Morphological and molecular data confirm the transfer of homostylous species in the typically distylous genus *Galianthe* (Rubiaceae), and the description of the new species *Galianthe vasquezii* from Peru and Colombia

Javier Elias Florentin ^{Corresp., 1} , **Andrea Alejandra Cabaña Fader** ¹ , **Roberto Manuel Salas** ¹ , **Steven Janssens** ² , **Steven Dessein** ² , **Elsa Leonor Cabral** ¹

1 Herbarium CTES, Instituto de Botánica del Nordeste Corrientes Argentina, Corrientes, Corrientes, Argentina

2 Plant systematic, Botanic Garden Meise, Meise, Belgium

Corresponding Author: Javier Elias Florentin
Email address: florentinjaviere@gmail.com

Galianthe (Rubiaceae) is a neotropical genus comprising 50 species divided into two subgenera, *Galianthe* subgen. *Galianthe,* with 39 species and *Galianthe* subgen. *Ebelia*, with 11 species. The diagnostic features of the genus are: usually erect habit with xylopodium, distylous flowers arranged in lax thyrsoid inflorescences, bifid stigmas, 2-carpellate and longitudinally dehiscent fruits, with dehiscent valves or indehiscent mericarps, plump seeds or complanate with a wing-like strophiole, and pollen with double reticulum, rarely with a simple reticulum. This study focused on two species that were originally described under *Diodia* due to the occurrence of fruits indehiscent mericarps: *Diodia palustris* and *D. spicata*. In the present study, classical taxonomy is combined with molecular analyses. As a result, we propose that both *Diodia* species belong to *Galianthe* subgen. *Ebelia*. The molecular position within *Galianthe*, based on ITS and ETS sequences, has been supported by the following morphological characters: thyrsoid, spiciform or cymoidal inflorescences, bifid stigmas, pollen grains with a double reticulum, and indehiscent mericarps. However, both species, unlike the remainder of the genus *Galianthe*, have homostylous flowers, so the presence of this type of flower significantly modifies the generic concept. In this framework, a third homostylous species, *Galianthe vasquezii*, from the Andean region is also described. Until now, this species remained cryptic under specimens of *Galianthe palustris* It differs however from the latter by having longer calyx lobes, the presence of dispersed trichomes inside the corolla lobes (vs. glabrous), fruits that are acropetally dehiscent (vs. basipetally dehiscent), and its Andean geographical distribution (vs. Paranaense). Additionally, a lectotype has been chosen for *Diodia palustris*, *Borreria pterophora* has been placed under synonymy of *Galianthe palustris*, and *Galianthe boliviana* is reported for the first time from Peru. A key of all

Galianthe species with indehiscent mericarps is also provided.

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2   **typically distylous genus *Galianthe* (Rubiaceae), and the description of the new species**

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4   **Javier E. Florentín[1], Andrea A. Cabaña Fader[1], Roberto M. Salas[1], Steven Janssens[2], Steven**

5   **Dessein[2] and Elsa Cabral[1,]**

6   [1]Herbarium CTES, Instituto de Botánica del Nordeste, Corrientes, Argentina

7   [2]Plant systematic, Botanic Garden Meise, Meise, Belgium

8   Author for correspondence:

9   Javier E. Florentín[1]

10 Email address: florentinjaviere@gmail.com

11 **ABSTRACT**

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13 *Galianthe* subgen. *Galianthe,* with 39 species and *Galianthe* subgen. *Ebelia*, with 11 species. The

14 diagnostic features of the genus are: usually erect habit with xylopodium, distylous flowers

15 arranged in lax thyrsoid inflorescences, bifid stigmas, 2-carpellate and longitudinally dehiscent

16 fruits, with dehiscent valves or indehiscent mericarps, plump seeds or complanate with a wing-

17 like strophiole, and pollen with double reticulum, rarely with a simple reticulum. This study

18 focused on two species that were originally described under *Diodia* due to the occurrence of

19 fruits indehiscent mericarps: *Diodia palustris* and *D. spicata*. In the present study, classical

20 taxonomy is combined with molecular analyses. As a result, we propose that both *Diodia* species

21 belong to *Galianthe* subgen. *Ebelia*. The molecular position within *Galianthe*, based on ITS and

22 ETS sequences, has been supported by the following morphological characters: thyrsoid,

23 spiciform or cymoidal inflorescences, bifid stigmas, pollen grains with a double reticulum, and

24 indehiscent mericarps. However, both species, unlike the remainder of the genus *Galianthe*, have

25 homostylous flowers, so the presence of this type of flower significantly modifies the generic

26 concept. In this framework, a third homostylous species, *Galianthe vasquezii*, from the Andean

27 region is also described. Until now, this species remained cryptic under specimens of *Galianthe*

28 *palustris* It differs however from the latter by having longer calyx lobes, the presence of

29 dispersed trichomes inside the corolla lobes (vs. glabrous), fruits that are acropetally dehiscent

30 (vs. basipetally dehiscent), and its Andean geographical distribution (vs. Paranaense).

31 Additionally, a lectotype has been chosen for *Diodia palustris*, *Borreria pterophora* has been

32   placed under synonymy of *Galianthe palustris*, and *Galianthe boliviana* is reported for the first

33   time from Peru. A key of all *Galianthe* species with indehiscent mericarps is also provided.

34   **INTRODUCTION**

35   *Galianthe* Griseb. is a neotropical genus belonging to tribe Spermacoceae (*Groeninckx et al.,*

36   *2009a*). The genus was revised by *Cabral* (*2002*) and divided into two subgenera (*Cabral &*

37   *Bacigalupo, 1997*): *Galianthe* subgen. *Galianthe*, from South America with 39 species, and

38   *Galianthe* subgen. *Ebelia* (Rchb.) E.L. Cabral & Bacigalupo, with 11 Mesoamerican and South

39   American species. Historically, *Galianthe* was associated with *Diodia* L., which has been

40   described based on only *D. virginiana* L. The type species has a palustrine habit, pauciflorous

41   axillary inflorescences, filiform corolla tube, bifid style with two long filiform stigmatic lobes,

42   and indehiscent fruits. Later, others authors (i.e. *Swartz 1788; Walter 1788, Candolle 1830, Small*

43   *1913*) added other species into this genus with diverse kinds of habits and inflorescences,

44   different floral morphology (e.g. distyly or homostyly, infundibuliform or campanulate corollas,

45   bifid or bilobate stigma), and 2-carpellate schizocarpic fruits, being currently comprised by ca.

46   180 names (called *Diodia s. lat.*). Later, *Bacigalupo & Cabral* (*1999*) revised the genus *Diodia*

47   and maintained only five species that were morphologically similar to the type species *D.*

48   *virginiana* L. (description as above, and constituting *Diodia s. str.*). Species that did not match

49   with these diagnostic features, were transferred to other genera as follows: eight species to

50   *Borreria* subgen. *Dasycephala* (DC.) Bacigalupo & E.L. Cabral (Bacigalupo & Cabral, 1996), 12

51   species to *Hexasepalum* Bartl. ex DC. (*Kirkbride 2014; Kirkbride & Delprete, 2015; Cabaña*

52   *Fader et al., 2016*), and 4 species to *Galianthe* subgen. *Ebelia* (*Cabral & Bacigalupo, 1997*). The

53   four *Galianthe* species are distylous, *Diodia bogotensis* (Kunth) Cham. & Schltdl. [= *Galianthe*

54   *bogotensis* (Kunth) E.L. Cabral & Bacigalupo]; *D. brasiliensis* Spreng. [= *G. brasiliensis*

55   (Spreng.) E.L. Cabral & Bacigalupo]; *D. cymosa* Cham. [=*G. cymosa* (Cham.) E.L. Cabral &

56   Bacigalupo], and *D. hispidula* A. Rich. ex DC. [=*G. hispidula* (A. Rich. ex DC.) E.L. Cabral &

57   Bacigalupo]. The remaining species with an uncertain position (ca. 150 names) are currently

58   under revision (*Cabaña Fader com. pers.*). In this sense, *Bacigalupo and Cabral* (*1996, 1998*)

59   transferred these species to *Borreria* subgen. *Dasycephala* because of their homostylous flowers

60   and indehiscent mericarps, while *Delprete et al. (2005)* and *Delprete (2007),* alluding to a broad

61   concept, transferred the two species to *Spermacoce* mainly based on fruit characters. *Dessein*

62   (*2003*) informally proposed to consider *Diodia spicata* as part of *Galianthe* based on molecular

63   data (ITS intron), palynological data (double reticulum), and fruit morphology. The aim of this

64 work is to confirm the taxonomic position of *D. palustris* and *D. spicata* based on morphological

65 and molecular data, and perform their formal combination in *Galianthe*. In addition, a third

66 homostylous species (*Galianthe vasquezii* R.M Salas & J. Florentín) is described and illustrated

67 based on specimens from Colombia and Peru (previously identified as *D. palustris)*. Additionally,

68 a lectotype has been chosen for *Diodia palustris* whereas *Borreria pterophora* has been placed

69 under synonymy of *Galianthe palustris*. Moreover *Galianthe boliviana* E.L. Cabral is for the

70 first time recorded in Peru. Finally, we provided a distribution map for the species investigated in

71 this study, as well as a dichotomous key for all taxa with indehiscent mericarps.

72 **MATERIALS AND METHODS**

73 **Morphological Study**

74 This study is based on classical taxonomy techniques. Collections deposited at the BA, BHCB,

75 CEPEC, CTES, ESA, FUEL, FPS, FURB, HAS, HOXA, HUT, IAC, IAN, IFFSC, IPA, K, LIL,

76 MBM, MO, NY, P, PR, SI, SP, UB, UFRN, USB, US, USM and UEC herbaria were analysed.

77 Furthermore, the databases of the 'Catálogo de plantas e fungos do Brazil' and 'Missouri

78 Botanical Garden' were examined. In order to carry out scanning electron microscope (SEM)

79 analyses, flowers were dehydrated using a graded series of ethanol solutions and afterwards

80 critically point dried and sputter-coated with gold-palladium. SEM images were obtained with a

81 JEOL 5800 LV scanning electron microscope. Pollen grains were acetolyzed according to

82 *Erdtman* (1966) and mounted in glycerine jelly for analysis by light microscopy (LM).

83 Conventional parameters (P = polar axis, E = equatorial axis) of at least 20 grains were measured

84 under LM, and the exine was analyzed using SEM. Pollen terminology follows *Punt et al.*

85 *(2007).* Species distribution maps were generated from distribution data that was present on the

86 herbarium labels for each specimen and subsequently georeferenced using *Google Earth* (*2015*)

87 and *DIVA-GIS* (*2013*).

88 "The electronic version of this article in Portable Document Format (PDF) will represent a

89 published work according to the International Code of Nomenclature for algae, fungi, and plants

90 (ICN), and hence the new names contained in the electronic version are effectively published

91 under that Code from the electronic edition alone. In addition, new names contained in this work

92 which have been issued with identifiers by IPNI will eventually be made available to the Global

93 Names Index. The IPNI LSIDs can be resolved and the associated information viewed through

94 any standard web browser by appending the LSID contained in this publication to the prefix

95   "http://ipni.org/". The online version of this work is archived and available from the following

96   digital repositories: PeerJ, PubMed Central, and CLOCKSS".

97   **Molecular study**

98   In total, 45 species (47 accessions) were included to infer the phylogenetic relationship of *Diodia*

99   *palustris* and *D. spicata*. The ingroup contains species from the *Borreria*, *Carajasia* R.M. Salas,

100   E.L. Cabral & Dessein*, Crusea* Cham. & Schltdl.*, Diodia, Emmeorhiza* Pohl ex Endl.*, Ernodea*

101   Sw.*, Galianthe, Hexasepalum, Mitracarpus* Zucc.*, Psyllocarpus* Mart. & Zucc.*, Richardia* L.,

102   *Schwendenera* K. Schum.*, Spermacoce*, and *Staelia* Cham. & Schldtl. genera, and *Bouvardia*

103   *ternifolia* (Cav.) Schltdl. as the outgroup. Leaf samples of these studies were obtained from silica

104   gel-dried material or herbarium materials. Forty-three species (44 accessions) were previously

105   used by *Salas et al.* (*2015*). Four accessions belonging to *D. palustris* has been added. All studied

106   species with geographical information, collector, herbarium and GenBank accession numbers are

107   provided in Appendix.

108   **Molecular Protocols**

109   Total genomic DNA was isolated from silica-dried leaf material using a modified CTAB protocol

110   (*Doyle and Doyle 1987*). Nuclear ribosomal ETS and ITS fragments were amplified following

111   *Baldwin and Markos* (*1998*) and *NegrónOrtiz and Watson* (*2002*), and *White et al.* (*1990*),

112   respectively. PCR reactions for both gene markers investigated in this study consisted of 2 min

113   initial denaturation at 94 °C and 30 cycles of 30 s denaturation at 94 °C, 30 s primer annealing at

114   primer specific temperature and 1 min extension at 72 °C. Primer annealing for ETS and ITS

115   were at 47°C and 48°C respectively. Amplification reactions were carried out on a GeneAmp

116   PCR system 9700 (Applied Biosystems). Purified amplification products were sent to Macrogen,

117   Inc. (Seoul, South Korea) for sequencing. Sequences obtained in this study were deposited at

118   GenBank [*Diodia palustris, Verdi et al. 1905*, ETS (MF166824), ITS (MF166826); *Miguel et al.*

119   *19*, ETS (MF166825), ITS (MF166827).

120   **Phylogenetic analyses**

121   Contiguous sequences were assembled using Geneious v7.0.6 (Biomatters, New Zealand).

122   Automatic alignments were carried out with MAFFT (*Katoh et al., 2002*) Subsequent manual

123   finetuning of the aligned dataset was done in Geneious v7.0.6. Congruency between the different

124   datasets was inferred using different methods. First, a series of incongruence length difference

125 tests (*ILD*; *Farris et al., 1995*) were carried out with PAUP* v.4. 0b10 (*Swofford, 2003*) using the

126 following parameters: simple taxon addition, TBR branch swapping and heuristic searches of

127 1000 repartitions of the data. Despite the well-known sensitivity of the ILD test (*Barker and*

128 *Lutzoni, 2002*), the results of this test were compared in light of the resolution and support values

129 of the obtained nuclear and nuclear ribosomal topologies. As a result, possible conflict between

130 data matrices was visually inspected, searching for conflicting relationships within each topology

131 that are strongly supported (hard vs. soft incongruence; *Johnson and Soltis, 1998).* Model

132 selection for the Bayesian inference analysis was conducted with ModelTest 3.06 (*Posada &*

133 *Crandall 1998*) under the Akaike Information Criterium (AIC). The GTR+G model was selected

134 for both ITS and ETS. Bayesian analyses of the concatenated dataset were carried out with

135 MrBayes 3.1 *(Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003)*. Four chains (one

136 cold, three heated), initiated from a random starting tree were run simultaneously for 10 million

137 generations. Every 1000 generations, a tree was sampled from the chain for a total of 10,000

138 trees. Due to the burn-in, 50% of the sample points were discarded. Convergence of the chains

139 was examined with TRACER 1.4 (*Rambaut & Drummond 2007*). This resulted in an effective

140 sampling size (ESS) parameter exceeding 100, which assumes a sufficient sampling and

141 acceptable mixing.

142 **RESULTS**

143 **Phylogenetic results**

144 The ingroup contains 14 genera represented by 45 species of the *Spermacoce* clade. Of these*,*

145 *Diodia spicata* and *D. palustris* are analysed for the first time in this context. ITS and ETS

146 datasets were analysed both separately and combined. Because topology of each gene marker is

147 very similar, we only present the results of the combined analysis (Fig. 1). Current results

148 indicate that most clades coincide with most currently accepted genera (e.g. *Crusea,*

149 *Emmeorhiza, Ernodea, Diodia s.s.* (sensu *Bacigalupo & Cabral, 1999*), *Mitracarpus,*

150 *Psyllocarpus, Richardia* and *Staelia*). *Spermacoce*, *Borreria* and *Hexasepalum* however, appear

151 unresolved or as polyphyletic genera being present in several different parts of the tree. In regard

152 of the species studied, we found that *G. spicata* and *G. palustris* fall intermingled among the

153 *Galianthe* species. The *Galianthe* clade, including both former *Diodia* species, is strongly

154 supported (Bayesian Posterior Probability (BBP): 100). The genus is divided into two strongly

155 supported subclades, one subclade consists of *G. grandifolia* E.L. Cabral, *G. eupatorioides*

156 (Cham. & Schltdl.) E.L. Cabral, and *G. peruviana* (Pers.) E.L. Cabral (BPP: 100), all from *G.*

157 subgen. *Galianthe*. The other subclade (BPP: 99) comprises *G. spicata*, *G. palustris*, and *G.*

158 *brasiliensis* (type species of *Galianthe* subgen. *Ebelia*). The clade of *Galianthe* and *Carajasia* is

159 only moderately supported (BBP: 79). The genera *Galianthe* and *Carajasia* share the occurrence

160 of pollen grains with a double reticulum, mostly associated with the distyly and bifid stigma. The

161 *Galianthe-Carajasia* clade forms an unsupported trichotomy with *Schwendenera* (also distylous)

162 and the remaining genera of the *Spermacoce* clade (all homostylous species never associated to

163 double reticulum pollen grains). Interestingly, all clades that coincide with generic concepts are

164 strongly supported [e.g. *Psyllocarpus* (BBP:99), *Spermacoce s.s.* (BBP:100), *Richardia*

165 (BBP:100), *Borreria s.s.* (BBP:100), *Mitracarpus* (BBP:97), *Hexasepalum s.s., Staelia* (BBP:99),

166 *Diodia s.s.* (BBP:100), *Borreria latifolia* group (BBP:100), and *Crusea* (BBP:100)]. The species

167 assigned to *Borreria* (sensu *Bacigalupo & Cabral, 1996*) are divided into two clades that are

168 intermingled with other morphologically well-defined genera. One of these clades, further

169 referred to as the *Borreria latifolia* group, comprises *Borreria alata*, *B. schumannii*, and *B.*

170 *latifolia* (BBP:100). The other clade comprises five *Borreria* species from North and South

171 America (*B. capitata*, *B. multibracteata*, *B. tenella*, *B. dasycephala*, and *B. verticillata*), as well

172 as the African *Spermacoce dibrachiata* (BBP: 100). *Spermacoce* is divided into two unrelated

173 branches, of which one clade comprises the type species *S. tenuior,* other American species with

174 similar flower morphology (*S. eryngioides*, *S. prostrata*, *S. incognita*, *S. confusa*, and S. *glabra*,

175 all with stamens and style included), and the Australian *S. breviflora* (support 100). As mentioned

176 above, the other species of *Spermacoce* (*S. dibrachiata*) falls among the species of *Borreria*.

177 *Hexasepalum* species are also divided into two clades, one of them is well supported (BBP: 99)

178 and contain *H. angustifolium* Bart. ex DC. (type species), *H. apiculatum* and *H. teres*. The other,

179 only represented by *H. sarmentosum* appears as sister species of the *Ernodea* (BBP: 100). The

180 genus *Ernodea*, represented by *E. taylori* and *E. littoralis*, constitutes a strongly supported clade

181 (BBP: 100). The results explained above allow us to support the following taxonomic changes.

182 Taxonomic Treatment

183 **Description of the new species**

184 **Galianthe vasquezii** R. M. Salas & J. Florentín, **sp. nov**. TYPE. PERU: Pasco, Oxapampa,

185 Parque Nacional Yanachaga-Chemillen, Quebrada Yanachaga, 2250 m, 10°24'S, 75°28'W, 14 Jun

186 2003, *R. Vásquez M. 28284* (holotype: HOXA!; isotypes MO!, HUT, USM).

187   *Description*

188   Herb decumbent or prostrate, stems quadrangular, angle strongly alate, with scabridous papillae,
189   more densely disposed near nodes. Leaves sessile or pseudopetiolate, pseudopetiole up to 4 mm
190   long, blades elliptic or obovate, apex acute, base attenuate, 12-32 × 5-17 mm, plicate-nervose,
191   adaxially glabrous or puberulous, abaxially scabridous on nerves, margin scabridous, with 3-5
192   secondary nerves; stipular sheath 3.2-5.6 mm long, with 7-9 linear fimbriae, glabrous, fimbriae
193   3.5-6.8 mm long. Inflorescences thyrsoid, partial inflorescences subglomeriform, multiflorous.
194   Flower pedicellate; pedicel 1-2 mm long; calyx (3-) 4-lobed, hypanthium 1.1-1.3 mm long,
195   glabrous or glabrescent, lobes narrowly triangular, 1-1.4 mm long, glabrous, apex acute; corolla
196   infundibuliform, 3-lobed, 1.75-2.1 mm long, white; lobes ovate, internally with hairs scattered at
197   base, tube internally with some dispersed hairs near its base and externally glabrous, straight;
198   stamens subincluded, anther 0.4-0.6 mm long, oblong, filament fixed immediately below
199   interlobular sinuses; pollen grains 7-8 zonocolpate, oblate-spheroidal to prolate spheroidal, small,
200   P= 31 μm, E= 29μm, colpi long, endoaperture an endocingulum, exine semitectate, reticulate,
201   muri nanospinose, 0,18-0,3 μm long; style bifid, 1.5-1.8 mm long, stigmatic branches ca. 0.2 mm
202   long, with conspicuous papillae. Fruit a capsule, cordate or deltoid in outline, 1.8-2 × 1.6-1.9
203   mm, glabrous, with two indehiscent mericarps which split from the base upwards, each valve
204   remains temporary attached in upper half, at maturity caduceus, seed 1.8-2 × 0.8-1 mm, ovoid,
205   ventral face longitudinally furrowed, partially covered by the strophiole; exotesta reticulate-
206   foveate. Fig. 2, 3

207   *Distribution*—Andes of Peru and Colombia.

208   *Observations*—All specimens of the *G. vasquezii* were previously identified as *Galianthe*
209   *palustris*. However, it differs from *G. palustris,* in having calyx lobes 1-1.4 mm long, with acute
210   apex (vs. calyx lobes 0.4-0.6 mm long, obtuse), corolla 1.75-2.1 mm long, lobes internally with
211   hairs scattered at base, tube internally with some dispersed hairs near its base (vs. corolla 1-1.5
212   mm long, internally glabrous), pollen grains with reticulate exine, muri nanospinose (vs. pollen
213   grains with bireticulate exine, suprareticulum psilate and incomplete, infrareticulum
214   nanospinose), fruit 1.8-2. mm long, deltoid in outline, acropetally dehiscent (vs. fruit 1.1-1.5 mm
215   long, oblong or obovate in outline, basipetally dehiscent), and seeds 1.8-2 mm long (vs. seeds 1-
216   1.42 mm long).

217 ***Ecology***—*Galianthe vasquezii* grows in Montane Forest of Peru and Colombia, which represents
218 a severely fragmented type of vegetation. It grows between 1800 and 2500 m altitude.

219 ***Conservation status***—The extent of occurrence (EOO) was calculated to be 397 km² (cell sized 2
220 km). Following the IUCN criteria (IUCN, 2001), this species should be considered as endangered
221 [EN B1 ab (ii, iii)], due to the continuing decline in area and quality of its habitat.

222 ***Additional Specimens Examined***—COLOMBIA: Antioquia, Monte del Diablo, 21 Jul. 1944,
223 *Bro. Daniel 3303* (US); Rio Negro, 16 Dec. 1933, *Bro. Daniel 165* (US). PERU: Distrito
224 Huancabamba, sector Grapanazú, límite Parque Nacional, Yanachaga-Chemillen, 10°26'S,
225 75°23'W, 15 Oct. 2003, *R. Rojas et al. 1892* (MO); idem, sector Tunqui, camino hacia María
226 Puñis, 1895 m, 10°16'31"S, 75°30'59"W, *M. Cueva 193* (HOXA, HUT, MO, USM);
227 Luispicanchi, Cuzco, Quincemil, 13°14'S, 70°45'W, Oct. 1950, *F. Marín 2731* (CTES, LIL);
228 Pasco, Oxapampa, carretera de Cochabamba, 10°33'42"S, 75°27'23"W, 11 Nov. 2004, *A.*
229 *Monteagudo et al. 7587* (CTES, MO).

230 ***New combinations***

231 **Galianthe palustris** (Cham. & Schltdl.) Cabaña Fader & E. L. Cabral, *comb. nov. Diodia*
232 *palustris* Cham. & Schltdl., Linnaea 3: 347. 1828. *Borreria palustris* (Cham. & Schltdl.)
233 Bacigalupo & E. L. Cabral, Hickenia 2: 264. 1998. *Spermacoce palustris* (Cham. & Schltdl.)
234 Delprete, Fl. Il. Catarin. (2): 740. 2005. TYPE: BRAZIL, Santa Catarina, "*Ad fretum St.*
235 *Catharinae Brasiliae ipsi legimus, in palustribus Brasiliae aequinoctiales*", s. d., *F. Sellow s.n.*
236 (holotype: B destroyed, lectotype here designed PR!).

237 *Borreria gymnocephala* DC., Prodr. 4: 549. 1830. *Diodia gymnocephala* (DC.) K. Schum., in
238 Martius, Fl. Bras. 6(6): 16. 1888. TYPE: BRAZIL. s. d., *J.P. Pohl s. n.* (holotype: G-DC!).

239 *Borreria pterophora* C. Presl., Abh. Königl. Böhm. Ges. Wiss. V, 3: 516. 1845. *nov. syn.* TYPE:
240 BRAZIL, Rio Janeiro, s.l., s.d., *Beske s.n.* (holotype: PR!).

241 *Diodia alata* Nees & Mart., Nova Acta Acad. Caes. Leop. Carol., Wied-Neuwied 12: 12. 1824.
242 *Dasycephala alata* (Nees & Mart.) Benth. & Hook. f. ex B.D. Jacks, Index Kew. 2: 719. 1893.
243 TYPE: BRAZIL, s.l., s.d., *M. Wied s.n.* (holotype BR!; isotypes: LD, LE, W!).

244 *Diodia microcarpa* K. Schum. ex Glaz., Bull. Soc. Bot. France 56 (Mém. 3d): 361. 1909. TYPE:
245 BRAZIL, "*Brasília*", *A.F.M. Glaziou 18283* (holotype B destroyed, photo F 867!).

246 *Description*

247 Herb stoloniferous, sometimes with ascendant stems. Stems quadrangular, angle strongly winged,

248 wing with long and slightly recurved fimbriae, or with scabridous and retrorse papillae, especially

249 near foliar nodes. Leaves subsessile or pseudopetiolate; pseudopetiole 1-4 mm long; blades

250 elliptic or obovate, rarely orbicular, 22-30 mm × 10-18.6 mm, apex obtuse, acute or acuminate,

251 base attenuate, plicate-nervose, with 3-4 secondary nerves, adaxially glabrous or scaberulous,

252 margin scaberulous, abaxially scabridous only on nerves; stipular sheath 3-4 mm long, basally

253 alate, 7-9 fimbriate, fimbriae linear, glabrous, 5-8 mm long. Inflorescences thyrsoid, partial

254 inflorescences congested and multiflorous, sometimes arranged on pleiochasium. Flowers shortly

255 pedicellate; pedicel 0.5-1 mm long; calyx 2(-3)-lobed, hypanthium glabrous, lobes triangular,

256 apex obtuse, succulent, 0.4-0.6 mm long; corolla 2-3-lobed, infundibuliform, white, 1-1.5 mm

257 long, tube internally and externally glabrous, scarcely papillate on apex of the dorsal face of

258 lobes; stamens 2-3, anthers 0.2-0.34 mm long, oblong, dorsal surface with a bullate connective,

259 immediately above the insertion of the filament; pollen grains 6-7(-8) zonocolporate, oblate-

260 spheroidal, small, P= 24 μm, E= 25.3μm, long colpi, endoaperture an endocingulum, exine

261 bireticulate, suprareticulum incomplete, muri psilate, infrareticulum complete, muri nanospinose,

262 0,15-0,28 μm long; style bifid, 1-1.5 mm long, stigmatic branches 0.2-0.46 mm long, notoriously

263 papillate. Fruit a capsule, which separates from the apex downwards into two indehiscent

264 mericarps, both mericarps remain basally united to the pedicel, tardily deciduous, oblong or ovate

265 in outline, glabrous; seeds 1-1.42 × 0.7-0.8 mm, ovate or obpiriforme in outline, ventral surface

266 with a longitudinal furrow covered by a persistent strophiole; exotesta reticulate-foveate. Fig. 3

267 *Distribution*—Brazil (Bahia, Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa

268 Catarina, and São Paulo), and Argentina, Misiones province.

269 *Ecology*—*Galianthe palustris* is a heliophilous plant that inhabits in swampy areas near lotic

270 water bodies, especially along main rivers and their tributaries.

271 *Additional Specimens Examined*—ARGENTINA. Misiones: San Pedro, Parque Provincial

272 Moconá, embarcadero, 7 Mar. 2013, *M. D. Judkevich et al. 46* (CTES); idem, borde de arroyo, 11

273 Dec. 2011, *L. M. Miguel et al. 19* (CTES). BRAZIL. Bahia: Belmonte, 23 Nov. 1970, *T. S. Santos*

274 *1124* (CEPEC); Minas Gerais: Camanducaia, Monte Verde, Estrada Camanducaia, 27 Apr. 2013,

275 *J. A. M. Carmo 125* (UEC); ídem, Monte Verde, 24 Jan. 2013, *J. A. M. Carmo 111* (UEC); ídem,

276 Mata dos Vargas, 22 Mar. 2000, *R. B Torres et al. 1176* (FUEL); Santos Dumont, *s. d.*, *H.L.M.*

Barreto 11339 (BHCB); São João do Manhuaçu, 19 km S of the intersection of Highway BR-116 & BR-262, just N of the village of São João do Manhuaçu, 27 Mar. 1976, *G. Davidse & W. G. D'Arcy 11434* (SP). Paraná: Mun. Bocaiúva do Sul, Serra da Bocaína, 31 Mar. 2001, *E. Barbosa et al. 654* (CTES, ESA, MBM); Serra de São Luís, BR 277, 19 Jan. 1985, *M.S. Ferrucci et al. 284* (CTES); Fazenda Reserva, 85 Km SW of Guarapuava, on bank of brook near Barbaquá, 17 Mar. 1967, *J. C. Lindeman et al. 4959* (CTES, MBM, NY, UB); Mun. Morretes, Serra Morumbi, picada ao Olimpo, 19 Jan. 1995, *O. S. Ribas et al. 761* (CTES); Mun. Piraquara, Floresta, 9 Mar. 1947, *G. Hatschbach 640* (CTES, LIL); BR-476, 7 Km E de Contendas, 26 Jan. 1985, *A. Krapovickas & C. L. Cristóbal 39632* (CTES); Fazenda de J. Rickli near Turvo, 40 km N of Guarapuava, forest, 9 May. 1967, *J. C. Lindeman et al. 5280* (CTES); Curitiba, 17 May. 2002, *J. Cordeiro 2233* (ESA). Rio Grande do Sul: Barracão, Parque Estadual de Espigão Alto, 1 Mar. 2001, *M. Sobral & J. Larocca s.n.*(FURB); Capivari, Viamão, 15 Mar. 1975, *Porto et al. 1389* (CTES); Esteio, 23 Mar. 1949, *B. Rambo 40638* (LIL); Leopoldo, on Monte Jacaré, 7 Dec. 1948, *B. Rambo 38588* (LIL); Pareci, Prope Montenegro, 31 Mar. 1950, *B. Rambo 46536* (CTES); Porto Alegre, 17 Dec. 1932, *B. Rambo s.n.* (P04541549); idem, Morro da Gloria, 16 Dec. 1931, *B. Rambo 577* (LIL); San Salvador, 14 Mar. 1947, *A. Sehnem 2676* (SI); ídem, 16 Dec. 1933, *B. Rambo 577* (SP); idem, Montenegro, 1 Mar. 1950, *A. Sehnem 4426* (SI); Santana, 6 Apr. 1974, *M.C. Sidia 27* (HAS, CTES). Rio de Janeiro: 17 Km from praça da Parati on road from Parati to Cunha, 26 Apr. 1972, *J. H. kirkbride 1729* (US); Nova Friburgo, 12 Nov. 1890, *A. Glaziou 18283* (P02088844); Petrópolis, vale Bonsucesso, 13 Apr. 1968, *B.D. Sucre 2738* (US); Serra da Mantiqueira, Maciço do Itatiaia, Parque Nacional do Itatiaia, 16 Apr. 1971, *I. Gottsberger et al. 110* (CTES); idem, *I. Gottsberger 110-16471*(CTES); Serra dos Orgãos, 11 Jan. 1905, *G. Gardner 445* (US). Santa Catarina: 6.5 KM NW de Aguas Mornas, caminho a Lourdes, 6 Feb. 1994, *A. Krapovickas et al. 44793* (CTES); Am Wege in del Velha bei Blumenau, Oct.1888, *E. H. G. Ule 1062* (US); Fazenda Farofa, trilha da estrada do meio, 6 Apr. 2007, *R. P. M., Souza 103* (ESA); Pilões, Palhoça, 6 Apr. 1956, *R. Reitz et al. 2997* (US, NY); Santa Terezinha, Urubici, 7 Apr. 2009, *M. Verdi et al. 1905* (IFFSC); São Bento do Sul, Rio Natal. Estrada rumo ao Xikavitska (Salto Seco), 19 Feb. 2011, *F. S. Meyer 982* (UFRN); São Bento do Sul, Trilha do Parque 23 de September, 14 Dec. 2014, *P. Schwirkowski 732* (FPS); Taió, Fazenda Tarumã, 18 Feb. 2010, *A. Korte & A. Kniess 1821* (FURB); Três Barras, Guruvá, San Francisco do Sul, 7 Nov. 1957, *R. Reitz et al. 5621* (NY, US); Urubici, Santa Terezinha, 7 Apr. 2009, *M. Verdi et al. 1905* (CTES); São Paulo: Barra do Turvo, 24 Mar. 2005, *M. Carboni, 110* (ESA); 10 km de Barra do Turvo em direção a Pariquera-Açu, 14 Feb. 1995, *J. P. Souza, et al. 96* (SP); Boracéia, 26 Mar.

310   1940, *N.G. Blanco s. n.* (SP); Campinas, Lago próximo ao parque ecológico da UNICAMP, 1

311   Jun. 1995, *L. Y. S Aona & A. D. Faria 95/50* (SP); Campos das Sete Lagôas, Fazenda

312   Campininha, just north of Rio Mogi-Guaçu 1,8 km NW of Pádua Sales, Mogi Guaçu, 4 Dec.

313   1961, *G. Eiten 3517* (SP); Cananéia, Serra do Tambor, Vale do Ribeira, sul do Estado de São

314   Paulo, 20 Nov. 2006, *M. A Pinho-Ferreira et al. 673* (UEC); Cunha, Trilha do Rio Bonito, Parque

315   Estadual da Serra do Mar, 19 Mar. 1996, *A. Rapini et al. 73* (UEC); Cunha, Parque Estadual da

316   Serra do Mar, Núcleo Cunha, 19 Mar. 1996, *A. Rapini, et al. 73* (SP); Estação Biológica, Alto da

317   Serra, 800-900 m, 6 Mar. 1929, *A. Smith 2076* (BA, NY); Estação Experimental, área nativa,

318   Pariquera-Açu, 2 Apr. 1997, *R. B. Torres et al. 182* (IAC); Eldorado, May. 2012, *A. Oriani, et al.*

319   *450* (ESA); Eldorado Paulista, P.E. Jacupiranga, Núcleo, Caverna do Diabo, Ilha da Caverna,

320   24°38'91''S, 48°23'31''W, 9 Feb. 1995, *Leitão Filho et al. 32980* (UEC); Estação Visconde do

321   Rio Claro, 12 Dec. 1888, *A.C.G.G. Loefgren 1220* (SP); Ilha do Cardoso, Jacareu, forest and

322   mangrove swamp, 8 Sep. 1976, *P.H. Davis et al. 60747* (UEC); Itapetininga, 9 Feb. 1976, *H. F.*

323   *Leitão Filho et al. 1630* (UEC); Itirapina, Ipiranga, 23 Mar. 1906, *A. Usteri s.n.* (SP); Juquiá Sitio

324   Areia Dourada, 29 Nov. 1994, *K. D. Barreto et al. 3290* (CTES); Paranapiacaba, 16 Jun. 1966,

325   *T.M. Pedersen 7795* (CTES, SI); Parque Estadual da Serra do Mar, Núcleo Curucutu, 13 Apr.

326   2001, *L. D. Meireles et al. 151* (UEC);   Pinheiros, 8 Jan. 193, *A. Gehrt s.n.* (IAC);

327   Pindamonhangaba, Fazenda São Sebastião do Ribeirão Grande, noroeste do talhão 10, 22 Feb.

328   1996, *S. A. Nicolau et. al. 1051* (SP); Ponta da Praia, 22 Dec. 1938, *E. Guimarães 5* (SP); Rio

329   Claro, 12 Dec 1988, *A.C.G.G. Loefgren 11782* (NY); Santo Amaro, Seminário do Espírito Santo,

330   20 Mar. 1943, *L. Roth 10317* (IPA); São Francisco Xavier, Caminho para Cachoeira das Couves,

331   14 Apr. 1995, *J.Y. Tamashiro et al. 902 (*UEC); São José dos Campos, Distrito de São Francisco

332   Xavier, 14 Apr. 1995, *J.Y. Tamashiro et al. 902* (SP); São Miguel Arcanjo, Parque Estadual Carlos

333   Botelho, 20 Mar. 2002, *S. Bortoleto et al. 31* (UEC); São Sebastião, 22 Apr. 2000, *J. P. Souza et*

334   *al. 3398* (UEC); Tapiraí, Reserva Particular da Votorantim, 26 Mar. 2013, *C. B. Virillo et al. 8*

335   (UEC).

336   ***Taxonomic notes***—Until the present, *Borreria pterophora* has been considered as an imperfectly

337   known but valid name, which is at present day also registered as an endemism of Rio de Janeiro

338   state, Brazil (BFG, 2015), however the examination of the holotype deposited at PR revealed us

339   that is a new synonym of *Galianthe palustris*.

340   ***Galianthe spicata*** (Miq.) Cabaña Fader & Dessein, *comb. nov. Diodia spicata* Miq., Stirp.

341   Surinam. Select. 179–180, t. 52. 1850. *Dasycephala spicata* (Miq.) Benth. & Hook. f. ex B.D.

342 Jacks., Gen. Pl. 2: 144. 1873. *Borreria spicata* (Miq.) Bacigalupo & E.L. Cabral, Opera Bot.

343 Belg. 7: 307. 1996. *Spermacoce spicata* (Miq.) Delprete, J. Bot. Res. Inst. Texas 1(2): 1028.

344 2007. TYPE: SURINAM: *Sylvarum prope Bergendaal*, *H.C. Focke s.n.* (holotype U!, isotypes

345 HAL0113849!, K000265575!).

346 *Diodia denudata* Standl., J. Wash. Acad. Sci. 15(5): 105. 1925. Type: PANAMÁ, on wet stream

347 bank along the Río Tapia, near sea level, 24 Dec 1923, *P. C. Standley 28123* (holotype:

348 US01154022!).

349 *Description*

350 Herb or subshrub 80-140 cm alt., erect, stems simple to much branched. Stems quadrangular,

351 fistulose, glabrous, angle weakly alate or without wings, glabrous. Leaves pseudopetiolate,

352 pseudopetiole 0.5-2 mm long, blades elliptic or narrowly elliptic, papery or subcoriaceous when

353 dry, adaxially glabrescent or scabridous, abaxially glabrous, only scabridous on nerves, base

354 acute or cuneate, apex acute or acuminate, 30-110 × 10-33 mm; 5-7 secondary nerves, visible on

355 both faces; stipular sheath 1.5-3 mm long, margin truncate or scarcely triangular, pilose, with 5-7

356 fimbriae, fimbriae 2-7 mm long, with some antrorse hairs. Inflorescences spiciform, partial

357 inflorescences glomeriform, axillary, (5)10-25 per flowering branch, with 3-20 flowers, bracts

358 foliaceous, decreasing in size towards the apex, sometimes up to the same size than the partial

359 inflorescence. Flowers homostylous, calyx 4-lobed, hypanthium obconic, 0.55-6 mm long,

360 puberulous, lobes 0.2-0.3 mm long, unequal, subtriangular, apex acute, margin ciliate; corolla

361 subtubular, slightly expanded to the apex, 1-1.2 mm long, white or greenish white, sometimes

362 with apex of lobes lilac, glabrescent outside, with a ring of moniliform hairs near insertion of the

363 filaments, tube 0.5-0.7 mm long, lobes ovate, apex acute, internally with some scattered hairs,

364 externally pilose and papillose, especially at the apex, 0.2-0.5 mm long, anthers 0.2-0.25 mm

365 long, oblong, sometimes with a theca visibly smaller than the other, filament 0.15 mm long;

366 pollen grains 7-zonocolpate, prolato-spheroidal, small, P= 30.3 μm, E= 28 μm, colpi long,

367 endoaperture an endocingulum, tectum bireticulate, microreticulate, suprareticulum psilate,

368 infrareticulum with muri nanospinose or psilate; stigma bifid, divided up to the half of its length,

369 with papillae only in the internal face of the stigmatic branches, scarcely exerted. Fruit a capsule,

370 1.2-1.4 × 1-1.2 mm, longitudinally separated from the pedicel upwards up to median portion of

371 the fruit, both mericarps remain attached to each other at the upper part, mericarps indehiscent,

372 subglobose, ventral face flat, slightly laterally compressed, dorsal face pubescent, hispidulous or

373 glabrescent; seeds 0.8-1 × 0.35-0.45 mm, oblong or ovate in outline, plane-convex, ventral face

374 with a ample groove, dark brown or nigrescent; exotesta reticulate-foveate, cells polygonal,

375 almost isodiametric. Fig. 4, 5

376 ***Distribution***—Brazil (Amazonas, Roraima, Rondônia, Para, Mato Grosso), Colombia (Chocó),

377 French Guaina, Panamá, Suriname, and Venezuela (Amazonas y Anzoátegui).

378 ***Ecology***—*Galianthe spicata* grows inside or edges of humid forests.

379 ***Additional Specimens Examined***—BRAZIL: Amazonas, vicinity of Camp Tucano, Rio Tucano,

380 3 Dec. 1965, *B. Maguire et al. 60319* (IAN, MO); Rondonia, Porto Velho, along hwy 364 92 km,

381 by road NE of junction with, 09°22'S 064°40'W, 20 Apr. 1987, *H. M. Nee 34960* (MO); Roraima,

382 Dormida, Serra do Lua, foothills of Serra da Lua, 13 Jan. 1969, *G. T. Prance 9271* (MO); Pará,

383 Conceição do Araguaia, near Corrego São João and Troncamento Santa Teresa, 8 Feb. 1980, *T. C.*

384 *Plowman 8524* (MO, NY); Altamira Gleba Curuaé, Jul. 2005. *M. Sobral et al. 10020* (BHCB).

385 COLOMBIA: Chocó, near Madurex Logging Campn above Teresita and below the rapids on Rio

386 Truando, Feb. 1967, *J. A. Duke 9977* (MO); ídem, logging road ca. 2-4km NW of Teresita, 100

387 m, 18 May. 1967, *J. A. Duke 11055* (MO). FRENCH GUIANA: Kamakusa, upper Mazaruni

388 River, 23-29 Nov. 1922, *J. S. de la Cruz 2808* (MO); Route de l'Est (N2), Montagne Maripa, c.

389 31km S of the Comte bridge, c. selectively logged forest, 04°26'N, 52°20'W, 3 Dec. 1994, *L.*

390 *Andersson 1961* (MO).

391 GUYANA: Rupununi, Kanuku Mts., Crabwood Cr. Camp 2 forest, on brown loamy sand, 3°07'N,

392 59°06'W, 260 m, 2 Apr. 1994, *M. J. Jansen-Jacobs et al. 3564* (MO); idem, E Kanuku Mts, NE of

393 Warimure, in forest, 03°05'N 059°20'W, 200-500 m, 23 Jan. 1991, *M. J. Jansen-Jacobs et al.*

394 *2189* (MO). PANAMA: Canal Area, Barro Colorado Island, 10-100 m, 9°09'17"N, 79°50'53"W,

395 16 Dec. 1967, *T. B. Croat 4373* (MO); idem, 9°9'17"N, 79°50'53"W, 18 Mar. 1969, *T. B. Croat*

396 *8738* (MO); idem, 9°9'0"N, 79°51'0"W, 1931, *S. Aviles s.n.* (MO); Cerro Azul, 700 m, 9°10'2"N,

397 79°24'59"W, 29 Jul. 1972, *W.G. D'Arcy 6199* (MO). idem, 700 m, 9°10'13"N, 79°25'13"W, 7 Jun.

398 1970, *A. Kant 46* (MO); Cerro Jefe, 1000 m, 9°14'0"N, 79°22'0"W, 12 Sep. 1994, *C. Galdames et*

399 *al. 1604* (MO); Colón: Santa Rita, 9°20'0"N, 79°47'0"W, 6 Apr. 1969, *W. H. Lewis et al. 5238*

400 (MO); idem, 9°20'0"N, 79°47'0"W, 6 Apr. 1969, *W. H. Lewis et al. 5238* (MO); idem, 9°20'0"N,

401 79°47'0"W, 6 Apr. 1969, *W. H. Lewis et al.* 5238 (MO); idem, 9°20'13"N 079°46'04"W, 31 Jan.

402 1971, *T. B. Croat 13191* (MO); idem, 9°19'42"N, 79°47'27"W, 9 Jul. 1971, *T. B. Croat & M. P.*

403 *Duncan 15339* (MO); Gamboa Pipeline Road, 90 m, 9°9'36"N, 79°44'44"W, 9 Feb. 1974, *M. H.*

404   *Nee 9577* (MO); Pipeline Road, 50-100 m, 9°10'0"N, 79°46'0"W, 23 May. 1969, *R. L. Lazor*

405   *3464* (MO). SURINAME: 1850, *F.A.W. Miquel s.n.* (K000265073). Brokopondo: Brownsberg

406   Nature Park, Trail to Mazaroni Val. Primary forest, 04°56'N, 55°11'W, 400-450 m, 24 Jan. 1999,

407   *P.G. Delprete 7083* (MO); Marowijne: Nassau Mts, Plataeu C, lateritic rocky soil, 4°49'N,

408   54°36'W, 500-550 m, 26 Jan. 2003, *M.J. Jansen-Jacobs et al. 6254* (MO). VENEZUELA:

409   Amazonas, Atabapo, 5km al Norte de la desembocadura del Rio Orinoco, 2°24'N, 64°24'W, 400

410   m, Oct. 1991, *E. Marín 1678* (MO); Anzoátegui, Cabeceras del Morichas Largo, entre Santa

411   Elena y San Pedro a unos 30 km Sur de la Viuda, 9 Nov. 1984, *R. A. Montes 2524* (MO).

412   ***First record from Peru***

413   ***Galianthe boliviana*** E.L. Cabral, Brittonia 57(2): 142, f. 1. 2005. TYPE. BOLIVIA: La Paz:

414   Inqusivi, Cerro Aguada, 2500-2800 m, 22 Nov 1991, *M. Lewis 40645* (holotype: LPB0000936!;

415   isotype MO!).

416   ***Distribution***—Sub-Andean foothills of Bolivia and Peru. *Galianthe boliviana* grows mainly on

417   eroded slopes between 1800 and 3150 m of altitude, especially in open forest of *Alnus acuminata*

418   Kunth. of Yungas at 2800-3000 m of altitude.

419   ***Taxonomic notes***—*Galianthe boliviana* is similar to *Galianthe dichasia* and *G. cymosa* in having

420   cymoidal inflorescences with subglomeriform partial inflorescence, but differs from these in

421   possessing stems 20-30 cm tall, with smooth, glabrous, and narrowly winged angles.

422   ***Additional Specimens Examined***—PERU: Cusco, Quispicanchis, Marcapata, 176 km from

423   Cusco on road to Maldonado, Marcapata to Cocha, 8 Mar. 1991, 13°25'S 070°54'W, 3150 m,

424   *Percy Núñez V. & C. Paycarmayta 13140* (MO).

425   Key to *Galianthe* species with indehiscent mericarps (modified from Cabral & Bacigalupo, 1997)

426   1. Stipular sheath tubular, prolonged above the insertion of the corresponding pairs of

427   leaves…………………………………………………………………………………......……...2

428   1'. Stipular sheath truncate, never surpassing the insertion of the corresponding pairs of

429   leaves…..…………………………………………………………………………….....…..3

430   2. Stipular sheath pilose; stems with strongly alate angles; Brazil…..…..……………………

431   ……………………………………………………………*G. vaginata* E.L. Cabral &

432   Bacigalupo

433 2'. Stipular sheath glabrous; stems without wings. Brazil……………………………………………….*G. polygonoides* E.L. Cabral & Bacigalupo

435 3. Leaves only with one nerve visible on abaxial face..…………………………..……..4

436 3'. Leaves plicate nervose.……………………………………………………………………7

437 4. Inflorescences pauciflorous, in lax cymoid, partial inflorescences 1-florous. Ecuador, Perù…………………………………………………….…………*G. dichotoma*

439 4'. Inflorescences multiflorous, partial inflorescences multiflorous, in fascicles or glomeruli.…………………………………………………………………….……………5

441 5. Inflorescences cymoid, partial inflorescences glomeriform, calyx 4-lobed, pollen with reticulate exine. Colombia. …………………….…...*G. bogotensis* (Kunth) E. L. Cabral & Bacigalupo

444 5'. Inflorescences thyrsoid, spiciform, or cymoid, partial inflorescences fasciculate, calyx 2-4-lobado, pollen grains with bireticulate exine…………………………...………………6

446 6. Inflorescences thyrsoid-spiciform or cymoid, primary axis shorter than the laterals, calyx 2 (-4) lobed, corolla of long-styled flowers with a fringe of hairs from apex of anthers to base of tube; pollen grains 6-7 zonocolpate, both reticula complete, fruit 2-3 times wider than long, . Argentina, SE, and S Brazil, Paraguay, and Uruguay…………..……………………….*G. brasiliensis* (Spreng.) E.L. Cabral & Bacigalupo

451 6'. Inflorescences thyrsoid-spiciform, primary axis longer than the laterals, calyx always 4-lobed, corolla of the long-styled flowers with ring of hairs, pollen grains 8-10 zonocolpate, suprareticulum incomplete, fruit as long as wide, , Mesoamerica………..............……...............……….....................*G. angulata* (Benth.) Borhidi

455 7. Partial inflorescences congested, glomeriform or subglomeriform……..…………………8

456 7'. Partial inflorescences pauciflorous, fasciculate…………………….…….……..……..…13

457 8. Inflorescences spiciform, con 5-20 partial inflorescences per flowering branch. Brazil, Colombia, French Guiana, Panama, Surinam, and Venezuela……..…………..……*G. spicata*

459 8'. Inflorescences thyrsoid or cymoid, with 3-5 partial inflorescences per flowering…………….9

461 9. Stems notoriously alate.…………………………………………………………….10

462 9'. Stems obscurely alate.……………………………………………………...…12

463 10. Inflorescences thyrsoid or with a simple axis, partial inflorescences glomeriform, flowers homostylous, calyx 2-3 lobed, corolla 2-3 lobed…………………………………….11

465 10'. Inflorescences cymoid, partial inflorescences subglomeriform, flowers distylous, calyx 4-
466   lobed, corolla 4-lobed, Argentina, Brazil, Paraguay, and Uruguay…….…………..
467   ……........ ………………………………………………*G. dichasia* (Sucre & C.G. Costa)
468   E.L. Cabral

469 11. Calyx lobes 1-1.4 mm long, with acute apex, corolla 1.75-2.1 mm long, corolla lobes
470   internally with hairs scattered at base, tube internally with some dispersed hairs near its base,
471   pollen grains with reticulate exine, muri nanospinose, fruit 1.8-2. mm long, deltoid in outline,
472   acropetally dehiscent, seeds 1.8-2 mm long…………..*G. vasquezii* R.M. Salas & J. Florentín

473 11'. Calyx lobes 0.4-0.6 mm long, obtuse, corolla 1-1.5 mm long, internally glabrous, pollen
474   grains with bireticulate exine, suprareticulum psilate and incomplete, infrareticulum
475   nanospinose, fruit 1.1-1.5 mm long, oblong or obovate in outline, basipetally dehiscent,
476   seeds 1-1.42 mm long………………………..………*G. palustris* (Cham. & Schltdl.) Cabaña
477   Fader & E. L. Cabral

478 12. Stems retrorse-scabridous on angles, leaves 1-7 mm lat.
479   Brazil…………………………………*G. cymosa* (Cham.) E.L. Cabral & Bacigalupo

480 12'. Stems glabrous, leaves 7-12 mm lat. Bolivia and Perú……………....*G. boliviana* E.L. Cabral

481 13. Stems scarcely branched; fruit sub-hemispherical, 1.6-2 mm long; Brazil, Paraguay, and
482   Argentina……........................……*G. hispidula* (A. Rich. ex DC.) E.L. Cabral & Bacigalupo

483 13'. Stems much branched; fruit turbinate, 5 mm long;
484   Brazil……………………………………
485   ………………………………………………………*G. humilis* E.L. Cabral & Bacigalupo

486 **DISCUSSION**

487 *Galianthe palustris* and *G. spicata* share the same taxonomic and nomenclatural history. First,
488 they were described under *Diodia*, later they were added to genus *Borreria* (*Bacigalupo &*
489 *Cabral, 1996, 1998*) due to the presence of homostylous flowers and type of fruit. Later they
490 were, transferred to the genus *Spermacoce* (*Delprete et al., 2005; Delprete, 2007*). In 1998,
491 *Bacigalupo & Cabral* (1998) observed that *G. palustris* (then still *Borreria palustris*) is
492 characterized by a thyrsoid inflorescence that is similar to that of *Galianthe*. Despite this
493 remarkable observation, the authors decided to transfer the species to genus *Borreria*. Nearly a
494 decade later, *Delprete et al.* (*2005*) and *Delprete* (*2007*) transferred both species to *Spermacoce* in
495 an attempt to create a broad genus concept for *Spermacoce*.

496   Despite overall molecular evidence, *Galianthe spicata* and *G. palustris* also share similar

497   morphological characteristics with the other *Galianthe* species (e.g. spiciform and thyrsoid

498   inflorescences, a bifid stigma and pollen grains with a double reticulum). This last character

499   appears in most species of *Galianthe,* except for *G. bogotensis* (Kunth) E.L. Cabral &

500   Bacigalupo, *G. dichotoma* (Willd. ex Roem. & Schult.) E. L. Cabral & Bacigalupo, and the new

501   species *G. vasquezii,* which have simple reticulum. *Pire* (*1997*) hypothesized that in a genus

502   mainly represented by species with double reticulum pollen grains, the simple reticulum is the

503   result of the absence of an infrareticulum persisting only a suprareticulum.

504   Current molecular data indicates that the phylogenetic position of *Diodia palustris* (*Galianthe*

505   *palustris)* and *D. spicata* (*G. spicata)* make *Galianthe* paraphyletic. The *Galianthe* clade,

506   including both former *Diodia* species, is strongly supported and has two molecularly well-defined

507   clades. The [*Diodia palustris* + *D. spicata*] + *G. brasiliensis* clade is composed only by species

508   with capsules separating into two indehiscent mericarps and which is a diagnostic character of

509   *Galianthe* subgen. *Ebelia*. The sister clade, [*G. eupatorioides* + *G. grandifolia*] + *G. peruviana*,

510   includes species of *Galianthe* subgen. *Galianthe,* and is characterized by fruits with dehiscent

511   valves. Both morphological and molecular data support the transfer of two former *Diodia* species

512   to *Galianthe*, and more specifically in subgen. *Ebelia*. Additionally, and according to present

513   sampling, the two subgenera described by *Cabral & Bacigalupo* (*1997*) seem to be monophyletic.

514   The transfer of *Diodia spicata* to *Galianthe* was originally proposed by *Dessein* (*2003*), based on

515   fruit, polynological and molecular features.

516   Even though morphological and molecular data show that three species share several

517   characteristics with *Galianthe* subgen. *Ebelia*, there is a significant difference with the other

518   species of the subgenus. The three species, unlike the remainder, have homostylous flowers. As a

519   result, these results demonstrate the presence of a new floral trait in *Galianthe* and therefore

520   strongly modify the generic concept of the genus.

521   According to *Groeninckx et al. (2009)*, distyly is often related with double reticulum pollen

522   grains in the tribe Spermacoce. Nevertheless, in the genus *Galianthe* there are some exceptions to

523   this generalization (e.g. *G. bogotensis* (distyly and simple reticulum), *G. spicata* and *G. vasquezii*

524   (homostyly and double reticulum), and *G. palustris* (homostyly and simple reticulum). *Cabral &*

525   *Bacigalupo (1997)* mentioned that *G. dichotoma* presents an intermediate state between

526   distyly/homostyly and pollen with simple reticulum. The authors defined this phenomenon as an

527   "unclear dimorphism" (in Spanish "dimorfismo poco manifiesto"). Future studies are necessary

528   in order to clearly define the floral morphs that are present in these species.

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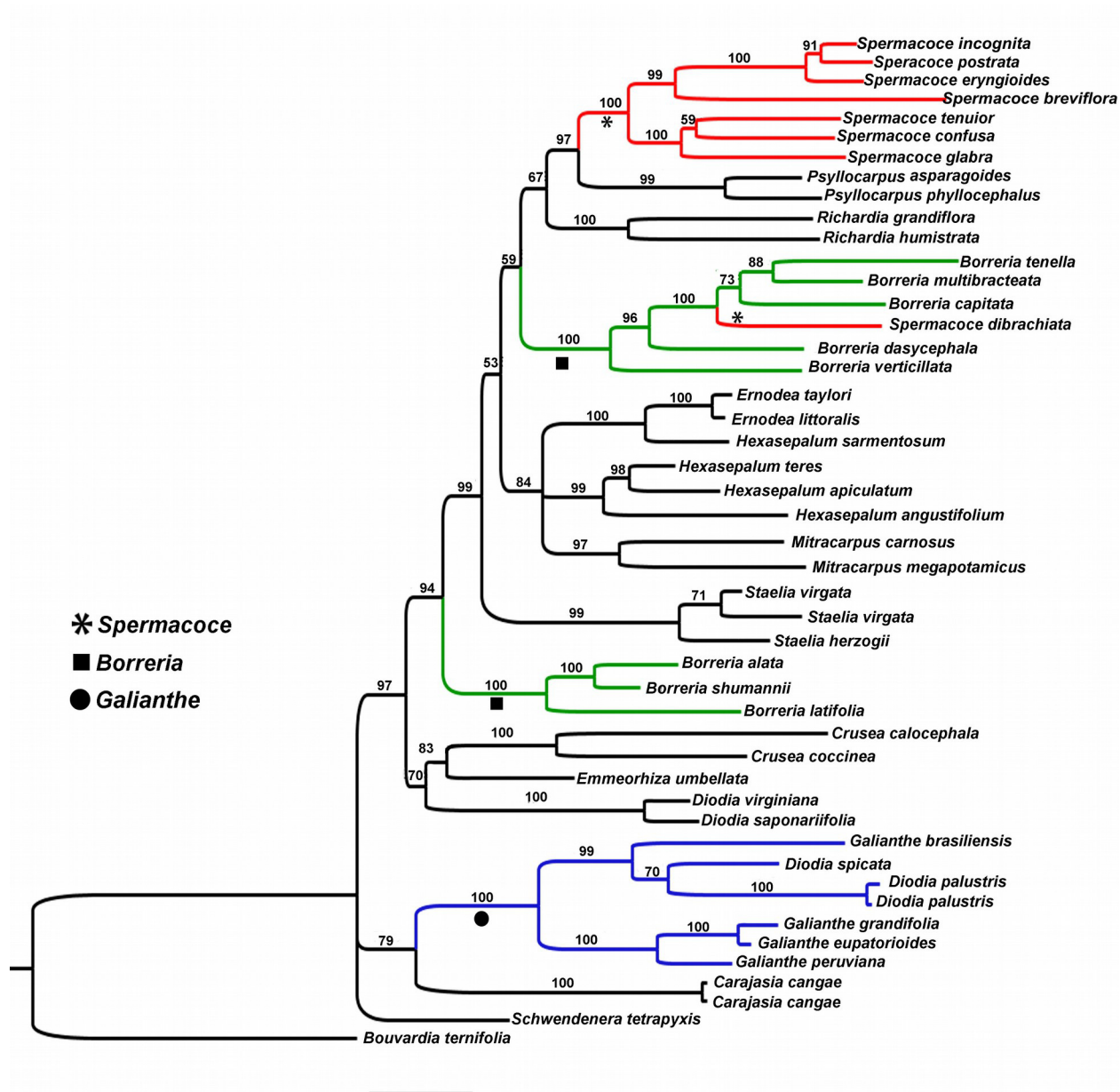
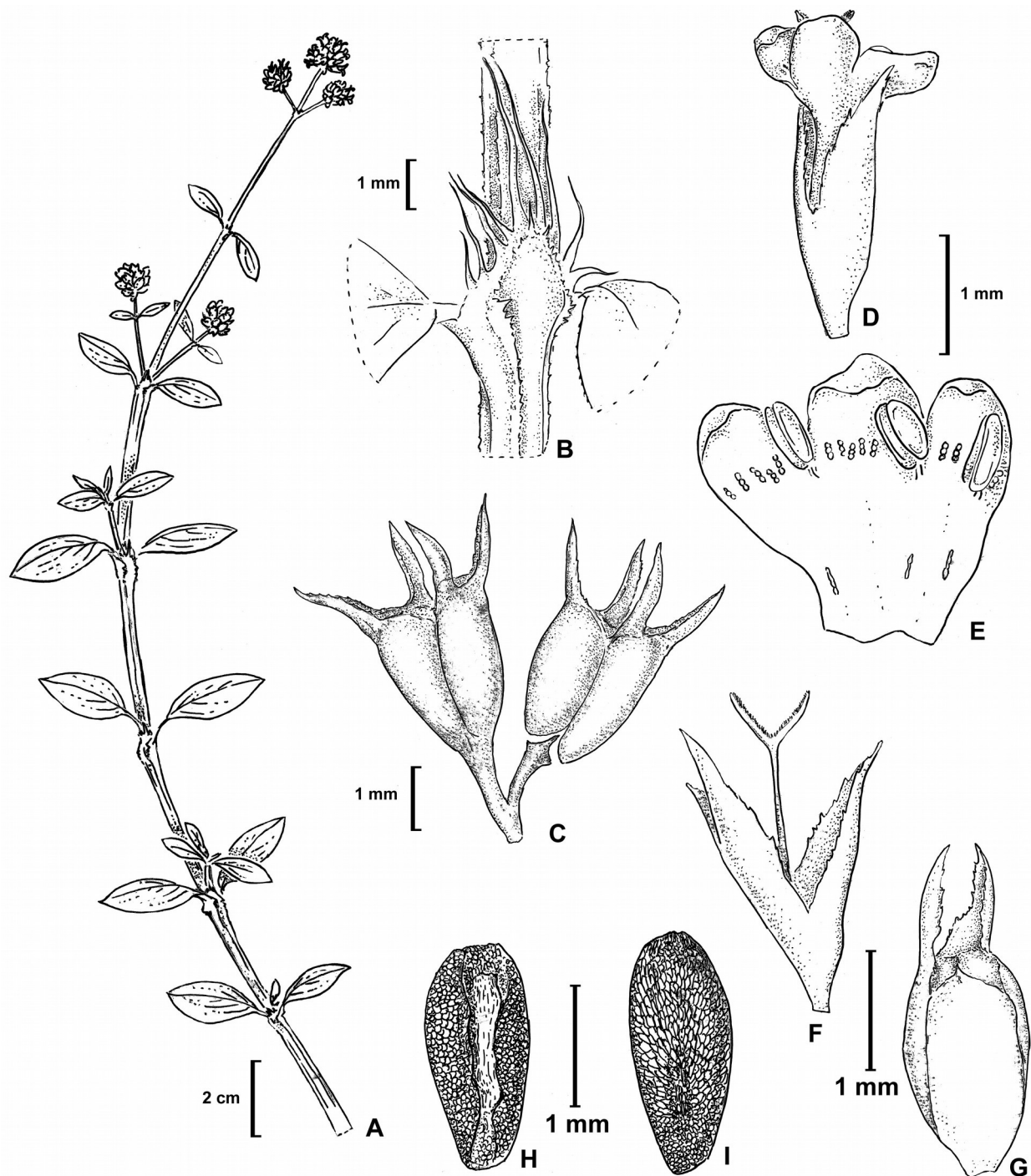
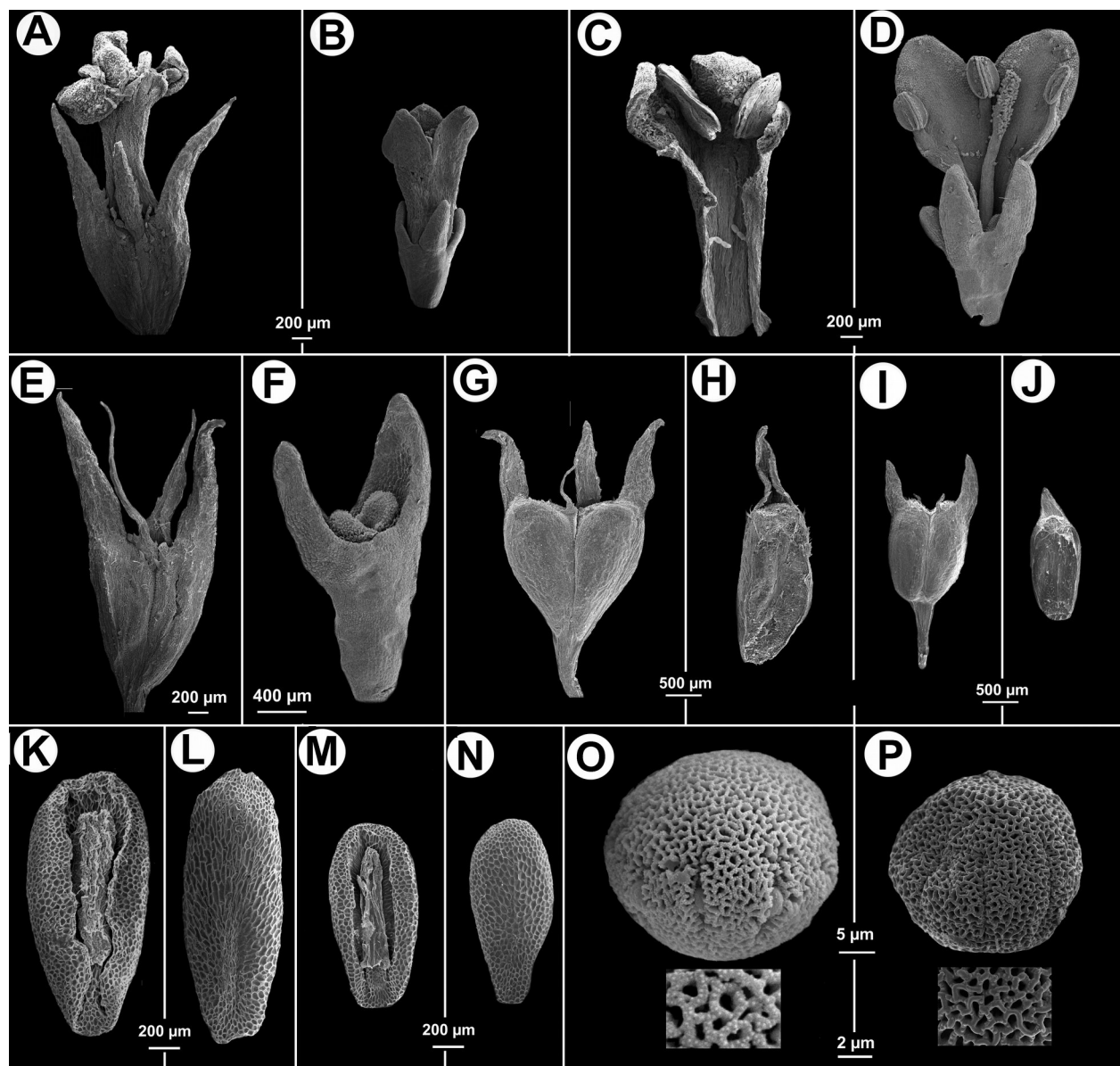
619   **FIGURE LEGENDS**

620   **Figure 1: Bayesian tree**

621   Showing the relationship of *Galianthe* with the remaining genera of *Spermacoce* clade.

622   **Figure 2:** *Galianthe vasquezii.*

623   (A) Apical part of flowering branch. (B) Stipular sheath. (C) Fruit. (D-F) Flower. (E) Inside of

624   corolla (F) Style, stigma and calyx. (G) Ventral view of indehiscent valve, calyx tube and lobes.

625   (H-I) Seeds. (H) Ventral view. (I) Dorsal. All from isotype (MO).

626   **Figure 3: Morphological characters distinguishing** *Galianthe vasquezii* (pictures A, C, E, G,

627   H, K, L, O from the isotype at MO). and *G. palustris* (pictures B, D, F, I, J, M, N, P from A. A.

628   Cabaña 19 at CTES). (A) Flower. (C) Inside the corolla with two stamens. (E) Hypanthium and

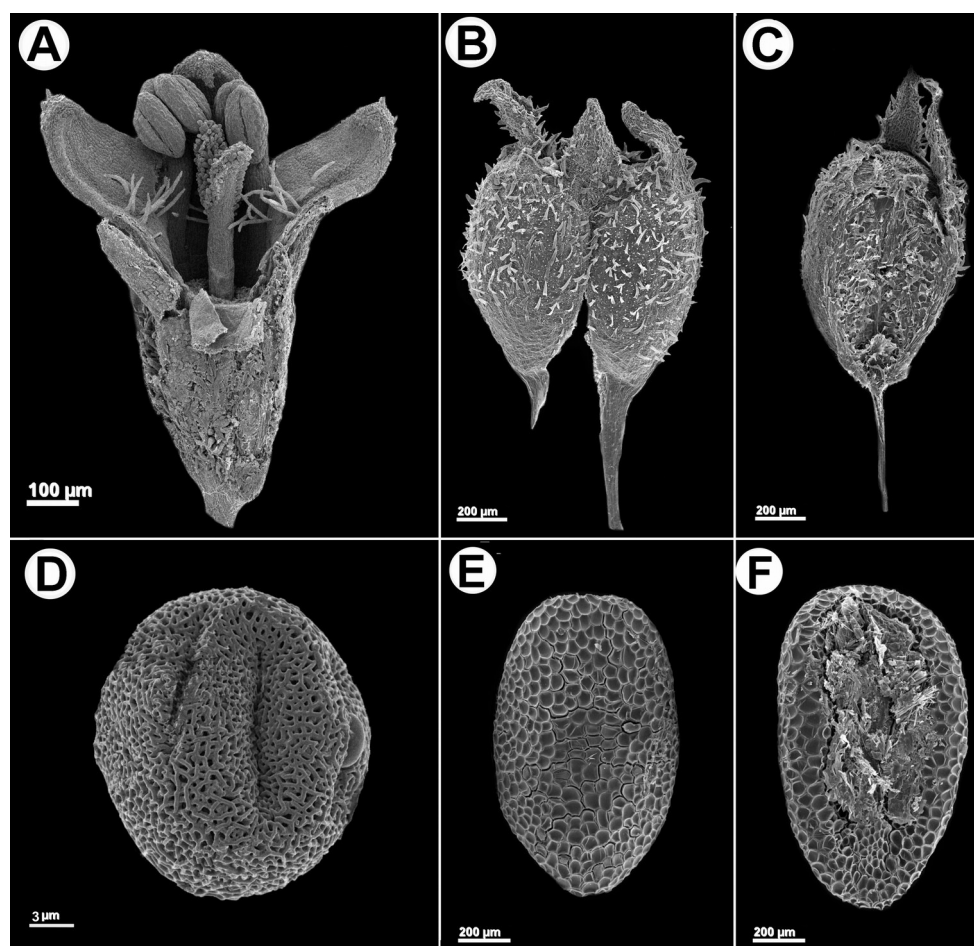
629   dimorphic calyx lobes. (G) Entire fruit. (H) indehiscent valve. (K) Ventral face of seed. (L)

630 Dorsal face of seed. (O) Equatorial view of pollen grains, with simple, below a detail of exine.

631 *Galianthe palustris.* (B) Flower. (D) Opened flower showing inside of corolla and three stamens,

632 style and stigma. (F) Hypanthium, nectariferous disc, and calyx lobes. (I) Entire fruit. (J)

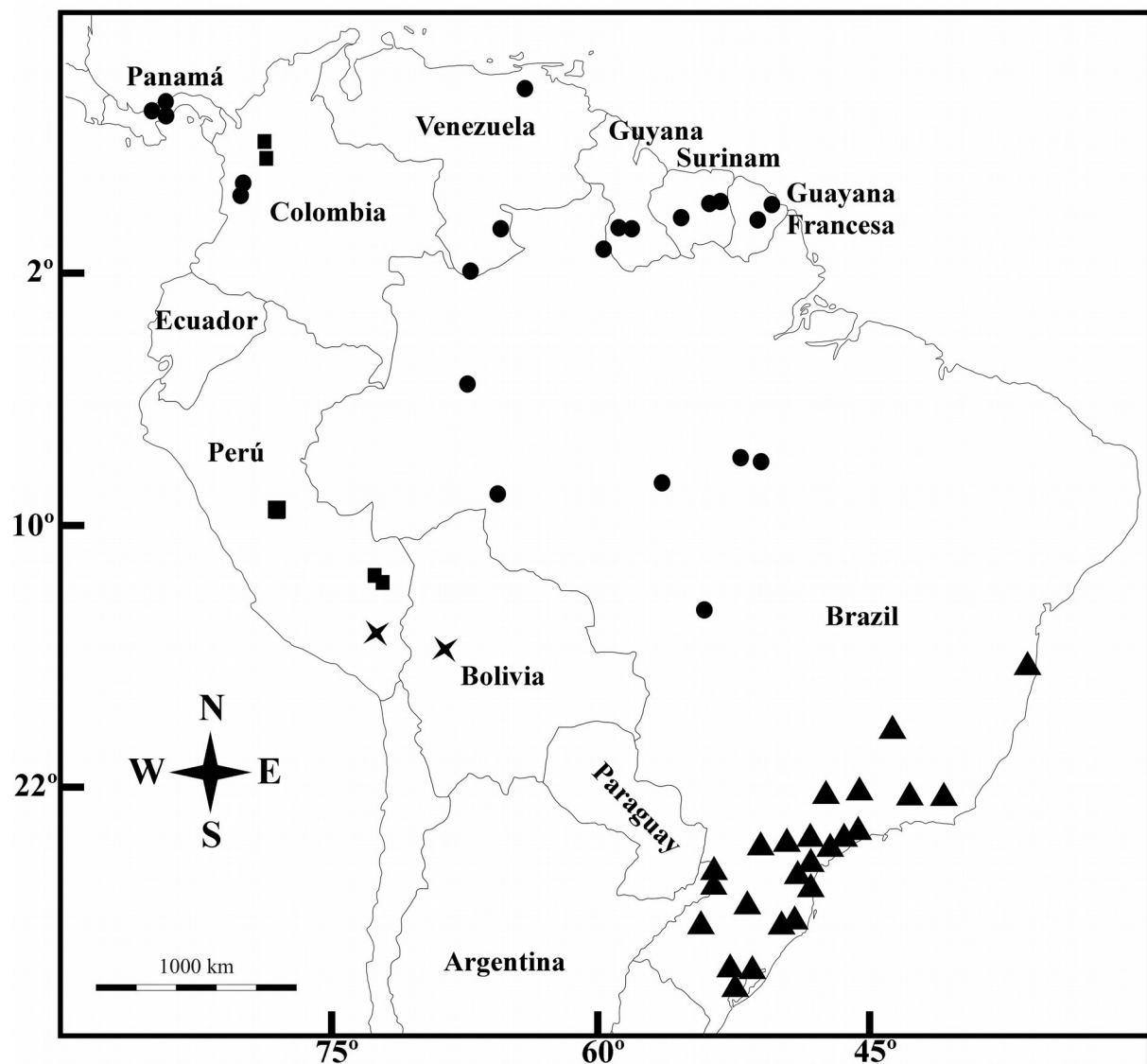
633 indehiscent valve. (M) Ventral face of seed. (N) Dorsal face of seed. (P) Pollen with double

634 reticulum, below a detail of exine showing the suprareticulum incomplete.

635 **Figure 4:** *Galianthe spicata.*

636 (A) Longitudinal section of flower. (B) Fruit with acropetal dehiscence. (C) Indehiscent valve.

637 (D) Equatorial view of pollen grains showing exine with double reticulum. (E) Dorsal face of

638 seed. (F) Ventral face of seed A: *From P. G. Delprete 11876* (CAY), B-D: from *M. Sobral et al.*

639 *10020* (BHCB).

640   **Figure 5: Geographic distribution**

641   *Galianthe boliviana* (X), *G. palustris* (triangle), *G. spicata* (dots) and *G. vasquezii* (square).

642   **Appendix**

643 List of taxa used in the molecular phylogenetic analysis with voucher information (geographical

644 origin, collector, collector number, herbarium, ITS and ETS accession number) and GenBank

645 accession numbers.

646 Ingroup. *Borreria* G. Mey. *B. alata* (Aubl.) DC., Brazil, Goiás, Queiroz et al. 14105 (CTES,

647 HUEFS; KF736995, KF737036); *B. capitata* (Ruiz & Pav.) DC., Brazil, Bahia, Queiroz et al.

648 13688 (CTES, HUEFS; KF736989, KF737031). *B. dasycephala* (Cham. & Schltdl.) Bacigalupo

649 & E.L. Cabral, Argentina, Misiones, Salas & Cabaña 388 (CTES; ITS KF73699); *B.*

650 *multibracteata* E.L. Cabral & Bacigalupo. Brazil, Goiás, Queiroz et al. 14261 (CTES, HUEFS;

651 KF736990, KF737032); *B. latifolia* (Aubl.) K. Schum., Brazil, Goiás, Queiroz et al. 14110

652 (CTES, HUEFS; KF736994, KF737035); *B. schumannii* (Standl. ex Bacigalupo) E.L. Cabral &

653 Sobrado, Argentina, Misiones, Cabral et al. 760 (CTES; KF736997, KF737038); *B. tenella*

654 (Kunth) Cham. & Schltdl., Brazil, Queiroz et al. 14252 (CTES, HUEFS; KF736988, KF737030);

655 *B. verticillata* (L.) G. Mey., Argentina, Corrientes, Salas 402 (CTES; KF736998, KF737039);

656 *Carajasia, C. cangae*, Brazil, Pará, Costa et al 588 (BHCB; KF737015, KF737057); Giorni et al

657 179 (BHCB; KF737016, KF737058). *Crusea* Cham. & Schltdl., *C. calocephala* DC., Mexico,

658 Oaxaca, Ochoterena et al. 456 (BR; KF737009, KF737051); *C. coccinea* DC., Mexico, Oaxaca,

659 Ochoterena et al. 461 (BR; KF737010, KF737052). *Diodia* L. *D. saponariifolia* Cham. &

660 Schltdl., Argentina, Misiones, Cabaña & Salas 22 (CTES; KF737007, KF737049). *D. virginiana*

661 L., USA, Missouri, Taylor 12758 (MO; KF737008, KF737050). *Emmeorhiza* Pohl ex Endl. *E.*

662 *umbellata* (Spreng.) K. Schum., Brazil, Bahia, Queiroz et al. 13746 (CTES, HUEFS; KF737000;

663 KF737042). *Ernodea* Sw. *E. littoralis* Sw., Cuba, Habana, Rova et al. 2286 (GB; KF737001,

664 KF737043). *E. taylori* Britton, North Bimini, Correll 44186 (NY; KF737002, KF737044).

665 *Galianthe* Griseb. *G. brasiliensis* (Spreng.) E.L. Cabral & Bacigalupo, Argentina, Misiones,

666 Cabral et al. 758 (CTES; KF737011, KF737053). *G. eupatorioides* (Cham. & Schltdl.) E.L.

667 Cabral, Brazil, Goiás, Queiroz et al. 14190 (CTES, HUEFS; KF737012, KF737054). *G.*

668 *grandifolia* E.L. Cabral, Brazil, Distrito Federal, Queiroz et al. 14015 (CTES, HUEFS;

669 KF737013, KF737055). *G. peruviana* (Pers.) E.L. Cabral, Brazil, Minas Gerais, Belo Horizonte,

670 Salas et al. 408 (BHCB, CTES; KF737014, KF737056). *G. palustris* (Cham. & Schltdl.) Cabaña

671 Fader & E. L. Cabral, Verdi et al. 1905 (CTES; MF166824, MF166826); Miguel et al. 19 (CTES;

672 MF166825, MF166827); *G. spicata* (Miq.) Cabaña Fader & Dessein, Brazil, French Guiana,

673 Andersson et al. 1961 (GB; AM939535, AM933008); *Hexasepalum* Bartl. ex DC. *H. apiculatum*

674 (Willd.) Delprete & J.H. Kirkbr., Brazil, Bahia. Queiroz et al. 13727 (CTES, HUEFS; KF737003,

675 KF737045). *H. angustifolium* Bartl. ex DC., Mexico, Rzedowski 17792 (NY; KF737004,

676   KF737046). *H. sarmentosum* (Sw.) Delprete & J.H. Kirkbr., Cameroon, Dessein et al. 1521 (BR;

677   KF737005, KF737047). *H. teres* (Walter) J.H. Kirkbr., Brazil, Goiás, Queiroz et al. 14089

678   (CTES, HUEFS; KF737048, KF737006). *Mitracarpus* Zucc. *M. carnosus* Borhidi & Lozada-

679   Pérez, Mexico, Oaxaca, Ochoterena et al. 516 (BR; KF736999, KF737040). *M. megapotamicus*

680   (Spreng.) Kuntze, Argentina, Corrientes, Salas & Cabaña 399 (CTES; ETS KF737041).

681   *Psyllocarpus* Mart. & Zucc. *P. asparagoides* Mart. ex Mart. & Zucc., Brazil, Minas Gerais,

682   Itacambira, Salas et al. 411 (BHCB, CTES; KF737018, KF737060). *P. phyllocephallus* K.

683   Schum., Brasil, Distrito Federal, Queiroz & al. 14016 (CTES; ETS KF737061). *Richardia* L. *R.*

684   *grandiflora* (Cham. & Schltdl.) Steud., Brazil, Bahia, Nova Roma, Queiroz et al. 14055 (CTES,

685   HUEFS; KF737027, KF737066). *R. humistrata* (Cham. & Schltdl.) Steud., Argentina, Misiones,

686   Bernardo de Irigoyen, Cabaña & Salas 17 (CTES; KF737028, KF737067). *Schwendenera* K.

687   Schum. *S. tetrapyxis* K. Schum., Brazil, Paraná, Marques et al. 83 (CTES; KF737017,

688   KF737059). *Spermacoce* L. *S. breviflora* F. Muell ex Benth., Australia, Harwood 1070 (BR;

689   KF737019, KF737062). *S. confuse* Rendle, Mexico, Ochoterena et al. 552 (BR; KF737020,

690   KF737063). *S. dibrachiata* Oliv., Zambia, Dessein et al. 626 (BR; ITS KF737021). *S.*

691   *eryngioides* (Cham. & Schltdl.) Kuntze., Argentina, Salas et al. 378 (CTES; KF736992,

692   KF737033). *S. glabra* Michx., USA, Missouri, Perry, Taylor 12757 (MO; KF737022, KF73706).

693   *S. incognita* (E.L. Cabral) Delprete., Brazil, Goiás, Queiroz et al. 14049 (CTES, HUEFS;

694   KF736993, KF737034); *S. prostrata* Aubl., Brazil, Goiás, Nova Roma, Queiroz et al. 14083

695   (CTES, CTES; KF736996, KF737037); *S. tenuior* L., México, Novelo et al. s/n (BR; KF737023,

696   KF737065). *Staelia* Cham. & Schltdl. *S. herzogii* (S. Moore) R.M. Salas & E.L. Cabral, Bolivia,

697   Santa Cruz, Soto et al. 1053 (CTES, USZ; ITS KF737024). *S. virgata* (Link ex Roem. & Schult.)

698   K. Schum., Brazil, Bahia, Salas et al. 423 (CTES, HUEFS; ITS KF737025); Brasil, Piauí, Salas

699   et al. 443 (CTES, HUEFS; ITS KF737026). Outgroup. *Bouvardia* Salisb. *B. ternifolia* (Cav.)

700   Schltdl., Mexico, Oaxaca, Ochoterena et al. 454 (BR; KF736987, KF737029).