



CELLULAR AND MOLECULAR BIOLOGY

Multi-loci phylogeny and morphological evidence support the recognition of *Januaria* (Spermacoceae, Rubiaceae), a new monospecific genus endemic to the North of Minas Gerais (Brazil)

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Abstract: *Januaria* is described as a new monospecific genus of Rubiaceae, based on material from Januária, northern Minas Gerais, Brazil. The new taxon is endemic to Brazil, occurring in a vegetation type that is known locally as “carrasco”, in the southern limit of the Caatinga biome. Morphological (including palynological and SEM analyses) and molecular phylogenetic analyses based on nuclear (ETS, ITS) and plastid (*atpB-rbcL*, *petD*, *rps16*, *trnL-trnF*) sequence data were performed in the *Spermacoce* clade (tribe Spermacoceae). The molecular position and morphological features (a unique fruit dehiscence type, and pollen exine with simple reticulum) support *Januaria* as a new genus, with *Mitracarpus* as sister group, from which it differs principally in calyx morphology, corolla shape, and fruit dehiscence. Additionally, a further comparison with other morphologically similar genera is presented. We provide a formal description of *Januaria*, together with a distribution map and comments on its conservation. In addition, a discussion about the Brazilian endemics of the *Spermacoce* clade is given, also with a key to all the genera of this group present in the country.

Key words: Caatinga, fruit, Brazilian Atlantic dry forest, *Januaria lombardii*.

INTRODUCTION

The Rubiaceae is a family that comprises mainly shrubs or trees, however a few lineages within the family include herbaceous species (Robbrecht & Manen 2006). The tribe Spermacoceae (sensu Andersson & Rova 1999) is the largest herbaceous lineage of the family, with over 1000 species in c. 80 genera (Groeninckx et al. 2009, Gibbons 2020, Nuñez-Florentin et al. 2022, Carmo et al. 2022). Tribe Spermacoceae s.s. (sensu Robbrecht 1988, coinciding with the classical definition) - currently known as *Spermacoce* clade - is historically one of the most challenging lineages in the fourth largest

family of flowering plants. From a morphological point of view, the *Spermacoce* clade is easily recognizable from the following combination of characters: herbaceous plants, presence of raphids, fimbriate stipules, uniovulate ovary locules, and pluri-aperturate pollen grains.

In America, the *Spermacoce* clade is currently represented by 23 genera, including the recently described genus *Paganuccia* R.M. Salas (Nuñez-Florentin et al. 2022). In 2015, Salas et al. published the first phylogenetic study focussing on the *Spermacoce* clade using nuclear markers (ITS and ETS), thereby describing the new genus, *Carajasia* R.M. Salas, E.L. Cabral & Dessein, based on morphological and molecular

evidence. Despite various taxonomic and phylogenetic studies carried out in the past to further revise and elucidate generic boundaries and phylogenetic relationships within the *Spermacoce* clade (Miguel & Cabral 2013, Salas et al. 2015, Florentín et al. 2017, Miguel et al. 2018), there are still different opinions among specialists on the delimitation of some genera (e.g. *Spermacoce-Borreria* complex).

The north of Minas Gerais (Brazil) is an area characterized by predominantly xerophytic and deciduous vegetation, which constitutes a mosaic of physiognomies, or vegetational complexes. It is also considered as the southern limit of the Caatinga biome, an area of transition between the Caatinga and the Cerrado (Velloso et al. 2002, Queiroz 2006, Queiroz et al. 2017, Fernandez et al. 2020). Lombardi et al. (2005) conducted a floristic survey in this area, specifically in the municipality of Januária. Among the arboreal/shrub Rubiaceae taxa founded, the authors identified one specimen as "*Borreria* sp." During a recent herbarium study, this specimen was analysed in greater depth and although the primary traits indicated that it undoubtedly belongs to the *Spermacoce* clade, a more detailed analysis revealed that it could not be considered as a member of the genus *Borreria* G. Mey (following the concept of Miguel & Cabral 2013) or another morphologically similar taxon (e.g. *Spermacoce* L. sensu Nuñez-Florentin et al. 2020).

Therefore, in order to elucidate the taxonomic position of this new taxon, a molecular phylogenetic analysis of the *Spermacoce* clade was carried out using molecular markers from the nuclear ribosomal (ITS and ETS) and chloroplast genomes (*atpB-rbcL*, *petD*, *rps16*, and *trnL-trnF*). By applying a complementary molecular and morphological analysis (e.g., fruit dehiscence, pollen, and seed observations), the evolutionary history of the new taxon and its relatives could

be inferred. In addition, a geographic overview of Brazilian *Spermacoceae*, focused on endemic genera of *Spermacoce* clade is given.

MATERIALS AND METHODS

Taxonomic treatment and conservation assessment

Conventional taxonomic techniques were followed for the description and analysis of the new monotypic genus and its species. Additional data was retrieved from herbarium specimens from BHCN and W [herbarium codes according to Thiers (2021, continuously updated)]. Information concerning the habitat, flowering period, and qualitative characteristics, such as the colour of the flowers, were obtained from the herbarium labels.

An assessment of the conservation status was carried out following the IUCN Standards and Petitions Committee (2019) recommendations.

Morphological analyses

For the morphological observations, floral and vegetative parts were rehydrated in warm soapy water and analysed under a stereomicroscope (SM) Leica MZ6 and measured using an electronic digital caliper (Schwyz). The morphological terminology follows Stearn (1986). For scanning electron microscopy (SEM) analyses, flowers were obtained from herbarium material and rehydrated for 12 hours in water at 60°C with a drop of detergent. After the preparation step, the material was dried to critical point with CO₂ and mounted on aluminium stubs. Fruits and seeds were mounted on aluminium stubs without any treatment. All material was sputter coated with 20 nm of gold-palladium. Observations were performed at 20 kV with a SEM Jeol LV 5800 at the Electron Microscopy unit of the Universidad Nacional del Nordeste (UNNE).

Palynological analyses

Pollen grains were acetolysed according to the technique described by Erdtman (1966) and mounted in glycerine jelly for analysis by light microscopy (LM). The shape of the pollen grains, the ratio of the polar axis (P) and the equatorial diameter (E) were studied by photographing at least 20 grains with a LM Leica DM LB2 microscope equipped with a digital camera and then measured afterwards using the program ImageJ v.1.51k (Rasband 2020). The exine structure

was analysed using SEM. The morphological terminology for pollen follows Punt et al. (2007).

Taxon and gene sampling

The sampling included 77 ingroup accessions from the *Spermacoce* clade (Appendix I; Fig. 1). The present sampling represents approximately 30% of the species and 80% of the genera of the *Spermacoce* clade. Two nuclear ribosomal (ITS, ETS) and four plastid (*atpB-rbcL*, *petD*, *rps16*, *trnL-trnF*) DNA regions were selected since they have proven to be phylogenetically informative within the Rubiaceae (Kårehed et al. 2008).

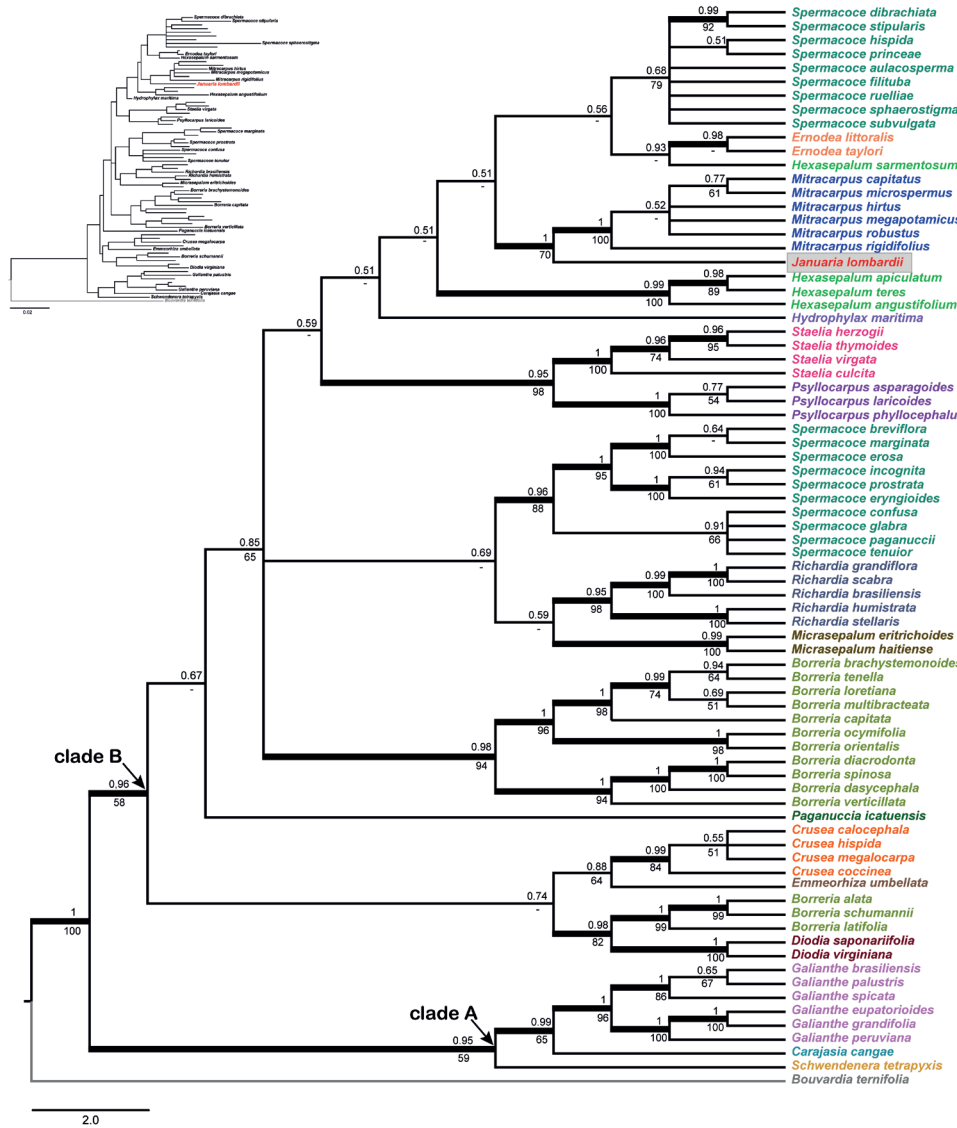


Figure 1. The 50% majority-rule consensus tree and respective phylogram obtained from the Bayesian analysis of the concatenated molecular datasets (ITS + ETS + *atpB-rbcL* + *petD* + *rps16* + *trnL-trnF*). Numbers above the branches indicate posterior probability values (PP), branches subtending nodes with PP > 0.95 are in bold type; values below branches indicate ML bootstrap support (BS) >50%. Capital letters A and B indicate clades discussed in the text. Same coloured species correspond to the same genus.

The new sequences were added to existing alignments used by Nuñez-Florentin et al. (2021, 2022). *Bouvardia ternifolia* Cav. was chosen as an outgroup taxon based on its placement in previous phylogenetic analyses (Kårehed et al. 2008, Salas et al. 2015). The full details of the vouchers used in the phylogenetic inference analysis are provided in Appendix I.

DNA extraction, amplification, purification, and sequencing

In order to assess the phylogenetic position of the new taxon within the *Spermacoce* clade, its genomic DNA was extracted from herbarium material, using a modified CTAB protocol (Doyle & Doyle 1987) according to the protocol outlined by Janssens et al. (2006). Amplification reactions were carried out on a GeneAmp PCR system 9700 (Applied Biosystems) for six markers, two nuclear (ITS, ETS), and four plastid (*atpB-rbcL*, *petD*, *rps16*, *trnL-trnF*). Primers and thermocycler programs used for the amplification of nuclear and plastid markers were those described in Nuñez-Florentin et al. (2022). Purified amplification products were sent to Macrogen, Inc. (Seoul, South Korea) for sequencing. DNA extraction, PCR and amplification were carried out at the molecular lab of Meise Botanic Garden.

Alignment and phylogenetic analyses

The sequences were edited and assembled de novo using Geneious v11.1.4 (Biomatters, Auckland, New Zealand). Automatic multiple alignments were carried out with AliView (Larsson 2014), using the Muscle algorithm, also with subsequent manual editing to improve homology for more variable regions. The combined matrix (nuclear + plastid markers) is available in Supplementary Material – Alignment. combined matrix.

Bayesian inference (BI) and Maximum likelihood (ML) were used to explore the phylogenetic relationships in the study group. The best-fit nucleotide substitution model for each nuclear and plastid dataset was selected with jModelTest v.2.1.4. using the Akaike information criterion (AIC; Posada 2008). The chosen models are shown in Table I. Selected models which are not implemented in MrBayes (for instance, TVM and TIM1) were substituted by the closest over-parameterized model.

BI analyses were run in the Cyber infrastructure for Phylogenetic Research (CIPRES Science Gateway; Miller et al. 2010) using MrBayes v3.1 (Huelsenbeck & Ronquist 2001), first on each individual data partition and then on the three combined data matrices constructed:

Table I. Properties of the datasets used for phylogenetic analyses.

DNA markers	Number of terminals	Alignment length	Variable sites	Parsimony-informative sites	Best-fit model
ITS	73	632	286	210 (33.2 %)	SYM+I+G
ETS	67	364	252	186 (51.1 %)	GTR+I+G
<i>atpB-rbcL</i>	48	805	120	33 (4.1 %)	TPM2uf+G
<i>petD</i>	43	1165	219	92 (7.9 %)	TIM1+I+G
<i>rps16</i>	44	640	135	45 (7 %)	GTR+G
<i>trnL-trnF</i>	43	458	106	42 (9.2 %)	TVM+G
combined nuclear (ITS + ETS)	74	996	538	396 (39.8 %)	ITS + ETS models
combined plastid	59	3068	580	212 (6.9 %)	<i>atpB-rbcL</i> + <i>petD</i> + <i>rps16</i> + <i>trnL-trnF</i> models
combined nuclear + plastid	78	4064	1118	608 (15 %)	ITS + ETS + <i>atpB-rbcL</i> + <i>petD</i> + <i>rps16</i> + <i>trnL-trnF</i> models

“the nuclear matrix”, “the plastid matrix”, and the “nuclear + plastid combined matrix” (in the absence of supported conflict between the resulting gene trees). For the combined analyses, a mixed model approach was used in which the data set was partitioned, and the models of evolution were applied to the different partitions. Two independent Metropolis coupled Markov chain Monte Carlo (MCMC) runs, each consisting of one cold and three heated chains, were started simultaneously from a random tree and run for 20 million generations, with the trees being sampled every 10,000 generations. At the end of the run, chain convergence and estimated sample size (ESS) parameters were assessed with Tracer v1.6.0 (Rambaut et al. 2014). Burn-in was set at 25% and the remaining posterior topologies summarized as a 50% majority-rule consensus tree, with branch support expressed as posterior probabilities (PP). PP values from 0.5 to 0.95 were considered as weak to moderate support, whereas posterior probabilities > 0.95 were considered as strong to very strong support (Suzuki et al. 2002, Alfaro et al. 2003).

ML analyses were performed using RAXML-MPI v.8.2 (Stamatakis et al. 2008), as implemented on the CIPRES Science Gateway web server (RAXML-HPC2 on XSEDE 8.1.11) (Miller et al. 2010), with the following settings: rapid bootstrap analysis with 1000 replicates and searching for the best-scoring ML tree starting with a random seed and utilizing the GTRGAMMA model. Rapid bootstrapping was performed on the ML tree using RAXML at 1000 replicates to determine branch support. Only the ML bootstrap (BS) values (≥ 0.5) are provided. Internodes with BS $\geq 75\%$ were considered statistically significant.

Congruency between the different datasets was inferred using different methods. Due to the known sensitivity issues of the ILD test (Barker & Lutzoni 2002), possible conflict between the marker datasets was also assessed by visually

inspecting the topologies, thereby searching for putatively conflicting relationships and the way those were supported within each topology (hard vs. soft incongruences; Johnson & Soltis 1998, Wiens 1998).

Geographic distribution

The geographic record obtained was plotted in QGIS 3.4.2-Madeira (QGIS Development Team 2018). The distribution of the species was superimposed with a layer of the Ecoregions derived from Olson’s classification (2001). To study the distribution of the species in relation to protected areas (PAs), we used spatial data from the world database on protected areas (WDPA <https://protectedplanet.net/>; visited in October 2021) and from Dinerstein et al. (2017).

RESULTS

Properties of the sequence data

Dataset characteristics and models of nucleotide substitution selected by AIC are presented in Table I. The nuclear ITS and ETS DNA regions were more phylogenetically informative than the four plastid markers. Of the 632 aligned ITS characters, 210 potentially informative sites (33.2%) were obtained, and ETS yielded 186 informative sites out of 364 characters (51.1%), for a total of 396 potentially informative nuclear characters, out of a total of 996 (39.8%). The aligned lengths of the four plastid markers ranged from 458 bp (*trnL-trnF*) to 1155 bp (*petD*), and all together they yielded 3068 characters, of which 212 (6.9%) were informative.

Separate analyses and assessment of incongruence

The individual nuclear and plastid gene datasets were first analysed separately (results available as Figures S1, S2). Most of them resulted in polytomies or only produced poorly resolved

clades, except for the ITS and ETS, which provided the highest proportion of well-resolved clades. No significant incongruence or decreased resolution was observed after analysing a combined nuclear (ITS + ETS) dataset versus a combined plastid (*atpB-rbcL* + *petD* + *rps16* + *trnL-trnF*) dataset.

Combined plastid and nuclear analyses

The concatenated ML trees (Figure S2) were very similar to the Bayesian topologies, therefore, only the 50% majority rule Bayesian consensus tree from the combined analysis (nuclear + plastid data) is shown and used for further discussion (Fig. 1).

Despite the poorly supported backbone of the *Spermacoce* clade, several well-supported clades have been recovered, most of these clades coinciding with genera such as *Crusea* Cham. & Schltld., *Micrasepalum* Urb., *Ernodea* Sw., *Psyllocarpus* Mart. & Zucc., *Richardia* L., and *Staelia* Cham. & Schltld., etc. which were recovered with high to maximum support values. *Borreria*, *Hexasepalum*, and *Spermacoce* however were found to be polyphyletic. Two major clades branch off in the early evolutionary history of the *Spermacoce* clade: clade “A” (PP = 0.95, BS = 59) including *Galianthe* Griseb. and the monotypic genera *Schwendenera tetrapyxis* K. Schum. and *Carajasia cangae* R.M. Salas, E.L. Cabral & Dessen, and clade “B” (PP = 0.96, BS = 58) comprising all the remaining genera, including the new taxon. The BI analysis (Fig. 1) show that the new taxon is a well-supported sister lineage of *Mitracarpus* (PP = 1), however in the ML analysis this relationship is not well supported (BS = 68). In addition, *Mitracarpus* is recovered as a strongly supported, monophyletic genus (PP = 1, BS = 100).

Taxonomic treatment

In the following paragraphs we present the formal description of the new genus and species based on morphological and phylogenetic evidence.

Januaria lombardii R.M. Salas & Nuñez Florentin, M. gen. et. sp. nov.

Type: BRAZIL. Minas Gerais, Januária, distrito de Fabião, 2 Km na estrada partindo do abrigo do Malhador [Parque Nacional Cavernas de Peruaçu], 15°07'58" S, 44°15'17" W, 23 May 1997, Lombardi J.A. & A. Salino 1674 (Holotype: BHCB37156!). Figs. 2-4.

Diagnosis

The genus *Januaria* (consisting of a single species *J. lombardii*) differs from all the other genera of the *Spermacoce* clade due to the particular fruit dehiscence (dehiscence longitudinal-transverse, with a longitudinal-septifragal dehiscence that starts in one of the carpels up to the middle of the fruit, and from there, it follows an transverse-loculicidal line, resulting in one indehiscent carpel remaining on the pedicel and another dehiscent carpel which falls off and releases the seed) and 8-zonoaperturate pollen grains with simple reticulate exine pattern.

Description

Shrub decumbent, height unknown. Stems simple, tetragonal to subcylindrical, glabrous in the basal branches to pubescent in the apical flowering branches; internodes 1.8–4.5 cm long. Stipular sheath 7.5–9.5 mm long, pubescent, with 5–7 fimbriae, 5.2–8.9 mm long, filiform, glabrous, colleter-tipped. Leaves opposite, pseudopetiolate to subsesile; petiole 1.2–1.7 mm long; leaf blade ovate to elliptic, 27.5–77 × 7.7–35.1 mm, concolorous, membranaceous, base attenuate, apex acute; adaxial surface hirsute, abaxial surface pubescent primarily on the middle

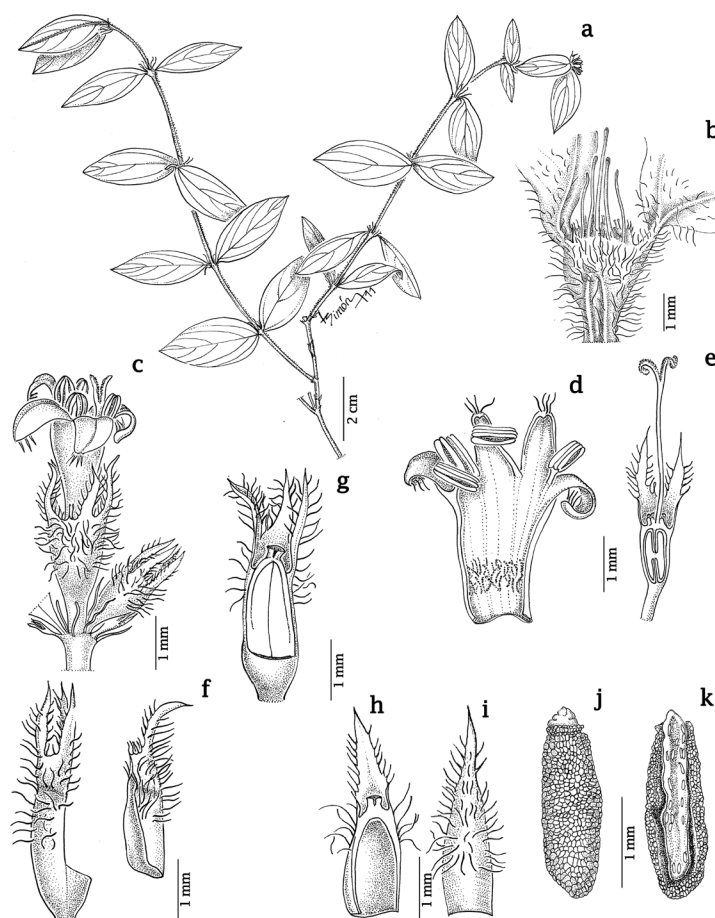


Figure 2. *Januaria lombardii*. **a**, Portion of the apex of a branch. **b**, Stipular sheath. **c**, Flower. **d**, Opened corolla. **e**, Longitudinal section of the hypanthium, calyx, style and bifid stigma. **f**, Dehiscent fruit, with indehiscent valve on the right and dehiscent valve on the left. **g**, Indehiscent valve, ventral view. **h-i**, Dehiscent valve. **h**, Ventral view. **i**, Dorsal view. **j-k**, Seed. **j**, Dorsal view. **k**, Ventral view. — Lombardi J.A. & A. Salino 1674 (BHCB). Drawing by Simón L.

vein and 2–3 secondary veins. Inflorescences with determinate growth; partial inflorescences compressed, axillar, 4–5 flowered. Flowers subsessile, homostylous; hypanthium 2.6–2.8 mm long, obconic, pubescent; calyx 4-lobate, calyx tube reduced; lobes triangular, 0.95–1.3 mm long, pubescent; corolla infundibuliform, white, 3.2–6.5 mm long, corolla tube 2.1–5 mm long, corolla lobes 1.1–3 mm long, 4-lobate; externally glabrous with trichomes on the apical portion of lobes, internally with a fringe of trichomes on the inferior half of corolla tube; stamens exserted, inserted at the sinuses of the corolla lobes, 3.1–3.4 mm long; anthers obovate to oblong, 0.6–0.8 mm long; orbicules absent; pollen grains (7–) 8 zonocolporate, small [$P = 23.3\text{--}26\ \mu\text{m}$, $E = 22.8\text{--}25.8\ \mu\text{m}$], oblate-spheroidal ($P/E = 0.9\text{--}1$); circular outline in polar view; ectocolpus long and narrow, 11–18.4 μm long; endoapertures

laterally fused, forming an endocingulum; exine 2.3–3 μm thick, reticulate, lumina 1.1–2.1 μm long, and muri 0.4–0.7 μm long, with nanospines uniformly present; ovary 2-carpellate, 2-locular, locules 1-ovulate; style exserted, 2.6–3 mm long, bifid. Fruits dry, subsessile, turbinate, 4.8–6.5 \times 1.5–2.3 mm, pubescent on the superior half; dehiscence longitudinal-transverse, with a longitudinal-septifragal dehiscence that starts in one of the carpels up to the middle of the fruit, and from there, it follows an transverse-loculicidal line, resulting in one indehiscent carpel remaining on the pedicel and another dehiscent carpel which falls off and releases the seed; calyx lobes persistent. Seeds ellipsoid, 1.9–2.1 \times 0.4–0.6 mm, dark castaneous, dorsal surface convex, ventral surface \pm plane, with longitudinal groove covered by the strophiole with abundant raphides; exotesta reticulate-foveate, cells

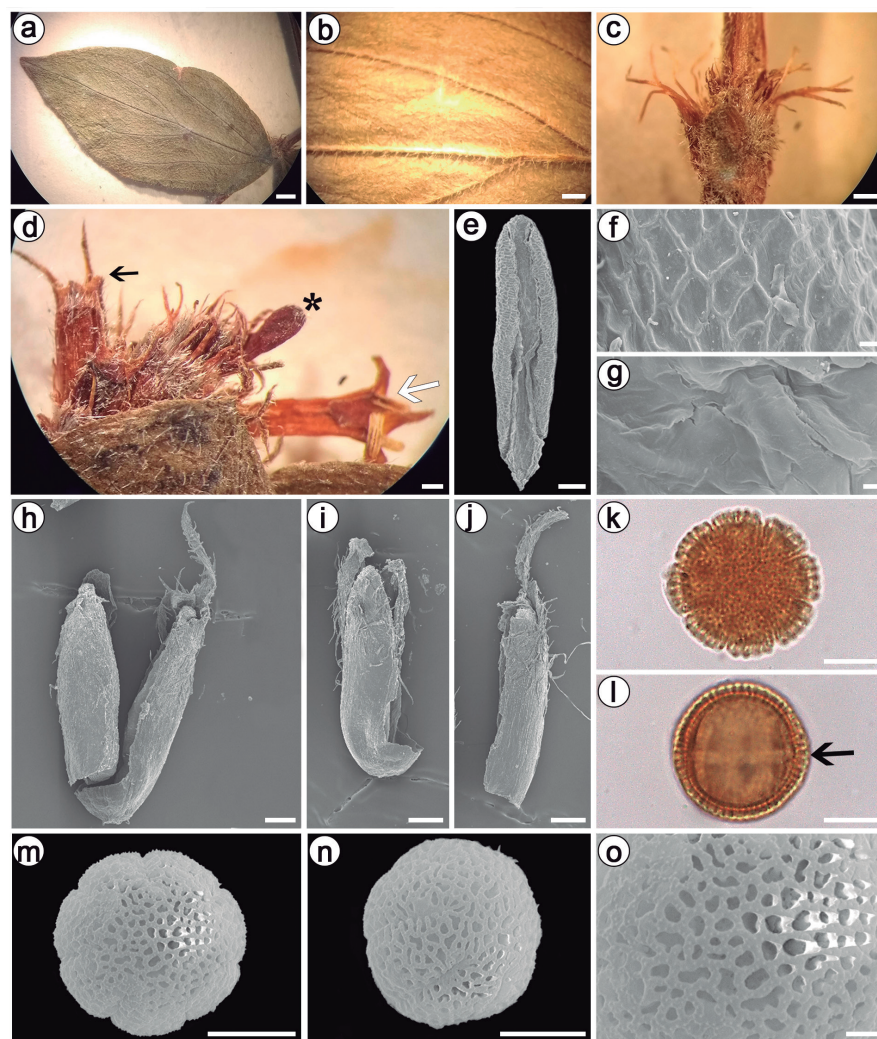


Figure 3. Vegetative and reproductive morphological and micro-morphological features of *Januaria lombardii*. a–d. Stereo microscope photographs. e–j, m–o. SEM photographs. k–l. Light microscopy photographs. a. Leaf, dorsal view. b. Detail of the ventral surface of a leaf. c. Detail of the stipular sheath. d. Detail of a floral node, with fruit (black arrow), floral bud (black asterisk), and flower (white arrow). e. Seed, ventral view. f. Detail of the exotesta. g. Interior surface of the anther wall (absence of orbicules). h. Fruit dehiscent. i. Indehiscent carpel, lateral view. j. Dehiscent carpel, lateral view. k–o. Pollen grains. k, m. Polar view. l. Equatorial view (endocingulum, black arrow). n. Sub-equatorial view. o. Detail of the reticulate exine. — Scales: a. 5 mm, b–c. 2 mm, d. 1 mm, e. 200 μ m, f. 20 μ m, g, o. 2 μ m, h–j. 500 μ m, k–n. 10 μ m. *Lombardi & Salino 1674* (BHCB).

polygonal, rectangular, periclinal walls concave, anticlinal walls straight.

Distribution and Habitat

Januaria is endemic from Minas Gerais (municipality of Januária), Brazil. Figure 4a represents the geographic distribution of this new taxon, which coincides with the ecoregion “Brazilian Dry Atlantic Forest” from Olson’s classification (2001). According to the information on the label of the collected specimen, this species occurs in a vegetation type that is known locally as “carrasco” (see further details in Discussion section).

Phenology

The sole specimen of this taxon was collected in May and contained flowers, floral buds and mature fruits, indicating that the fertile period of this species could cover the months from December to May-June approximately, coinciding with the rainy season.

Etymology

The generic name refers to the locality where the holotype was collected, Januária, Minas Gerais, Brazil. The specific epithet honours Julio Antonio Lombardi, who collected the holotype of *Januaria lombardii*, and has made significant contributions to the study of the Brazilian Flora,

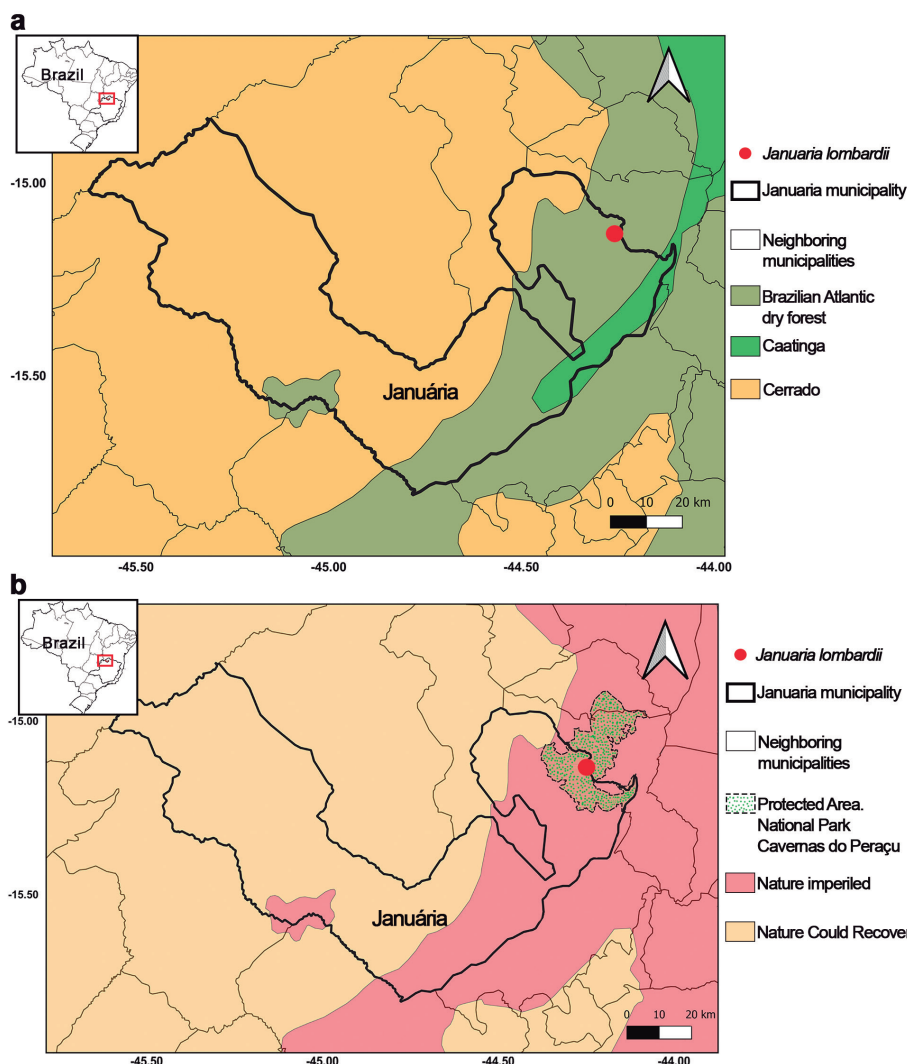


Figure 4. a. Geographical distribution of *Januaria lombardii*. Coloured areas represent the distribution of three ecoregions near *J. lombardii*. The regionalization follows Olson’s classification (2001). **b.** Geographical distribution of *Januaria lombardii* in relation to the protected areas (National Park Cavernas do Peruaçu) and the protection status of the ecoregions according to Dinerstein et al. (2017). The ecoregion protection categories are defined as follows: Nature Could Recover, the sum of the amount of natural habitat remaining and the amount of the total ecoregion that is protected is less than 50% but more than 20%; Nature Imperiled, the sum of the amount of natural habitat remaining and the amount of the total ecoregion that is protected is less than or equal to 20%.

especially in the taxonomy of Celastraceae, Oleaceae, and Vitaceae.

Preliminary conservation status

Januaria is known from a single collection from the Cavernas do Peruaçu National Park, a conservation unity of 564.48 km², located in the municipalities of Itacarambi, Januária, and São João das Missões. Even though the vegetation cover within the limits of the park is relatively well preserved, its adjacent areas, as well as most of the north of Minas Gerais are in constant transformation due to increased anthropogenic land use (Fig. 4b). Although extensive field work was carried out in April-May

2012, no additional populations of the species were found. Accordingly, based on the available information, and following the criteria and recommendations of the IUCN Red List Status (2019) and Callmänder et al. (2005), we consider this new taxon as Vulnerable (VU), under criterion D2, for the time being.

Notes

Due to its morphological characteristics, especially compressed, axillary, pauciflorous, inflorescences with homostylous flowers the new taxon resembles some species of *Borreria*, *Hexasepalum*, and *Spermacoce*. However, it differs from each of them due to the unique

dehiscence of its fruits, by the 8-zonoaperturate pollen grains with simple reticulum (vs. primarily perforate to perforate-microreticulate, and eutectate in *Borreria*, *Hexasepalum*, and *Spermacoce*) and the presence of a shortly branched, bifid stigma (vs. capitate-bilobate to bilobate in *Borreria*, *Hexasepalum*, and *Spermacoce*). For a further comparison between these taxa see Table II. In the vegetative state, they are very similar, and maybe this is the reason for the scarce collections and/or misidentification.

Additional specimens analysed (paratype)

BRAZIL: sine loco, sine data, *Gardner 2191* (W, proparte).

DISCUSSION

The phylogenetic position of *Januaria* in the *Spermacoce* clade, and its distinctive morphology

The topologies recovered in the current study agree with previous studies of the *Spermacoce* clade that were obtained from nuclear data only (Salas et al. 2015, Florentín et al. 2017, Miguel et al. 2018) or from a combined nuclear and plastid data matrix (Nuñez-Florentin et al. 2021, 2022).

Using molecular sequence data, we demonstrated that new described taxon, *Januaria lombardii*, belongs to the *Spermacoce* clade, one of the most taxonomically complex groups within the tribe Spermacoaceae (for discussion see Groeninckx et al. 2009, Salas et al. 2015). Specifically, the new taxon is recovered within clade “B”, in closed relationship with the genus *Mitracarpus* (Fig. 1).

Mitracarpus is a morphologically and phylogenetically well circumscribed Neotropical genus (Souza et al. 2010), strongly supported, but largely unresolved in relationship with the other genera of the *Spermacoce* clade (Salas et al. 2015,

Nuñez-Florentin et al. 2021, 2022). In the present analyses, *Januaria*, results a monospecific genus sister to *Mitracarpus*, from which it differs by its calyx morphology (4 lobes of the same length vs. 2 smaller lobes and 2 larger lobes), corolla shape (infundibuliform vs. hypocrateriform), seed morphology (ellipsoid seed with a longitudinal ventral groove vs. ovoid or ellipsoid seed with a quadrangular or rectangular, “X-shaped” or “inverted Y-shaped” ventral groove), and fruit dehiscence (longitudinal-transverse dehiscence with one dehiscent carpel and one indehiscent carpel vs. circumscissile dehiscence, with both dehiscent carpels). For a further comparison with *Mitracarpus* and other morphologically similar genera see Table II.

One of the most striking features of *Januaria* is the fruit dehiscence, which is unique within the *Spermacoce* clade. The taxonomic value of the fruit morphology in the *Spermacoce* clade has already been shown in the description of the tribe Spermacoaceae by Berchtold & Presl (1820). Subsequently, classic taxonomists, such as Candolle (1830) or Schumann (1888, 1891), considered the type of dehiscence as the only criterion to support or conserve genera. There are some genera in which the fruit morphology is so unique that their circumscription is based on only that particular character (e.g. *Mitracarpus*, *Ernodea*, *Crusea*). The main role attributed to the type of dehiscence has resulted in several taxa that have been considered artificial groups, not supported by morphological characters other than such features (e.g. *Diodia* L., *Borreria*, *Spermacoce*, *Staelia*, etc. Cabaña Fader 2013, Miguel 2016, Salas 2011). Therefore, in the case of the genus *Januaria*, the longitudinal-transverse dehiscence in combination with the specific morphological pollen characters and the phylogenetic position support its recognition as a new genus.

Table II. Comparison between *Januaria* and the morphological and phylogenetic closely related genera *Borreria*, *Hexasepalum*, *Mitracarpus*, and *Spermacoce*.

Character/ Taxon	<i>Januaria</i>	<i>Borreria</i>	<i>Hexasepalum</i>	<i>Mitracarpus</i>	<i>Spermacoce</i> (American taxa)
Inflorescence	Terminal and axillary, with determinate growth, pauciflorous, congested	Terminal and axillary, with determinate growth (rarely indeterminate growth, and pseudoaxillary), pluriflorous, congested	Axillary, with indeterminate growth (rarely determinate), pauciflorous, congested	Terminal and axillary, with determinate growth, pluriflorous, congested	Terminal and pseudoaxillary, with determinate growth, pauciflorous (o rarely pluriflorous), congested
Calyx	calyx 4-lobed, equal	calyx 2 or 4-lobed, equal	calyx 4-lobed, equal (rarely subequal)	calyx 4-lobed, 2 large and 2 small ones, rarely almost equal.	calyx 4-lobed (rarely 2), equal
Corolla shape	infundibuliform	infundibuliform, campanuliform, or cyathiform	infundibuliform	hypocrateriform	urceolate, infundibuliform
Stigma	bifid	bilobate, rarely shortly bifid or capitate-bilobate	capitate-bilobate	bifid	bilobate, shortly bifid, capitate-bilobate
Style and stamens position	both exserted	both exserted	both exserted	style exserted, stamens subserted	both included
Fruit dehiscence	dehiscence longitudinal-transverse. One carpel indehiscent and one carpel dehiscence that separates from the plant	dehiscence longitudinal, septicidal with remnants of the septum remaining attached to the separated valves; or fruits splitting into two indehiscent valves (both remaining attached)	dehiscence longitudinal, splitting into two indehiscent mericarps.	dehiscence circumscissile, the upper fruit half remaining entire, fruit base and basal part of septum remaining on the pedicel	dehiscence longitudinal, septicidal with remnants of the septum remaining attached to the separated valves; or fruits splitting into one dehiscent and one indehiscent valve (both remaining attached); or indehiscent fruits eventually tardily separated at the apex into two indehiscent carpels that remain attached to the pedicel
Seeds	ellipsoid, longitudinal ventral groove; exotesta reticulate-foveate	ellipsoid, ovoid, obovoid or oblong, longitudinal ventral groove, invariable present, variably in width; ruminant seeds may be present as well as elaisome; exotesta reticulate-foveate, reticulate-areolate, or reticulate-coliculate-papillate	obovoid, longitudinal groove, invariable present; ruminant seeds may be present (dorsal surface); exotesta reticulate-foveate or reticulate-areolate	ovoid or ellipsoid, ventral groove quadrangular or rectangular, "X-shaped" or "inverted Y-shaped"; exotesta reticulate-foveate or reticulate-areolate	ellipsoid, ovoid, or obovoid, longitudinal ventral groove, invariable present, variably in width; exotesta reticulate-foveate
Pollen	(7)–8 zonocolporate, spheroidal, small in size, ectocolpi long, endoaperture endocingulum; exine reticulate; equally covered with microspines	6–10 zonocolporate, zonoporolate, or pantoaperturate, spheroidal or sub-oblate, small, medium, or large in size, ectoaperture short colpi or pore, endoaperture alongate, endocingulum or pore; exine perforate, perforate-microreticulum, reticulate; equally covered with microspines	8–20 zonocolporate, spheroidal, or suboblate, large to very large, ectocolpi short, endoaperture endocingulum; exine perforate; equally covered with microspines	5–7 zonocolporate or zonocolporate, spheroidal, medium in size, ectolongi long, endoaperture (when present) endocingulum; exine perforate or microreticulate; equally covered with microspines	3–4, 7–10, zonocolporate, spheroidal or sub-prolate, small to very small in size, ectocolpi long, endoaperture endocingulum; exine eutectate or perforate; microspines restricted to zone around the apertures

The reticulate simple exine, which is relatively uncommon within the *Spermacoce* clade, is characteristic for *Januaria lombardii*. Apart from the new taxon, a micro-reticulate to reticulate exine is observed in *Staelia catolensis* R. M. Salas & E.L. Cabral (Salas & Cabral 2014), *Richardia brasiliensis* Gomes (Pire 1997), three species of *Mitracarpus* [*M. brasiliensis* M. Porto & Waechter and *M. diversifolius* Souza & E.L. Cabral (Souza 2008), *M. robustus* Souza & E.L. Cabral (Nuñez-Florentin, unpublished data)], four Australian *Spermacoce* species [e.g. *S. congestanthera* Harwood, *S. graniticola* Harwood, etc. (Dessein et al. 2005)] and three *Borreria* species from *Borreria* subsect. *Latifolia*, also known as the “*Borreria latifolia* group” [e.g., *B. bradei* Standl., *B. dimorpha* J.H. Kirkbr, and *B. shumannii* (Pire 1996, Sobrado 2015)]. According to the pollen size, shape, number of apertures, and colpi length, the pollen grains of *Mitracarpus robustus*, *Spermacoce congestanthera*, and *Staelia catolensis* are those that most closely resemble *J. lombardii*, yet they differ from the latter in a few slightly different features. *Mitracarpus robustus* has 9–10 aperturate pollen grains, whereas *Januaria* is (7–) 8 aperturate. Furthermore, while *Staelia catolensis* has a micro-reticulate to reticulate ornamentation pattern with a lumina size of 0.1–1.2 μm , *Januaria* has a reticulate exine with larger lumina (1.1–2.1 μm). In addition, *Spermacoce congestanthera* has colpi that are middle sized to short in length, whereas the ectocolpi of *Januaria* pollen are long. As a result, *J. lombardii* pollen has a morphological affinity with pollen type 20, as proposed by Dessein et al. (2005) and then expanded by Salas & Cabral (2014). Table III presents a detailed comparison of the palynological characteristics between *J. lombardii* and other taxa with a reticulate exine.

The endemism and habitat of *Januaria*

Following the regionalization by Olson et al. (2001), the only georeferenced location known for *J. lombardii* shows that inhabits the Brazilian Atlantic Dry Forest, in an ecotonal region between Caatinga and Cerrado ecoregions (Fig. 4a). The Carrasco (a local name for sedimentary Caatinga) is sometimes referred to as being composed of a mix of seasonally dry tropical forests and woodlands (SDTFW sensu Queiroz et al. 2017) and savanna elements (Araújo et al. 1998, Fernandez et al. 2020). According to the information on the label, *J. lombardii* was collected in this vegetation type called “Carrasco”, characterized by a mainly deciduous shrub-tree vegetation composed of plants that are mostly no higher than 5m tall, with only a few species reaching 10m. Herbs and climbers are common in the forest margins or along roads but are rare in the interior of the vegetation (Lombardi et al. 2005).

The floristic study of Lombardi et al. (2005) highlights the floristic and physiognomic diversity of Januária, northern of Minas Gerais, and emphasizes the importance and urgency of inventories for the region as it is of high priority for conservation due to the enormous anthropogenic pressure caused by the continuously advancing agricultural frontier and the new mining concessions. In this sense, Fernandez et al. (2020) noted the importance of the border areas of the Caatinga and stated that “the adjacency and connectivity of different biomes within such a small area provide geographic opportunities for ecologically labile species to expand their ranges and be recorded in different biomes even within a small geographic area”.

Even though the new genus *Januaria* occurs within the limits of a protected area (National Park Carvernas do Peraçu, Fig. 4b), the national park and adjacent areas are currently considered

Table III. Comparison of pollen grains features between *Januaria* and similar species with reticulate exine of *Spermacoce* clade.

Characters/ Taxa	<i>Januaria lombardii</i>	<i>Borreria bradei</i>	<i>Mitracarpus robustus</i>	<i>Richardia brasiliensis</i>	<i>Spermacoce congestanthera</i>	<i>Staelia catolensis</i>
Size	small	large	small	large	small	small
Shape	spheroidal	oblate spheroidal	spheroidal	sub-oblate	spheroidal	spheroidal
N° apertures	(7)–8	10–11	9–10	11–12	7–8	7–(8)
Colpi length	long	short	long	short	medium (-short)	long
Endoaperture	endocingulum	endocingulum	endocingulum	endocingulum	endocingulum	endocingulum
Exine	reticulate (lumina 1.1–2.1 μm width; muri 0.4–0.7 μm width)	reticulate (lumina 0.3–1.7 μm width; muri 0.5–1 μm width)	reticulate (lumina 1–2.1 μm width; muri 0.4–1 μm width)	reticulate	micro- reticulate	micro-reticulate to reticulate (lumina 0.1–1.2 μm width; muri 0.4–0.6 μm width)
Reference	Present study	Sobrado (2015) Pire (1996)	Unpublished data	Pire (1997)	Dessein et al. (2005)	Salas & Cabral (2014)

as “*Nature imperiled*” areas by Dinerstein et al. (2017). Dinerstein and coauthors organized the 846 ecoregions recognized worldwide into four distinct categories, defined by the extent of the remaining natural habitat and protected land. The category “*Nature imperiled*” refers to regions in which the protected area is less or equal to 20% where the remaining habitat exists as a mosaic of isolated fragments insufficient in size and orientation to adequately conserve biodiversity. In addition, more recently, Peixoto Teixeira et al. (2021) using GIS, quantified the total area of Caatinga encompassed by fully protected and sustainable use reserves. The authors found that less than 8% of the Caatinga is legally protected under Brazil’s national nature reserve legislation (SNUC law), and only 1.3% is in reserves with full legal protection. Therefore, Dinerstein et al. (2017) and Peixoto Teixeira et al. (2021) agreed with previous authors (e.g. Miles et al. 2006, Queiroz et al. 2006), that the tropical dry forest is one of the most endangered biomes in the world, and despite a recent expansion of the protected area network, only small portions

of the Brazilian semiarid region are effectively safeguarded.

Endemism of *Spermacoce* clade in Brazil

As stated by Lombardi et al. (2005) the wide variety of vegetation types in a relatively small sample area, such as in the north of Minas Gerais, is probably conditioned by edaphic factors, including the capacity of the soil to retain water. The whole Brazilian territory shows similar conditions, thereby exhibiting a large expanse of several biomes, with different types of vegetation and remarkable plant diversity.

Studies on the ancestral area reconstruction and diversification are still lacking in the *Spermacoce* clade, but it has been hypothesized that the Neotropics is the centre of origin for the *Spermacoce* clade (Dessein 2003, Janssens et al. 2016). According to our knowledge, Brazil comprises 16 of the 24 currently recognized genera of the *Spermacoce* clade. Of these, seven genera are endemic to the country: *Carajasia*, *Denscantia* E.L. Cabral & Bacigalupo, *Paganuccia*, *Planaltina* R.M. Salas & E.L. Cabral, *Psyllocarpus*,

Schwendenera K. Schum., and the new genus *Januaria*. *Denscantia* is a climbing subshrub endemic to Alagoas, Bahia, Espírito Santo, and Rio de Janeiro, growing in different biomes: only four species inhabit the Atlantic Forest biome of Brazil, in areas of Restinga, while *D. calcicola* R.M. Salas & E.L. Cabral grows in a seasonally dry region in the Caatinga, north-eastern Brazil (Salas & Cabral 2012). *Planaltina* is a genus with four species endemic from the central Brazilian highlands in Goiás, Minas Gerais, and Federal district states, growing between 800–1200 m (Salas & Cabral 2010). *Psyllocarpus* is divided in two sections (Kirkbride (1979): *P.* sect. *Psyllocarpus*, inhabits the Cerrado in the states of Bahia, Goiás, Minas Gerais, and the Distrito Federal; whereas *P.* sect. *Amazonica* J.H.Kirkbr. is restricted to white-sand Amazonian campinas in the states of Amazonas, Pará, and Rondônia. *Schwendenera* is endemic to the Atlantic Forest, occurring in the interior or along the margin of the humid forest biome in the south of Brazil (São Paulo and Paraná states) (Salas et al. 2020). *Paganuccia* is a recently described monospecific genus (Nuñez-Florentin et al. 2022), endemic to the dune areas of the mid São Francisco River valley (Bahia), occurring in the Caatinga. *Carajasia* is also a monotypic genus with a restrictive distribution, being endemic to Pará in the north of Brazil, where it only grows in ferric soil (or Canga) on the top of the Carajás mountain range (Salas et al. 2015).

Paganuccia and *Carajasia* are similar to *Januaria* in that they are all recently described genera based on new or hitherto unstudied, or unidentified, herbarium material from areas with limited access. Their limited access could explain why these areas are poorly explored botanically resulting in large collecting deficits. As Fernandez et al. (2020) argues: “further botanical exploration with increased collecting efforts and taxonomic revisions of plant diversity, especially

understudied families, may potentially reveal larger numbers of flowering plants in the dry forests, especially in the Caatinga; and that the discovery of new taxa is inevitable in light of the large number of unidentified specimens in Brazilian herbaria”. In this sense, Bebbier et al. (2010) mentioned that only 16% of the flowering plants are described within five years of being collected for the first time. The description of the remaining 84% involves much older specimens, with nearly 25% of new species descriptions involving specimens that are over 50 years old. Extrapolation of these results suggests that, of the estimated 70,000 species still to be described, more than half have already been collected and are stored in herbaria. *Januaria lombardii* is indeed a good example of this interesting scenario. This taxon was collected twice, one collection by Julio Lombardi in 1997, and the oldest specimen probably collected in the middle of the nineteenth century (Gardner’s paratype), i.e., the genus remained indeterminate in a herbarium for more than 100 years. Supporting Bebbier et al. (2010), a study also performed in the *Spermacoce* clade, resulted in the recently described new species of *Staelia* overlooked for more than a century (*Staelia schumannii* R.M. Salas & E.L. Cabral, Carmo et al. 2021).

In view of the above, we provide an updated key to all the genera of the *Spermacoce* clade in Brazil (adapted from Nuñez-Florentin et al. 2022).

Key to the genera of *Spermacoce* clade occurring in Brazil

Genera distributions (in Brazil) are presented within brackets.

1. Inflorescences lax, thyrsoid or pleiothyrsoid, with partial inflorescences glomeriform or subglomeriform. **2**

1. Inflorescences mostly congested; flowers in glomerules, axillary or pseudoaxillary, pauciflorous to multiflorous, or with 1-flowered axillary inflorescences. **5**

2. Plants erect, rarely climbing. Flowers heterostylous, or rarely homostylous. Seeds with a persistent or deciduous strophiole [widespread]. **Galianthe**

2. Plants climbing. Flowers always homostylous. Seeds always with a persistent strophiole. **3**

3. Fruits indehiscent or fruits tardily separated at the apex into two indehiscent carpels. Seeds wingless or shortly winged [Bahia]. **Paganuccia**

3. Fruits septicidally dehiscent, dividing into two valves. Seeds winged, wing derived from a strophiole that exceeds the base and apex of the seed or from an extension of the exotesta **4**

4. Flowers pedicellate, pedicel 1.7–5.5 mm long. Fruit with an apical portion of the carpels exceeding the hypanthium and forming a “beak” or “rostrum”. Strophiole developed and exceeding the length of the seed apically and basally [widespread] **Emmeorhiza**
Pohl ex Endl.

4. Flowers subpedicellate or shortly pedicellate, pedicel 0.5–1.2 mm long. Fruit without an apical portion that exceeds the hypanthium. Strophiole as long as, or shorter than, the length of the seed [Alagoas, Bahia, Espírito Santo, Paraíba, Pernambuco, Rio de Janeiro, Sergipe]. **Denscantia**

5. Fruits dry or fleshy, indehiscent, or dry schizocarpic. **6**

5. Fruits with longitudinal, circumscissile, longitudinal-oblique or longitudinal-transverse dehiscence. **12**

6. Flowers heterostylous. Stigma 4-fid [Paraná, São Paulo]. **Schwendenera**

6. Flowers homostylous. Stigma bifid, 2-lobed, 3-fid or 4-lobed, never 4-fid **7**

7. Ovary 3- or 4-carpellate. Stigma 3-fid, 4-lobed, rarely 2-lobed [widespread].

Richardia

7. Ovary 2-carpellate. Stigma bifid, 2-lobed, or obscurely 2–4-lobed. **8**

8. Pauciflorous inflorescences (1–5 flowers per node) **9**

8. Multiflorous inflorescences (40–100 flowers per node). **11**

9. Herbs rupicolous, with stems and leaves reddish. Calyx lobes 0.15–0.3 mm long. Schizocarp fruits with septicidal dehiscence into two mericarps leaving a basal carpophore [Pará]. **Carajasia**

9. Herbs or subshrubs, generally psammophilous or growing in wet soils, rarely rupicolous, with green stems and leaves. Calyx lobes 0.6–8 mm long. Schizocarp fruits with septicidal dehiscence into two mericarps without leaving a basal carpophore, or indehiscent fruits (tardily separated at the apex into two indehiscent carpels). **10**

10. Growing in marshy soils. Corolla hypocrateriform, corolla tube internally glabrous, lobes internally pubescent. Stigma and style bifid [widespread] **Diodia** s.s.

10. Mostly on sandy soils, not marshy. Corolla infundibuliform, tube with a ring of moniliform trichomes internally in the inferior part of the tube. Stigma 2-lobed or obscurely 2–4-lobed [widespread]. **Hexasepalum**

11. Inflorescences in axillary (bilateral) glomeruli. Corolla with a ring of trichomes internally on the middle of the corolla tube, lobes glabrous. Stamens and style exerted. Seeds 1(2) per capsule [widespread]. **Borreria** p.p.

11. Inflorescences in pseudoaxillary (unilateral) glomeruli, rarely terminal and with 1–2 axillary glomeruli [e.g. *Spermacoce decipiens*

(K. Schum.) Kuntze]. Corolla with a fringe of trichomes internally on the inferior half of the corolla lobes or in the tube. Stamens and style included. Seeds 2 per capsule [widespread].

Spermacoce p.p.

12. Calyx 2- or 4-lobed, if 4-lobed then with 2 lobes smaller than the other 2. Fruit with circumscissile or longitudinal-oblique dehiscence. **13**

12. Calyx 2- or 4-lobed, lobes equal to subequal in size. Fruit with longitudinal dehiscence, or longitudinal-transverse dehiscence. **14**

13. Calyx 4-lobed, with 2 larger and 2 smaller lobes, rarely exceptions. Corolla mostly hypocrateriform. Fruit with circumscissile dehiscence; the fruit separates into two parts after dehiscence, the superior part in the shape of a "mitre" formed by the upper portion of the carpels and persistent calyx lobes, and the inferior part formed of the basal portion of the carpels and basal part of the septum [widespread]. **Mitracarpus**

13. Calyx 2-lobed (rarely 3–4), with lobes equal or subequal. Corolla infundibuliform. Fruit with longitudinal-oblique dehiscence, fruit separated into three parts after dehiscence, two apical caducous valves, and a basal portion formed of the basal portion of the carpels, and an intercarpellar septum that remains intact and persists on the plant [widespread]. **Staelia** p.p

14. Fruit divides into three parts, two caducous valves and a persistent intercarpellar septum. **15**

14. Fruit divides into two dehiscent valves, or one dehiscent valve and the other indehiscent. **17**

15. Flowers homostylous with included stamens, or distylous with included stamens in long-styled flowers and exerted in short-styled flowers. Fruit strongly compressed laterally

[Amazonas, Bahia, Distrito Federal, Goiás, Minas Gerais, Pará, Rondônia]. **Psyllocarpus** p.p.

15. Flowers always homostylous. Stamens and style exerted. Fruit obovoid or subglobose. **16**

16. Calyx 2-lobed. Stigma bifid. Pollen grains 7–10-aperturate, small (P = 25.7, E = 25.3 μm), with long colpi. Ventral surface of seeds without ruminations [widespread]. **Staelia** p.p.

16. Calyx 4–7 lobed. Stigma bilobate or obscurely 2–4-lobed. Pollen grains 10–11(13)-aperturate, large (P = 61.5–65, E = 60–64.5 μm), with short colpi. Ventral surface of seeds ruminant [Distrito Federal, Goiás, Minas Gerais] **Planaltina**

17. Flowers heterostylous or homostylous. Fruit strongly compressed, with a persistent membranous septum parallel to the valves [Amazonas, Bahia, Distrito Federal, Goiás, Minas Gerais, Pará, Rondônia]. **Psyllocarpus** p.p.

17. Flowers always homostylous. Fruit obconic, turbinate, obovoid, not compressed, without a persistent membranous septum parallel to the valves. **18**

18. Pollen with a reticulate simple exine. Longitudinal-transverse dehiscence, with one indehiscent carpel and one dehiscent valve that separates from the pedicel [Minas Gerais].

Januaria

18. Pollen with an eutectate, perforate, rarely microreticulate exine. Longitudinal dehiscence, with both carpels dehiscent or both indehiscent, or one carpel indehiscent and one valve dehiscent, in all cases both valves remain together and persist on the pedicel. **19**

19. Inflorescences in axillary (bilateral) glomeruli. Stamens and style exerted, stamens attached at the sinuses of the corolla lobes [widespread]. **Borreria** p.p

19. Inflorescences in pseudoaxillary (unilateral) glomeruli, rarely terminal and 1–2 axillary. Stamens and style included, stamens

attached in the middle or at the base of the corolla tube, sometimes near to interlobular sinuses but inside the tube [widespread].

Spermacoce p.p

CONCLUSION

This study further unravels the complex phylogenetic relationships within the *Spermacoce* clade as part of an ongoing global revision of the group. It also further improves the taxonomic classification of the *Spermacoce* clade. In this opportunity, we present the description of an endemic, and probably endangered, new genus, with a combination of phylogenetic and morphological evidence. Nevertheless, many issues remain unsolved in the classification of the *Spermacoce* clade (tribe Spermacoceae) and further sampling will be required (especially of those poorly known taxa) in order to provide further improvements. To prevent an irreversible loss of biodiversity, more attention and funding must be devoted to the protection of the Brazilian dry forest and the many endemic species that are characteristic of these notable ecosystems.

Furthermore, we believe that the description of this new taxon, despite being a unicate, is extremely important considering that the area it inhabits is clearly threatened. This is a contribution to the recent checklists of floristic information about the Caatinga, considered as a first large step in the increase in scientific knowledge, fundamental for establishing conservation priorities, information on land use management, or even conducting further biogeographic studies.

Acknowledgments

MNF, JEF, RMS thank the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET) for the grants awarded that supported this work. This study was also partially funded by the Agencia Nacional de

Promoción Científica y Técnica (FONCYT PICT-2016-3517) and the Universidad Nacional del Nordeste (PI A009-17 and PI 16P001 grants). We are grateful to Elsa Leonor Cabral for her valuable comments and suggestions, and to Laura Simón for her help with the illustration of the species. We also thank the staff of the molecular laboratory of Meise Botanic Garden, Belgium, especially Pieter Asselman and Wim Baert.

REFERENCES

- ALFARO ME, ZOLLER S & LUTZONI F. 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molec Biol Evol* 20: 255-266. <https://doi.org/10.1093/molbev/msg028>.
- ANDERSSON L & ROVA JHE. 1999. The *rps16* intron and the phylogeny of the Rubioideae (Rubiaceae). *Plant Syst Evol* 214: 161-186. <https://doi.org/10.1007/BF00985737>.
- ARAÚJO FS, SAMPAIO EVSB, FIGUEIREDO MA, RODAL MN & FERNANDES AG. 1998. Composição florística da vegetação de carrasco, Novo Oriente, CE. *Braz J Bot* 21: 105-116. <https://doi.org/10.1590/S0100-84041998000200001>.
- BARKER FK & LUTZONI FM. 2002. The utility of the incongruence length difference test. *Syst Biol* 51: 625-637. <https://doi.org/10.1080/10635150290102302>.
- BEBBER DP ET AL. 2010. Herbaria are a major frontier for species discovery. *Proc Natl Acad Sci USA* 107(51): 22169-22171. <https://doi.org/10.1073/pnas.1011841108>.
- BERCHTOLD F & PRESL KB. 1820. *Spermacoceae*. *Prir. Rostlin. Krala Wiljma Endersa, Praga*, 256 p.
- CABAÑA FADER AA. 2013. *Estudios biosistemáticos en especies americanas de Diodia s. lat. (Rubiaceae)*. Ph.D. Dissertation. Universidad Nacional del Nordeste, Corrientes, Argentina.
- CALLMANDER MW, SCHATZ GE & LOWRY II PP. 2005. IUCN Red List assessment and the Global Strategy for Plant Conservation: taxonomists must act now. *Taxon* 54(4): 1047-1050. <https://doi.org/10.2307/25065491>.
- CANDOLLE AP. 1830. *Prodromus Systematis Naturalis Regni Vegetabilis* 4: 561. Paris: Treuttel & Würts.
- CARMO JAM, REGINATO M, FLORENTÍN JE, NUÑEZ-FLORENTIN M, SALAS RM & SIMÕES AO. 2022. One more piece to the puzzle: *Diadorimia*, a new monotypic genus in the Spermacoceae (Rubiaceae), endemic to the campo rupestre of Minas Gerais, southeastern Brazil. *Taxon* 71 (2): 396-419. <https://doi.org/10.1002/tax.12643>.

- CARMO JAM, SOBRADO SV, CABRAL EL & SALAS RM. 2021. *Staelia schumannii* (Rubiaceae, Spermaceae): an old new species from Bahia, Brazil, overlooked for more than a century. *Syst Bot* 46(3): 844-851. <https://doi.org/10.1600/036364421X16312067913525>.
- DESSEIN S. 2003. Systematic studies in the Spermaceae (Rubiaceae). Ph.D. Dissertation. Katholieke Universiteit Leuven, Belgium.
- DESSEIN S, HARWOOD B, SMETS E & ROBBRECHT E. 2005. Pollen of the *Spermaceae* (Rubiaceae) species from the Northern Territory of Australia: morphology and taxonomic significance. *Aust Syst Bot* 18(4): 367-382. <https://doi.org/10.1071/SB03025>.
- DINERSTEIN E ET AL. 2017. An ecoregion-based approach to protecting half the terrestrial realm. *BioScience* 67(6): 534-545. <https://doi.org/10.1093/biosci/bix014>.
- DOYLE JJ & DOYLE JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull Bot Soc Amer* 19: 11-15.
- ERDTMAN G. 1966. An introduction to palynology, vol. 1, Pollen morphology and plant taxonomy; Angiosperms. New York & London: Hafner, 541 p.
- FERNANDES MF, CARDOSO D & QUEIROZ LP. 2020. An updated plant checklist of the Brazilian Caatinga seasonally dry forests and woodlands reveals high species richness and endemism. *J Arid Environ* 174: 104079. <https://doi.org/10.1016/j.jaridenv.2019.104079>.
- FLORENTÍN JE, CABAÑA FADER AA, SALAS RM, JANSSENS S, DESSEIN S & CABRAL EL. 2017. 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. *Peer J* 5: e4012. <https://doi.org/10.7717/peerj.4012>.
- GIBBONS KL. 2020. *Hedyotis*, *Oldenlandia* and related genera (Rubiaceae: Spermaceae) in Australia: New genera and new combinations in an Asian-Australian-Pacific lineage. *Taxon* 69: 515-542. <https://doi.org/10.1002/tax.12236>.
- GROENINCKX I, DESSEIN S, OCHOTERENA H, PERSSON C, MOTLEY TJ, KÅREHED J, BREMER B, HUYSMANS S & SMETS E. 2009. Phylogeny of the herbaceous tribe Spermaceae (Rubiaceae) based on plastid DNA data. *Ann Missouri Bot Gard* 96: 109-132. <https://doi.org/10.3417/2006201>.
- HARWOOD B & DESSEIN S. 2005. Australian *Spermaceae* species (Rubiaceae: Spermaceae). I. Northern Territory. *Aust Syst Bot* 18: 297-365. <https://doi.org/10.1071/SB03024>.
- HUELSENBECK J & RONQUIST F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755. <https://doi.org/10.1093/bioinformatics/17.8.754>.
- IUCN STANDARDS AND PETITIONS COMMITTEE. 2019. Guidelines for Using the IUCN Red List Categories and Criteria. Version 14. Prepared by the Standards and Petitions Committee. Retrieved from <http://www.iucnredlist.org/documents/RedListGuidelines.pdf>.
- JANSSENS S, GEUTEN K, YUAN YM, SONG Y, KÜPFER P & SMETS E. 2006. Phylogenetics of *Impatiens* and *Hydrocera* (Balsaminaceae) using chloroplast *atpB-rbcL* spacer sequences. *Syst Bot* 31: 171-180. <https://doi.org/10.1600/036364406775971796>.
- JANSSENS SB, GROENINCKX I, DE BLOCK P, VERSTRAETE B, SMETS E & STEVEN D. 2016. Dispersing towards Madagascar: biogeography and evolution of the Madagascan endemics of the Spermaceae tribe (Rubiaceae). *Mol Phylogenetics Evol* 95: 58-66. <https://doi.org/10.1016/j.ympev.2015.10.024>.
- JOHNSON LA & SOLTIS SE. 1998. Assessing congruence: Empirical examples from molecular data. In: SOLTIS DE, SOLTIS PS & DOYLE JJ (Eds), *Molecular systematics of plants, vol. 2, DNA sequencing*. Boston: Kluwer Academic Publishing, p. 297-348.
- KÅREHED J, GROENINCKX I, DESSEIN S, MOTLEY TJ & BREMER B. 2008. The phylogenetic utility of chloroplast and nuclear DNA markers and the phylogeny of the Rubiaceae tribe Spermaceae. *Molec Phylogen Evol* 49: 843-866. <https://doi.org/10.1016/j.ympev.2008.09.025>.
- KIRKBRIDE JH. 1979. Revision of the Genus *Psyllocarpus* (Rubiaceae). *Smithsonian Contributions to Botany* 41: 1-32.
- LARSSON A. 2014. AliView: A fast and lightweight alignment viewer and editor for large datasets. *Bioinformatics* 30(22): 3276-3278. <https://doi.org/10.1093/bioinformatics/btu531>.
- LOMBARDI JA, SALINO A & TEMONI LG. 2005. Diversidade florística de plantas vasculares no município de Januária, Minas Gerais, Brasil. *Lundiana* 6(1): 3-20.
- MIGUEL ML. 2016. *Estudios biosistématicos en especies sudamericanas de Borreria subsecc. Borreria y filogenia de las especies del complejo Spermaceae-Borreria (Rubiaceae)*. Ph.D. Dissertation. Universidad Nacional del Nordeste, Corrientes, Argentina.
- MIGUEL LM & CABRAL EL. 2013. *Borreria krapocarmeniana*, a new cryptic species recovered through taxonomic analyses of *Borreria scabiosoides* and *Borreria linoides*

(Spermacoaceae, Rubiaceae). *Syst Bot* 38: 769-781. <https://doi.org/10.1600/036364413X670368>.

MIGUELLE, SOBRADOSV, JANSSENS, DESSEINS & CABRALEL. 2018. The monotypic Brazilian genus *Diacrodon* is a synonym of *Borreria* (Spermacoaceae, Rubiaceae): Morphological and molecular evidences. *An Acad Bras Cienc* 90: 1397-1415. <https://doi.org/10.1590/0001-3765201820170314>.

MILES L, NEWTON AC, DEFRIES RS, RAVILIOUS C, MAY I, BLYTH S, KAPOS V & GORDON JE. 2006. A global overview of the conservation status of tropical dry forests. *J Biogeogr* 33: 491-505. <https://doi.org/10.1111/j.1365-2699.2005.01424.x>.

MILLER MA, PFEIFFERW & SCHWARTZ T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE)*, New Orleans, Louisiana, 14 Nov 2010. Piscataway: IEEE, p. 45-52 <https://doi.org/10.1109/GCE.2010.5676129>.

NUÑEZ-FLORENTIN M, FLORENTÍN JE & SALAS RM. 2020. Integrative taxonomic analyses sheds light on three historically disputed American *Spermacoce* species, and a key to the American species of *Spermacoce* (Spermacoaceae, Rubiaceae). *Syst Bot* 45(3): 585-606. <https://doi.org/10.1600/036364420X15935294613464>.

NUÑEZ-FLORENTIN M, SALAS RM, CARMO JAM, CABRAL EL, DESSEIN S & JANSSENS SB. 2022. *Paganuccia icatuensis* (Rubiaceae), a new genus and species from Bahia, Brazil, with a key to all the genera of the tribe Spermacoaceae in the Americas. *Taxon* 71(3): 630-649. <https://doi.org/10.1002/tax.12651>.

NUÑEZ-FLORENTIN M, SALAS RM, JANSSENS SB, DESSEIN S & CARDOSO D. 2021. Molecular-based phylogenetic placement and revision of *Micrasepalum* (Spermacoaceae-Rubiaceae). *Taxon* 70(6): 1300-1316. <https://doi.org/10.1002/tax.12593>.

OLSON DM ET AL. 2001. Terrestrial ecoregions of the world: a new map of life on earth. *Bioscience* 51: 933-938.

PEIXOTO TEIXEIRA L, NIC LUGHADHA E, VINICIUS CHAGAS DA SILVA, M & FREIRE MORO M. 2021. How much of the Caatinga is legally protected? An analysis of temporal and geographical coverage of protected areas in the Brazilian semiarid region. *Acta Bot Brasilica* 35(3): 473-485. <https://doi.org/10.1590/0102-33062020abb0492>.

PIRE SM. 1996. Palynological study of American species of *Borreria* (Rubiaceae-Spermacoaceae). *Opera Bot* 77: 413-423.

PIRE SM. 1997. El polen de especies brasileñas de *Richardia* L. (Rubiaceae-Spermacoaceae). *Geociencias II* (n° especial): 184-191.

POSADA D. 2008. jModelTest: Phylogenetic model averaging. *Molec Biol Evol* 25: 1253-1256. <https://doi.org/10.1093/molbev/msn083>.

PUNT WS, HOEN PP, BLACKMORE S, NILSSON S & LE THOMAS A. 2007. Glossary of pollen and spore terminology. *Rev Palaeobot Palynol* 143: 1-81. <https://doi.org/10.1016/j.revpalbo.2006.06.008>.

QGIS DEVELOPMENT TEAM. 2018. QGIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>.

QUEIROZ LP. 2006. The Brazilian caatinga: phytogeographical patterns inferred from distribution data of the Leguminosae. In: PENNINGTON RT, LEWIS GP & RATTER JA (Eds), *Neotropical savannas and seasonally dry forests: plant diversity, biogeography, and conservation*. Boca Raton: CRC Press, Taylor & Francis Group, p. 121-157.

QUEIROZ LP, CARDOSO DBOS, FERNANDES MF & MF MORO. 2017. Diversity and Evolution of Flowering Plants of the Caatinga Domain. In: SILVA JMC, LEAL IR & TABARELLI M (Eds), *Caatinga: the Largest Tropical Dry Forest Region in South America*. Springer, Cham. 23-63. <https://doi.org/10.1007/978-3-319-68339-3>.

RAMBAUT A, SUCHARD MA, XIE D & DRUMMOND AJ. 2014. Tracer, version 1.6. Retrieved from <https://doi.org/10.1111/j.1438-8677.1996.0Atb00515.x>.

RASBAND WS. 2020. Image J, version 1.51k. Bethesda, MD: U.S. National Institutes of Health. <http://imagej.nih.gov/ij/>. (accessed 30 Sept 2021).

ROBBRECHT E. 1988. *Tropical woody Rubiaceae. Characteristic features and progressions. Contributions to a new subfamilial classification*. Opera Botanica Belgica 272. Meise: Natl. Bot. Gard. Belgium.

ROBBRECHT E & MANEN JF. 2006. The major evolutionary lineages of the coffee family (Rubiaceae, Angiosperms). Combined analysis (nDNA and cpDNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on *rbcl*, *rps16*, *trnL-F* and *atpB-rbcl* data. A new classification in two subfamilies, Cinchonoideae and Rubioideae. *Syst Geogr Plants* 76: 85-146.

SALAS RM. 2011. *Revisión de Staelia s.l. (Rubiaceae)*. Ph. D. Dissertation. Universidad Nacional del Nordeste. Corrientes, Argentina.

SALAS RM ET AL. 2020. *Schwendenera* in Flora do Brasil 2020. Jardim Botânico do Rio de Janeiro. Disponível em: <http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB14285>. Access: 13 Oct 2021.

SALAS RM & CABRAL EL. 2010. *Planaltina* nuevo género de la tribu Spermacoaceae (Rubiaceae), endémico del Planalto central de Brasil y una nueva especie del estado de Goiás, Brasil. *J Bot Res Inst* 4(1): 193-206.

SALAS RM & CABRAL EL. 2012. *Denscantia calcicola* (Rubiaceae), a new species from limestone outcrops in the Brazilian Caatinga. *Syst Bot* 37(3): 807-810. <https://doi.org/10.1600/036364412X648742>.

SALAS RM & CABRAL EL. 2014. Morfología polínica de *Staelia* s.l. (Rubiaceae). *Bol Soc Argent Bot* 49(1): 51-65. <https://doi.org/10.31055/1851.2372.v49.n1.7821>.

SALAS RM, CABRAL EL, VIANA PL, DESSEIN S & JANSSENS S. 2015. *Carajasia* (Rubiaceae), a new and endangered genus from Carajás mountain range, Pará, Brazil. *Phytotaxa* 206: 14-29. <http://dx.doi.org/10.11646/phytotaxa.00.0.0>.

SCHUMANN K. 1888. Rubiaceae-Spermacoaceae. *Flora Brasiliensis* 6(6): 6-99.

SCHUMANN K. 1891. Rubiaceae. In: ENGLER A & PRANTL K (Eds), *Die naturlichen Pflanzenfamilien*. Leipzig: Engelmann. 1-156.

SOBRADO SV. 2015. *Estudios biosistemáticos en especies de Borreria (Spermacoaceae-Rubiaceae) con énfasis en Borreria subsecc. Latifoliae*. Ph.D. Dissertation. Universidad Nacional del Nordeste, Corrientes, Argentina.

SOUZA EB. 2008. *Estudos sistemáticos em Mitracarpus (Rubiaceae-Spermacoaceae) com ênfase em espécies brasileiras*. Ph. D. Dissertation. Universidade Estadual de Feira de Santana, Bahia, Brazil.

SOUZA EB, CABRAL EL & ZAPPIDC. 2010. Revisão de *Mitracarpus* (Rubiaceae-Spermacoaceae) para o Brasil. *Rodriguésia* 61(2): 319-352. <https://doi.org/10.1590/2175-7860201061213>.

STAMATAKIS A, HOOVER P & ROUGEMONT J. 2008. A rapid bootstrap algorithm for the RAxML web-servers. *Syst Biol* 75: 758-771. <https://doi.org/10.1080/10635150802429642>.

STEARN WT. 1986. *Botanical Latin*. Ed. 3. London: David and Charles Publishers plc, 289 p.

SUZUKI Y, GLAZKO GV & NEI M. 2002. Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. *Proc Natl Acad Sci USA* 99: 16138-16143. <https://doi.org/10.1073/pnas.212646199>.

THIERS B. 2021. (continuously updated). Index Herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium Available at <http://sweetgum.nybg.org/science/ih>. (accessed on 03 March 2021).

VELLOSO AL, SAMPAIO EVSB & PAREYN FGC (Eds). 2002. *Ecorregiões - Propostas para o bioma Caatinga*.

Recife, Associação Plantas do Nordeste/Instituto de Conservação Ambiental The Nature Conservancy do Brasil.

WIENS JJ. 1998. Combining data sets with different phylogenetic histories. *Syst Biol* 47: 568-581. <https://doi.org/10.1080/106351598260581>.

SUPPLEMENTARY MATERIAL

Figures S1, S2.

Alignment. Combined matrix (ITS + ETS + *atpB-rbcL* + *petD* + *rps16* + *trnL-trnF*).

APPENDIX I

Voucher information and GenBank accession number for taxa used in the present study.

Species, geographic origin, voucher information (collector and number, and herbarium of deposition), and GenBank accession numbers for ITS, ETS, *atpB-rbcL*, *petD*, *rps16*, *trnL-trnF*. Newly generated sequences are indicated by an asterisk (*). – : information not applicable.

OUTGROUP: *Bouvardia ternifolia* (Cav.)

Schltdl., Oaxaca, Mexico, *Ochoterena et al.* 454 (BR), KF736987, KF737029, –, –, –, –; National Botanical Garden, Meise, origin unknown S2928 (BR), –, –, –, AF002758, –; Mexico, *Spencer et al.* 363 (NY), –, –, –, –, –, EU642537. — **INGROUP:**

Borreria alata (Aubl.) DC., Goiás, Brazil, *Queiroz et al.* 14105 (CTES), KF736995, KF737036, –, –, –, –; Misiones, Argentina, *Miguel 64* (CTES), –, –, MZ064144, MZ064115, MZ064094, MZ064059; ***Borreria brachystemonoides*** Cham. & Schltdl., Corrientes, Argentina, *Miguel et al.* 26 (CTES), MF166821, MF166810, MZ064145, MZ064116, MZ064095, MZ064060; ***Borreria capitata*** (Ruiz & Pav.) DC., Minas Gerais, Brazil, *Sobrado et al.* 135 (CTES), MF166822, MF166811, –, –, –, –; French Guiana, *Andersson 1908* (GB), –, –, EU543007, EU557764, EU543069, EU543158; ***Borreria dasycephala*** (Cham. & Schltdl.) Bacigalupo & E.L.Cabral, Misiones, Argentina, *Salas et Cabaña*

388 (CTES), KF736991, -, -, -, -, -; Misiones, Argentina, Miguel et al. 18 (CTES), -, MF166807, MZ064146, -, -, -; **Borreria diacrodonta** L.M.Miguel & E.L.Cabral, Ceará, Brazil, Bolland s.n. (K), MF166816, MF166805, -, -, -, -; Brazil, Salas 432 (CTES), -, -, MZ064147, -, -, -; **Borreria latifolia** (Aubl.) K.Schum., Goiás, Brazil, Queiroz et al. 14110 (CTES), KF736994, KF737035, -, -, -, -; Minas Gerais, Brazil, Sobrado 143 (CTES), -, -, MZ064148, MZ064117, MZ064096, MZ064061; **Borreria loretiana** E.L.Cabral, Misiones, Argentina, Keller et Paredes 9918 (CTES), MF166820, MF166809, MZ064149, MZ064118, -, MZ064062; **Borreria multibracteata** E.L.Cabral & Bacigalupo, Goiás, Brazil, Queiroz et al. 14261 (CTES), KF736990, KF737032, -, -, -, -; **Borreria ocymifolia** (Willd. ex Roem. & Schult.) Bacigalupo & E.L.Cabral, French Guiana, Andersson et al. 2040 (GB), AM939463, -, EU542952, EU557712, -, EU543108; Ecuador, Bremer 3340 (UPS), -, AM932951, -, -, -, -; **Borreria orientalis** E.L.Cabral, R.M.Salas et L.M.Miguel, Misiones, Argentina, Sobrado et Salas 158 (CTES), MF166823, MF166812, MZ064150, MZ064119, -, MZ064063; **Borreria schumannii** (Standl. ex Bacigalupo) E.L.Cabral & Sobrado, Misiones, Argentina, Cabral et al. 760 (CTES), KF736997⁴, KF737038, -, -, -, -; Corrientes, Argentina, Medina 398 (CTES), -, -, MZ064151, MZ064120, MZ064097, MZ064064; **Borreria spinosa** Cham. & Schltld. ex DC., Minas Gerais, Brazil, Viana et al. 5917 (BHCB), MF166817, MF166806, MZ064152, MZ064121, -, MZ064065; **Borreria tenella** (Kunth) Cham. & Schltld., Misiones, Argentina, Miguel et al. 15 (CTES), MF166819, MF166808, -, MZ064122, -, MZ064066; Tocantins, Brazil, Fonseca 6547 (IBGE), -, -, MZ064153, -, -, -; **Borreria verticillata** (L.) G.Mey., Corrientes, Argentina, Salas 402 (CTES), KF736998, KF737039, -, -, -, -; Minas Gerais, Brazil, Oliveira 43 (CESJ), -, -, -, MZ064123, -, MZ064067; **Carajasia cangae** R.M.Salas, E.L.Cabral & Dessenin, Pará, Brazil, Costa et al. 588 (BHCB), KF737015, KF737057, -, -, -, -; Brazil, Mota 1972 (BHCB), -, -,

MZ064154, MZ064124, MZ064098, MZ064068; **Crusea calocephala** DC., Oaxaca, Mexico, Ochoterena et al. 456 (BR), KF737009, KF737051, -, -, -; Guatemala, Gustafsson et al. 215 (GB), -, -, EU542930, EU557690, -, EU543088; **Crusea coccinea** DC., Oaxaca, Mexico, Ochoterena et al. 461 (BR), KF737010, KF737052, -, -, -, -; **Crusea hispida** (Mill.) B.L.Rob., Tabasco, Mexico, Chase 2913 (K), -, -, -, AF002759, -; **Crusea megalocarpa** (A.Gray) S.Watson, Mexico, Pringle 3852 (S), AM939439, AM932929, EU542931, EU557691, EU543025, EU543089; **Diodia aulacosperma** K.Schum., Kenya, Luke 9029 (UPS), AM939444, AM932934, EU542934, EU557695, EU543026, EU543092; **Diodia saponariifolia** Cham. & Schltld., Misiones, Argentina, Cabaña & Salas 22 (CTES), KF737007, KF737049, -, -, -, -; Misiones, Argentina, Miguel et al. 20 (CTES), -, -, -, MZ064125, MZ064099, MZ064069; **Diodia virginiana** L., Missouri, U.S.A., Taylor 12758 (MO), KF737008, KF737050, -, -, -, -; U.S.A., Vincent 4296 (GB), -, -, -, AY764288, -; **Emmeorhiza umbellata** (Spreng.) K.Schum., Bahia, Brazil, Queiroz et al. 13746 (CTES), KF737000, KF737042, MZ064143, MZ064126, MZ064100, MZ064070; **Ernodea littoralis** Sw., Cuba, Rova et al. 2286 (GB), KF737001, KF737043, EU542937, EU557698, AF002763, EU543095; **Ernodea taylori** Britton, North Bimini, Correll 44186 (NY), KF737002, KF737044, -, -, -, -; **Galianthe brasiliensis** (Spreng.) E.L.Cabral & Bacigalupo, Misiones, Argentina, Cabral et al. 758 (CTES), KF737011, KF737053, MZ064156, -, -, -; Misiones, Argentina, Miguel 32 (CTES), -, -, -, MZ064128, MZ064102, MZ064072; **Galianthe eupatorioides** (Cham. & Schltld.) E.L.Cabral, Goiás, Brazil, Queiroz et al. 14190 (CTES), KF737012, KF737054, -, -, -, -; Argentina, Schinini et Cristóbal 9811 (GB), -, -, EU542939, EU557700, EU543028, EU543097; **Galianthe grandifolia** E.L.Cabral, Distrito Federal, Brazil, Queiroz et al. 14015 (CTES), KF737013, KF737055, -, -, -, -; Brazil, Viana et al. 5860 (BHCB), -, -, MZ064157, MZ064129, MZ064103, MZ064073;

Galianthe palustris (Cham. & Schltdl.) Cabaña Fader & E.L.Cabral, Misiones, Argentina, *Miguel et al.* 19 (CTES), MF166825, MF166827, MZ064158, MZ064130, MZ064104, –; ***Galianthe peruviana*** (Pers.) E.L.Cabral, Minas Gerais, Brazil, *Salas et al.* 408 (BHCB, CTES), KF737014, KF737056, –, –, –, –; Brazil, *Salas et al.* 413 (CTES, HUEFS), –, –, MZ064159, MZ064131, MZ064105, MZ064074; ***Galianthe spicata*** (Miq.) Cabaña Fader & Dessein, French Guiana, *Anderson et al.* 1961 (GB), AM939535, AM933008, EU542935, EU557696, EU543027, EU543093; ***Hexasepalum angustifolium*** Bartl. ex DC., Mexico, *Rzedowski et al.* 17792 (MEXU), KF737004, KF737046, –, –, –; ***Hexasepalum apiculatum*** (Willd.) Delprete & J.H.Kirkbr., Bahia, Brazil, *Queiroz et al.* 13727 (CTES), KF737003, KF737045, –, –, –, –; Brazil, *Salas et al.* 457 (CTES), –, –, MZ064160, –, –, MZ064075; Bahia, Brazil, *Queiroz et al.* 14601 (HUEFS), –, –, –, MZ064132, –, –; ***Hexasepalum sarmentosum*** (Sw.) Delprete & J.H.Kirkbr., Cameroon, *Dessein et al.* 1521 (BR), KF737005⁴, KF737047, –, –, –, –; French Guiana, *Andersson et al.* 2071 (GB), –, –, –, –, AF002762¹, –; ***Hexasepalum teres*** (Walter) J.H.Kirkbr., Goiás, Brazil, *Queiroz et al.* 14089 (CTES), KF737006, KF737048, –, –, –, –; ***Hydrophylax maritima*** L., Sri Lanka, *Lundqvist* 8945 (UPS), –, –, EU567457, –, –, –; ***Januaria lombardii*** R.M. Salas & Nuñez-Florentin, M., Brazil, Minas Gerais, *Januaria*, *Lombardi & Salino* 1674 (BHCB), OP921300*, OP902588*, OP902589*, –, –, OP902590*; ***Micrasepalum eritrichoides*** (C. Wright ex Griseb.) Urb., Cuba, *León* 12997 (US), MZ064088, –, MZ064161, MZ064133, MZ064106, MZ064076; ***Micrasepalum haitiense*** Urb. & Ekman, Dominican Republic, *Liogier* 14859 (US), MZ064089, –, –, –, –, –; ***Mitracarpus capitatus*** Lozada-Pérez & Borhidi, *Ochoterena* 543 (BR), KM215366, KM215328, –, –, KM215470, –; ***Mitracarpus hirtus*** (L.) DC., Brazil, *Souza* 1228 (HUEFS), KM215374, –, –, –, –, –; Argentina, *Keller* 11863 (CTES), –, MZ064084, –, –, MZ064107–; Argentina, *Miguel* 67 (CTES), –, –,

MZ064162, MZ064134, –, MZ064077; ***Mitracarpus megapotamicus*** (Spreng.) Kuntze, *Beck* 26027 (LPB), KM215361, –, –, –, –, –; Corrientes, Argentina, *Salas et Cabaña* 399 (CTES), –, KF737041, –, –, –, –; *Bueno* 2617 (HAS), –, –, –, –, KM215465, –; ***Mitracarpus microspermus*** K.Schum., Brazil, *Queiroz* 14122 (HUEFS), KM215351, KM215313, –, –, –, –; Guyana, *Jansen-Jacobs et al.* 4785 (GB), –, –, EU542975, EU557732, EU543044, –; Brazil, *Viana et al.* 5888 (BHCB), –, –, –, –, MZ064078; ***Mitracarpus rigidifolius*** Standl., Brazil, *Souza* 911 (HUEFS), KM215352, KM215314, –, –, –, –; Brazil, *Salas et al.* 452 (CTES, HUEFS), –, –, MZ064163, MZ064135, MZ064108, –; ***Mitracarpus robustus*** E.B.Souza & E.L.Cabral, Brazil, *Salas* 410 (CTES, HUEFS), –, –, MZ064164, MZ064136, MZ064109, MZ064079; ***Paganuccia icatuensis*** R. M. Salas, Bahia, Brazil, *Salas et al.* 434 (BR, CTES, K, MO, NY, SI), MZ703642, MZ703643, MZ703645, MZ703646, –, MZ703644; ***Psyllocarpus asparagoides*** Mart. ex Mart. & Zucc., Brazil, *Salas et al.* 411 (CTES, HUEFS), KF737018, KF737060, MZ064165, MZ064137, –, –, Bahia, Brazil, *Harley et al.* 20077 (AAU), –, –, –, –, AF003611, –; ***Psyllocarpus laricooides*** Mart. ex Mart. & Zucc., Brazil, *Andersson et al.* 35750 (UPS), AM939531, –, –, –, –, –; Minas Gerais, Brazil, *Mota* 2662 (BHCB), –, MZ064085, –, –, MZ064110, –; Minas Gerais, Brazil, *Monteiro* 245 (SPF), –, –, MZ064166, MZ064138, –, –; ***Psyllocarpus phyllocephalus*** K.Schum., Brazil, *Queiroz et al.* 14016 (CTES), –, KF737061, –, –, –, –; Minas Gerais, Brazil, *Viana* 5885 (BHCB), MZ064090, –, MZ064167, MZ064139, MZ064111, MZ064080; ***Richardia brasiliensis*** Gomes, Brazil, *Souza* 966 (HUEFS), KM215369, KM215334, –, –, KM215474 –; ***Richardia grandiflora*** (Cham. & Schltdl.) Steud., Goiás, Brazil, *Queiroz et al.* 14055 (CTES, HUEFS), KF737027, KF737066, MZ064168, MZ064140, –, –, –; Brazil, *Souza* 967 (HUEFS), –, –, –, –, KM215475, –; ***Richardia humistrata*** (Cham. & Schltdl.) Steud., Misiones, Argentina, *Cabaña et Salas* 17 (CTES), KF737028, KF737067, –, –, –, –; Argentina, *Keller* 5268 (CTES),

–, –, MZ064169, MZ064141, –, MZ064081; **Richardia scabra** L., Minas Gerais, Brazil, Lombardi et al. 3771 (CTES), MZ064091, –, MZ064170, MZ064142, –, –; Minas Gerais, Brazil, Viana et al. 5912 (CTES), –, MZ064086, –, –, –, Goiás, Brazil, Fonseca et al. 4078 (CTES), –, –, –, –, MZ064112, –; **Richardia stellaris** (Cham. & Schltdl.) Steud., Australia, Egeröd 85343 (GB), AM939534, –, EU543006, EU557763, EU543068, EU543157; **Schwendenera tetrapyxis** K.Schum., Paraná, Brazil, Marques et al. 83 (CTES), KF737017, KF737059, –, –, –, –; **Spermacoce breviflora** F.Muell ex Benth., Northern Territory, Australia, Harwood 1070 (BR), KF737019, KF737062, –, –, –, –; **Spermacoce confusa** Rendle, Oaxaca, Mexico, Ochoterena et al. 552 (CTES), KF737020, KF737063, –, –, –, –; Colombia, Andersson et al. 2074 (GB), –, –, –, AF003619, –; **Spermacoce dibrachiata** Oliv. Zambia, Dessein et al. 626 (BR), KF737021, –, –, –, –; **Spermacoce erosa** Harwood, Australia, Harwood 1148 (BR), AM939537, AM933009, EU543008, EU557765, EU543070, EU543159; **Spermacoce eryngioides** (Cham. & Schltdl.) Kuntze, Corrientes, Argentina, Salas et al. 378 (CTES), KF736992, KF737033, –, –, –, –; **Spermacoce flituba** (K.Schum.) Verdc., Kenya, Luke 9022 (UPS), AM939539, AM933011, EU543009, –, EU543071, EU543160; **Spermacoce glabra** Michx., Missouri, U.S.A., Taylor 12757 (MO), KF737022, KF737064, –, –, –, –; **Spermacoce hispida** L., Sri Lanka, Wanntorp et al. 2667 (S), AM939540, AM933017, EU543011, EU557768, EU543073, EU543162; **Spermacoce incognita** (E.L.Cabral) Delprete, Goiás, Brazil, Queiroz et al. 14049 (CTES), KF736993, KF737034, –, –, –, –; **Spermacoce marginata** Benth., Dessein s.n. (BR), –, –, KT252890, KT252886, KT252880, KT252883; **Spermacoce paganucci** E.L.Cabral & Bacigalupo, Brazil, Queiroz 14609 (HUEFS), –, KM215324, –, –, –, –; **Spermacoce princeae** (K.Schum.) Verdc., Kenya, Luke & Luke 8371 (UPS?), HM042452, HM042507, –, –, HM042566, HM042585;

Spermacoce prostrata Aubl., Goiás, Brazil, Queiroz et al. 14083 (CTES), KF736996, KF737037, –, –, –, –; Colombia, Andersson et al. 2078 (GB), –, –, EU543012, EU557769, –, EU543163; **Spermacoce ruelliae** DC., Gabon, Andersson & Nilsson 2296 (GB), AM939543, AM933014, EU543014, EU557771, EU543074, EU543165; **Spermacoce sphaerostigma** (A.Rich.) Oliv., Zambia, Dessein et al. 555 (BR), MF166813, MF166801, –, –, –, –; **Spermacoce stipularis** Dessein, Zambia, Dessein et al. 368 (BR), MF166814, MF166802, –, –, –, –; **Spermacoce subvulgata** (K.Schum.) J.G.García, Zambia, Dessein et al. 216 (BR), MF166815, MF166803, –, –, –, –; **Spermacoce tenuior** L., Tabasco, Mexico, Novelo et al. 4160 (MO), KF737023, KF737065, –, –, –, –; **Staelia culcita** R.M.Salas & E.L.Cabral, Minas Gerais, Brazil, Viana et al. 5891 (BHCB), MZ064092, –, MZ064171, –, MZ064113, MZ064082; **Staelia herzogii** (S.Moore) R.M.Salas & E.L.Cabral, Santa Cruz, Bolivia, Soto et al. 1053 (CTES, USZ), KF737024, –, –, –, –, –; **Staelia thymoides** Cham. & Schltdl., Misiones, Argentina, Cabral et al. 754 (CTES), MZ064093, –, –, –, –, –; **Staelia virgata** (Link ex Roem. & Schult.) K.Schum., Bahia, Brazil, Salas et al. 423 (CTES), KF737025⁴, MZ064087, MZ064172, –, MZ064114, MZ064083.

How to cite

NUÑEZ-FLORENTIN M, FLORENTÍN JE, SALAS RM, DESSEIN S & JANSSENS SB. 2023. Multi-loci phylogeny and morphological evidence support the recognition of *Januaria* (Spermacoceae, Rubiaceae), a new monospecific genus endemic to the North of Minas Gerais (Brazil). *An Acad Bras Cienc* 95: e20211601. DOI 10.1590/0001-3765202320211601.

Manuscript received on January 04, 2022;
accepted for publication on August 19, 2022

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Author contributions

MNF designed the project, took the lead in writing the first draft, which was enhanced and complemented by all the authors. She analysed the herbarium materials, conducted the phylogenetic analyses, and produced the scanning electron microscopy images. JEF performed the distributional geographic maps and contributed with a description of the new taxon's habitat. JEF & SD conducted the preliminary conservation status assessment. RMS contributed to the morphological and habitat descriptions of the new taxon. SBJ, SD & MNF performed the molecular laboratory work. All authors contributed to and accepted the final manuscript.

