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Phylogenetics of Gratioleae (Plantaginaceae): paraphyly of *Stemodia* and its implications for generic circumscriptions, with insights from floral evolution

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Gratioleae are the most species-rich tribe of Plantaginaceae in the tropics, spanning *c.* 30 genera and > 300 species, with a wide range of morphological variation and ecological strategies. As a first effort towards a phylogenetic classification of Gratioleae we generated a new phylogenetic hypothesis with improved taxa sampling, based on one nuclear and three plastid markers, using maximum parsimony and Bayesian inference approaches, and performed ancestral state reconstructions of ten morphological characters of flowers. The paraphyly of *Stemodia s.l.* was corroborated with members being retrieved in four clades; *Bacopa*, *Conobea*, *Leucospora* and *Schistophragma* are non-monophyletic. As actions towards recognizing only monophyletic genera in Gratioleae, we propose *Stemodia s.s.* as a monophyletic and morphological cohesive group, describe *Umbraria* as a new genus to accommodate two species segregated from *Stemodia*, transfer *Stemodia vandelliodes* to *Darcya*, merge *Conobea* with *Bacopa* and re-establish the names *Chodaphyton ericifolium* and *Geochorda glechomoides*. Traits used to define *Stemodia s.l.* are plesiomorphic in the tribe; the flower type (composed of seven floral characters) constitutes a good diagnostic set of traits for almost all genera in the tribe. Our study sheds light on the urgent need to reassess generic circumscriptions towards a unified classification in Gratioleae.

ADDITIONAL KEYWORDS: androecium – classifications – flower symmetry – generic limits – ITS1– morphology – *rps16* intron – Scrophulariaceae – *trnL–trnF* – typification.

INTRODUCTION

In the last three decades, angiosperms have undergone major rearrangements in several levels due to the use of DNA sequence-based phylogenetic studies as the basis for classifications (Chase *et al.*, 1993; APG, 1998; APG II, 2003; APG III, 2009; APG IV, 2016), but an apparent stability was achieved at ordinal and familial levels (see differences among the iterations of the APG system). At infrafamilial levels, however, this desired stability is still a challenge, and new classifications are sometimes controversial (Frodin, 2004; Humphreys & Linder, 2009), especially regarding large and traditionally recognized genera with cultural, economic and political importance, but which are indisputably non-monophyletic in their

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broader concepts, such as *Acacia* Mill. (Fabaceae; Miller, Seigler & Mishler, 2014, and references therein), *Aster* L. (Asteraceae; see Li *et al.*, 2012, for historical background), *Psychotria* L. (Rubiaceae; Nepokroeff, Bremer & Sytsma, 1999; Razafimandimbison *et al.*, 2014) and *Salvia* L. (Lamiaceae; Drew *et al.*, 2017), among others

Gratioleae are the most species-rich tribe of Plantaginaceae (Lamiales; *sensu* APG IV, 2016) in the tropical region, with *c*. 30 genera and > 300 species and a wide range of morphological variation and ecological strategies (Estes & Small, 2008; Scatigna *et al.*, 2018a) (Fig. 1A–L). Gratioleae include specialized aquatic herbs (e.g. *Dopatrium* Buch.-Ham. ex Benth. and *Hydrotriche* Zucc.), rupicolous subshrubs (e.g. *Lapaea* Scatigna & V.C.Souza) and even carnivorous plants (e.g. *Philcoxia* P.Taylor & V.C.Souza) (Fischer *et al.*, 2004; Pereira *et al.*, 2012; Scatigna *et al.*, 2020). They also include species with economic importance such as the ambulias and marshweeds [e.g. *Limnophila sessiliflora* Blume and *L. aromatica* (Lam.) Merr.], and the ornamental Brazilian snapdragon [*Matourea azurea* (Linden) Colletta & V.C.Souza], in addition to species of pharmacological interest, such as the Indian Ayurvedic brahmi [*Bacopa monnieri* (L.) Pennell], *Limnophila heterophylla* (Roxb.) Benth., *Matourea scutellarioides* (Benth.) Colletta & V.C.Souza and *Stemodia foliosa* Benth. (Gorai, Jash & Singh, 2014; Kongkeaw *et al.*, 2014; Le *et al.*, 2018; Oliveira *et al.*, 2018).

Despite the remarkable diversity and economic importance in Gratioleae, comprehensive taxonomic studies of the group are scarce and outdated, with competing classifications. The circumscription of the tribe has changed significantly over time, with the number of recognized genera ranging from 16 to 40 (Bentham, 1846; Wettstein, 1891; Thieret, 1967; Fischer, 2004; Albach, Meudt & Oxelman, 2005; Rahmanzadeh *et al.*, 2005). Some authors have suggested recognizing the group at the family level (Gratiolaceae: Rahmanzadeh *et al.*, 2005; Souza & Lorenzi, 2012), but since this could entail the recognition of additional smaller families, including Angeloniaceae (Souza & Lorenzi, 2012), there is a tendency to retain the tribal status (Tank *et al.*, 2006; Schäferhoff *et al.*, 2010; Souza & Lorenzi, 2019). Phylogenetic studies in Lamiales (Schäferhoff *et al.*, 2010) and in Plantaginaceae (Albach *et al.*, 2005; Rahmanzadeh *et al.*, 2005) have demonstrated that Gratioleae, in its traditional circumscriptions (Bentham, 1846; Wettstein, 1891), is paraphyletic. The phylogenetic studies of Albach *et al.* (2005) and Rahmanzadeh *et al.* (2005) provided new proposals for the circumscription of Gratioleae, with a list of included genera, but the sampling in both studies accounted for only up to six of the 25–32 genera recognized. Furthermore, some of those genera are widely treated as synonyms [e.g. *Amphiantus* Torr. and *Gratiola* L., *Benjaminia* Vell. and *Bacopa* Aubl., *Morgania* R.Br. and *Stemodia* L., *Otacanthus* Lindl. and *Achetaria* Cham. & Schltdl. (= *Matourea* Aubl.), among others; Barker, 1990; Estes & Small, 2008; Souza & Giulietti, 2009; Colletta, Scatigna & Souza, 2020]. Other genera included in Gratioleae of Plantaginaceae by Albach *et al.* (2005) and/or Rahmanzadeh *et al.* (2005) are currently treated in distinct families [e.g. *Capraria* L. and *Limosella* L. in Scrophulariaceae *s.s.*, *Lindenbergia* Lehm. and *Schizosepala* G.M.Barroso (= *Agalinis* Raf.) in Orobanchaceae; Tank *et al.*, 2006]. Hence, it seems clear that Gratioleae, even in the circumscriptions partially based on phylogenetic studies (Albach *et al.*, 2005; Rahmanzadeh *et al.*, 2005), remain paraphyletic and the number of genera is over-estimated.

Although phylogenetic studies in Gratioleae are scarce, they provide evidence for the non-monophyly of the largest genera of the tribe, namely *Bacopa*, *Gratiola* and *Stemodia* (Fritsch *et al.*, 2007; Estes & Small, 2008; Scatigna *et al.*, 2018a; Gonzalez-Socoloske *et al.*, 2020). This led to proposals for taxonomic changes such as merging *Amphianthus* Torr. with *Gratiola* (Estes & Small, 2008) and segregating part of *Stemodia* into the newly described genus *Lapaea* Scatigna & V.C.Souza (Scatigna *et al.*, 2020). Nevertheless, the sampling in all those studies represents only *c*. 50% of the genera and < 15% of the species recognized in Gratioleae (*sensu* Albach *et al.*, 2005), and questions regarding the paraphyly of *Bacopa* and *Stemodia* remain unresolved.

Stemodia is one of the largest and most heterogeneous genera in Gratioleae, encompassing *c*. 50 species, distributed in the tropics, especially in open and wet areas of the Americas and Australia (Minod, 1918; Turner & Cowan, 1993a; Fischer, 2004; Souza & Giulietti, 2009). The genus, in a broad sense (called *Stemodia s.l.* from hereon), includes amphibian herbs to terrestrial subshrubs with crawling to erect habit, sessile to clearly petiolate leaves, sessile to long-pedicellate flowers, with or without bracteoles, resupinate or non-resupinate flowers, among other variable characters (Souza & Giulietti, 2009; Scatigna *et al.*, 2018a; Sosa & O'Leary, 2018). Because of this variation, the delimitation of *Stemodia* has been historically controversial. The genus was first described by Browne (1756) as *Stemodiacra* P.Browne, based exclusively on *Stemodiacra maritima* P.Browne, which was characterized by a bifid filament (Fig. 2A). Subsequently, Linnaeus (1759) published *Stemodia* L., based on Browne's (1756) *Stemodiacra*, and *Stemodia maritima* L., based on *Stemodiacra maritima*. During the following century, various authors (Kunth, 1818; Saint-Hilaire, 1824; Chamisso & Schlechtendal, 1828; Bentham, 1835) described *c*. 50 species under

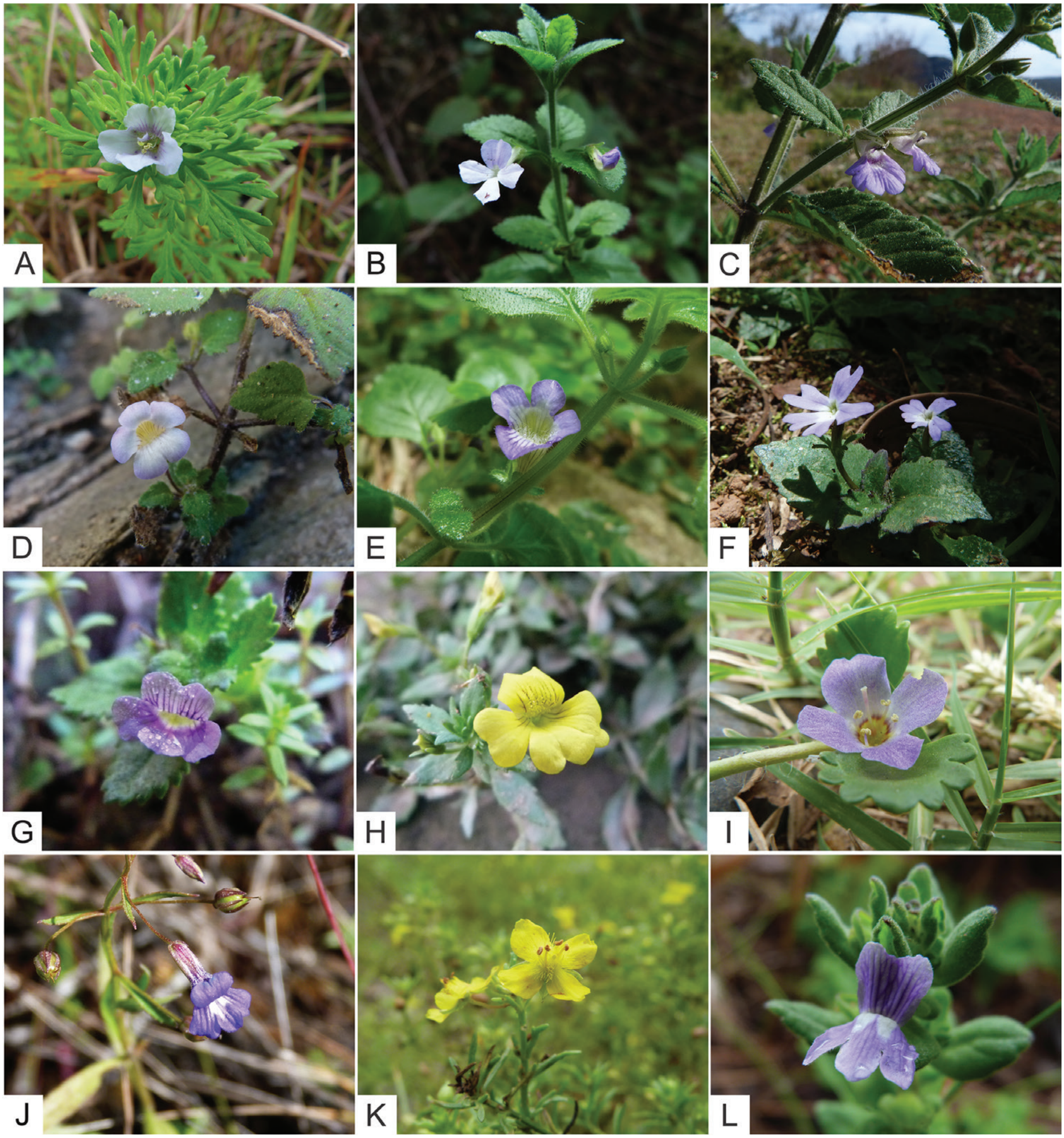


Figure 1. Flower diversity in Gratioleae. A, *Stemodia hassleriana*. B, *Stemodia trifoliata*. C, *Stemodia foliosa*. D, *Stemodia microphylla*. E, *Stemodia veronicoides*. F, *Stemodia vandellioides*. G, *Stemodia verticillata*. H, *Mecardonia procumbens*. I, *Conobea glechomoides*. J, *Conobea scoparoides*. K, *Scoparia montevidensis*. L, *Matourea erecta*. Photographs by A. V. Scatigna.

Stemodia, which would later be conserved over *Stemodiacra* (Briquet, 1906). Bentham (1846) made a drastic change in its circumscription, excluding 24 species from *Stemodia*, several of which he had described earlier (Bentham, 1835); he transferred 13 of them to *Limnophila* R.Br. and the other 11 species to six different genera, namely *Conobea* Aubl., *Gratiola*, *Lindenbergia*, *Mazus* Lour., *Pterostigma* Benth. and *Vandellia* L. These seven genera shared, in part, anthers with stipitate thecae, but differed in

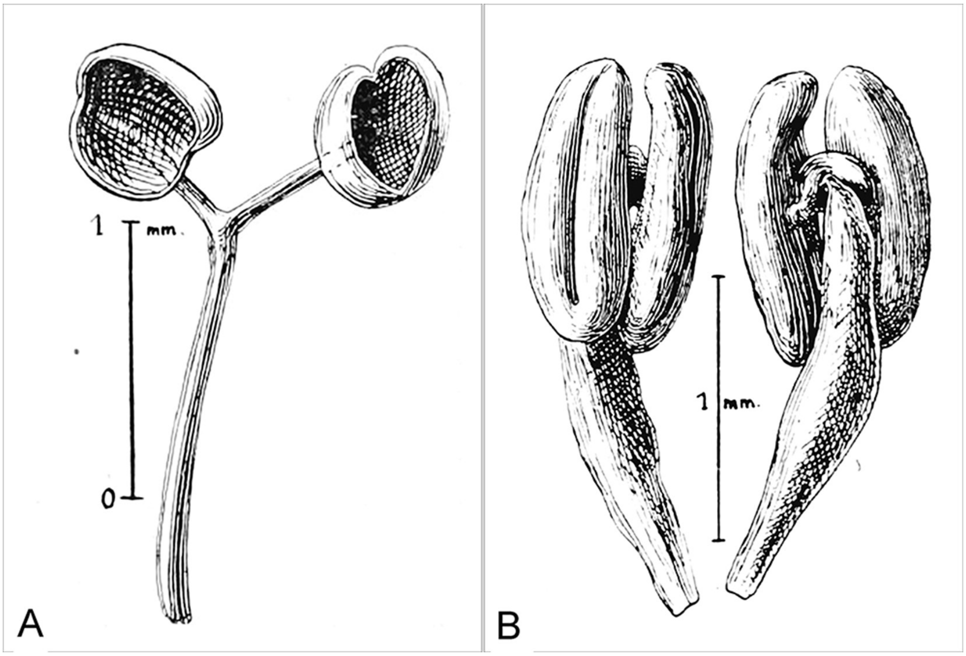


Figure 2. Stamens of *Stemodia* with thecae separated by arms of the connective. A, *Stemodia maritima*. B, *Stemodia lanceolata*. Adapted from Minod (1918), available at the Biodiversity Heritage Library (not in copyright).

calyces (sepal fusion), fruit (dehiscence) and stamens (presence of appendage, number of stamens, numbers of thecae) (Bentham, 1846). Later, Minod (1918) used morphological differences in habit, indument, style and fruits to segregate five species from *Stemodia* and place them into the five monotypic genera, *Chodaphyton* Minod, *Lendneria* Minod, *Stemodiacra*, *Valeria* Minod and *Verena* Minod. However, this classification was not followed by subsequent authors, who adopted a broader concept of the genus (Barroso, 1952; D'Arcy, 1979; Turner & Cowan, 1993a, b; Fischer, 2004; Souza & Giulietti, 2009; Sosa & O'Leary, 2018; Scatigna & Souza, 2020). Other genera currently include species previously treated under *Stemodia*, i.e. *Darcya* B.L.Turner & C.C.Cowan (Turner & Cowan, 1993b), *Anamaria* V.C.Souza (Souza, 2001) and *Lindernia* All. (Fischer, Schäferhoff & Müller, 2013). On the other hand, Barker (1990) merged *Morgania* with *Stemodia*, arguing that there were no significant differences between them. More recently, Scatigna *et al.* (2020) described *Lapaea* as a new genus to accommodate five species segregated from *Stemodia*. Emphasis on different sets of morphological characters resulted in competing classifications and delimitations of *Stemodia*, whereas the narrow sampling of the genus (*c*. 20% of species) in phylogenetic studies may obscure the need to segregate even more species from *Stemodia s.s.* (Scatigna *et al.*, 2020.). Consequently, an effort to resolve the circumscription of *Stemodia* is imperative to achieve a stable generic classification in Gratioleae.

Even though *Stemodia s.l.* has a broad geographical distribution and exhibits high morphological variation, it has been widely defined by the possession of four fertile stamens and anthers with two thecae separated by arm-like projections of connective (frequently referred to as stipitate or stalked thecae) (Fig. 2B), in addition to the calyx with equal to subequal sepals (Bentham, 1846; Minod, 1918; D'Arcy, 1979; Turner & Cowan, 1993a; Souza & Giulietti, 2009; Scatigna, Souza & Simões, 2017, 2018b). Characters from the calyx (i.e. sepals eight, equal to subequal or unequal) and androecium (i.e. number of fertile stamens two, four or five; number of thecae per anther and thecae stipitate or sessile) were used by early authors for differentiation of genera in Gratioleae (Bentham, 1846; Bentham & Hooker, 1876; Wettstein, 1891), but were never assessed from an evolutionary perspective. Accordingly, investigating questions regarding the distribution of these features among genera and how they have changed during the evolution may help to support a phylogenetic classification of the tribe.

Taxonomic classifications should meet basic principles such as the monophyly of taxa, nomenclatural stability and ease in group recognition [Backlund & Bremer, 1998; Stevens, 2001 (onwards)]. One important step towards meeting these is to provide a reliable and comprehensive phylogenetic tree. Additionally, evolutionary assessment of morphological traits may help to support taxonomic decisions. As a first effort towards a phylogenetic classification in Gratioleae we aimed to: (1) infer the phylogenetic relationships of members of *Stemodia s.l.*; (2) assess the taxonomic implications of a potential rearrangement in *Stemodia* and (3) search for potential morphological synapomorphies and/or diagnostic features that support the recognized genera. For that, we generated a new DNA sequence-based phylogenetic hypothesis with improved taxa sampling in both *Stemodia* and Gratioleae, followed by ancestral state reconstruction of morphological characters of flowers, including some traditionally used to define *Stemodia*.

MATERIAL AND METHODS

PHYLOGENETIC STUDY

Taxon sampling

We improved on the datasets published by Scatigna *et al.* (2020) by including newly generated and previously published sequences available at GenBank. We sampled 95 taxa, including 24 species of *Stemodia s.l.* and 64 species representing 16 other genera of Gratioleae. To test the monophyly of Gratioleae, we included six species of its sister group, Angelonieae, and a member of Plantagineae was used as to root the trees, following the results of Schäferhoff *et al.* (2010). Voucher information and respective GenBank accession numbers are available in Supporting Information, Appendix S1.

DNA isolation, amplification and sequencing

Genomic DNA was extracted from leaf samples (silica-gel dried or herbarium material) following the protocol of Tel-Zur *et al.* (1999) adapted to 2-mL tubes. Four DNA markers were sequenced: the plastid *rps16* and *trnL* introns and the *trnL–trnF* intergenic spacer and the nuclear ribosomal ITS1 intergenic spacer. Primers and polymerase chain reaction (PCR) conditions followed Scatigna *et al.* (2018a) and are presented in Supporting Information, Appendix S2. Reactions were performed with the GoTaq Green Master Mix (Promega), with a final volume of 12.5 μL, composed of 6.25 μL of Master Mix, 0.75 μL of each primer at 5 μM, 1 μL of template DNA, 1 μL of bovine serum albumin at 10 mg/mL, 2 μL of dimethyl sulphoxide (DMSO) 5% and nuclease-free water to 12.5 μL. PCR products were generally purified with ExoSAP-IT PCR Product Cleaner (Thermo Fischer Scientific, Waltham, MA, USA); when necessary, gel bands were purified with the Wizard SV Gel and PCR Clean-Up System (Promega). Sanger sequencing procedures were conducted at the Laboratório Multiusuário de Genotipagem e Sequenciamento of the Biology Institute of the University of Campinas. Reactions were performed using *c.* 10 ng of DNA, 0.5 μL of primer (same as used in the PCRs) in a concentration of 5 pmol/μL, 2.0 μL of sequencing buffer, 0.4 μL of ABI Prism Big Dye Terminator v.3.1 (Applied Biosystems, Foster City, California) and distilled water in q.s.p. for 10 μL. Sequence products were cleaned using 2.5 μL of ethylenediamine tetra-acetic acid (EDTA) at 125 mM and ethanol 100%, followed by a second cleaning with 30 μL of 70% ethanol and further drying overnight at room temperature. Sequences were visualized on a 3500xL Genetic Analyzer (Applied Biosystems).

Sequence assembly and alignment

Newly generated sequences were assembled and edited with Geneious R11 (Kearse *et al.*, 2012). Data matrices of each DNA region was extracted from Scatigna *et al.* (2018a) and expanded with both newly generated and previously published (available at NCBI database) sequences, and then aligned with MAFFT v.7 (Katoh & Standley, 2013) using the *Auto* algorithm, followed by minor manual adjustments with Mesquite v.3.10 (Maddison & Maddison, 2016). Individual matrices were also merged into two datasets with Mesquite v.3.10 (Maddison & Maddison, 2016), one with the three plastid DNA regions (partial combined, PC) and the other with the ITS1 added to the PC dataset (total combined, TC).

Phylogenetic analyses

Each DNA region separately and both PC and TC datasets were analysed using maximum parsimony (MP) and Bayesian inference (BI) approaches. Best-fit nucleotide substitution models for each matrix were selected by using the Bayesian information criterion (BIC) in jModelTest2 (Guindon & Gascuel, 2003; Darriba *et al.*, 2012) on the CIPRES Science Gateway (Miller, Pfeiffer & Schwartz, 2010). Selected models were TVM+G for *rps16* and *trnL–trnF*, GTR+G for *trnL* and GTR+I+G for ITS1. Bayesian inference was performed with MrBayes v.3.2.6 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003), also on CIPRES; two parallel runs with four Markov chain Monte Carlo (Yang & Rannala, 1997) chains were run for 20 000 000 generations and sampled every 2000 generations. Convergence of runs was tested with Tracer v.1.6 (Rambaut *et al.*, 2014) with effective sample size (ESS) ≥ 200 as a cut-off point for convergence. The first 25% of sampled trees were discarded as burn-in (Huelsenbeck & Ronquist, 2001), and clade posterior probabilities (PP) were assessed by a 50% majority-rule consensus tree generated with the remaining trees in TreeAnnotator v.1.5.4 (Rambaut & Drummond, 2007). Support values were considered high for PP ≥ 0.95, and low for PP < 95.

Maximum parsimony analyses were performed with PAUP* v. 4.0 beta 10 (Swofford, 2002). A heuristic search for most-parsimonious trees included an initial round of tree searches with 1000 random addition sequence replicates (RASR). Ten trees were held at each step with tree bisection-reconnection (TBR) branch swapping, MULTREES and steepest descent in effect; maximum of 100 trees were saved for each replicate. A second round of heuristic search used all trees retained in memory. Relative support for each node was estimated with the bootstrap (BS) resampling procedure (Felsenstein, 1985) as implemented in PAUP* by using a full heuristic search with 1000 replicates, 250 RASR and three trees held at each step, TBR branch swapping with steepest descent and MULTREES all in effect; ten trees were saved for each RASR. Support values were considered strong, moderate and weak for BS ≥ 75%, BS = 50–74% and BS < 50%, respectively. Tree files were opened and edited with FigTree v.1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

ANCESTRAL CHARACTER STATE RECONSTRUCTION

To search for potential morphological synapomorphies for recovered clades, we selected ten floral characters considered diagnostic for *Stemodia* and variable among other genera of the tribe (Table 1). Character states were based on the examination of herbarium specimens housed at ALCB, BHCB, BHZB, CEN, CEPEC, CTES, CVRD, DIAM, EAC, ESA, HCJS, HRB, HRCB, HUEFS, IAC, IAN, ICN, MAC, MAR, MBM, MBML, MG, NY, OUPR, R, RB, S, SLUI, SP, SPF, UB, UEC, UFG and UPCB and consultation of digital images from G, K, LE, MO and P databases (herbarium acronyms follow Thiers, 2021), complemented with data from the literature (Bentham, 1846; Wettstein, 1891; Minod, 1918; Thieret, 1967; D'Arcy, 1979; Turner & Cowan, 1993a, b; Fischer, 2004; Souza & Giulietti, 2009). Character states were organized in a matrix including all sampled species, except *Limnophila* sp. because of missing morphological data, and with accessions reduced to one per species. Character histories were traced with maximum likelihood in Mesquite under the Markov k-state 1 parameter model (Mk1; Schluter *et al.*, 1997; Pagel, 1999) onto the majority-rule consensus tree generated by the BI of the TC dataset.

Diagnostic features

To search for potential diagnostic features, either independently or in sets, we placed six characters in columns with its respective states next to each terminal taxon in Figure 3. Characters 5 to 10 from Table 1 plus number of petals were combined to constitute the floral types (Figs 4 and 5). Besides floral characters, we included leaf attachment as a potential diagnostic character with three features: (1) clearly petiolate; (2) obscurely petiolate (petiole obscured by the attenuate base of leaf blade) and (3) clearly sessile (frequently clasping). This vegetative character was used by Minod (1918), Souza & Giulietti (2009) and Scatigna *et al.* (2017, 2018b) to define morphological assemblages of *Stemodia* spp.

DATA MATRICES

We generated 173 new DNA accessions from 47 specimens representing 44 species. Our analyses included all the newly generated accessions plus 197 previously published sequences of representatives of Gratioleae, Angelonieae and *Plantago* L. available at GenBank. The TC matrix consisted of 102 terminals and 2924 aligned characters, of which 870 (29.7%) were potentially parsimony informative, 1668 were constant and 386 were variable but uninformative. Missing taxa in each matrix represented 6.8% (7/102) for *rps16* and *trnL–trnF*, 5.9% (6/102) for *trnL* and

Table 1. Morphological characters, possible states and codifications

Character	States
1. Flower attachment	0. Sessile to subsessile; 1. Pedicellate
2. Bracteoles	0. Absent; 1. Present
3. Sepals	0. Equal to subequal; 1. Strongly unequal
4. Flower resupination	0. Absent; 1. Present
5. Anterior pair of stamens	0. Present; 1. Reduced to staminodes; 2. Absent
6. Number of fertile thecae on anterior pair of anthers	0. Zero; 1. One; 2. Two
7. Posterior pair of stamens	0. Present; 1. Reduced to staminodes; 2. Absent
8. Number of fertile thecae on posterior pair of anthers	0. Zero; 1. One; 2. Two
9. Arm of connective subtending thecae	0. Present; 1. Absent
10. Anther fusion	0. Free; 1. Adherent

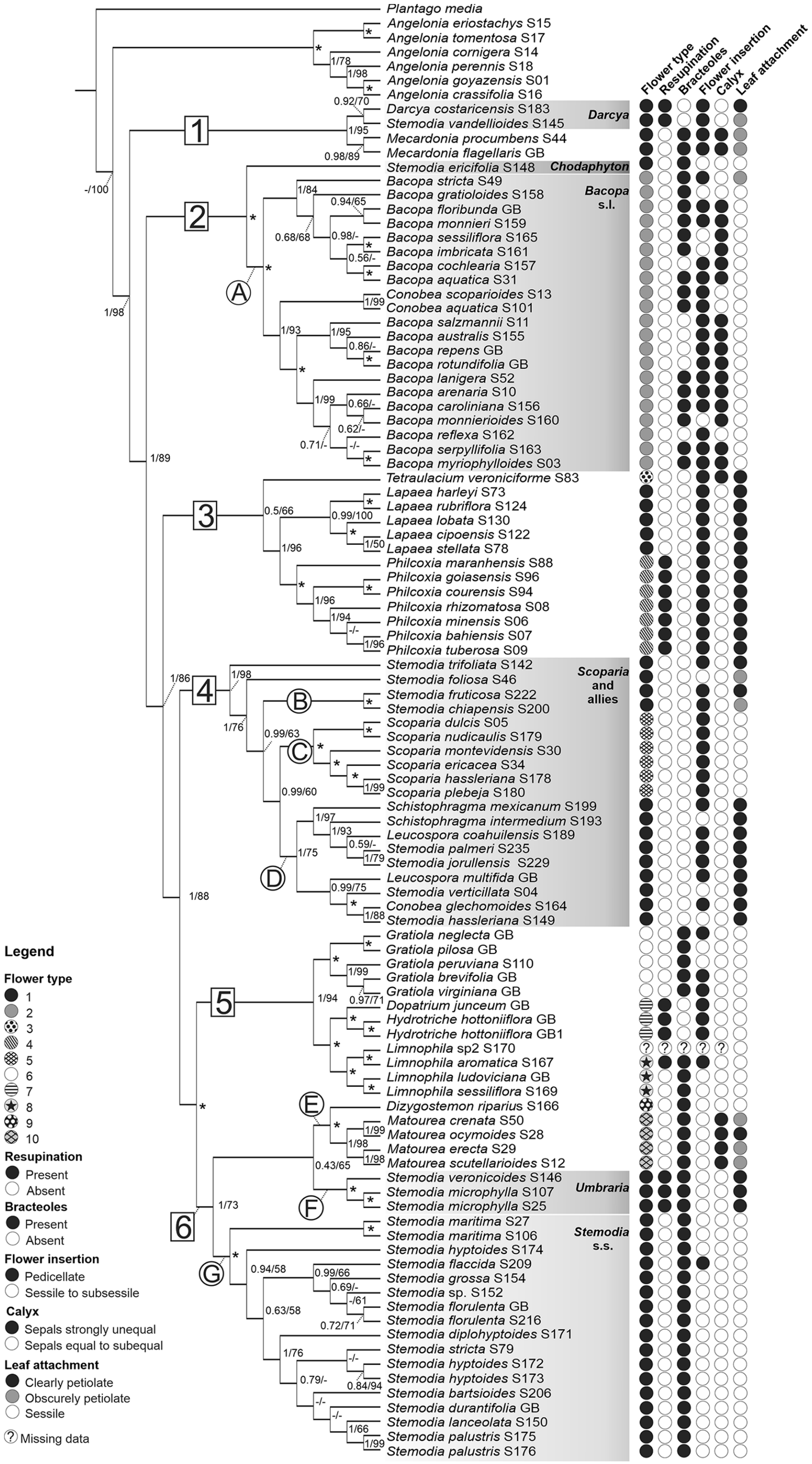
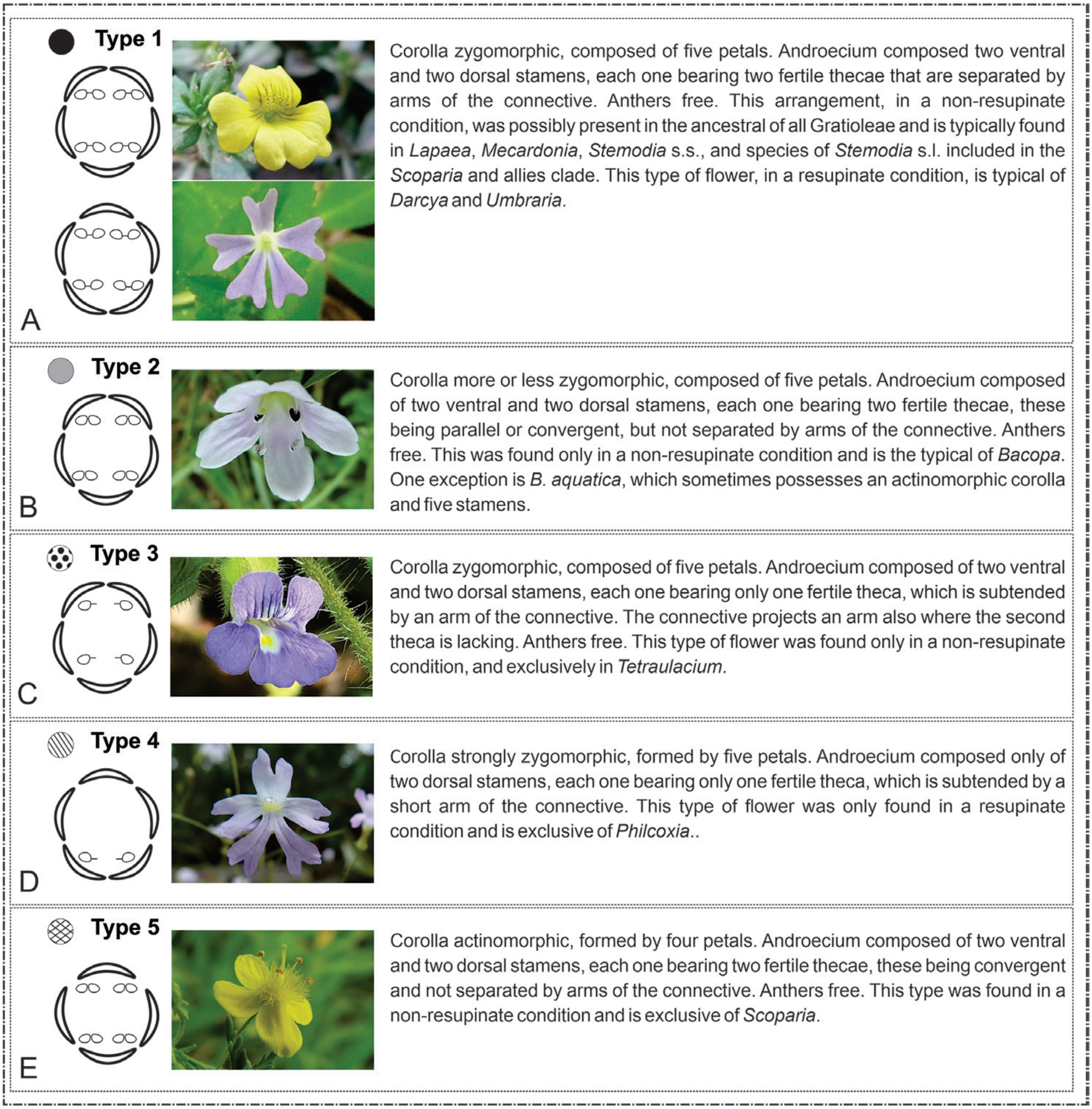


Figure 3. Majority-rule consensus tree obtained from the Bayesian inference analyses of the total combined dataset. Columns next to terminal taxa are potential diagnostic features. Clades denoted by letters and numbers are described in Results section and discussed in Discussion section.



Corolla zygomorphic, composed of five petals. Androecium composed two ventral and two dorsal stamens, each one bearing two fertile thecae that are separated by arms of the connective. Anthers free. This arrangement, in a non-resupinate condition, was possibly present in the ancestral of all Gratioleae and is typically found in *Lapaea*, *Mecardonia*, *Stemodia* s.s., and species of *Stemodia* s.l. included in the *Scoparia* and allies clade. This type of flower, in a resupinate condition, is typical of *Darcya* and *Umbraria*.

Corolla more or less zygomorphic, composed of five petals. Androecium composed of two ventral and two dorsal stamens, each one bearing two fertile thecae, these being parallel or convergent, but not separated by arms of the connective. Anthers free. This was found only in a non-resupinate condition and is the typical of *Bacopa*. One exception is *B. aquatica*, which sometimes possesses an actinomorphic corolla and five stamens.

Corolla zygomorphic, composed of five petals. Androecium composed of two ventral and two dorsal stamens, each one bearing only one fertile theca, which is subtended by an arm of the connective. The connective projects an arm also where the second theca is lacking. Anthers free. This type of flower was found only in a non-resupinate condition, and exclusively in *Tetraulacium*.

Corolla strongly zygomorphic, formed by five petals. Androecium composed only of two dorsal stamens, each one bearing only one fertile theca, which is subtended by a short arm of the connective. This type of flower was only found in a resupinate condition and is exclusive of *Philcoxia*..

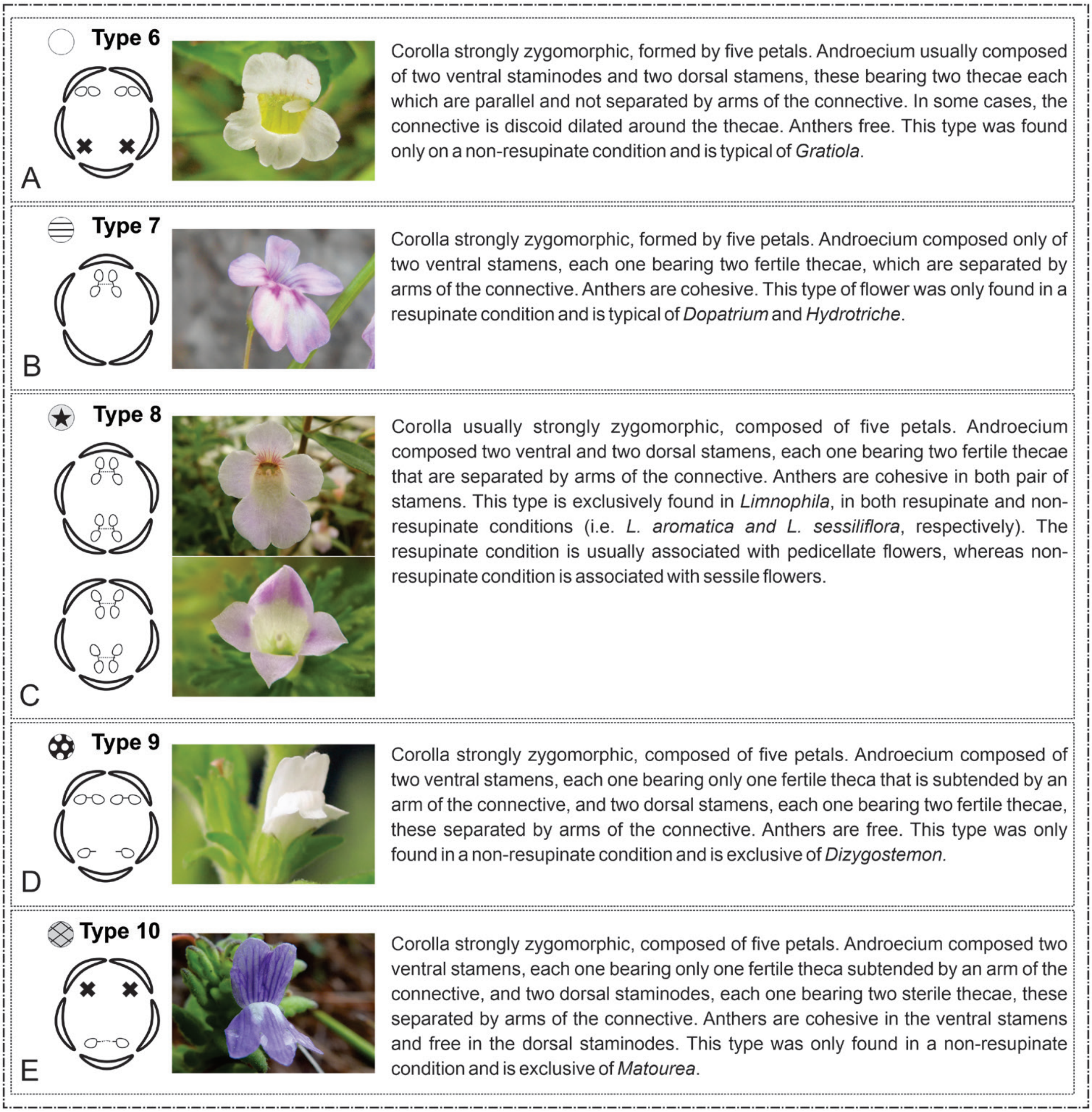
Corolla actinomorphic, formed by four petals. Androecium composed of two ventral and two dorsal stamens, each one bearing two fertile thecae, these being convergent and not separated by arms of the connective. Anthers free. This type was found in a non-resupinate condition and is exclusive of *Scoparia*.

Figure 4. Flower types 1–5, their descriptions and examples. A, Type 1, *Mecardonia procumbens* (upper) and *Stemodia vandellioides* (lower). B, Type 2, *Bacopa monnieri*. C, Type 3, *Tetraulacium veroniciforme*. D, Type 4, *Philcoxia goiasensis*. E, Type 5, *Scoparia montevidensis*. Photographs: A (lower) by L. Funez; C by E. Messias; all others by A. V. Scatigna.

17.6% (18/102) for ITS1. Detailed information about each dataset is presented in Table 2.

PHYLOGENETIC ANALYSES

There were no visual significantly conflicting topologies (with BS ≥ 75% in the MP analysis or PP ≥ 0.95 in the Bayesian analysis) among trees generated by each approach in each matrix, except one incongruence in the *Lapaea* clade in the ITS1 dataset, which has been discussed in detail by Scatigna *et al.* (2020) and does not affect our discussion. The majority-rule consensus tree generated by the BI analysis of the TC dataset was completely resolved, and most nodes had strong support values (PP ≥ 0.95); it is therefore the only one graphically presented



Corolla strongly zygomorphic, formed by five petals. Androecium usually composed of two ventral staminodes and two dorsal stamens, these bearing two thecae each which are parallel and not separated by arms of the connective. In some cases, the connective is discoid dilated around the thecae. Anthers free. This type was found only on a non-resupinate condition and is typical of *Gratiola*.

Corolla strongly zygomorphic, formed by five petals. Androecium composed only of two ventral stamens, each one bearing two fertile thecae, which are separated by arms of the connective. Anthers are cohesive. This type of flower was only found in a resupinate condition and is typical of *Dopatrium* and *Hydrotriche*.

Corolla usually strongly zygomorphic, composed of five petals. Androecium composed two ventral and two dorsal stamens, each one bearing two fertile thecae that are separated by arms of the connective. Anthers are cohesive in both pair of stamens. This type is exclusively found in *Limnophila*, in both resupinate and non-resupinate conditions (i.e. *L. aromatica and L. sessiliflora*, respectively). The resupinate condition is usually associated with pedicellate flowers, whereas non-resupinate condition is associated with sessile flowers.

Corolla strongly zygomorphic, composed of five petals. Androecium composed of two ventral stamens, each one bearing only one fertile theca that is subtended by an arm of the connective, and two dorsal stamens, each one bearing two fertile thecae, these separated by arms of the connective. Anthers are free. This type was only found in a non-resupinate condition and is exclusive of *Dizygostemon*.

Corolla strongly zygomorphic, composed of five petals. Androecium composed two ventral stamens, each one bearing only one fertile theca subtended by an arm of the connective, and two dorsal staminodes, each one bearing two sterile thecae, these separated by arms of the connective. Anthers are cohesive in the ventral stamens and free in the dorsal staminodes. This type was only found in a non-resupinate condition and is exclusive of *Matourea*.

Figure 5. Flower types 6–10, their descriptions and examples. A, Type 6, *Gratiola peruviana*. B, Type 7, *Dopatrium junceum*. C, Type 8, *Limnophila aromatica* (upper) and *Limnophila sessiliflora* (lower). D, Type 9, *Dizygostemon riparius*. E, Type 10, *Matourea erecta*. Photographs: A by L. Funez; B by M. Prasad; D by E. Martins; all others by A. V. Scatigna.

and described next (Fig. 3). Trees generated by each approach for each dataset separately are available in Supporting Information, Appendix S3, Figs S1–18; corresponding taxa of terminal labels are presented in Supporting Information, Appendix S1.

All representatives of Gratioleae were retrieved in a monophyletic group (Gratioleae clade – PP = 1.00; BS = 98) sister to a clade formed by all members of Angelonieae (PP = 1.00; BS = 100). In the Gratioleae clade, we identified six major clades (clades 1–6; Fig. 3).

Clade 1 (PP = 1.00; BS = 95) was composed of *Darcya*, *Mecardonia* Ruiz & Pav. and one *Stemodia* sp. This clade was formed by two subclades, one including the two sampled representatives of *Mecardonia* (PP = 0.98; BS = 89) and the other with *Stemodia vandellioides* (Benth.) V.C.Souza and *Darcya*

Table 2. Maximum parsimony scores and evolutionary model for each dataset (BIC = Bayesian inference criterion)

Dataset	*rps16*	*trnL*	*trnLF*	ITS1	Partial combined	Total combined
Terminals	95	96	95	84	102	102
Missing taxa (%)	6.8	5.9	6.8	17.6	—	—
Number of characters	1164	674	619	467	2457	2924
Constant characters	644	464	335	225	1443	1668
Variable, uninformative characters	171	76	90	49	337	386
Potentially parsimony informative characters	349	134	194	193	677	870
Percentage informative (%)	29.9	19.9	31.3	41.3	27.5	29.7
Tree length	989	323	465	1304	1798	3147
Consistency index (CI)	0.556	0.638	0.641	0.329	0.586	0.471
Retention index (RI)	0.845	0.904	0.888	0.680	0.866	0.799
Evolutionary model (BIC)	TVM+G	GTR+G	TVM+G	GTR+I+G	Mixed	Mixed

costaricensis (B.L.Turner) B.L.Turner (PP = 0.92; BS = 70). This major clade was retrieved as sister to the clade (PP = 1.00; BS = 83) formed by clades 2 to 6.

Clade 2 (PP = 1; BS = 100) was formed by *Stemodia ericifolia* K.Schum. as sister to a subclade (subclade A; PP = 1; BS = 100) formed by all representatives of *Bacopa* and part of *Conobea*. This clade was recovered as sister to a group (PP = 1; BS = 89) comprising the remaining clades of Gratioleae; subclade A was composed by a subclade comprising two *Conobea* spp. (PP = 1.00; BS = 99), including the type species *C. aquatica* Aubl., deeply nested in the sampled representatives of *Bacopa*.

Clade 3 (PP = 0.5; BS = 64) was formed by *Tetraulacium veroniciforme* Turcz. as sister to a clade (PP = 1.00; BS = 96) composed of the sister clades *Lapaea* (PP = 0.99; BS = 100) and *Philcoxia* (PP = 1.00; BS = 100).

Clade 4 (PP = 1.00; BS = 98) included members of *Conobea*, *Leucospora*, *Schistophragma*, *Scoparia* and *Stemodia*. This clade was formed by *Stemodia trifoliata* (Link) Rchb., *Stemodia foliosa* and a clade (PP = 0.99; BS = 63) comprising three subclades (B–D, Fig. 3). Subclade B (PP = 1.00; BS = 100) included *Stemodia fruticosa* Lundell and *Stemodia chiapensis* B.L.Turner and was recovered as sister to another clade (PP = 0.99; BS = 60), which in turn was formed by subclades C and D. Subclade C (PP = 1.00; BS = 100) was composed of all representatives of *Scoparia*. Subclade D (PP = 1.00; BS = 75) was composed of two subclades, the first (PP = 1.00; BS = 97) included the two representatives of *Schistophragma* Benth. forming a grade, plus *Leucospora coahuilensis* Henrickson, *Stemodia jorullensis* Kunth. and *S. palmeri* A.Gray, whereas the second (PP = 0.99; BS = 75) was formed by *L. multifida* (Michx.) Nutt., *Stemodia verticillata* (Mill.) Hassl., *Stemodia hassleriana* Chodat and *Conobea glechomoides* (Spreng.) V.C.Souza.

Clade 5 (PP = 1.00; BS = 94) included *Dopatrium*, *Gratiola*, *Hydrotriche* and *Limnophila*. This clade was composed of all members of *Gratiola* forming a monophyletic group (PP = 1.00; BS = 100) sister to another clade (PP = 1.00; BS = 98) comprising a smaller group formed by *Dopatrium junceum* (Roxb.) Benth. and *Hydrotriche hottoniiflora* Zucc. (PP = 1.00; BS = 100) as sister to a clade composed of all sampled representatives of *Limnophila* (PP = 1.00; BS = 100).

Clade 6 (PP = 1.00; BS = 73) included *Dizygostemon* (Benth.) Wettst., *Matourea* and *Stemodia*. This clade was formed by three subclades (E – G, Fig. 3). Subclade E (PP = 1.00; BS = 100) was composed of *Dizygostemon* as sister to a clade formed by all sampled representatives of *Matourea* (PP = 1.00; BS = 98). Subclade F (PP = 1.00; BS = 100) was formed by two accessions of *Stemodia microphylla* in a clade (PP = 1.00; BS = 100) plus *Stemodia veronicoides* J.A.Schmidt. Subclades E and F were weakly supported as sister groups (PP < 0.5; BS = 65). Subclade G (PP = 1.00; BS = 100) was composed of *Stemodia maritima*, type species of the genus, as sister to a clade (PP = 0.94; BS = 58) comprising all remnant representatives of *Stemodia*. In the clade sister to *S. maritima* (in subclade F), all sampled representatives of *Stemodia* from Australia formed a well-supported monophyletic group (PP = 0.99; BS = 66) as did the representatives of *Stemodia* from the Americas (PP = 1.00; BS = 76), except for one accession of *S. hyptoides* Cham. & Schltdl., for which placement was not clear and, therefore, obscured the relationship between clades from Australia and Americas; relationships in these clades were generally unresolved.

ANCESTRAL CHARACTER STATE RECONSTRUCTION

Potential apomorphies discussed throughout the text are mapped onto a simplified tree based on the BI majority-rule consensus tree (Fig. 6). Traced history of each character from Table 1 is presented individually in Supporting Information, Appendix S4D (Figs S19–28). The state of nine of the ten studied characters were unambiguously reconstructed for the ancestor of all Gratioleae. Most characters were potentially homoplastic but useful in defining clades, such as the independent resupination of flowers (character 4: state 1) in *Darcya, Philcoxia* and subclade F. Fewer characters yielded potential synapomorphies, like the loss of the anterior pair of stamens (5: 2) in *Philcoxia* and the reduction of the anterior pair of stamens to staminodes (5: 1) in *Gratiola*. Other character states, ambiguously reconstructed for some clades, could be useful as diagnostic features, such as the sessile flowers (1: 0) in subclades E (*Matourea* + *Dizygostemon*) and G (*Stemodia s.s.*).

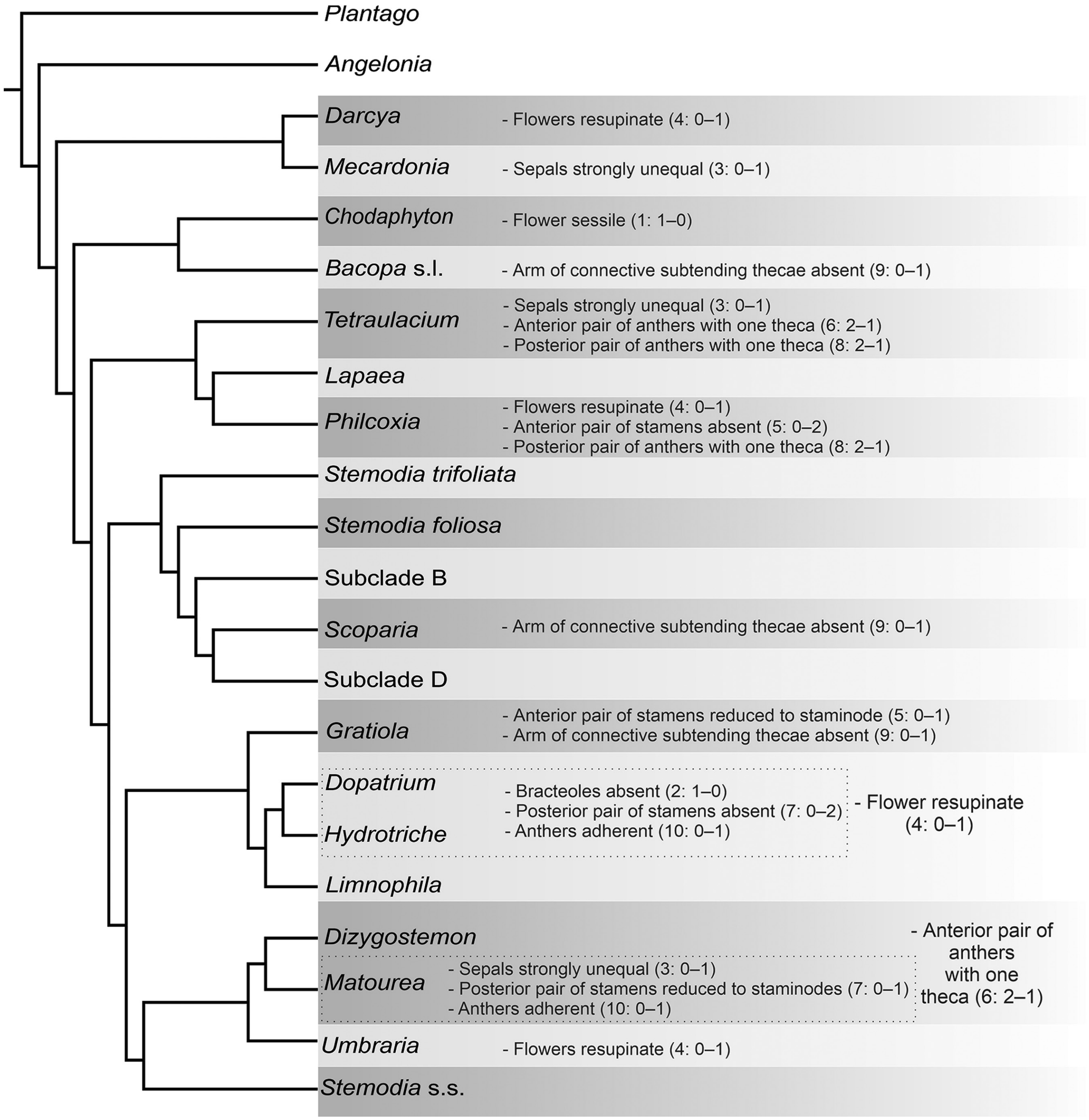


Figure 6. Summary of relationships among recognized genera and clades of Gratioleae, showing their morphological synapomorphies (character: character state change).

DISCUSSION

The circumscription of Gratioleae and some of the genera have been long controversial due to the scarcity of phylogenetic studies published so far (Albach *et al.*, 2005; Scatigna *et al.*, 2018a). Our results corroborate the placement of sampled members of Gratioleae (*sensu* Albach *et al.*, 2005) in this tribe, in accordance with previously published contributions (Fritsch *et al.*, 2007; Estes & Small, 2008; Scatigna *et al.*, 2018a) and provide further evidence of non-monophyly of *Bacopa*, *Conobea*, *Leucospora* Nutt., *Schistophragma* and *Stemodia*, highlighting the urgent need to reassess their circumscriptions.

FLORAL EVOLUTION

According to our reconstructions, the ancestor of all Gratioleae unequivocally presented flowers pedicellate (character 1: state 1; Supporting Information, Fig. S19), calyx with equal to subequal sepals (3: 0; Supporting Information, Fig. S21), flowers non-resupinate (4: 0; Supporting Information, Fig. S22), anterior pair of stamens present (5: 0; Fig. S23), two thecae per anther in the anterior pair (6: 2; Supporting Information, Fig. S24), posterior pair of stamens present (7: 0; Supporting Information, Fig. S25), two thecae per anther in the posterior pair (8: 2; Supporting Information, Fig. S26), thecae subtended by the connective arms (9: 1; Supporting Information, Fig. S27) and anthers free (10: 0; Supporting Information, Fig. S28). As for the bracteoles (character 2; Supporting Information, Fig. S20), the reconstruction was ambiguous, and neither its presence (state 0) or absence (state 1) was unequivocally reconstructed for the ancestor of Gratioleae.

Outer flower characters

Pedicellate flower (pedicel > 2 mm long; character 1, state 1) is the plesiomorphic condition in Gratioleae, with several independent shifts to sessile (pedicel < 2 mm long; character 1, state 0) within clades 2, 4 and 5. The weakly supported relationships among subclades E, F and G in clade 6 make the reconstructions ambiguous, that is, it is possible that the flower shifted to sessile in the ancestor of clade 6, with a reversal in subclade F, or, alternatively, the pedicellate condition was plesiomorphic in clade 6 with two independent shifts to sessile in subclades E and G. Although sessile flowers could not yield an unequivocal synapomorphy for *Stemodia s.s.* (subclade G), it is a potential diagnostic feature, present in almost all species of this assemblage (Souza & Giulietti, 2009; Scatigna *et al.*, 2018b).

The history of presence of bracteoles (character 2) could not be unambiguously reconstructed for almost any of the major clades. Nevertheless, the lack of bracteoles (character 2, state 0) is a potential diagnostic feature for *Darcya* plus *Stemodia vandellioides*, and for clades 3 and 4, whereas presence of bracteoles is typical of clades 2 and 6. In clade 5, the loss of bracteoles is a potential apomorphy for the clade *Dopatrium* + *Hydrotriche*.

The calyx morphology (character 3) has long been used to define some genera such as *Bacopa*, *Matourea* and *Mecardonia*, which have strongly unequal sepals, in contrast with *Stemodia s.l.*, with equal to subequal sepals (Bentham, 1846; D'Arcy, 1979; Souza & Giulietti, 2009; Sosa, Moroni & O'Leary, 2018). According to our reconstruction, the ancestral state of Gratioleae is sepals equal to subequal (state 0), which independently shifted to strongly unequal (state 1) at least four times, one in the common ancestor of *Mecardonia* (in clade 1), one in *Tetraulacium veroniciforme* (in clade 3), one in the common ancestor of *Matourea* (in subclade F). In clade 2, in which the reconstruction of the ancestral state was ambiguous, the strongly unequal sepals may have emerged in one or two independent events; this is because of the clade composed of *Conobea aquatica* and *Conobea scoparioides*, both with subequal sepals (state 0) nested in *Bacopa*. Clearly, the definition of equal to subequal sepals may be subjective, whereas the calyx with strongly unequal sepals may exhibit several distinct conformations. For instance, the calyx of *Matourea* is composed of a posterior (dorsal) sepal much wider than the other four, which are equal among themselves; the calyx of *Tetraulacium* is composed of three sizes of sepals: a large posterior one, two slightly shorter lateral sepals and two much shorter abaxial sepals; in *Bacopa* and *Mecardonia,* three external sepals present three distinct sizes and shapes, and two internal sepals are much narrower and equal between each other (Souza & Giulietti, 2009). We believe that calyx symmetry and aestivation could yield more reliable diagnostic characters.

The plesiomorphic condition of the flower in Gratioleae is non-resupinate (character 4, state 0), with three unequivocal shifts to resupinate (character 4, state 1), one in the clade *Darcya* + *Stemodia vandellioides* (in clade 1), one in *Philcoxia* (in clade 3) and one in subclade F (in clade 6). In clade 5, resupinate flowers may have emerged in the most recent common ancestor of *Dopatrium*, *Hyrotriche* and *Limnophila*, reverting to non-resupinate in a subclade of *Limnophila*; alternatively, the non-resupinate flower may be the plesiomorphic condition in this clade, and the resupinate flower emerged independently in the most recent common ancestor of *Dopatrium* and *Hydrotriche* and in a subclade of *Limnophila*. Because our sampling in these three genera is limited and most extant species have resupinate flowers (Philcox, 1970; Fischer, 1997, 2004), we believe that the first hypothesis is more plausible. Resupination of flowers in Gratioleae occur only in species with pedicellate flowers.

Androecium

Lamiales are typically characterized by flowers with bilateral symmetry (Reeves & Olmstead, 1998; Endress, 2012). Zygomorphic flowers present an asymmetry along a dorsoventral axis, that is, abaxial (anterior or ventral) organs, usually petals and stamens, are morphologically different from the adaxial (posterior or dorsal) ones. Furthermore, the most adaxial stamen is usually lacking or reduced to a rudimentary staminode, which is associated to pollinator access to the remaining stamens (Hileman & Cubas, 2009). In Gratioleae and in other groups of Plantaginaceae and even other families of Lamiales, shifts in stamen number have occurred several times (Reeves & Olmstead, 1998; Hileman, 2014a). Consequently, stamen number has been used as a single, potentially diagnostic character, regardless of which pair of stamens, either abaxial or adaxial, was aborted (Scatigna *et al.*, 2018a). However, there is evidence that the genetic mechanism for abortion or reduction of ventral stamen, at least in *Gratiola officinalis* L., is different from that of dorsal stamen abortion found in other genera of Plantaginaceae (Hileman & Cubas, 2009; Preston & Hileman, 2009). Therefore, we decided to interpret the abaxial pair of stamens as a distinct character from the adaxial pair, as well as its respective number of fertile thecae.

The presence of the anterior (abaxial) pair of stamens (character 5: state 0) is the plesiomorphic condition in Gratioleae. The complete loss of the anterior pair of stamens (character 5: state 2) is a potential synapomorphy for *Philcoxia*, whereas its reduction to staminodes (character 5: state 1) is a potential synapomorphy for *Gratiola*. However, our sampling in *Gratiola* did not encompass the whole variation of development in anterior pair of stamens, which ranges from well-developed staminodia in some species to the complete loss of these organs in others (Estes, 2008), explaining that the interspecific relationships of more species could change our understanding about stamen evolution in this genus. In Gratioleae, the ancestral condition regarding the number of fertile thecae in the anterior pair of stamens is two (character 6: state 0), with two independent shifts to one fertile theca (character 6: state 1), one in *Tetraulacium* (in clade 3) and other in the common ancestor of *Dizygostemon* and *Matourea* (subclade E in clade 6). The genetic mechanism associated with the reduction or loss of the anterior pair of stamens in Gratioleae is still obscure (Hileman & Cubas, 2009; Preston & Hileman, 2009).

Regarding the posterior (adaxial) pair of stamens, the plesiomorphic condition in Gratioleae is present (character 7: state 0). The complete loss of the posterior pair of stamens (character 7: state 2) is a potential synapomorphy for the clade *Dopatrium* + *Hydrotriche* (in clade 5), whereas its reduction to staminodes is a potential synapomorphy for *Matourea* (in clade 6). The posterior pair of stamens bearing two fertile thecae per anther (character 8: state 0) is the ancestral condition for Gratioleae, whereas the posterior pair of stamens with only one fertile theca (character 8: state 1) emerged twice, one in *Tetraulacium* and other in *Philcoxia* (in clade 3). The genetic programme related to the abortion of the posterior pair of stamens in Plantaginaceae is possibly associated with that of bilateral symmetry in the majority of Lamiales (Hileman, 2014a, b.)

Anther coherence (or adherence) is a rare feature in Lamiales that has been documented in some genera of Plantaginaceae, e.g. *Antirrhinum* L. (Fischer, 2004), *Limnophila* (Philcox, 1970) and *Matourea* (Scatigna *et al.*, 2019). According to our reconstructions, the ancestral condition of the anthers in Gratioleae is free (character 9: state 0), with two independent shifts to adherent, one in the common ancestor of the *Dopatrium*, *Hydrotriche* and *Limnophila* and other in the common ancestor of *Matourea*. We did not sample *Dizygostemon floribundus* (Benth.) Radlk., type species of its genus, which presents anther coherence in both anterior and posterior pair of anthers; its inclusion could show a different history of anthers coherence in subclade E.

Last, Bentham & Hooker (1876) characterized subtribe Stemodiineae by the anthers with the thecae separated by arms of the connective (Fig. 2A, B), a feature frequently described as stipitate or stalked thecae. This subtribe included *Stemodia* along with other seven genera (see notes under *Clade 6*, *subclade E*). According to our reconstructions, the presence of arms of the connective subtending the thecae (character 10: state 0) is the plesiomorphic condition in Gratioleae, with three independent shifts to absence of arms (state 1), one in the common ancestor of *Bacopa s.l.* (in clade 2), one in the common ancestor of *Scoparia* (in clade 4) and one in the common ancestor of *Gratiola* (in clade 5).

As previously mentioned, the combination of equal to subequal sepals with four fertile stamens with two thecae subtended by arms of the connective was used to define *Stemodia s.l.* According to our reconstructions, all these features are symplesiomorphies, which helps to explain the paraphyly of this genus in its broad sense.

PHYLOGENETIC RELATIONSHIPS AND TAXONOMIC IMPLICATIONS

Paraphyly of Stemodia s.l.

We were able to include *c*. 50% of the members *Stemodia s.l.* in our analysis, including the type species *S. maritima* and representatives of the genus from the Americas and Australia, covering a wide range of both morphological variation and geographical distribution. Sampled representatives of *Stemodia s.l.* were recovered in four main clades (clades 1, 2, 4 and 6), apart from that one containing the recently segregated genus *Lapaea* (clade 3; Scatigna *et al.*, 2020). Furthermore, in clades 4 and 6, representatives of *Stemodia* did not form monophyletic groups.

Clade 1 – Darcya *and* Mecardonia

Stemodia vandellioides is a Brazilian endemic species that occurs in open and wet, often disturbed areas of the Atlantic Forest. This species has been traditionally recognized in Gratioleae, first under the genus *Conobea* (Bentham, 1846), a placement followed by Wettstein (1891), then under *Lindernia* All. (Barroso, 1952). Subsequently, Souza (2003) placed it under *Stemodia*, due to the anthers with the thecae separated by the connective arms. More recently, Fischer *et al.* (2013), in a phylogenetic study of Linderniaceae, suggested that *S. vandellioides* should belong to *Lindernia s.s.*, even though they did not sample this species in their analysis. Until now, *S. vandellioides* had never been included in a phylogenetic study. Our results corroborate the placement of *S. vandellioides* in Gratioleae, but closely related to *Darcya costaricensis*, in our clade 1, rather than in *Stemodia s.s.* (subclade G in clade 6).

Darcya is a Central American genus composed of three species originally described under *Stemodia* (D'Arcy, 1979; Fernandez-Alonso, 1987; Turner, 1992; Turner & Cowan, 1993c). According to Turner & Cowan (1993c), it is characterized by leaves with main veins 3–5-digitate, terminal or axillary bracteate racemes, androecium composed of a longer pair of stamens with two separate, pubescent thecae in each anther and a short pair of stamens with two glabrous, reduced thecae in each anther. In a preliminary comparison between *Darcya* spp. and *Stemodia vandellioides*, we noticed that both taxa share angulate, slightly winged stems, leaves with five-digitate main veins, absence of bracteoles, corolla salverform and lower pair of stamens with reduced (but fertile) thecae. After detailed study of herbarium material, we observed that the three-lobed lower lip of the corolla of *Darcya*, as described by D'Arcy (1979), Fernandez-Alonso (1987) and Turner (1992), is in fact composed of two lateral lobes and a central lobule that in turn is formed by two fused petals, whereas the upper lip is formed by a single, emarginate corolla lobe; this means that, like *Stemodia vandellioides*, *Darcya* has resupinate flowers. Furthermore, the corolla tube throat, both in *S. vandellioides* and *Darcya* spp., has a densely tufted ring, resembling the corollas of species of *Verbena* L. (Verbenaceae) and *Buchnera* L. (Orobanchaceae). The only diverging features in *S. vandellioides* relatively to the delimitation of *Darcya* are the glabrous thecae (vs. pubescent) and the leaf-like floral bracts (vs. differentiated). We were only able to generate sequence of *trnL* from a single accession of *Darcya*, which may explain the low support for the clade *S. vandellioides* + *D. costaricensis*. Nevertheless, because of the morphological consistency and the shared possession of a unique combination of diagnostic features (Fig. 3), that is, flower type 1 and resupinate, bracteoles absent, flowers pedicellate and calyx with equal sepals, we propose transferring *S. vandellioides* to *Darcya*.

Mecardonia is a relatively small genus of tribe Gratioleae, with *c*. ten species distributed in the Americas, especially in southern South America (Greppi, Sosa & Dematteis, 2017). Although our sampling in this genus is too narrow to test its monophyly, this is a morphologically consistent group, being characterized by flower of type 1 and non-resupinate, bracteoles present and calyx with slightly to strongly unequal sepals. The usually yellow, sometimes white, corolla is also a diagnostic feature of this genus (Souza & Giulietti, 2009). The calyx with unequal sepals has led authors to consider *Mecardonia* and *Bacopa* as congeneric (Pennell, 1946), but several phylogenetic studies have refuted this hypothesis, recovering *Mecardonia* as sister group to a clade comprising all other Gratioleae, including *Bacopa* (Albach *et al.*, 2005; Fritsch *et al.*, 2007; Estes & Small, 2008; Schäferhoff *et al.*, 2010; Scatigna *et al.*, 2018a).

Clade 2 – Bacopa s.l., Conobea *and* Chodaphyton

Bacopa, in its current circumscription, is one of the most diverse genera in Gratioleae, comprising 50–60 spp. mainly distributed in the tropics, especially in America (Sosa, Moroni & O'Leary, 2018). The high morphological variation in this genus has led to the description of several genera now under the synonymy of *Bacopa* (Pennell, 1946; D'Arcy, 1979; Souza & Giulietti, 2009; Sosa *et al.*, 2018). Our results corroborate the placement of sampled species of *Bacopa* in this genus, but also point to its paraphyly due to the placement of members of *Conobea* nested in it, as previously demonstrated by Scatigna *et al.* (2018a) and Gonzalez-Socoloske *et al.* (2020).

Bacopa is defined by its unequal sepals, being three broader and inclosing the two, equally narrower, inner sepals, and by the anther thecae being closely sessile (not subtended by arms of the connective) and converging at least at the apex (Pennell, 1946; D'Arcy, 1979; Sosa *et al.*, 2018). Nevertheless, some species of *Bacopa* [*B. gratioloides* (Cham.) Edwall, *B. depressa* (Benth.) Edwall, *B. reflexa* (Benth.) Edwall] have the sepals alike (Souza & Giulietti, 2009). *Bacopa reflexa* has been treated either under the monotypic genus *Benjaminia* Mart. ex Benj., because of the equal sepals and pinnatifid leaves (D'Arcy, 1979; Barringer & Burger, 2000; Backsh-Comeau *et al.*, 2016), or under *Bacopa*, due to the presence of a ring of bristles around the ovary, a feature exclusive of this genus, although not present in all species (Pennell, 1946; Souza & Giulietti, 2009; Scatigna & Mota, 2017). Our results corroborate retaining *Bacopa reflexa* in this genus.

Conobea sensu Wettstein (1891) was composed of three sections: (1) *Conobea* section *Conobea* ('*Sphaerotheca*'), with globose capsules; (2) *C.* section *Leucospora* (Nutt.) Benth. with ovoid capsules and (3) *C.* section *Schistophragma* (Benth.) Benth. & Hook., with linear capsules. Thieret (1967) proposed the recognition of these sections at the generic level and provided additional diagnostic characters such as seed surface and disposition, leaf shape and indument, among others. The first group is composed of *C. aquatica* (type of the genus), *C. scoparioides* (Cham. & Schltdl.) Benth. and *C. punctata* Nees & Mart., and is here referred to as *Conobea s.s.* It is characterized by the sepals alike, by the converging thecae and by the globose capsule (Souza & Giulietti, 2009). According to our results, retaining *Conobea* as a genus would result in the paraphyly of *Bacopa* and in the need for the recognition of at least another genus, with the creation of several new combinations, jeopardizing the nomenclatural stability in Gratioleae. Therefore, we propose merging *Conobea s.s.* and *Bacopa* in a single genus. This enlarged concept of *Bacopa* is generally characterized by the flower of type 2 and non-resupinate, bracteoles usually present (rarely absent) and anthers with thecae contiguous at least in part. *Leucospora* and *Schistophragma* were retrieved in clade 4, which is discussed under the section Clade 4 – Scoparia and allies.

Stemodia ericifolia, recovered as sister to *Bacopa* and distantly related to *Stemodia s.s.*, was already segregated from this genus by Minod (1918) and transferred to the monotypic genus *Chodaphyton*, under the combination *C. ericifolium* (Kuntze) Minod. Our results corroborate Minod's (1918) proposal and, therefore his combination is resurrected here. *Chodaphyton* is characterized by flowers of type 1 and non-resupinate, bracteoles present, flowers sessile, calyx with equal sepals and sessile leaves, a set of traits that is also present in *Stemodia s.s.* However, *Chodaphyton* differs from *Stemodia s.s.* in the bifid stigma (vs. entire and obdeltoid), and in the presence of unique bristle-like, unicellular and non-glandular trichomes over surfaces of vegetative parts, which are lacking in other species of *Stemodia s.l.* (Minod, 1918; Sosa, 2005). Alternatively, *C. ericifolium* could be transferred to a broadened concept of *Bacopa*. In this case, *Bacopa s.l.* would not be characterized by a morphological synapomorphy, i.e. thecae not separated by arms of the connective, hindering its morphological delimitation.

Clade 3 – Tetraulacium, Philcoxia *and* Lapaea

This clade is composed of the monotypic genus *Tetraulacium*, the carnivorous genus *Philcoxia* and the recently described *Lapaea*. The relationships in *Philcoxia* were discussed in detail by Scatigna *et al.* (2018a), whereas those of *Lapaea* were presented by Scatigna *et al.* (2020). Although there is an incongruence between the ITS1 and PC datasets regarding the relationships among *L. cipoensis* (Scatigna) Scatigna, *L. lobata* (J.A.Schmidt) Scatigna and *L. stellata* (B.L.Turner) Scatigna, the composition of the clade is the same in both datasets and therefore we combined them in the TC dataset.

Clade 4 – Scoparia *and allies*

This is the most taxonomically complex clade recovered in our analyses. It is formed by representatives of five genera, namely *Conobea*, *Leucospora*, *Schistophragma*, *Scoparia* and *Stemodia*. Furthermore, only members of *Scoparia* were retrieved in a monophyletic group. The only features shared by all members of clade 4 are the flower non-resupinate, bracteoles absent and calyx with equal to subequal sepals, a set of traits also present in other clades (e.g. *Lapaea*).

Scoparia is a cohesive and distinct assemblage of species characterized mainly by its rotaceous and apparently tetramerous corolla. Although its circumscription is relatively stable, its taxonomic position has been controversial. The genus was first assigned to tribe Sibthorpieae by Bentham (1846). Subsequently, Bentham & Hooker (1876) placed *Scoparia* in tribe Digitaleae, subtribe Sibthorpieae, along with *Sibthorpia* L., *Hemiphragma* Wall. and *Capraria* L.; this tribe was characterized by the rotaceous to subrotaceous corolla with short tube, number of stamens equalling that of the corolla lobes and sagitate anthers (Souza & Giulietti, 2009). The placement of *Scoparia* in Digitaleae was followed by Wettstein (1891) and Barroso (1952), but not by Thieret (1967), who finally transferred the genus to Gratioleae. This placement has been followed by subsequent authors (Ichaso, 1978; Fischer, 2004;

Albach *et al.*, 2005) and corroborated by phylogenetic studies based on DNA sequence data (Albach *et al.*, 2005; Fritsch *et al.*, 2007; Estes & Small, 2008; Scatigna *et al.*, 2018a). Our results corroborate the monophyly of *Scoparia* and its placement in Gratioleae.

Although Souza and Giulietti (2009) recognized some of Brazilian species of *Stemodia* in two morphological groups (see notes under *Clade 6*, *subclade F*), some species (i.e. *S. foliosa*, *S. trifoliata*, *S. vandellioides* and *S. verticillata*) could not be assigned to a consistent assemblage. In our phylogenetic tree, all these species (except *S. vandellioides*) were recovered in clade 4, along with *S. chiapensis*, *S. hassleriana*, *S. jorullensis* and *S. palmeri*, which were not treated by Souza & Giulietti (2009), but they did not form a monophyletic group. Fritsch *et al.* (2007) and Estes & Small (2008) recovered *S. suffruticosa* in a clade with *S. verticillata*, *Leucospora multifida* and *Scoparia* spp. Part the species of *Stemodia* recovered in our clade 4 were included by Bentham (1846) in section *Diamoste* Cham. & Schltdl., group '*Petiolatae*', which was characterized by the clearly petiolate leaves and short to long-pedicellate, axillary flowers. The same species were included by Minod (1918) in the informal infrageneric division '*Ebracteolatae*', and some of them were transferred to monotypic genera (i.e. *Lendneria*, *Valeria* and *Verena*; Minod, 1918).

Schistophragma and *Leucospora* are characterized by divided leaf blades, but the distinction between them is not clear (Turner & Cowan, 1993a). According to Thieret (1967), *Leucospora* was composed exclusively of *L. multifida*, being characterized by the longitudinally sulcate, scalariform-reticulate seeds and ovoid capsule, whereas *Schistophragma* should include *S. pusilla* Benth. (= *S. mexicanum* Benth. ex D.Dietr.) and *C. intermedia* A.Gray [= *S. intermedium* (A.Gray) Pennell], being characterized by the spirally furrowed seeds and the elongate to linear capsules. Subsequently, Henrickson (1989) published *L. coahuilensis* as a new species resembling *S. intermedium* in the habit, in the pinnatifid leaves and in the fruit being lance-acuminate, but being characterized by white, longitudinally sulcate seeds, which in turn resemble those of *L. multifida*. The intermediate traits found in *L. coahuilensis* would be an indicative of the fragility of their generic boundaries (Henrickson, 1989). Dr. David Keil (pers. comm.) was preparing an account in which he would merge *Schistophragma* with *Leucospora* and make the appropriate combinations (Henrickson, 1989; Turner & Cowan, 1993a), but the suspicion of more complex phylogenetic relationships led him not to proceed with these taxonomic changes (Keil, pers. comm.). In our study, both genera were recovered as non-monophyletic groups, with *S. mexicanum*, *S. intermedium* and *L. coahuilensis* forming a grade related to *Stemodia jorullensis* and *S. palmeri*, in a subclade sister to another subclade formed by *L. multifida*, *Stemodia hassleriana* and *Stemodia verticillata*.

Conobea glechomoides was first described under *Herpestis* C.F.Gaertn. by Sprengel (1827) and subsequently transferred by Chamisso & Schlechtendal (1828) to the monotypic genus *Geochorda* Cham. & Schltdl., under the illegitimate name *G. cuneata* Cham. & Schltdl. Kuntze (1898) provided the correct name *G. glechomoides* (Spreng.) Kuntze. Later, Souza & Giulietti (2009) claimed that there were not sufficient differences between *Geochorda* and *Conobea* and therefore created the combination *C. glechomoides*. However, these authors examined only one specimen [*Sellow 3623* (K)] and, probably because of that, they missed the nature of the anthers with two thecae separated by arms of the connective (appropriately described and illustrated by Descole & Borsini, 1954), and the lack of bracteoles beneath the calyx, being clearly divergent from our concept of *Conobea s.s.*; instead, this set of features approximates *C. glechomoides* to *S. verticillata*. Furthermore, the shortly campanulate corolla is similar to that of *S. hassleriana* (Sosa, 2010). In our study, *C. glechomoides* was recovered in a clade with *S. hassleriana*, *S. verticilata* and *Leucospora multifida*, relatively distantly related to its congeners. Because we are proposing the transfer of all species of *Conobea s.s.* to *Bacopa*, the use of the name *C. glechomoides* should be avoided and replaced by the next available combination, that is, *Geochorda glechomoides*, until other taxonomic and nomenclatural changes are made to this clade.

Considering the monophyly of taxa as a basic principle of the current systematics (Backlund & Bremer, 1998; Stevens, 2001 onwards), we could recognize clade 4 as a large and heterogeneous *Scoparia*, which is the oldest generic name in the group. This would imply in the loss of morphological cohesion and prediction of a well-established and known genus, which are also principles of good systematics (Humphreys & Linder, 2009). The alternative would be to retain *Scoparia* in its current concept and to recognize subclade D as a larger and more diverse *Leucospora*, including *Schistophragma*, *C. glechomoides* and part of *Stemodia* in its circumscription; in this case, we would have to recognize at least other three additional genera, represented by *Stemodia trifoliata*, *S. foliosa* and subclade B, respectively. The latter approach would imply in increasing the number of generic names and in recognizing monotypic genera, which could increase confusion in an already complex group.

DNA source of several taxa recovered in clade 4 was herbarium samples, and for most of them we were only able to sequence one or other marker, sometimes even partially. On the other hand, several species that could be assigned to this heterogeneous group (i.e. *Stemodia peduncularis* Benth., *S. macrantha* B.L.Rob., *S. pusilla* Benth., *S. tenuifolia* Minod, among others) were not sampled.

Because of all that, we believe that any taxonomic changes at this time would be precipitated. Increased sampling and additional sequenced regions (e.g. complete ITS and *ndhF* gene) is essential for elucidating relationships in clade 4, whereas the study of additional morphological characters, such as seed surface, could provide support for taxonomic decisions.

Clade 5 – Dopatrium, Gratiola, Hydrotriche *and* Limnophila

This clade encompasses four genera of mainly semi-aquatic or aquatic herbs with frequently specialized structures, such as leaf dimorphism and cleistogamous flowers (Philcox, 1970; Fischer, 2004). The Neotropical genus *Gratiola* was recovered here as a monophyletic group, in accordance with Estes & Small (2008), sister to a clade formed by *Dopatrium* Buch.-Ham. ex Benth., *Hydrotriche* and *Limnophila*, all exclusive to the Eastern Hemisphere. Our results indicate that *Dopatrium* and *Hydrotriche* are sister groups, but our limited sampling does not allow us to infer their monophyly. *Limnophila* was included by Bentham & Hooker (1876) in Stemodieae, along with *Stemodia* and other genera that have anthers with separated thecae. Our results corroborate a closer relationship with members of Dopatriineae (*sensu* Fischer, 2004). Philcox (1970) used the capsule dehiscence to differentiate *Limnophila* from *Stemodia*, but we observed additional diagnostic features such as flower resupination (at least in long-pedicellate species) and coherence of anthers in *Limnophila*, which are both lacking in *Stemodia s.s*. Some *Limnophila* spp. have leaf dimorphism, i.e. submerged leaves that are pinnatisect and aerial leaves that are entire; this dimorphism is also lacking in *Stemodia s.s*.

Clade 6, subclade E – Matourea *and* Dizygostemon

Bentham (1846) considered *Achetaria* and *Dizygostemon* as two distinct sections of *Beyrichia* Cham. & Schltdl. of Gratioleae, differing in the posterior pair of stamens being sterile in *Achetaria* and fertile in *Dizygostemon* and in the capsule dehiscence being two-valved in *Achetaria* and four-valved in *Dizygostemon*. Subsequently, Wettstein (1891), based on the differences presented by Bentham (1846), elevated *Dizygostemon* to the generic level. Souza & Giulietti (2009) adopted a broad concept of *Achetaria* (currently at the generic level; Pennell, 1940), including *Beyrichia* and *Otacanthus* in its synonymy, but retaining *Dizygostemon* as a monotypic genus; they also discussed a possible close relationship between these two genera due to similarity in the calyx (with a much larger dorsal sepal relatively to the other four), in the corolla shape (with the tube being occluded by the inflated palate), and in the androecium (with the anterior pair being monothecous). More recently, Colletta *et al.* (2020) included *Achetaria* (*sensu* Souza & Giulietti, 2009) in the synonymy of *Matourea*. The putative phylogenetic relationship hypothesized by Souza & Giulietti (2009) was corroborated by our results; subclade E, in clade 6, is formed by *Dizygostemon riparius* Scatigna & Colletta as sister to the clade composed of *Matourea* spp.

Stemodiineae (*sensu* Bentham & Hooker, 1876) were characterized by the anthers with separated thecae and comprised, besides *Beyrichia* (including *Matourea* and *Dizygostemon*), seven other genera: *Adenosma*, *Hydrotriche* and *Limnophila* (recovered here in clade 5, along with *Gratiola*), *Lindenbergia* (currently in Orobanchaceae; Young, Steiner & Depamphilis, 1999; Olmstead *et al.*, 2001), *Morgania* (= *Stemodia s.s.*; Barker, 1990), *Stemodia s.l.* (here recovered as paraphyletic) and *Tetraulacium* Turcz (recovered in clade 3 along with *Lapaea* and *Philcoxia*). *Adenosma*, with *c.* 15 species distributed in Asia and Australia (Fischer, 2004), is the only genus of this subtribe that has never been included in a molecular phylogenetic study. It has a wide variation in vegetative characters, but is characterized by the bi-bracteolate flowers, calyx with the posterior sepal larger than the other four, and androecium with four stamens with separated thecae and at least the anterior pair monothecous (Fischer, 2004). This set of traits suggests a close relationship of *Adenosma* with *Matourea* and *Dizygostemon*.

Clade 6, subclade F – Umbraria

Souza & Giulietti (2009) recognized two morphologically consistent groups of *Stemodia* spp. One of them, characterized by prostrate habit, petiolate leaves and axillary and long-pedicellate flowers (Souza & Giulietti, 2009), included some of the species transferred to *Lapaea* by Scatigna *et al.* (2020) plus *Stemodia microphylla* J.A.Schmidt. and *S. veronicoides*. These last two species, according to Scatigna *et al.* (2017, 2020), differ from *Lapaea* in the opposite leaves (vs. usually three-whorled), in the presence of bracteoles on pedicels (vs. absence) and in the resupinate flower (vs. non-resupinate). Molecular evidence provided by Scatigna *et al.* (2018a, 2020) corroborated the segregation of *S. microphylla* from *Lapaea* and suggested a closer relationship with *Stemodia maritima* and *Matourea* (then as *Achetaria*). In this contribution, we corroborate the close relationship between *S. microphylla* and *S. veronicoides* (subclade F) and its exclusion from both the *Lapaea* and *Stemodia s.s.* clades. These two species present a unique set of traits, that is, flowers of type 1 and resupinate, bracteoles present, flowers pedicellate, calyx with equal to subequal sepals, leaves clearly petiolate and crawling to ascending habit. Retaining these species in our concept of *Stemodia s.s.* would drastically expand its morphological delimitation and preclude the ease in genera recognition; therefore, we propose the segregation of *S. microphylla* and *S. veronicoides* from *Stemodia s.s.* and their transfer to the new genus *Umbraria* gen. ined. described here.

Clade 6, subclade G – Stemodia s.s.

The second morphological assemblage of *Stemodia* spp. recognized by Souza & Giulietti (2009), referred to as *Stemodia* s.s. by Scatigna *et al.* (2020), is characterized by the erect habit, sessile leaves with frequently clasping base and bi-bracteolate, usually short-pedicellate and non-resupinate flowers. According to Sosa & Dematteis (2013, 2014) and Scatigna *et al.* (2018b), this assemblage includes *S. maritima*, type species of the genus, in addition to *S. diplohyptoides* M.M.Sosa & Dematt., *S. durantifolia* (L.) Sw., *S. hyptoides*, *S. lanceolata* Benth., *S. palustris* A.St.-Hil., *S. perfoliata* Scatigna & V.C.Souza, *S. scoparioides* Minod and *S. stricta* Cham. & Schltdl. This group of species is also consistent with Bentham's (1846) *Stemodia* section *Stemodia* ('*Diamoste*' *sensu* Bentham, 1846), subgroups '*Axillares*' and '*Spiciflorae*', and with Minod's (1918) informal subgeneric group '*Spiciflorae bracteolatae*' of *Stemodia*. All sampled species belonging to this group were recovered in a well-supported clade (subclade G) along with other American representatives and all accessions from Australia, which also exhibit a similar set of morphological traits. Fritsch *et al.* (2007) obtained similar results, recovering *S. durantifolia, S. schottii* Holz. and *S. florulenta* W.R.Barker (wrongly identified as *S. glabra*) in an unresolved topology, but closely related to *Achetaria* and *Otacanthus* (= *Matourea*); *S. schottii* occurs in the southern USA and northern Mexico and exhibits morphological traits consistent with *Stemodia s.s.*, except for the unusual crawling habit. Estes & Small (2008) also recovered *S. schotti* and *S. florulenta* (identified as *S. glabra*) in a clade with *S. maritima* and sister to a clade formed by *Achetaria* and *Otacanthus*.

Stemodia serrata Benth., an exclusively African species, and *S. viscosa* Roxb., from south-eastern Asia and Australia, were not included in any previous studies nor in our analyses, but their morphology is consistent with our concept of *Stemodia s.s.* and therefore are here assigned to this group.

Stemodia s.s. (Fig. 7A–G), as here proposed, is characterized by the erect habit (Fig. 7D), clearly sessile leaves with usually clasping base (Fig. 7G), bi-bracteolate, non-resupinate and usually sessile or short-pedicellate flowers (Fig. 7E) that are often concentrated at the apex of flowering branches (Fig. 7A–C), calyx well-divided into five equal sepals and androecium composed of four fertile stamens with two equal and glabrous separated thecae in each anther. This encompasses *c.* 25 species, including some of the American *Stemodia* spp. plus *S. serrata* from Africa, *S. viscosa* from Southeast Asia and Australia and all exclusively Australian species, except *S. debilis* Benth., of which the overall aspect is closer to the concept of *Adenosma* Nees (petiolate leaves and dorsal sepal much larger than the others; Bentham, 1846).

CONCLUSIONS

With an expanded sampling in *Stemodia s.l.* and Gratioleae, we advanced the understanding of the phylogenetic history and floral evolution in the tribe. The paraphyly of *Stemodia s.l.* was corroborated, with members of this assemblage being retrieved in four distinct main clades, apart from that one containing the recently segregated genus *Lapaea*. Furthermore, some representatives of *Stemodia* that are grouped in two of these clades did not form monophyletic groups. Besides *Stemodia s.l.*, *Bacopa*, *Conobea*, *Leucospora* and *Schistophragma* were recovered as non-monophyletic. As an action towards recognizing only monophyletic genera, *Stemodia s.s.* is proposed as a monophyletic and morphologically cohesive group, *Umbraria* is described as a new genus to accommodate two species segregated from *Stemodia*, *Stemodia vandelliodes* is transferred to *Darcya*, *Conobea s.s.* is merged with *Bacopa*, and the names *Chodaphyton ericifolium* and *Geochorda glechomoides* are re-established. Traits traditionally used to define *Stemodia s.l.* (i.e. calyx with equal to subequal sepals, androecium composed of four fertile stamens and anthers with two thecae separated by arms of the connective) are plesiomorphic in the tribe, as expected for traits that define paraphyletic taxa. Meanwhile, the flower type (composed of seven floral characters combined) constitutes a good diagnostic set of traits for almost all genera in the tribe, except *Stemodia s.l.*, for which other characters such as flower resupination, presence of bracteoles and leaf attachments must be considered. Further systematic studies in Gratioleae should focus on the '*Scoparia* and allies' clade and expand sampling in extra-American taxa, e.g. *Adenosma*, *Dopatrium* and *Limnophila*. Additionally, characters from calyx (symmetry and aestivation), gynoecium, seed and pollen may help in taxonomic changes such as those required in clade '*Scoparia* and allies'. Although this is the first effort towards a phylogenetic classification of Gratioleae,

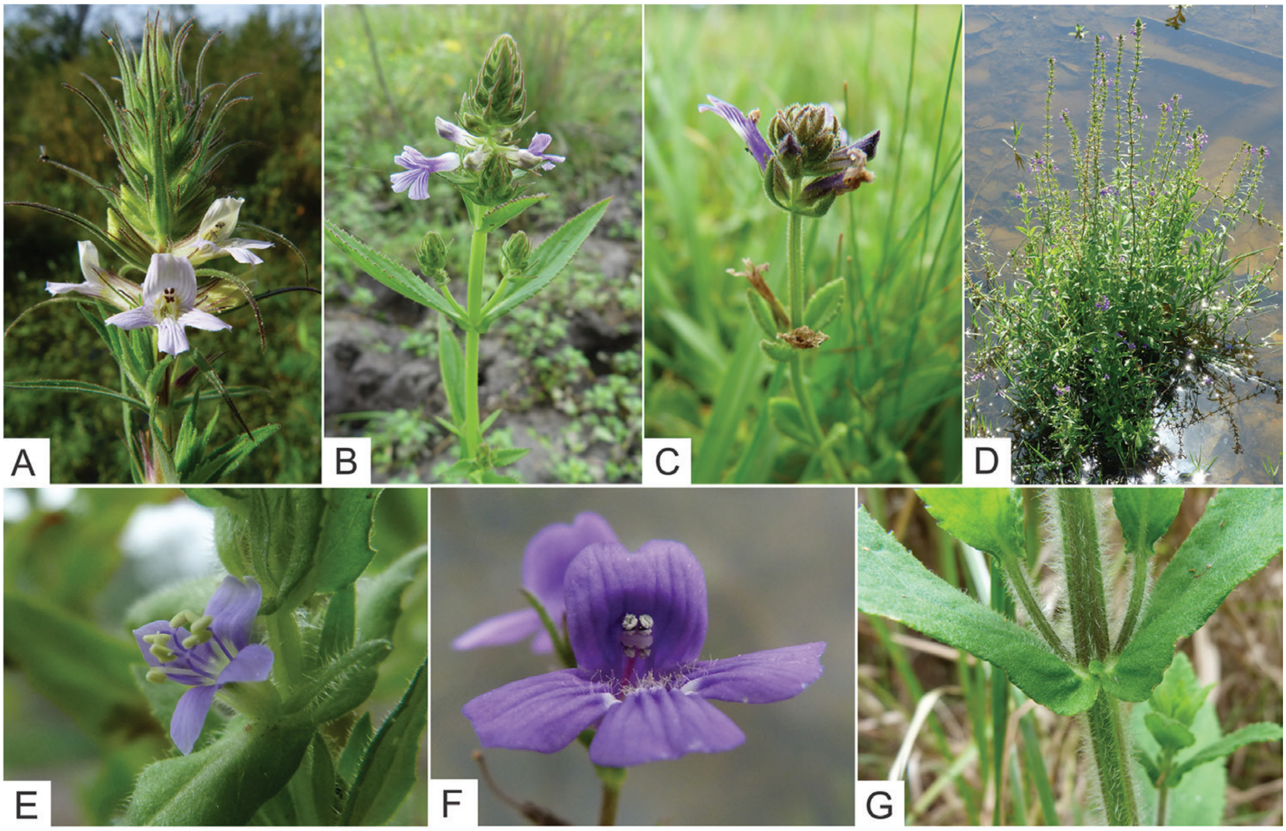


Figure 7. Representatives of *Stemodia sensu stricto* and some diagnostic features. A, Terminal spiciform inflorescence of *S. lanceolata*. B, Terminal and axillary spiciform inflorescences of *S. hyptoides*. C, Terminal inflorescence of *S. stricta*. D, Erect and palustrine habit of *S. lanceolata*. E, Sessile flower of *S. maritima*. F, Strongly zygomorphic flower of *S. palustris*. G, Sessile leaves with auriculate base of *S. hyptoides*. Photographs by A. V. Scatigna.

the evidence gathered yields strong support for the morphological delimitation of monophyletic genera and for the taxonomic changes proposed here.

TAXONOMIC OUTCOMES

A NEW GENUS SEGREGATED FROM *STEMODIA*

Umbraria Scatigna & V.C.Souza, **gen. nov.** Type: *U. microphylla* (J.A.Schmidt) Scatigna (≡ *Stemodia microphylla* J.A.Schmidt).

Umbaria is characterized by the following exclusive set of traits: crawling habit; leaves opposite, clearly petiolate; flowers axillary, long-pedicellate, resupinate, two-bracteolate; calyx with subequal sepals; and androecium composed of four fertile stamens and anthers free with two thecae separated by a two-brachiate connective.

Herbs terrestrial to rupicolous, perennial, indument on vegetative parts composed of short, capitate trichomes, interspersed with non-glandular trichomes, sometimes glandular-punctate. Stems crawling to ascending, terete to sub-quadrangular, branched. Leaves opposite clearly petiolate. Flowers axillary, resupinate by torsion of pedicel, single, 2-bracteolate, long-pedicellate, pentamerous, bisexual; sepals subequal; corolla bilabiate, lobes whitish, lilac, purple or blue, tube greenish, yellow or white, upper lip three-lobed, lower lip two-lobed. Stamens four, didynamous, included; anthers with two thecae, separated by a two-brachiate connective, glabrous. Ovary superior, syncarpous, bicarpelar, bilocular, ovoid, glabrous; placentation axillary, ovules numerous; style terminal, solitary, apex obdeltoid, curved, glabrous. Capsule ovoid, apex acute, rounded or mucronate, seeds wrinkled to ribbed, dark to light brownish.

The name *Umbraria* alludes to *umbra,* Latin word for shadow, in a reference to the shady habitat of both species recognized in this new genus.

Umbraria consists of two species, one of which occurs in the campos rupestres from eastern Minas Gerais, especially in the Espinhaço Range and Serra do Ibitipoca, between 700 and 1500 m a.s.l., whereas the other occurs in the Atlantic Forest from Rio de

Janeiro to Bahia, around 400 m a.s.l. Both species grow on edges of relatively humid and shaded fractures of quartzite or granite outcrops. *Umbraria* is similar to *Lapaea* in the crawling habit, petiolate leaves and long-pedicellate flowers, but differs by the presence of bracteoles (vs. absence), by the flowers resupinate (vs. non-resupinate) and by the leaves opposite (vs. usually three-whorled).

Umbraria microphylla (J.A.Schmidt) Scatigna, **comb. nov.** ≡ *Stemodia microphylla* J.A.Schmidt in Mart., Fl. bras. 8(1): 298. 1862. Type: Brazil, in rupestribus Serra da Lapa [Serra do Cipó], jusdem prov. [Minas Gerais], *Riedel 1109* [lectotype, designated by Turner & Cowan, 1993b: K barcode K000528930 (digital image!); isolectotypes: G barcode G00343872 (digital image!), GH barcode 00091758 (digital image!), LE barcodes LE 00004906 (digital image!) and LE 00004907 (left specimen; digital image!)] (Fig. 1D).

The correct collector number according to the labels from specimens housed at LE is *1109* and not *1107* as cited by Turner & Cowan (1993b) and Souza & Giulietti (2009).

Umbraria veronicoides (J.A.Schmidt) Scatigna, **comb. nov.** ≡ *Stemodia veronicoides* J.A.Schmidt in Mart., Fl. bras. 8(1): 298. 1862. Type: Brazil, in humidis saxosis umbrosis prope Ouro Preto [Minas Gerais] et prope Aldea de Almada in distr. dos Ilheos [Ilhéus], prov. Bahiensis [Bahia], *Riedel s.n.* (syntypes possibly in HBG and/or LE) (Fig. 1E).

NEW COMBINATION IN *DARCYA*

Darcya vandellioides (Benth.) Scatigna, **comb. nov.** ≡ *Conobea vandellioides* Benth. *in* DC., Prodr., 10: 391. 1846. ≡ *Lindernia vandellioides* (Benth.) Pennell ex G.M.Barroso, Rodriguesia, 27: 44. 1952. ≡ *Stemodia vandellioides* (Benth.) V.C.Souza in Wanderley *et al.* (ed.) Flora Fanerogâmica do Estado de São Paulo vol. 3: 316. 2003. Type: Brazil, Minas Gerais, Perna de Páo, *Gardner 5057* [lectotype, first-step designated by Souza & Giulietti (2009), second-step here designated: K barcode K000529083!; isolectotypes: BM barcode BM000953382!, K barcode K000529081!]. [Figs 1F, 4A (lower)].

In the protologue, Bentham (1846) cited two gatherings: one is *Langsdorff s.n*, from Rio de Janeiro, and the other is *Gardner 5057*, from Minas Gerais. Souza & Giulietti (2009) designated a specimen of *Gardner 5057* house at K as lectotype. However, there are two duplicates from this gathering at K and we believe that the lectotypification should be narrowed according to Art. 9.17. of the International Code of Nomenclature (Turland *et al.*, 2018). Hence, we selected the specimen K barcode K000529083 as second-step lectotype because it is well preserved and bears the stamp from the Herbarium Benthamianum.

NEW MAMES AND COMBINATIONS IN *BACOPA*

Bacopa and *Conobea* were published in the same work, at the same time (Aublet, 1775), having no priority of one over the other. Including *Conobea* in the synonymy of *Bacopa* requires substantially fewer new names and/or new combinations than the other way around. Furthermore, the morphological delimitation of *Bacopa* has been historically more stable than that of *Conobea s.l*. Hence, we expand here *Bacopa* to include *Conobea*.

Bacopa aubletiana Scatigna, **nom. nov**. ≡ *Conobea aquatica* Aubl., Hist. pl. Guiane 2: 639. 1775. Type: French Guyana, *Aublet s.n.* [lectotype, designated by Souza & Giulietti (2009): BM barcode BM000953384!; isolectotype: LINN-HS No. 1082.1 (digital image!)].

Because the combination *Bacopa aquatica* is already occupied (i.e. *Bacopa aquatica* Aubl.), we propose a new name honouring Jean Baptiste Christophe Fusée Aublet (1720–1778), who described both *Bacopa* and *Conobea.*

Bacopa scoparioides (Cham. & Schltdl.) Scatigna, **comb. nov.** ≡ *Sphaerotheca scoparioides* Cham. & Schltdl., Linnaea 2: 606. 1827. ≡ *Conobea scoparioides* (Cham. & Schltdl.) Benth. in DC., Prodr. 10: 391. 1846. Type: Brazil, Brasilia equinoctiali, *Sellow s.n.* [lectotype, first-step designated by Souza & Giulietti (2009), second-step here designated: K barcode K000529087!; isolectotypes: B (destroyed; photographs at F neg. n° 12424! and PH barcode 00027486!), HAL barcode HAL0098474 (digital image!), K barcode K000529085!] (Fig. 1F).

In the protologue, Chamisso & Schlechtendal (1827) cited a gathering by Sellow from Brazil without designating any herbarium. Later, Souza & Giulietti (2009) selected a specimen from this gathering housed at K as lectotype. However, there are two specimens of that gathering in K and following Art. 9.17 (Turland *et al.*, 2018), we designate the specimen K barcode K000529087 as second-step lectotype because it is more complete and better preserved.

Bacopa neuwiedii Scatigna, **nom. nov**. ≡ *Conobea punctata* Nees & Mart., Nov. act. nat. cur. 11: 43. 1822. Type: Brazil, *M.A.P. zu Wied-Neuwied s.n.* [isotype: BR barcode 0000006589325 (digital image!)].

Several authors that studied Scrophulariaceae *s.l.* from Brazil did not have success in finding the original material of *Conobea punctata* and, therefore, its identity has long been obscure (Bentham, 1846; Schmidt, 1862; Barroso, 1952; Souza & Giulietti, 2009). We found a specimen housed at BR (BR barcode 0000006589325), identified as *Conobea punctata*, that was collected by Wied-Neuwied and belonged to Martius' personal herbarium. The specimen is cited as an isotype by Moraes (2013) and has a morphology consistent with the original description made by Nees von Esenbeck

& Martius (1822). Its general morphology is also consistent with our concept of *Conobea s.s*. Hence, we are confident in transferring this species to *Bacopa*.

Because the combination *Bacopa punctata* is already occupied (i.e. *Bacopa punctata* Engl.), we propose a new name honouring the collector of the type, the German explorer and naturalist Maximilian Alexander Philipp zu Wied-Neuwied.

RE-ESTABLISHED NAMES

Chodaphyton ericifolium (K.Schum.) Minod (≡ *Stemodia ericifolia* K.Schum.).

Geochorda glechomoides (Spreng.) Kuntze [≡ *Herpestis glechomoides* Spreng. ≡ *Conobea glechomoides* (Spreng.) V.C.Souza] (Fig. 1I).

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DATA AVAILABILITY

Data underlying this article, i.e. nucleotide sequence alignments, tree files and the morphological data matrix, are available in FigShare at http://doi.org/10.6084/m9.figshare.17185397. DNA sequences are available in GenBank at https://www.ncbi.nlm.nih.gov/genbank/, and can be accessed with accession numbers provided in Supporting Information, Appendix S1.

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Sytsma KJ, **Michaels HJ**, **Kress WJ**, **Karol KG**, **Clark WD**, **Hedren M**, **Gaut BS**, **Jansen RK**, **Kim KJ**, **Wimpee CF**, **Smith JF**, **Furnier GR**, **Strauss SH**, **Xiang Q-Y**, **Plunkett GM**, **Soltis PS**, **Swensen SM**, **Williams SE**, **Gadek PA**, **Quinn CJ**, **Eguiarte LE**, **Golenberg E**, **Learn Jr GH**, **Graham SW**, **Barrett SCH**, **Dayanandan S**, **Albert VA. 1993.** Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden* **80:** 528–580.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. List of taxa and respective voucher data, GenBank accession numbers for sequenced markers and labels of taxa in the supplementary files.
Appendix S2. List of DNA regions, primers used in our study, respective sequences and references and specific amplification conditions.
Appendix S3. **Figs S1–S18.** Majority-rule consensus trees and strict consensus trees generated by Bayesian inference and maximum parsimony, respectively, based on each individual dataset.
Appendix S4. **Figs S19, S28.** Ancestral character state reconstructions by maximum likelihood onto the majority-rule consensus trees generated by Bayesian inference of the total combined dataset. Respective character and states are given in the box on the left. Circles represent probabilities.