



## ***Carajasia* (Rubiaceae), a new and endangered genus from Carajás mountain range, Pará, Brazil<sup>#</sup>**

ROBERTO M. SALAS<sup>1\*</sup>, PEDRO L. VIANA<sup>2</sup>, ELSA L. CABRAL<sup>1</sup>, STEVEN DESSEIN<sup>3</sup> & STEVEN JANSSENS<sup>3</sup>

<sup>1</sup>Instituto de Botánica del Nordeste, CONICET. FACENA-UNNE. Sargento Cabral 2131, cc. 209, CP. 3400. Corrientes, Argentina.

<sup>2</sup>Museu Paraense Emílio Goeldi. Avenida Magalhães Barata, 376, São Braz, Belém - PA, 66040-170, Brazil.

<sup>3</sup>Botanic Garden Meise, Nieuwelaan 38, BE-1860 Meise, Belgium.

\*Author for Correspondence: [robertoymanus@gmail.com](mailto:robertoymanus@gmail.com)

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### **Abstract**

*Carajasia* is described as a new genus of Rubiaceae. It is so far known only from the mountain summits of Serra dos Carajás (Pará, Brazil), where it is part of a shrubby vegetation surrounded by tropical rainforest. The new genus belongs to the tribe Spermacoceae and is positioned within it to the *Spermacoce* clade. *Carajasia* is unique within the clade in having a very particular combination of characters: flowering branches with two axillary flowers per node, homostylous flowers, corollas with a fringe of moniliform hairs, pubescent styles with distinct stigma lobes, bilobed nectariferous discs covered by triangular papillae, pollen with a double reticulum and fruits with a peculiar type of dehiscence. A detailed description of *Carajasia* is presented, including observations of the fruit and pollen, along with distribution maps and images of the plant in its habitat. A dichotomous key to distinguish *Carajasia* from other genera with deeply divided stigmas is provided. A molecular phylogenetic study was carried out using ITS and ETS sequences to determine the phylogenetic position of the new genus within the *Spermacoce* clade. The results of the combined analyses demonstrated that *Carajasia* is sister to *Galianthe* with moderate to high support. Both genera form a weakly supported clade with *Schwendenera*. This clade is sister to the other genera of the *Spermacoce* clade studied in this work. *Galianthe* and *Schwendenera* share with *Carajasia* pollen with a double reticulum, but they are clearly differentiated by suffruticose habit, heterostylous flowers and the pattern of fruit dehiscence. To clarify the phylogenetic position of *Carajasia*, some morphological characters are discussed based on the molecular results: division of the stigma, pollen types and floral syndrome.

**Key words:** *Galianthe*, *Spermacoce* clade, Spermacoceae, phylogeny, taxonomy, pollen

### **Introduction**

During the last two decades, the delimitation of the tribe Spermacoceae has undergone through considerable changes. Currently, three different concepts are recognized. The first coincides with the classical definition (Robbrecht, 1988), including about 20 genera and is still defended by some scientists on the basis of morphological characters (Terrell & Wunderlin 2002). The two other tribal concepts are mainly based on molecular studies. Bremer (1996) proposed the broadest concept of Spermacoceae, including the former tribes Knoxieae, Triainolepideae, Hedyotideae and Manettieae. Andersson & Rova (1999) redefined and narrowed this concept by recognizing Knoxieae (including Triainolepideae and the ‘*Pentas* group’ of the tribe Hedyotideae) as a separate tribe. The proposal of Andersson & Rova (1999), often referred to as Spermacoceae *s.l.*, was followed with minor modifications by other Rubiaceae specialists (Dessein 2003; Robbrecht & Manen 2006; Groeninckx *et al.* 2009), and is also accepted in this paper. Robbrecht & Manen (2006) included 33 genera in Spermacoceae *s.l.*, to which Groeninckx *et al.* (2009) added other taxa; the tribe now comprises 61 genera with an estimated 1235 species. Several new genera were described in Spermacoceae *s.l.* based on morphological (Terrell 1987; 2001a, b, Terrell & Lewis, 1990, Terrell & Robinson, 2009, Borhidi 2012, Borhidi & Lozada, 2010, 2011) or combined morphological and molecular evidence (Groeninckx *et al.*, 2010a, b, c). Despite the consensus among specialists about Spermacoceae *s.l.*, morphologically it is difficult to define because no single morphological synapomorphy supports the tribe (Groeninckx *et al.* 2009).

The classical concept of the tribe Spermaceae, here named the *Spermaceae* clade, which forms a monophyletic group deeply nested among genera of the former tribe Hedyotideae, is morphologically easy to recognize. The often-herbaceous plants are generally characterized by the following combination of characters: presence of raphides, fimbriate stipules, uniovulate ovary locules and pluri-aperturate pollen grains. In America, the *Spermaceae* clade is currently represented by 22 genera, ten of which have recently been revised [*Crusea* Chamisso & Schlechtendal (1830: 165), *Denscantia* Cabral & Bacigalupo (2001: 353), *Diodia* Linnaeus (1753: 104), *Ernodea* Swartz (1788: 2), *Galianthe* Grisebach (1879: 156), *Psyllocarpus* Martius & Zuccarini (1824: 130), *Planaltina* Salas & Cabral (2010: 198–199), *Richardia* Linnaeus (1753: 330), *Staelia* Chamisso & Schlechtendal (1828: 364), and *Tessiera* Candolle (1830: 57)], three are under revision [*Borreria* Meyer (1818: 79), *Spermaceae* Linnaeus (1753: 102), and *Diodella* Small (1913a: 177, 200)] and the remaining genera are monospecific [*Anthospermopsis* (Schumann 1888: 72) Kirkbride (1997: 373), *Diacrodon* Sprague (1928: 34), *Emmeorhiza* Pohl ex Endlicher (1838: 565), *Nodocarpea* Gray (1883: 79), *Micrasepalum* Urban (1913: 548), *Schwendenera* Schumann (1886: 157), *Tobagoa* Urban (1916: 341–342), and *Tortuella* Urban (1927: 89)]. Within the *Spermaceae* clade, molecular evidence questions the generic boundaries based mainly on morphological data. For this reason, there are different points of view among specialists about the limits of some genera, especially in the *Spermaceae-Borreria* complex (Salas *et al.* 2011). On the other hand, there is a clear consensus on the status of other morphologically well-defined genera; some of these are also strongly supported by molecular data: *Crusea*, *Denscantia*, *Ernodea*, *Emmeorhiza*, *Galianthe*, *Mitracarpus*, *Psyllocarpus*, *Richardia*, and *Staelia* (Dessein 2003, Kårehed *et al.* 2008).

As part of an environmental impact assessment study conducted at the Serra Sul Mountain Range, in the Carajás region, Pará state, Brazil (Golder Associates Brasil 2010), several specimens of a plant with a unique combination of characters were collected growing on iron outcrops with a peculiar open physiognomy usually referred to as “canga” vegetation (e.g. Secco & Mesquita 1983). Based on their herbaceous habit, fimbriate stipules, uniovulate ovary locules, and capsular fruits, the specimens were assigned to the *Spermaceae* clade. Their assignment to a particular genus of the *Spermaceae* clade was not that easy though. In fact the specimens were characterized by a peculiar combination of characters: 1-flowered axillary inflorescences, homostylous flowers, corollas with a fringe of moniliform hairs, pubescent styles with distinct stigma lobes, 2-lobed nectariferous discs, pollen with a double reticulum and fruits with a peculiar type of dehiscence. Based on this unique set of morphological characters, the specimens were designated as a new genus here described and named as *Carajasia* R.M. Salas, E.L. Cabral & Dessein to highlight the importance of the Carajás mountain range as a reservoir of endemic species (Secco & Mesquita 1983, Morelato & Rosa 1991). In addition, a recent study of material preserved at the MG herbarium revealed two collections from the Serra Sul Mountain Range collected nearly 30 years ago. To test the generic status and phylogenetic position of the new taxon, a molecular study was carried out. Their morphological and phylogenetic affinities are discussed, and an artificial key to distinguish *Carajasia* from the other taxa of the *Spermaceae* clade with a deeply divided stigma is provided.

## Materials and Methods

### Taxonomy

*Borreria*, *Spermaceae* and *Diodella* are considered to be problematic genera with regards to their generic delimitation. For the American taxa, Bacigalupo & Cabral (1996, *Borreria*) and Bacigalupo *et al.* (2010, American *Spermaceae*) were followed. For the African *Spermaceae* species, Verdcourt (1975) and Dessein (2003) were used, whereas for the Australian *Spermaceae* Dessein (2003) and Harwood & Dessein (2005) were followed. The generic concept of *Diodella* used in this study was derived from Bacigalupo & Cabral (1999, 2006).

Pollen morphological descriptions of the genera analyzed were obtained from Kirkbride (1979, *Psyllocarpus*), Pire (1996, *Borreria*, 1997, *Galianthe*, 1998, *Richardia*), Dessein *et al.* (2002, African *Spermaceae*), Dessein (2003, *Ernodea*), Dessein & Harwood (2005, Australian *Spermaceae*), Souza (2008, *Mitracarpus*), Cabaña (unpubl. data, *Diodella* and *Diodia s.s.*) and Salas (2012, *Staelia*). Information about the unlisted genera corresponds to unpublished data of Cabral *et al.* (unpubl. data).

### Molecular Study

Nuclear ribosomal ITS and ETS datasets were analyzed both separately and combined. To evaluate whether the data matrices provided different signal in the combined analyses, a partition homogeneity test (implemented in PAUP\* 4.0b10a) was conducted (Swofford *et al.* 2002).

Maximum parsimony analyses were conducted using PAUP\* v.4.0b10a (Swofford *et al.* 2002). Heuristic searches were conducted with TBR branch swapping on 10,000 random addition replicates with five trees held at each step. Non-parametric bootstrap analysis was carried out to calculate the relative support for individual clades found in the parsimony analysis. For each of 1000 bootstrap replicates, a heuristic search was conducted with identical settings as in the original heuristic analysis.

Model selection for the Bayesian inference analysis was conducted with ModelTest 3.06 (Posada & Crandall 1998) under the Akaike Information Criterion (AIC). The GTR+G model was selected for both ITS and ETS. Bayesian analyses of the concatenated dataset were carried out with MrBayes 3.1 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003). Four chains (one cold, three heated), initiated from a random starting tree were run simultaneously for 10 million generations. Every 1000 generations, a tree was sampled from the chain for a total of 10,000 trees. Due to the burn-in, 50% of the sample points were discarded. Convergence of the chains was examined with TRACER 1.4 (Rambaut & Drummond 2007). This resulted in an effective sampling size (ESS) parameter exceeding 100, which assumes a sufficient sampling and acceptable mixing.

Maximum Likelihood analyses were carried out using the RaxML search algorithm (Stamatakis *et al.* 2005) under the GTRGAMMA approximation of rate heterogeneity for each gene (Stamatakis 2006). Two hundred bootstrap trees were inferred using the RaxML Rapid bootstrap algorithm (ML-BS) to provide support values for the best-scoring ML tree. The studied species with geographical information, collector, herbarium and GenBank accession numbers are provided in Appendix.

## Results

### Taxonomic treatment

*Carajasia cangae* R.M. Salas, E.L. Cabral & Dessein, *gen. et sp. nov.*

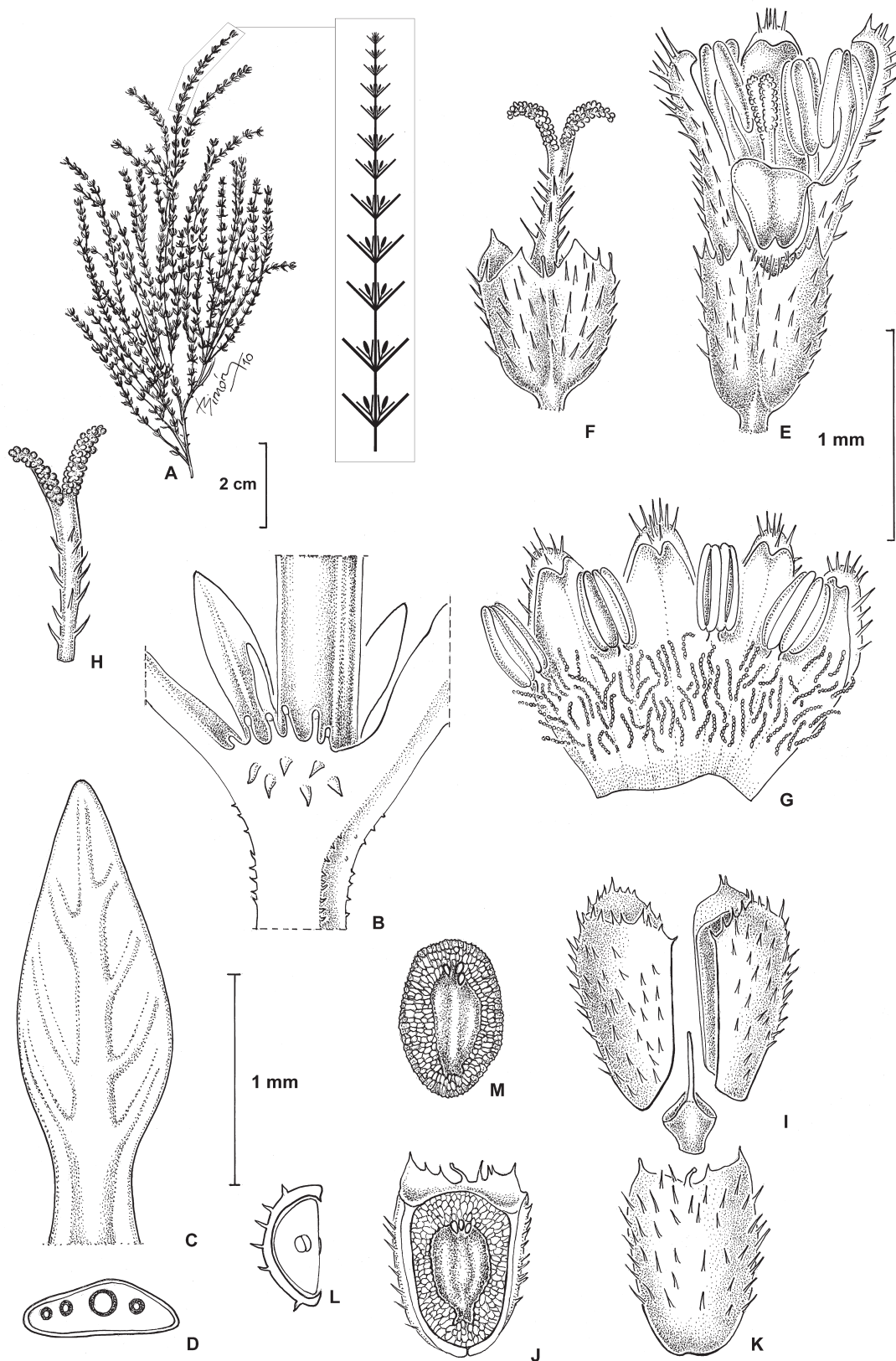
*Carajasia* and *C. cangae* differ from the remaining genera of *Spermacoceae* in having flowering branches with two axillary flowers each node, homostylous flowers, corolla with a fringe of moniliform hairs, pubescent styles with distinct stigma lobes, 2-lobed nectariferous discs, bireticulate pollen grains, dry fruits with septicidal dehiscence into two caducous mericarps leaving a basal carpophore and each mericarp covered by a hyaline wall of the intercarpelar septum.

Type:—Brazil: Pará: Canaã dos Carajás, Flona de Carajás, Serra Sul, S11-C, 6°22'19"S, 50°23'5"W, 723 m, 22 March 2012, *Viana P.L., Santos F.M., Arruda A.J., Jorge T.B. & P.M. Burkowski* 5263 (holotype BHCB, isotype CTES, MG). (Figs. 1–7)

Herbs 2–10 cm tall, perennial, with main branches erect. Stem tetragonal, glabrous, papillate on angles, wine-colored. Stipules fimbriate, basal sheath 0.1–0.15 mm long, glabrous or with a few scattered short and triangular hairs, with 6–7 fimbriae; fimbriae 0.1–0.18 mm long, glabrous, with apex glandular. Leaves pseudoverticillate (due to the presence of axillary brachyblasts), sessile; blades narrowly ovate, 1.8–2.1 x 0.5–0.7 mm, attenuate at the base, acuminate at the apex, slightly succulent, glabrous; primary veins visible on the adaxial side; secondary veins 3–4 on each side of the main vein, alternate and visible on abaxial side. Flowering branches with two axillary flowers on each node; each flower subtended by 2 foliaceous bracts; bracteoles inconspicuous. Flowers sessile, homostylous; hypanthium obovoid, pubescent; calyx tube 0.05 mm long; calyx 4-lobed, lobes triangular, 0.15–0.17 mm long, margin ciliate; corolla 1–1.2 mm long, tube funnel-shaped, equal or slightly shorter than the corolla lobes, internally with a fringe of moniliform hairs from the base of the stamens to near the base of the tube, externally pubescent, lobes internally glabrous; stamens shorter than the corolla lobes, anthers 0.45–0.48 mm long, pollen 5–7 colpi, bireticulate, spheroidal, E 20.6–21 µm, P 19–21 µm, ectocolpi 12–12.7 µm long, endoaperture an endocingulum, infrareticulum complete, muri spinose, suprareticulum reduced, muri mostly psilate (Fig. 2 H–J); style 1.1–1.2 mm long; nectariferous disc 2-lobed, with certain triangular papillae taller than the remaining cells of the disc, each papilla with striated cuticle; ovary 2-locular, obovate, placenta attached near the middle of the septum, each locule 1-ovulate. Dry fruit obovoid, 0.9–1.1 mm long, pubescent, with septicidal dehiscence into two caducous mericarps leaving a basal carpophore, each mericarp covered by a hyaline wall of the intercarpelar septum; seeds plano-convex, ovate in outline, wing absent, 0.7–0.75 mm long; ventral face slightly furrowed, partially covered by the strophiole, with numerous raphides; testa reticulo-areolate.

**Etymology:**—The genus is named after “Floresta Nacional de Carajás”, especially to the Carajás mountain range,

Pará, Brazil, the only region where the plant was found. The specific epithet refers to Canga vegetation, which is the only habitat in which the species was found (Fig. 5).

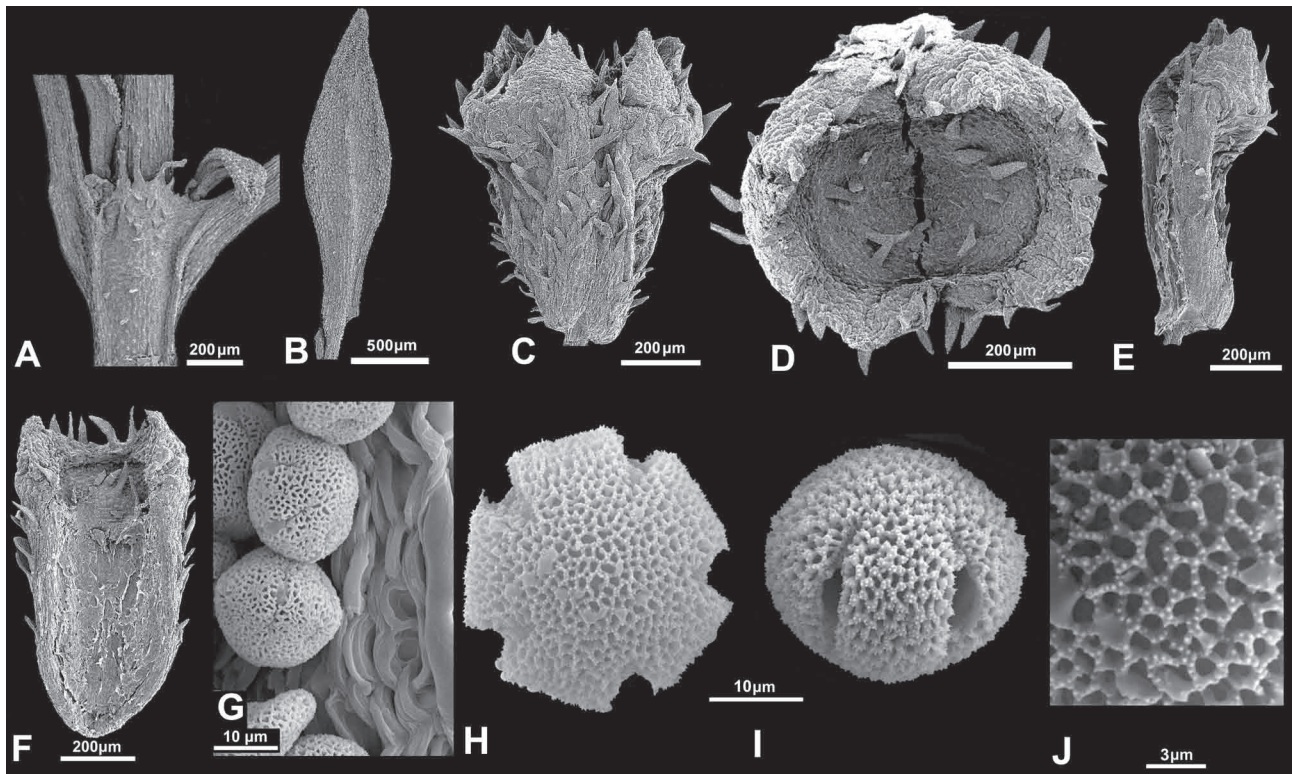


**FIGURE 1.** *Carajasia cangae*. **A**, habit, in rectangle a diagram of flowering branch; **B**, stipule with fimbriate edge. **C**, abaxial face of leaf; **D**, transverse section of leaf; **E–H**, flower; **E**, flower with a creased corolla lobe; **F**, hypanthium, style and stigma; **G**, inside of corolla; **H**, style and stigma; **I–L**, fruit; **I**, dehiscent fruit; **J**, ventral view of mericarps without a hyaline intercarpelar septum; **K**, dorsal view of mericarps; **L**, transverse section of mericarps; **M**, seed. Drawn by Laura Simón from the holotype [Viana et al. 5263 (BHCB)].



**Distribution and habitat:**—All collections of this species are from the municipality of Canaã dos Carajás, state of Pará, Brazil. They inhabit ferric soil (or Canga) only on the top of the Carajás mountain range (Fig. 3, 4). These mountains are situated at 580–850 m elevation and surrounded by Amazonian forest at lower elevations.

**Conservation Status:**—*Carajasia cangae* should be classified as endangered [EN B2ab(ii, iv)] according to IUCN Red List criteria (IUCN 2001): area of occupancy estimated to be less than 50 km<sup>2</sup> in a severely fragmented area, besides the species is only known from fourteen collections, all from one location. Planned mining activities form the main threat to the species.



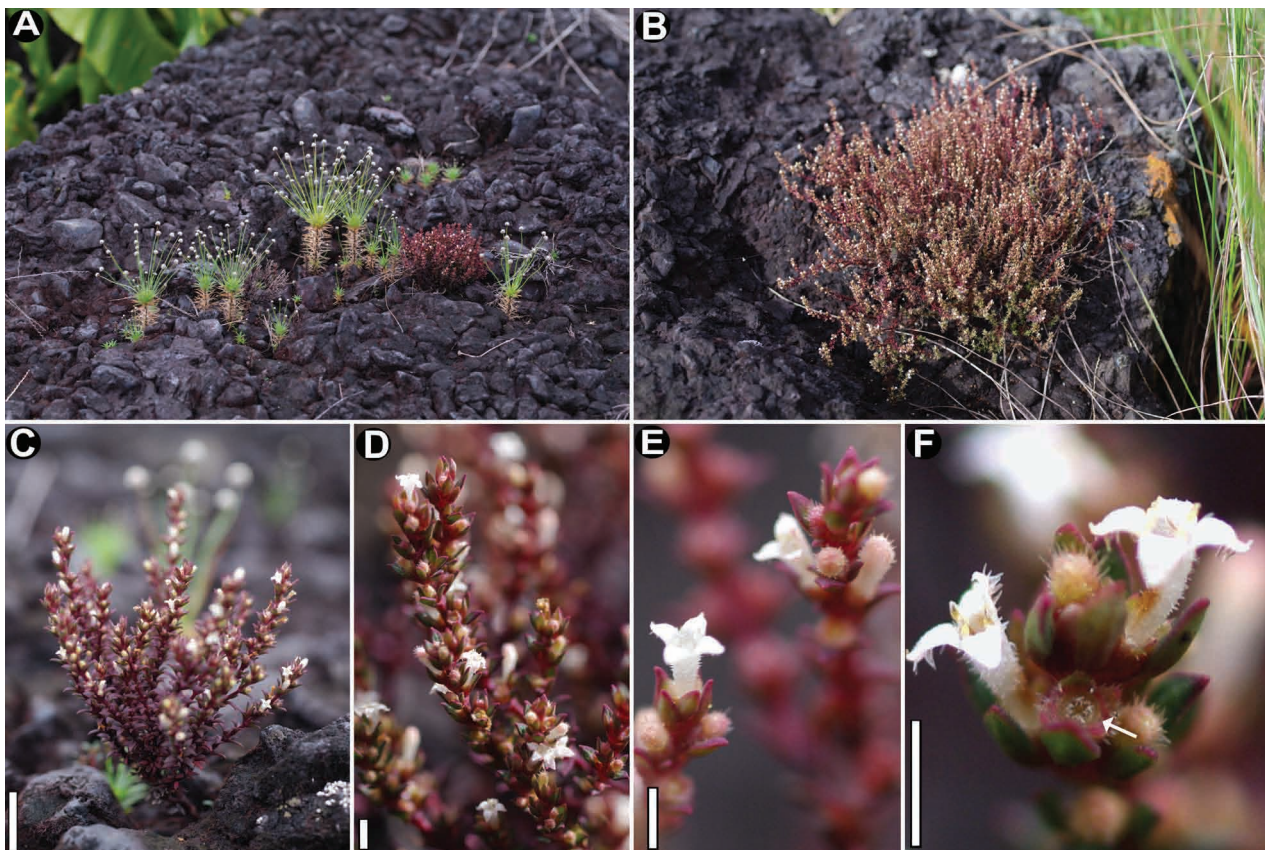
**FIGURE 2.** Microphotographs from the scanning microscope of *Carajasia cangae*. **A**, stipule; **B**, leaf; **C**, immature fruit; **D**, apical view of fruit; **E**, lateral view of mericarp with the septum at the base; **F**, internal view of mericarp; **G**, inside view of anther locule without orbicules; **H–J**, bireticulate pollen grains; **H**, polar view; **I**, equatorial view; **J**, exine. A–J, from holotype [Viana *et al.* 5263 (BHCB)].

**Conservation of habitat in the future:**—The Serra do Carajás is located in the southeastern portion of Pará State in northern Brazil. It is an isolated mountain range in the eastern Amazon region. The rugged relief of the region bears a rich mosaic of vegetation types and consists of one of the most important forest remnants in the eastern Amazon. It is partially situated within the limits of a conservation unit, the FLONA Carajás (Carajás National Forest). The diversity of endemic species of the canga from the Serra do Carajás region is noteworthy. Although the flora is still not satisfactorily inventoried, several endemic plant species are known from the canga vegetation of this region (Secco & Mesquita 1983, Morelato & Rosa 1991).

The distribution of *Carajasia cangae* coincides with the extension of the Serra Sul mountain, which covers an area of approximately 50 km<sup>2</sup> located within a single municipality. For organizational purposes this mountain used to be divided in four different areas, known as S11-A, S11-B, S11-C and S11-D (Golder Associates Brasil 2009). This region harbors one of the most important iron deposits in the world and is part of the Carajás Mineral Province (Lindenmayer *et al.* 2001). The vegetation on the top of Serra Sul is composed of patches of saxicolous scrubby vegetation, which occur on the iron outcrops, and is commonly referred to as “canga” (Secco & Mesquita 1983). In this area, *C. cangae* occurs as small populations distributed along storm drains on consolidated iron outcrops. One of the areas mentioned above, S11-D, will become in 2016 one of the largest open-pit mine of the world (Golder Associates Brasil 2010). Despite many efforts during the period 2010–2012 looking for populations of *C. cangae* in localities outside Serra Sul, no other localities were found. Half of the known collections of *C. cangae* came from “area S11-D” of Serra Sul. For this reason, conservation efforts for this monotypic genus are highly needed to ensure its survival. In a first phase, seeds need to be collected and conserved in a seed bank for ex-situ conservation. Ideally, this would be combined with cultivating the plant in botanical gardens worldwide. In parallel, the natural populations should be monitored, and the



influence of the mining activities evaluated, which would support the development of effective strategies to insure the survival of this taxon in the wild.

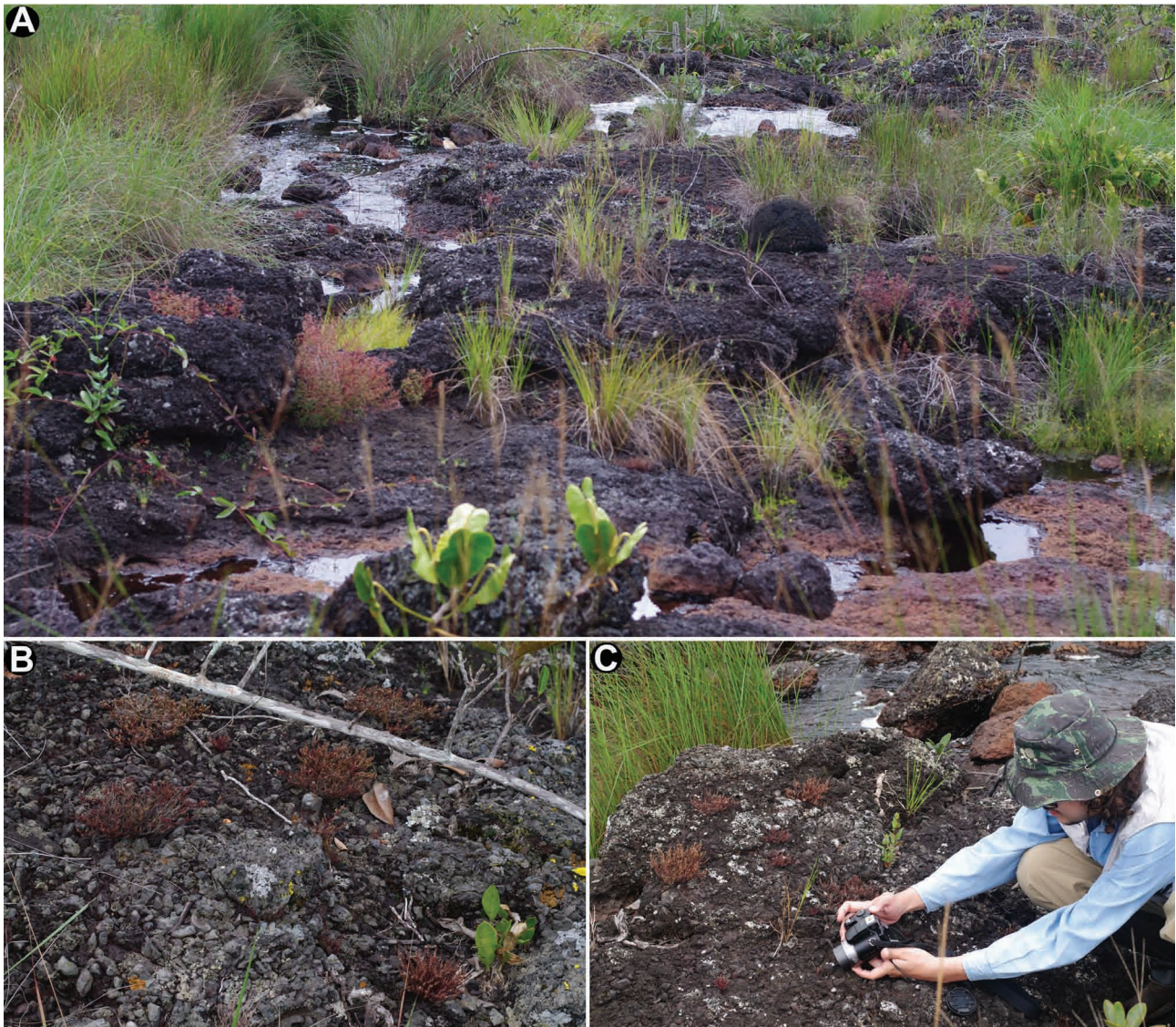


**FIGURE 3.** Images of *Carajasia cangae*. **A**, *Carajasia cangae* and *Paepalanthus fasciculoides* Hensold growing on the Canga soil; **B**, habit of well-developed plant; **C**, reduced habit; **D**, detail of the inflorescence; **E**, apex of the inflorescence; **F**, flowers, flowers bud and immature fruit, the arrow indicating the papillae over the fruit. Scale bars: C: 1 cm, D, E and F 1 mm. Photographs by P.L. Viana.

**Additional specimens examined (paratypes):**—BRAZIL: Pará: Canaã dos Carajás, Flona de Carajás, Serra Sul, S11-A, drenagem em meio a vegetação rupestre sobre canga, 6°17'30,54"S, 50°28'8,19"W, 710 m, 21 July 2012, *Arruda A.J., Paula L.F.A., Silva L.V.C. & Jorge T.B. 1199* (BHCB); S11-B, vegetação rupestre, 6°20'36"S, 50°25'25"W, 747 m, 27 January 2012, *Silva L.V.C., Pivari M.O., Jorge T.B. & Gontijo M. 1127* (BHCB); S11-B, vegetação rupestre, 6°20'32"S, 50°25'4"W, 724 m, 25 April 2012, *Arruda A.J., Santos F.M., Arruda L.J. & Jorge T.B. 1094* (BHCB); S11-C, vegetação rupestre, 6°22'23"S, 50°23'24"W, 700 m, 24 January 2012, *Silva L.V.C., Arruda A.J., Pivari M.O., Jorge T.B., Paula L.F.A., Gontijo M. & Ranieri B.D. 1081* (BHCB); S11-C, campo graminoso associado a drenagem com muitas pedras, 6°22'22"S, 50°23'03"W, 723 m, 20 March 2012, *Arruda A.J., Viana P.L., Santos F.M., Burkowski P.M., Jorge T.B. & Arruda L.J. 737* (BHCB); S11-D, vegetação rupestre sobre canga, 6°24'28"S, 50°21'5"W, 819 m, 24 April 2012 (small population, less than 30 individuals), *Arruda A.J., Santos F.M., Arruda L.J. & Jorge T.B. 1085* (BHCB); S11-D, mata baixa sobre canga, 6°23'31"S, 50°19'9"W, 604 m, 23 May 2012, *Arruda A.J. Salino A. & Arruda L.J. 1172* (BHCB); S11-D, campo graminoso alagado sobre canga, 6°24'5"S, 50°21'33"W, 797 m, 24 April 2012, *Arruda A.J. Santos F.M., Arruda L.J. & Jorge T.B. 1078* (BHCB); S11-D, mata baixa, 6°24'25.26"S, 50°18'55.02"O"W, 720 m, 1 July 2010, *Almeida T.E., Marino F., Megale M. & Arruda, A.J. 2463* (BHCB); S11-D, vegetação rupestre, 820 m, 18 May 2010, *Pivari M.O., Silva L.V.C. & Santos A.O. 1504* (BHCB); S11-D, 6°23'08"S, 50°23'05"W, 16 April 2009, *Giorni V.T., Viana P.L., Versieux L.M., Garcia L.C., Silva L.V.C. & Silva, D.S. 173* (BHCB); S11-D, vegetação rupestre, 753 m, 11 October 2008, *Silva L.V.C. 558* (BHCB); Serra Sul, ocorre em córregos sobre canga, lajeado, nas rochas que permanecem secas, 22 March 2012, 6°17'02"S, 50°20'13"W, 580 m, *Burkowski P.M., Arruda A.J., Viana P.L., Santos F.M., Jorge, T.B. & Arruda L.J. 1152* (BHCB, CTES); vegetação de canga, 16 April 1986, *Secco R.S., Silva M.F.F., Carreira L. & Salomão R.P. 714* (MG); 28 May 1987, *Silva M.F.F. & N.A. Rosa 2455* (MG).

**Taxonomic notes:**—The dehiscence of the *Carajasia* fruits is most similar to some species of *Crusea*; in both genera the dehiscence is septicidal and separates the fruit into two caducous mericarps leaving a basal carpophore on the pedicel. The ventral face of each mericarp is covered by a hyaline wall that belongs to the intercarpelar septum, which in Fig. 1 I–L was removed to shows the seed and in the Fig. 2 F remains complete.





**FIGURE 4.** Image of Canga vegetation, the natural environment of *Carajasia cangae*. **A**, temporary streams with xerophytic vegetation, with some caespitose grasses, the *Philodendron wulfschlaegelii* Schott and some scattered ferruginous plants of *C. cangae*. **B**, **C**, some plants of different stature of *C. cangae*. Photographs by P.L. Viana.

Besides *Carajasia*, there are eleven supraspecific taxa known within the *Spermacoce* clade that are characterized by deeply divided stigmas [*Borreria* subsect. *Latifoliae* (Schumann 1888: 143), Bacigalupo & Cabral 1996: 306), *Diodia*, *Emmeorrhiza*, *Galianthe*, *Mitracarpus*, *Psyllocarpus* sect. *Amazonica* Kirkbride (1979: 13), *Richardia*, *Schwendenera*, *Staelia*, *Tobagoa* and *Tortuella*]. An artificial key to distinguish *Carajasia* from the remaining taxa with deeply divided stigmas is presented below.

**Key to taxa with deeply divided stigma of Spermacoceae s.s.**

- 1 Inflorescence lax, thyrsoid or pleiothyrsoid; flowers pedicellate.....2
- Inflorescence mostly congested; sometimes thyrsoid or dichasial, and with sessile or subsessile flowers .....4
- 2 Fruit 4-carpellate; mericarps indehiscent ..... *Schwendenera*
- Fruit 2-carpellate; mericarps dehiscent .....3
- 3 Inflorescence umbellate, flowers homostylous; fruit with an apical extension of the carpel, longer than the length of the calyx lobes; seed strophiole longer than the seed length .....*Emmeorrhiza*
- Inflorescences thyrsoid, flowers heterostylous; fruit without an apical extension of the carpel; seed strophiole equal or shorter than the seed length ..... *Galianthe* subgen. *Galianthe*
- 4 Fruit indehiscent, dry or fleshy.....5
- Fruit completely or partially separated into 2–4 dry mericarps when mature.....7
- 5 Herbs prostrate, rooting at the nodes; flowers homostylous, corolla tube filiform, internally glabrous.....*Diodia*



- Subshrubs or small shrubs, erect, without roots at the nodes; flowers heterostylous, corolla tube funnel-shaped, internally with a fringe of moniliform hairs .....6
- 6 Plants without fetid smell; leaf blades linear or narrowly elliptic, 5–10 × 0.3 mm, coriaceous when dry; corolla 4–4.5 mm long; drupe reddish-yellow ..... *Tortuella*
- Plants with fetid smell; leaf blades ovate or elliptic, 40–110 × 25–30 mm long, papery when dry; corolla 1.5–2 mm long; capsule brown-colored..... *Tobagoa*
- 7 Ovary (2-) 3- or 4-carpellate; calyx lobes deciduous at fruit maturity..... *Richardia*
- Ovary 2-carpellate; calyx lobes persistent on the fruit.....8
- 8 Herbs rupicolous, with stems and leaves reddish; inflorescences heads axillary, 1-flowered; calyx lobes 0.15–0.17 mm long; corolla 1–1.2 mm long; style pubescent; nectariferous disc with scattered hairs ..... *Carajasia*
- Herbs or subshrubs, generally psammophilous, rarely rupicolous, with green stems and leaves, sometimes with reddish leaf margins; inflorescences heads terminal and axillary, mostly many-flowered; calyx lobes 0.5–10 mm long; corolla 2–15 mm long; style glabrous; nectariferous disc with glands.....9
- 9 Fruit with longitudinal dehiscence; calyx 4-lobed, lobes equal in size.....10
- Fruit with circumscissile or longitudinal-oblique dehiscence; calyx 2- or 4-lobed, when 4-lobed then with 2 lobes visibly smaller than the other 2 .....12
- 10 Mericarps indehiscent; inflorescences thyrsoid or dichasial with partial inflorescences congested..... *Galianthe* subgen. *Ebelia*
- Mericarps dehiscent; inflorescences partial inflorescences in glomerules.....11
- 11 Flowers heterostylous; fruit with intercarpelar septum persistent on the pedicel and mericarps caducous, strongly compressed parallel to the septum ..... *Psyllocarpus* sect. *Amazonica*
- Flowers homostylous; fruit with septum and mericarps persistent on the pedicel, without compression, commonly obovoid..... *Borreria* subsect. *Latifoliae*
- 12 Calyx lobes with 2 larger and 2 smaller lobes; fruits with circumscissile dehiscence; mature anthers usually yellow, rarely blackish ..... *Mitracarpus*
- Calyx lobes equal or subequal; fruits with longitudinal-oblique dehiscence in both carpels; mature anthers blue, creamy or whitish..... *Staelia*

## Molecular Study

The ingroup contains 14 genera represented by 35 species of the *Spermacoce* clade whereas the outgroup consists of *Bouvardia ternifolia* (Cavanilles 1797: 305) Schlechtendal (1853: 98) (*Spermacoceae s.l.*, see Groeninckx *et al.* 2009). The majority of the species were not molecularly analyzed before. Previously sequenced species are indicated in Appendix. ITS and ETS datasets were analyzed both separately and combined. Topologies for either method of analysis were highly similar. As a result the discussion is focused on the results of the combined ML analysis.

The results of the combined phylogenetic analyses show that *Carajasia* is sister to *Galianthe* with moderate to high support. In addition, *Carajasia* and *Galianthe* form a weakly to unsupported clade with *Schwendenera*. Most species of this clade have pollen grains with a double reticulum, which is associated with heterostylous flowers (except for *Carajasia* flowers that are homostylous). The clade consisting of *Galianthe*, *Carajasia* and *Schwendenera* is sister to the remaining genera of the *Spermacoce* clade analyzed in this study (Fig. 6).

Based on present results, most clades coincide with currently accepted genera: *Crusea*, *Emmeorrhiza*, *Ernodea*, *Diodia s.s.* (sensu Bacigalupo & Cabral 1999), *Mitracarpus*, *Psyllocarpus*, *Richardia* and *Staelia*. *Diodella*, *Borreria* and *Spermacoce* however are bi- or polyphyletic.

*Diodella* species, as delimited by Bacigalupo & Cabral (2006), fall into two clades. Whereas *D. apiculata* (Willdenow 1818: 531) Delprete (2004: 169–174), *D. teres* Small (1913b: 271) and *D. crassifolia* (Bentham 1845: 108) Borhidi (2006: 186) form a well-supported clade on their own (here referred to as *Diodella teres* Group), *D. sarmentosa* (Swartz 1788: 30) Bacigalupo & Cabral (2006: 100) appears as a well supported sister group of the genus *Ernodea*. In this study, *Ernodea* is represented by *E. taylori* Britton (1908: 208) and *E. littoralis* Swartz (1788: 26), which form a strongly supported clade. Although the *Diodella sarmentosa*—*Ernodea* clade is sister to the *Diodella teres* group, this relationship is only very weakly supported (Fig. 6).

*Mitracarpus* is represented by only two species, *M. megapotamicus* (Sprengel 1827: 40) Kuntze (1931: 331) and *M. carnosus* Borhidi & Lozada-Pérez (2007: 34–36), and as expected the two taxa fall together in a single moderate to well supported clade. The relationship of *Mitracarpus* with the other genera of the *Spermacoce* clade remains largely unresolved (Fig. 6, 7).

The species assigned to *Borreria* by Bacigalupo & Cabral (1996) are divided over three different clades intermingled with other morphologically well-defined genera: 1) the clade of *Borreria latifolia* (Aublet 1775: 55–57) Schumann (1888: 61), *B. alata* (Aublet 1775: 60–61) Candolle (1830: 544) and *B. schumannii* (Standley ex Bacigalupo) Cabral & Sobrado (Cabral *et al.* 2011: 266), which is well supported (100/1000/1) and composed of three species with bifid



stigmas; 2) *Spermacoce dibrachiata* Oliver (1873: 87) falls into a clade comprising five American species of *Borreria* [*Borreria virgata* Chamisso & Schlechtendal (1828: 324), *B. capitata* (Ruiz & Pavon 1798: 61) Candolle (1830: 545), *B. tenella* (Kunth 1818: 345) Chamisso & Schlechtendal (1828: 317), *B. dasycephala* (Chamisso & Schlechtendal 1828: 348–350) Bacigalupo & Cabral (1996: 306) and *B. verticillata* (Linnaeus 1753: 102) Meyer (1818: 83)], and all these species have exerted stamens and style and bilobed stigma; 3) the strongly supported clade of *Borreria eryngioides* Chamisso & Schlechtendal (1828: 316), *B. prostrata* (Aublet 1775: 58) Miquel (1850: 177–178) and *B. incognita* E.L. Cabral (Bacigalupo & Cabral 1996: 322) characterized by stamens and style included and stigma shortly bilobed and Pollen type 1 (see Pire 1996 and Dessein *et al.* 2002). As for *Spermacoce*, its species fall into three different clades: 1) the African *Spermacoce dibrachiata*, as mentioned above, is part of the clade mainly constituted by American *Borreria* species; 2) *Spermacoce tenuior* Linnaeus (1753: 102), *S. confusa* Rendle (1936: 12) and *S. glabra* Michaux (1803: 82) form a well-supported clade on their own; 3) the Australian *Spermacoce breviflora* Mueller ex Benthham (1867: 442) is sister to *Borreria eryngioides*, *B. prostrata* and *B. incognita* thereby forming a well-supported clade.

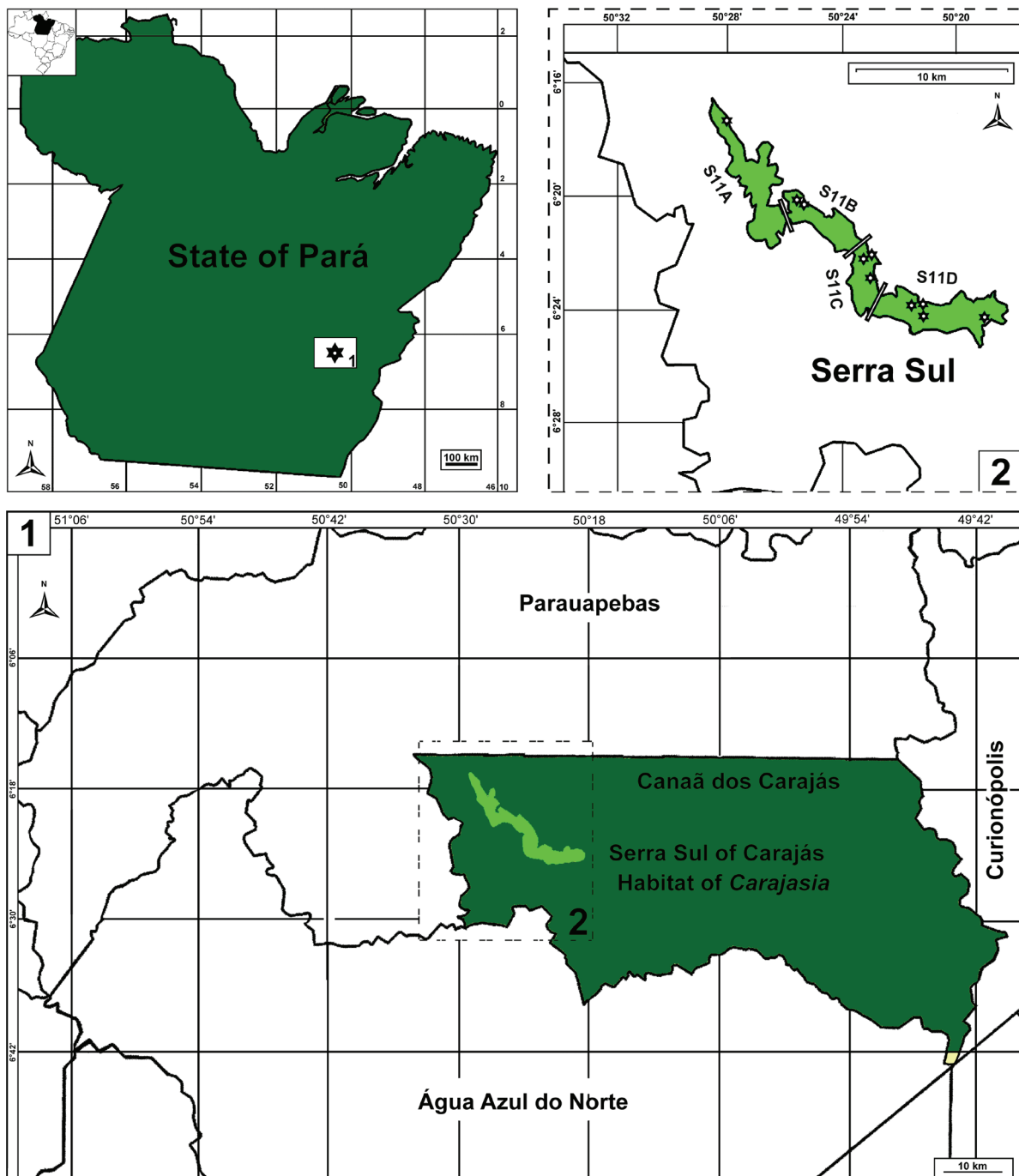


FIGURE 5. Geographic distribution of *Carajasia cangae* with collections represented by stars.

## Discussion

Morphological characters, such as flowering branches with two axillary flowers, the presence of homostylous flowers associated with double reticulum of the pollen, a deeply bifid stigma, a pubescent style and nectariferous disc with triangular papillae, provide sufficient support to describe the new genus *Carajasia*. Additionally, molecular analyses also support the recognition of this new genus as closely related with the only two genera of the *Spermacoce* clade with double-reticulate pollen grains included in our analysis, i.e. *Galianthe* and *Schwendenera* (Fig. 6, 7). Homostylous flowers associated with a double reticulum are also present in the Amazonian *Diodia spicata* Miquel (1850: 179–180) (Dessein 2003). This species differs from *Carajasia* in having a taller habit, larger leaves, a 3–12 flowered inflorescence, and dry fruits that longitudinally split from the base into two indehiscent mericarps. Based on these morphological features, and also supported by molecular analyses, Dessein (2003) proposed to transfer *Diodia spicata* into *Galianthe*. The monospecific genus *Tobagoa* (of which no molecular data is available however) also shares a bifid stigma and double reticulum, yet differs in having a taller habit, larger leaves, many-flowered inflorescences, heterostylous flowers and an indehiscent capsule. The morphological comparison of *Carajasia* and these related taxa are summarized in table 1.

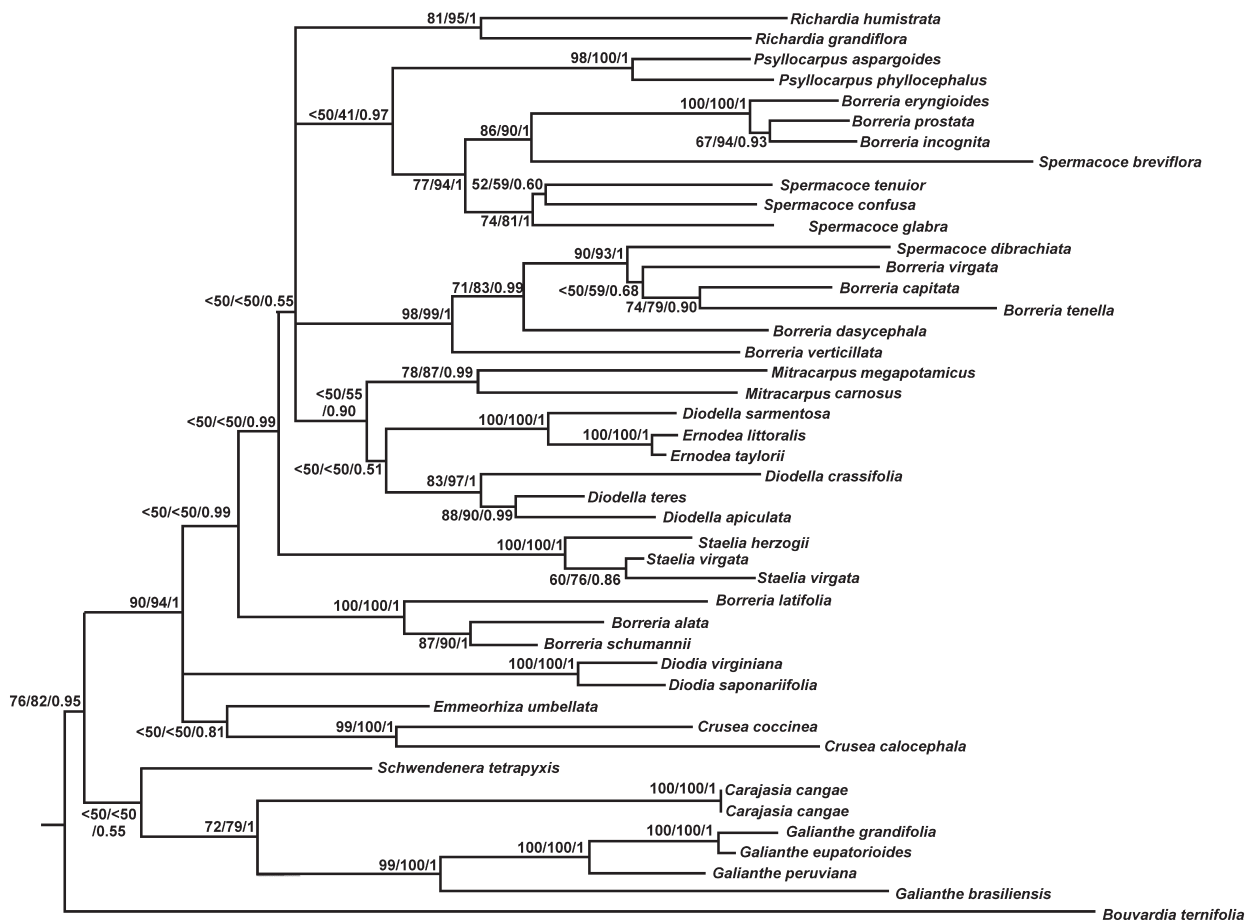
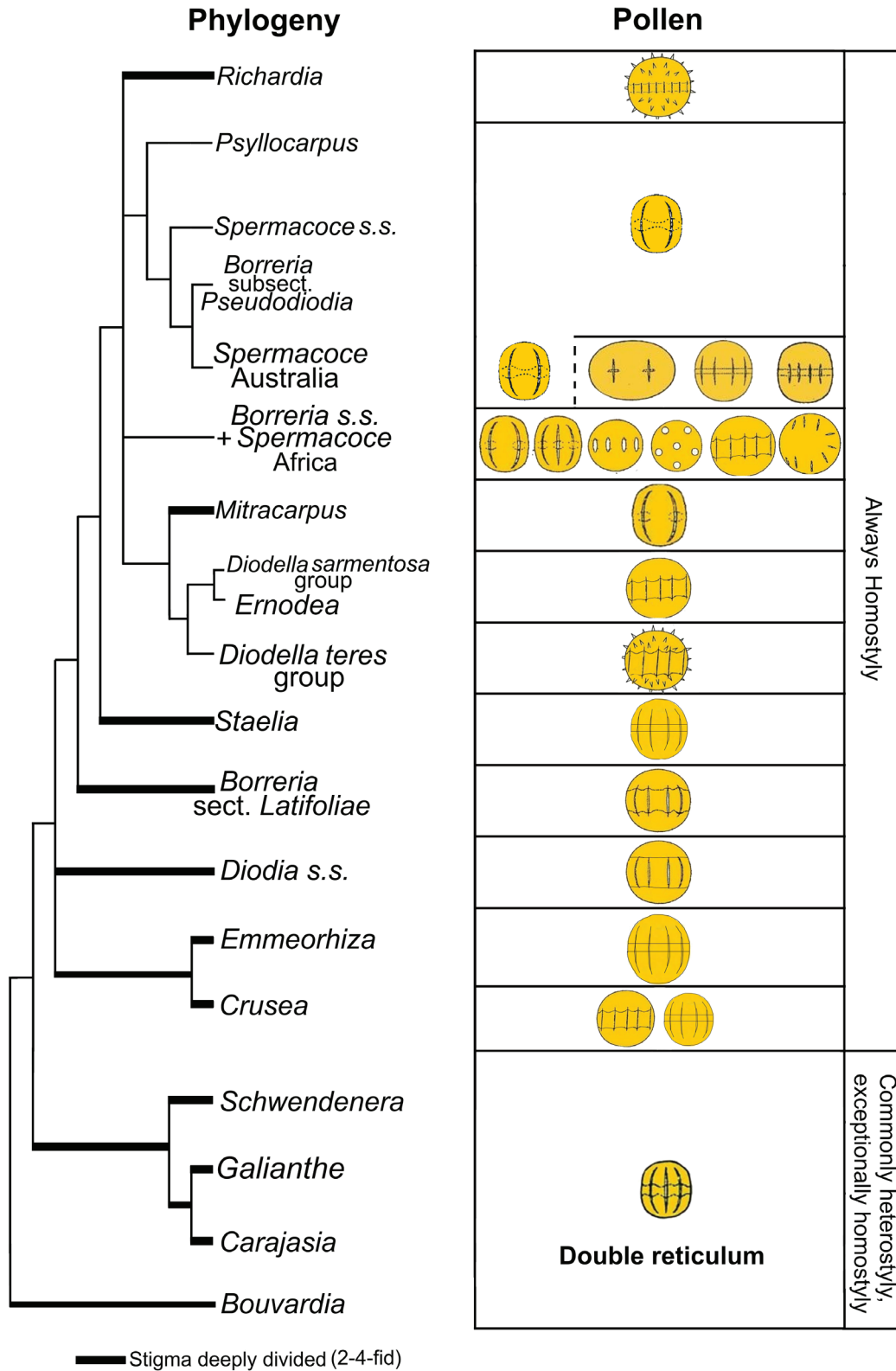


FIGURE 6. Phylogenetic tree showing the relationship of *Carajasia* with the remaining genera of *Spermacoce* clade.

Even though the topology is rather well resolved for the most early diversified nodes, one needs to remain cautious in drawing conclusions about relationships between taxa due to the rather low number of species analyzed per genus, especially for the most species-rich lineages (i.e. *Borreria*, *Diodella* and *Spermacoce*). Nevertheless, morphological characters can be used to interpret the phylogenetic position of *Carajasia*: heterostylous vs. homostylous flowers, stigma deeply divided vs. 2-lobed stigma, pollen with bireticulate exine vs. other type of exine ornamentations (Fig. 7). As a result, heterostylous flowers and bireticulate exine may be considered as plesiomorphic characters in *Schwendenera* and *Galianthe*, whereas *Carajasia* shows a combination of homostylous flowers and bireticulate pollen exine. With respect to the stigma, the bifid stigma also occurs in the heterostylous genera and in some lineages basal



to homostylous genera [*Emmeorrhiza*, *Diodia* (sensu Bacigalupo), *Borreria* subsect. *Latifoliae* (e.g. *Borreria latifolia*) and *Staelia*]. In *Schwendenera*, the 3- or 4-lobed stigma is only shared by the homostylous genus *Richardia*, being one of the most recently diversified genera in the analysis. Furthermore, *Crusea* and *Mitracarpus* are rather exceptional as the first lineage is characterized by a bilobed stigma yet being sister to *Emmeorrhiza*, and the second lineage having a bifid stigma but being positioned as sister to *Diodella-Ernodea* group (the latter characterized by a bilobed or subcapitate stigma).



**FIGURE 7.** Comparison between phylogenetic results with pollinic and morphologic data. The same tree of the figure 7 with the replacement of the terminals by its taxonomic equivalent (genera or infrageneric taxa) or informal groups (as *Diodella teres* group).

**TABLE 1. Morphologically distinctive characters of *Carajasia*, *Diodia spicata*, *Galianthe*, *Schwendenera* and *Tobagoa*.**

	<i>Carajasia</i>	<i>Diodia spicata</i>	<i>Galianthe</i>	<i>Schwendenera</i>	<i>Tobagoa</i>
Stature (cm)	2–10	100–150	30–250	30–80	30–130
Leaves (shape and size, mm)	Spatulate, 1.8–2.1 x 0.5–0.7	Elliptic, 30–100 x 10–30	Linear, elliptic or oblong, 5–130 x 2.5–40	Elliptic or ovate, 10–25 x 5–12	Elliptic or oblong, 50–110 x 8–30
Inflorescence	Flowering branch with axillary flower heads, with 2 flowers per node	Flowering branch with axillary flower heads, 3–20 flowered, spicate	Terminal thyrsoïd or pleiothyrsoïd many-flowered	Flowering branch with axillary flower heads,, 9–11 flowered	Flowering branch with axillary flower heads,, 8–20 flowered
Floral pedicel, (long)	Absent	Absent	Present, mostly long pedicellate, rarely absent	Present (0.5–1.5 mm)	Absent
Flowers	homostylous	homostylous	Heterostylous, rarely homostylous	heterostylous	heterostylous
Corolla (mm long)	1–1.2	1.2–1.3	2.5–7	2.5–3	2–3
Nectariferous disc	2-lobed	2-partite	Entire, 2-lobed and 2-partite	Entire	2-lobed
Nectary surface	Papillose, with some triangular papillae larger than the rest	Uniformly papillose	Uniformly papillose, rarely with some cylindrical papillae near the style insertion	Uniformly papillose	Uniformly papillose
Fruit dehiscence	Septicidal, into 2 indehiscent mericarps separating from the top.	Septicidal, into 2 indehiscent mericarps separating from the base	Septicidal and loculicidal, into 2 dehiscent (subgen. <i>Galianthe</i> ) or indehiscent carpels (subgen. <i>Ebelia</i> ).	Septicidal, into 4 indehiscent mericarps	Indehiscent
Pollen	5–7 colpi, bireticate, supracolpate incomplete	7-colpate, bireticate, both reticula complete	6–10 colpi, bireticate, rarely supracolpate incomplete or simple and microreticulate	7–8 colpi, bireticate, both reticula complete	5–7 colpi, bireticate, both reticula complete
Geographic distribution	Pará, Brazil, endemic	Brazil, Colombia, French Guiana, Panamá, Suriname and Venezuela	Central and South America	São Paulo and Paraná, Brazil	Mesoamerica, Colombia, Venezuela and Tobago

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## Appendix

List of taxa used in the molecular phylogenetic analysis with voucher information (geographical origin, collector, collector number, herbarium, ITS and ETS accession number) and GenBank accession numbers.

Ingroup. *Borreria* G. Mey. *B. alata* (Aubl.) DC., Brazil, Goiás, *Queiroz et al. 14105* (CTES, HUEFS; KF736995, KF737036); *B. dasycephala* (Cham. & Schltdl.) Bacigalupo & E.L. Cabral, Argentina, Misiones, *Salas & Cabaña 388* (CTES; ITS KF73699); *B. capitata* (Ruiz & Pav.) DC., Brazil, Goiás, *Queiroz et al. 14261* (CTES, HUEFS; KF736990, KF737032); *B. eryngioides* Cham. & Schltdl., Argentina, *Salas et al. 378* (CTES; KF736992, KF737033); *B. incognita* E.L. Cabral., Brazil, Goiás, *Queiroz et al. 14049* (CTES, HUEFS; KF736993, KF737034); *B. latifolia* (Aubl.) K. Schum., Brazil, Goiás, *Queiroz et al. 14110* (CTES, HUEFS; KF736994, KF737035); *B. prostrata* (Aubl.) Miq., Brazil, Goiás, Nova Roma, *Queiroz et al. 14083* (CTES, CTES; KF736996, KF737037); *B. schumannii* (Standl. ex Bacigalupo) E.L. Cabral & Sobrado, Argentina, Misiones, *Cabral et al. 760* (CTES; KF736997, KF737038); *B. tenella* (Kunth) Cham. & Schltdl., Brazil, *Queiroz et al. 14252* (CTES, HUEFS; KF736988, KF737030); *B. verticillata* (L.) G. Mey., Argentina, Corrientes, *Salas 402* (CTES; KF736998, KF737039); *B. virgata* Cham. & Schltdl., Brazil, Bahia, *Queiroz et al. 13688* (CTES, HUEFS; KF736989, KF737031). *Carajasia*, *C. cangae*, Brazil, Pará, *Costa et al. 588* (BHCB; KF737015, KF737057); *Giorni et al. 179* (BHCB; KF737016, KF737058). *Crusea* Cham. & Schltdl., *C. calocephala* DC., Mexico, Oaxaca, *Ochoterena et al. 456* (BR; KF737009, KF737051); *C. coccinea* DC., Mexico, Oaxaca, *Ochoterena et al. 461* (BR; KF737010, KF737052). *Diodella* Small. *D. apiculata* (Willd. ex Roem. & Schult.) Delprete, Brazil, Bahia. *Queiroz et al. 13727* (CTES, HUEFS; KF737003, KF737045). *D. crassifolia* (Benth.) Borhidi, Mexico, *Rzedowski 17792* (NY; KF737004, KF737046). *D. sarmentosa* (Sw.) Bacigalupo & E.L. Cabral, Cameroon, *Dessein et al. 1521* (BR; KF737005, KF737047). *D. teres* (Walter) Small., Brazil, Goiás, *Queiroz et al. 14089* (CTES, HUEFS; KF737048, KF737006). *Diodia* L. *D. saponariifolia* Cham. & Schltdl., Argentina, Misiones, *Cabaña & Salas 22* (CTES; KF737007, KF737049). *D. virginiana* L., USA, Missouri, *Taylor 12758* (MO; KF737008, KF737050). *Emmeorrhiza* Pohl ex Endl. *E. umbellata* (Spreng.) K. Schum., Brazil, Bahia, *Queiroz et al. 13746* (CTES, HUEFS; KF737000; KF737042). *Ernodea* Sw. *E. littoralis* Sw., Cuba, Habana, *Rova et al. 2286* (GB; KF737001, KF737043). *E. taylori* Britton, North Bimini, *Correll 44186* (NY; KF737002, KF737044). *Galianthe* Griseb. *G. brasiliensis* (Spreng.) E.L. Cabral & Bacigalupo, Argentina, Misiones, *Cabral et al. 758* (CTES; KF737011, KF737053). *G. eupatorioides* (Cham. & Schltdl.) E.L. Cabral, Brazil, Goiás, *Queiroz et al. 14190* (CTES, HUEFS; KF737012, KF737054). *G. grandifolia* E.L. Cabral, Brazil, Distrito Federal, *Queiroz et al. 14015* (CTES, HUEFS; KF737013, KF737055). *G. peruviana* (Pers.) E.L. Cabral, Brazil, Minas Gerais, Belo Horizonte *Salas et al. 408* (BHCB, CTES; KF737014, KF737056). *Mitracarpus* Zucc. *M. carnosus* Borhidi & Lozada-Pérez, Mexico, Oaxaca, *Ochoterena et al. 516* (BR; KF736999, KF737040). *M. megapotamicus* (Spreng.) Kuntze, Argentina, Corrientes, *Salas & Cabaña 399* (CTES; ETS KF737041). *Psyllocarpus* Mart. & Zucc. *P. asparagoides* Mart. ex Mart. & Zucc., Brazil, Minas Gerais, Itacambira, *Salas et al. 411* (BHCB, CTES; KF737018, KF737060). *P. phyllocephallus* K. Schum., Brasil, Distrito Federal, *Queiroz & al. 14016* (CTES; ETS KF737061). *Richardia* L. *R. grandiflora* (Cham. & Schltdl.) Steud., Brazil, Bahia, Nova Roma, *Queiroz et al. 14055* (CTES, HUEFS; KF737027, KF737066). *R. humistrata* (Cham. & Schltdl.) Steud., Argentina, Misiones, Bernardo de Irigoyen, *Cabaña & Salas 17* (CTES; KF737028, KF737067). *Schwendenera* K. Schum. *S. tetrapyxis* K. Schum., Brazil, Paraná, *Marques et al. 83* (CTES; KF737017, KF737059). *Spermacoce* L. *S. breviflora* F. Muell ex Benth., Australia, *Harwood 1070* (BR; KF737019, KF737062). *S. confusa* Rendle, Mexico, *Ochoterena et al. 552* (BR; KF737020, KF737063). *S. dibrachiata* Oliv., Zambia, *Dessein et al. 626* (BR; ITS KF737021). *Spermacoce glabra* Michx., USA, Missouri, Perry, *Taylor 12757* (MO; KF737022, KF73706). *S. tenuior* L., México, *Novelo et al. s/n* (BR; KF737023, KF737065). *Staelia* Cham. & Schltdl. *S. herzogii* (S. Moore) R.M. Salas & E.L. Cabral, Bolivia, Santa Cruz, *Soto et al. 1053* (CTES, USZ; ITS KF737024). *S. virgata* (Link ex Roem. & Schult.) K. Schum., Brazil, Bahia, *Salas et al. 423* (CTES, HUEFS; ITS KF737025); Brasil, Piauí, *Salas et al. 443* (CTES, HUEFS; ITS KF737026). Outgroup. *Bouvardia* Salisb. *B. ternifolia* (Cav.) Schltdl., Mexico, Oaxaca, *Ochoterena et al. 454* (BR; KF736987, KF737029).