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The monotypic Brazilian genus *Diacrodon* is a synonym of *Borreria* (Spermacoceae, Rubiaceae): morphological and molecular evidences

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ABSTRACT

Diacrodon is a monotypic genus of the tribe Spermacoceae (Rubiaceae), endemic to northeastern Brazil. *Diacrodon compressus* is frequently misidentified with a two lobed calyx species of *Borreria, B. verticillata*. Traditionally, in Spermacoceae the fruit type was considered a diagnostic character among the genera. In this sense, *D. compressus* presents a strongly compressed, one seeded and indehiscent fruit (*vs.* globose, two seeded and dehiscent fruit in *B. verticillata*). In this work, we address two objectives: evaluate the systematic position and determine the identity of *Diacrodon* in respect to other taxa. Molecular analyses using ITS and ETS indicate that *D. compressus* is strongly related to other species of *Borreria*. The morphological results revealed that *D. compressus,* despite of its type of fruit, is identical to *Borreria* in floral and palynological features. As conclusion, the new combination *Borreria diacrodonta* is made and a lectotype is designated. An updated description of the species and a key to the *Borreria* species with a two lobed calyx are provided. The distribution of *B. diacrodonta* is extended to Brazilian states Goiás and Minas Gerais, and Paraguay. By this taxonomical change it has become clear that the dehiscence of the fruits lack taxonomic value in the delimitation of *Borreria*.

Key words: flower, fruit, ITS-ETS dataset, morphology, pollen, seed.

INTRODUCTION

Diacrodon is a monotypic genus that is endemic to Brazil. Its sole species *Diacrodon compressus* Sprague was described from two dried specimens that were collected by *Bolland* in Ceará, Brazil (Sprague 1928). Since its description, *Diacrodon* was recorded in species lists only for the original locality (Robbrecht 1988, Andersson 1992). Recently, Delprete and Jardim (2012) and Souza (2015) included *D. compressus* in new Brazilian floristic works. Moreover, *Diacrodon* is scarcely treated in other bibliographic manuscripts probably due to its very restricted distribution and its large similarity with *Borreria verticillata* (L.) G. Mey., with which it is often confused.

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In the original description, Sprague mentioned that the specimens analyzed resemble *B. verticillata* based on general aspects as well as for the occurrence of flowers with a two lobed calyx. Morphologically, *Diacrodon* has been defined primarily based on its fruits, described as strongly compressed, indehiscent and single-seeded. In this sense, fruit dehiscence was classically considered a character with taxonomical value to separate genera in the Spermacoceae tribe. Nowadays however, it is a questioned treat in the delimitation of related genera such as *Borreria* and *Spermacoce*. Dessein (2003) treated *Diacrodon* as part of the *Borreria-Spermacoce* complex and argued that the difference in morphology of the fruits is a result of a recent evolutionary event, also he mentioned that this character is not enough to treat *Diacrodon* as a separate genus.

In South America, several taxonomic and morphological studies recognize *Spermacoce* and *Borreria* as two separate genera mainly by floral and palynological characters which are valuable to distinguish *Borreria* from *Spermacoce* (Miguel and Cabral 2013, Florentin et al. 2016). They also mention that fruit dehiscence is not a valuable character to delineate the two genera. Moreover, they indicate that *Borreria* can be distinguished by a set of characters such as axillary glomerules (with bilateral development, completely rounding the stem), flowers with exserted stamens and stigma, campanulate, infundibuliform, ciatiform or subhypocrateriform corolla, and spheroidal or oblate-spheroidal, porate, colporate or colpate with endocingulum pollen grains, and tectate perforate exine (Miguel and Cabral 2013, Miguel 2016, Sobrado 2016).

As part of a larger study on *Borreria* and because *Diacrodon* is often compared with one of its species, we address two objectives in this work: (1) evaluate the systematic position of *Diacrodon* with respect to other genera of the tribe Spermacoceae, and (2) determine the identity of *D. compressus*. For this reason, we analyze the phylogenetic position of *Diacrodon* using ITS and ETS sequence data. Additionally, we study flower, fruit, seed and pollen morphology of *Diacrodon* and make a comparison with two morphologically similar species of *Borreria* that also have a two lobed calyx (*B. spinosa* Cham. & Schltdl. ex DC., and *B. verticillata*).

MATERIALS AND METHODS

TAXONOMICAL AND MORPHOLOGICAL STUDIES

Herbarium material was consulted at ASE, CTES, HUEFS, K, NY, RB, SI, UB, UEC, UFRN and US (Herbarium acronyms follow Thiers 2016). Measurements, colors and other details given in the descriptions are based on herbarium specimens. We used conventional taxonomic methods.

Description of pollen and seed micromorphology is based on following specimens: ***Diacrodon compressus***: Brazil, Bahia, Estrada de Barra para Olho d´Agua, 11º22’55.8” S 43º20’32.8” W, 423 m, 19 May 2010, *R. Salas et al. 424* (CTES, HUEFS, SI); Brazil, Bahia, Pilão Arcado, a 2 km de la ciudad, camino Remanso, 9º51’55.5” S, 42º18’38.2” W, 409 m, 23 May 2010, *R. Salas et al. 442* (CTES); ***Borreria spinosa***: Argentina, Chaco, Villa Ángela, 24 Feb 1964, *A.G. Schulz 13668* (CTES); Argentina, Chaco, Dpto. San Fernando, Colonia Benítez, 27°21’34.5” S 58°57’36.9” W, 58 m, 26 Feb 2017, *S. Sobrado & J. Valdés 180* (CTES); Argentina, Salta, Anta, 25º29’49.5” S 63º38’23.9” W, 312 m, 7 Dec 2012, *H.A. Keller et al. 11160* (CTES); and ***Borreria verticillata***: Argentina, Misiones, Loreto, 27°20’27.24” S 55°32’24.06” W, 9 Dec 2011, *S. Sobrado et al. 116* (CTES). Palynological data is based on observations of acetolyzed pollen grains following the method of Erdtman (1960). Observations were made with a Jeol JSM 5800 LV scanning electron microscope (SEM) and with an Olympus BX51 light microscope (LM) with an × 100

oil immersion lens. For LM observations, pollen grains were mounted in glycerin jelly, whereas for SEM observations, pollen was put on stubs and sputter-coated with gold. LM measurements were conducted on at least 20 mature pollen grains. Seeds were examined without any treatment using SEM. The terminology of pollen follows Punt et al. (2007) and that of seeds follows Stearn (1986).

In order to analyze the fruit morphology of *D. compressus* using LM, fruits were fixed in FAA (formaldehyde, acetic acid, 70% alcohol, 90:5:5) and subsequently dehydrated using tertiary butyl alcohol series. After dehydration, fruits were embedded in paraffin (Johansen 1940), transversely sectioned with a rotary microtome, and finally stained with safranin-astra blue (Luque et al. 1996).

MOLECULAR STUDY

In total 55 ingroup taxa are included in the molecular analysis. *Bouvardia ternifolia* (Cav.) Schltdl. is used as outgroup taxon. The ingroup contains representatives of 14 genera of the *Spermacoce* clade (Kårehed et al. 2008, Salas et al. 2015) of which the majority has been analyzed before (Kårehed et al. 2008, Groeninckx et al. 2009, Neupane et al. 2015, Salas et al. 2015). For this study, we included representatives of *Diacrodon compressus* and increased the sampling of the American *Borreria* species from 8 to 12. Selected DNA markers for this study are the nuclear ribosomal ITS and ETS. Methods used for DNA extraction, PCR amplification, sequencing and alignment follow Janssens et al. (2006, 2007, 2016). Model selection for the Bayesian inference analysis was conducted with ModelTest 3.06 (Posada and Crandall 1998) under the Akaike Information Criterium (AIC). The GTR+G model was selected for both ITS and ETS. Maximum Likelihood analyses were conducted using the RAxML search algorithm (Stamatakis et al. 2005) under the GTRGAMMA model (Stamatakis 2006). Support values for the best-scoring ML tree were obtained by analyzing five hundred bootstrap trees using the RAxML Rapid bootstrap algorithm (ML-BS). Appendix provides a list of all taxa analyzed with inclusion of localities, voucher information and GenBank accession numbers.

RESULTS

MOLECULAR STUDY (FIG. 1)

Most of the clades obtained coincide with the taxonomic delineation established for the following genera: *Carajasia* R.M. Salas, E.L. Cabral & S. Dessein, *Crusea* Cham. & Schltdl., *Diodia* L. (*sensu* Bacigalupo and Cabral 1999), *Emmeorhiza* Pohl ex Endl., *Ernodea* Sw., *Galianthe* Griseb., *Mitracarpus* Zucc., *Psyllocarpus* Mart. & Zucc., *Richardia* L., and *Staelia* Cham. & Schltdl. and their relation were discussed previously (Kårehed et al. 2008, Groeninckx et al. 2009, Salas et al. 2015).

Diacrodon compressus falls into a clade together with American *Borreria* species and is strongly supported (**Clade I**, ML-BS=87). In this large clade, we can distinguish two subclades. The first subclade (**subclade IA**) shows *Borreria spinosa* and *Diacrodon* as sister group, which are closely related to *B. dasycephala* (Cham. & Schltdl.) Bacigalupo & E.L. Cabral. The two lobed calyx and zonocolporate pollen grains (type III *sensu* Pire 1996) characterize these three species. The second subclade (**subclade IB**) contains *B. orientalis* E.L. Cabral, R.M. Salas & L.M. Miguel, *B. capitata* (Ruiz & Pav.) DC., *B. multibracteata* E.L. Cabral & Bacigalupo, *B. loretiana* E.L. Cabral, *B. brachystemonoides* Cham. & Schltdl., and *B. tenella* (Kunth) Cham. & Schltdl., which are all species with a four lobed calyx and porate pollen (except *B. orientalis*, which is characterized by zonocolporate grains). The sister taxa of these two

subclades (IA and IB) is *B. verticillata*, another species with a two lobed calyx but which is not closely related to *D. compressus.*

As for *Borreria*, the species are grouped into two strongly supported clades, those grouped with *Diacrodon* in Clade I; and the species of *Borreria* subsect. *Latifoliae* grouped in Clade II. In the latter group (**Clade II**, ML-BS=100) there are three species present, *B. alata* (Aubl.) DC., *B. latifolia* (Aubl.) K. Schum., and *B. schumannii* (Standl. ex Bacigalupo) E.L. Cabral & Sobrado. These species are morphologically characterized by long stigma lobes.

Additionally, the species of the related genus *Spermacoce* are divided in two clades. **Clade III,** containing species of the related genus *Spermacoce*, is strongly supported (ML-BS=95) and consists of two subclades. One of this subclades contains the American *Spermacoce* taxa, *S. confusa* Rendle, *S. glabra* Michx., *S. tenuior* L., and the only Australian species included in this work, *S. breviflora* F. Muell. ex Benth. Whereas the other clade consists of the American species, *S. eryngioides* (Cham. & Schltdl.) Kuntze*, S. incognita* (E.L. Cabral) Delprete, and *S. prostrata* Aubl. The latter species were initially treated under *Borreria* sect. *Pseudodiodia* (*sensu* Bacigalupo and Cabral 1996), but recently they were designated as *Spermacoce* (Florentin et al. 2016). Furthermore, this clade is related to species of *Psyllocarpus* yet only with moderate support (ML-BS=67).

Clade IV (ML-BS=96) is formed exclusively by African *Spermacoce* species: *S. dibrachiata* Oliv., *S. sphaerostigma* (A. Rich.) Oliv., *S. stipularis* Dessein, and *S. subvulgata* (K. Schum.) J.G. García. This clade is weakly related to a lineage containing species of *Hexasepalum* (*H. sarmentosum* (Sw.) Delprete & J.H. Kirkbr.) and two species of *Ernodea* (*E. littoralis* Sw. and *E. taylori* Britton).

MORPHOLOGICAL ANALYSES

Morphological characters were compared between *Diacrodon compressus* and either the species it was formerly confused with, *Borreria verticillata*, as well as the species it is closely related to, *B. spinosa* (Table I).

Flower morphology (Fig. 2)

Except for the campanulate corolla and the spatulate calyx lobes in *B. verticillata* (*vs.* infundibuliform and narrowly triangular respectively in *D. compressus*) there are no significant differences in the morphological structures of the flowers between these similar species (Fig. 2a-d, i-l). On the other hand, *B. spinosa* differs mainly in the calyx lobes indumenta, the morphology and papillae coverage of the stigma, and the form and length of the nectariferous disc cells (Fig. 2e-h).

Palynology (Fig. 3).

Borreria spinosa, *B. verticillata*, and *Diacrodon* are identical in pollen morphology. In general, these three taxa are characterized by oblate-spheroidal, medium-sized, and zonoaperturate grains, with 6-8 compound apertures, colpus as ectoaperture and a lalongate endoaperture. The exine is tectate-perforate and uniformly spinulate (Fig. 3c, f, i).

Fruit morphology (Fig. 4a-h)

Fruit morphology (fruit form and dehiscence pattern of capsules and valves) can be used to distinguish the three species investigated here. *Diacrodon compressus* can be easily distinguished from *Borreria spinosa*

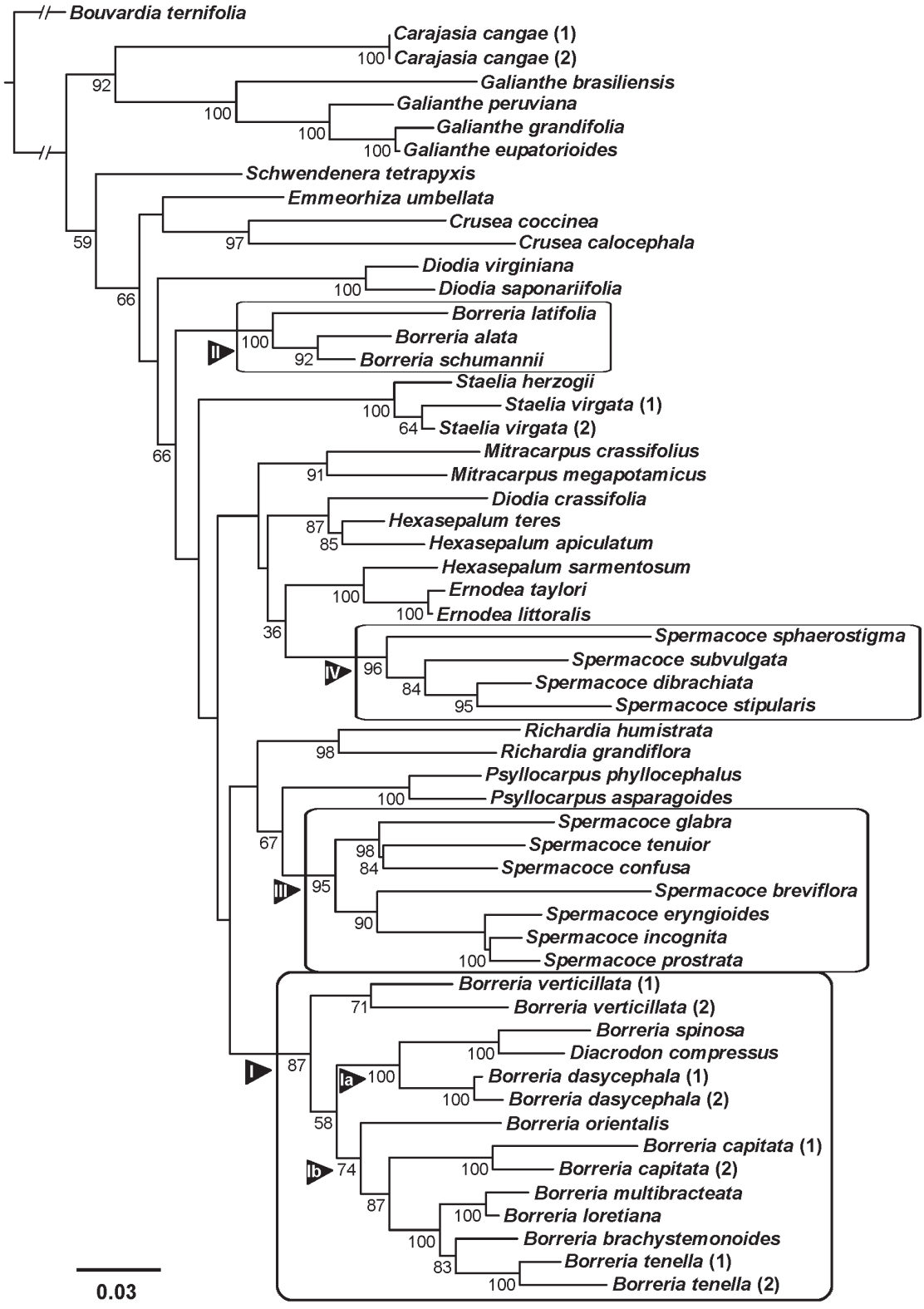

Figure 1 - ML tree showing phylogenetic position of *Diacrodon* in Spermacoce clade based on nuclear (ITS, ETS) data. Values at the nodes represent bootstrap support (ML-BS).

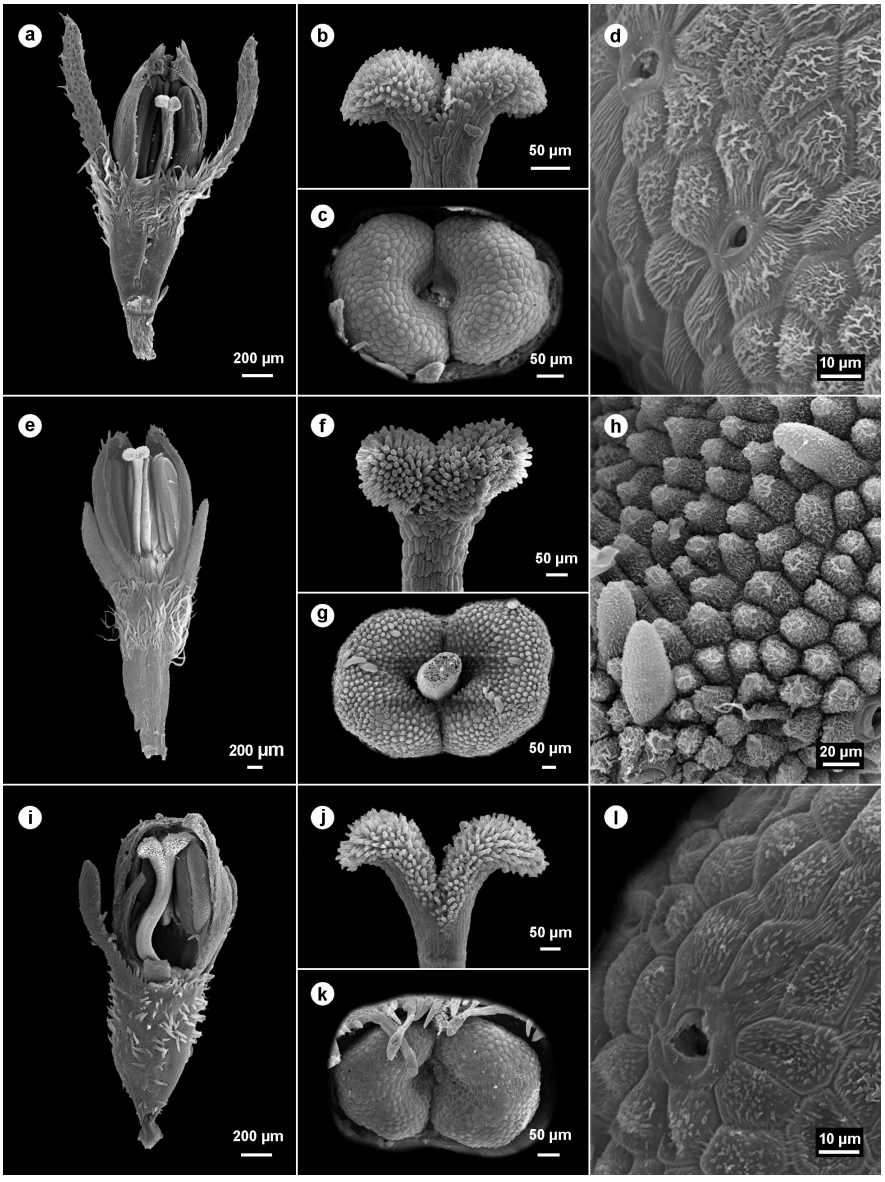

Figure 2 - Flower morphology. **a**-**d**. *Diacrodon compressus,* **a**. Flower bud. Note the narrowly triangular calyx lobes and the hypanthium pilose in the upper part; **b**. Shortly bifid stigma, showing short papillae at the inner surface; **c**. Nectariferous disc; **d**. Isodiametric and uniform disc cells; **e**-**h**. *Borreria spinosa,* **e**. Flower bud. Note the narrowly triangular calyx lobes and the hypanthium puberulous in the upper part; **f**. Bilobed stigma, showing fully surface covered with papillae; **g**. Nectariferous disc; **h**. Conical and not uniform disc cells. Note some of them with a greater length; **i**-**l**. *B. verticillata*, **i**. Flower bud. Note the spatulate calyx lobes and the hypanthium pilose in the upper part; **j**. Shortly bifid stigma, showing papillae at the inner surface; **k**. Nectariferous disc; **l**. Isodiametric and uniform disc cells.

and *B. verticillata* by having cuneiform to obovate capsules and strongly compressed to the septum (Fig. 4a). *B. spinosa* and *B. verticillata* present ovate to narrowly obovate and globose or subglobose capsules (Fig. 4g, h). Also, *D. compressus* has indehiscent fruits, however sometimes the capsules split tardily into two indehiscent valves (mericarps). Transverse sections reveal a region between the two locules where

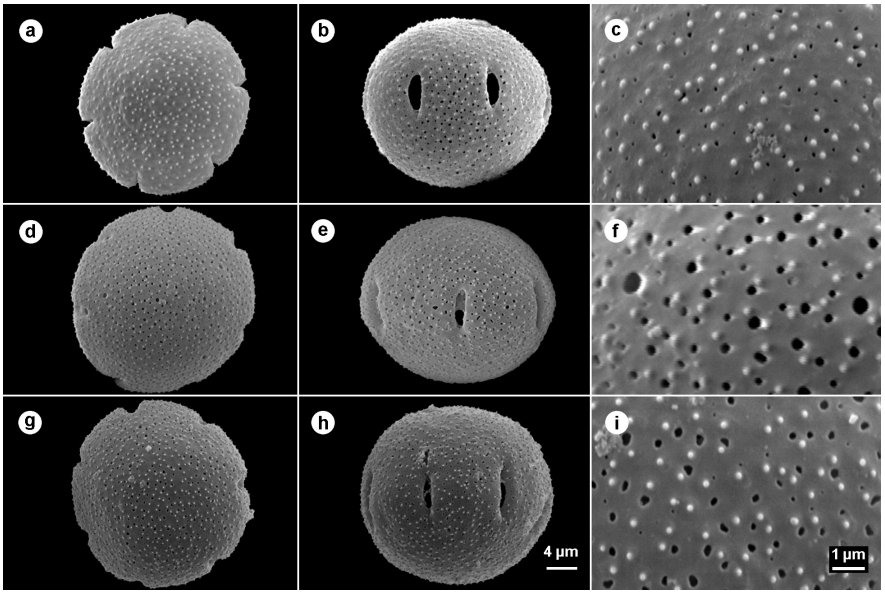

Figure 3 - Pollen morphology. **a-c**. *Diacrodon compressus,* **a**. Polar view; **b**. Equatorial view; **c**. Detail of the exine; **d-f**. *Borreria spinosa*, **d**. Polar view; **e**. Equatorial view; **f**. Detail of the exine; **g-i**. *Borreria verticillata*, **g**. Polar view; **h**. Equatorial view; **i**. Detail of the exine. Scales: **a-b**, **d-e**, **g-h** 4 µm (on **h**); **c**, **f**, **i** 1 µm (on **i**).

the septum cells are thinner, indicating the area where the dehiscence occurs (Fig. 4f). In comparison, the capsules of *B. spinosa* and *B. verticillata* show always the same dehiscence pattern, except when the valves split. In *B. verticillata* both valves can be dehiscent (Fig. 1h), resembling *B. spinosa* (Fig. 4g), yet can also present two indehiscent valves similar to *D. compressus* (Fig. 4a); but in the last case the capsule and seed are not compressed. Moreover, the seeds always undergo a normal development in each locule in both *Borreria* species analyzed here. To date, it was always assumed that *D. compressus* as one seeded (Sprague 1928). However, SEM and LM images of transverse sections of fruits reveal the presence of capsules with one normal and one aborted seed as well as capsules with two normal seeds (one per locule) [Fig. 4b-f].

Seed morphology (Fig. 4i-k)

In spite of the similarities in seed morphology between the three species, *Diacrodon* is somehow distinct as it is characterized by medium sized seeds, which are laterally compressed and narrowly elliptic in outline (ventral view). Seeds of *B. spinosa* and *B. verticillata* are elliptic to obovate, whereas *B. spinosa* has larger seeds and *B. verticillata* has smaller seeds. In addition, *Borreria* species have seeds with a wider longitudinal groove covered by strophiole compared to those of *Diacrodon*.

DISCUSSION

PHYLOGENETIC POSITION OF *Diacrodon*

Our phylogenetic analysis supports the inclusion of *Diacrodon* in *Borreria* in order to form a monophyletic and natural group. Its sole species, *D. compressus*, is intermingled in a group with some American *Borreria* species. In agreement with previous results, we found that *Borreria* currently represent a polyphyletic

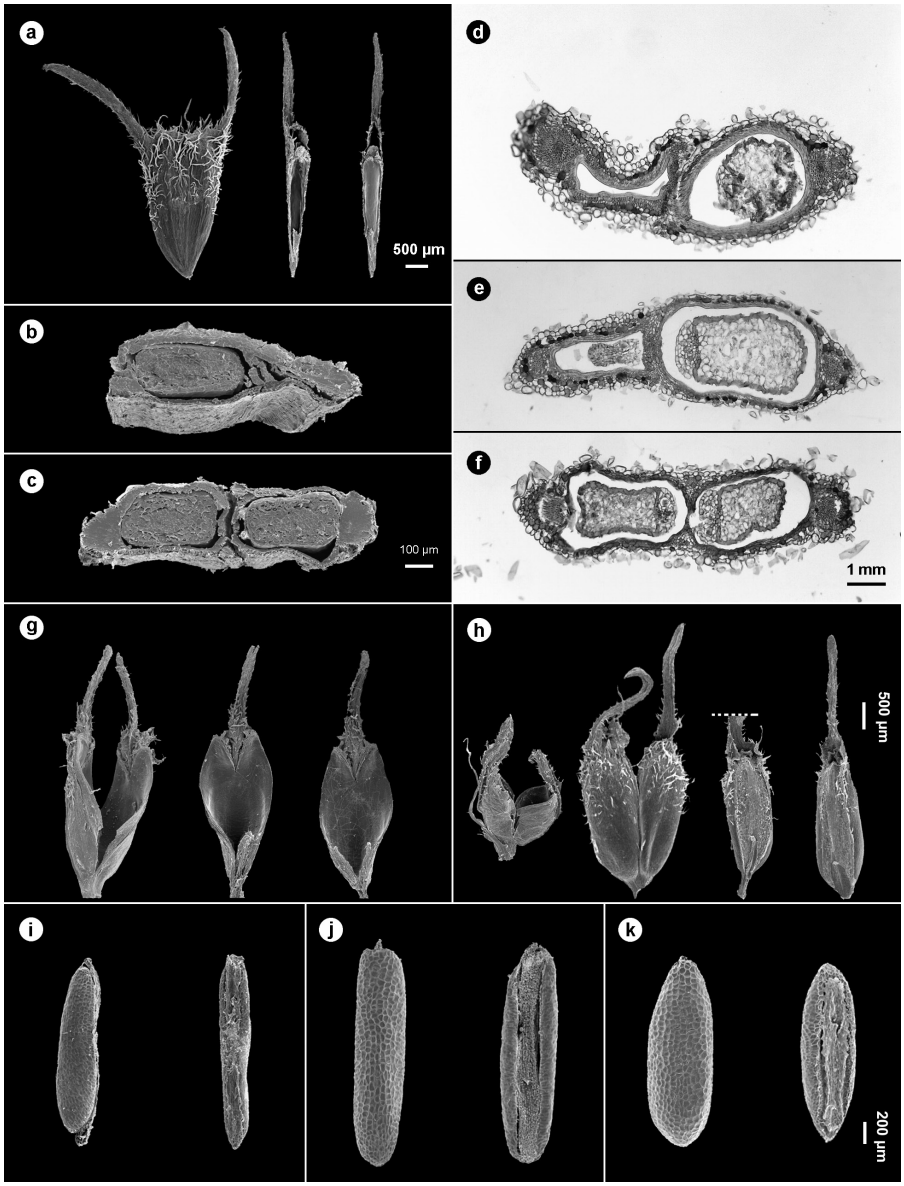

Figure 4 - Fruit and seed morphology. **a**-**h**. Fruits. **a**-**f**. *Diacrodon compressus,* **a**. Fruit and indehiscent valves; **b**. Cross section of a fruit with one seed (SEM); **c**. Cross section of a fruit with two seed (SEM); **d**. Cross section of fruit with one seed (MO); **e**. Cross section of fruit with a mature seed and with an aborted seed (MO); **f**. Cross section of fruit with two seeds (MO); **g**. *Borreria spinosa,* Fruit and valves, both dehiscent; **h**. *Borreria verticillata,* Fruits and valves. Note the dehiscence pattern variation of the valves; **i**-**k**. Seeds. **i**. *Diacrodon compressus,* lateral and narrowly elliptic ventral view. Note the lateral compression of the seed and the narrowly groove; **j**. *Borreria spinosa*, dorsal and elliptic to obovate ventral view. Note the wide groove; **k**. *B. verticillata*, dorsal and obovate ventral view. Note the wide groove.

genus. Dessein (2003) and Salas et al. (2015) obtained similar results, based on a more limited number of American *Borreria* species. First, some species that currently are *Borreria* subsect. *Latifoliae* (with deeply bifid stigma) fell in their study in a different and well supported clade indicating that this group is probably a monophyletic clade (Sobrado 2016). Second, in their results others American *Borreria* species

TABLE I
Comparison of morphological features in *Diacrodon compressus*, *Borreria spinosa,* and *B. verticillata*.

	Diacrodon compressus	*Borreria spinosa*	*Borreria verticillata*
Stem pubescence	Glabrous to glabrescent	Glabrescent to pubescent with papillae or long hairs in the angles	Glabrous to glabrescent, rarely winged
Leaf shape and dimension	Narrowly elliptic to narrowly obovate	Linear to narrowly obovate	Narrowly elliptic to elliptic
	8-50 × 2-7 mm	12-65 × 2-15 mm	13-40 x 2-30 mm
Leaf pubescence	Glabrous or glabrescent in the middle vein	Glabrescent and with papillae in the middle vein	Glabrous and with papillae in the middle vein
Stipular sheath pubescence and bristles number	Glabrescent	Puberulous or pubescent	Glabrous or puberulous
	6-8 bristles	5-8 bristles	5-7 bristles
Glomerules	1 apical until 3 axillary, the apical hemispherical	1 apical until 5 axillary, the apical hemispherical	1 apical until 3 axillary, the apical spherical
Calyx lobes	2	2 or 4	
	narrowly triangular		spatulate
	scabridulous or ciliate	glabrous or pilose in the base	puberulous in the margin
	1.5-2.3 mm long	0.9-2.3 mm long	0.4-1.2 mm long
Hypanthium pubescence	Pilose in the upper part	Pilose in the upper part	Puberulous in the upper part
Corolla	Infundibuliform		Campanulate
	2.5-4 mm long	3-4 mm long	1.5-3.2 mm long
Stamens and stigma position	Exserted	Exserted	Exserted
Stigma	Shortly bifid	Bilobed	Shortly bifid
	1.92-2.87 mm long	1.96-2.76 mm long	2.11-3.89 mm long
	Papillae covering only the inner surface	Papillae covering the entire surface	Papillae covering only the inner surface
Nectariferous disc cells	Isodiametric and uniform	Conical, some with a greater length, like a finger-form and not uniform	Isodiametric and uniform
Capsule	Cuneiform to obovate, laterally compressed to the septum	Subglobose, narrowly obovate to obovate	Globose to subglobose, ovate to obovate
	2.1-3.5 × 1.2-1.7 mm	1.6-2.7 × 0.96-1.4 mm	0.89-2.10 × 0.75-1.36 mm
	Indehiscent or tardily dehiscent	Dehiscent	Dehiscent
	Pilose at the upper part	Pubescent at the upper part	Puberulous or pubescent at the upper part
Valves dehiscence	Indehiscent	Dehiscent	Dehiscent or indehiscent
Number of seed per fruit	1 or 2	2	2
Seed	Narrowly elliptic	Elliptic to obovate	Obovate
	1.77-1.97 x 0.25-0.45 mm	1.49-1.71 x 0.49-0.68 mm	0.96-1.11 x 0.39-0.52 mm
Ventral groove	Narrowly elliptic (136-140 µm widht)	Wide (156-200 µm widht)	
	Reticulate-foveate	Reticulate-foveate	Reticulate-foveate
Testa	Tetragonal and isodiametric to elongated longitudinally at the center cells; anticlinal wall slightly straight; periclinal wall striate and cleaved	Isodiametric cells; anticlinal wall slightly straight; periclinal wall smooth	
Pollen	6-7 zonocolporate, oblate-spheroidal (P= 16.5-22.6 µm, E= 19.15-25.92 µm)	7 zonocolporate, oblate-spheroidal (P= 28-30 µm; E= 30-34 µm)	6-8 zonocolporate, oblate-spheroidal (P= 20.2-23.5 µm; E= 21.2-26)
	Tectate-perforate, spinulate exine	Tectate-perforate, spinulate exine	Tectate-perforate, spinulate exine
Distribution	Brazil and Paraguay	Wide distribution in America, from Mexico to Argentina	Wide distribution in America, from United State to Argentina, and introduced in Africa

(with capitate, bilobed or slightly bifid stigma) which are placed in *Borreria* subsect. *Borreria* formed a clade with African species of the related genus *Spermacoce*. However, this last relation is refuted by our study, because African *Spermacoce* representatives form a strongly support clade, which is weakly related to *Ernodea* and *Hexasepalum*. In addition, American and Australian *Spermacoce* species are nested in a different monophyletic group related to *Psyllocarpus* and distinct to *Borreria*.

Although, we consider that these results partially elucidate the controversy regarding the generic status between *Borreria* and *Spermacoce*, we believe that it is important to find synapomorphies that can support a generic rearrangement. To further assess the generic limitation of *Borreria* and *Spermacoce*, and the relation between American, Australian and African species, a more thorough morphological and molecular study that includes more gene markers and a larger sampling is needed.

IDENTITY OF *Diacrodon compressus*

Most of the morphological characters analyzed in this study support the inclusion of *Diacrodon compressus* in the genus *Borreria*.

Distinction of *Diacrodon* into a genus of its own was based on the lack of dehiscence in the fruit and on the development of a single seed per fruit. In this sense, the presence of completely indehiscent capsules corresponds with the fruit morphological description of some *Spermacoce* species. Also, we notice that Sprague´s species has a tardily dehiscent capsule opening in two indehiscent valves. However, this type of dehiscence should be evaluated whether it is an adaptation to flooded environments because some specimens of *Diacrodon* were recorded to grow on margins of streams and rivers. This hypothesis could be supported by the fact that some species of *Borreria* which also have capsules with both valves being indehiscent are occurring in similar conditions [eg. *B. dasycephala, B. schumannii* and *B. hyssopifolia* (Willd. ex Roem. & Schult.) Bacigalupo & E.L. Cabral]. Additionally, the abnormal development of seeds is also mentioned in the original description of *B. noronhensis* Sucre (Sucre 1969), and *B. diamantinae* R.M. Salas & E.L. Cabal (Cabral et al. 2011). Even Dessein (2003) observed a tendency to abort one of the ovules in *Spermacoce hockii* (De Wild.) Dessein. One Australian species, *S. gibba* Harwood is also characterized by an indehiscent and one-seeded capsule (Harwood and Dessein 2005). These examples show that the fruit characteristics referred by Sprague are not exclusive to *Diacrodon*. Also, it is according with that fruit dehiscence is not valuable character to differentiate *Borreria* from *Spermacoce*.

However, *Borreria* is currently defined by a distinct set of floral and palynological characters (Miguel 2016, Miguel and Cabral 2013). In this sense, *Diacrodon compressus* with its flowers with exserted stamens and stigma, infundibuliform corolla, oblate-spheroidal, colporate pollen grains, and tectate perforate exine, is identical to *Borreria*. In addition, the shape of the stigma that defines *Borreria* subsect. *Borreria,* is capitate, bilobed or shortly bifid. According to *Diacrodon* is characterized by a short bifid stigma as in subsection *Borreria*, which also includes others species with calyx two lobed. Pollen morphology in *Diacrodon*, *B. spinosa* and *B. verticillata* corresponds to the pollen type III described by Pire (1996), which is exclusively found among American *Borreria* species.

In conclusion, by combining morphological and molecular evidence we suggest to transfer the monotypic genus *Diacrodon* to the species-rich genus *Borreria*. This taxonomical change implies a different fruit morphology for *Borreria,* and makes clear that the dehiscence of fruits alone contains no taxonomic value to delineate *Borreria* from *Spermacoce*. Both genera have at least 4 types of fruits: (1) Capsules

with both dehiscent valves [eg. *B. spinosa*, *S. eryngioides*]; (2) Capsules with a dehiscent valve and the other indehiscent [eg. *B. ocymifolia* (Wild. Ex Roem. & Schult.) Bacigalupo & E.L. Cabral, *S. tenuior*]; (3) Capsules with two indehiscent valves (mericarps) [eg. *B. dasycephala*, *S. natalensis* Hochst.]; and (4) completely indehiscent fruit [eg. *B. diacrodonta*, *S. glabra* Michx.].

TAXONOMIC TREATMENT

Borreria diacrodonta* L. M. Miguel & E. L. Cabral, *nom. nov., comb. nov. *Diacrodon compressus* Sprague, Kew Bull. 1: 33-34. 1928, non *Borreria compressa* Hutch. & Dalziel, Fl. W. Trop. Afr. 2: 135. 1931.

Type: BRAZIL: Ceará, Fortaleza, 20 miles inland, on the plains, 1926, *B.G.C. Bolland s.n.* [**Lectotype here designated: K (barcode 173612, JSTOR image!);** isolectotypes K (barcode 16439, JSTOR image!), K (barcode16442), HUEFS (barcode 42204), HUEFS (barcode 42206), IPA (barcode 41338), IPA (barcode 41339)]. **Figs. 5 and 6a, b.**

Erect to decumbent suffrutex, 8-50 cm tall, ramose; stem quadrangular, notable angles sometimes whitish in distal internodes, glabrous to glabrescent; internodes 4.5-58.5 mm long. Leaves sessile, pseudoverticillate; blade narrowly elliptic to narrowly obovate, 8-50 × 2-7 mm, base obtuse, apex acute, papery, glabrous or glabrescent in the middle vein, secondary veins inconspicuous; stipular sheath 2-4 mm long, glabrescent, with 6-8 bristles, 0.79-5.36 mm long, filiform, glabrous. Floriferous axis with 1 apical and until 3 axillary bilateral glomerules, 7-18 mm wide, multi-flowered, bracts 4-8. Flower sessile; hypanthium turbinate, 1.3-2.4 mm long, pilose at the upper part; calyx 2-lobed, lobes narrowly triangular, 1.4-2.2 mm long, scabridulous or ciliate; corolla 4-lobed, infundibuliform, 2.5-4 mm long, white, externally glabrous with some papillae at the apex of the lobes, internally with a ring of moniliform hairs at the middle of the tube; stamens exserted, anthers 1-1.2 mm long, filaments 1.5-2 mm long, pollen 6-7-zonocolporate, medium sized (P= 16.5-22.6 μm, E= 19.15-25.92 μm) and oblate-spheroidal (P/E= 0.89), ectocolpi 4-6 μm long, endoaperture lalongate with margins poorly outlined, exine tectate-perforate, uniformly spinulate (nano-spinules 0.1-0.17 μm long), 1.63-2.48 μm thick, nexine inner surface finely granular with narrow and distinct endocracks, perforations circular (0.4-0.6 μm diam); style exserted, 2.2-4 mm long, stigma shortly bifid, each lobe 1.3-2 mm long, papillate at the inner surface; nectariferous disc bipartite, with uniform, striate and isodiametric cells. Capsule sessile, cuneiform to obovate, strongly compressed perpendicular to the septum, 2.1-3.5 × 1.2-1.7) mm long, pale yellow or castaneous, albo-pilose at the upper part, with 2-3 visible ribs, crowned by persistent and patent ciliate calyx lobes, indehiscent or tardily dehiscent in two indehiscent valves; seeds one or two by capsule, strongly laterally compressed, narrowly elliptical, 1.77-1.97 x 0.25-0.45 mm, brownish, ventral face with a longitudinal groove covered by a hyaline strophiole with numerous raphide bundles; testa reticulo-foveate, cells isodiametrical to elongate longitudinally at the center, anticlinal wall slightly straight, periclinal wall striate and cleaved.

Specimens examined: BRAZIL. *S.l.*, 25 August 1980, *P.P.D. 029* (RB 00334547). **Bahia:** ca. 4 km NE from Gentido do Ouro along the road towards Central, ca. 1000 m, 42°30'W, 11°24'S, 22 February 1977, *R.M. Harley 18942* (K); Chapadão Ocidental da Bahia, 12 km N of Correntina, on the road to Inhaúmas, 44°40'W, 13°15'S, 28 April 1980, *R.M. Harley 21867* (only left stem, RB); 3km de Morro do Chapéu, 11º33'S, 41º11'W, 1112 m, 26 August 1981, *L.M. Gonçalves 126* (RB); Caetité, Tucano, 15 March 1995, *G. Hatschbach et al. 61930* (CTES); Boquira, BR-122, km 54, 20 April 1996, *G. Hatschbach*

et al. 65070 (CTES); Oliveira dos Brejinhos, BR-122, km 8, 20 April 1996, *G. Hatschbach et al. 65082* (CTES); Estrada de Barra para Olho d´Agua, 11º22'55.8"S, 43º20'32.8"W, 423 m, 19 May 2010, *R. Salas et al. 424* (CTES, HUEFS, SI); Barra, Olho d´Agua, 10º48'31.4"S, 43º21'29.9"W, 400 m, 19 May 2010, *R. Salas et al. 426* (CTES, HUEFS, SI); Barra, Ibiraba, 10º47'26.8"S, 42º48'58"W, 402 m, 20 May 2010, *R. Salas et al. 432* (CTES, HUEFS, SI); Buritirama, Altamira, 10º49'39.3"S, 43º33'34.1"W, 444 m, 21 May 2010, *R. Salas et al. 436* (CTES, HUEFS, SI); Pilão Arcado, a 2 km de la ciudad, camino Remanso, 9º51'55.5"S, 42º18'38.2"W, 409 m, 23 May 2010, *R. Salas et al. 442* (CTES, HUEFS, SI); Bom Jesus da Lapa, basin of the Upper São Francisco River, 4 Km N of Bom Jesus da Lapa, 13º13'S, 43º24'W, 20 April 1980, *R.M. Harley et al. 21585* (HUEFS, K, RB, UB, UEC, US); Bom Jesus da Lapa, estrada para Ibotirama, 13º15'0"S, 43º25'0"W, 530 m, 11 April 2005, *J.G. Carvalho-Sobrinho 511a* (HUEFS); Casa Nova, Fazenda Santarém-Sítio Morrinho, área vazante do lago de Sobradinho, 9º36'38"S, 41º19'43"W, 410 m, 10 October 2004, *L.P. Queiroz et al. 9664* (HUEFS); Ilhéus, cidade de Ilhéus, praia do Pontal, 30 July 2001, *J.G. Jardim et al. 3724* (RB); Pilão Arcado, sitio do Poção, 10°07'24"S, 42°33'00"W, 10 November 2009, *A.P. Prata et al. 2544* (ASE). **Distrito Federal:** Samambaia, Parque Boca da Mata, 10 November 1995, *J.M. Rezende 226* (CTES); **Goiás:** São Domingos, Rod. GO-110, Serra Geral de Goiás, 15 May 2000, *G. Hatschbach et al. 71119* (CTES); São Domingos, Gruta Angélica, 550 m, 16 May 2000, *G. Hatschbach et al. 71183* (CTES); Alto Paraíso, 14º06'21.3"S, 47º29'21.9"W, 1081 m, 25 April 2009, *L.P. Queiroz et al. 14258* (HUEFS); idem, 14°07'15"S, 47°30'15"W, 1115-1300 m, 25 April 2009, *L.P. Queiroz et al. 14288* (HUEFS). **Minas Gerais:** São Romão, Clube Urucuia, 24 August 1990, *E. Tameirão Neto 515* (CTES); Januária, distrito Tejuco, 15º39'59.5"S, 44º37'58.2"W, 16 May 2002, *J. Lombardi et al. 4771* (CTES); Januaria, 15°29'26"S, 44°21'13"W, 18 April 2012, *P.L. Viana et al. 5897* (CTES). **Pernambuco:** Petrolina, area do banco ativo de germoplasma de plantas forrageiras do CPATSA/EMBRAPA, 9º04'S, 40º18'W, 450 m, 23 June 1983, *L. Coradin et al. 5970* (CEN, CTES, K, SI); idem, 25 July 1984, *G.C. Pinto 125/84* (RB). **Piauí:** Disceu Arco Verde, Camino a São Raimundo Nonato, 9º15'40.5"S, 42º29'23.9"W, 420 m, 23 May 2010, *R. Salas et al. 445* (CTES, HUEFS, SI); Itaueira, Estrada São Raimundo Nonato para Floriano, 7º20'57"S, 43º7'23.6"W, 278 m, 23 May 2010, *R. Salas et al. 448* (CTES, HUEFS, SI). **Rio Grande do Norte:** Caicó, Serra da Formiga, 6°21'18"S, 36°57'17"W, 370 m, 15 August 2009, *J.L. Costa-Lima et al. 201* (UFRN); Caraúbas, 5º36'S, 37º33'W, 6 September 1984, *G.C.P. Pinto et al. 294/84* (HUEFS, RB). **Sergipe**: Gararu, 14 December 1981, *G. Viana 313* (ASE). **PARAGUAY. Canindeyú:** refúgio biológico Mbaracayú (Salto del Guaira), 250 km NE de Hernandarias, 18 February 1993, *G. Caballero Marmori 3054* (CTES).

Distribution: *Borreria diacrodonta* used to be an endemic Brazilian species. It was initially only known from Ceará, Bahia, Pernambuco, Piauí, Rio Grande do Norte, and Sergipe. According to Souza (2015) it is also distributed in Alagoas (Brazil) and Paraíba (Brazil). The specimens here examined from Goiás and Minas Gerais (Brazil) and Paraguay represent new distribution records for this species and as such the species is not endemic to Brazil anymore (**Fig. 6c**).

Phenology: Specimens with flowers and fruits have been collected almost throughout the entire year, except in September and October.

Habitat and ecology: *Borreria diacrodonta* occurs in open areas, savannas and grasslands, in sandy soil, which characterizes the Caatinga vegetation. Also, according to the label of the examined specimen from Goiás, the species also occurs in Cerrado vegetation, yet its location is situated at the border with Bahia and probably this can be regarded as a transition zone between Caatinga and Cerrado. *Borreria*

diacrodonta is also reported from anthropogenic environments such as roadsides, yet also occurs at streams and river margins.

Conservation status: According to the IUCN (2016) criteria, *Borreria diacrodonta* is considered Least Concern (LC). The extent of occurrence (EOO) of this taxon is estimated in 1,871,842.309 km^2, whereas its area of occupancy (AOO) is estimated to be 140.000 km^2 (using a grid cell of 2 km), which falls within endangered status. Nevertheless, in this work, the distribution of *Borreria diacrodonta* was considerably amplified; formerly this taxon was restricted to northern Brazil, whereas now it is known from two countries, Brazil and Paraguay, and eight additional localities. Even more, some species observations indicate that *B. diacrodonta* acts as invasive plant (*E. Tameirão Neto 515*).

Notes: The basonym *Diacrodon compressus* was described based on two dried specimens made by *Bolland* from Ceará (Sprague 1928). At the online database of the Kew herbarium, three specimens of *Bolland s.n.* as type of this species are present, but only two of them have high-resolution images available. The herbarium sheet K 173612 is chosen here as lectotype because it is a specimen in good condition with many flowers and fruits that help to understand and characterize the species.

Taxonomical affinities: In addition to *B. spinosa* and *B. verticillata*, analyzed previously by affinity with *Borreria diacrodonta*, in Brazil and Paraguay are another 12 species of *Borreria* with calyx two lobed. These taxa are included in the following identification key.

KEY TO THE *Borreria* SPECIES WITH TWO LOBED CALYX FROM BRAZIL AND PARAGUAY

1. Plant with filiform stem, stems up to 1 mm wide, leaves 0.3 mm wide, corolla glabrous internally. . . . . ***B. delicatula*** E.L. Cabral

1’. Plant with stems more than 1 mm wide, leaves more than 0.3 mm wide, corolla with a ring of moniliform hairs on the tube internally, sometimes with scattered hairs at the lobes 2

2. Yellow-green plants when dry, leaves opposite without axillary brachiblats 3

2’. Green or green-nigrescent plants when dry, leaves pseudo-verticillate with axillary brachiblast. 5

3. Leaves filiform or narrowly elliptic, 0.5-2 mm wide, secondary veins inconspicuous, stipular sheath with 4-6 filiform bristles, 8-10 bracts . . . . . ***B. limae*** Sucre

3’. Leaves elliptic, oblong or obovate, 4-28 mm wide, secondary veins notorious, stipular sheath with 2-4 triangular or narrowly triangular bristles, never filiform, 4-6 bracts 4

4. Leaves elliptic or obovate with glabrous and coriaceous margin, stipular sheath with triangular bristles of 1-3 mm long . . . . . ***B. runkii*** K. Schum.

4’. Leaves narrowly oblong with scabrid margin, stipular sheath with narrowly triangular bristles of 4-9 mm long . . . . . ***B. viridiflora*** Chodat & Hassl.

5 (2’). Leaves succulent, always glabrous. Exclusively island species 6

5’. Leaves membranaceous, glabrous, puberulous or pilose. Continental species 7

6. Suffrutex up to 80 cm tall, leaves pseudo-petiolate, up to 3 glomerules per floriferous axis rounded by 2 bracts. Fernando de Noronha Island, Brazil . . . . . ***B. noronhensis*** Sucre

6’. Herb up to 9 cm tall, leaves sessile, only with apical glomerule rounded by 4-6 bracts. Trindade Island, Brazil. . . . . ***B. evenia*** Standl.

7. Leaves linear or narrowly elliptic, up to 2.3 mm wide, seeds with transversal grooves and pantoporate pollen grains 8

7’. Leaves narrowly elliptic, elliptic or oblong, 2-10 mm wide, seeds without transversal grooves and zonocolporate pollen grains 9

8. Suffrutex 7-20 cm tall, corolla 1.5-2 mm long, always withe, seeds without elaiosome. . . . ***B. paraensis*** E.L. Cabral & Bacigalupo

8’. Suffrutex 20-50 cm tall, corolla 3-4 mm long, vinaceous, vinaceous turning to white, rare white, seeds with elaiosome (remarkable after rehydrate) . . . . . ***B. elaiosulcata*** E.L. Cabral & L.M. Miguel

9. Leaves always pubescent underneath, without papillae on the mid-vein 10

9’. Leaves glabrous or glabrescent underneath, with papillae on the mid-vein 11

10. Corolla salmon and stamens bluish. . . . . ***B. bahiana*** E.L. Cabral

10’. Corolla and stamens white. . . . . ***B. marticrovettiana*** E.L. Cabral

11. Corolla 2, 3 or 4-lobed in the same glomerule, fruits with caduceus calyx to maturity . . . . ***B. dasycephala*** (Cham. & Schltdl.) Bacigalupo & E.L. Cabral

11’. Corolla always 4-lobed, fruits with persistent calyx to maturity. 12

12. Stems glabrous, fistulose, green-nigrescent plant when dry. Growing always in flooded grassy fields . . . . . ***B. sulcata*** (Bacigalupo) E.L. Cabral

12’. Stems puberulous or pubescent, not fistulose, green plant when dry. Growing in high lands, sometimes in margin of rivers or streams 3

13. Glomerules always globose, bracts adppressed to the stem, calyx lobes spatulate with rounded apex, corolla campanulate . . . . . ***B. verticillata*** (L.) G. Mey

13’. Glomerules hemispherical, bracts spreading, calyx lobes narrowly triangular with acute apex, corolla infundibuliform. 4

14. Fruit subglobose, narrowly obovate to obovate, dehiscent septicidally splitting in two dehiscent valves, always 2-seeded, seeds convex, elliptic to obovate in outline (ventral view) . . . . . ***B. spinosa*** Cham. & Schltdl. ex DC.

14’. Fruit cuneiform to obovate, laterally compressed to the septum, indehiscent or tardilly dehiscent, splitting in two indehiscent valves (mericarps), 1(-2)-seeded, seeds laterally compressed, narrowly elliptic in outline (ventral view) . . . . . ***B. diacrodonta*** L.M. Miguel & E.L. Cabral

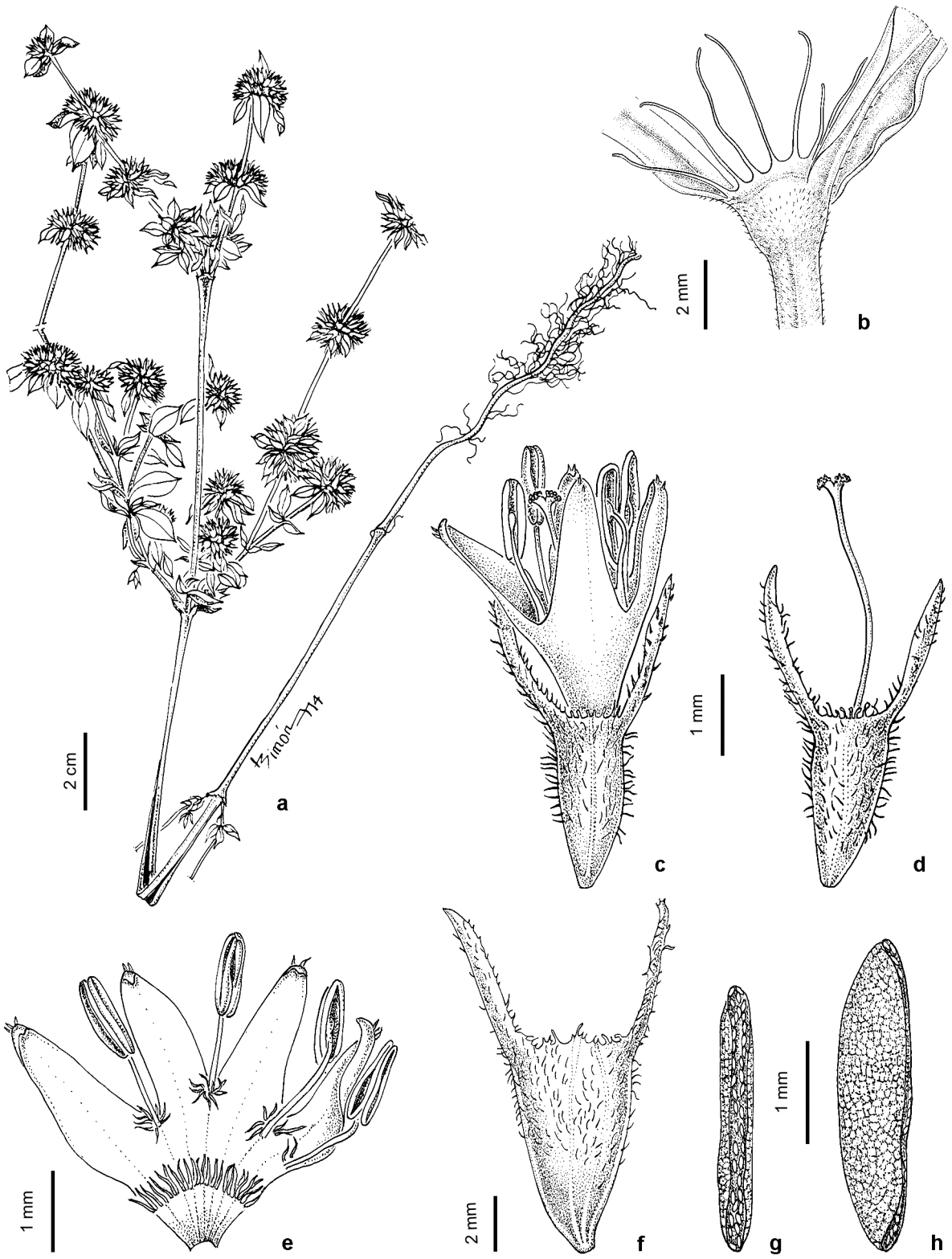

Figure 5 - *Borreria diacrodonta*, **a**. Habit; **b**. Stipular sheath; **c**. Flower; **d**. Hypanthium, calyx lobes, style, and stigma; **e**. Open corolla; **f**. Fruit; **g**. Seed, ventral view; **h**. Seed, lateral view. (Illustration: *L. Simón*).

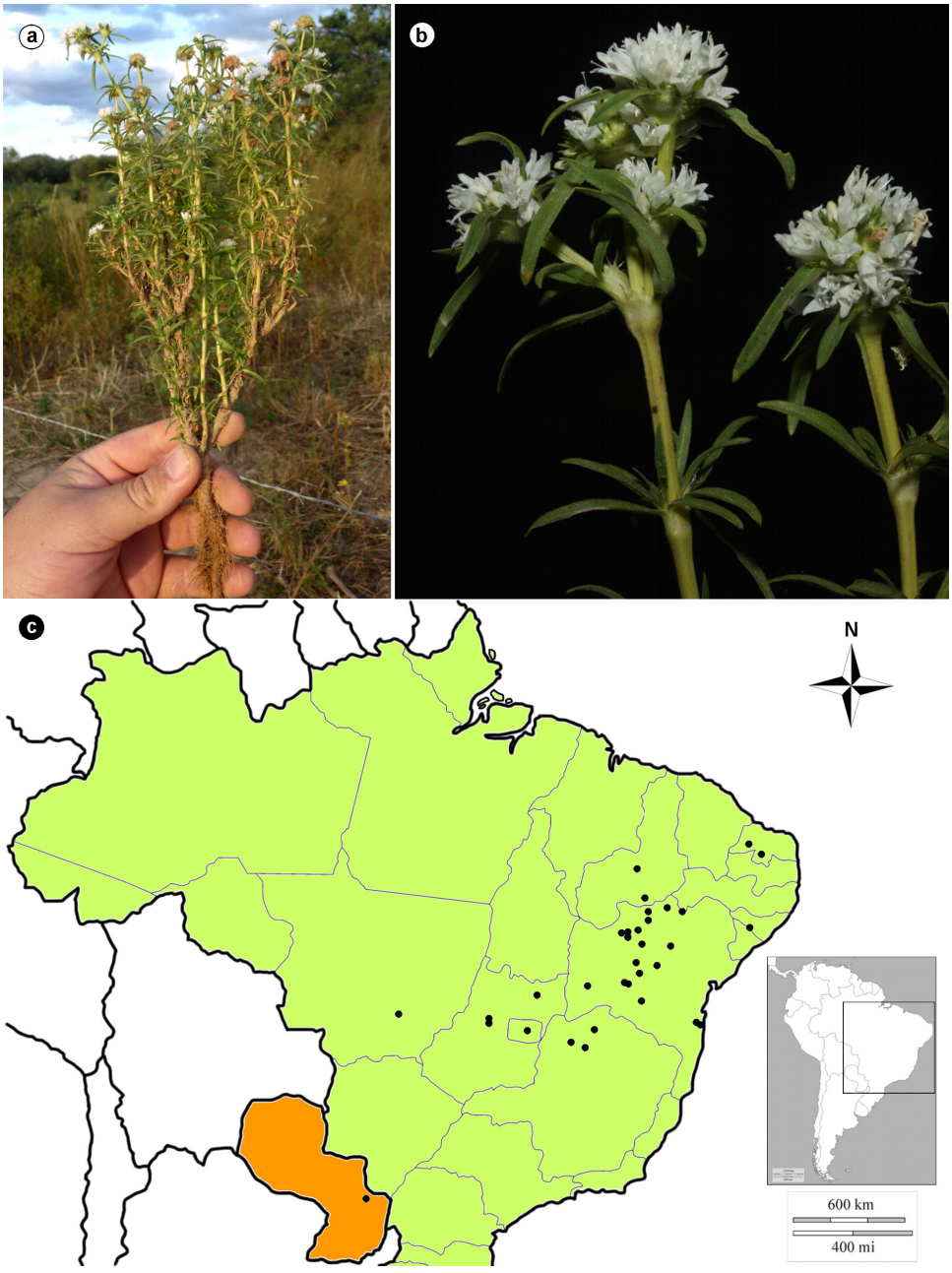

Figure 6 - *Borreria diacrodonta*. **a**. Habit; **b**. Detail of the inflorescence; **c**. Distribution map. (Photographs: *R.M. Salas*).

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APPENDIX

Accession numbers, voucher data and origin of plant material for taxa included in the DNA analyses.

Borreria G. Mey., *B. alata* (Aubl.) DC., Brazil, Goiás, *Queiroz et al. 14105* (CTES, HUEFS; KF736995, KF737036); *B. brachystemonoides* Cham. & Schltdl., Argentina, Corrientes, *Miguel et al. 28* (CTES; MF166821, MF166810); *B. capitata* (Ruiz & Pav.) DC., [1] Brazil, Minas Gerais, *Sobrado et al. 135* (CTES; ITS MF166822); [2] Brazil, Bahia, *Queiroz et al. 13688* (HUEFS; ETS MF166811); *B. dasycephala* (Cham. & Schltdl.) Bacigalupo & E.L. Cabral, [1] Argentina, Misiones, *Salas & Cabaña 388* (CTES; ITS KF73699); [2] Argentina, Misiones, *Miguel et al. 18* (CTES; MF166818, MF166807); *B. latifolia* (Aubl.) K. Schum., Brazil, Goiás, *Queiroz et al. 14110* (CTES, HUEFS; KF736994, KF737035); *B. loretiana* E.L. Cabral, Argentina, Misiones, *Keller & Paredes 9918* (CTES; MF166820, MF166809); *B. multibracteata* E.L. Cabral & Bacigalupo, Brazil, Goiás, *Queiroz et al. 14261* (CTES, HUEFS; KF736990, KF737032); *B. orientalis* E.L. Cabral, R.M. Salas & L.M. Miguel, Argentina, Misiones, *Sobrado & Salas 158* (CTES; MF166823, MF166812); *B. schumannii* (Standl. ex Bacigalupo) E.L. Cabral & Sobrado, Argentina, Misiones, *Cabral et al. 760* (CTES; KF736997, KF737038); *B. spinosa* Cham. & Schltdl. ex DC., Brazil, Minas Gerais, Diamantina, *Viana et al. 5917* (BHCB; MF166817, MF166806); *B. tenella* (Kunth) Cham. & Schltdl., [1] Argentina, Misiones, *Miguel et al. 15* (CTES; MF166819, MF166808); [2] Brazil, Goiás, *Queiroz et al. 14252* (HUEFS, KF736988, KF737030); *B. verticillata* (L.) G. Mey., [1] Centroamérica, *Ochoterena s.n* (CTES; ETS MF166804); [2] Argentina, Corrientes, *Salas 402* (CTES; KF736998, KF737039). ***Carajasia*** R.M. Salas, E.L. Cabral & Dessein, *C. cangae*, [1] Brazil, Pará, *Giorni et al. 179* (BHCB; KF737015, KF737057); [2] idem, *Costa et al. 588* (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). ***Diacrodon*** Sprague, *D. compressus*, Brazil, Ceará, *Bolland s.n* (K; MF166816, MF166805). ***Diodia*** L., *D. saponariifolia* Cham. & Schltdl., Argentina, Misiones, Cabaña & Salas 22 (CTES; KF737007, KF737049); *D. virginiana* L., USA, Missouri, *Taylor 12758* (MO; KF737008, KF737050). ***Emmeorhiza*** 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). ***Hexasepalum*** Bartl. ex DC.; *H. apiculatum* (Willd. ex Roem. & Schult.) Delprete & J.H. Kirkbr., Brazil, Bahia, *Queiroz et al. 13727* (CTES, HUEFS; KF737003, KF737045); *H. sarmentosum* (Sw.) Delprete & J.H. Kirkbr., Cameroon, *Dessein et al. 1521* (BR; KF737005, KF737047); *H. teres* (Walter) J.H. Kirkbr., Brazil, Goiás, *Queiroz et al. 14089* (CTES, HUEFS; KF737048, KF737006). ***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. phyllocephalus* K. Schum., Brazil, Distrito Federal, *Queiroz et 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). ***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); *S. eryngioides* (Cham. & Schltdl.) Kuntze, Argentina, *Salas et al. 378* (CTES; KF736992, KF737033); *Spermacoce glabra* Michx., USA, Missouri, Perry, Taylor 12757 (MO; KF737022, KF73706); *S. incognita* (E.L. Cabral) Delprete, Brazil, Goiás, *Queiroz et al. 14049* (CTES, HUEFS; KF736993, KF737034); *S. prostrata* Aubl., Brazil, Goiás, Nova Roma, *Queiroz et al. 14083* (CTES, CTES; KF736996, KF737037); *S. sphaerostigma* (A. Rich.) Oliv., Zambia, *Dessein et al. 555* (BR; MF166813, MF166801); *S. subvulgata* (K. Schum.) J.G. García, Zambia, *Dessein et al. 216* (BR; MF166815, MF166803); *S. stipularis* Dessein, Zambia, *Dessein et al. 368* (BR; MF166814, MF166802); *S. tenuior* L., México, *Novelo et al. s.n.* (BR; KF737023, KF737065). ***Schwendenera*** K. Schum., *S. tetrapyxis* K. Schum., Brazil, Paraná, *Marques et al. 83* (CTES; KF737017, KF737059). ***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., [1] Brazil, Piauí, *Salas et al. 443* (CTES, HUEFS; ITS KF737026); [2] Brazil, Bahia, *Salas et al. 423* (CTES, HUEFS; ITS KF737025).

Outgroup. *Bouvardia* Salisb., *B. ternifolia* (Cav.) Schltdl., Mexico, Oaxaca, *Ochoterena et al. 454* (BR; KF736987, KF737029).