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A new species and palinotaxonomy of *Besleria* (Gesneriaceae) from the cloud forest of Valle del Cauca, Colombia

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Abstract

We describe and illustrate a new species of *Besleria* (Gesneriaceae) from the department of Valle del Cauca, Colombia. This new species is morphologically similar to *B. florida, B. microphylla*, *B. silverstoneana,* and *B. solanoides*. We include a morphological key to distinguish *B. brevisepala* from similar species. We also studied pollen morphology to provide support for taxonomic differences among *Besleria* species from the Andean forest of Colombia. We investigated the pollen characteristics of the genus and assessed their use for taxonomic differentiation in this group through light and electron microscopy. Pollen grains were subjected to acetolysis, measured, photographed, and described qualitatively. We analyzed qualitative and quantitative data using descriptive and multivariate statistics. The pollen grains of *Besleria* were monad, isopolar, small, with circular amb; oblate to suboblate, 3-porate, with circular to oval pores, without annulus, operculate, and with vestibulum. The exine was tectate, perforate, and microreticulate with narrow muri and small lumina of slightly different shape and dimension. Our results confirm that *Besleria* is a eurypalynous genus, which allowed the identification of pollen morphological characteristics that are important for species differentiation.

Introduction

Besleria Linnaeus (1753: 619) is a neotropical genus of Gesneriaceae, distributed from southern Mexico to Bolivia and Brazil (Skog 1979, 1996, Kvist *et al.* 1998). In Colombia, *Besleria* is found in the humid forest of the Andean and Sub Andean regions, and the Chocó biogeographic region; a few species are also found in the Amazonian region (Cortés 2013, 2019).

The genus *Besleria* has more than 170 described species and is one of the most diverse in the family Gesneriaceae (Wiehler 1975, 1983, Kvist *et al*. 1998, Weber 2004, Cortés 2019). Colombia has the highest diversity of *Besleria* worldwide, with over 50% of the species recorded for the genus (Cortés 2013, 2019). In addition, new species of *Besleria* have been recently discovered in the cloud forest of Colombia (Cortés *et al.* 2017).

Several *Besleria* species are very similar morphologically; this is the case for *B. brevisepala* (species described in this article), *B. florida* Morton (1944: 23), *B. microphylla* Fritsch (1922: 8), *B. silverstoneana* Cortés (2017: 185), and *B. solanoides* Kunth (1818: 398). Given this morphological similarity, pollen grains could provide valuable information to support taxonomic differences among *Besleria* species. The shape and structure of pollen grains is an important diagnostic characteristic in Gesneriaceae and can help delimit the genera taxonomically (Fritze & William 1988).

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Some Gesneriaceae genera are stenopalynous, *i.e.* with a constant pollen type (especially as to the type of aperture and ornamentation), but many genera are eurypalynous or of variable morphology (Williams 1978, Luegmayr 1993a, 1993b). This allows infrageneric differentiations; qualitative and quantitative pollen data help in the delimitation of species (Souza *et al.* 2018). Therefore, pollen is a significantly informative characteristic in Gesneriaceae taxonomy.

There have been few studies on the pollen morphology of *Besleria* species (*i.e.* Roubik & Moreno 1991, Fourny *et al.* 2010, Gasparino *et al*. 2011, Cortés *et al*. 2017); considering the eurypalynous character of *Besleria,* differences in pollen morphology have been detected, mainly in the ornamentation of the exine, as well as in the shape, size, and characteristics of the type and size of the apertures. These studies have therefore contributed to differentiate very similar species, such as *B. longimucronata* Hoehne (1958: 41)*, B. selloana* Klotzsch & Hanstein (1864: 398), and *B. umbrosa* Martius (1829: 44). These species can be distinguished only by the characteristics of the inflorescence, which is pedunculate to subsessile in *B. longimucronata*; it has 6–12 flowers in *B. selloana* (Chautems & Kiyama 2003), and 2–3 flowers in *B. umbrosa*. In addition to morphological characteristics, the exine ornamentation of pollen grains can separate clearly the species: *B. longimucronata* has a perforate-psilate exine, *B. selloana* has a microreticulate exine, and *B. umbrosa* has a rugulate exine (Gasparino *et al*. 2011).

Based on collected specimens, field observation, morphological studies of herbarium specimen, and pollen morphology, we aim to describe a new species of *Besleria* and differentiate it from species morphologically very similar.

Materials and methods

Plant material:—Specimens were collected in the district of San Bernardo, in the municipality of Cali, Colombia (Fig. 1). This area is classified as tropical montane cloud forest, according to Morales & Armenteras (2013); the landscape was fragmented and included a secondary forest.

Morphological descriptions of new species were based on dried exsiccata housed in the CUVC herbarium, Universidad del Valle, Cali, Colombia, and on flowers conserved in 70% ethanol. A Nikon SMZ 1500 dissecting stereomicroscope was used for observations. Photographs of the flowers were obtained with a Nikon DS-Ri1 U3 Digital Camera Controller at the Universidad del Valle.

The morphological key to identify *Besleria brevisepala*, *B. florida, B. microphylla*, *B. silverstoneana*, and *B. solanoides* was based on dried material from 40 exsiccata obtained from the CAUP, COL, CUVC and HUA herbaria (Supplementary Material 1). We used tag data to create a distribution map using Esri's ArcGIS® 10.3 software (ArcGIS 2020).

The pollen grains of five *Besleria* species native to Colombia were studied: *B. brevisepala*, *B. florida*, *B. microphylla*, *B. silverstoneana,* and *B. solanoides*. Pollen grains were obtained from dried herbarium exsiccata supplied by the COL, CUVC and HUA herbaria.

Pollen morphology:—We analyzed the pollen grains of 13 specimens (Supplementary Material 2) using light microscopy (LM) and scanning electron microscopy (SEM). Pollen samples were hydrated in a solution of water and 50% glycerin for 24 hours, then acetolyzed according to the method described by Erdtman (1960) and Melhem *et al*. (2003), with the following modification: the pollen material was left in the acetolysis solution for 10 minutes in a water bath at 60–65°C. Pollen diameters were measured (n=25) under LM within seven days of sample preparation (Salgado-Labouriau *et al*. 1965). Other measurements (polar area, sexine, nexine thickness, and apertures) were taken from 10 pollen grains per specimen. Non-acetolyzed pollen grains were used for SEM analysis; they were hydrated in a 50% glycerin and water solution for 24 hours, washed twice in acetic acid for 3 minutes, rinsed twice with distilled water, and dehydrated in a series of alcohol concentrations (30%, 50%, 70%, and 90%) for 3 minutes. Specimens were then assembled on carbon tape and allowed to dry in a sealed Petri dish containing silica gel for 2 to 3 days.

Statistical analyses were conducted to obtain the mean (χ), standard deviation (S_x), standard error (S), 95% confidence intervals (CI), and coefficient of variability (V) (Zar 1999) using the program R (R Core Team 2017). A principal component analysis (PCA) and a cluster analysis with Euclidean distances and complete linkage were performed to verify whether the quantitative pollen data permitted the grouping of the species included here (PCA analysis) and to establish the relationship among the species (cluster analysis) using the program Minitab (Minitab LLC 2020). For these analyses, we used seven metric variables that are present in all the analyzed species: polar axis in equatorial view (PLEV), equatorial diameter in equatorial view (EDEV), shape (SHAP), exine thickness (EXIN), polar area index (PAI), pore length (PL), and pore width (PW).

The pollen photomicrographs were obtained with differential interference contrast (DIC) using a Nikon Eclipse Ni-U 90 light microscope, a Nikon DS-Ri1 U3 Digital Camera Controller, and a JEOL model JSM-6490 LV scanning electron microscope at the Universidad del Valle. The pollen terminology follows Barth & Melhem (1988) and Punt *et al*. (2007), and pollen descriptions follow Bellonzi *et al.* (2020). We followed Faegri & Iversen (1964) and Salgado-Labouriau (1965) for the polar area index and shape, respectively.

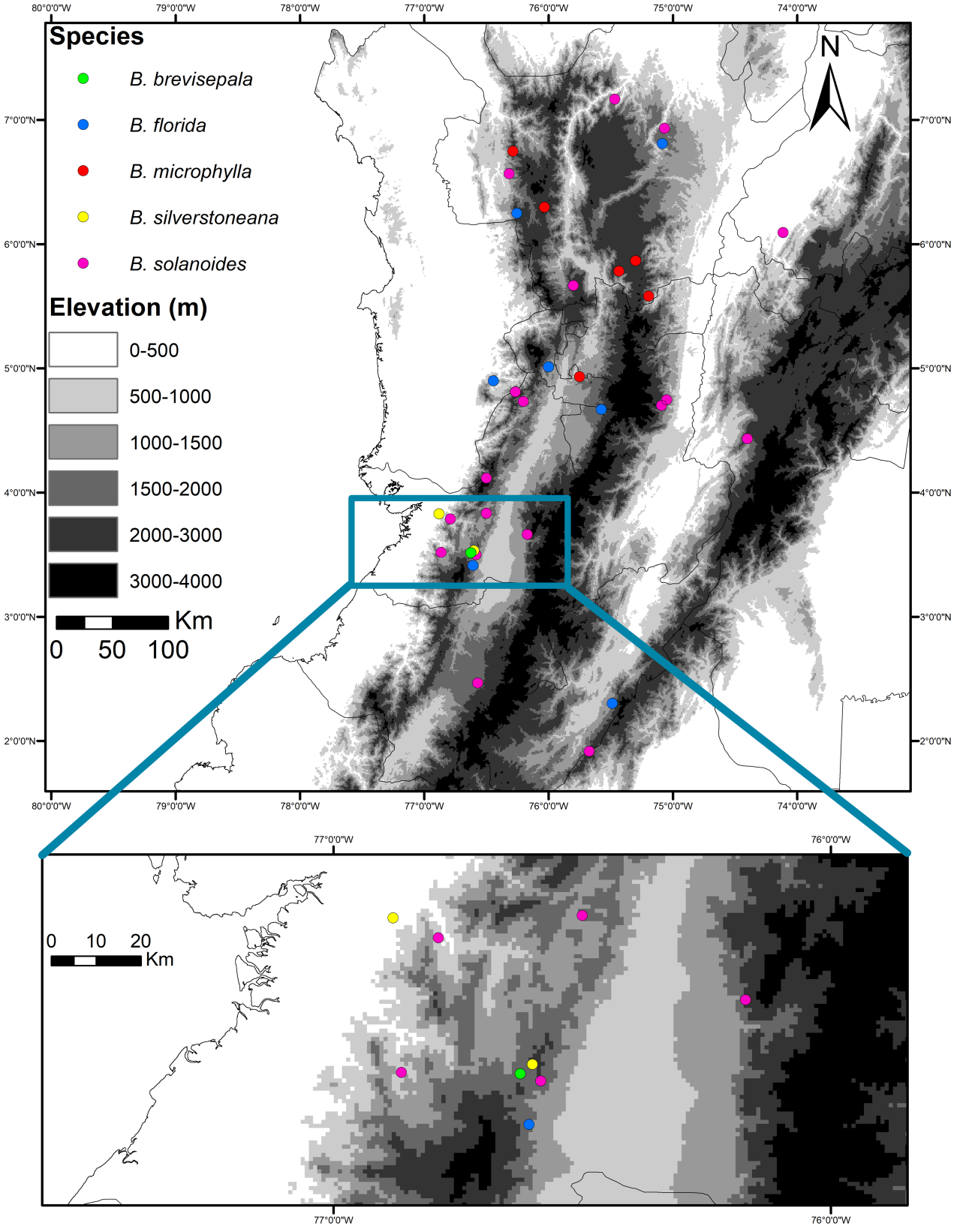

FIGURE 1. Geographical distribution of *B. brevisepala, B. florida, B. microphylla, B. silverstoneana,* and *B. solanoides* in Colombia.

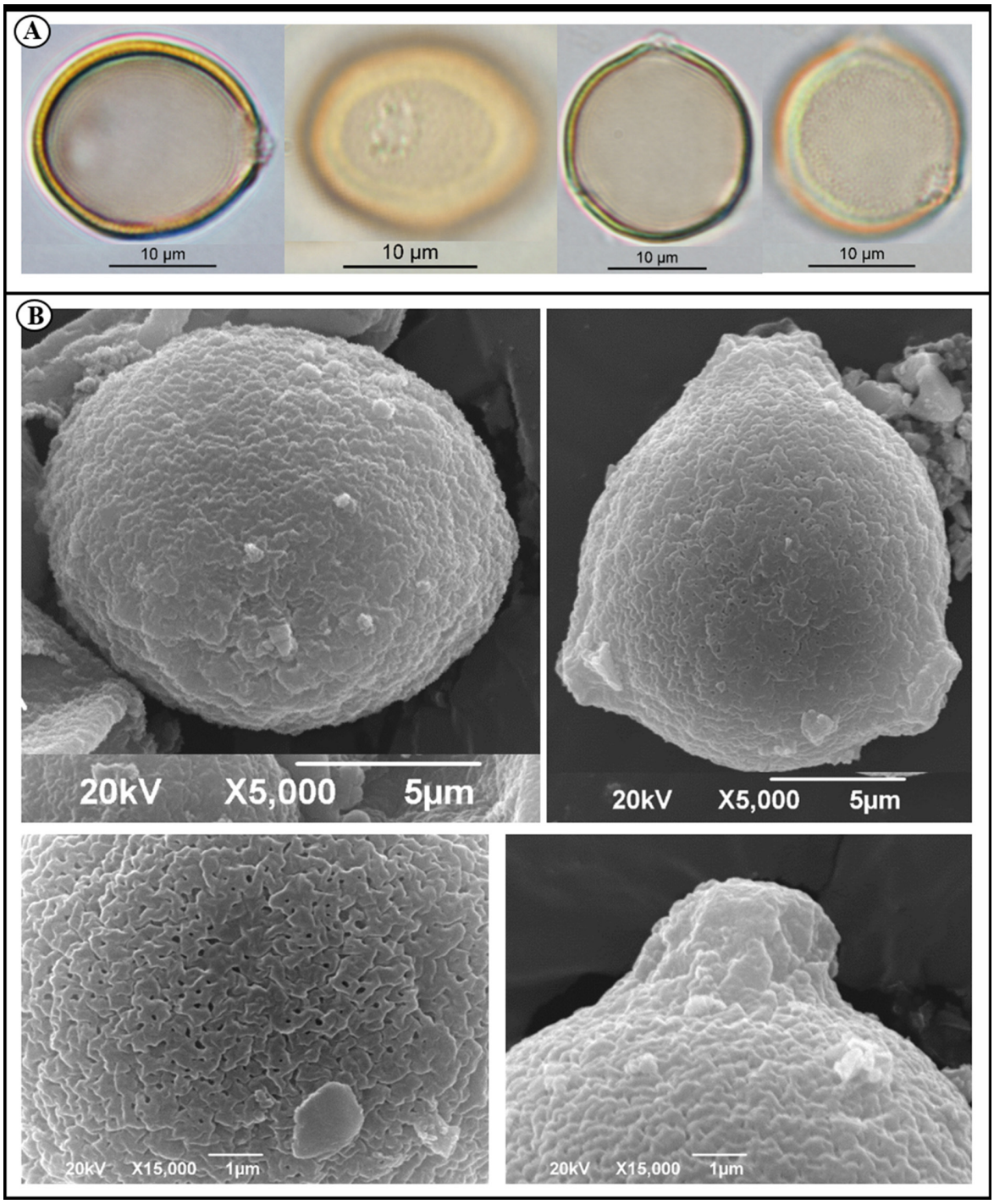

FIGURE 2. Pollen micromorphology. **Plate 1**. *Besleria brevisepala*. A. LM DIC: equatorial and polar middle and surface views (×1000). B. SEM: equatorial and polar views (×5000); surface and operculum details (×15000).

Results

Taxonomic treatment

Besleria brevisepala O.L.Cortés, *sp. nov*., (Fig. 2: Plates 1, 5, 6).

Type:—COLOMBIA. Valle del Cauca: Dagua municipality, district of San Bernardo, road to Buenaventura, western cordillera, eastern slope, 03°30’52.0’’N, 76°37’27.8’’W, 1992 m, 2 March 2016, *O.L. Cortés-Ceballos 258 & A. Giraldo-Rodríguez* (holotype CUVC!, isotypes COL! HUA! MO!).

This species differs from Besleria microphylla in having 1–2-flowers (vs. inflorescence in fascicles in B. microphylla), longer leaf blades (4.5–8.5 cm long vs. 3–4 cm long; although some specimens had leaves up to 6.5 cm long in B. microphylla), an ovate and unequal calyx, the dorsal sepal longer than the other sepals (5–6.5 mm long and 5–5.1 mm wide vs. triangular-lanceolate and equal, 4–5 mm long and 1.5–2 mm wide in B. microphylla), glabrous corolla and slightly larger (12–19 mm long, 6–8 mm in the widest part vs. corolla short pilose to pubescent, 12–15 mm long and 4–5 mm in the widest part in B. microphylla) and the flowers presented a glabrous ovary vs. a puberulous ovary in B. microphylla. It differs from B. solanoides in having 1–2-flowers (vs. inflorescence in fasciculus in B. solanoides), and in the petiole (villous-strigose, obsolete or up to 1 cm long vs. glabrous, 1–5 cm long in B. solanoides), leaf (slightly strigose on adaxial surface, 4.5–8.5 × 1.4–2 cm vs. glabrous on adaxial surface, 4–15 × 5.5–5.6 cm in B. solanoides), calyx (villous-strigose, and unequal, dorsal sepal longer than other sepals, 5–6.5 × 5–5.1 mm vs. glabrous, and equal, 3–5 × 1.5–2.5 mm in B. solanoides), and flowers (pilose style vs. glabrous style in B. solanoides).

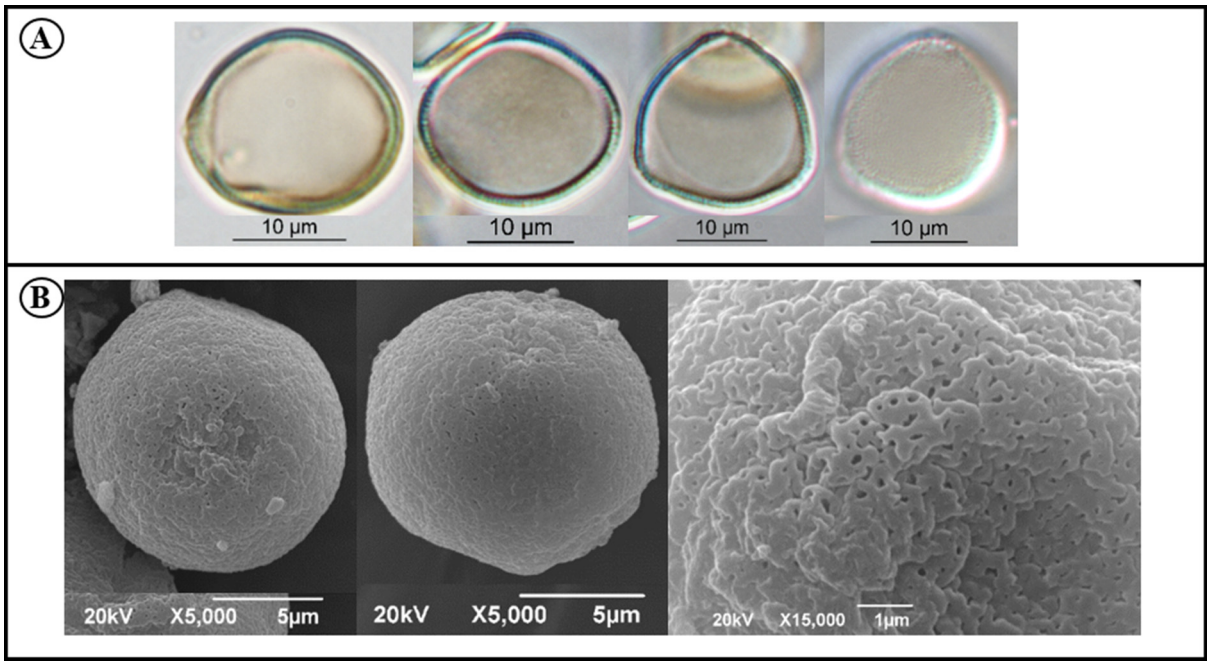

FIGURE 2. Pollen micromorphology. **Plate 2**. *Besleria florida.* A. LM DIC: equatorial and polar middle and surface views (×1000). B. SEM: equatorial and polar views (×5000) and surface detail (×15000).

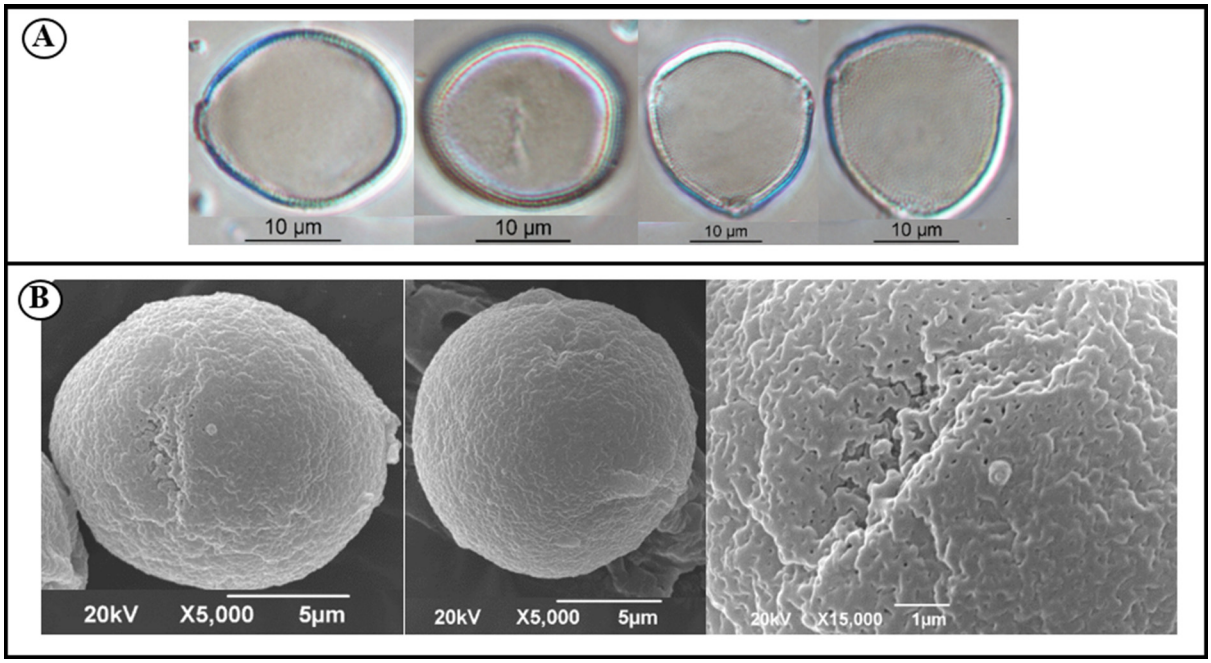

FIGURE 2. Pollen micromorphology. **Plate 3.** *Besleria microphylla.* A. LM DIC:equatorial and polar middle and surface views (×1000). B. SEM: equatorial and polar views (×5000) and surface detail (×15000).

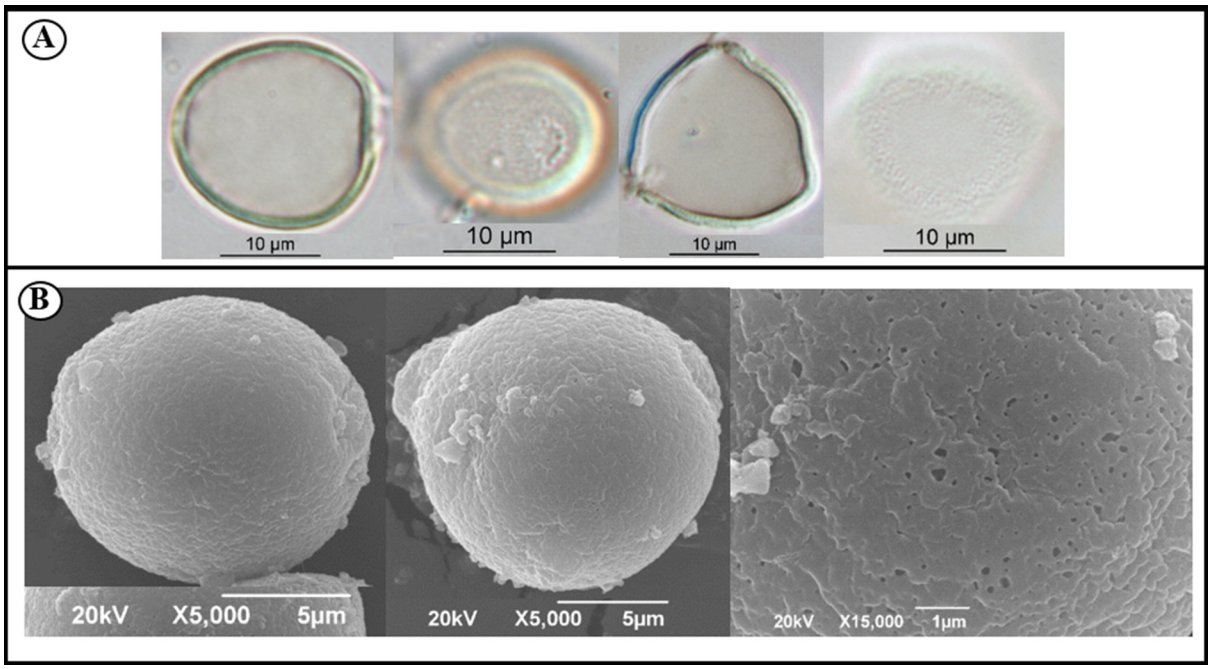

FIGURE 2. Pollen micromorphology. **Plate 4.** *Besleria silverstoneana*. A. LM DIC: equatorial and polar middle and surface views (×1000). B. SEM: equatorial and polar views (×5000) and surface detail (×15000).

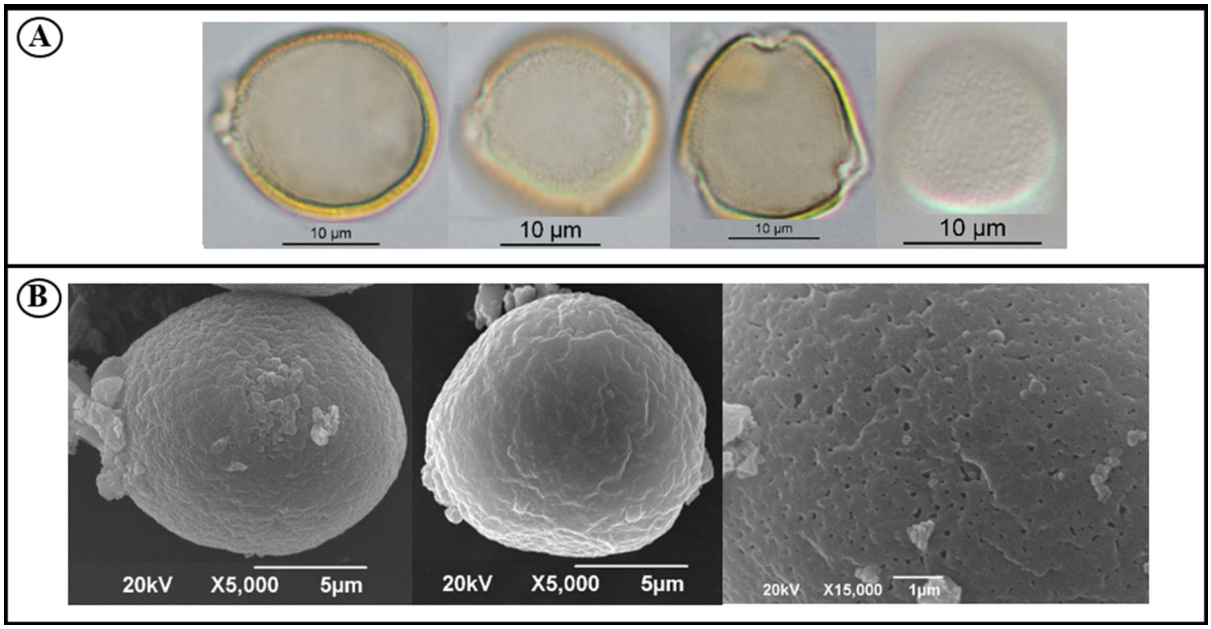

FIGURE 2. Pollen micromorphology. **Plate 5.** *Besleria solanoides.* LM DIC: equatorial and polar middle and surface views (×1000). B. SEM: equatorial and polar views (×5000) and surface detail (×15000).

Description:—Terrestrial shrub; stem erect, 1.5–2.5 m tall, terete, villous, pubescent toward apex; branches arising from base or 10 cm above base, 0.5–3 cm diameter. Leaves opposite, decussate, members of each pair almost equal in length; petiole villous, obsolete or up to 1 cm long; blades elliptic to oblanceolate, 4.5–8.5 × 1.4–2 cm, apex acuminate, base cuneate, margin entire, ciliate, 6–8 pairs of veins, villous-glabrous on adaxial surface, villous along veins of abaxial surface. Inflorescence 1–2-flowered, axillary, pedicel 0.8–1.5 cm long, villous. Calyx free, sepals five, adnate to corolla, unequal, ovate, apex attenuated, margin entire and sometimes slightly serrate at the base, ciliate, pale green, sometimes purple at base, villous-strigose on adaxial surface, glabrous on abaxial surface, sepals of two sizes, dorsal sepal longer than ventral, 5–6.5 × 5–5.1 mm, other sepals 5–5.2 × 3.5–4 mm. Corolla orange, tube cylindrical, at a 45° angle to the stem, ventricose ventrally, 1.5–1.9 cm long, constricted at throat and middle, tube 4.2–4.3 mm broad at throat, 6–8 mm broad at venter, 4–4.2 mm broad at middle, 6–6.2 mm broad at base, aperture 4–4.1 × 2.2–2.3 mm, glabrous on the outer surface, internally with glandular trichomes up to 1 mm long, becoming sparse toward base, limb

with five unequal lobes, which are orbicular, erect or spreading, margin ciliate and slightly revolute, three lobes (two lateral and one ventral) 4–4.1 × 4–4.1 mm, two lobes dorsal 2–2.5 × 3–3.1 mm. Stamens didynamous, staminode one, dorsal, filaments spiral, adnate to corolla, up to 4 mm above base, glabrous, anthers connivent. Ovary 3–3.2 × 2.5–2.6 mm, glabrous, style pilose, 7–7.5 mm long, bilobed, brown stigma. Disc annular, complete, glabrous. Fruit a fleshy berry, calyx persistent.

Palynology:—Pollen grains isolated, triporate, suboblate (16–)16.97(–17.50) × (18–)20.04(–21) µm, P/E index- approx. 0.85, radially symmetrical, isopolar, circular in polar view. Exine 0.95 µm thick. Pores 1.51 × 1.50 µm, apoporium 14.60 µm long. Exine with microreticulate ornamentation (Fig. 2: Plate 1).

Distribution:—This species is known only from the type locality, *i.e.*, Colombia, Valle del Cauca, Dagua municipality, district of San Bernardo (Fig. 1). This area is located at the eastern slope of the Western Cordillera of the Colombian Andes. The elevation varies between 1800–2200 m. The site has a bimodal precipitation regime, with the highest precipitation during April–May and October–November (Kattan *et al.* 1984). Since 1938, the Colombian government established this region as a natural protected area, and there are few or no farming activities (RUNAP 2010). This declaration stopped the extraction of wood from the forests of this area, used for construction and making charcoal. Land use changed to recreational farms with forest fragments from which firewood and cape land are extracted (Kattan *et al.* 1984).

Besleria brevisepala shares habitat with other herbs such as *B. florida, B. physaloides, B. silverstoneana, B. vestita, Carludovica palmata*, *Columnea dimidiata, Drymonia teuscheri, Glossoloma ichthyoderma, Justicia chlorostachya, Paradrymonia metamorphophylla, Pilea gallowayana* and *Solanum anceps*. *The shrub stratum of this habitat is dominated by Acalypha macrostachya, Gonzalagunia cornifolia, Palicourea angustifolia, Palicourea demissa, Piper aduncum, Piper crassinervium*, *Phenax hirtus Saurauia brachybotrys* y *Siparuna aspera,*. And the arboreal stratum is dominated by *Alchornea glandulosa, Beilschmiedia costaricensis*, *Cinchona pubescens, Croton mutisianus, Elaeagia utilis, Guettarda crispiflora, Hedyosmum bonplandianum, Meriania speciosa, Miconia caudata, Roupala monosperma* and *Tournefortia scabrida. In addition, the palms Chamaedorea pinnatifrons* and *Geonoma orbignyana are part of Besleria brevisepala* habitat.

Etymology:—The specific epithet is given by the size of the sepals, as they are very small compared with what is commonly found in the other species of this genus.

Taxonomic remarks

Besleria brevisepala is morphologically similar to *B. florida, B. microphylla*, *B. silverstoneana,* and *B. solanoides*. These species are terrestrial shrubs with erect stem, 1.5–2.5 m tall, terete, short petiolated, with elliptic to oblanceolate leaf blades, acuminate apex, cuneate base, margin entire, calyx adnate to corolla, orange corolla, and ventricose or slightly ventricose. These species are differentiated in the key below. Moreover, *Besleria brevisepala* is sympatric with *B. silverstoneana* and *B. solanoides* (Fig. 1, Table S1). *Besleria florida* has been found in the department of Valle del Cauca, Colombia, in the township of Yanaconas, in the municipality of Cali (*A. Giraldo 9055,* Fig. 1, Table S1). The townships of Yanaconas and San Bernardo are located in the cloud forest of the Andean region. *Besleria florida* has also been found in the Department of Huila, in the Tres Esquinas rural settlement in Andean forest. This implies that this species´ distribution encompasses almost the entire Andean cordillera, like that of *B. solanoides* (Fig. 1, Table S1). Therefore, the four species mentioned above grow in the cloud forest of Valle del Cauca, and it is highly probable that increasing the intensity of herbarium collections would result in confirming the sympatry with *B. florida*. *Besleria microphylla* has a more restricted distribution than *B. florida* and *B. solanoides*; it has only been reported in the Department of Antioquia and the coffee area of the Andean region of Colombia (Fig. 1, Table S1). It is highly probable that *B. microphylla* is not sympatric with *B. brevisepala* nor with the other three species cited above.

Morphological key for *Besleria brevisepala* and similar species

1 Stem glabrous; petiole 1–5 cm long, glabrous; sepals glabrous; ovary and style glabrous ..... *B. solanoides*
- Stem villous-strigose or hirsute; petiole 0.3–2 cm long, villose-strigose or hirsute; sepals pilose or villose-strigose; ovary and style glabrous or pilose 2
2 Stem hirsute; petiole 1–2 cm long, hirsute; sepals pilose; ovary and style glabrous ..... *B. florida*
- Stem villous-strigose; petiole 0.3–1 cm long, villose-strigose; sepals villose-strigose; ovary glabrous or pilose, style pilose 3

3 Leaf blade 3–4 cm long; sepals lanceolate up to 5 mm long; corolla pubescent on the outer surface..... *B. microphylla*
- Leaf blade 4–15 cm long; sepals oblong, 5–15 mm long; corolla glabrous to slightly pilose on the outer surface 4
4 Sepals free with attenuated apex, 5–6.5 mm long; corolla ventricose; ovary glabrous, style pilose .....*B. brevisepala*
- Sepals connate at the base and sepal teeth with acuminate apex, 11–15 mm long; corolla slightly ventricose; ovary and style pilose.....*B. silverstoneana*

Palynological remarks

The pollen grains of the studied *Besleria* species are monads, isopolar, small, ranging from 14.4–17.5 × 18.8–21.0 µm (in *B. silverstoneana*) to 17.5–22.5 × 22.5–25.0 µm (in *B. microphylla*) (Table 1, Fig. 2), with circular amb and a large to very large polar area, suboblate to oblate spheroidal (only in *B. solanoides*), 3-porate, without annulus, operculate, and with vestibulum (Fig. 2, Table 2). The pores are circular to oval (only in *B. florida* and *B. microphylla*). The exine is semitectate and microreticulate, with narrow muri and small lumina discernible only under SEM observations; only *B. silverstoneana* present tectate exine with perforate ornamentation. The sexine is thicker than the nexine (Fig. 2).

TABLE 1. Quantitative data of pollen grains of *Besleria* spp. from Colombia. PLEV: Polar length in equatorial view, EDEV: equatorial diameter in equatorial view, χ: means, Sχ: standard deviation, S: standard error, CI: confidence intervals, V: coefficient of variability, IV: range.

Taxon	PLEV (µm)					EDEV (µm)				
	IV	$\chi \pm S_\chi$	S	V(%)	IC	IV	$\chi \pm S_\chi$	S	V(%)	IC
B. brevisepala	16.00–17.50	16.97±0.07	3.09	2.26	16.82–17.12	18.00–21.00	20.04±0.17	0.86	4.30	19.70–20.38
B. florida	15.40–18.00	16.44±0.13	0.67	4.06	16.18–16.71	18.00–21.00	19.26±0.16	0.81	4.21	18.94–19.58
B. microphylla	17.50–22.50	19.65±0.23	1.18	5.95	19.19–20.11	22.50–25.00	23.01±0.18	0.93	4.04	22.64–23.37
B. silverstoneana	14.40–17.50	16.00±0.17	0.83	5.22	15.67–16.33	18.80–21.00	20.10±0.160	0.82	4.07	19.78–20.42
B. solanoides	17.5–21.25	19.65±0.19	0.99	5.03	19.26–20.04	20.00–22.50	21.12±0.22	1.14	5.41	20.67–21.57

TABLE 2. Quantitative data of aperture, exine thickness, shape, and polar area index (PAI) of pollen grains of *Besleria* spp. from Colombia. AP: apoporium, P/E: ratio between polar diameter and equatorial diameter, SO: suboblate, OS: oblate-spheroidal.

Taxon	AP(µm)	PAI(µm)	Pore (µm)		Exine (µm)			P/E	Shape
			Length	Width	Total	Sexine	Nexine		
B. brevisepala	14.06	0.70	1.51	1.50	0.95	0.68	0.27	0.81	SO
B. florida	14.56	0.77	2.83	1.94	1.02	0.61	0.41	0.85	SO
B. microphylla	17.37	0.75	3.13	2.60	1.25	1.02	0.22	0.85	SO
B. silverstoneana	14.68	0.73	1.54	1.44	0.92	0.64	0.28	0.79	SO
B. solanoides	14.22	0.69	2.97	2.85	1.72	1.20	0.51	0.93	OS

Variations among *Besleria* species were explained mainly by the quantitative characteristics of the pollen grains in the PCA analysis, which summarized 87.8% of the total variance (Fig. 3, Table 3). The first axis of the PCA explained 68.7% of variability and accounted for the distribution of all groups along this axis. All characteristics contributed to variability among species. However, the most relevant variables for species grouping were pore width (PW), polar length in equatorial view (PLEV), exine thickness (EXIN), and shape (SHAP, Table 3). *B. brevisepala, B. florida,* and

B. silverstoneana were grouped on the negative side of the first axis; these species presented the highest values of polar area index and lowest values of other pollen metrics. The pollen grains of *B. microphylla* and *B. solanoides* had the highest values of the analyzed metric variables (except PAI); they were located on the positive side of axis 1 (Fig. 3). The second axis of the PCA only explained 19.1% of variability. The most relevant variables on this axis were polar area index (PAI) and pore length (PL, Fig. 3, Table 3).

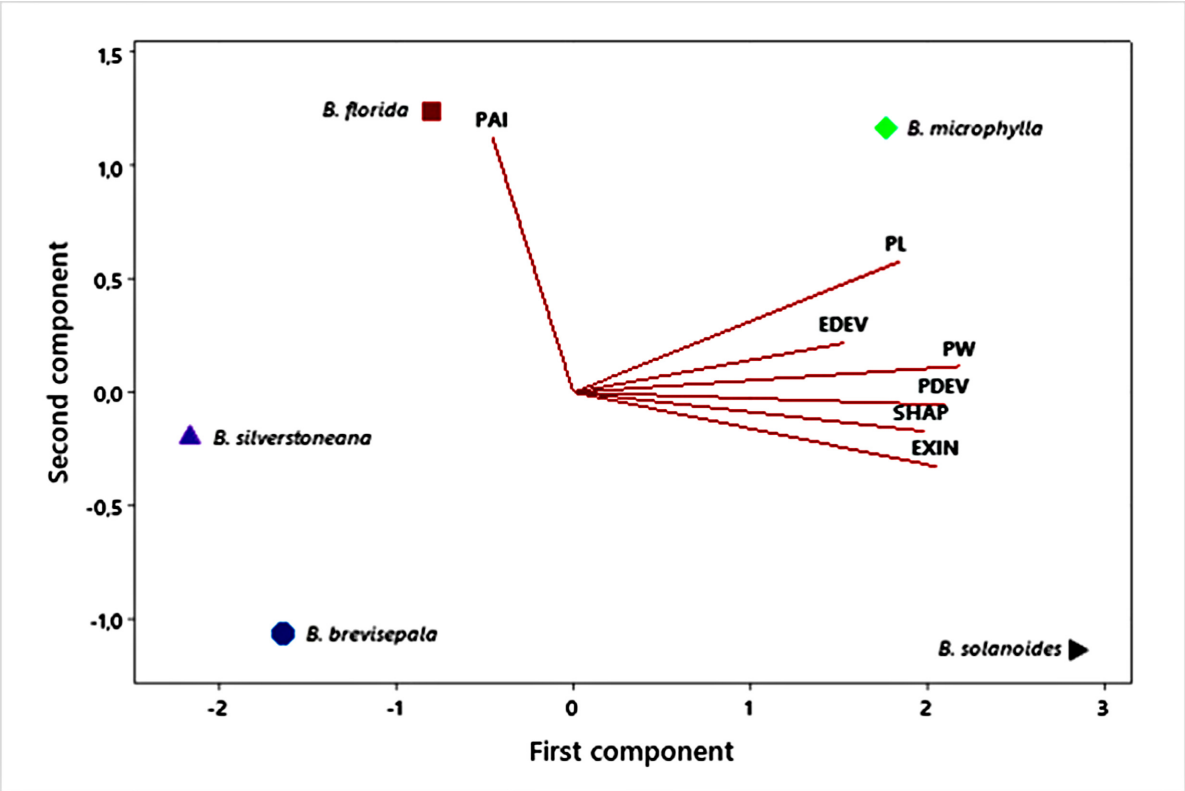

FIGURE 3. PCA analysis performed with the pollen metrical variables of *Besleria* spp. PDEV: Polar diameter in equatorial view, EDEV: equatorial diameter in equatorial view, EXIN: exine, P/E: ratio between polar diameter and equatorial diameter, PAI: polar area index, PL: pore length, PW: pore width.

TABLE 3. Pearson and Kendall correlation coefficients for pollen grains metric variables of the first and the second axis of PCA ordination in *Besleria* spp. from Colombia. PLEV: Polar length in equatorial view, EDEV: equatorial diameter in equatorial view, EXIN: exine thickness, P/E: ratio between polar diameter and equatorial diameter, PAI: polar area index, PL: pore length, PW: pore width.

Variables	Principal components	
	Axis 1	Axis 2
PLEV	0.436	-0.041
EDEV	0.317	0.162
PL	0.382	0.430
PW	0.453	0.086
PAI	-0.095	0.839
EXIN	0.426	-0.245
SHAP	0.410	-0.128

The cluster analysis yielded a dendrogram forming two groups (CA, Fig. 4). One group was composed of *Besleria microphylla* and *B. solanoides*, with a similarity of 61.14%. The species in this group had a greater pollen size and pore

diameter. The other group comprised *B. brevisepala, B. florida,* and *B. silverstoneana* with 66.94% similarity. Among them, *B. brevisepala* and *B. silverstoneana* were the most similar (80.83%). These two species had a lower diameter, pore size, and exine values than *B. florida*, which had a small diameter. However, the pore size of *B. florida* was larger than that of *B. brevisepala* or *B. silverstoneana*.

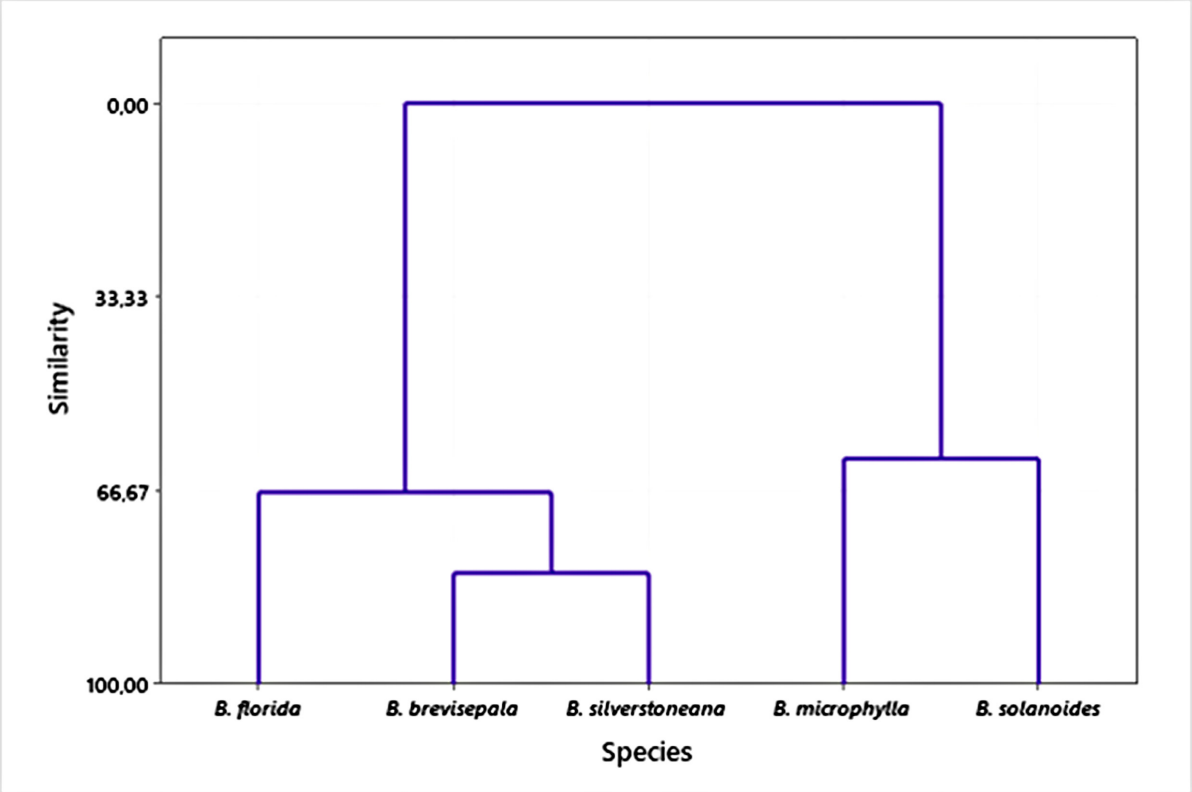

FIGURE 4. Cluster analysis with Euclidean distances and complete linkage for pollen metrical variables in *Besleria spp*. from Colombia.

Artificial pollen key to *Besleria* species from the Andean forests of Colombia

1 Pollen exine perforate ..... *B. silverstoneana*
- Pollen exine microreticulate 2
2 Pollen shape oblate spheroidal ..... *B. solanoides*
- Pollen shape suboblate 3
3 Pores circular ..... *B. brevisepala*
- Pores oval 4
4 PLEV < 18 µ × EDEV <21 µ ..... *B. florida*
- PLEV > 18 µ × EDEV >21 µ ..... *B. microphylla*

Discussion

The five *Besleria* species studied here were morphologically similar, as explained in the description of *B. brevisepala* and the morphological key. Qualitative data and multivariate analyses showed that there were significant variations in pollen morphology, confirming that the genus is eurypalynous and indicating the usefulness of pollen grains in the taxonomic differentiation of *Besleria* (Cortés 2019). The taxonomic use of palynology will be helpful because this genus presents high sympatry and morphological similarity among species.

We found that the five *Besleria* species had 3-porate pollen grains, which coincides with *B. physalloides* and *B. silverstoneana* (Cortés *et al*. 2017), the only species reported for Colombia with this trait. While *B. laxiflora, B. longimucronata, B. sellanoa*, and *B. umbrosa* have 3-colporate pollen grains (Roubik & Moreno 1991, Gasparino *et al*. 2011), *B. macahensis* and *B. melancholica* have 3-colpate pollen grains (Fourny *et al.* 2010). Like all studied *Besleria* species, the five species in this study also presented small pollen grains except for *B. melancholica* which had medium-

size pollen grains (Fourny *et al*. 2010). The dominant conditions were microreticulate exine and suboblate shape, which coincides with reports for *Besleria* by Cortés *et al*. (2017). Only *B. silverstoneana* had perforate exine, and only *B. solanoides* had oblate-spheroidal pollen grains. These two characteristics, sculpture and shape, varied significantly among the species reported in previous studies. The exine varies as follows among species: psilate in *B. laxiflora* (Roubik & Moreno 1991), psilate-perforate in *B. longimucronata* (Gasparino *et al*. 2011), regulate in *B. umbrosa* (Fourny *et al*. 2010, Gasparino *et al.* 2011), rugulate-perforate in *B. melancholica* and *B. macahensis* (Fourny *et al.* 2010), perforate in *B. physalloides*, and microreticulate in *B. sellanoa* (Gasparino *et al.* 2011). On the other hand, the pollen shape was oblate-spheroidal in *B. longimucronata, B. sellanoa,* and *B. umbrosa*, suboblate in *B. physalloides*, subprolate in *B. macahensis* and *B. melancholica*, and prolate in *B. laxiflora*.

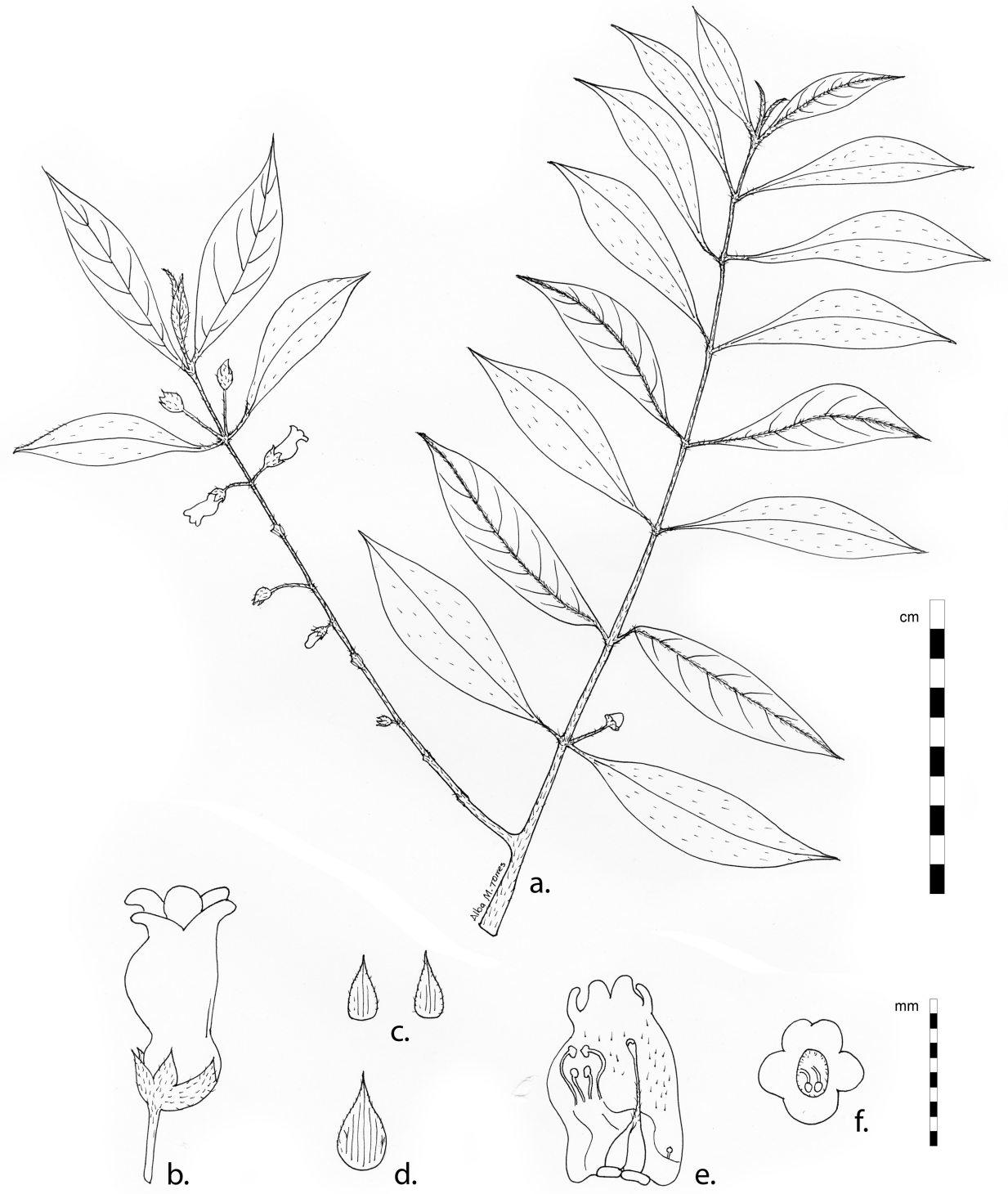

FIGURE 5. *Besleria brevisepala*. A. habit. B. flower outer view. C. lateral and ventral sepal. D. dorsal sepal. E. corolla inner view. F. corolla (front view). Drawn from the holotype by Alba Marina Torres-Gonzalez.

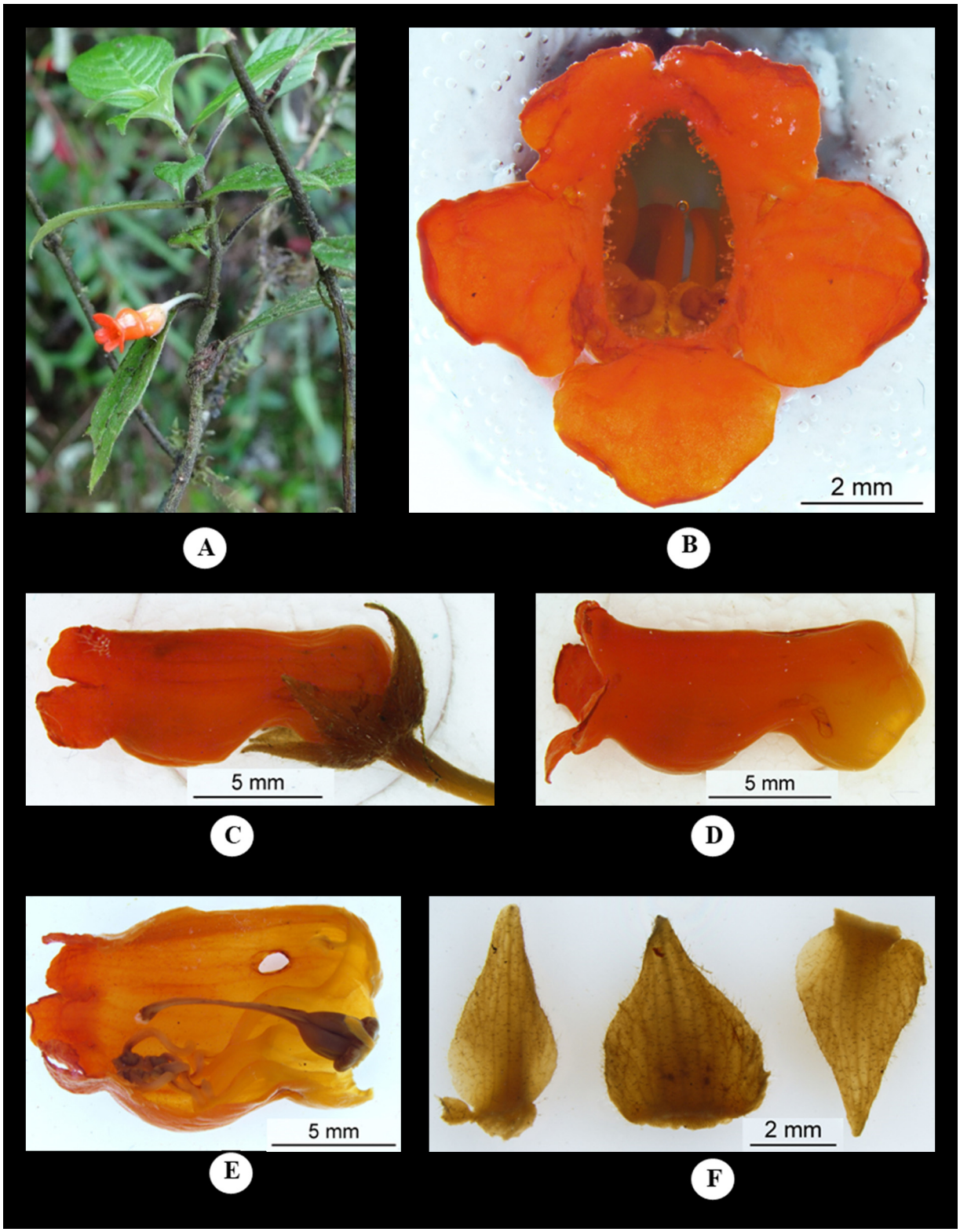

FIGURE 6. *Besleria brevisepala*. A. habit. B. flower outer view. C. corolla inner view. D. flower front view. E. corolla front view. F. lateral, dorsal, ventral sepals. Photos: O.L. Cortés.

Acknowledgements

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S1: Table 1. Voucher specimens of *Besleria* spp. used for morphological analysis from Colombia. COL: Herbarium Nacional Colombiano, Universidad Nacional de Colombia. CUVC: Herbarium Luis Sigifredo Espinal Tascón, Universidad del Valle, Colombia. HUA: Herbarium Universidad de Antioquia, Colombia. CAUP: Herbario de la Universidad del Cauca.

Taxon	**Locality**
B. brevisepala	Colombia, Valle del Cauca: municipio Dagua, corregimiento San Bernardo, road to Buenaventura, cordillera occidental, vertiente oriental. 1992 m. 03°30'52.0''N, 76°37'27.8''W. 02 March 2016. *O.L. Cortés & A. Giraldo-Rodríguez 260* CUVC!
B. florida	Colombia, Antioquia: Amalfi, quebrada Guayabito. 1600 m. 15 February 1993. *D. Tuberquia & G. Gómez 140* COL!
	Colombia, Antioquia: Urrao, vereda Ocaidó Arriba, cuenca del rio Ocaidó. 1720-1750 m. 6°14.9'N, 76°15.3'W. 15 December 2017. *J. Betancur et al. 12734* COL!
	Colombia, Risaralda, Municipio: Santuario, vereda San Rafael. 2100 m. 5°07'N, 76°00'W. 7 March 2006. *J. Betancur et al. 12117* COL!
	Colombia, Chocó: Novita, Vereda Llanadas, ladera norte del Cerro Torrá. 22 February 1977. *E. Forero et al. 3113* COL!
	Colombia, Quindío, Municipio Salento, Verda Boquía, finca los Andes. 2474 m. 5 February 2010. *E. Quintana & J.P. Gómez 54* COL!
	Colombia, Valle, El Silencio, Yanaconas. 1900-2200 m. 28 February 1939. *E.P. Killip & H. Garcia 33753* COL!
	Colombia, Huila, Municipio Gigante, Vereda Alto Tres Esquinas. 2040 m. 2°76'56.13''N, 75°28''32.25''W. 21 July 1997. *LM. Alvarez & C.M. Ospina 2315* CAUP!
	Colombia, Valle, Buenaventura, vereda Alto del Oso. 940 m. 3°47'17.350''N, 76°47'21.630''W. 16 March 2016. *A. Giraldo-Rodríguez 8688* CUVC!
	Colombia, Huila, Municipio Guadalupe, vereda Alto Resinas. 1900 m. 1°55'03.1''N, 75°40'20.1''W. 31 August 2016. *A. Giraldo-Rodríguez 9055* CUVC!
B. microphylla	Colombia, Antioquia: Nariño, bosque de Paramitos. 2100 m. 3 January 1949. *L. Uribe 1873* COL!
	Colombia, Antioquia, Municipo: Urrao, vereda La Ná. 2100-2150 m. 27 February 1989. *A. Cogollo et al. 4243* COL!
	Colombia, Antioquia: Abejorral, quebrada Minitas, vereda Pantano Negro. 2100 m. 05°47'N, 75°26'W. 7 November 2000. *J. C. Marrugo 2* COL!
	Colombia, Antioquia, Municipio La Unión. 2300 m. 05°52'N, 75°18'W. 4 October 1987. *A.E. Brent & F.J. Roldán 6269* COL!
	Colombia, Antioquia, Municipio de Frontino, Corregimiento Nutibara, cuenca alta del rio Cuevas. 1510 m. 14 July 1986. *D. Sánchez et al. 393* COL!
	Colombia, Risaralda, Municipo de Marcella, Corregimieto de la Nona, Convención, Reserva la Nona. 1700-2000 m. 17 December 1999. *W. Bargas 5400* HUA!
B. silverstoneana	Colombia, Valle del Cauca: municipio Dagua, corregimiento San Bernardo, road to Buenaventura, cordillera occidental, vertiente oriental. 1992 m. 03°30'52.0''N, 76°37'27.8''W. 02 March 2016. *O.L. Cortés & A. Giraldo 259* CUVC!
	Colombia, Valle del Cauca: Dagua, corregimiento San Bernardo, vía a Buenaventura. 1992 m. 03°30'52.0''N, 76°37'27.8''W. 9 March 2013. *A. Giraldo-Rodríguez 7961* HUA!
	Colombia, Valle del Cauca: Dagua, corregimiento San Bernardo, vía a Buenaventura. 1992 m. 03°30'52.0''N, 76°37'27.8''W. 2 March 2016. *O.L. Cortés & A. Giraldo-Rodríguez 260* CUVC!
	Colombia, Buenaventura, reserva forestal protectora de los ríos escalerete y San Cipriano. 100 m. 3°49'41.22"N, 76°52'49.74"W. 29 August 2020. *A. Giraldo-Rodríguez 9985* CUVC!
	Colombia, Valle del Cauca, eastern slope of the Western Cordillera of the Colombian Andes, finca Zingara. 3°32'N, 76°36'W. July 2016. *O.L. Cortés 300* CUVC!

B. solanoides	Colombia, Valle del Cauca, Santa Helena, encima del Topacio, borde del Parque Nacional Los Farallones. 1920 m. 3°30'N, 76°35'W. 10 December 1985. *A. Gentry et al. 53064* CUVC!
	Colombia, Valle del Cauca, El Cerrito, finca Buenos Aires, corregimiento Castillo. 2126 m 3°39'48.1''N, 76°10'16.3''W. 23 February 2013. *P. Silverstone-Sopkin & M. Llano-Almario 11671* CUVC!
	Colombia, Valle del Cauca, Yumbo, Finca la Samaria. 1700-1750 m. 4°7'N, 76°30'W. 14 february 1984. *A. Juncosa 2175* CUVC!
	Colombia, Chocó, San José del Palmar, Cerro del Torrá, vertiente nororiental. 1850-1930 m. 13 August 1982. *P.A. Silverstone-Sopkin 1336* CUVC!
	Colombia, Valle del Cauca, Yotoco. 1500 m. 3°50'N, 76°30'W. 14 July 1984. *A. Gentry et al. 48072* CUVC!
	Colombia, Valle del Cauca, Cordillera Occidental hoya del rio Calima, el Cairo, etre Darien y Media Canoa. 1650-1750 m. 6-7 January 1943. *J. Cuatrecasas 13906* COL!
	Colombia, Antioquia, PNN Las Orquídeas. 1090-1215 m. 6°34'N, 76°19'W. 28 July 1988. *A. Cogollo et al. 3579* COL!
	Colombia, Antioquia, municipio Amalfi. 1550 m. 6°56'N, 75°4'W. 28 September 1988. *F.J. Roldan & O. Escobar 781* COL!
	Colombia, Antioquia, municipio Jardín. 2220 m. 5°40'N, 75°48'W. 10 June 1987. *R. Callejas et al. 3975* COL!
	Colombia, Antioquia, municipio Yurumal. 1890-2000 m. 7°10'N, 75°28'W. 4 June 1989. *JL. Luteyn & O. Escobar 13196* COL!
	Colombia, Chocó, Cerro el Torrá, vertiente nordeste, abajo pista de helicopteros. 1850-1940 m. 13 August 1982. *P. Silverstone 1336* COL!
	Colombia, Cauca, Pisojé, al noreste de Popayán, carretera de los Ollos (Yambitará). 2000 m. 25 January 1976. *T.C. Polwman & D.Vaughan 5286* COL!
	Colombia, Cundinamarca, carretera entre Fusagasugá y Bogotá. 3000 m. 4°26'N, 74°24'W. 8 December 1980. *T.B.Croat 51991* COL!
	Colombia, Santander, Bolivar, cueva de las Guacamayas. 1900-2000 m. 28 March 1948. *L. Uribe 1673* COL!
	Colombia, Tolima, municipio Snta Isabel, finca la Cima, cordillera central vertiente oriental. 1860 m. 6 August 1980. *A.M. Cleef et al. 10634* COL!
	Colombia, Tolima, municipio Santa Isabel, vereda la Pava, finca la Pavita, cordillera central vertiente oriental. 2030 m. 4 August 1980. *J.M. Idrobo et al. 10511* COL!
	Valle del Cauca, Dagua, vereda Loma Alta, cordillera occidental, vertiente oriental, 1900-2000 m. 1 February 2004. *A. Ospina 114* COL!
	Valle del Cauca, Dagua, vereda Yatacue, PNN Farallones, alto Anchicayá. 642 m. 03°33'3.4''N, 76°53'24.8''W. 16 November 2014. *A. Vasquez 322* CUVC!
	Valle del Cauca, cordillera occidental, vertiente oriental, finca Zíngara. 3°32'N, 76°36'W. 9 June 2015. *M. Ramírez 139* CUVC!

S2: Table 2. Voucher specimens of *Besleria* spp. used for palynological analysis. from Colombia. COL: Herbarium Nacional Colombiano, Universidad Nacional de Colombia. CUVC: Herbarium Luis Sigifredo Espinal Tascón, Universidad del Valle, Colombia. HUA: Herbarium Universidad de Antioquia, Colombia. *material used as a pattern for descriptions.

Taxon	**Locality**
**B. brevisepala*	Colombia, Valle del Cauca: Dagua, corregimiento San Bernardo, vía a Buenaventura. 02 March 2016. *O.L. Cortés & A. Giraldo-Rodríguez 260* CUVC!
**B. florida*	Colombia, Antioquia: Amalfi, quebrada Guayabito. 15 February 1993. *D. Tuberquia & G. Gómez 140* COL!
B. florida	Colombia, Antioquia: Urrao, vereda Ocaidó Arriba, cuenca del rio Ocaidó. 15 December 2017. *J. Betancur et al. 12734* COL!
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**B. microphylla*	Colombia, Antioquia: Abejorral, quebrada Minitas, vereda Pantano Negro. 7 November 2000. *J. C. Marrugo 2* COL!
B. microphylla	Colombia, Antioquia: Urrao, vereda La Ná. 27 February 1989. *A. Cogollo et al. 4243* COL!
B. microphylla	Colombia, Antioquia: Nariño, bosque de Paramitos. 3 January 1949. *L. Uribe 1873* COL!
**B. silverstoneana*	Colombia, Valle del Cauca: Dagua, corregimiento San Bernardo, vía a Buenaventura. 02 March 2016. *O.L. Cortés & A. Giraldo-Rodríguez 259* CUVC!
B. silverstoneana	Colombia, Valle del Cauca: Dagua, corregimiento San Bernardo, vía a Buenaventura. 9 March 2013. *A. Giraldo-Rodríguez 7961* HUA!
B. silverstoneana	Colombia, Valle del Cauca: Dagua, corregimiento San Bernardo, vía a Buenaventura. 2 March 2016. *O.L. Cortés & A. Giraldo-Rodríguez 260* CUVC!
**B. solanoides*	Colombia, Valle del Cauca: Santa Helena, encima del Topacio, borde del Parque Nacional los Farallones. 10 December 1985. *A. Gentry et al. 53064* CUVC!
B. solanoides	Colombia, Valle del Cauca: El Cerrito, finca Buenos Aires, corregimiento Castillo. 23 February 2013. *P. Silverstone-Sopkin & M. Llano-Almario 11671* CUVC!