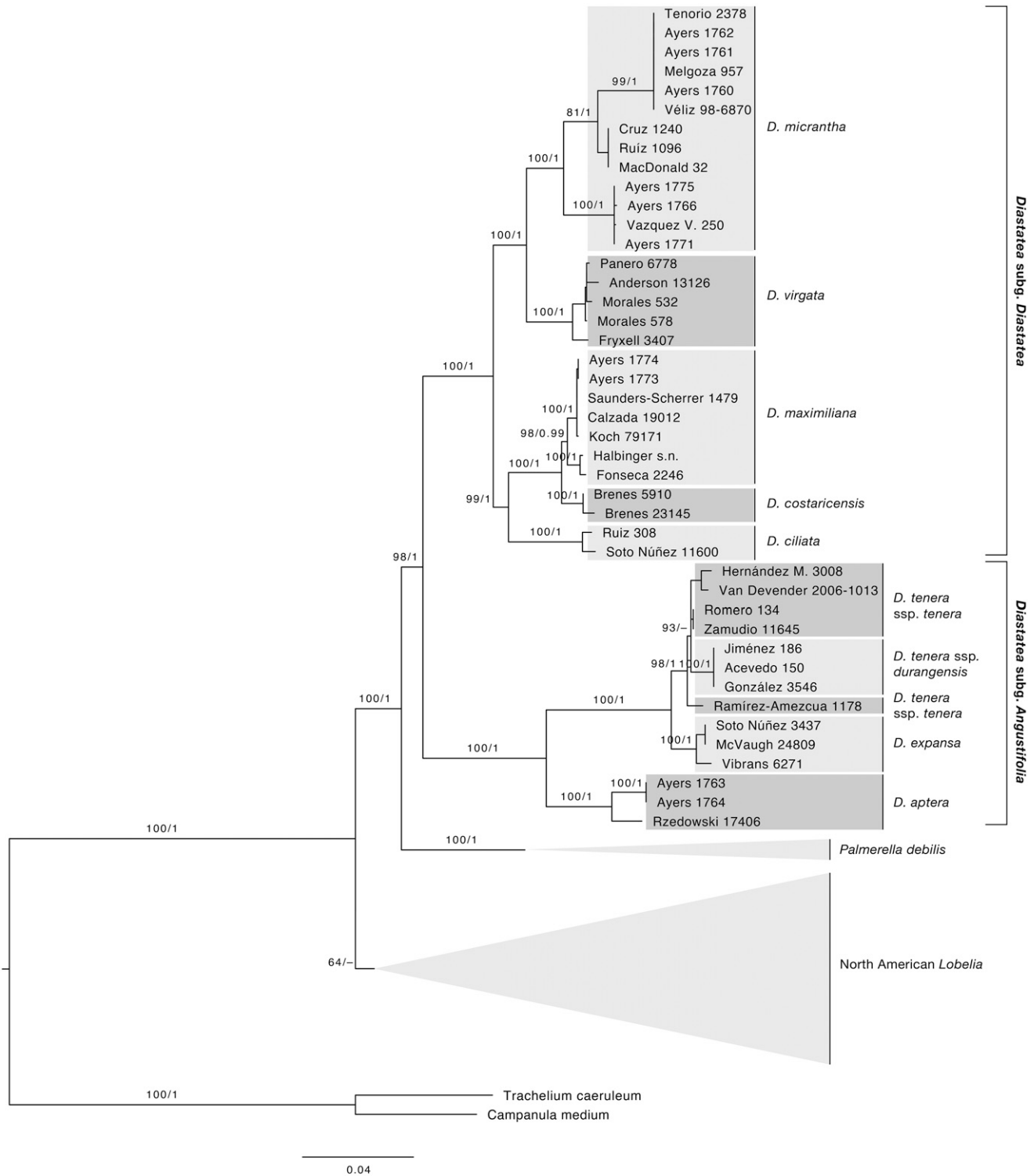


FIG. 1. Maximum likelihood tree for the concatenated dataset showing branch lengths. Log likelihood = -14286.226 and a total tree length of 1.728. Numbers above branches represent bootstrap values followed by posterior probabilities. Posterior probabilities below 0.95 are represented as dashes.

distribution of *D.* subg. *Diastatea* trends to the south (Figs. 5, 6), while *D.* subg. *Angustifolia* trends to the north (Fig. 7).

DISCUSSION

This study set out to determine the monophyly of *Diastatea* and to clarify the species level relationships and the number

of species within the genus. Monophyly was confirmed by our study and our results indicate that there are two well-resolved clades within *Diastatea* (Fig. 1), which are the basis for the recognition of two new subgenera, *Diastatea* subg. *Diastatea* and *Diastatea* subg. *Angustifolia*. Additionally, our new understanding of *Diastatea* supports recognition of new taxa at both species and subspecies levels. In particular,

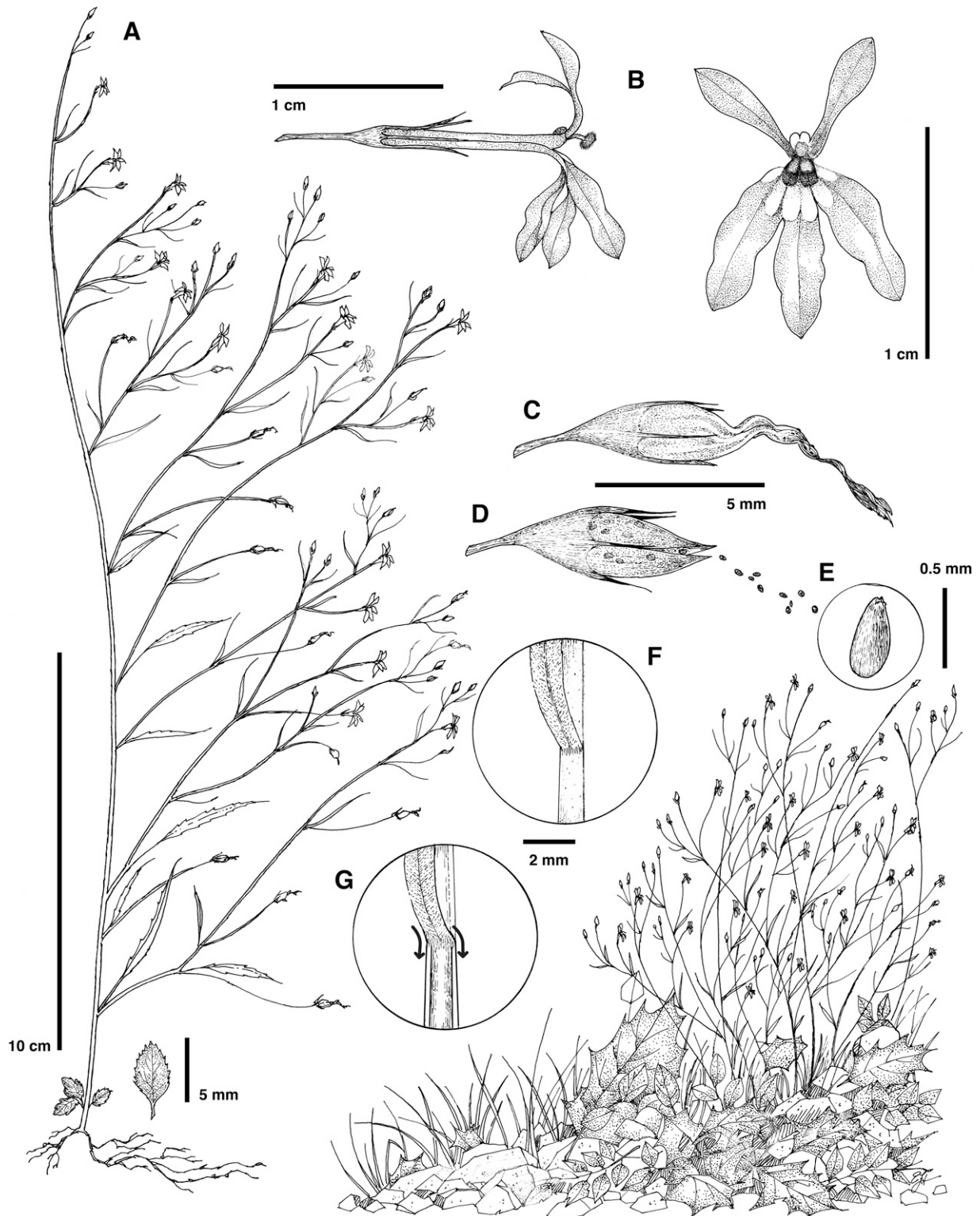


FIG. 2. Illustration of *Diastatea aptera* with inset (F, G) showing contrasting stem of *D. tenera* (representative of all other species of *Diastatea*). A–F. *Diastatea aptera*. A. Habit with enlargement of basal leaf. B. Lateral and frontal views of flower. C. Fruit with persistent withered corolla tube. D. Fruit dehiscing loculicidally via apical valves. E. Seed. F. Stem lacking the winged projections seen in all other species within the genus. G. Winged stem of *D. tenera*. Wings formed from decurrent leaf bases as highlighted by arrows. Illustration by Elaine Hultgren.

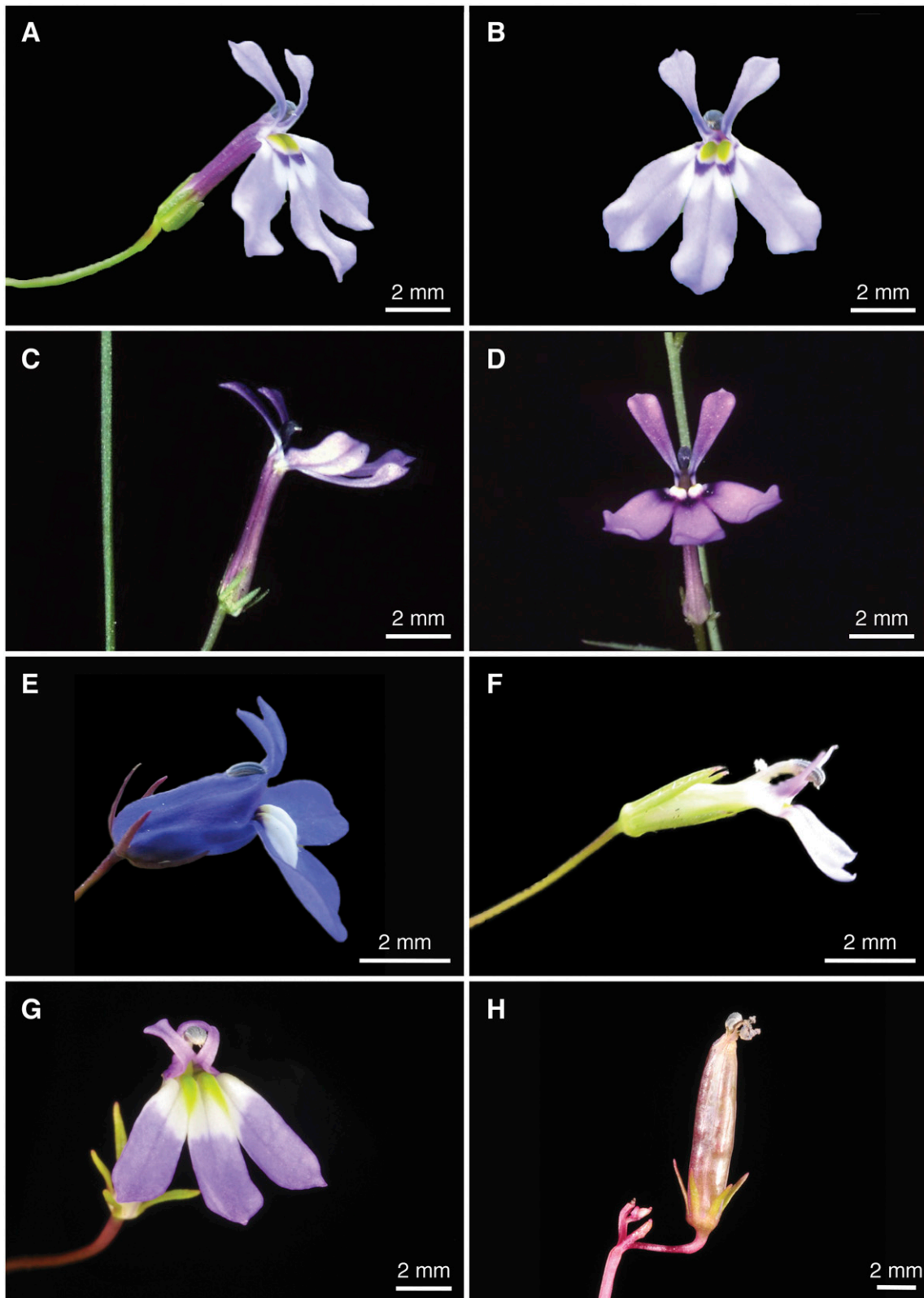


FIG. 3. A–B. *Diastatea aptera*. A. Lateral view of flower showing superior ovary. B. Frontal view of flower showing corolla lobe shape and nectar guides. C–D. *Diastatea tenera* subsp. *tenera*. C. Lateral view showing small gibbous projection at base of floral tube. D. Frontal view showing corolla lobe shape and nectar guides. E. Lateral view of *D. maximiliana* showing broad floral tube compared to *D. micrantha*. F. Lateral view of *D. micrantha* flower showing arching staminal column and relatively small floral tube as compared to *D. maximiliana*. G–H. *Diastatea virgata*. G. Frontal view of flower showing nectar guides. H. Fruit showing hyaline corolla tube. Photos by T. J. Ayers.

D. virgata was found to be polyphyletic and specimens identified as *D. tenera* included an unrecognized species and two subspecies. Due to these findings and some doubtful and excluded taxa uncovered by our taxonomic work, the genus *Diastatea* now includes eight species in two subgenera: subg. *Diastatea*: *D. ciliata*, *D. costaricensis*, *D. maximiliana*, *D. micrantha*, and *D. virgata*; subg. *Angustifolia*: *D. aptera*, *D. expansa*, *D. tenera* subsp. *tenera*, and *D. tenera* subsp. *durangensis*.

Species recognition within the Lobelioideae has historically been based on fixed, consistent morphological characters or suites of characters (McVaugh 1940b; Ayers 1990; Crespo et al. 1998; Lammers 1999). In *Diastatea*, species boundaries may be maintained due to minute habitat preferences. In our limited fieldwork we never saw two species co-occur. Variation in floral morphology and nectar guides might also suggest differences in pollinators leading to the maintenance of species boundaries.

In species that have a very broad distribution, subspecies and varieties have been recognized using morphological traits of less importance such as changes in vestiture that often vary over the geographical range of the taxon. McVaugh based his recognition of the former *D. virgata* subsp. *ciliata* on distinctive vestiture as well as on the geographical distribution of the collections (McVaugh 1940b). Wimmer, who had a less conservative view, recognized subspecific taxa such as *D. micrantha* var. *ovatifolia* (Wimmer 1948) based on minute morphological differences without any geographical basis and most Lobelioid systematists have chosen not to recognize these. In their work on *Solenopsis*, Crespo et al. (1998) highlighted the importance of unique morphological characters coupled with distinct geographical distributions when defining subspecies for that genus.

Our recognition of species within *Diastatea* is based primarily on fixed, qualitative morphological differences and molecular characters and secondarily on geographical distribution. Species are defined as monophyletic lineages of populations. Subspecies are based on quantitative morphological differences coupled with geographical distribution primarily and molecular characters secondarily. Subspecies may not represent monophyletic units, which may indicate continued gene flow among populations.

Individual analyses were run on the ITS and the combined chloroplast regions and they resulted in similar topologies when compared to the total combined analysis (Figs. 1, S1, S2, S3). Topologies of the ITS, chloroplast, and combined analyses differed in the relationship between *D.* subg. *Angustifolia* and some individuals in *D.* subg. *Diastatea* (Fig. S2), but the bootstrap support for this node on the ITS tree is relatively low (58 BS). When more individuals and loci were included in the total combined analysis these relationships were resolved (Fig. 1).

As discussed in the introduction, certain species of *Lobelia* do share one or two morphological traits with *Diastatea*. Our analysis has determined that despite these shared characters, *Diastatea* is distinct from the several sections of *Lobelia* where these shared characters occur. All representative species of *Lobelia* grouped together, never with *Palmerella debilis* (synonym *Lobelia dunnii* Greene) or *Diastatea* (Figs. 1, S1). The BS value of 64 (0.62 PP) is likely an artifact of missing taxa and missing sequence data as some species of *Lobelia* were only represented by one locus within our combined dataset (Appendix 1; Table S1).

Relationships Within subg. *Diastatea*—*Diastatea* subgenus *Diastatea* is composed of five species and was strongly supported in our analysis. The species now include *D. ciliata*, *D. costaricensis*, *D. maximiliana*, *D. micrantha*, and *D. virgata* (Figs. 1, 3E–H). This subgenus can be distinguished from the other by the pubescent, lanceolate to ovate and sometimes narrowly elliptic leaves, a flattened disc-shaped hypanthium that never envelops the base of the capsule in fruit, and the lack of a gibbous projection at the base of the corolla tube.

The combined data shows that *D. micrantha* forms one strongly supported monophyletic group sister to *D. virgata* (Fig. 1). Prior to this treatment *D. micrantha* included the synonym *D. maximiliana*. Our analysis strongly supports *D. maximiliana* as a distinct species more closely related to *D. ciliata* and *D. costaricensis*, than to *D. micrantha*.

Morphologically, *D. micrantha* is recognized by its small flowers (Fig. 3F) although some large-flowered individuals have been collected in Oaxaca. Examination of herbarium and greenhouse specimens show the style of *D. micrantha* rarely, if ever, emerges from the anther tube. Instead, the stigmatic lobes diverge and become receptive within the tube and are self-pollinated. The tendency to self-pollinate, and the genetic isolation that may result, suggests that there is more to learn about this widely distributed and morphologically variable species.

Diastatea virgata, as previously recognized with two subspecies, is polyphyletic, necessitating the elevation of *D. virgata* subsp. *ciliata* to specific rank. Now *Diastatea virgata* forms a monophyletic group in our analysis (Fig. 1) and is more closely related to *D. micrantha* than to its former subspecific taxon. Unlike *D. micrantha*, the stigma of *D. virgata* always emerges from the anther tube suggesting that it is an out-crossing species. The fruits of this species tend to be longer and narrower than all other species and the flowers are large by comparison. Both the corolla tube and the fruit are two to three times longer than those of the diminutive *D. micrantha* (Fig. 3F–H).

Diastatea ciliata was first recognized as a variety of *D. virgata* in McVaugh's 1940b treatment and was later changed to a subspecies by Lammers (2006). McVaugh (1940b) likely recognized *D. ciliata* as a variety of *D. virgata* because they share similarities in flower size and leaf shape. Both the molecular and morphological evidence support our decision to elevate this taxon to specific rank. Molecularly, it was strongly supported as sister to *D. maximiliana* and *D. costaricensis* rather than *D. virgata* (Fig. 1). Morphologically, it can be distinguished from *D. virgata* by its ciliate calyx lobes and slightly broader fruit, and from *D. micrantha*, *D. maximiliana*, and *D. costaricensis* by its corolla tube which is two to three times as long.

Diastatea maximiliana, recognized by Wimmer in his 1953 treatment, is currently treated as a synonym of *D. micrantha* (McVaugh 1940a; Lammers 2007), but both the molecular evidence and its unique morphology support it as a distinct taxon sister to *D. costaricensis* and *D. ciliata* (Fig. 1). Its broad corolla tube and large, charismatic corolla lobes are two to three times as wide as, and easily distinguishable from, *D. micrantha* (Fig. 3E, F).

Diastatea costaricensis is underrepresented in our study due to a paucity of collections. Nevertheless, *D. costaricensis* can be distinguished from *D. maximiliana* by strongly ciliate calyx lobes, which are equal in length to the fruit, increased density and length of pubescence on the leaves and stem, and

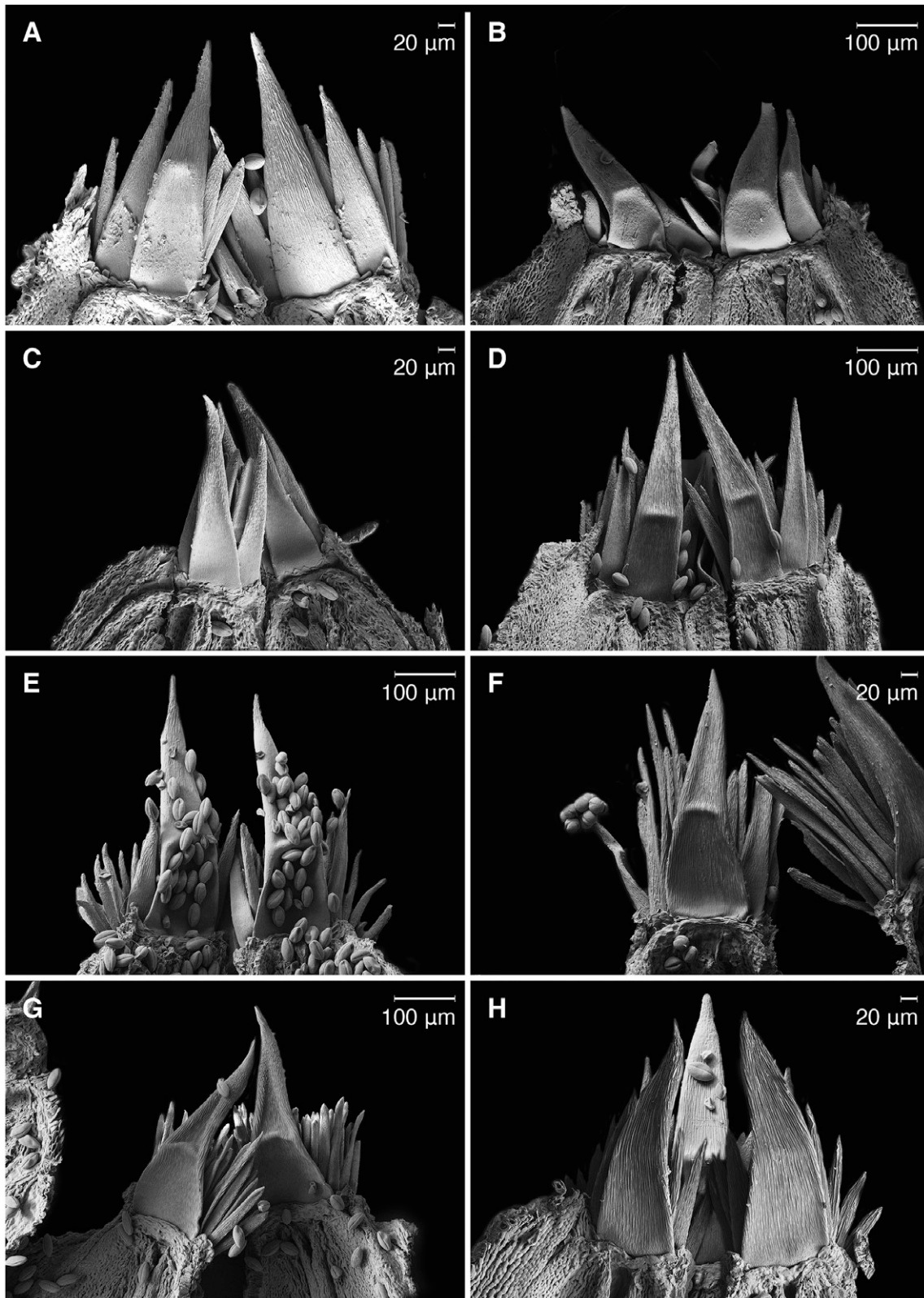


FIG. 4. Scanning electron micrograph images of anther trichomes in *Diastatea*. A–D. *Diastatea* subgenus *Angustifolia*. A. *Diastatea aptera* (Soto 13195). B. *Diastatea expansa* (Rosiles M. s.n.). C. *Diastatea tenera* subsp. *tenera* (Ayers et al. 1777). D. *Diastatea tenera* subsp. *durangensis* (Gonzalez 3546). E–H. *Diastatea* subgenus *Diastatea*. E. *Diastatea ciliata* (Reveal et al. 4183). F. *Diastatea costaricensis* (Smith 1435). G. *Diastatea maximiliana* (Ayers et al. 1774). H. *Diastatea virgata* (Ayers et al. 1772).

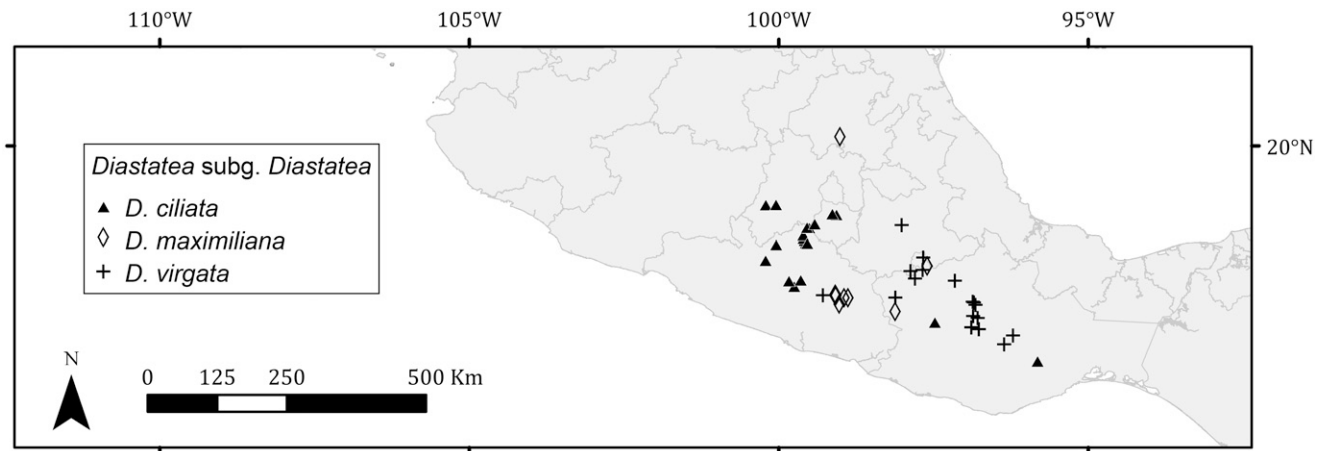


FIG. 5. Distribution map of *Diastatea ciliata*, *D. maximiliana*, and *D. virgata* in *Diastatea* subgenus *Diastatea*.

much-reduced corolla lobes, which are two to three times smaller than *D. maximiliana* in both length and width. Two morphological intermediaries between *D. maximiliana* and *D. costaricensis* have been found and are represented in the study under *D. maximiliana* (E. Halbinger s.n. and R. M. Fonseca 2246). Future collections of this species should focus between the Mexican state of Guerrero and Guatemala to determine whether more intervening populations of either taxon exist.

Relationships Within *Diastatea* subgenus *Angustifolia*—*Diastatea* subgenus *Angustifolia* comprises *D. aptera*, *D. expansa*, *D. tenera* subsp. *tenera*, and *D. tenera* subsp. *durangensis*, (Fig. 1). This subgenus is morphologically distinguished by its glabrous, narrowly elliptic to linear leaves, an obconic hypanthium in flower that may swell to partially envelop the base of the capsule in fruit, and a small gibbous projection on the ventral side of the corolla tube in *D. tenera* and *D. expansa*.

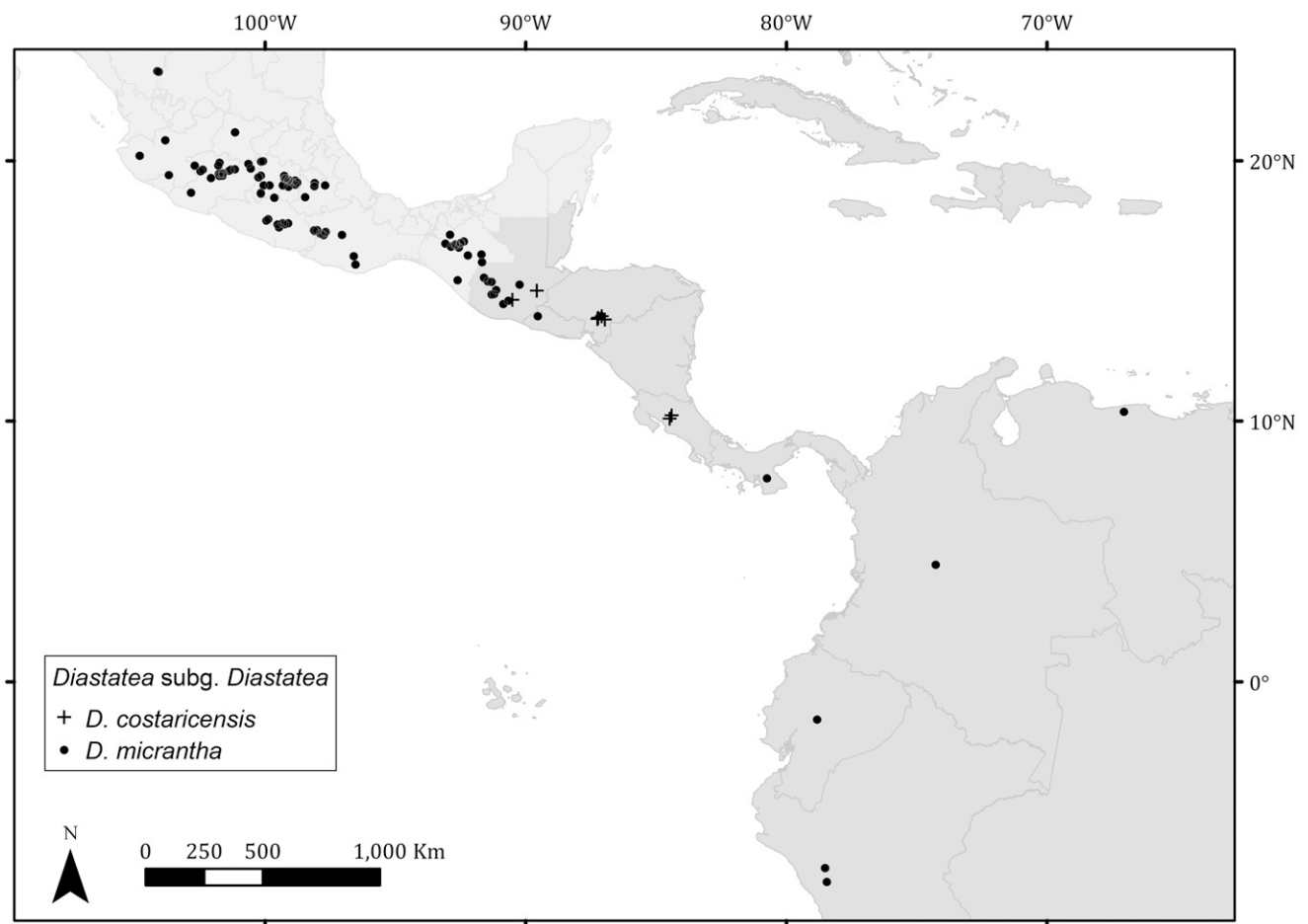


FIG. 6. Distribution map of *Diastatea costaricensis* and *D. micrantha* in *Diastatea* subgenus *Diastatea*.

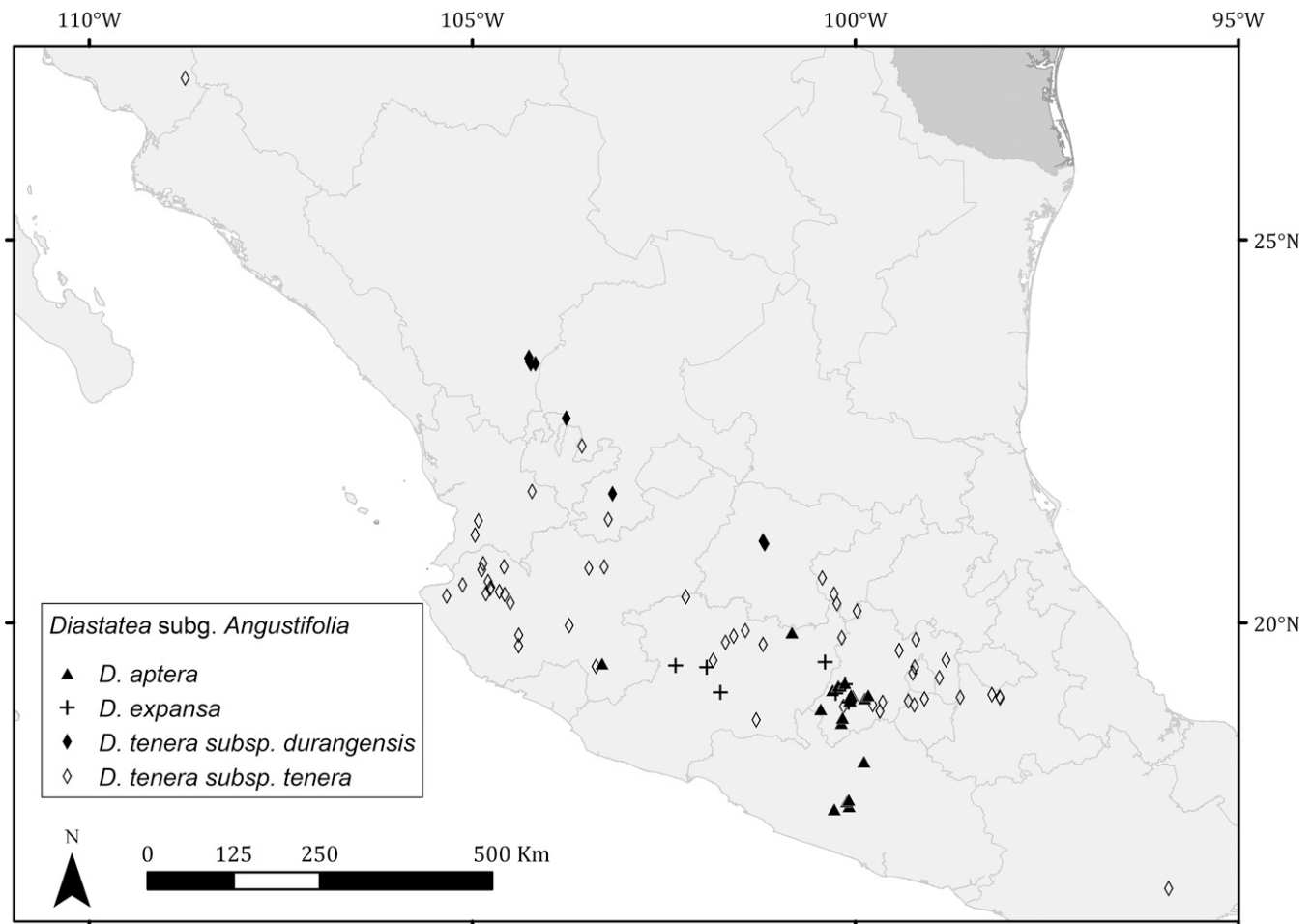


FIG. 7. Distribution map of all taxa in *Diastatea* subg. *Angustifolia*.

Diastatea tenera is the most widely distributed species in *D.* subg. *Angustifolia* and the most morphologically variable. It is strongly supported as monophyletic and is sister to *D. expansa* (Fig. 1). *Diastatea tenera* subsp. *tenera* (Fig. 3C, D) is easily distinguished from the other widespread and variable species, *D. micrantha* (Fig. 3F). Its flowers are two to three times the size of *D. micrantha* in both corolla tube length and corolla lobe length and width. It has glabrous, narrowly elliptic to linear cauline leaves and the base of the corolla in most *D. tenera* has a small gibbous projection on the ventral surface (Fig. 3C). No species in *D.* subg. *Diastatea* possess this trait. Of the 76 collections of *D. tenera* subsp. *tenera* that we examined, 14 collections were found to lack a gibbous corolla. Further molecular work should include these individuals.

The large flowers of *D. tenera* subsp. *durangensis* (Fig. 8) are so visually striking that we were tempted to recognize it as a new species. After close examination of 89 collections of both subspecies, it became clear that flower size was the only character separating *D. tenera* subsp. *durangensis* from *D. tenera* subsp. *tenera*. Flower size increases northwestward from the states of Puebla and Querétaro but is particularly evident in populations from Guanajuato northwest to Durango. Because the large-flowered populations are geographically isolated near the southwestern edge of the Central Mexican Plateau they are described here as a geographical subspecies. This decision is bolstered by the placement of three representative specimens of this subspecies in the phylogenetic analysis (Fig. 1).

McVaugh described *D. expansa* in 1940 and it can be distinguished from *D. tenera* based on a funnellform corolla, reflexed calyx lobes, and very prominent teeth on the leaf margin. It is sister to *D. tenera* and has high support values in our analysis (Fig. 1).

The morphological and phylogenetic analysis also revealed an undescribed taxon, *D. aptera*. This taxon is sister to the other taxa in *D.* subg. *Angustifolia* (Fig. 1). When examining herbarium sheets for the taxonomic treatment, we noticed that some sheets determined as *D. tenera* had nearly half inferior fruit. Although it is not unusual for *D. tenera* to have a slightly expanded hypanthium in fruit, these specimens were exceptional (Fig. 2C, D). McVaugh noted these exceptional specimens in his treatment, but grouped them with *D. tenera* (1940b). Interestingly, their ovaries are completely superior in flower (Figs. 2B, 3A, B). Close examination of the specimens also revealed an autapomorphy for the group; the new species, unlike all other species of *Diastatea*, has no winged projections on the stem (Fig. 2F). In all other species of *Diastatea* the leaf bases are decurrent, creating a small, winged projection for the length of the stem (Fig. 2G). In *D. aptera* the stems are wingless with very short pubescence arising uniformly at the base of the stem. In other species the pubescence is more or less confined to the winged projection or the stem is completely glabrous as is the case with *D. tenera*. *Lobelia xalapensis*, which is often mistaken for *D. micrantha*, also has winged projections on the stem, so this trait is not confined to *Diastatea*.

Future Directions—There is sufficient evidence of admixture of characters in certain individuals to warrant further study of this genus. The large-flowered *D. micrantha* of Oaxaca, the populations of *D. maximiliana* that overlap geographically with *D. costaricensis*, and the individuals of *D. tenera* lacking the characteristic gibbous corolla deserve further examination. It will be important to include species from both subgenera of *Diastatea* in any future work in the Mexican lineages of the subfamily Lobelioideae.

TAXONOMIC TREATMENT

DIASTATEA Scheidw., Allg. Gartenzeitung (Otto & Dietrich) 9: 396. 1841. TYPE: *Diastatea virgata* Scheidw.

Herbaceous annuals. **Roots** branching, fibrous. **Stems** ascending to erect, simple to highly branched, often minutely winged, green to often purple below, glabrous to pubescent, especially on wings. **Leaves** alternate, cauline, petiolate or sessile; lowermost blades smaller and often broadly ovate or round, increasing in size upward; middle and upper blades broadly ovate to narrowly elliptic or linear; bases often decurrent; apex round to acuminate; margins regularly or irregularly serrate, sometimes serrulate, crenate, or erose, the teeth tipped with white or purple thickened callosities, or reduced to marginal callosities; glabrous, or the margins, veins, and pseudo-petioles (if present) ciliate-pubescent. **Inflorescences** weakly to strongly secund terminal raceme with few to many flowers; bracts leaf-like, becoming smaller and linear distally, ascending to appressed. **Flowers** resupinate at anthesis; pedicels filiform, ebracteolate, ascending, glabrous to scabrous, green to purple, elongating in fruit, the distal portion often bent toward stem so that mature fruit is erect; hypanthium either obconic or somewhat flattened and disc-shaped in flower, sometimes enveloping lower portion of capsule in fruit, glabrous to minutely scabrous; calyx lobes subequal to equal, narrowly triangular, linear, or narrowly elliptic, apex acute, margins entire or with 1–2 pairs of teeth, glabrous or ciliate; corollas distinctly bilabiate, white to purplish-blue, glabrous; tube broadly to narrowly cylindrical or funnel-form, with or without a gibbous projection at base on ventral side, not cleft dorsally, or with slight cleft such that dorsal sinus exceeds the lateral sinuses; dorsal lobes two, broadly triangular or distal portion elliptic to obovate with a claw attaching to deltoid base; ventral lobes three, rounded to oblong with nectar guides at throat; filaments white to purple, connate distally up to half their length, distinct at base and adnate to corolla; anther tube exerted, dark purple or striped with tan to white at anther margins, usually with a white downy covering, sometimes with stiff hairs on back of dorsal anthers, apex of two shorter ventral anthers with two large triangular trichomes attached interior to additional smaller ones; pollen white to yellow, ovoid when dry; ovary superior in flower, bilocular, placentation axile; style purple and tipped with a ring of white trichomes before splitting into two recurved stigmatic lobes. **Fruit** a capsule, superior to apparently half-inferior due to expanding hypanthium, glabrous, enclosed in persistent shiny, hyaline corolla tube, dehiscing loculicidally by apical valves; seeds ellipsoid, 0.4–0.6 mm

long, orange to brown, smooth and shiny with faint striations.

Notes—Michael Josef Francois Scheidweiler collected and proposed the name for *Diastatea* in 1841 based upon the species *D. virgata* Scheidw. (Otto and Dietrich 1841). The etymological origin stems from the Greek word diastatos, which means “having extension, severed, disunited, or torn by faction” (Quattrocchi 2000). The characters he felt defined the genus included the free (superior) ovary and capsule, as well as the entire corolla tube and the manner in which it enveloped the mature capsule. In his discussion on the plant’s merits as an ornamental in the greenhouses of the Belgian king, he alluded to its flowering time; he said that it would be a pleasant addition to the *Lobelia* currently under cultivation, because although it didn’t grow as large as them, it bloomed in the fall when they had stopped blooming (Otto and Dietrich 1841).

Diastatea subg. *Angustifolia* E.P.Johnson and T.J.Ayers, subg. nov. TYPE: *Diastatea tenera* (A.Gray) McVaugh, Bull. Torrey Bot. Club 67: 143. 1940.

Middle and upper cauline leaves narrowly elliptic to linear, glabrous. Corolla tube cylindrical or with gibbous projection at base.

Distribution—The southern range limit of *Diastatea* subg. *Angustifolia* is in central Guerrero (Fig. 7). The subgenus has a center of distribution across the Trans-Mexican Volcanic Belt mostly from Jalisco to Puebla, but there are also populations of the most widespread species, *D. tenera*, near the edge of the Central Mexican Plateau from Zacatecas to Durango with outliers in Sonora and Oaxaca. The disjunct population of *D. tenera* in Sonora may be derived from the Jalisco population as suggested by its placement in the phylogeny.

1. *Diastatea aptera* E.P.Johnson & T.J.Ayers, sp. nov. TYPE: MÉXICO. México: Amatepec; just N of Amatepec, 14.2 mi S of MEX 134 at Tejuipilco, 4.5 mi S of Río Bejucos bridge on highway; 18°45.648' N, 100°09.972' W, 6 Nov 2006, T. J. Ayers, R. Scott & A. Scott 1767 (holotype: MEXU!; isotypes: ASC!, NY!).

Stems 8.7–45 cm tall, simple or with few to many ascending branches, 0.3–1.9 mm wide at base, minutely pubescent below decreasing distally, round, wingless. **Leaves** sessile or subsessile below, not decurrent; blades purple to green, base rounded, apex acute; lowermost blades ovate, 4–7.1 mm long, 2.5–3.8 mm wide, margins regularly serrate, glabrous or minutely scabrous; middle to upper blades narrowly elliptic to linear, 7–71 mm long, 0.4–2.4 mm wide, marginal teeth reduced to callosities, glabrous. **Inflorescence** strongly secund, with few to 20 flowers per stem; bracts narrowly linear, 4–40 mm long, 0.2–1.2 mm wide, ascending to appressed, glabrous. **Flowers** with pedicels 2.5–37 mm long in flower, ascending and bent distally in fruit; hypanthium obconic in flower, 0.5–1.2 mm in diameter, 0.7–2 mm in length from the calyx sinus to attachment point with pedicel, expanded in fruit enveloping from one-third to one-half of the mature capsule, glabrous; calyx lobes equal to subequal, three-quarters or nearly equal the length of the fruit, narrowly triangular to linear-elliptic, 1–3.8 mm long in flower, green to purple, margins entire or with 1–2 pair of teeth near base, glabrous; corolla purple, the nectar guides of yellow or yellowish green spots surrounded by a dark purple band on white area at



FIG. 8. Isotype of *Diastatea tenera* subsp. *durangensis* held at ASC (F. Acevedo 366).

throat; tube cylindrical, sometimes minutely gibbous at base, (4–)6–11 mm long from calyx sinus to lateral corolla sinus, 0.3–1.2 mm wide at middle; dorsal lobes oblanceolate to obovate with a narrowed claw attaching distal portion to deltoid base, (2–)4–6.7 mm long, 0.5–2.5 mm wide; ventral lobes oblong, (2–)5.2–8.5 mm long, (0.6–)1.4–2.5 mm wide; filaments (4.5–)8.5–10.6 mm long; anthers (1–)1.5–2 mm long, purple with downy white covering on dorsal anthers, plus two large, narrow trichomes and several slightly smaller secondary trichomes at apex of ventral anther pair. **Fruits** narrowly ellipsoid with an unequal base mirroring gibbous corolla, 3.7–6.6 mm long, 1–3 mm wide, light brown; seeds ellipsoid, 0.5 mm long, medium to dark orange-brown. Figures 2, 3A, B, 4A, S4A.

Chromosome Number— $2n = 14$ (Fig. S5A).

Distribution—Mexican states of Guerrero and México (Fig. 7).

Etymology—We chose the specific epithet *aptera* meaning “without wing” because this taxon lacks the minutely winged stems created by the decurrent leaf bases that are present in all other species of *Diastatea*.

Notes—This new species of *Diastatea* is most often incorrectly identified as *D. tenera*, but can be easily distinguished from *D. tenera* by a wingless, minutely pubescent stem, vs. a winged mostly glabrous stem in *D. tenera*; long, linear leaves vs. more or less narrowly elliptic leaves in *D. tenera*; a one-third to one-half inferior fruit capsule vs. a mostly superior or only one-third inferior capsule in *D. tenera*; calyx lobe teeth at the base vs. just below the middle in *D. tenera*; and a non-gibbous corolla base vs. a gibbous corolla base in *D. tenera*. Our molecular work supports the recognition of *D. aptera* as a new species in a clade separate and sister to the one containing both *D. tenera* and *D. expansa*.

Additional Specimens Examined—MÉXICO. —GUERRERO: Campo Morado. District of Mina, 2 Jan 1938, *Hinton 11162* (GH); Villa Xochitl, 10.3 km al O; [17.69°, -100.09°], 17 Jan 1999, *Soto 13195* (MEXU); Local.: Toro Muerto; Distr.: Mina, 15 Nov 1939, *Hinton et al. 14860* (MO, TEX); Petalcala; Distrito Mina; Sierra Madre del Sur, 15 Dec 1937, *Mexia 8959* (MO, NY, LL); Tlacotepec, 10.7 km al SO; [17.67°, -100.11°], 31 Oct 1998, *Soto 11807* (MEXU). —JALISCO: Llano verde, cerca de Los Corales, municipio de Tecalitlán, Sierra de Los Corales, 24 Oct 1963, *Rzedowski 17406* (MEXU). —MÉXICO: San Diego, 15 km al NNE de Tejupilco, sobre el camino a Temascaltepec, 19 Nov 1967, *Rzedowski 25197* (WIS); Temascaltepec, 1.8 mi S of Temascaltepec on road to Tejupilco; [18.9893°, -100.0628°], 6 Nov 2006, *Ayers et al. 1763* (ASC); Temascaltepec; 7.5 mi S of Temascaltepec on MEX 134, road to Tejupilco; [18.98623°, -100.06817°], 6 Nov 2006, *Ayers et al. 1764* (ASC); Temascaltepec; 2 mi W of Temascaltepec on road to Valle de Bravo; [19.05353°, -100.05653°], 7 Nov 2006, *Ayers et al. 1768* (ASC); Amatepec; just N of Amatepec, 14.2 mi S of MEX 134 at Tejupilco, 4.5 mi S of Río Bejucos bridge on highway; [18.7608°, -100.1662°], 6 Nov 2006, *Ayers et al. 1767* (ASC, MEXU, NY); Mpio. Temascaltepec; 6.5 km northeast of Temascaltepec on hwy 130; [19.1°, -100.0°], 10 Nov 1985, *Reisfield 1250* (MO, TEX); Valle de Bravo, 23 Nov 1952, *Matuda et al. 27317* (MEXU); Loc.: La Labor; Distr.: Temascaltepec, 7 Nov 1935, *Hinton et al. 8625* (TEX); Cajones, Temascaltepec, 17 Nov 1935, *Hinton et al. 8661* (MEXU, MO, TEX); Amatepec y cercanías, 27 Dec 1953, *Matuda et al. 29847* (MEXU); Tejupilco de Hidalgo; 15 km (9.4 mi) NE de Tejupilco de Hidalgo o 18.9 km (11.8 mi) al SE de Temascaltepec sobre ruta 130 (134?), 14 Dec 1984, *Cowan 4816* (MEXU, TEX); 5 km al Suroeste de Nanchititla; Mun. Tejupilco, 27 Dec 1972, *Medrano et al. 5022* (MEXU); Pelon de Tres Reyes, Tezoloapán (Otzoloapán?), 22 Nov 1970, *Matuda et al. 38220* (LL); 3 km al S de Colorines, municipio de Valle de Bravo, 29 Dec 1966, *Rzedowski 23743* (TEX, WIS). —MICHOCÁN: Cerro Blanco de Coro, municipio de Zinapécuaro, 29 Oct 1991, *Escobedo 2257* (MEXU).

2. *DIASTATEA EXPANSA* McVaugh, Bull. Torrey Bot. Club 67: 787. 1940. TYPE: MÉXICO. México: Correría, Dist. Temascaltepec, 1230 m, 19 Nov 1932, G. B. *Hinton 2644* (holotype: GH!; isotypes: K-image!, NY-image!).

Stems 7.5–47 cm tall, simple or with few to many ascending branches, 0.7–1.6 mm wide at base, winged; wings minutely ciliate. **Leaves** subsessile or pseudo-petiolate below, sessile above; blades green to purple, base truncate to cuneate and decurrent into narrow wings, apex acute; lowermost blades deltoid to elliptic, 2.3–12 mm long, 2.2–5.7 mm wide; middle to upper blades narrowly elliptic, 4.8–25 mm long, 1.2–5.2 mm wide; margins regularly serrate with prominent teeth; glabrous. **Inflorescence** strongly secund with few–20 flowers per stem, bracts narrowly elliptic to linear, 5.2–25 mm long, 0.8–3.1 mm wide, ascending to somewhat appressed. **Flowers** with pedicels 3–17 mm long in flower, glabrous, spreading to ascending, often bent distally in fruit; hypanthium oblique, obconic in flower, 0.6–1.5 mm in diameter, 0.5–0.7 mm in length from the calyx sinus to attachment point with pedicel, often expanded in fruit enveloping up to one-third of the mature capsule, glabrous; calyx lobes subequal, from one-half to three-quarters the length of the fruit, narrowly triangular to linear-elliptic, 1.2–3.3 mm long in flower, green or purple, margins entire or with 1–2 pair of teeth, glabrous; corolla purplish-blue, nectar guides yellow with raised projections at throat; tube funnellform, flaring from base to end of tube, and gibbous at base on ventral side, 3.0–4.6 mm long from calyx sinus to lateral corolla sinus, 1.5–2.5 mm wide at distal end; dorsal lobes oblanceolate with a narrowed claw attaching distal portion to deltoid base, 2.7–4.0 mm long, 0.7–1.5 mm wide; ventral lobes ovate, obovate or oblong, with or without a mucronate tip, 3.2–4.7 mm long, 1.7–2.4 mm wide; filaments 3.0–4.5 mm long; anthers 1.1–1.5 mm long, purple with downy white covering increasing at apex of dorsal anthers, plus two large, narrow trichomes and several slightly smaller secondary trichomes at apex of ventral anther pair. **Fruits** narrowly ellipsoid and oblique, 4.0–7.0 mm long, 2.0–3.0 mm wide, light brown seeds ellipsoid, 0.5 mm long, medium orange-brown. Figure 4B.

Chromosome Number—Unknown.

Distribution—Mexican states of Guerrero, México, and Michoacán (Fig. 7).

Notes—*Diastatea expansa* may be distinguished from *D. tenera* using both the leaf margins and the funnellform corolla as diagnostic traits. The corolla is not always as expanded as the specific epithet suggests. The leaf margins provide a reliable second diagnostic trait. *Diastatea expansa* has regularly serrate margins with very sharp and prominent teeth tipped with callosities while *D. tenera* has minute teeth that are often reduced to mere callosities.

Additional Specimens Examined—MÉXICO. —GUERRERO: Chirilagua, Dist.: Mina, 18 Nov 1936, *Hinton 9852* (MEXU). —MÉXICO: Moist ravine by Río Molino in pine-oak woods above reservoir, Valle del Bravo, 1–2 Nov 1949, *Moore, Jr. & Cetto 5486* (GH); Desviación de la carretera Temascaltepec-Tejupilco a Tenería y Río Grande, [18.9°, -100.1°], 16 Oct 1996, *Vibrans 6271* (MEXU); Zacazonapan, 3 Dec 1981, *Rosiles M. s. n.* (MEXU, TEX). —MICHOCÁN: Zitácuaro-Zirahuato, Distr.: Zitácuaro, 21 Oct 1938, *Hinton 13393* (MEXU); 12 km al E de Uruapan, carretera a Pátzcuaro, 23 Jan 1977, *Rzedowski 34603* (MEXU); Northwestern foothills of Cerro Tancitaro, 13–14 km south of Peribán de Ramos, 29 Nov 1970, *McVaugh 24809* (MEXU); A 3 km al SO de Ario de Rosales, carr. a La Huacana, 24 Oct 1981, *Núñez 3437* (MEXU).

3. *DIASTATEA TENERA* (A.Gray) McVaugh, Bull. Torrey Bot. Club 67: 143. 1940. *Palmerella tenera* A. Gray, Proc. Amer. Acad. Arts 22: 433. 1887. *Lobelia palmeri* Greene, Pittonia 1(6): 297. 1889; non *Lobelia tenera* Kunth, Nov. Gen. Sp. [H.B.K.] 3: 314. 1819. TYPE: MÉXICO. Jalisco: Río Blanco, 17

September 1886, *E. Palmer* 552 (holotype: GH!; isotypes: P-image!, YU-image!).

Laurentia pinetorum Brandege, Univ. Calif. Publ. Bot. 4: 92. 1910. TYPE: MÉXICO. Popocatepetl, dry sandy soil in pine forests, October 1908, C. A. Purpus 3665 (holotype: UC-image!; isotypes: F-image!, GH!, MO!, NY-image!, US-image!).

Stems simple or with few to many ascending branches, 0.2–1.2 mm wide at base, winged, glabrous, or the wings minutely ciliate. **Leaves** sessile, decurrent into narrow wings on stem; blades green or purple throughout or green adaxially and purple abaxially, base rounded to cuneate, apex rounded to narrowly acute; lowermost leaves deltoid to ovate; middle to upper leaves narrowly elliptic to linear; margins regularly serrate to serrulate or teeth reduced to marginal callosities; glabrous. **Inflorescence** strongly secund with few to 20 flowers per stem; bracts leaf-like, becoming smaller and linear distally, ascending or appressed. **Flowers** with glabrous or slightly scabrous pedicels, spreading to ascending, in fruit often bent distally; hypanthium obconic in flower, 0.3–1.2 mm in diameter, 0.3–2.0 mm in length from calyx sinus to attachment point with pedicel, expanded in fruit to envelop up to one-third of the mature capsule, glabrous; calyx lobes equal to subequal, about one-third to two-thirds the length of the fruit, green or purple; corolla purple, purplish-blue, or white, tube cylindrical and gibbous at base on ventral side; anthers dark purple with downy white covering and short stiff hairs at apex of dorsal anthers, plus two large, narrow trichomes and several slightly smaller secondary trichomes at apex of ventral anther pair. **Fruits** light-brown to purplish; seeds ellipsoid, 0.4–0.5 mm long, orange-brown.

3a. DIASTATEA TENERA (A.Gray) McVaugh subsp. TENERA

Stems 3.1–43.5 cm tall, simple or with a few weak side branches, rarely many ascending branches. **Leaves**, the lowermost ovate to narrowly ovate, rarely reniform, 1.5–5.5 mm long, 1–4 mm wide; middle to upper leaves narrowly elliptic to linear, 3.3–24 mm long, 0.5–3.8(–4.2) mm wide. **Inflorescence** of few to 20 flowers; bracts 3–26 mm long, 0.4–2.7 mm wide. **Flowers** with pedicels 1.2–29 mm long in flower; calyx lobes equal to subequal, narrowly triangular to narrowly elliptic, (0.5–)0.9–3 mm long in flower, margins entire or with 1–2 pair of teeth near the middle (rarely at base), glabrous; corolla purple to purplish-blue rarely white, the nectar guides yellow and dark purple with yellow projections at throat in purple morphs, the nectar guides purple with green streaks in the throat in white morphs; tube 3.9–8 mm long from calyx sinus to lateral corolla sinus, 0.2–1.4 mm wide at middle; dorsal lobes elliptic to obovate with pointed apex, narrowed claw attaching distal portion to deltoid base, 2.8–5.1(–6.2) mm long, 0.8–2(–2.5) mm wide; ventral lobes oblong sometimes distal end expanded, mucronate tip, (1.8–)3.5–6 mm long, 1.0–2.7(–3) mm wide; filaments 4–10 mm long; anthers 0.7–1.5 mm long. **Fruits** narrowly ovoid to narrowly ellipsoid, with an unequal base mirroring the gibbous corolla, 3.7–7.0(–8.5) mm long, 1.2–3 mm wide. Figures 3C, D, 4C.

Chromosome Number—Unknown.

Distribution—Mexican states of Guerrero, Jalisco, México, Michoacán, Morelos, Nayarit, Puebla, Querétaro, Sonora, Zacatecas, and the Distrito Federal (Fig. 7).

Notes—Small white or pale, purple-flowered individuals of *D. tenera* appear to self-pollinate. This is unusual because the styles of most *D. tenera*, as seen on both herbarium sheets and in a small greenhouse study, emerge from the anther tube before the stigmatic lobes open and become receptive. We examined one very small, autogamous, white individual from Sonora. It was collected at 1244 m, which is on the lower end of the range for this species. Ayers (1990) found a similar phenomenon between flower color, reproductive mechanism, and elevation in individuals of *Lobelia cordifolia*. The flowers from lower elevations were white and autogamous as compared to the flowers from higher elevation that were dark purple and allogamous.

Fourteen of the seventy-six collections examined do not have gibbous projections at the base of their corollas but in all other respects are morphologically consistent with *D. tenera*.

Additional Specimens Examined—MÉXICO. —DISTRITO FEDERAL: Por la entrada del Parque Tarango, por el sendero hacia la barranca norte, [19.35°N, -99.25°W], 11 Oct 2001, *César G. 210* (MEXU); Lomas, Feb 1937, *Lyonnet 1466* (MEXU). —GUERRERO: Mpio. Chilpancingo, 8.9 mi. E of the western boundary of Chilapa, 5.6 mi. E Río Atempa on highway 93, 26 Oct 1984, *Saunders-Scherrer 1481* (TEX). —JALISCO: Mascota, 17.2 mi N of Mascota on road to Puerto Vallarta, [20.69347°N, -104.87467°W], 14 Nov 2006, *Ayers et al. 1777B* (ASC); Mascota, 17.2 mi N of Mascota on road to Puerto Vallarta, [20.69347°N, -104.87467°W], 14 Nov 2006, *Ayers et al. 1777A* (ASC); Mpio. Tecalitlán: Puente San Jose sobre el Río Las Juntas, a ca. 8 km al S de Tecalitlán, carretera Colima-Pihuamo, 22 Nov 1981, *Lott 818* (MEXU); Road from Ameca to Mascota, 2.5 mi SE of Mascota, 20 Oct 1983, *Ayers et al. 262* (TEX); Mpio. Zapopan, ca. 2 km al E de Pinar de La Venta sobre carr. Guadalajara-Tepic, 10 Nov 1989, *Koch & Fryxell 8988* (TEX); Hills near Guadalajara, 28 Oct 1889, *Pringle 2374* (MO); Road from Ameca to Mascota, 6 mi SE of Mascota, 20 Oct 1983, *Ayers et al. 268* (TEX); Mountains north of Autlán, 3–5 miles above Mina San Francisco (Cia. Minera de Autlán), 5 Oct 1960, *McVaugh 19934* (MEXU, WIS); Pine forest 1–2 miles east of Tapalpa, 1 Nov 1960, *McVaugh 20565* (MEXU); San Sebastián Arroyo del Cura, Sierra Madre Mts., 5 Jan 1927, *Mexia 1378* (MO); Precipitous south-facing mountainsides 4 miles north-northeast of Talpa de Allende, 12–13 Oct 1960, *McVaugh 20108* (MEXU, WIS); Mpio. Puerto Vallarta, 14 km al SE de P. Vallarta, por la carretera que va a la Estación Biológica de la U.N.A.M., 10 Nov 1978, *García P. & Delgado S. 923* (TEX); 4.3 mi E of Talpa de Allende on road to Los Volcanes and Ayutla, 22 Oct 1983, *Ayers et al. 313* (TEX); Carretera Chamela-P. Vallarta, “El Tuito”, unos 20 ó 25 km antes de P. Vallarta, 24 Nov 1976, *Hernández M. et al. 3008* (MEXU); 20 km al S de Talpa de Allende, Mpio. de Talpa de Allende, 4 Oct 1982, *Hernández M. et al. 9072* (MEXU); Sierra de San Esteban, 13 Oct 1903, *Pringle 8756* (MO); 12 km NW of Los Volcanes, 30 Oct 1973, *Breedlove 35780* (MEXU); Road from Ameca to Mascota, 3.1 mi E of El Jacal, 20 Oct 1983, *Ayers et al. 255* (MEXU, TEX); Road to Mascota, 6 miles northwest of Cuautla, 21 Oct 1952, *McVaugh 13634* (MEXU); Collected along stream near small village of La Venta just W of Guadalajara, 19 Oct 1983, *Ayers et al. 195* (TEX); 4.4 mi E of intersection of MEX 15 & 70, 6.8 mi W of Periférico in Guadalajara on road to Tepic, 3 Jan 1985, *Ayers et al. 600* (TEX); El Tuito, a 4.5 km al N de la carretera Barra de Navidad–Puerto Vallarta, por el camino a “La Mina”, 13 Dec 1985, *Ayala 463* (MEXU); 20 km al NE de Mascota, Mpio. de Mascota, 5 Oct 1982, *Hernández M. et al. 9095* (MEXU); 8.7 mi S of Autlán on road to Barra de Navidad; at summit, 22 Oct 1983, *Ayers et al. 317* (MEXU, TEX); 9.5 mi SSW of Talpa de Allende on “old” road (right fork) to La Cuesta and coast, 21 Oct 1983, *Ayers et al. 283* (TEX); Mpio. Zapopan, a 1 km al E de la Mesa Tapon, Sierra de la Primavera, 7 Nov 1990, *Mendoza 5282* (MEXU). —MÉXICO: Tejuipulco, District of Tamascaltepec, 24 Sep 1935, *Hinton 8488* (GH); Mpio. Coyotepec. 1.5 km al O de Coyotepec, Sierra Alcaparrosa, 16 Nov 1980, *García P. 1398* (MEXU, TEX); Parte alta de la Sierra de Alcaparrosa, 5 km al W de Coyotepec, 16 Nov 1980, *Rzedowski 37098* (MEXU); Ixtapan de la Sal, 12 Oct 1952, *Matuda et al. 27084* (MEXU); Mun. Coatepec Harinas, Rancho Santo Tobias near Villa Guerrero, 19 Nov 1943, *Gilly 107* (TEX); Highway 115 at Chalco, 3 Nov 1985, *Saunders-Scherrer & Dieringer 1567* (LL); Near Coatepec Harinas; open grassy fields, 10 Nov 1964, *Ripley & Barneby 13758* (NY); San Miguel Tlaixpán, municipio Texcoco, 23 Oct 1983, *Ventura V. 1538* (MEXU); 5 km al W de Progreso Industrial, sobre la carretera a Villa del Carbón, 18 Sep 1977, *Rzedowski s. n.* (MEXU). —MICHOCÁN: Cerro Blanco, municipio de Pátzcuaro, 18 Oct 1985, *Escobedo 439* (MEXU); Comanja, municipio de Coeneo, 21 Oct 1985, *Escobedo 441* (MEXU); Vicinity of Morelia, Cerros

San Miguel, 15 Sep 1910, *Arséne 5565* (MO); Vicinity of Morelia, Cerros San Miguel, 15 Nov 1911, *Arséne 6064* (MO); Vicinity of Morelia, Loma Sta. María, 28 Oct 1910, *Arséne 5861* (MEXU); Vicinity of Morelia, Loma Sta. María, 28 Oct 1910, *Arséne 5865* (MO); Vicinity of Morelia, Cerros San Miguel, 15 Nov 1911, *Arséne 6070* (MEXU, MO); Cerca de Palmas, municipio de Epitacio, 28 Oct 1990, *Rzedowski 50517* (MEXU); Morelia, Loma Sta. María, 11 Oct 1909, *Arséne 3113* (MEXU); Morelia, Loma Sta. María, 1 Nov 1912, *Arséne 8907* (MEXU); 16 km al oeste-suroeste de Coalcomán, sobre la carretera a Villa Victoria, 5.5 km al norte de El Tejón, [18.74°, -101.29°], 2 Nov 2007, *Ramírez-Amezcuca & Steinmann 1178* (ASC); Transmarraán, municipio de Coeneo, 15 Oct 1991, *Escobedo 2218* (MEXU); 5 km al S de Tende-paracua, municipio de Huaniqueo, 21 Oct 1990, *Rzedowski 50334* (MEXU); Parte alta del Cerro El Chivo, municipio de Tingambato, 7 Sep 1989, *Díaz-Barriga 5979* (MEXU); Aprox. 0.5 km al SE de San Francisco de Los Reyes, municipio de Tlalpujahua, 25 Nov 1992, *García et al. 3577* (MEXU). —MORELOS: Salto de S. Antonio, cerca de Cuernavaca, 18 Nov 1951, *Gold 25883* (MEXU); 500 m al N de la caseta de cobro de Tepoztlán, sobre la carr. Cuatla-Cuernavaca, Mpio. Tepoztlán, 4 Nov 1987, *Cabrera C. & Flores 14705* (MEXU); Sierra de Ocuila, rumbo Mexicapa, 16–18 Dec 1938, *Lyonnet 2922* (MEXU); Barranca del Tecolote, 30 Oct 1970, *Vázquez 2738* (MEXU); Mountainside above Cuernavaca, 6 Dec 1907, *Pringle 15028* (MO). —NAYARIT: About 3 km west of Mazatán, 20 Nov 1963, *Feddema 2671* (MEXU); 25 km by rd S of Tepic, [21.3°, -104.9°], 18 Oct 1970, *Webster & Breckon 15685* (MEXU); Mun. La Yesca, 23 km al SE de Puente de Camotlán, brecha a Huajimic, [21.7°, -104.2°], 26 Oct 1989, *Tenorio L. 16702* (MEXU). —OAXACA: Distrito: Yauteppec, Municipio: San Juan Lajarcia, 1070 m línea recta al S-SE del punto trino de Nejapa-Camarón-Lajarcia, meseta del Cerro Gavilán, [16.534°, -95.907°], 2 Nov 2001, *Salas M. et al. 4299* (MEXU). —PUEBLA: Vicinity of Puebla, Tepoxuchilt, 1 Oct 1910, *Arséne 5509* (MO); San Baltasar, 28 Oct 1906, *Arséne 2138* (MEXU); Tepoxuchilt, 1 Oct 1910, *Antonio s. n.* (MEXU); Parque Estatal Gral. Lázaro Cárdenas, [19.02°, -98.11°], 1 Nov 1994, *Romero 586* (MEXU); Popocatepetl, Oct 1908, *Purpus 3665* (MO); Parque Estatal Gral. Lázaro Cárdenas, [19.0°, -98.1°], 3 Nov 1993, *Romero 134* (MEXU). —QUERÉTARO: El Batán, camino a Amealco, 29 Sep 1984, *Argüelles 2185* (MEXU); Camino entre carr a México y Amealco, km 19 aprox., 10 Nov 1976, *Argüelles 630* (MEXU); Cerca 8 km al NW de Amealco, por la carretera a San Juan del Río, municipio de Amealco, 14 Oct 2000, *Zamudio & Becerra 11645* (TEX); Camino a Amealco, pasando Huimilpan a mano izquierda, 18 Nov 1992, *Argüelles 3429* (MEXU). —SONORA: Municipio de Álamos, 6.2 km below (southwest) Rancho Santa Bárbara, 34.6 km east-northeast of Álamos (by road), [27.12°, -108.75°], 1 Oct 2006, *Van Devender et al. 2006–1013* (ASC). —ZACATECAS: Aprox. 2–3 km NW of Monte Escobedo, 6 Nov 1963, *Feddema 2481* (MEXU, WIS); Mpio. Juchipila, 4 km al oeste de Pueblo Viejo, Sierra de Morones, Cerro de Piñones, ladera este, por el camino al rancho de Lorenzo Magallanes, [21.35°, -103.22°], 12 Oct 1999, *Balleza C. & Adame G. 11502* (MEXU).

3b. *Diastatea tenera* subsp. *durangensis* E.P.Johnson & T.J.Ayers, subsp. nov. TYPE: MÉXICO. Durango: Mpio. Súcil. Laguna de Mogotes, alrededores, 8 km de El Alemán, Reserva de la Biosfera La Michilía, [23.47°, -104.26°], 17 Nov 1986, *F. Acevedo 366* (holotype: CIIDIR; isotypes: ASC!, IBUG, MEXU).

Stems 9.5–37 cm tall, simple or a few weak side branches. **Leaves**, the lowermost deltoid, 2–4 mm long, 1.2–1.8 mm; middle to upper leaves linear, 6–24 mm long, 0.5–1.6 mm wide. **Inflorescence** with few to 12 flowers; bracts 5.4–20 mm long, 0.4–0.9 mm wide. **Flowers** with pedicels 3.7–17.5 mm long in flower; calyx lobes subequal, narrowly triangular or linear, 1.1–2.5 mm long in flower, margins with 1–2 pair of teeth at or below the middle, glabrous or ciliolate; corolla purplish-blue or purple, the nectar guides pale yellow, gradually brighter toward throat, with projections at throat; tube 8.8–13.5 mm long from calyx sinus to lateral corolla sinus, 0.8–1.6 mm wide at middle; dorsal lobes obovate, apex acute to mucronate, with narrowed claw attaching distal portion to deltoid base, 6.1–9.0 mm long, 1.8–4.0 mm wide; ventral lobes broadly oblanceolate with mucronate tip, 7.1–11.3 mm long, 2.2–4.3 mm wide; filaments 9.5–13 mm long; anthers 1.7–2.0 mm long. **Fruits** narrowly ellipsoid, with an unequal base

mirroring gibbous corolla, 5.2–10 mm long, 2.2–3.6 mm wide. Figures 4D, 8, S4B.

Chromosome Number—Unknown.

Etymology—The new subspecific epithet reflects that the center of distribution of this taxon is in the Mexican state of Durango.

Distribution—Mexican states of Durango, Guanajuato, Jalisco, and Zacatecas (Fig. 7).

Notes—Exceptionally large-flowered individuals of *D. tenera* have been found in Durango. They appear to be the end point of a cline stretching from Puebla north to Durango, in which the flowers of *D. tenera* increase dramatically in size. The populations in Zacatecas and Guanajuato have the long corolla tubes of *D. tenera* subsp. *durangensis* but their lower corolla lobes are slightly shorter than the populations in Durango and Jalisco.

Because of the distinct difference in size between the individuals found mainly in Durango and the rest of *D. tenera*, and the fact that the collections appear to have a distinctive range adjacent to the central Mexican Plateau, they are described here as a new subspecies. We chose subspecific rank over varietal rank because varietal rank in the Campanulaceae has been dismissed by Lammers (2006). Future examination of the subspecies should look at habitat and pollinator differences as possible drivers of floral differentiation.

Additional Specimens Examined—MÉXICO. —DURANGO: San Juan de Michis, Mpio. de Súcil, 25 Oct 1984, *Acevedo 150* (TEX, WIS); Mpio. Súcil. San Juan de Michis, 3.5 km al S del poblado, Reserva de la Biosfera La Michilía, [23.47°, -104.26°], 21 Oct 1985, *González & Acevedo 3546* (ASC); Mpio. Súcil, Sierra de Michis, San Agustín, Reserva de la Biosfera La Michilía, [23°, -104°], 12 Oct 1984, *Jiménez 186* (ASC); Mpio. Súcil, El Alemán, 8 km cerca del Arroyo Las Joaquinas, Reserva de la Biosfera La Michilía, 18 Nov 1986, *Acevedo 376* (ASC); Mpio. Súcil, Reserva de la Biosfera La Michilía, Portrero Mesa Larga, [23.5°, -104.3°], 17 Nov 1993, *García A. & Alvarado 1911* (ASC); Mpio. Súcil, Portrero Los Anegados, 3 km de el Alemán, Reserva de la Biosfera La Michilía, [23.47°, -104.26°], 11 Oct 1986, *Acevedo 306* (ASC); Mpio. Súcil, Potrero El Taray, Reserva de la Biosfera La Michilía, [23.41°, -104.25°], 12 Sep 1983, *Acevedo s. n.* (ASC); Mpio. Súcil, Reserva de la Biosfera La Michilía, camino Súcil-Charcos, km 51, entrada al Rancho La Peña, [23.4°, -104.2°], 11 Oct 1992, *García A. 1700* (ASC). —GUANAJUATO: 8 km northeast of Santa Rosa, 10 Nov 1970, *McVaugh 24150* (MEXU); Mpio. San José, Cañada Las Peras 3 km al SE de El Zorrillo, 6 Nov 2003, *Gutiérrez G. et al. 787* (MEXU). —JALISCO: Rancho Viejo, about 10 km east-northeast of Huejuquilla el Alto, 31 Oct 1963, *Feddema 2310* (NY, WIS). —ZACATECAS: Plus or minus 35 km al W de Jalpa, sobre la carretera a Tlaltenango; 27 km del entronque con la carretera Jalpa-Juchipila, 23 Oct 1970, *Rzedowski & McVaugh 1039* (MEXU, MO, NY, LL).

DIASTATEA Scheidw. subg. DIASTATEA

Middle and upper cauline leaves ovate to lanceolate and sometimes narrowly elliptic, glabrous to pubescent. Corolla tube cylindrical, without gibbous projection at base.

Distribution—The species within this subgenus are found in the following Mexican states and Central and South American countries: Chiapas, Colima, Durango, Guanajuato, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Nuevo Leon, Oaxaca, Puebla, and within the Distrito Federal; Costa Rica, El Salvador, Guatemala, Honduras, and Panama; Argentina, Bolivia, Colombia, Ecuador, Peru, and Venezuela.

4. DIASTATEA MICRANTHA (Kunth) McVaugh, Bull. Torrey Bot. Club 67: 143. 1940. *Lobelia micrantha* Kunth, Nov. Gen. Sp. [H.B.K.] 3: 316. 1818. *Rapuntium micranthum* (Kunth) C. Presl, Prodr. Monogr. Lobel.: 25. 1836. *Dortmannia micrantha* (Kunth) Kuntze, Revis. Gen. Pl. 2: 972. 1891 as 'Dortmannia.' *Laurentia micrantha* (Kunth) Zahlbr., Bull.

- Torrey Bot. Club 24(8): 386. 1897; non *Laurentia micrantha* (E.Mey.) A.D.C., Prodr. [A. P. de Candolle] 7(2): 411. 1839. TYPE: ECUADOR. "in Regni Quitensi, prope pagum Puembo, alt. 1300 hex. Floret Julio." A. Humboldt & A. Bonpland s. n. (holotype: P-image! isotypes: B-W [presumed destroyed], F-image! [photo of isotype at B, photo 9110]).
- Lobelia subtilis* Kunth, Nov. Gen. Sp. [H.B.K.] 3: 317. 1819. *Rapuntium subtile* (Kunth) C. Presl, Prodr. Monogr. Lobel.: 25. 1836. TYPE: ECUADOR. Quito, Puembo. A. Humboldt & A. Bonpland s. n. (holotype: P-image!; isotype: P-image!).
- Lobelia ruderalis* Willd. ex Roem. & Schult., Syst. Veg., ed. 15 bis [Roemer & Schultes] 5: 56. 1819. TYPE: PERU. "Habitat in Peru." A. Humboldt & A. Bonpland s. n. (holotype: B-W-image!).
- Lobelia draba* Willd. ex Roem. & Schult., Syst. Veg., ed. 15 bis [Roemer & Schultes] 5: 67. 1819. TYPE: no locality or collector on specimen (holotype: B-W-image!).
- Lobelia parviflora* M.Martens & Galeotti, Bull. Acad. Roy. Sci. Brux. 9(2): 41. 1842. *Dortmannia parviflora* (M.Martens & Galeotti) Kuntze, Revis. Gen. Pl. 2: 973. 1891 as 'Dortmannia.' TYPE: MÉXICO. Oaxaca, Yavezia, cordillera [from description: "On trouve cette espèce au bord des ruisseaux peu ombragés de la cordillère orientale d'Oaxaca, près de Yavezia, de Socorro et de Castrasana, de 6,500–7,500 pieds d'élévation"], Jan 1840, H. G. Galeotti 1970 (holotype: BR-image!; isotypes: BR-image!, K-image!, US-image!, W-image!).
- Laurentia ovatifolia* B.L.Rob., Proc. Amer. Acad. Arts 26: 166. 1891. *Laurentia micrantha* var. *ovatifolia* (B.L.Rob.) E.Wimm. in J.F.Macbr., Publ. Field Mus. Nat. Hist., Bot. Ser. 13, pt. 6: 476. 1937. *Diastatea micrantha* var. *ovatifolia* (B.L.Rob.) E.Wimm., Ann. Naturhist. Mus. Wien 56: 332. 1948. LECTOTYPE, here designated from two syntypes: MÉXICO. San Luis Potosi: hills near Cardenas, 8 Sep 1890, C. G. Pringle 3302 (lectotype: GH!; isolectotypes: BR, COLO, PH, S, NDG, K, GOET, W-image!).
- Laurentia michoacana* B.L.Rob., Proc. Amer. Acad. Arts 26: 167. 1891, nomen nudum.
- Laurentia pedunculata* Brandege, Univ. Calif. Publ. Bot. 6: 73. 1914. *Laurentia micrantha* var. *longibracteata* E.Wimm., Revista Sudamer. Bot. 2: 104. 1935. *Diastatea micrantha* var. *longibracteata* (E.Wimm.) E.Wimm., Ann. Naturhist. Mus. Wien 56: 332. 1948. TYPE: MÉXICO. Finca Covadonga, Jul 1913, C. A. Purpus 6705 (holotype: UC-image!; isotypes: GH!, MO-image!, US-image!).
- Diastatea serrata* Standl. & L.O.Williams, Ceiba 1: 91. 1950. TYPE: HONDURAS. Department of Morazán. Drainage of the Río Yeguaré, at about Longitude 87°W and Latitude 14°N, Alt. 850 m, 26 Nov 1948, L. O. Williams 14786 (holotype: US-image!).

Stems 2.7–80 cm tall, simple to branched, 0.2–3.0 mm wide at base, minutely winged, pubescent with soft hairs evenly distributed around stem, often becoming less pubescent distally, rarely glabrous. **Leaves** pseudo-petiolate; pseudo-petioles 1–22 mm long; margins ciliolate and decurrent into narrow wings on stem; blades purple to green, sometimes green adaxially and purplish abaxially, base subcordate to narrowly cuneate, apex round to acuminate; lowermost blades round, ovate, lanceolate to narrowly elliptic, 2.5–11 mm long, 2–8 mm wide, the margins regularly serrate to erose, glabrous or with sparsely scattered stiff hairs adaxially and on veins abaxially; upper blades ovate to narrowly

lanceolate, 4.5–60 mm long, 2.0–26 mm wide, the margins regularly or irregularly serrate, ciliolate; pubescence on veins abaxially. **Inflorescence** weakly to strongly secund with few to 30 flowers per inflorescence; bracts linear to elliptic, 2–53 mm long, 0.2–12 mm wide, spreading. **Flowers** with pedicels 1.5–25(–57) mm long in flower, glabrous, spreading to ascending, often bent upward distally in fruit; hypanthium obconic in flower, 0.5–0.9 mm in diameter, 0.3–0.5 mm in length from calyx sinus to attachment point with pedicel, not much expanded in fruit, glabrous, calyx lobes subequal to equal, from one-third to two-thirds the length of the fruit, narrowly triangular or linear, 1.1–3.5(–5) mm long in flower, green, margins entire, glabrous or ciliate; corolla purplish-blue or white, if white, then lobes lavender or blue, nectar guides yellow sometimes with green raised projections at throat; tube cylindrical, 2.4–4.5 mm long from calyx sinus to lateral corolla sinus, 0.7–1.1 mm wide at middle; dorsal lobes linear to linear spatulate, 1–2 mm long, 0.2–0.3 mm wide; ventral lobes oblong, sometimes with a mucronate tip, 0.9–2.5(–3.5) mm long, 0.4–1.3(–1.6) mm wide; filaments 2.8–4.6 mm long; anthers 0.5–1(–1.5) mm long, dark purple, either with downy white covering or appearing glabrous, sometimes with short hairs near apex of dorsal anthers, plus two large and many small white trichomes at apex of ventral anther pair. **Fruits** narrowly to broadly ellipsoid sometimes slightly curved at tip, 3–6.5(–8) mm long, 1.4–2.6 mm wide, light brown or purplish-brown; seeds ovoid to ellipsoid, 0.4–0.5 mm long, orange to dark-orange. Figures 3F, S4D.

Chromosome Number— $2n = 14$ (Fig. S5C).

Distribution—Mexican states of: Chiapas, Colima, Durango, Guanajuato, Guerrero, Hidalgo, Jalisco, México, Michoacán, Morelos, Nuevo Leon, Oaxaca, Puebla and the Distrito Federal; Central and South American countries of: Costa Rica, El Salvador, Guatemala, Honduras, and Panama; Argentina, Bolivia, Colombia, Ecuador, Peru, and Venezuela (Fig. 6).

Notes—*Diastatea micrantha* can be distinguished by its minute corolla tube and corolla lobes, and its tendency to self-pollinate. Eight collections from Oaxaca have unusually large flowers for *D. micrantha*. Because Oaxaca is the center of distribution for *D. virgata*, these large-flowered specimens warrant further examination and should be included in future morphological and molecular work.

The type of *D. micrantha* was originally collected in Ecuador, but its center of distribution is in the Trans-Mexican Volcanic Belt. Its broad geographic range and morphological variation have led to it being described as at least ten different species.

Lobelia xalapensis and *L. diastateoides* can be easily confused with *D. micrantha* because their ovaries in flower are essentially nearly superior. *Lobelia xalapensis* can be distinguished from *D. micrantha* by a shorter more ovoid fruit, corolla split dorsally to the base, and prickly pubescent pedicels. *Lobelia diastateoides* can be distinguished from *D. micrantha* by a perennial habit and corolla split nearly to the base.

McVaugh noted in his 1940b revision of *Laurentia* that *Laurentia michoacana* B.L.Rob. is a nomen nudum and we want to re-emphasize that here. B. L. Robinson (1891) makes it clear that Pringle 3337, despite being distributed under the name *Laurentia michoacana* B.L.Rob., "should be for the present doubtfully referred to as *Lobelia parviflora* Mart. & Gal., since it is highly probable that the type of this species will be found on investigation to be a *Laurentia*." It was, in fact, found to be

Pátzcuaro, 3 Nov 1986, *Mayo 578* (MEXU); Lado Sureste del Cerro El Águila subiendo por Huatzanguio, municipio de Morelia, [19.62°, -101.38°], 10 Nov 2007, *García 379* (MEXU); A 5 km al S de Uruapan, camino a Nueva Italia. Mpio. Uruapan, 15 Nov 1983, *Martínez S. et al.* 5322 (MEXU); Mun. Los Reyes, a 18.5 km al SE de Tinguidin, [19.66°, -102.40°], 19 Nov 2001, *Martínez 34663* (MEXU); Sparsely forested slopes of Cerro Potrerillos, ca. 5 miles north of Cotija and 2 miles south of Jiquilpan, 5–9 Oct 1961, *King & Soderstrom 4611* (TEX); Mpio. de Ciudad Hidalgo, Mil Cumbres, a 2.8 km al E de Mil Cumbres, 14 Nov 1988, *Ramomoorthy 4519* (MEXU); 2 km al SE del Cerro del Estribo, municipio de Pátzcuaro, 8 Dec 1986, *Garduño 2437* (MEXU); Lado NW del Cerro San Miguel, municipio de Pátzcuaro, 13 Nov 1985, *Barriga 1748* (MEXU); Guanatini-Pamatacuaro; municipio Los Reyes, 16 Oct 1984, *Labat 1320* (MEXU); Bonilla, municipio de Pátzcuaro, 23 Oct 1991, *Escobedo 2238* (MEXU); Pátzcuaro, Cerro Estribo, [19.51463°, -101.63725°], 1 Nov 2006, *Ayers et al.* 1760 (ASC, MEXU); El Cerrito, 1.5 km al E de Jeráhuaro, municipio de Zinapécuaro, 20 Oct 1989, *Jasso 1710* (MEXU); Los Tres Cerritos, municipio de Zacapu, 19 Oct 1988, *Núñez 355* (MEXU). —MORELOS: 2–3 km al N de Tepoztlán, sobre la cañada de la zona arqueológica de Tepoztlán, Mpio. Tepoztlán, [19.0°, -99.10°], 5 Oct 1986, *Flores F. & Cabrera 162* (MEXU). —NUEVO LEÓN: Rancho San Luis Aculco, Chalco, 8 Nov 1980, *Hinton 18109* (TEX). —OAXACA: La Sirena, Miahuatlán, 23 Oct 1995, *Hinton 26399* (TEX); En Puente Jaltengo a 15 km al N de Candelaria Loxichia, camino Pochutla-Oaxaca, *Martínez S. et al.* 2470 (ASC, MEXU); Tlaxiaco, 22 Oct 1957, *MacDougall s. n.* (MEXU); Sto. Tomas Ocotepic, Tlaxiaco, 17 Nov 1972, *MacDougall H518* (NY); Teposcolula, 1 Dec 1895, *Robinson 1506* (GH); Santiago Juxtahuaca, San Sebastián Tecomaxtlahuaca, km 8 de la carretera Tecomaxtlahuaca-San Martín Peras, [17.310°, -98.098°], 2 Nov 1994, *Panero 5427* (NY, TEX); A 35.5 km al NE de Putla, camino a Tlaxiaco, Distr. de Putla, 27 Nov 1983, *Martínez S. & Barrie 5808* (MEXU); Barranca de Pájaro on the road to Tlaxiaco, 27 Nov 1983, *Barrie 754* (TEX); A 5 km al S de Tlaxiaco, Distrito de Tlaxiaco, 27 Oct 1980, *Tellez 3869* (MEXU); Santiago Juxtahuaca, San Martín Peras, km 16 de la desviación a Cañada de Lobos sobre el nuevo camino a campos de fresas, [17.329°, -98.121°], 24 Oct 1994, *Panero et al.* 5283 (TEX); Juxtahuaca, Santiago Juxtahuaca, 22 Oct 1997, [17.2674°, -98.0°], *Calzada 22292* (MEXU). —PUEBLA: Parque Estatal General Lázaro Cárdenas, [19.03°, -98.11°], 1 Nov 1994, *Romero 584* (MEXU); Between Acatzingo and El Seco, 10 Oct 1944, *Sharp 441275* (MEXU); Moist steep bank above river near La Vente, 15 Nov 1944, *Sharp 441660* (MEXU); Parque Estatal General Lázaro Cárdenas, [19.01°, -98.11°], 25 Oct 1994, *Romero 556* (MEXU); Tepoxuchilt, 27 Oct 1910, *Antonio s. n.* (MEXU); Parque Estatal General Lázaro Cárdenas, [19.03°, -98.11°], 1 Nov 1994, *Romero 598* (MEXU); Malinche San Miguel Canoa, 20 Oct 1968, *Boege 1011* (MEXU); Matamoros, 2 Oct 1942, *Miranda 2254* (MEXU). **Panama.** —PROV. HERRERA: Roadside ca 3 km N of Las Minas, 19 Jan 1981, *D'Arcy & Sytisma 14287* (MEXU). **Peru.** —PROV. CONTUMAZÁ: Dept. Cajamarca, 28 Jun 1992, *Sagástegui et al.* 14748 (ASC). —PROV. OTUZZO: Dpto. La Libertad, Huaranchal, 6 Jun 1958, *López et al.* 2657 (NY). **Venezuela.** —ESTADO DE MIRANDA: Los Teques, 29 Nov 1938, *Alston 5262* (LL).

5. *Diastatea ciliata* (McVaugh) E.P.Johnson & T.J.Ayers, comb. nov. *Diastatea virgata* var. *ciliata* McVaugh, Bull. Torrey Bot. Club 67: 793. 1940. *Diastatea virgata* subsp. *ciliata* (McVaugh) Lammers, Novon 16(1): 69. 2006. TYPE: MÉXICO. Guerrero: Mountains above Iguala, 24 Oct 1900, C. G. Pringle 8375 (holotype: GH!; isotypes: F, G, GOET, ISC, JE-image!, MO!, NY-image!, PH-image!, POM, RM, S, US-image!).

Stems 15–54 cm tall, simple or with few to many ascending branches, 0.5–4.0 mm wide at base, minutely winged, pubescent with soft hairs especially near base, the hairs evenly distributed around stem, not confined to wings. **Leaves** sessile or pseudo-petiolate, the faintly winged pseudo-petioles decurrent into narrow wings on stem; blades green, base rounded and attenuate into poorly defined winged petioles, apex rounded, acute, or acuminate; lowermost blades suborbicular to broadly ovate, 8–20 mm long, 4–16 mm wide; middle to upper blades ovate to broadly lanceolate, 17–63 mm long, (6–)10–25(–46) mm wide; margins irregularly serrate, sometimes crenate; glabrous throughout or

ciliate on margins with sparse hairs adaxially and on the veins abaxially. **Inflorescences** weakly secund with few to 20 flowers per stem; bracts lanceolate and leafy at base of inflorescence becoming narrowly linear distally, 2.7–60 mm long, 0.5–25 mm wide, spreading to ascending. **Flowers** with pedicels 3–15 mm long in flower, minutely pubescent, ascending and often bent distally in fruit; hypanthium a flattened disc in flower, 1–1.5 mm in diameter, 0.5–0.9 mm in length from calyx sinus to attachment point with pedicel, becoming minutely obconic in fruit, pubescent with short, stiff hairs; calyx lobes subequal to equal, ca. three-quarters the length of the fruit, narrowly triangular to linear-elliptic, (3–)4–5(–6.1) mm long in flower, green, purple, or magenta, margins entire and prominently white ciliate; corolla purple to purplish-blue, nectar guides blue-white with two small raised projections at throat; tube cylindrical, (5–)6.2–8.8 mm long from calyx sinus to lateral corolla sinus, (0.3–)0.7–1.6 mm wide at middle; dorsal lobes linear to elliptic-oblancheolate, usually with a narrowed claw attaching distal portion to deltoid base, 3.5–5.0 mm long, 0.4–0.6 mm wide; ventral lobes oblong with mucronate tip, 4.5–5.7 mm long, 1.6–2.7 mm wide; filaments (5–)5.7–8.6 mm long; anthers 1.2–1.8 mm long, dark purple with downy white covering, two large and many small white trichomes at apex of ventral anther pair. **Fruits** narrowly ovoid to ellipsoid and slightly curved at tip, (4.8–)5.9–12.0 mm long, 1.6–3.2 mm wide, light brown or purplish; seeds round to ovoid, 0.4–0.5 mm long, orange to orange-brown. Figures 4E, S4C.

Chromosome Number—Unknown.

Distribution—Mexican states of Guerrero, México, Morelos, Oaxaca, and Puebla (Fig. 5).

Notes—We elevated this taxon from the rank of subspecies due to a combination of morphological traits and molecular evidence. Morphologically, it has characters that are distinct from *D. virgata*, such as ciliate calyx margins and narrowly ovoid capsules. *Diastatea virgata*, in contrast, has glabrous calyx margins and narrowly cylindrical fruits. Molecular evidence suggests that the closest relatives of *D. ciliata* are *D. maximiliana* and *D. costaricensis* rather than *D. virgata*.

Additional Specimens Examined—MÉXICO. —GUERRERO: 14.7 miles west of México Highway 95 and 7 miles east of Xochipala, 15 Oct 1975, *Reveal et al.* 4183 (MO, TEX); Los Ranchos (Rancho Viejo), 15 miles west of Teloloapan, 17 Dec 1963, *Porter 1358* (GH); Mpios. de Iguala y Buenavista, Cañón de La Mano, entre Los Amates y El Naranjo, 10 km al N de Iguala por el ferrocarril, 5 Oct 1986, *Catalán H.* 289 (MEXU); Taxco, 5 Oct 1960, *Paray 3084* (MEXU); 19 miles north of Iguala, 16 Nov 1958, *Jones 23329* (MEXU, LL); 14 km al SW de Xochipala carr. a filo de Caballo, 17 Oct 1983, *Núñez & Martínez 5668* (MEXU); Temisco; Barranca del Consuelo, 15 Nov 1937, *Mexia 8813* (MO); Mpio. General Heliodoro Castillo (Tlacoapa), Tlacotepec, 3.73 km al N, [17.82°, -99.84°], 29 Oct 1998, *Soto 11600* (MEXU); Mpio. Taxco de Alarcón, Mexcaltepec, 0.4 km al NE, [18.43°, -99.54°], 25 Oct 1997, *Gordillo 2024* (MEXU); El Mogote, 2 km al E, [18.68°, -99.54°], 22 Oct 1997, *Gordillo 2021* (MEXU); Piedras Negras, cerca de Cacahuamilpa, 15 Nov 1953, *Matuda et al.* 29731 (MEXU). —MÉXICO: Vigas, Temascaltepec, 9 Oct 1935, *Hinton et al.* 8538 (MEXU, MO, LL); Tenayac, Temascaltepec, 19 Oct 1935, *Hinton et al.* 8374 (GH). —MORELOS: Cuernavaca-Yautepec road, near Yautepec, km 22, 25 Oct 1943, *Lundell 12611* (LL); Near Yautepec, 21 Oct 1902, *Pringle 11005* (MO); km 126 of Cuernavaca-Taxco Highway, 19 Oct 1943, *Lundell & Lundell 12565* (LL); Cañón del Lobo, cerca de Yautepec, 19 Sep 1954, *Gold 699* (MEXU). —OAXACA: Distrito: Yautepec, Municipio: San Carlos Yautepec. Rancho Los Ruices, Sta. Ma. Lachixonace, [16.524°, -95.820°], 21 Oct 2010, *Ruiz & Sánchez 308* (ASC); 6 km W of Tlaxiaco on Mex 125, [17.15°, -97.48°], 28 Nov 2003, *Yahara et al.* 2837 (MO). —PUEBLA: Cerro Tlacoctli, 6 Oct 1942, *Miranda 2319* (MEXU).

6. *DIASTATEA VIRGATA* Scheidw., Allg. Gartenzeitung (Otto & Dietrich) 9: 396. 1841. NEOTYPE, here designated: MÉXICO.

Oaxaca: Teotitlán, Santa María Ixcatlán, aproximadamente 6 km al NNW de Santa María Ixcatlán sobre la brecha a San Pedro Nodón [17.825°, -97.156°], 28 Oct 1996, J. L. Panero & J. I. Calzada 6778 (neotype: NY!; isoneotypes: TEX!, MEXU!).

Lobelia ramosissima M.Martens & Galeotti, Bull. Acad. Roy. Sci. Brux. 9(2): 42. 1842. *Laurentia ramosissima* (M.Martens & Galeotti) Benth. & Hook.f. ex Hemsl., Biol. Cent.-Amer., Bot. 2(10): 265. 1881. TYPE: MÉXICO. Oaxaca: Cordillera 1840, H. G. Galeotti 1971 (holotype: BR-image!).

Stems 6.2–43.5 cm tall, simple or with few to many ascending branches, 0.4–3.9 mm wide at base, glabrous or minutely hirtellous on the prominent wings. **Leaves** subsessile or pseudo-petiolate; pseudo-petioles 3–8 mm long, margins winged, ciliate, decurrent into narrow wings on stem; blades green or suffused with purple, base rounded to narrowly cuneate, apex rounded or blunt, tipped with callosity; lowermost blades ovate to lanceolate, 6–28 mm long, 3.5–15 mm wide; middle to upper blades ovate, lanceolate to narrowly elliptic, (4.5–)8.4–55 mm long, (2.2–)3.8–25 mm wide; margins irregularly serrate to erose with callosities often occurring between teeth; mostly glabrous or with sparsely scattered stiff hairs. **Inflorescence** weakly to strongly secund with few to 20 flowers per stem; bracts narrowly lanceolate to linear, 2.5–30 mm long, 0.5–15 mm wide, ascending to somewhat appressed. **Flowers** with pedicels 2–30 mm long in flower, ascending and often bent distally in fruit, glabrous; hypanthium a flattened disc in flower, 0.8–1.3 mm in diameter, 0.3–1.0 mm in length from calyx sinus to attachment point with pedicel, not much expanded in fruit, glabrous; calyx lobes subequal to equal, from one-third to one-half the length of the fruit, linear, 2.1–4.8 mm long in flower, green, purple, or green with purple tip and teeth, margins entire or with 1–2 pair of gland-tipped teeth near the middle, glabrous; corolla purple to purplish-blue, the nectar guides pale yellow with small bright yellow raised projections at throat; tube cylindrical, 6.8–9.7 mm long from calyx sinus to lateral corolla sinus, 0.6–1.3 mm wide at middle; dorsal lobes elliptic, obovate, or oblanceolate, usually with a narrow claw attaching distal portion to deltoid base, 3.3–6.0 mm long, 0.6–1.9 mm wide; ventral lobes oblong to oblanceolate with mucronate tip, (3.8–)4.1–7.5(–8.8) mm long, 1.4–2.8 mm wide; filaments (6.5–)7.2–10 mm long; anthers 1.2–2.0 mm long, dark purple with downy white covering and short stiff hairs throughout, plus two large and many small white trichomes at apex of ventral anther pair, sometimes with slightly lighter margins between adjacent anthers. **Fruits** cylindrical sometimes slightly curved at tip, 6.0–13.2 mm long, 1.2–2.5 mm wide, light brown or purplish-brown; seeds ellipsoid, 0.5 mm long, orange to dark orange. Figures 3G, H, 4H.

Chromosome Number— $2n = 14$ (Fig. S5B).

Distribution—Well-documented in the Mexican state of Oaxaca and less frequently in Puebla and Guerrero (Fig. 5).

Notes—McVaugh (1940b) noted in his treatment of the genus that Scheidweiler's original material of *D. virgata* had not been located. Despite this, it does not appear that McVaugh ever chose a neotype for *D. virgata*. We also conducted searches of herbaria where Scheidweiler's collections are known to be deposited, including the National Botanic Garden of Belgium Vascular Plant Herbarium (BR), the Botanic Garden and Botanical Museum Berlin-Dahlem (B), and the Nationaal Herbarium Nederland at Leiden (L), and did

not locate his original material. Therefore, a neotype for *D. virgata* has been designated here.

Subspecific taxa are no longer recognized within *D. virgata*. *Diastatea virgata* subsp. *ciliata* has been elevated to specific rank based on both distinct morphological traits and molecular evidence.

Additional Specimens Examined—MÉXICO. —GUERRERO: Mpio. Chilpancingo, 4.7 mi E of turnoff to Omeapa on highway 93 Tixtla-Chilapa, 26 Oct 1984, Saunders-Scherrer et al. 1440 (MEXU, LL); Mpio. Chilapa, 7 mi W of Chilapa on road (MEX 93) to Chilpancingo, 2.5 mi E of Zoquiapa, 17°33.519'N, 99°15.007' W, 8 Nov 2006, Ayers et al. 1772 (ASC, IBUG, MEXU, NY). —OAXACA: Distrito de Huajuapán de León, Mpio. de Santiago Chazumba: 54 km. al SO. de Tehuacán (Puebla) sobre la carretera a Huajuapán, 2 km. al O. del limite con Puebla, Lat. 18°12'N, Long. 97°40'W, 18 Oct 1979, Wendt et al. 2243 (LL); Alrededores de Monte Albán, Mpio. Monte-Albán, Dto. Centro, 8 Oct 1985, López et al. 722 (MEXU); 18.4 miles SE of Tlacolula, 13 Nov 1980, Fryxell & Lott 3407 (MEXU, MO, NY); Jayacatlán, 4 Nov 1894, Smith 284 (GH); El Paria, Etlá, Nov 1898, Conzatti & González 904 (GH); Monte Albán, S of Oaxaca City, 14 Oct 1983, Anderson 13126 (NY); 20 km south of Nochixtlán along Highway 190 to Oaxaca, 4 Nov 1973, Breedlove 35859 (MO); North of Jayacatlán along road towards Nacaltepec, 4 Nov 1973, Breedlove 35904 (MO); Monte Albán, near Oaxaca, 30 Oct 1894, Smith 721 (GH, MO); "Vuelta Grande" 9 km al E de Nacaltepec, brecha a La Unión, Cuicatlán [17.5°, -96.9°], 7 Dec 1991, Tovar et al. 6586-b (MO); 1.6 km E of Highway 125 on the road to Guadalupe Cuautepac at km 85 from Tehuacán on Highway 125, 18 Oct 1985, Bartholomew et al. 3092B (GH); Distrito: Etlá, Municipio: San Felipe Tejalapa. Loma de la Mina [17.069°, -96.890°], 17 Oct 2011, Morales 578 (ASC); Distrito: Etlá, Municipio: San Felipe Tejalapa. Barranca Boca de León [17.072°, -96.889°], 17 Oct 2011, Morales 532 (ASC); Mun. Tlacolula, 5 km al S de Matatlán, Nueve Puntas, Estación Microondas, Dec 1980, Ramamoorthy et al. 1188 (MEXU); Mpio. Silacayoapan, Dto. Silacayoapan, 10 km al SE de San Martín del Estado hacia Silacayoapan alrededores del cerro la Cabra [17.55°, -98.117°], 7 Dec 1992, Colín 14118 (MEXU). —PUEBLA: 65 miles southeast of Izucar de Matamoros (jct. at south end of town), 6 Oct 1984, Lavin 5169 (NY, TEX); 12 miles southeast of Petlalcingo, 7 Nov 1964, Ripley & Barneby 13681 (NY); Rancho San Antonio, 10 km al NW de Molcaxac, Brecha a Huatlatlauca [18.7°, -98.0°], 15 Oct 1984, Tenorio L. et al. 7681 (MEXU).

7. DIASTATEA MAXIMILIANA E.Wimm., Ann. Naturhist. Mus. Wien 56: 332. 1948. *Laurentia maximiliana* E.Wimm., Repert. Spec. Nov. Regni Veg. 38: 78. 1935. NEOTYPE, here designated: MÉXICO. Guerrero: Chilapa de Álvarez, 11.5 mi E of Chilapa on MEX 93, road to Atlixac, [17.59222°, -99.09123°], 9 Nov 2006, T. J. Ayers, R. Scott & A. Scott 1773 (neotype: MEXU!; isoneotypes: ASC!, IBUG!, NY!).

Stems 13–46 cm tall, simple or with few to many ascending branches, 0.5–2.7 mm wide at base, winged, glabrous except for the ciliate wings. **Leaves** sessile to subsessile below, sessile above, decurrent into narrow wings on stem; blades purple to green, base truncate to cuneate, apex subacute to narrowly acute; lowermost blades ovate to elliptic, 1.6–12 mm long, 1.7–7 mm wide, margins regularly serrate, mostly glabrous but with short hairs near base; upper blades narrowly elliptic to linear, 7.5–35 mm long, 0.9–4.5 mm wide, margins regularly or irregularly serrate, glabrous or ciliate on margins and at base. **Inflorescence** strongly secund with few to 18 flowers per stem, bracts linear to elliptic, 6–25.5 mm long, 0.5–1.8 mm wide, ascending to appressed. **Flowers** with pedicels 3–15 mm long in flower, glabrous or minutely pubescent near attachment with hypanthium, ascending to arcuate in fruit; hypanthium a flattened disc in flower, 1–1.7 mm in diameter, 0.1–0.6 mm in length from calyx sinus to attachment point with pedicel, not much expanded in fruit, glabrous; calyx lobes equal, one-half to two-thirds the length of the fruit and rarely exceeding it, narrowly triangular to linear, 1.8–4.8 mm long in flower, green, purple, or green with

purple apex, margins entire, rarely with 1 pair of teeth at base, glabrous or finely ciliate; corolla purplish-blue to purple, some with pale yellow streaking especially along veins, nectar guides white at ventral lobe sinuses leading to yellow and dark blue projections at throat, sometimes with yellow streaks in the tube; tube broadly cylindrical and sometimes minutely funnelliform, 2.9–4.6 mm long from calyx sinus to lateral corolla sinus, 1.3–3.0 mm wide at middle; dorsal lobes oblanceolate, 2.5–4.0 mm long, 0.6–1.3 mm wide; ventral lobes obovate to oblong with a mucronate tip, 2.6–4.8 mm long, 1.5–3.1 mm wide; filaments 3.2–4.5 mm long; anthers (0.8–)1.1–1.5 mm long, dark purple with downy white covering and a few short hairs on dorsal anthers, two large and many small white trichomes at apex of ventral anther pair, the margins between adjacent anthers white. **Fruits** ovoid, 4.1–5.6 mm long, 2.5–3.5 mm wide, light brown or purplish; seeds ellipsoid, 0.5–0.6 mm long, orange-brown. Figures 3E, 4G.

Chromosome Number—Unknown.

Distribution—Mexican state of Guerrero although several specimens with affinities to *D. maximiliana* have been collected in the states of Hidalgo and Oaxaca (Fig. 5).

Notes—We have been unable to locate the specimens noted under Wimmer's (1935) description of *Laurentia maximiliana*, namely *L. Schultze* no. 163 and 113, which were held at the herbarium of the Botanic Garden and Botanical Museum Berlin-Dahlem. It is likely that specimens seen by Wimmer were destroyed during WWII; therefore a neotype has been designated here.

McVaugh (1940b) placed this taxon in synonymy with *D. micrantha*. It has a broad corolla tube and fruit, both of which are minute in *D. micrantha*. Additionally, its cauline leaves are narrowly elliptic in contrast to the ovate to lanceolate leaves seen in *D. micrantha*. In our molecular phylogeny, *D. maximiliana* was more closely related to *D. costaricensis* and the newly designated *D. ciliata*, than to *D. micrantha*.

While preparing this treatment, two unusual specimens came to our attention: *E. Halbinger* s. n. (MEXU) and *R. M. Fonseca* 2246 (MEXU). These specimens fall within the geographic range of *D. maximiliana*, but they share morphological traits with both *D. maximiliana* and *D. costaricensis*. Both their pubescence and their large corolla lobes mimic that of *D. maximiliana*, but their calyx lobes are toothed, and the lobes are equal in length to the fruit, which are diagnostic traits of *D. costaricensis*. This odd mixture of morphology warrants further study in any future work on these species.

Additional Specimens Examined—MÉXICO. —GUERRERO: Mpio. Atlix-tac, 24 km al E de Chilapa por la carretera a Tlapa, 29 Oct 1979, Koch et al. 79171 (MEXU, NY); Mpio. Chilpancingo, 8.9 mi E of the western boundary of Chilapa, 5.6 mi E Río Atempa on highway 93, 26 Oct 1984, Saunders-Scherrer et al. 1479 (LL); Mpio. Chilapa, Estación de Microondas Pozo Largo, carretera Chilapa a Tlapa, 27 Dec 1993, Calzada 19012 (MEXU); Chilapa de Álvarez, 11.5 mi E of Chilapa on MEX 93, road to Atlix-tac, 9 Nov 2006, Ayers et al. 1773 (ASC, IBUG, MEXU, NY); Chilapa de Álvarez, 9.8 mi E of Chilapa on MEX 93, road to Atlix-tac, [17.60793°, -99.08788°], 9 Nov 2006, Ayers et al. 1774 (ASC); Mesones, a un lado de la Carr. Chilapa-Tlapa, 27 Oct 1995, Fonseca 2246 (MEXU); Hueycantenango, 15 Nov 1975, Halbinger s. n. (MEXU). —HIDALGO: Mun. Ajacuba, aprox. 1 km al SE del "puerto del gachupín", ladera W de "La mesa grande", vertiente S de la sierra de Chicavasco, ejido San Nicolás Tecomatlán, al NE de Emiliano Zapata, [20.15°, -99.01°], 27 Oct 1989, Vilchis et al. 633 (MEXU). —OAXACA: Santiago Juxtahuaca, San Martín Peras, km 16 de la desviación a Cañada de Lobos sobre el nuevo camino a campos de fresas, [17.329°, -98.121°], 24 Oct 1994, Panero et al. 5283 (TEX); Mun. Zapouquila Rincón del Tecolote, al NW de Guadalupe Membrillos, Distrito Huajuapán, 17 Oct

1991, Tenorio L. et al. 17678 (MO); Mun. Zapouquila Piedras Paradas, Cerro Chicamole al N de Guadalupe Membrillos, Distrito Huajuapán, 22 Nov 1991, Tenorio L. 18142 (MO).

8. *DIASTATEA COSTARICENSIS* McVaugh, Bull. Torrey Bot. Club 67: 789. 1940. TYPE: COSTA RICA. Prope San José, November 1846, A. S. Ørsted 9238 (holotype: C-image!; isotype: C-image!).

Stems 5.5–48 cm tall, simple or with few to many ascending branches, 0.8–2.5 mm wide at base, minutely winged; wings ciliate with flattened trichomes. **Leaves** sessile or pseudo-petiolate below, sessile above; blades green to purple, base rounded to cuneate, decurrent into narrow wings, apex rounded to narrowly acute; lowermost blades ovate, 2.8–5.5 mm long, 2.5–3.5 mm wide, the margins regularly serrate with prominent teeth; middle to upper blades narrowly lanceolate to narrowly elliptic, 8–33 mm long, 1.1–4.2(–5.5) mm wide, margins irregularly serrate; glabrous throughout or ciliate on margins with sparse hairs adaxially and on the veins abaxially. **Inflorescences** strongly secund with few to 10 flowers per stem; bracts narrowly elliptic to linear, 4.5–20 mm long, 0.6–2 mm wide, ascending to appressed. **Flowers** with pedicels 2.2–10 mm long in flower, minutely pubescent, ascending and often bent distally in fruit; hypanthium a flattened disc in flower, 1–1.5 mm in diameter, 0.4–1 mm in length from calyx sinus to attachment point with pedicel, not much expanded in fruit, glabrous; calyx lobes equal, more or less equaling fruit in length, narrowly triangular, 2.5–4.7 mm long in flower, green, purple, or green with purple tip and teeth, margins entire or rarely with 1–2 pair of teeth below middle, strongly ciliate; corolla purplish-blue, nectar guides yellow with raised projections at throat; tube broadly cylindrical, 3–4 mm long from calyx sinus to lateral corolla sinus, 1.2–2 mm wide at middle; dorsal lobes broadly triangular, 1.3–1.5 mm long, 0.5 mm wide; ventral lobes rounded spatulate, 1.5–2 mm long, 1 mm wide; filaments 2.8–3.7 mm long; anthers 0.7–1 mm long, purple-brown with a few scattered hairs near apex of dorsal set, two large and many small white trichomes at apex of ventral anther pair, the margins between adjacent anthers white. **Fruits** ovoid, 4.5–6.2 mm long, 2.0–3.2 mm wide, light brown or purplish; seeds ellipsoid, 0.5 mm long, orange-brown. Figure 4F.

Chromosome Number—Unknown.

Distribution—Costa Rica, Guatemala, and Honduras. Not documented from southern Mexico (Fig. 6).

Notes—All of the sheets examined for this study lacked stigmatic lobes exerted from the anther tube. This species may be autogamous with only rare outcrossing events. A discussion of individuals with intermediate morphologies is included under *D. maximiliana*.

As McVaugh noted in his revision of *Laurentia* (McVaugh 1940b), Wimmer erroneously identified *Oersted* 9238, the type collection of *D. costaricensis*, as conspecific with *Oersted* 9246, the type of *Lobelia irasuensis* Planch. & Oerst., collected on Volcán Irazú at the same time. Because of this error in identification, Wimmer made two subsequent combinations first in *Laurentia* as *L. irasuensis* (Planch. & Oerst.) E.Wimm. (Standley 1938) and then in *Diastatea* as *D. irasuensis* (Planch. & Oerst.) E.Wimm. (Wimmer 1948). Therefore, *D. irasuensis* ≡ *Lobelia irasuensis* and thus is treated here as an excluded taxon.

Additional Specimens Examined—Costa Rica. —PROV. ALAJUELA: Loc. Zarcero, Cantón Alfaro Ruiz, 11 Jan 1939, *Smith* 1435 (NY); La Palma de San Ramón, 23–26 Oct 1922, *Brenes* 3777 (NY); San Rafael de San Ramón, 23 Dec 1927, *Brenes* 5910 (NY). **Guatemala**. —DEPT. GUATEMALA: Cerro del Carmen, Nov 1865, *Bernoulli* 146 (NY). —DEPT. SANTA ROSA: Estanzuela, Nov 1892, *Smith* 4257 (GH, NY). **Honduras**. —DEPT. MORAZÁN: Mountains between Montaña Uyuca and La Montañita, 27 Dec 1962, *Williams et al.* 23239 (GH, LL, MO, NY); In the fields on the west slope of Mt. Uyuca, 29 Dec 1949, *Williams* 17045 (GH); Bosque mixto entre Cuesta de Los Muertos y Monte Oscuro, La Montañita, 27 Dec 1962, *Molina R.* 11169 (NY). —DEPT. EL PARAÍSO: 2 km northwest of Güinope, near Manzaragua road, 27 Feb 1949, *Standley* 17257 (MO, NY); 3 km NW of Güinope, 27 Feb 1949, *Williams & Merrill* 15693 (MO); 2 miles NW of Güinope, 5 Jan 1947, *Williams & Molina R.* 11523 (GH, MEXU, MO).

Doubtful and Excluded Taxa—*Diastatea lemairei* E. Wimm., *Pflanzenr.* (Engler) 4, 276b: 386. 1953. *Diastatea ghiesbreghtii* (Lem.) E. Wimm., *Ann. Naturhist. Mus. Wien* 56: 333. 1948. *Lobelia ghiesbreghtii* Lem., *Ill. Hort.* 1: pl. 34. 1854. non Decaisne. 1848. *Dortmanna ghiesbreghtii* (Lem.) Kuntze, *Revis. Gen. Pl.* 2: 972. 1891 as *Dortmannia*. TYPE: unknown.

Notes—This species was included in Wimmer's (1953) treatment as *Diastatea lemairei* E. Wimm., originally described under the name *Lobelia ghiesbreghtii* Lem. by Lemaire (1854). Descaisne had already used the name *Lobelia ghiesbreghtii* in 1848 to describe a different plant, rendering Lemaire's name illegitimate in the genus *Lobelia*.

Lemaire's original description included an illustration, a discussion on its merits as a cultivated plant, and instructions on how to cultivate it. He described it as having pink-lilac corollas, which set it apart in the greenhouse from its congeners that had blue corollas. In addition to the pink corollas, he described the pedicels as being bibracteolate towards the base and the corolla being split to the middle. Otto Kuntze (1891) later transferred Lemaire's *L. ghiesbreghtii* to *Dortmannia ghiesbreghtii*.

Wimmer first recognized *Diastatea ghiesbreghtii* in 1948 and later changed the name to *D. lemairei*. Subsequently, in his 1953 treatment (Wimmer 1953) and then again in his supplement in 1968 (Wimmer 1968), Wimmer expressed doubt about whether the plant belonged in *Diastatea*. He finally concluded that it was probably *Lobelia stenosisiphon* (Adamson) E. Wimm.

The characters included in Lemaire's description and original illustration, as well as Wimmer's doubt, have us convinced that *Diastatea lemairei* E. Wimm. is not a *Diastatea* and it is relegated to the status of doubtful and excluded taxon.

Diastatea irasuensis (Planch. & Oerst.) E. Wimm., *Ann. Naturhist. Mus. Wien* 56: 332. 1948 as irazuensis. *Laurentia irasuensis* (Planch. & Oerst.) E. Wimm. ex. Standl., *Field Mus. Publ. Bot.* 18: 1415. 1938 as irazuensis \equiv *Lobelia irasuensis* Planch. & Oerst.

Notes—Wimmer mistakenly based the name of a new species he described (now known as *Diastatea costaricensis* McVaugh) on the type of *Lobelia irasuensis* Planch. & Oerst., a true *Lobelia*, therefore, *Diastatea irasuensis* \equiv *Lobelia irasuensis* and thus is treated here as an excluded taxon. See *Diastatea costaricensis* for further discussion.

Lobelia minutiflora Kunze, *Linnaea* 16: 318. 1842. as 'minutiflorum.' *Dortmanna minutiflora* (Kunze) Kuntze, *Revis. Gen. Pl.* 2: 972. 1891 as 'Dortmannia.' TYPE: unknown (no original material found. If at LZ, presumed destroyed in WWII).

Notes—McVaugh (1940b) treated *Lobelia minutiflora* as a heterotypic synonym of *D. micrantha* when he recognized *Diastatea* in his revision of *Laurentia*. However, Kunze describes the corolla as reddish and barely split in half "flores rubelli, vix ad dimidium fissi" thus it is doubtful this specimen represented *D. micrantha* and is not recognized here as a heterotypic synonym of that taxon.

KEY TO THE GENUS *DIASTATEA*

1. Middle and upper cauline leaves narrowly elliptic to linear, glabrous; corolla often gibbous at base, if not gibbous, then leaf bases not decurrent and hypanthium expanded to encompass one-third to one-half of capsule in fruit 2 (*Diastatea* subg. *Angustifolia*)
 2. Stems evenly and minutely puberulent below, leaf bases not decurrent *D. aptera*
 2. Stems glabrous, decurrent leaf bases forming small, winged projections on stems (visible at 20 \times magnification) 3
 3. Teeth on leaf margins prominent and more or less regularly spaced 1–1.5 mm apart; corolla tube funnellform, flaring distally *D. expansa*
 3. Teeth on leaf margins minute or reduced to marginal callosities, usually more than 2 mm apart; corolla tube narrowly cylindrical 4 (*D. tenera*)
 4. Corolla tube 3.9–8 mm long, lower corolla lobes (1.8–)3.6–6.7 mm long, stamens 4.7–11.5 mm long *D. tenera* subsp. *tenera*
 4. Corolla tube 8–13.5 mm, lower corolla lobes 6–11.3 mm long, stamens 11.2–15 mm long *D. tenera* subsp. *durangensis*
1. Middle and upper cauline leaves ovate or lanceolate, if narrowly elliptic then pubescent at least proximally; corolla not gibbous at base, hypanthium not expanded 5 (*Diastatea* subg. *Diastatea*)
 5. Cauline leaves broadly ovate to broadly lanceolate 6
 6. Corolla minute, corolla tube 2.4–4.5 mm long; fruit usually less than 6 mm long (rarely 8 mm) *D. micrantha*
 6. Corolla conspicuous, corolla tube (5–)6.2–9.7 mm long; fruit 6 mm or longer 7
 7. Calyx margins prominently ciliate *D. ciliata*
 7. Calyx margins glabrous *D. virgata*
 5. Cauline leaves narrowly elliptic, 0.5 cm wide or less 8
 8. Calyx lobes glabrous or only minutely ciliate, one-half to two-thirds length of fruit or less (rarely nearly reaching end of fruit), found in the Mexican states of Guerrero and Oaxaca *D. maximiliana*
 8. Calyx lobes strongly ciliate, more or less equal to fruit in length, found from Guatemala south to Costa Rica. *D. costaricensis*

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AUTHOR CONTRIBUTIONS

EPJ wrote the manuscript and the descriptions and conducted the phylogenetic and morphological research guided by TJA. TJA conducted the fieldwork, performed and wrote the methods for the chromosome count and SEM studies, edited drafts of the manuscript, and took all photographs of *Diastatea*.

LITERATURE CITED

- Antonelli, A. 2008. Higher level phylogeny and evolutionary trends in Campanulaceae subfamily Lobelioideae: Molecular signal overshadows morphology. *Molecular Phylogenetics and Evolution* 46: 1–18.
- Ayers, T. J. 1990. Systematics of *Heterotoma* (Campanulaceae) and the evolution of nectar spurs in the new world Lobelioideae. *Systematic Botany* 15: 296–327.
- Chernomor, O., A. von Haeseler, and B. Q. Minh. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology* 65: 997–1008.
- Chiang, T.-Y., B. A. Schaal, and C.-I. Peng. 1998. Universal primers for amplification and sequencing a noncoding spacer between the *atpB* and *rbcL* genes of chloroplast DNA. *Botanical Bulletin of Academia Sinica* 39: 245–250.
- Crespo, M. B., L. Serra, and A. Juan. 1998. *Solenopsis* (Lobeliaceae): A genus endemic in the Mediterranean region. *Plant Systematics and Evolution* 210: 211–229.
- Devitt, T. J. 2006. Phylogeography of the western lyresnake (*Trimorphodon biscutatus*): Testing aridland biogeographical hypotheses across the nearctic-neotropical transition. *Molecular Ecology* 15: 4387–4407.
- Doyle, J. J. and J. L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- Gouy, M., S. Guindon, and O. Gascuel. 2010. SeaView version 4: A multi-platform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution* 27: 221–224.
- Hoang, D. T., O. Chernomor, A. von Haeseler, B. Q. Minh, and L. S. Vinh. 2017. UFBoot2: Improving the ultrafast bootstrap approximation. *Molecular Biology and Evolution* 35: 518–522.
- Jeppesen, S. 1981. *Diastatea*. Pp. 122–124 in *Flora of Ecuador*, eds. G. Harling and B. Sparre. Stockholm: Swedish Natural Science Research Council.
- Johnson, E. P. and T. J. Ayers. 2022. Data from: Molecular phylogeny and taxonomic revision of the genus *Diastatea* (Campanulaceae: Lobelioideae). Dryad Digital Repository. <https://doi.org/10.5061/dryad.rr4xgxd89>.
- Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. von Haeseler, and L. S. Jermini. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* 14: 587–589.
- Katoh, K. and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772–780.
- Katoh, K. and H. Toh. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298.
- Knox, E. B., A. M. Muasya, and N. Muchhala. 2008. The predominantly-South American clade of Lobeliaceae. *Systematic Botany* 33: 462–468.
- Koopman, M. M. and T. J. Ayers. 2005. Nectar spur evolution in the Mexican Lobelias (Campanulaceae: Lobelioideae). *American Journal of Botany* 92: 558–562.
- Kuntze, O. 1891. *Revisio Generum Plantarum* 2: 972.
- Lammers, T. G. 1992. Circumscription and phylogeny of the Campanulales. *Annals of the Missouri Botanical Garden* 79: 388–413.
- Lammers, T. G. 1993. Chromosome numbers of Campanulaceae. III. Review and integration of data for subfamily Lobelioideae. *American Journal of Botany* 80: 660–675.
- Lammers, T. G. 1999. A new *Lobelia* from Mexico, with additional new combinations in world Campanulaceae. *Novon* 9: 381–389.
- Lammers, T. G. 2006. Validation of names at subspecific rank in North American Campanulaceae. *Novon: A Journal for Botanical Nomenclature* 16: 69–73.
- Lammers, T. G. 2007. *World Checklist and Bibliography of Campanulaceae*. Richmond: Royal Botanic Gardens, Kew.
- Lammers, T. G. 2011. Revision of the infrageneric classification of *Lobelia* L. (Campanulaceae: Lobelioideae). *Annals of the Missouri Botanical Garden* 98: 37–62.
- Lemaire, C. 1854. *Lobelia Ghiesbreghtii*. *L'illustration Horticole: journal special des serres et des jardins, ou choix raisonne des plantes les plus interessantes sous le rapport ornemental, comprenant leur histoire complete, leur description comparee, leur figure et leur culture* 1: Planche 34.
- Marshall, C. J. and J. K. Liebherr. 2000. Cladistic biogeography of the Mexican transition zone. *Journal of Biogeography* 27: 203–216.
- Matuda, E. 1950. A contribution to our knowledge of the wild and cultivated flora of Chiapas. I. Districts Soconusco and Mariscal. *American Midland Naturalist* 44: 513–616.
- McVaugh, R. 1940a. A key to the North American species of *Lobelia* (sect. Hemipogon). *American Midland Naturalist* 24: 681–702.
- McVaugh, R. 1940b. A revision of *Laurentia* and allied genera in North America. *Bulletin of the Torrey Botanical Club* 67: 778–798.
- McVaugh, R. 1943. *Diastatea*. Pp. 26–30 in *North American Flora*, 32A. New York: The New York Botanical Garden.
- McVaugh, R. 1949. Studies in South American Lobelioideae (Campanulaceae) with special reference to Colombian species. *Brittonia* 6: 450–493.
- McVaugh, R. 1965. South American Lobelioideae new to science. *Annals of the Missouri Botanical Garden* 52: 399–409.
- Murata, J. 1995. Revision of the infrageneric classification of *Lobelia* (Campanulaceae: Lobelioideae) with special reference to seed coat morphology. *Journal of the Faculty of Science* 15: 349–371.
- Nash, D. L. and L. O. Williams. 1976. *Diastatea*. Pp. 408–411 in *Flora of Guatemala*. Chicago: Field Museum of Natural History.
- Nguyen, L.-T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution* 32: 268–274.
- Otto, F. and A. Dietrich. 1841. *Allgemeine Gartenzeitung* 9: 396–397.
- Quattrocchi, U. 2000. *CRC World Dictionary of Plant Names: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology*. Vol II. Boca Raton: CRC Press.
- Robinson, B. L. 1891. *Proceedings of the American Academy of Arts* 26: 166.
- Ronquist, F., M. Teslenko, P. van der Mark, D. L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M. A. Suchard, and J. P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
- Rzedowski, J. 2018. Tres novedades de Campanulaceae-Lobelioideae del Estado de Oaxaca (México). *Phytoneron* 2018–15: 1–9.
- Rzedowski, J. 2019. Updated inventory of the Mexican species of Campanulaceae. *Botanical Sciences* 97: 110–127.
- Rzedowski, J. and G. Rzedowski. 1997. *Diastatea*. Pp. 2–9 in *Flora Del Bajío Y de Regiones Adyacentes: Fascículo 58*. Pátzcuaro: Instituto de Ecología A. C. Centro Regional del Bajío.
- Senterre, B. and G. Castillo-Campos. 2008. A new rare species of *Lobelia* (Campanulaceae, Lobelioideae) from Mexican lowland rainforest. *Novon* 18: 378–383.
- Serra, L. and M. B. Crespo. 1997. An outline revision of the subtribe Siphocampylinae (Lobeliaceae). *Lagascalia* 19: 881–888.
- Serra, L. and M. B. Crespo. 1999. *Wimmerella*, a new South African genus of Lobelioideae (Campanulaceae). *Novon* 9: 414–418.
- Slanis, A. C., M. C. Perea, and A. A. García. 2009. *Diastatea* (Campanulaceae, Lobelioideae), nuevo género para la flora Argentina. *Darwiniana* 47: 349–352.
- Shaw, J. and E. B. Lickey. 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142–166.
- Shaw, J. and E. B. Lickey. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. *American Journal of Botany* 94: 275–288.
- Standley, P. C. 1938. Flora of Costa Rica. *Botanical Series, Field Museum of Natural History*, vol. 18, Part IV. *Publication* 429: 1415–1416.
- Thiers, B. 2019. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/science/ih/> (last accessed December 2019).
- Trifinopoulos, J., L.-T. Nguyen, A. von Haeseler, and B. Q. Minh. 2016. W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis. *Nucleic Acids Research* 44: W232–W235.
- White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in *PCR Protocols: A Guide to Methods and Applications* eds. M. Innis, D. Gelfand, J. Sninsky, and T. White. San Diego: Academic Press.
- Wilbur, R. L. 1976. Flora of Panama. *Annals of the Missouri Botanical Garden* 63: 593–595, 623–635.
- Wilbur, R. L. 2001. *Diastatea*. Pp. 560–561 in *Flora de Nicaragua 1*, eds. W. D. Stevens, C. Ulloa Ulloa, A. Pool, and O. M. Montiel. St. Louis: Missouri Botanical Garden Press.
- Wimmer, F. E. 1935. Repertorium specierum novarum regni vegetabilis. *Feddes Repertorium Specierum Novarum Regni Vegetabilis* 38: 78.

Paz to Coroico, 17 km E of cordillera summit, 1 Jan 1994, T. Ayers 1203 (ASC), MT664839, —, MT661730. *L. sinaloae* México. —DURANGO: Mpio. Durango. Parque El Tecuán, Bajío El Chanate, 14 Oct 1996, S. Acevedo 1062 (ASC), MT664837, —, —. *L. siphilitica* —/—/DQ0060151*. *L. volcanica* México. —VERACRUZ: 2.2 mi S of Ten-ejapán on road from Orizaba to Tequila, 16 Jan 1984, T. Ayers 334 (ASC), —, —, MT661722. *L. xalapensis* Bolivia. —LA PAZ: Nor Yungas. Ca 3 km SW of Coroico on road to Carmen Pampa, 15 May 2007, T. Ayers 1790 *cult.* (ASC), MT664840, MT622137, MT661731. *Palmerella*

debilis USA. —CALIFORNIA: Ventura County, Hwy 33 6.9 mi N of Ojai on the road to Lockwood Valley, Los Padres National Forest, just S of Wheeler Gorge Campground and Visitor Center, 34.50868, -119.27454, 7 June 2012, *Johnson et. al.* 21 (ASC), MT664841, MT622138, MT661732; Los Angeles County, San Gabriel Mtns., San Antonio Canyon, Mt Baldy Rd 2.9 miles past junction with Mountain Ave inside canyon on W side of road, 8 June 2012, *Johnson et. al.* 22 (ASC), MT664842, MT622139, MT661733. *Trachelium caeruleum* EU090187*, EU090187*, DQ304570*.