Morphological and molecular evidence in the delimitation of *Behria* and *Bessera*, two genera of the *Milla* complex (Themidaceae)

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Abstract: Among the taxa of the *Milla* complex (Themidaceae), a monocot group of perennial petaloid geophytes, there are two genera with controversial taxonomic status: *Behria* and *Bessera*. Tree-based and character-based analyses were conducted on 14 populations to determine if they should be considered different taxa. In addition, ecological separation was taken into account. As outgroups representative taxa of *Dandya*, *Jaimehintonia*, *Milla* and *Petronymphe*, i.e. the rest of the genera in the *Milla* clade, were used. *Behria* is a monotypic genus restricted to Baja California and *Bessera* includes two species from the Pacific slopes of Mexico and the Trans-Mexican Volcanic Belt. The chloroplast intergenic spacer *psbK-psbI* was sequenced and 37 morphological characters were coded. Tree-based analyses retrieved all populations of *Behria* and all populations of *Bessera* as monophyletic groups, both forming part of a more inclusive clade. Character-based analysis detected six diagnostic characters, all of which were floral. The conclusion of recognizing *Behria* and *Bessera* as independent genera was based on these results and also on their different distributional ranges.
Key words: chloroplast intergenic spacer *psbK-psbI*, *Dandya*, *Jaimehintonia*, petaloid geophytes, *Petronymphe*.

Resumen: Entre los taxones del complejo *Milla* (Themidaceae), un grupo de monocotiledóneas petaloides geófitas, existen dos géneros con estatus taxonómico incierto: *Behria* y *Bessera*. Con el objetivo de determinar si estos géneros pueden considerarse independientes, se realizaron análisis filogenéticos y de atributos diagnósticos con base en atributos morfológicos y moleculares de 14 poblaciones. Adicionalmente se consideraron individuos representativos del resto de los géneros del complejo, tales como *Dandya*, *Jaimehintonia*, *Milla* y *Petronymphe*. *Behria* es un género monotípico restringido al sur de la Península de Baja California. *Bessera* comprende dos especies de la vertiente pacífica de México y la Faja Volcánica Transmexicana. Se secuenció el espaciador intergénico *psbK-psbI* del cloroplasto y se codificaron 37 atributos morfológicos. Los análisis filogenéticos identificaron a las poblaciones de *Behria* y *Bessera* como grupos monofiléticos independientes, formando parte de un clado más inclusivo. Los resultados del análisis de atributos diagnósticos reconocieron seis atributos florales. Por lo tanto se concluye que estos taxones pueden ser identificados claramente por su morfología floral, además tienen diferentes patrones de distribución y se deben considerar como géneros independientes.
Palabras clave: espaciador intergénico del cloroplasto *psbK-psbI*, *Dandya*, geófitas petaloides, *Jaimehintonia*, *Milla* y *Petronymphe*.

Themidaceae is a plant family of perennial petaloid geophytes found mainly in western North America (Fay and Chase, 1996). The genera currently included in Themidaceae were formerly recognized as tribe *Brodiaeae* in the Alliaceae (Dahlgren *et al*., 1985). In previous taxonomic studies they were divided into two complexes: the *Milla* complex, centered in Mexico, and the *Brodiaea* complex, centered in the western United States (Moore, 1953). More recently, Pires *et al*. (2001) and Pires and Sytsma (2002) found that the genera are grouped into four clades: (1) the *Milla* complex, (2) *Brodiaea-Dichelostemma-Triteleiopsis*, (3) *Triteleia-Bloomeria-Muilla clevelandii*, and (4) *Androstephium*-other members of *Muilla*.

The *Milla* clade is supported by two synapomorphic character states: an ovary stipe adnate to the perianth tube, and a membranous corm (Pires and Sytsma, 2002). It is comprised of six genera (*Behria*, *Bessera*, *Dandya*, *Jaimehintonia*, *Milla*, and *Petronymphe*). *Behria* is a monotypic genus endemic to Baja California (Greene, 1886). In contrast, *Bessera* encompasses two species from western and

central Mexico (Ramírez-Delgadillo, 1992; Moore, 1953). *Dandya* has four species with a restricted geographic distribution in western and northern Mexico (Espejo-Serna and López-Ferrari, 1992; Lenz, 1971b; Villarreal-Quintanilla and Encina-Domínguez, 2005). *Milla* includes ten species (Lenz, 1971a; Moore, 1953; Howard, 1999) with *M. biflora* Cav., the most widely distributed member of the group, found from Arizona to Guatemala (Espejo-Serna and López-Ferrari, 2003; McNeal, 2003). Lastly, *Jaimehintonia* and *Petronymphe* are both monotypic. The first is restricted to a couple of small areas in Nuevo Leon (Turner, 1993) and the latter is known from a single locality in Guerrero (Moore, 1951).

Greene (1886) described *Behria* as a new genus when he was writing the monograph on *Brodiaea*. Later, Macbride (1918) transferred *Behria tenuiflora* to *Bessera arguing* that both *Bessera elegans* Schult. f. and *Bessera* (*Behria*) tenuiflora possess a red perianth and exerted stamens fused at the base. According to him, the only difference between the two taxa was the degree of fusion of floral segments. The dispute on the status of *Behria* continued, some authors treat *Behria tenuiflora* as part of *Bessera* (Moore, 1953; Ramírez-Delgadillo, 1992; Espejo-Serna and López-Ferrari, 1992; Pires *et al*., 2001), while others place this taxon in its own genus (Krause, 1930; Lenz, 1971b; Shreve and Wiggins, 1964; León de la Luz and Pérez-Navarro, 2004).

Whether *Behria* and *Bessera* are separate genera has not yet been demonstrated. A study of Themidaceae based on morphological and cpDNA data (*trnL-trnF and rbcL*) retrieved *Bessera elegans* and *B.* (*Behria*) tenuiflora as sister species, but *B. tuitensis* was not considered (Pires *et al*., 2001). Another study based solely on cpDNA (*trnL-F, rpl16, and ndhF*) retrieved *B. elegans* and *B. tuitensis* as sisters, but that study did not include *B.* (*Behria*) tenuiflora (Pires and Sytsma, 2002). To date, no study has included the two species in *Bessera* and *Behria* tenuiflora simultaneously.

Wiens and Penkrot (2002) proposed a novel method for delimiting species, based on morphological and molecular characters. Tree-based and character-based analyses are performed separately and distribution patterns are also considered. This method utilizes populations as terminals rather than individuals to avoid a biased treatment of the polymorphisms shared between populations as homoplasies rather than synapomorphies in the tree-based morphological analyses. The character-based analysis finds diagnostic character states representing differences among the putative species. This procedure was designed to delimit closely related taxa.

The method of Wiens and Penkrot (2002) is utilized in this study to clarify whether *Behria* and *Bessera* are independent taxa. Thus, tree-based and character-based analyses were performed with populations of these closely associated groups, together with representative species of genera in the *Milla* clade as the study units, employing morphological characters and chloroplast DNA sequences of *psbK-psbI*.

Table 1. Morphological data matrix. Character and character state number as in Appendix 2. Letters and numbers after the species name are the population abbreviation, indicated in Table 2. "?" = missing data, "-" = not applicable. Polymorphisms: (0, 1) = A; (0, 1, 2) = B; (0, 2) = C; (1, 2) = D; (1, 3) = E; (2, 3) = F; (2, 3, 4) = G

Terminals / Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
Behria tenuiflora BCS1	A	1	0	1	1	1	C	0	0	0	D	1	2	1	1	1	0	0	2	0	0	0	2	0	1	1	0	0	0	0	2	-	-	-	0	1	2
B. tenuiflora BCS2	1	1	0	1	1	1	B	0	0	0	1	1	2	1	1	1	0	0	2	0	0	0	2	0	1	1	0	0	0	0	2	-	-	-	0	1	2
B. tenuiflora BCS3	?	1	0	1	1	1	0	0	0	0	1	?	2	1	?	1	0	0	2	0	0	0	2	0	1	1	0	0	0	0	2	-	-	-	0	1	2
B. tenuiflora BCS4	A	1	0	1	1	1	0	0	0	0	1	?	2	1	?	1	0	0	2	0	0	0	2	0	1	1	0	0	0	0	2	-	-	-	0	1	F
Bessera elegans MOR1	1	1	0	1	1	1	0	0	0	0	2	1	2	1	1	1	1	2	1	1	1	-	2	1	0	1	2	0	2	0	2	0	1	1	0	1	2
B. elegans GRO1	0	1	0	1	1	1	0	1	0	1	1	E	2	1	1	1	1	2	1	1	1	-	2	0	0	0	1	0	2	0	2	0	1	1	0	1	2
B. elegans GRO2	1	1	0	1	1	1	0	1	0	0	3	1	2	1	2	1	1	2	1	1	1	-	2	1	0	1	1	0	2	0	3	0	1	1	0	1	3
B. elegans COL1	D	1	0	1	1	1	A	A	0	A	D	E	2	1	1	1	1	2	1	1	1	-	4	0	0	0	1	0	2	0	3	1	0	0	0	1	3
B. elegans JAL1	1	1	0	1	1	1	0	0	1	0	1	1	2	1	2	1	1	2	1	1	1	-	2	1	1	1	2	0	2	0	3	0	1	1	0	1	3
B. elegans JAL2	?	1	0	1	1	1	?	?	?	?	?	?	2	1	?	1	1	2	1	1	1	-	2	?	?	?	?	0	2	0	?	?	?	?	0	1	?
B. elegans JAL3	0	1	0	1	1	1	0	0	0	0	1	1	2	1	1	1	1	2	1	1	1	-	G	0	1	0	1	0	2	0	2	0	1	1	0	1	3
B. elegans JAL4	?	1	0	1	1	1	?	?	1	?	?	?	2	1	1	1	1	2	1	1	1	-	G	1	1	1	2	0	2	0	2	0	1	1	0	1	3
B. elegans MEX1	1	1	0	1	1	1	A	0	A	0	1	1	2	1	2	1	1	2	1	1	1	-	G	1	1	1	2	0	2	0	2	0	1	1	0	1	3
B. tuitensis JAL5	D	1	0	1	1	1	0	0	0	0	1	1	2	1	2	1	1	2	1	1	1	-	3	0	1	0	0	0	1	0	0	-	-	-	0	1	0
Dandya balsensis MOR	A	0	1	0	0	0	2	1	0	0	1	?	2	1	1	1	1	3	0	1	0	-	0	0	1	0	0	1	0	0	0	-	-	-	0	1	0
D. thadhowardii GRO	D	0	1	0	0	0	2	1	0	0	1	?	2	1	1	1	1	3	0	1	0	-	0	0	1	0	0	1	0	0	0	-	-	-	0	0	0
Jaimehintonia gypsophila NL	A	1	0	1	1	1	D	1	1	1	F	F	2	0	A	1	0	1	2	1	0	2	5	0	0	0	0	1	3	1	4	-	-	-	0	1	4
Milla biflora JAL	1	1	0	1	1	1	0	1	0	1	1	1	0	0	0	1	0	1	2	1	1	1	0	0	1	0	0	1	3	1	0	-	-	-	1	0	0
M. bryani COAH	1	1	0	1	1	1	D	0	0	1	D	D	1	0	0	1	0	1	2	1	1	1	0	0	1	0	0	1	3	1	0	-	-	-	1	0	0
M. magnifica GRO	1	1	0	1	1	1	0	0	0	1	0	0	1	0	1	0	0	1	2	1	1	1	0	0	1	0	0	1	3	1	0	-	-	-	1	0	0
M. mexicana PUE	?	1	0	1	1	1	0	0	0	1	3	1	1	0	0	0	0	1	2	1	1	1	0	0	1	0	0	1	3	1	0	-	-	-	1	0	0
Milla sp. QRO	1	1	0	1	1	1	2	0	0	1	0	0	1	0	0	0	0	1	2	1	1	1	0	0	1	0	0	1	3	1	0	-	-	-	1	0	0
Petronymphe decora UC	2	0	1	0	0	0	2	0	0	1	0	?	2	1	1	1	0	0	2	1	0	1	1	0	1	1	0	1	3	1	1	-	-	-	0	1	1

Materials and methods

Taxon sampling and outgroup selection. Populations were considered as terminals in the analyses. The ingroup was comprised of a total of 14 populations: nine populations of *Bessera elegans*, the single known population of *B. tuitensis* and four populations of *Behria tenuiflora* (table 1; figures 1, 2). As outgroups, a single individual each of *Dandya thadhowardii*, *D. balsensis*, *Jaimehintonia gypsophila*, *Milla biflora*, *M. bryani*, *M. magnifica*, *M. mexicana*, *Milla* sp., and *Petronymphe decora* were used, as suggested in the procedure of Wiens and Penkrot (2002). The total number of individuals was 76 (table 1). *Petronymphe decora*, the most distantly related taxon according to previous phylogenetic studies, was used as the functional outgroup (Pires and Sytsma, 2002). Fresh leaves were collected from each individual and dried in silica and the vouchers were deposited at XAL and HCIB.

Morphological data set. Morphological characters were scored from living material and herbarium specimens and, complemented with information from the literature where necessary (Moore, 1951, 1953; Lenz, 1971b; López-Ferrari and Espejo-Serna, 1992). Vouchers and specimens are listed in Appendix 1. The morphological matrix included 37 characters (12 vegetative and 25 floral). Morphological characters were scored as polymorphic if they varied within populations. Characters and character states are listed in Appendix 2; the data matrix is shown in Appendix 3.

Molecular data set. Total genomic DNA was isolated from silica-gel-dried leaf tissue using the modified 2× CTAB method (Cota-Sánchez *et al*., 2006). The intergenic spacer between chloroplast genes *psbK* and *psbI* was amplified and sequenced using primers and protocols of Lahaye *et al*. (2008). All amplified products and total DNA were purified using the QIAquick PCR purification kit (Qiagen, California, U.S.A.) following the protocols provided by the manufacturer. Clean products were sequenced using the *Taq* BigDye Terminator Cycle Sequencing Kit (Perkin Elmer Applied Biosystems, Foster City, California, U.S.A.) and analyzed with an ABI 310 automated DNA sequencer (Perkin Elmer Applied Biosystems, Foster City, U.S.A.). Electropherograms were edited and sequences were assembled using Sequencher 4.1 (GeneCodes, Ann Arbor, Michigan). Sequences were manually aligned with Se-Al v. 2.0a11 (Rambaut, 2002). All sequences were deposited in GenBank (accession numbers are included in Appendix 1).

Tree-based analyses. Data matrices were constructed on WinClada (Nixon 2002). Three sets of phylogenetic analyses were performed with the 23 populations as terminals: 1) A morphological analysis, 2) A molecular analysis, including DNA sequences of *psbK-psbI*, and 3) A combined analysis.

Table 2. Studied populations.

Population	Species	Collector and collector number	No individuals per population	Locality/State	Population abbreviation
1	*Behria tenuiflora*	E. Gándara 2030	5	Todos Santos, BCS	BCS1
2	*B. tenuiflora*	E. Gándara 2031	5	Pichilingue, BCS	BCS2
3	*B. tenuiflora*	J.L. León de la Luz 07031	5	Los Cabos, BCS	BCS3
4	*B. tenuiflora*	J.L. León de la Luz 08130	4	Sierra de la Laguna, BCS	BCS4
5	*Bessera elegans*	E. Gándara 1994	5	Tlaquiltenango, MOR	MOR1
6	*B. elegans*	E. Gándara 1999	5	Taxco, GUE	GRO1
7	*B. elegans*	E. Gándara 2000	5	Tetipac, GUE	GRO2
8	*B. elegans*	E. Gándara 2003	5	Campo Cuatro, COL	COL1
9	*B. elegans*	E. Gándara 2007	3	Mascota, JAL	JAL1
10	*B. elegans*	E. Gándara 2009	5	La Venta, JAL	JAL2
11	*B. elegans*	E. Gándara 2024	5	Puerto Valalrta, JAL	JAL3
12	*B. elegans*	E. Gándara 2025	5	San Sebastián del Oeste, JAL	JAL4
13	*B. elegans*	E. Gándara 2029	5	Tejupilco, EDO MEX	MEX1
14	*B. tuitensis*	E. Gándara 2006	5	El Tuito, JAL	JAL5
15	*Dandya balsensis*	E. Gándara 1993	1	Tlaquiltenango, MOR	MOR
16	*D. thadhowardii*	E. Gándara 1998	1	Iguala, GUE	GRO
17	*Jaimehintonia gypsophila*	E. Gándara 2011	1	Aramberri, NL	NL
18	*Milla biflora*	E. Gándara 2008	1	Mascota, JAL	JAL
19	*M. bryani*	E. Gándara 2017	1	Cuatro Ciénegas, COA	COAH
20	*M. magnifica*	E. Gándara 1996	1	Cacahuamilpa, GUE	GRO
21	*M. mexicana*	E. Gándara 1991	1	Izúcar de Matamoros, PUE	PUE
22	*Milla* sp.	E. Gándara 2017	1	Cadereyta, QUE	QRO
23	*Petronymphe decora*	E. Gándara 2023	1	Cultivated in Berkeley	UC

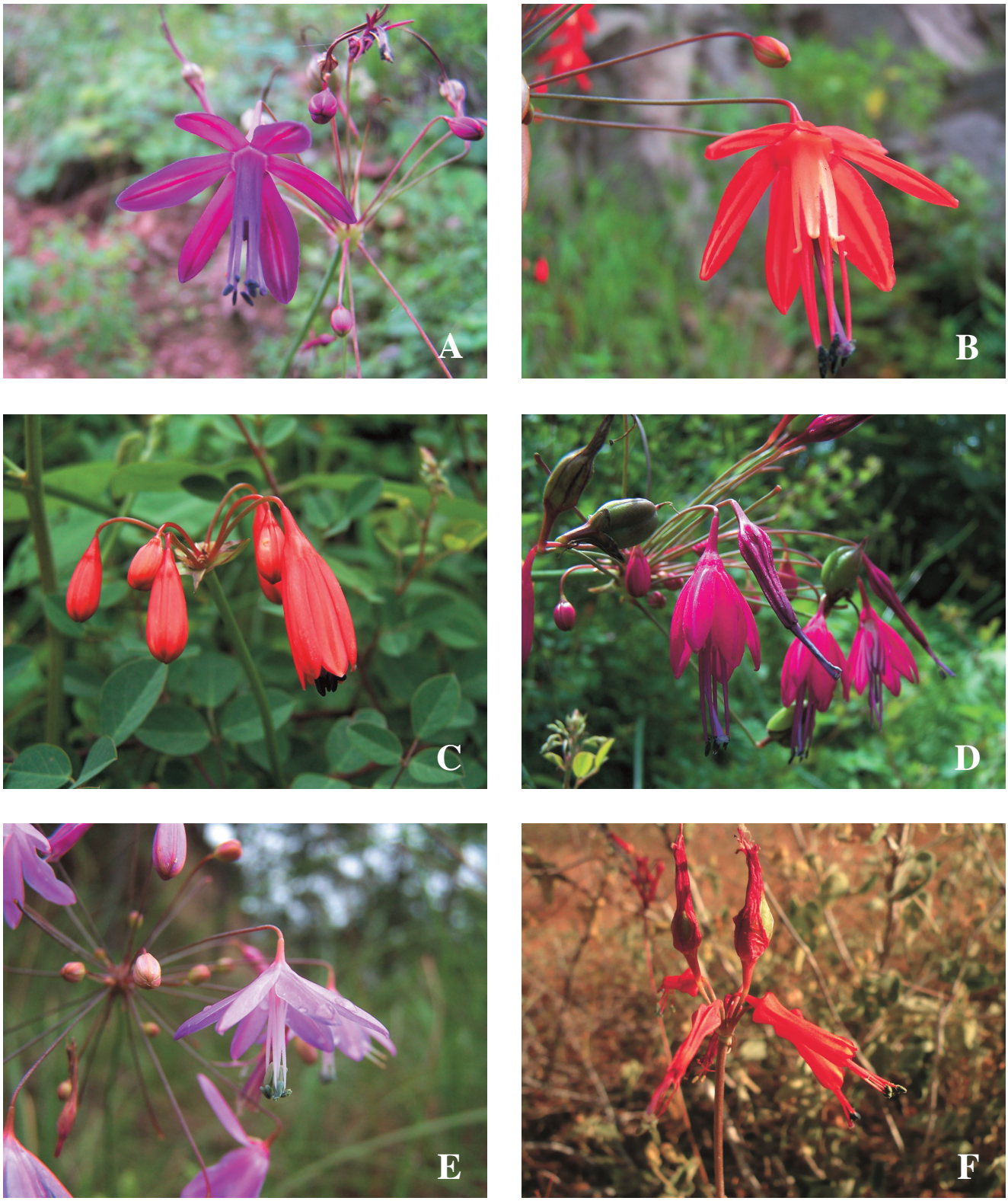

Figure 1. Morphological variation in *Bessera* and *Behria*. A-D: *Bessera elegans*. A: specimen from Campo Cuatro, Colima, *E. Gándara 2003*. B: specimen from the Mascota, Jalisco, *E Gándara 2007*. C-D: specimens from a polymorphic population in Tejupilco, Estado de México, *E. Gándara 2029*. E: *Bessera tuitensis* from El Tuito, Jalisco, *E. Gándara 2006*. F: *Behria tenuiflora* from Todos Santos, Baja California Sur, *E. Gándara 2030*.

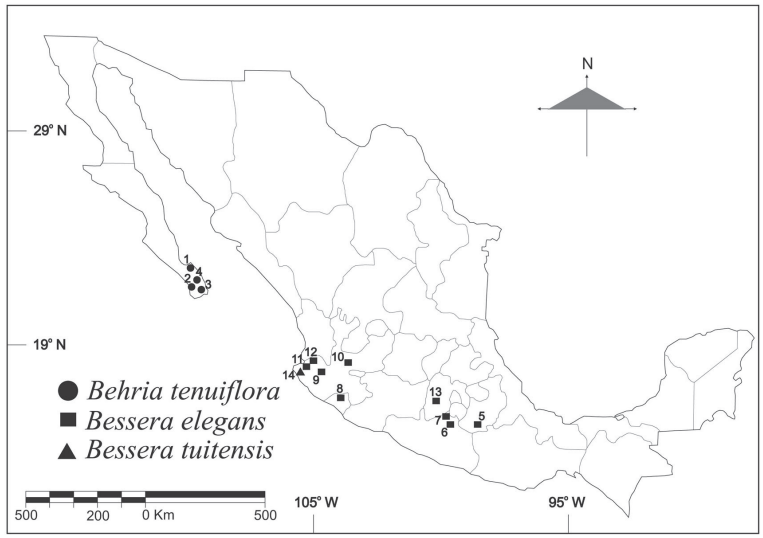

Figure 2. Geographic distribution of studied populations of *Behria tenuiflora*, *Bessera elegans* and *B. tuitensis*. The numbers are the population numbers (table 1).

Parsimony analyses were run in TNT (Goloboff *et al*., 2003) under equal weights; gaps were taken as missing data, with 200 iterations of parsimony ratchet (Nixon, 1999). Clade support was estimated by Jackknife, with 1,000 replicates with 30% deletion on a traditional search in TNT (Goloboff *et al*., 2003). Bremer support (Bremer, 1994) was calculated using the BS5 option on 10,000 trees held in memory in NONA (Goloboff, 1999). The potential incongruence of the

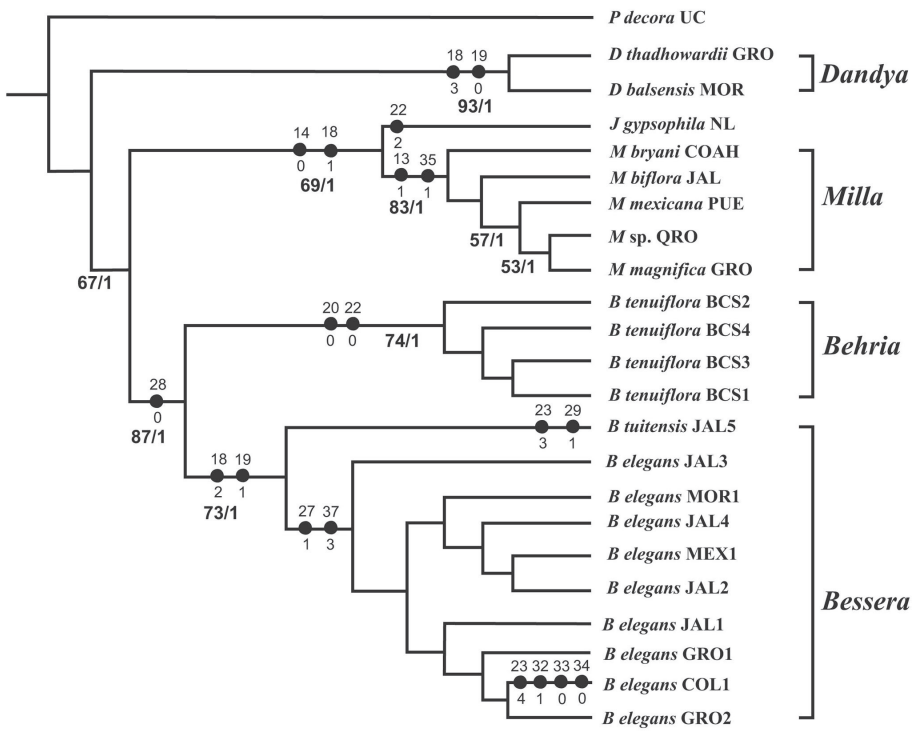

Figure 3. One of five MPT inferred from morphological data (L = 86 steps, CI = 0.69, RI = 0.85). Numbers below branches indicate Jackknife/Bremer support. Character numbers and character states as in Appendix 2. Filled circles are synapomorphic character states. Numbers above filled circles indicates the character. Numbers below filled circles indicates the character states. Letters and numbers after the species name are the population abbreviation, indicated in table 1.

molecular and morphological data sets was tested using the incongruence length difference (ILD) test of Farris *et al*. (1995) as implemented in WinClada (Nixon, 2002).

Character-based analysis. Diagnostic character states that represent seemingly fixed differences between the putative taxa have to be detected in the morphological data matrix. The character-based approach was implemented by comparing the frequencies of qualitative characters and the range of trait values for quantitative continuous and meristic characters across all populations to search for potentially diagnostic characters. Characters were considered diagnostic for a species or a set of populations if they were invariant for alternative character states or showed no overlap in trait values as indicated by Wiens and Penkrot (2002).

Results

Tree based-analyses. Morphological analysis.- Parsimony analysis retrieved five most parsimonious trees (MPT) (L = 86 steps, CI =0. 69, RI = 0.85), and one of them is shown in figure 3. MPT shows that the four populations of *Behria tenuiflora* were retrieved as a monophyletic group with moderate support (Jackknife, jk = 74%, Bremer support, brs = 1) and supported by two synapomorphic character states: a discoid base neck of the perianth tube (character 20) and lobes less than 1/4 of the length of the perianth tube (character 22). The nine *Bessera* populations were also retrieved as a monophyletic group with moderate support (jk = 73%, brs = 1). Two synapomorphic character states supported this group: campanulate flowers (character 18), and perianth tube enclosing part of ovary (character 19). A population of *B. elegans* from Colima showed four autapomorphies: purple tepals (character 23), purple staminal tube of the same color as filaments, and a membrane apex truncated (characters 32-34). *Bessera tuitensis* population showed two autapomorphic character states: pinkish tepals (character 23), and staminal ring 1-1.5 mm length (character 29). The *Behria*/*Bessera* clade received moderate support (jk = 87%, brs = 1) and is marked by a single synapomorphy: filaments longer than tepals (character 28) (figure 4). Consensus tree topology was identical to the combined consensus (figure 5). Molecular analysis.- The *psbK-psbI* data matrix included 494 base pairs (bp) with nine (1.91%) being parsimony informative, and 38 (7.69%) variable. Parsimony analysis retrieved a single MPT displayed in figure 4 (L = 37 steps, CI = 1, RI = 1), in which only a few groups received support: the four populations of *Behria tenuiflora* (jk = 68 %), the two representative species of *Dandya* (jk = 96 %) and two *Milla* species: *Milla* sp. and *M. bryani* (jk = 67 %).

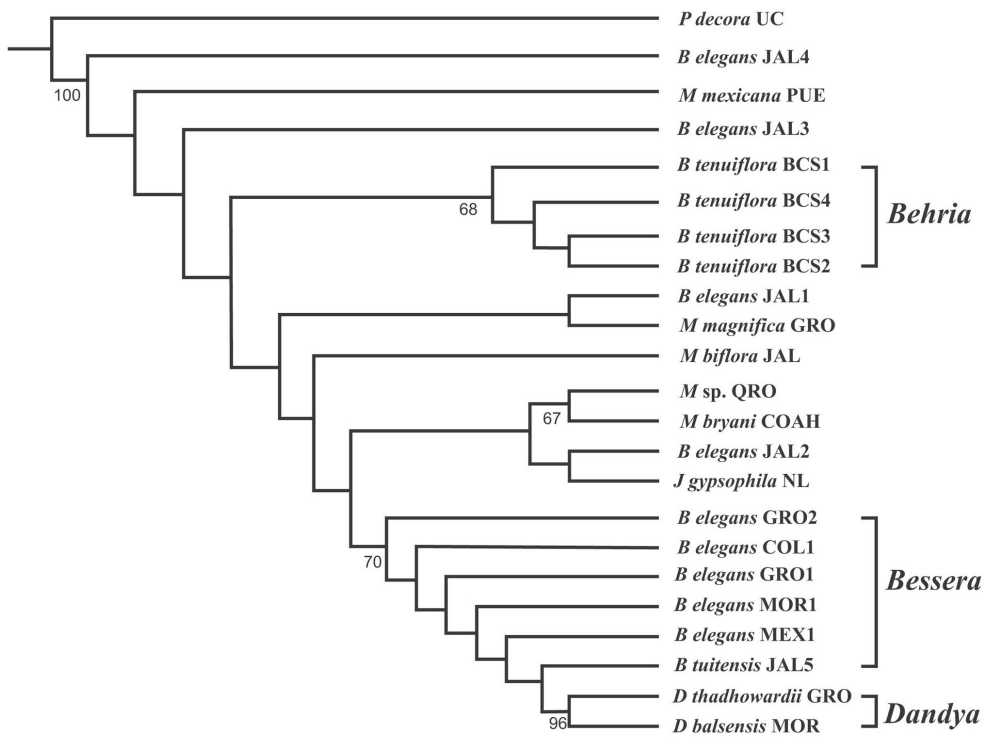

Figure 4. Single MPT inferred from molecular data (L = 37 steps, CI = 1, RI = 1). Numbers below branches indicate Jackknife support. Letters and numbers after the species name are the population abbreviation, indicated in table 1.

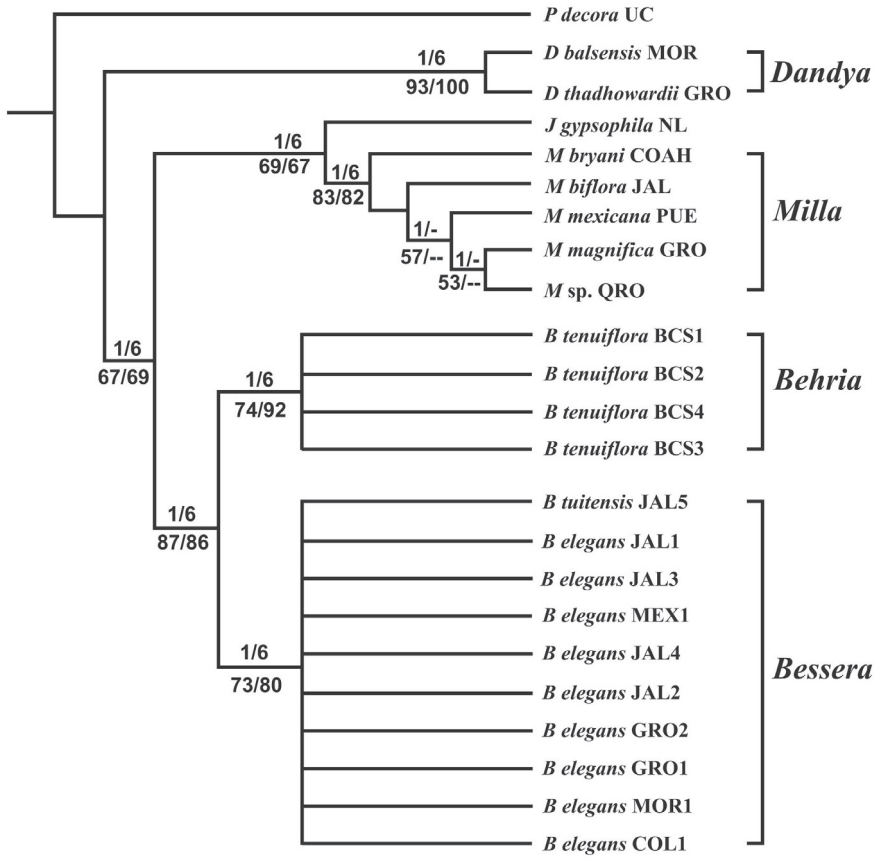

Figure 5. Strict consensus of five MPT inferred from analyses of the morphological (L = 86 steps, CI = 0.69, RI = 0.85) and total evidence data (L = 128 steps, CI = 0.75, RI = 0.84). Both analyses retrieved the same clades. Numbers below branches indicate morphological/total evidence Jackknife support. Numbers above branches indicate morphological/total evidence Bremer support. Letters and numbers after the species name are the population abbreviation, indicated in table 1.

Combined analysis.- The combined analysis of *psbK-psbI* DNA sequences and morphology retrieved five MPT (L = 128 steps, CI = 0.75, RI = 0.84). The strict consensus is shown in figure 5. The same groups were retrieved as in the morphological analysis, though the clades received higher jackknife and Bremer support. The four populations of *Behria* were retrieved as monophyletic with strong support (jk = 92%, brs = 6). *Bessera* populations were also supported (jk = 80%, brs = 6). *Behria* appears as the sister group to *Bessera* and this clade received support (jk = 86%, brs = 6). Likewise there was another supported clade formed by several *Milla* species (jk = 82%, brs = 6) which was sister to *Jaimehintonia* (jk = 67%, brs = 6). Finally, *Dandya* species were in a highly supported clade (jk = 100%, brs = 6).

Character based analysis. The morphological character-based approach supports the recognition of two different related taxa. *Bessera* can be distinguished from *Behria* by five diagnostic characters: 1) polytepalous flowers (vs gamotepalous) (character 17); 2) base neck of the perianth tube elongated (vs discoid) (character 20); 3) three lobes of flower different in shape and size (vs all equal shape) (character 21); 4) stamen filaments connate and 5) forming a membranous tube 10-25 mm length (vs filament base connate and forming a membranous cup to 0.6 mm length) (character 29). In addition *Behria* has tubular perianth (character 18); 3) which totally encloses the ovary (character 19).

Discussion

Tree based analyses. Our results show that the single MPT retrieved from the molecular analysis is mostly unresolved. We utilized the chloroplast intergenic spacer *psbK-psbI* because it is a chloroplast region that was included as a candidate locus for DNA barcoding for its evolutionary rates and has a high ability to differentiate independently evolving entities corresponding to taxonomic species (Pennisi, 2007; Lahaye *et al*., 2008). However it was not variable enough to allow resolution in our MPT. Further loci must be added to improve resolution. Chloroplast DNA loci such as *trnL-F, rpl16* and *ndhF* have been sequenced in Themidaceae but they did not have enough variation to resolve the

relationships within this family (Pires and Sytsma, 2002). A large number of markers are known to be variable in angiosperms, like *trnS-trnG*, *rpl32-trnL* and *trnQ-rps16* proposed by Shaw *et al*. (2005; 2007), and these could be tested for resolving relationships within Behria and Bessera, and in even in the *Milla* clade.

In contrast, the combined and morphological analyses recovered the same clades, but the combined analysis received higher Jackknife and Bremer support. Morphological and combined phylogenetic analyses identified the four populations of *Behria* as a monophyletic group. The nine populations of *Bessera elegans* and the single population of *B. tuitensis* were also identified by these analyses as a monophyletic group. These two clades form part of a more inclusive group. Although populations of *Bessera* and *Behria* were recovered by the morphological and combined analyses as sister groups, this grouping is supported by a single synapomorphic character state: filaments longer than tepals.

Character based analysis. Five diagnostic characters were detected in the character analysis and all of them are floral attributes. Vegetative morphology is similar in *Behria* and *Bessera* and also in the genera of the entire family. *Bessera* species have campanulate flowers, with a perianth tube partially enclosing the ovary. In contrast *Behria* has tubular flowers and the perianth tube completely encloses the ovary. Thus, the floral characters pointed out by previous authors (Schultes, 1829; Greene 1886; Moore, 1953; Lenz 1971b; León de la Luz and Pérez-Navarro, 2004) for differentiating *Bessera* from *Behria* (campanulate flowers vs. tubular flowers) were detected by us as diagnostic.

Based on the tree-morphology and on the character-based results we conclude that *Behria* and *Bessera* should be recognized as separate genera. Populations of these taxa were retrieved as monophyletic groups, forming part of a more inclusive clade. There is still controversy about "how to chop up a tree" for assigning taxonomic rank and it has been suggested that recognition of the non-monophyletic and nomenclaturally redundant monotypic genera be avoided (Brumitt, 2002). Changes in generic delimitation practice have been influenced by new sources of data, such as molecular characters. Technological advances that have allowed widespread incorporation of molecular data into taxonomic studies have also facilitated a return to a global approach to the study of plant genera (Humphreys and Linder, 2009). Decisions on the identification of independent genera which are sister taxa differ depending on the plant group and on the author. For example, Tang and Lu (2005) decided to recognize *Zabelia* (Caprifoliaceae) as independent from *Abelia*. Although molecular analyses found these taxa as sisters they based their decision on morphological and ecological evidence. Devos *et al*. (2006) decided that the monotypic genus *Coeloglossum* (Orchidaceae) was separate from *Dactylorhiza*, even though these genera were retrieved as sister groups of a more inclusive clade. In another example Specht and Stevenson (2006), based on morphological and molecular evidence, considered *Monocostus* (Costaceae) as a genus independent of *Dimerocostus*, though they were retrieved as sister groups.

We also based our decision to recognize *Behria* and *Bessera* as separate genera on differences in their distribution ranges. As mentioned above, *Behria* is restricted to the Baja California Peninsula in the southern area of La Paz-Los Cabos while *Bessera* occurs on continental Mexico on the Pacific slopes and along the Transmexican Volcanic Belt. (Moore, 1953; Ramírez-Delgadillo, 1992; León de la Luz and Pérez-Navarro, 2004). The species delimitation method of Wiens and Penkrot (2002) favors recognizing taxa as independent if their populations are geographically separated. Moreover, Martínez-Gutiérrez and Sethi (1997) indicated that the La Paz-Los Cabos morphotectonic subprovince (Ferrusquía-Villafranca, 1993) was connected to the Pacific slope, in Jalisco, during the lower Miocene, between 16-13 my before present. An interesting hypothesis that could be addressed in the future would include dating the divergence of *Behria* and *Bessera* to determine if it coincides with the separation of these two areas. Both are part of North America, but the Baja California Peninsula is rifting with the Pacific plate.

In Amaryllidaceae (Meerow *et al*., 1999) and in Iridaceae (Taylor *et al*., 2009), two groups of monocots that are closely related to Themidaceae, high floral diversity has been attributed to floral modification induced by pollinator-mediated selection, driven by changes in one or only a few genes. It has been documented that *Behria* is pollinated by hummingbirds (Arriaga *et al*., 1990). The pollination vectors for *Bessera* are unknown, though we did observe hummingbirds, bees, bumble bees and butterflies visiting flowers in the populations where we collected plants of this genus. It is important to determine if pollination systems have played a role in floral diversification in this group as it has in other monocot groups.

Behria, *Bessera* and the rest of the *Milla* clade represent an interesting group for evolutionary biology studies, such as determining time of divergence and factors influencing speciation, such as shifts in soil type and pollinators. The majority of the taxa in the *Milla* clade are restricted to a single or to a few localities, and each taxon grows on a different kind of soil, and is also pollinated by different vectors.

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Appendix 1. Vouchers and specimens studied.

***Behria tenuiflora* Greene:**
Baja California Sur: municipio de Los Cabos, camino a Los Naranjos, *J.L. León de la Luz 07030* (HCIB; GenBank GU142856); municipio de La Paz, 2 km después de Todos Santos, carretera Todos Santos-Los Cabos, *E. Gándara 2030* (XAL; GenBank GU142856); Sierra de La Laguna, cerca del Valle 2, La Cieneguita, *J.L. León de La Luz 08130* (HCIB; GenBank GU142856); Bahía de Pichilingue, *E. Gándara 2031* (XAL; GenBank GU142856).

***Bessera elegans* Shult.:**
Colima: municipio de Comala, 7.8 km después de Campo Cuatro, camino de Campo Cuatro hacia el crucero de Minatitlán, *E. Gándara 2003* (XAL; GenBank GU142858). **Guerrero:** municipio de Tetipac, 3 km después de Tetipac, brecha desde Tetipac hacia Almoloya de Alquisiras, por el camino hacia El Ranchito, *E. Gándara 2000* (XAL; GenBank GU142858); municipio de Taxco, camino Taxco-Teotipac, aprox. 2 km después de Taxco, *E. Gándara 1999* (XAL; 5 GenBank GU142858. **Jalisco:** municipio de Mascota, carretera Mascota-Ameca, entre los kilómetros 111-112, *E. Gándara 2007* (XAL; GenBank GU142853); municipio de San Sebastián del Oeste, carretera Mascota Puerto-Vallarta, 2 km después del puente Progreso, desde Mascota *E. Gándara 2025* (XAL; GenBank GU142853); municipio de Puerto Vallarta, Ejido Santa Cruz de Quelitán, La Laguna Seca, aprox. 3 km al NW de Santa Cruz, brecha hacia El Vertedero, después de la Puerta de La Pedrera, *E. Gándara 2024* (XAL; GenBank GU142853); municipio de Zapopan, Pinar de la Venta, *E. Gándara 2009* (XAL; GenBank GU142853). **Estado de México:** municipio de Tejupilco, km 94 Toluca-Tejupilco sobre el km 134 de la carretera, 2.6 km después de la desviación de Almoloya de Alquisiras a Tejupilco, *E. Gándara 2029* (XAL; GenBank GU142858). **Morelos:** municipio de Tlaquiltenango, km 4.3 sobre el camino Valle de Vazquéz-Chimalacatlán, *E. Gándara 1994* (XAL; GenBank GU142858).

***Bessera tuitensis* R. Delgad.:**
Jalisco: municipio de Cabo Corrientes, El Tuito, 5 km después de El Tuito hacia Puerto Vallarta, sobre la desviación a la Mina Zimapán, km 175-177, *E. Gándara 2006* (XAL; GenBank GU142857).

***Dandya balsensis* López-Ferr. and Espejo:**
Morelos: municipio de Tlaquiltenango, km 3.5 sobre el camino Valle de Vazquéz-Chimalacatlán. *E. Gándara 1993* (XAL; GenBank GU142859).

***Dandya thadhowardii* Lenz:**
Guerrero: municipio de Iguala, carretera federal 95 Iguala-Chilpancingo, 42.8 km después de Iguala, sobre el km 168.5 hacia Chilpancingo *E. Gándara 1998* (XAL; GenBank GU142860).

***Jaimehintonia gypsophila* Turner:**
Nuevo León: municipio de Aramberri, 1 km NW de la desviación, sobre el km 7.5 km de la carretera, hacia Arambrerri de La escondida, aprox. a 500 m de la Mina de Cuarzo Hierba Anís, Los Cerritos Blancos, *E. Gándara 2011* (XAL; GenBank GU142852).

***Milla biflora* Cav.:**
Jalisco: municipio de Mascota, carretera Mascota-Ameca, entre los kilómetros 107-108, *E. Gándara 2008* (XAL; GenBank GU142849).

***Milla bryani* I.M. Johnst.:**
Coahuila: municipio de Cuatro Ciénegas de Carranza, km 24 sobre la brecha Ocampo-La Esmeralda, hacia La Esmeralda, SW Ocampo, Cuesta Los Güeros, Cañón de Palos Blancos, ladera oeste de La Sierra de la Madera, *E. Gándara 2017* (XAL; GenBank GU142854).

***Milla magnifica* H.E. Moore:**
Guerrero: municipio de Taxco, 2 km después de las grutas de Cacahuamilpa, hacia Taxco, carretera grutas de Cacahuamilpa-Taxco, *E. Gándara 1996* (XAL; GenBank GU142850).

***Milla mexicana* T.M. Howard:**
Puebla: carretera Izúcar de Matamoros-Coatzintla, entre los kilómetros 37-38, 25 km después de San Juan de Epantla, *E. Gándara 1991*(XAL; GenBank GU142851).

***Milla* sp.:**
Querétaro: municipio de Cadereyta, 1 km después de la desviación Querétaro-San Juan del Río desde Pinal de Amoles, entre los kilómetros 80-70 de la carretera 120, *E. Gándara 2027* (XAL; GenBank GU142852).

***Petronymphe decora* Moore:**
Material obtenido a partir de especímenes cultivados en "Berkeley Botanical Garden, University of California", U.S.A., *E. Gándara 2023* (XAL; GenBank GU142848).

Appendix 2. Morphological characters and character states utilized in phylogenetic analyses.

Corm:
1. Corm diameter: (0) 0.5-10 mm; (1) 11-20; (2) 21-40 mm.

Leaves:
2. Leaf shape: (0) flattened; (1) terete.
3. Leaf shape in cross-section: (0) circular; (1) triangular.
4. Leaf in cross section: (0) solid; (1) hollow.
5. Leaf abaxial surface: (0) keeled; (1) not keeled.
6. Leaf adaxial surface: (0) channeled; (1) not channeled.
7. Leaf number: (0) 1-2; (1) 3-4; (2) 5 or more.
8. Leaf color (adaxial and abaxial surface): (0) green; (1) purple at base.

Scapose inflorescence:
9. Number: (0); solitary; (1) two or more.
10. Length: (0) longer than leaves; (1) shorter than leaves.
11. Color: (0) green; (1) purple at base; (2) purple at least to middle; (3) purple up to involucres.
12. Pubescence: (0) glabrous; (1) scabrous at the base; (2) scabrous up to middle; (3) scabrous up to involucre.

Inflorescence:
13. Pedicel length: (0) absent; (1) smaller than perianth tube; (2) longer than perianth tube.
14. Flower orientation: (0) erect-spreading; (1) pendant.
15. Number of flowers per inflorescence: (0) 1-4; (1) 5-15; (2) 20 or more.
16. Flowers: (0) nocturnal; (1) diurnal.

Perianth:
17. Type: (0) gamotepalous; (1) polytepalous.
18. Shape: (0) tubular; (1) salverform; (2) campanulate; (3) subcampanulate.
19. Tube length: (0) less than 1 mm; (1) enclosing part of ovary; (2) totally enclosing ovary.
20. Base neck of tube: (0) discoid; (1) not discoid.
21. Lobes: (0) six, equal shape: (1) three, different shape.
22. Lobe length, relative to perianth tube: (0) less than 1/4; (1) 1/4 to 1/2; (2) more than 1/2.
23. Tepal general color: (0) white; (1) yellow; (2) reddish to orange; (3) pinkish; (4) purple; (5) bluish.
24. Tepal color: (0) same color on abaxial and adaxial surface; (1) different color.
25. Tepal midvein: abaxial surface: (0) concolorous; (1) discolorous.
26. Tepal midvein: adaxial surface: (0) concolorous; (1) discolorous.
27. Tepal pattern color, adaxial surface: (0) uniform color; (1) with two stripes of different color, respect to surface, around the midvein; (2) midvein and edge concolorous with the abaxial surface.

Stamens:
28. Filaments: (0) longer than tepals; (1) shorter than tepals.
29. Filament base: (0) connate and forming a membranous cup up to 0.5 mm length; (1) connate and forming a membranous ring 1-9 mm length; (2) connate and forming a membranous tube 10-25 mm length; (3) free.
30. Filament attachment: (0) lower part of perianth; (1) mid-upper part of perianth.
31. Filament color: (0) white; (1) yellow; (2) red (3) purple; (3) bluish.
32. Staminal tube color: (0) white; (1) purple.
33. Staminal tube color: (0) same color as filaments; (1) different color.
34. Staminal tube membrane: (0) truncated; (1) acute or winged.
35. Anthers: (0) dorsifixed; (1) basifixed.
36. Anthers: (0) connate; (1) free.

Gynoecium:
37. Style color: (0) white; (1) yellow; (2) red; (3) purple; (4) bluish.