



UNIVERSIDADE ESTADUAL DE CAMPINAS
INSTITUTO DE BIOLOGIA

ANDRÉ VITO SCATIGNA

SYSTEMATICS OF GRATIOLEAE (PLANTAGINACEAE): REDEFINING *STEMODIA* L.

SISTEMÁTICA DE GRATIOLEAE (PLANTAGINACEAE): REDEFININDO *STEMODIA* L.

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RESUMO

Nas últimas três décadas, as Angiospermas sofreram rearranjos drásticos em sua classificação em vários níveis hierárquicos devido à implementação de estudos filogenéticos baseados em dados moleculares na sistemática. Embora uma aparente estabilidade tenha sido alcançada nos níveis de Ordem e Família, as circunscrições genéricas ainda estão longe de serem completamente avaliadas a partir de uma perspectiva filogenética. Gratioleae é uma tribo predominantemente tropical das Plantaginaceae, apresentando alta diversidade morfológica e ecológica. *Stemodia* sensu lato é um dos gêneros mais diversificados e heterogêneos das Gratioleae (Plantaginaceae), sendo tradicionalmente definido pelo cálice com sépalas iguais a subiguais e quatro estames férteis com duas tecas, separadas por um conectivo 2-braquiado, em cada antera. O gênero apresenta distribuição pantropical e abrange ca. 60 espécies. Estudos filogenéticos recentes em Gratioleae indicam que *Stemodia*, como atualmente circunscrito, é um gênero polifilético. Neste estudo, investigamos a filogenia de Gratioleae, com ênfase em *Stemodia* s.l., baseada em sequências de três regiões do cpDNA (introns *rps16* e *trnL* e espaçador intergênico *trnL-trnF*) e um do nrDNA (espaçador ITS1); usamos tanto a parcimônia quanto a inferência bayesiana para reconstruir a filogenia. Testamos o monofiletismo do gênero e seu posicionamento na tribo e, com base nisso, propomos uma nova circunscrição e delimitação morfológica de um grupo monofilético denominado *Stemodia* sensu stricto. Como aqui proposto, *Stemodia* s.s. engloba 26 espécies e é caracterizado pelo hábito ereto, folhas claramente sésseis com base geralmente auriculada, flores bi-bracteoladas, não-ressupinadas e geralmente curto-pediceladas e concentradas no ápice dos ramos, além do cálice bem dividido em cinco sépalas iguais e do androceu composto por quatro estames férteis com duas tecas iguais e glabras, separadas pelo conectivo 2-braquiado. Além disso, propomos *Lapaea* como o novo gênero segregado de *Stemodia* s.s. para acomodar as espécies do chamado grupo *S. stellata*; descrevemos e ilustramos uma nova espécie notável de flor vermelha, pertencente a este grupo; testamos o monofiletismo do novo gênero descrito e avaliamos suas relações interespecíficas; e apresentamos uma sinopse taxonômica das espécies reconhecidas no novo gênero, fornecendo uma chave de identificação, fotos e notas sobre sua morfologia, distribuição geográfica, habitat, ecologia e estado de conservação.

ABSTRACT

In the past three decades, the Angiosperms have undergone drastic rearrangements in their classification at various hierarchic levels due to the implementation of phylogenetic studies based on molecular. Although an apparent stability has been achieved at Ordinal and Familial levels, generic circumscriptions are still far from being completely assessed from a phylogenetic perspective. Gratioleae is a mainly tropical tribe of Plantaginaceae presenting high morphological and ecological diversity. *Stemodia* sensu lato is one of the most diverse and heterogeneous genera in Gratioleae, being traditionally defined by the calyx with equal to subequal sepals and four fertile stamens with two thecae, separated by a 2-brachiate connective, in each anther. It has pantropical distribution and encompasses ca. 60 species. Recent phylogenetic studies in Gratioleae indicate that *Stemodia*, as traditionally circumscribed, is a polyphyletic genus. In this study, we investigate the phylogeny of Gratioleae with emphasis on *Stemodia* s.l. based on sequences of three regions from cpDNA (*rps16*, and *trnL* introns and the *trnL-trnF* intergenic spacer) and one from nrDNA (ITS1 spacer); we used both parsimony and Bayesian inference to reconstruct the phylogeny. We tested the monophyly of the genus and its placement within the tribe and, based on that, proposed a new circumscription and morphological delimitation of a monophyletic group here called *Stemodia* sensu stricto. As herein proposed, *Stemodia* s.s. encompasses 26 species and is characterized by the erect habit, clearly sessile leaves with usually clasping base, bi-bracteolate, non-ressupinate and usually short-pedicellate flowers that are often concentrated at the apex of flowering branches, in addition to the calyx well-divided into five equal sepals and the androecium composed of four fertile stamens with two equal and glabrous thecae, separated by a 2-brachiate connective, in each anther. Furthermore, we propose *Lapaea* as new genus segregated from *Stemodia* s.s. to accommodate species of the called *S. stellata* group; describe and illustrate a remarkable red-flowered new species, belonging to this group, based on morphological and micromorphological evidence; test the monophyly of the herein described new genus and assess its interspecific relationships; and present a synoptic revision of the species recognized in the new genus, providing an identification key, photos, and notes on their morphology, geographic distribution, habitat, ecology and conservation status.

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INTRODUÇÃO GERAL

A SISTEMÁTICA FILOGENÉTICA E A CLASSIFICAÇÃO

A tarefa dos biólogos sistematas de classificar organismos em níveis supraespecíficos, embora não siga uma cartilha de diretrizes clara, busca encontrar alguns princípios básicos como: 1) monofiletismo de grupos; 2) estabilidade nomenclatural, ou seja, a manutenção de grupos tradicionalmente reconhecidos; 3) minimização de redundância, por exemplo grupos monoetípicos desnecessários; 4) prioridade para grupos com maior suporte para o monofiletismo; e 5) facilidade no reconhecimento dos grupos (Backlund and Bremer 1998; Stevens 2001 onwards).

Nas últimas três décadas, as bases da classificação dos organismos sofreram mudanças profundas devido ao uso de filogenias moleculares. As Angiospermas, especialmente, vêm sofrendo grandes rearranjos, em diversos níveis hierárquicos, com base em hipóteses filogenéticas geradas a partir de dados de sequências de DNA (Chase et al. 1993; APG 1998; APG II 2003; Frodin 2004; Humphreys and Linder 2009; APG III 2009; APG IV 2016). Uma aparente estabilidade foi alcançada nos níveis superiores (ordem e família), principalmente nos últimos dez anos (Cronquist 1965; Stevens 2002; APG III 2009; APG IV 2016). No nível hierárquico genérico, entretanto, essa almejada estabilidade é ainda um desafio e as classificações são por vezes bastante controversas, especialmente para grupos tradicionalmente reconhecidos e de importância cultural, econômica e até política, mas que são comprovadamente não monofiléticos em suas circunscrições mais abrangentes, como é o caso de *Acacia* Mill. (Fabaceae), *Aster* L. (Asteraceae), *Senecio* L. (Asteraceae), *Psychotria* L. (Rubiaceae) e *Salvia* L. (Lamiaceae), entre tantos outros (Drew et al. 2017).

A DESINTEGRAÇÃO DE SCROPHULARIACEAE

Um dos casos mais icônicos de rearranjos a nível de família em Angiospermas talvez tenha sido a desintegração de Scrophulariaceae sensu lato. Como tradicionalmente delimitada, a família chegou a ser uma das maiores dentro de Lamiales, sendo basicamente reconhecida pela falta de caracteres diagnósticos, os quais estavam presentes em famílias relacionadas como Gesneriaceae, Bignoniaceae e Lamiaceae, entre outras (Bentham 1846; Bentham and Hooker 1876; Wettstein 1891; Olmstead and Reeves 1995; Olmstead et al. 2001; Tank et al. 2006). Essa grande e heterogênea família incluía desde ervas aquáticas, *Hydrotriche* Zucc. e *Limnophila* R.Br., a subarbustos hemiparasitas,

como *Striga* Lour., *Pedicularis* L. e *Orthocarpus* Nutt. Outros grupos de plantas bastante especializadas como Lentibulariaceae (plantas carnívoras aquáticas), Plantaginaceae sensu stricto (com flores altamente adaptadas à anemofilia) e Orobanchaceae (holoparasitas) foram consideradas derivadas das Scrophulariaceae (Cronquist 1981) ou mesmo como integrantes desta família (Hallier 1903).

A falta de coesão morfológica e ecológica, de sinapomorfias ou então de características diagnósticas das Scrophulariaceae em sua circunscrição tradicional levantou a suspeita de se tratar de um grupo não monofilético (Olmstead and Reeves 1995). De fato, o parafiletismo dessa família foi investigado e corroborado por diversos estudos filogenéticos com base em dados de sequências de DNA, e vários de seus membros foram transferidos para ao menos outras dez famílias (Olmstead and Reeves 1995; Olmstead et al. 2001; Oxelman et al. 2005; Rahmanzadeh et al. 2005).

PLANTAGINACEAE, GRATIOLEAE

Plantaginaceae é uma das 25 famílias atualmente reconhecidas na ordem Lamiales (Albach et al. 2005; Refulio-Rodriguez and Olmstead 2014) cuja circunscrição foi drasticamente aumentada após a inclusão de diversos gêneros anteriormente posicionados em Scrophulariaceae s.l., como a maior parte da tribo Gratiroleae sensu Wettstein (1891), além de Callitrichaceae e gêneros previamente incluídos em Globulariaceae, Hippuridaceae e Pedaliaceae (Albach et al. 2005; Mabberley 2008).

Gratiroleae é uma das tribos de Plantaginaceae com distribuição predominantemente tropical, compreendendo entre 16 e 40 gêneros, dependendo das diferentes propostas de classificação para tribo e gênero (Wettstein 1891; Fischer 2004; Albach et al. 2005), e mais de 300 espécies (Estes and Small 2008). A tribo exhibe amplo espectro de morfologia e estratégias ecológicas, incluindo ervas anuais especializadas a ambientes aquáticos, como *Bacopa* Aubl., *Gratiola* L., *Hydrotriche* e *Limnophila*, subarbustos perenes rupícolas, como *Achetaria* Cham. & Schltldl., *Scoparia* L. e *Stemodia* L. em parte, e até mesmo plantas carnívoras como *Philcoxia* P.Taylor & V.C.Souza (Taylor et al. 2000; Albach et al. 2005; Souza and Giuletta 2009; Pereira et al. 2012). Estudos sistemáticos contemporâneos dentro da tribo são bastante escassos e com amostragem limitada, especialmente com relação a táxons do Velho Mundo, o que talvez, junto com a variação citada, justifique sua circunscrição controversa (Albach et al. 2005). Contudo, os poucos estudos em filogenia molecular indicaram o não monofiletismo de *Bacopa*, *Gratiola* e

Stemodia, três dos principais gêneros da tribo que ocorrem nos neotrópicos (Fritsch et al. 2007; Estes and Small 2008; Scatigna et al. 2018a).

STEMODIA: UM GÊNERO POLIFILÉTICO

Stemodia sensu lato é um dos mais diversos gêneros de Gratioleae, com ca. 60 espécies e distribuição pantropical, ocorrendo em áreas abertas e com solo úmido, especialmente no Novo Mundo e Austrália (Turner and Cowan 1993a; Fischer 2004). Apesar de exibir grande variação nos caracteres vegetativos, a presença de quatro estames férteis com duas tecas separadas pelo conectivo 2-braquiado em cada antera e o cálice com sépalas iguais a subiguais entre si têm sido historicamente usados para definir o gênero (Bentham 1846; Minod 1918; D'Arcy 1979; Turner and Cowan 1993a; Souza and Giulietti 2009). O grupo foi tradicionalmente reconhecido como membro de Gratioleae (Bentham 1846; Bentham and Hooker 1876; Wettstein 1891), posicionamento este corroborado por estudos filogenéticos com base em sequências de DNA plastidial e nuclear (Fritsch et al. 2007; Estes and Small 2008; Schäferhoff et al. 2010).

No entanto, a delimitação de *Stemodia* foi e ainda é controversa. Linnaeus (1759) descreveu o gênero baseado exclusivamente em *Stemodia maritima* L., sendo caracterizado por anteras com duas tecas separadas entre si por um filete bifido. Cerca de um século mais tarde, o gênero chegou a contar com aproximadamente 60 espécies, até que Bentham (1846) transferiu 13 delas para *Limnophila*, um gênero predominantemente do Velho Mundo, e outras 11 para outros seis gêneros da tribo que também apresentam anteras com tecas separadas. Mais tarde, Minod (1918), seguindo a proposta de Bentham (1846), transferiu as seguintes espécies para gêneros monotípicos: *Stemodiacra maritima* (L.) P.Browne (= *Stemodia maritima* L.), *Lendneria humilis* (Sol.) Minod (= *S. verticillata* [Mill.] Hassl.), *Verena hassleriana* (Chodat) Minod (= *S. hassleriana* Chodat), *Valeria trifoliata* (Link) Minod (= *S. trifoliata* [Link] Reichb.) e *Chodaphyton ericifolium* (Kuntze) Minod (= *S. ericifolia* K.Schum.); esta classificação não foi seguida por autores subsequentes, que adotaram um conceito mais abrangente do gênero (D'Arcy 1979; Turner and Cowan 1993a, 1993b; Fischer 2004; Souza and Giulietti 2009; Sosa and Dematteis 2013; Scatigna and Souza 2018; BFG 2018). Alguns outros gêneros como *Darcya* B.L.Turner & C.Cowan, *Anamaria* V.C.Souza e *Lindernia* All. incluem espécies previamente reconhecidas em *Stemodia* de acordo com diferentes autores (respectivamente Turner and Cowan 1993c; Souza 2001; Fischer et al. 2013). Por outro lado, Barker (1990) incluiu as quatro espécies de *Morgania* R.Br., todas da Austrália, na

circunscrição de *Stemodia*, alegando falta de diferenças significativas entre os gêneros. Turner and Cowan (1993a) especularam que *Stemodia* s.l. representava um grupo monofilético, ainda que bastante heterogêneo, enquanto Souza and Giulietti (2009) reconheceram as espécies brasileiras de *Stemodia* em ao menos dois grupos morfológicamente bem definidos e por eles denominados “grupos naturais”. O primeiro grupo seria caracterizado pelo hábito herbáceo e ereto, folhas claramente sésseis e usualmente amplexicaules e flores sésseis ou subsésseis, bracteoladas e concentradas em inflorescências espiciformes; este grupo é composto por *Stemodia durantifolia* (L.) Sw., *S. hyptoides* Cham. & Schltl., *S. maritima* (espécie tipo do gênero), *S. palustris* A.St.Hil., *S. perfoliata* Scatigna & V.C.Souza e *S. stricta* Cham. & Schltl (Scatigna et al. 2018c). O segundo grupo seria caracterizado pelo hábito subarbuscivo e procumbente, folhas claramente pecioladas, e flores longamente pediceladas, ebracteoladas e inseridas nas axilas das folhas, não formando inflorescências espiciformes; este grupo, aqui chamado grupo *Stemodia stellata*, é composto por *S. cipoensis* Scatigna, *S. harleyi* B.L.Turner, *S. lobata* J.A.Schmidt e *S. stellata* B.L.Turner (Scatigna et al. 2017), além de uma espécie nova com flores vermelhas que está sendo descrita no primeiro capítulo desta tese (Scatigna et al. in prep. [Capítulo 1]).

Estudos filogenéticos moleculares em Gratioleae apontam para o polifiletismo de *Stemodia* em sua circunscrição tradicional. Fritsch et al. (2007) usaram dados de sequências de DNA plastidial e nuclear e incluíram cinco espécies de *Stemodia* em suas análises, recuperando-as em duas linhagens distintas, sendo a primeira constituída por *Stemodia verticillata* e *S. suffruticosa* Kunth, formando um grado relacionado com *Leucospora multifida* (Michx.) Nutt. e membros de *Scoparia*, e a segunda contendo *S. durantifolia*, *S. glabra* Oerst. e *S. schottii* Holz. em uma topologia não resolvida, mas relacionadas com *Achetaria* e *Otacanthus* Lindl. (= *Achetaria*). Em seguida, Estes and Small (2008), usando dados de sequências de DNA apenas plastidial, também incluíram cinco espécies de *Stemodia*, mas com *S. maritima* no lugar de *S. durantifolia*; estes autores encontraram as mesmas relações que Fritsch et al. (2007). Mais recentemente, Scatigna et al. (2018a), com dados de sequências de DNA plastidial e nuclear, amostraram cinco espécies de *Stemodia* e obtiveram resultados apenas em parte semelhantes aos anteriores; *S. verticillata* formou um grado com *S. foliosa* Benth. relacionado com *Scoparia* spp., e *Stemodia maritima* formou um grado com *Stemodia microphylla* J.A.Schmidt relacionado com *Achetaria* spp., enquanto *Stemodia stellata* foi recuperada como grupo irmão de *Philcoxia* e relacionada com *Tetraulacium veroniciforme* Turcz. em um clado

distinto e não relacionado com os dois previamente recuperados (Figura 1). Um estudo filogenético da tribo Gratioleae, com ênfase em *Stemodia*, é apresentado no segundo capítulo desta tese (Scatigna et al. in prep. [Capítulo 2]).

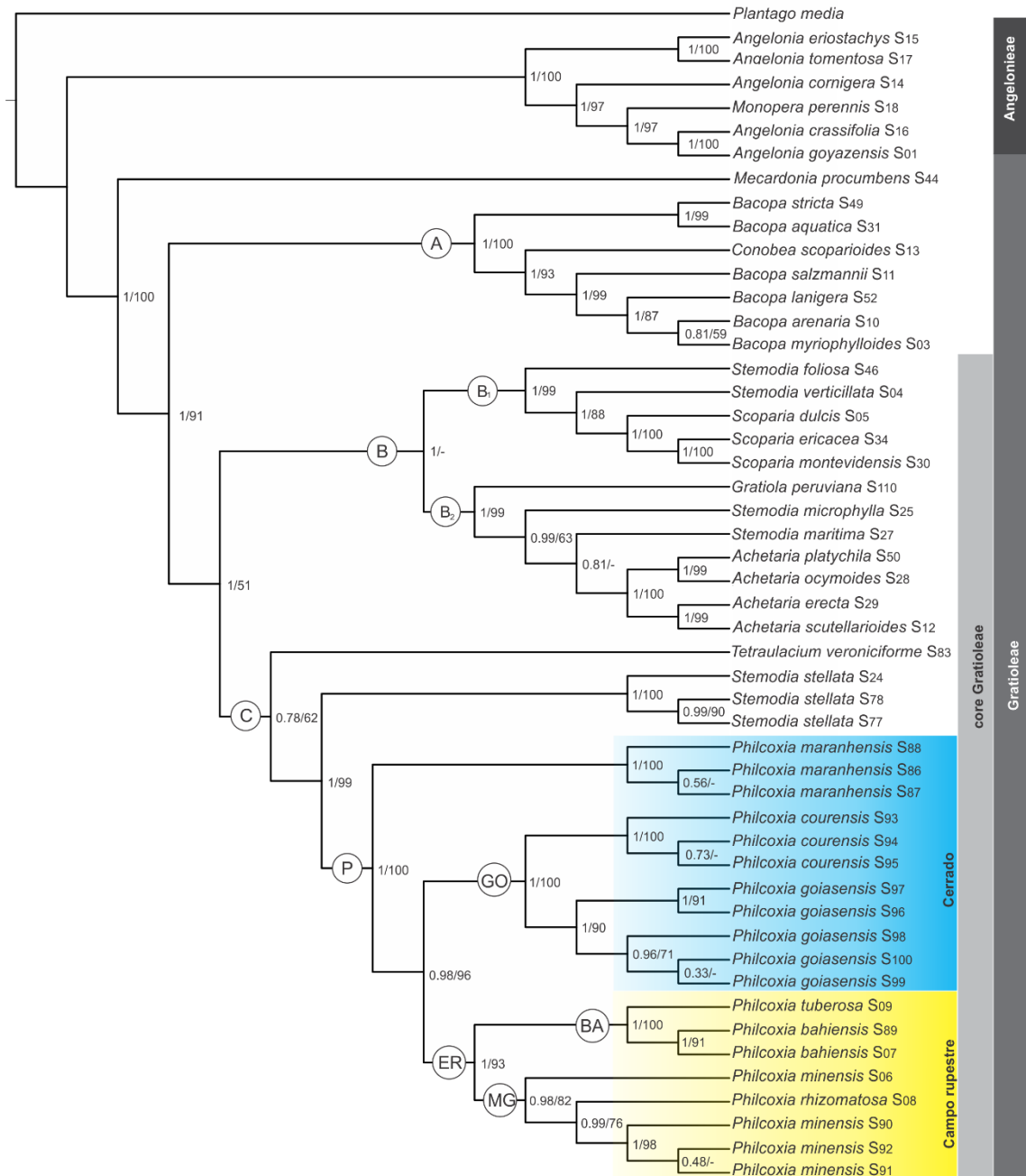


Figura 1. Filogenia de Gratioleae adaptada de Scatigna et al. 2018a.

MOTIVAÇÃO E OBJETIVOS

Embora os únicos três estudos filogenéticos focados em membros de Gratioleae tenham trazido novas evidências sobre o polifiletismo de *Stemodia* e o parafiletismo de gêneros relacionados, suas amostragens somadas representam menos de 20% da diversidade de *Stemodia* e menos ainda em relação à tribo como um todo (Fritsch et al. 2007; Estes and Small 2008; Scatigna et al. 2018a). Até agora, nenhuma classificação genérica em Gratioleae seguiu evidências filogenéticas, seja com dados morfológicos ou moleculares (sequências de DNA), com exceção de um único trabalho que resultou na inclusão de *Amphianthus* Torr., um gênero monotípico, em *Gratiola* (Estes and Small 2008). Por outro lado, a maioria dos trabalhos taxonômicos que tratam de membros de Gratioleae consistem em floras ou revisões genéricas com limitação geográfica (Minod 1918; Pennell 1920, 1923, 1943; D'Arcy 1979; Turner and Cowan 1993a, 1993b; Souza and Giulietti 2009; Sosa et al. 2018), enquanto apenas poucos estudos focaram em gêneros por inteiro (Pennell 1935; Philcox 1970; Ronse 2001). Tendo em vista a distribuição pantropical de alguns gêneros da tribo, é provável que a falta de trabalhos de síntese tenha contribuído para a manutenção de grupos possivelmente relacionados filogeneticamente em gêneros distintos, como talvez seja o caso de *Stemodia*, *Morgania* R.Br. e *Limnophila* (Philcox 1970), ou então *Conobea* Aubl. (em parte), *Leucospora* Nutt. e *Schistophragma* Benth. (Thieret 1967; Turner and Cowan 1993a). Uma classificação mais estável, baseada em estudos filogenéticos, poderia facilitar a escolha de grupos bem delimitados como alvo de estudos de revisão taxonômica, com chaves de identificações e descrições para todos seus representantes reunidas em uma mesma obra. Além disso, estudos filogenéticos robustos contribuem para um entendimento mais preciso acerca das características morfológicas relacionadas à evolução das linhagens.

Tudo isso motivou a execução deste projeto, que teve como objetivo geral investigar a filogenia de Gratioleae, com base em uma amostragem bastante mais abrangente, como um subsídio para novas propostas de classificação genérica dentro da tribo, com ênfase em *Stemodia* s.l. Em cada capítulo desta tese, buscamos cumprir os seguintes objetivos específicos:

Capítulo 1 – 1) propor um novo gênero segregado de *Stemodia* s.s. para acomodar as espécies do chamado grupo *Stemodia stellata*; 2) testar o monofiletismo deste novo gênero e investigar suas relações interespecíficas; e 3) apresentar uma sinopse taxonômica das espécies reconhecidas no novo gênero, fornecendo uma chave de

identificação, fotos e notas sobre sua morfologia, distribuição geográfica, habitat, ecologia e status de conservação.

Capítulo 2 – 1) redefinir a circunscrição de *Stemodia* s.s., apresentando uma nova delimitação morfológica do grupo; e 2) testar o posicionamento filogenético das demais espécies de *Stemodia* s.l. e discutir as implicações taxonômicas para sua acomodação em outros gêneros.

Capítulo 3 – Apresentar as novidades taxonômicas em *Stemodia* s.l. como contribuição para o conhecimento do gênero no âmbito do projeto Flora do Brasil Online 2020.

DIVULGAÇÃO DE RESULTADOS

O manuscrito apresentado no Capítulo 1 desta tese será submetido ao periódico *Systematics and Biodiversity*; o Capítulo 2 será submetido preferencialmente aos periódicos *Molecular Phylogenetics and Evolution* ou *Botanical Journal of the Linnean Society*; os três artigos apresentados no Capítulo 3 foram publicados nas revistas *Systematic Botany*, *Brittonia* e *Phytotaxa*, respectivamente, e seus resultados foram incluídos na monografia para o gênero *Stemodia* no site da Flora do Brasil 2020.

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CAPÍTULO 1¹

***Lapaea* (Plantaginaceae, Gratioleae), a new genus endemic to the Espinhaço Range, Brazil, with a remarkable new species**

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Abstract: The Espinhaço Range is a vast mountain chain in eastern Brazil that shelters the core area of the *campo rupestre*, a very peculiar open vegetation mosaic associated with rocky formations known for its high biodiversity and endemism rates. *Stemodia* sensu lato is one of the most diverse genera of the Gratioleae (Plantaginaceae). Recent phylogenetic studies corroborated the polyphyly of *Stemodia* in its traditional circumscription. Here we propose *Lapaea* as new genus segregated from *Stemodia* s.s. to accommodate the species of the *S. stellata* group; describe and illustrate a remarkable red-flowered new species, belonging to this group, based on morphological and micromorphological evidence; investigate the monophyly of the herein described new genus and its interspecific relationships; and present a synopsis of the species recognized in the new genus, providing an identification key, photos, and notes on their morphology, geographic distribution, habitat, ecology and conservation status. *Lapaea* is a monophyletic genus with five species, each one restricted to a specific endemism center in the Espinhaço Range; its interspecific relationships are consistent with the geographic distribution.

Key words: *Campo rupestre*, Gratiolaceae, Phylogenetics, Polyphyly, *Stemodia*

Introduction

The Espinhaço Range (ER) is a vast mountain chain of ca. 1200 km north-south range in eastern Brazil, in the states of Bahia and Minas Gerais (Silveira et al. 2016). It is bordered by three Brazilian domains, the *Caatinga* to the North, the *Cerrado* to the West and the Atlantic to the East and South (Bitencourt and Rapini 2013). The ER shelters the core area of the *campo rupestre*, an azonal vegetation mosaic characterized by a grass-shrubby physiognomy associated with rocky formations of quartzite, sandstone and

¹ Este capítulo não corresponde à versão submetida para publicação em periódico e, portanto, não passou por editoração nem revisão por pares. Os novos nomes e novas combinações não têm validade nomenclatura ou taxonômica.

ironstone, usually interspersed with white sandy patches, and that occurs in elevations higher than 900 m above sea level (a.s.l.) (Alves and Kolbek 2010; Alves et al. 2014; Silveira et al. 2016). The *campo rupestre*, with an estimated area of 66,500 km², represents less than 0.8% of the Brazilian territory (Fernandes et al. 2014); yet, it harbors over 15% of the species of the Angiosperms native in Brazil (Flora do Brasil 2020 in construction; BFG 2018). Furthermore, 40% of the more than 5,000 species native to the *campo rupestre* are composed of endemics (Flora do Brasil 2020 in construction). The Espinhaço Range have two major centers of endemism, one in the Chapada Diamantina (CD), in Bahia, and other in the Espinhaço Range in Minas Gerais (ERMG), which in turn are subdivided in several centers of endemism (Echternacht et al. 2011; Bitencourt and Rapini 2013; Ribeiro et al. 2014). The high levels of diversity and endemism in the *campo rupestre* are likely related to the heterogeneity of soil and habitat, the isolation at several scales and the influence of the three adjacent domains, in addition to the wide altitudinal and latitudinal range within a tropical area (Conceição and Pirani 2005; Bitencourt and Rapini 2013; Silveira et al. 2016).

Stemodia sensu lato is one of the largest and genera of the tribe Gratioleae of the Plantaginaceae, comprising ca. 60 species and distributed in the tropics, especially in the New World and Australia. It is morphologically heterogeneous, being generally characterized by the calyx with equal to subequal sepals, by strongly zygomorphic, usually blue to lilac corolla, and by the possession of four fertile stamens with two thecae in each anther separated by a 2-brachiate connective, often described as stalked/stipitate thecae (Minod 1918; D'Arcy 1979; Turner and Cowan 1993a; Souza and Giulietti 2009). Phylogenetic studies based on plastidial and nuclear DNA sequence data suggest that *Stemodia*, in its current circumscription, is polyphyletic (Fritsch et al. 2007; Estes and Small 2008; Scatigna et al. 2018a). In the most recent phylogenetic study in Gratioleae (Scatigna et al. 2018a), the five sampled species of *Stemodia* were retrieved in three different clades; *Stemodia foliosa* formed a grade with *S. verticillata* related to *Scoparia* spp. (clade B1 in Scatigna et al. 2018a), *S. microphylla* formed a grade with *S. maritima*, related to *Achetaria* spp. (clade B2 in Scatigna et al. 2018a), and *Stemodia stellata* was recovered as sister group of *Philcoxia* and related to *Tetraulacium* (clade C in Scatigna et al. 2018a).

Stemodia maritima, the type species of the genus, was assigned by Souza and Giulietti (2009) to a morphological assemblage of species characterized by the erect habit, by the sessile (frequently clasping) leaves and by the sessile to sub-sessile, bracteolate

flowers frequently concentrated at the apex of stems; this group of species, which includes *S. durantifolia* (L.) Sw., *S. hyptoides* Cham. & Schltdl., *S. palustris* A.St.Hil., *S. perfoliata* Scatigna & V.C.Souza and *S. stricta* Cham. & Schltdl. (Scatigna et al. 2018b), should be referred to as *Stemodia* sensu stricto. Meanwhile, *Stemodia stellata* was assigned by Scatigna et al. (2017) into a second cohesive group of species of *Stemodia*, along with *S. cipoensis*, *S. harleyi* and *S. lobata*, being characterized by a procumbent, suffruticose and generally rupicolous habit, with usually 3-whorled and clearly petiolate leaves, and axillary, long-pedicelate and ebracteolate flowers; this assemblage of species, herein called *Stemodia stellata* group, is restricted to the quartzite outcrops of the Espinhaço Range in Minas Gerais and Bahia (Scatigna et al. 2017). During systematic studies for a more comprehensive study of *Stemodia* s.l. (Scatigna et al. in prep. [Cap. 2]), we came across some specimens from the Chapada Diamantina which are similar to the *Stemodia stellata* group in several aspects, but with a remarkable trait, that is, flowers with red-colored corolla; these specimens clearly represent a new species.

Given the phylogenetic placement of *Stemodia stellata* (Scatigna et al. 2018a) and its morphological affinities (Souza and Giulietti 2009; Scatigna et al. 2017), in this study we aim to: 1) propose a new genus segregated from *Stemodia* s.s. to accommodate the species of the *S. stellata* group; 2) test the monophyly of the herein described new genus and assess its interspecific relationships; and 3) present a synopsis of the species recognized in the new genus, including a herein described new species, providing an identification key, photos, and notes on their morphology, geographic distribution, habitat, ecology and conservation status.

Material and methods

Taxonomic study:—The descriptions and morphological comparisons were based primarily on field observations and examination of herbarium specimens and complemented with data from literature (Schmidt 1862; Turner and Cowan 1993b; Souza and Giulietti 2009; Scatigna et al. 2017); Morphological terminology follows Harris and Harris (2001). We studied all Gratioleae collections housed at the following herbaria: ALCB, BHCN, BHZB, CEN, CEPEC, CVRD, DIAM, EAC, ESA, HCJS, HRB, HRCB, HUEFS, IAC, IAN, ICN, MBM, MBML, MG, OUPR, R, RB, SP, SPF, UB, UEC, UFG and UPCB, in addition to the digital images from G, K, MO, NY, and P databases; all abbreviations follow Thiers (2018). Conservation status assessments were based on the IUCN Red List categories and criteria (IUCN 2012) and subsequent guidelines (IUCN

Standards and Petitions Subcommittee 2017). We estimated the extent of occurrence (EOO) and area of occupancy (AOO) with the Geospatial Conservation Assessment Tool (GeoCAT; Bachman et al. 2011) using a cell width of 2 km.

Micromorphological study:—Leaf and stem samples of the new species were fixed in formol-acetic acid alcohol (FAA) 50% and stored in 70% ethanol. The material was critical point dried, mounted on aluminum stubs with double-sided adhesive tape and covered with 50 nm of gold in a Bal-Tec SCD 050 sputter coater. Images were observed in a Jeol JSM 5800LV Scanning Electron Microscope, at 10kV, and captured with SemAfore 5.21 software. Microorophological terminology follows Harris and Harris (2001).

Phylogenetic study:—For the phylogenetic analyses, we extracted the datasets of the cpDNA *rps16*, and *trnL* introns and *trnL-trnF* intergenic spacer, and the nrDNA ITS1 intergenic spacer, with 50 samples representing by 28 species and eight genera of Gratioleae, six species and two genera of Angelonieae (sister group of Gratioleae; Schäferhoff et al. 2010) and one species of *Plantago* L. of the tribe Plantagineae, from Scatigna et al. (2018a). We reduced the sampling of *Philcoxia* to one accession for each species and included newly generated sequences of two or more accessions of each of the five species recognized in the new genus. All new sequences were generated as described by Scatigna et al. (2018a). We performed Parsimony (MP) and Bayesian inference (BI) analysis for each data matrix separately and then for a combined dataset with the three plastid regions (Partial Combined; PC). Parameters for phylogenetic analysis followed Scatigna et al. (2018a). We considered as strong support the values of Bootstrap (BS) \geq 75% in the MP analysis, and of Posterior Probability (PP) \geq 0.95 in the Bayesian analysis. *Plantago media* L. was used as rooting taxon.

Results and discussion

Taxonomic treatment

Lapaea Scatigna & V.C.Souza, **gen. nov.**—TYPE: *Lapaea stellata* (B.L.Turner) Scatigna

Lapaea differs from *Stemodia* sensu stricto by being crawling to procumbent subshrubs (vs. erect herbs); by having clearly petiolate leaves (vs. sessile); by the long-pedicellate,

axillary flowers (vs. sessile to sub-sessile, frequently in spiciform inflorescences); by lacking bracteoles (vs. present); and in the sepals being connate at the basal third (vs. free). It also differs from its sister genus, *Philcoxia*, in the absence of peltate leaves (vs. presence); in the aerial stems and petioles (vs. underground); and in the non-resupinate flowers (vs. resupinate). *Lapaea* is the only genus of Gratiioleae that includes a red-flowered species.

Description:—Suffruticose, terrestrial to rupicolous perennials, up to ca. 1 m high, with variable indument on vegetative parts, usually aromatic. Stems crawling to ascending, terete to sub-quadrangular, branched. Leaves, 3-whorled, sometimes opposite in younger plants, clearly petiolate, broadly-ovate to elliptical, margin crenate to serrate. Flowers axillary, non-resupinate, single or geminate, ebracteolate, pentamerous, bisexual; pedicels 0.5 – 3.0 cm long, clearly ascending after maturation of fruits; sepals equal to sub-equal, clearly connate at base; corolla slightly to strongly bilabiate, dark blue, purple, lilac or red, upper lip 2-lobed, lower lip 3-lobed. Stamens 4, didynamous, included; anthers with 2 thecae, separated by a 2-brachiate connective, glabrous; staminode 1, much reduced. Ovary superior, syncarpous, 2-carpelar, 2-locular, ovoid, glabrous; placentation axillary, ovules numerous; style terminal, solitary; stigma obdeltoid, curved, glabrous. Capsule ovoid, dehiscence both loculicidal and septicial on apical portion, functionally poricidal, apex zygomorphic, glabrous; seeds cylindrical to ovoid, stipitate, reticulate, longitudinally ribbed.

Distribution:—*Lapaea* is restricted to the Espinhaço Range in the states of Minas Gerais and Bahia, Brazil. All known species of this genus occur in areas of *campo rupestre*, at elevations of ca. 900 – 2000 m a.s.l., growing on edges of relatively humid and shaded fractures of quartzitic outcrops that are regionally called "lapas".

Etymology:—The name *Lapaea* alludes to “lapa”, a Brazilian word for natural shelters provided by fractures on rocks and boulders (Figs. 2C, 3F) and that are common in the *campo rupestre* of the ER. Rock paintings made by ancestral peoples are often found in these “lapas”, which highlights the significant importance for both biodiversity and human history and culture of these rocky formations.

Notes:—Souza and Giulietti (2009) recognized an assemblage of species of *Stemodia* that included the *Stemodia stelatta* group (sensu Scatigna et al. 2017) in addition to *S. microphylla* and *S. veronicoides*, two species that also have crawling or prostrate habit, with petiolate leaves and long-pedicelate flowers. *Lapaea* differs from these two species in the absence of bracteoles on pedicels (vs. presence) and in the non-resupinate flower (vs. resupinate). Molecular evidence provided by Scatigna et al. (2018a) and this contribution corroborate the separation of *S. microphylla* from *Lapaea*.

Some specimens of *Lapaea* spp. may be confused with *Stemodia trifoliata* (Link) Rchb., in the usually 3-whorled leaves, especially in herbarium material, but they are rapidly differentiated from it by the crawling to procumbent habit (vs. erect), by the curved, obdeltoid stigma (vs. erect, strongly bilobed), by the corolla being infundibular and bilabiate (vs. salverform and cruciform) and by the leaf margins being simple crenate to serrate (vs. usually double-serrate). In addition, herbarium specimens tend to be blackish in *Lapaea* spp. and greenish in *S. trifoliata*.

Key to the species of *Lapaea*

Espinhaço Range portion of occurrence given in brackets: CD=Chapada Diamantina; ERMG=Espinhaço Range in Minas Gerais.

1. Dentrific trichomes present.....*Lapaea stellata* (ERMG)
1. Dentrific trichomes absent
 2. Stem and pedicel densely glandular-puberulent, covered exclusively with short-capitate (glandular) trichomes; corolla throat glabrous.....*Lapaea lobata* (ERMG)
 2. Stem and pedicel hirsute to villous, covered with long, non-capitate (eglandular) or minutely capitate trichomes, in addition to short, capitate trichomes; corolla throat pubescent or villous
 3. Leaves hispid, mainly covered with long, stiff, strongly curved, eglandular trichomes, in addition to short, capitate trichomes; corolla throat pubescent.....*Lapaea cipoensis* (ERMG)
 3. Leaves villous, mainly covered with long, flexible, minutely capitate trichomes, in addition to short, capitate trichomes; corolla throat villous
 4. Corolla blue to purplish, with white and yellow nectar guide, tube 7 – 8 mm long; leaves ovate to ovate-elliptical, margin flat, petioles 0.4 – 1.3 cm; sepals 6 – 8 mm long long;*Lapaea harleyi* (CD)

4. Corolla red, without nectar guide, tube 15 – 18.2 mm long; leaves elliptic, margin revolute, petioles 0.2 – 0.5 cm long; sepals 7.5 – 10.1 mm long;*Lapaea rubriflora* (CD)

Lapaea cipoensis (Scatigna) Scatigna, **comb. nov.** *Stemodia cipoensis* Scatigna in Scatigna et al. Systematic Botany 42: 373. 2017. TYPE:—BRAZIL. Minas Gerais: Santana do Riacho, RPPN Brumas do Espinhaço, base do morro Ermo Açu, 43°42'50"W, 19°02'09"S, alt. 1430m, 30 April 2016, A.V. Scatigna & C.A. Ferreira Junior 1120 (holotype: UEC!; isotypes: K!, NY!, SPF!). Fig. 4A.

Lapaea cipoensis is a very aromatic plant characterized by a hispid texture in its vegetative parts due to the presence of stiff, strongly curved, eglandular trichomes, in addition to short, capitate trichomes. It exhibits a more robust habit relatively to the two other species of *Lapaea* from the ERMG, *L. lobata* and *L. stellata*. In addition, it differs from *L. lobata* in the hispid indument of stems, petioles and pedicels (vs. glandular-puberulent), in the deep blue to purple corolla with a white patch in the pubescent throat (vs. entirely violet, with glabrous throat) and in the dorsiventrally compressed corolla tube (vs. not compressed); and from *L. stellata* in the lack of dendritic trichomes (vs. present) and in the upper corolla lip being patent to ascendant (vs. reflexed).

Representative specimens examined:—BRAZIL. Minas Gerais: Santana do Riacho, RPPN Brumas do Espinhaço, sopé do Ermo Açu, 11 April 2014, C.A. Ferreira Junior et al. 1373 (BHZB[photo]!, UEC!); *ibid.*, base do morro Ermo Açu, 43°42'50"W, 19°02'09"S, Alt. 1430 m, 29 April 2016, A.V. Scatigna & C.A. Ferreira Junior 1116 (UEC!); *idem* Scatigna & Ferreira Junior 1119 (BHCB!, UEC!).

Distribution, habitat and ecology:—*Lapaea cipoensis* is restricted to the Brazilian state of Minas Gerais, being only known from the Reserva Particular do Patrimônio Natural (RPPN) Brumas do Espinhaço, a private Natural Reserve, at elevations around 1400 m a.s.l., in Serra do Cipó, part of the central portion of the ERMG. It occurs in the edges of "lapas" in an area of *campo rupestre*. Scatigna et al. (2017) observed that, like in related species, *L. cipoensis* exhibits capsules oriented upwards, due to the curvature of the pedicels, with functionally poricidal dehiscence that may be associated with seed

dispersal by raindrops and/or wind. The few records were collected with flowers and fruits on April.

Conservation status:—*Lapaea cipoensis* has been assessed as CR (critically endangered) by Scatigna et al. (2017), meeting criteria B1ab(iii) and B2ab(iii). The species has the AOO and EOO equal to 4 km² and is known to occur in a single location. The suitable habitat is under continuing decline of area and quality due to fire and cattle grazing, invasion of exotic grasses and erosion caused by tourist activities such as hiking, as observed by Ribeiro et al. (2005), Gualtieri-Pinto et al. (2008) and Echternacht et al. (2010).

Lapaea harleyi (B.L.Turner) Scatigna, **comb. nov.** *Stemodia harleyi* B.L.Turner in Turner and Cowan. *Phytologia* 75: 292. 1993. TYPE:—BRAZIL. Bahia: Mucugê, about 5 km along Andaraí road, 41°20'W, 12°58'S, Alt. ca. 900 m, *R.M.Harley et al. 20667* (holotype: CTES!; isotypes: CEPEC!, K[digital image]!, NY[digital image]!, SPF!). Fig. 4B.

Lapaea harleyi is very similar to *L. rubriflora* in the habit and villose indument on vegetative parts, but differs from it in the in the corolla tube being much shorter (7 – 8 mm vs. 15 – 18.2 mm long) and blue to purplish, with white and yellow nectar guide (vs. red, without nectar guide); in the longer petioles (0.4 – 1.3 cm vs. 0.2 – 0.5 cm long); in the flat leaf blade margin (vs. revolute); in the shorter sepals (6 - 8 mm vs. 7.5 – 10.1 mm long); and in the leaf shape (ovate to ovate-elliptical vs. elliptic), texture (membranous vs. sub-coriaceous) and surface aspect (smooth vs. bullate). See comments under *L. rubriflora* for micromorphological comparison.

Representative specimens examined:—BRAZIL. Bahia: Andaraí, Igatu, 19 February 2011, *A.A.C. Miranda & Chiquinho 14* (HUEFS); *ibid.*, Distrito de Igatu, Grota do Brejão, 43°42'50"W, 19°02'09"S, Alt. 1430 m, 15 December 1999, *J.G. Jardim et al. 2321* (CEPEC, NY[digital image]); *ibid.*, Serra do Sincorá, approx. 15 km North of Mucugê on road to Andaraí, approx. 41°20'W, 12°57'S, Alt. ca. 1100 m, 18 February 1977, *R.M. Harley et al. 18865* (CEPEC, K[digital image], SPF, UEC); Lençóis, Fazenda Salobrinho, 41°23'W, 12°32'S, *R. Funch 7* (HUEFS); *ibid.*, Ca. 1 km do início da estrada lateral que sai da Rodovia Lençóis-Seabra, a 23 km NW de Lençóis, Alt. 900-1000 m, 16 October

1994, V.C. Souza et al. CFCR14118 (ESA, K[digital image], NY); *ibid.*, Chapadinha, 41°26'25"W, 12°27'35"S, Alt. 910 m, 27 October 1994, A.M. Carvalho et al. PCD1079 (ALCB, CEPEC, ESA, HUEFS, HRB, K[digital image]); Mucugê, Cemitério Bizantino, trilha para o cruzeiro, 41°22'40"W, 13°00'25"S, 19 January 2015, A.V. Scatigna & J.A.M. Carmo 651, 652, 653 (UEC); *ibid.*, Pico do Gobira, 41°22'40"W, 13°05'05"S, 20 January 2005, R.M. Castro & S.M. Lambert 1088 (ESA, HUEFS); *ibid.*, Serra do Esbarrancado, no topo da serra, 41°30'33"W, 12°43'51"S, 01 November 2011, R.P. Oliveira et al 1969 (HUEFS); *ibid.*, 10 km N na rod. para Andaraí, 41°19'08"W, 12°57'57"S, 27 January 2001, J.G. Jardim et al. 2538 (CEPEC, ESA, NY[digital image]); *ibid.*, encosta da Serra na subida para o campo do Gobira, 24 January 2000, L.P. Queiroz et al. 5648 (CEPEC, ESA, HUEFS); *ibid.*, 10-15 km ao NW de Mucugê na estrada para Andaraí, 41°20'W, 12°57'S, Alt. 1000m 27 July 1979, S. Mori et al. 12688 (CEPEC, K[digital image], NY); *ibid.*, campo defronte ao cemitério, 20 July 1981, Giuliatti et al. CFCR1397 (ESA, K, SPF); Palmeiras, Morro da Mão Inácia, 41°23'W, 12°34'S, 18 November 2006, Novais et al. 39 (HUEFS); *ibid.*, Morro do Pai Inácio, km 224 da rodovia BR 242, ca. 41°27'W, 12°30'S, 19 December 1981, G.P. Lewis et al. 899 (CEPEC, K[digital image], RB); *ibid.*, Pai Inácio, 41°28'W, 12°27'S, 19 November 1983, L.R. Noblick & A. Pinto 2794 (HUEFS, UEC); *ibid.*, Pai Inácio, BR242, W of Lençóis at km 232, 12 June 1981, S.A. Mori & B.M. Boom 14396 (CEPEC, K[digital image], NY[digital image]); *ibid.*, Morro do Pai Inácio, 16 November 1983, G.C. Pereira Pinto et al. 443/83 (HRB); *ibid.*, Próximo ao Rio Mucugezinho, Rod. Lençóis-Seabra, ca. 21 km NW de Lençóis, 41°27'10"W, 12°27'27"S, Alt. 900-1100 m, 17 February 1994, V.C. Souza et al. CFCR14168 (ESA, K, NY); *ibid.*, Morro do Pai Inácio, 41°28'15"W, 12°27'20"S, Alt. 1080 m, 25 October 1994, A.M. Carvalho et al. PCD1005 (ALCB); *ibid.*, Pai Inácio, 41°28'17"W, 12°27'31"S, Alt. 1150 m, 21 November 1994, E. Melo et al. PCD1145 (ALCB, CEPEC, ESA, K[digital image]); *idem*, E. Melo et al. PCD1166 (ALCB, ESA).

Distribution, habitat and ecology:—*Lapaea harleyi* is restricted to the Brazilian state of Bahia, being known from the Chapada Diamantina, in the municipalities of Andaraí, Lençóis, Mucugê and Palmeiras. It grows at the shady bases of large rocky blocks in areas of *campo rupestre*. Specimens were collected with flowers and fruits especially between October and March; fewer records were made in June and July.

Conservation status:—*Lapaea harleyi* was assessed as VU (vulnerable) by the CNCFlora Red List Project (Souza et al. 2013) and included in the same category in the last official list of threatened species of the Brazilian Flora (MMA 2014). The EOO (15.378,91 km²) was overestimated by Souza et al. (2013) probably because they included specimens of *L. rubriflora* wrongly identified as *L. harleyi* in their analysis. More recently, the species was included in the official list of threatened species of the Flora of Bahia under the category VU (vulnerable; SMA 2017). According to our evaluation, *L. harleyi* should be considered endangered (EN), meeting criteria B1ab(iii) and B2ab(iii), with an AOO = 56 km² and EOO = 971.241 km², populations severely fragmented and observed continuing decline of area and quality of suitable habitat due to historical mining activities and growing cattle grazing, in addition to invasion of exotic grasses facilitated by constant fire (Ganzen and Viana 2006).

Lapaea lobata (J.A.Schmidt) Scatigna, **comb. nov.** *Stemodia lobata* J.A.Schmidt in Mart., Fl. Bras. 8(1): 299. 1862. TYPE:—BRAZIL. Minas Gerais: Monte Itacolomi, Riedel *s.n.* (types not located). Fig. 4C.

Stemodia damaziana Beauv., Bull. Herb. Boissier Ser. 2. 7: 151. 1907. TYPE:—BRAZIL. Morro de São Sebastião, Damazio 294 (types: G).

Lapaea lobata is also a very aromatic plant, characterized by its glandular-puberulent indument formed exclusively by short, capitate trichomes on vegetative parts and by its broadly and deeply crenate, sometimes almost lobate leaf blades. *Lapaea lobata* differs from the other species of the genus from the ERMG, *L. cipoensis* and *L. stellata*, in its entirely deep violet corolla with glabrous throat and in its not dorsiventrally compressed tube (vs. pale lilac to deep blue or purple with white patch on throat and dorsiventrally compressed tube).

Representative specimens examined:—BRAZIL. Minas Gerais: Mariana, Parque Estadual do Itacolomi, Sertão, 43°27'49"W, 20°26'25"S, Alt. 1429 m 23 April 2010, E.S. Cândido et al. 399 (OUPR); *ibid.*, Serrinha, 7 February 2003, M.C.T.B. Messias 769 (OUPR); [Ouro Branco], Serra de Itatiaia prope Chapada, 12 May 1895, Schwacke 11492 (P); Ouro Preto, Andorinhas, 11 June 1975, J. Badini *s.n.* (OUPR); *ibid.*, Parque Municipal Cachoeira das Andorinhas, 43°29'38"W, 20°21'39"S, 02 May 2016, Scatigna et al. 1142, 1143, 1144 (UEC); *ibid.*, Andorinhas, *s.col.* 09 May 1971 (OUPR); *ibid.*,

Camarinhas, 07 June 1973, *J. Badini s.n.* (OUPR); *ibid.*, Camarinhas, 1940, *J. Badini s.n.* (OUPR); *ibid.*, Serra do Itacolomi, 1937, *J. Badini, s.n.* (OUPR); *ibid.*, Alto do Itacolomi, 1938, *Badini s.n.* (R); *ibid.*, Morro de São Sebastião, *A.P. Alves s.n.* (R); *ibid.*, Morro de São Sebastião, *L. Damazio 763* (OUPR); *ibid.*, Morro de São Sebastião, 1904, *L. Damazio s.n.* (BHCB); *ibid.*, Serra de Lavras Novas, 10 April 1972, *J. Badini s.n.* (OUPR); Serra de Lavras Novas, 11 May 1974, *J. badini s.n.* (OUPR); Serra das Lavras Novas, localidade de Venda do Campo, 27 March 2001, *M. Groppo Jr. & M. Ulwin 686* (SPF); *ibid.*, Lavras Novas, Serra do Buieie, próx. estrada, 43°31'32"W, 20°27'47"S, 03 May 2016, *A.V. Scatigna & T.V. Bastos 1145, 1146, 1147, 1148* (UEC); Lavras Novas, *s.col.* (OUPR).

Dubious location: Minas Gerais: Serra do Cipó, 3 March 1958, *Heringer & Castellanos 5991* (UB, R).

Distribution, habitat and ecology:—*Lapaea lobata* is restricted to Minas Gerais, Brazil, being mainly collected in the municipalities of Mariana and Ouro Preto, in the Southernmost portion of the ERMG, at elevations around 1400 m a.s.l. The only record from Serra do Cipó (*Heringer & Castellanos 5991* [R!, UB!]) was collected in 1958 and despite the extensive field work in the location during the next ca. 60 yr. the species has never been recollected there; in addition, the herbarium sheet of R bears the observation “Et. Horto Florest. Paraopeba” meaning that it is possible that the collected specimen was in cultivation, which brings into question the correct provenance of the gathering of *Heringer & Castellanos*. It grows on humid and shaded edges of quartzite boulders in areas of *campo rupestre*. Specimens with flowers and fruits were collected mainly between March and June, with one record in October and one in December.

Conservation status:—We assessed *Lapaea lobata* as CR (critically endangered), meeting criterion B1ab(i,ii,iii). The species has the EOO equal to 35.575 km², with severely fragmented population and observed continuing decline of AOO, EOO and area and quality of habitat. The region has historically suffered intense degradation because of gold mining activities and is currently under pressure of iron mining activities and human occupation (Carmo & Kamino 2015). Recently, the region of Mariana has suffered the largest environmental tragedy in Brazil due to the rupture of a dam in a mining company area, with serious damage to local vegetation (Lopes 2016).

Lapaea rubriflora Scatigna & V.C.Souza, **sp. nov.** TYPE:—BRAZIL. Bahia: Abaíra, Campo de Ouro Fino, 41°54'W, 13°15'S, Alt. 1600 – 1700 m, 10 Jan 1992, *Harley, R.M., Giulietti, A.M., Lughadha, E.N. & Freire Fierro, A. H50738* (holotype: HUEFS!; isotypes: CEPEC!, ESA!, K[digital image]!, NY[digital image]!, SP!, SPF!). Figs. 1–3, 4E.

Lapaea rubriflora is characterized by the unique red-colored flower. It is similar to *L. harleyi* in general aspect, but differs in the elliptic leaves (vs. ovate to ovate-elliptical) with shorter petioles (0.2 – 0.5 cm vs. 0.4 – 1.3 cm long) and revolute margin (vs. flat); in the longer sepals (7.5 – 10.1 mm vs. 6 – 8 mm long); and in the corolla tube being much longer (15.0 – 18.2 mm vs. 7 – 8 mm long) and red, without nectar guide (vs. blue to purplish, with white and yellow nectar guide).

Description:—*Suffruticose* perennials, 30 – 80 cm tall. *Stems* terete to sub-quadrangular, branched, covered with short capitate trichomes (< 0.2 mm long) interspersed with long minutely capitate trichomes (0.5 – 1 mm long) densely towards apex. *Leaves* usually 3-whorled, rarely opposite; petioles 0.2 – 0.5 cm long, densely covered with short capitate trichomes and long minutely capitate trichomes; blades ovate to elliptical, 1.8 – 4.1 cm × 1.0 – 2.5 cm, sub-coriaceous bullate, apex acute to obtuse, round, base cuneate to obtuse, margin crenate to serrate, clearly revolute, abaxial surface densely covered with short capitate trichomes and longer minutely capitate trichomes, these concentrated on prominent veins and on margin, adaxial surface covered with short capitate trichomes interspersed with longer minutely capitate trichomes throughout the surface. *Flowers* axillary, single or geminate, ebracteolate; pedicel 1.5 – 2.5 cm long, covered with short and long capitate trichomes, densely towards flower, upcurved after fruiting; sepals sub-equal, connate at base, lanceolate, 7.5 – 10.1 mm × 1.8 – 2.2 mm., apex acute to acuminate, glandular-pubescent and with few longer minutely capitate trichomes on veins and margins, denser on base; corolla slightly bilabiate, red; tube 15.0 – 18.2 mm long, cylindrical, slightly gibbous at anthers region, externally covered with short glandular trichomes and longer minutely capitate trichomes scattered over the surface, glabrous at base, internally sparsely pubescent, denser on filaments insertion; upper lip shallowly 2-lobed, ca. 4.5 – 5.0 mm × 7.5 – 8.0 mm, apex emarginated, externally glandular-pubescent; lower lip 3-lobed, throat glabrous, lobes orbicular, ca. 3.5 – 4 mm diam., externally glandular pubescent. *Stamens* 4, didynamous, included, filaments filiform,

apex capitate, anterior pair ca. 8.0 mm long, posterior ca. 6.0 mm long, glabrous; anthers with two thecae, separated, minutely stipitate, ca. 2 mm long, glabrous, dehiscence longitudinal; staminode 1, minute, slightly capitate. *Carpels* 2, sub-equal; ovary superior, syncarpous, 2-locular, ovoid, ca. 3.5 × 1.5 mm, glabrous; placentation axilar, ovules numerous; style terminal, solitary, filiform, 6.5 mm long, glabrous, apex obdeltoid, ca. 0.5 mm long, curved downwards, glabrous. *Capsule* ovoid, ca. 7.5 × 4 mm, dehiscence both loculicidal and septicidal, functionally poricidal, glabrous; seeds ovoid, slightly compressed, 0.8 mm × 0.2 mm, base acuminate, shallowly foveolate.

Additional specimens examined (paratypes):—BRAZIL. Bahia: Abaíra, Serra ao Sul do Riacho da Taquara, 41°55'W, 13°15'S, Alt. 1890 m, 10 Jan 1992, R.M. Harley et al. H51257 (CEPEC, E, ESA, HUEFS, K[digital image], NY[digital image]); *ibid.*, Campo do Cigano, 41°55'W, 13°15'S, Alt. 1700-1800 m, 25 February 1992, P.T. Sano H52323 (CEPEC, ESA, HUEFS, K[digital image]); *ibid.*, Campo de Ouro Fino, 41°54'W, 13°15'S, Alt. 1600-1700 m, 24 January 1992, J.R. Pirani et al. H50778 (ESA, HUEFS, K[digital image]); *ibid.*, Catolés, Serra do Barbado, between 41°54'06"W, 13°17'27"S and 41°54'29"W, 13°17'50"S, Alt. 1750-2035 m, 26 February 1994, V.C. Souza et al. CFCR14590 (CEPEC, E, ESA, HUEFS, K[digital image], MO, NY); *ibid.*, Distrito de Catolés, Encosta da Serra do Atalho, subida pela boca do Leão, 20 April 1998, L.P. Queiroz et al. 5075 (HUEFS[2 sheets], K[digital image], NY[digital image]); *ibid.*, Catolés, Trilha para o Campo do Ouro Fino, 41°54'07"W, 13°03'08"S, 11 January 2007, A.K.A. Santos & Sr. Raimundo 961 (HUEFS); *ibid.*, Catolés, descida para Mata dos Frios, 41°53'08"W, 13°18'57"S, Alt. 1450 m, 23 October 1999, E.B. Miranda et al. 292 (HUEFS); *ibid.*, Catolés, Trilha para Pico do Barbado, Forquilha, 41°54'15"W, 13°17'27"S, Alt. 1670 m, 17 January 2016, A.V. Scatigna & J.A.M. Carmo 1035, 1042 (UEC).

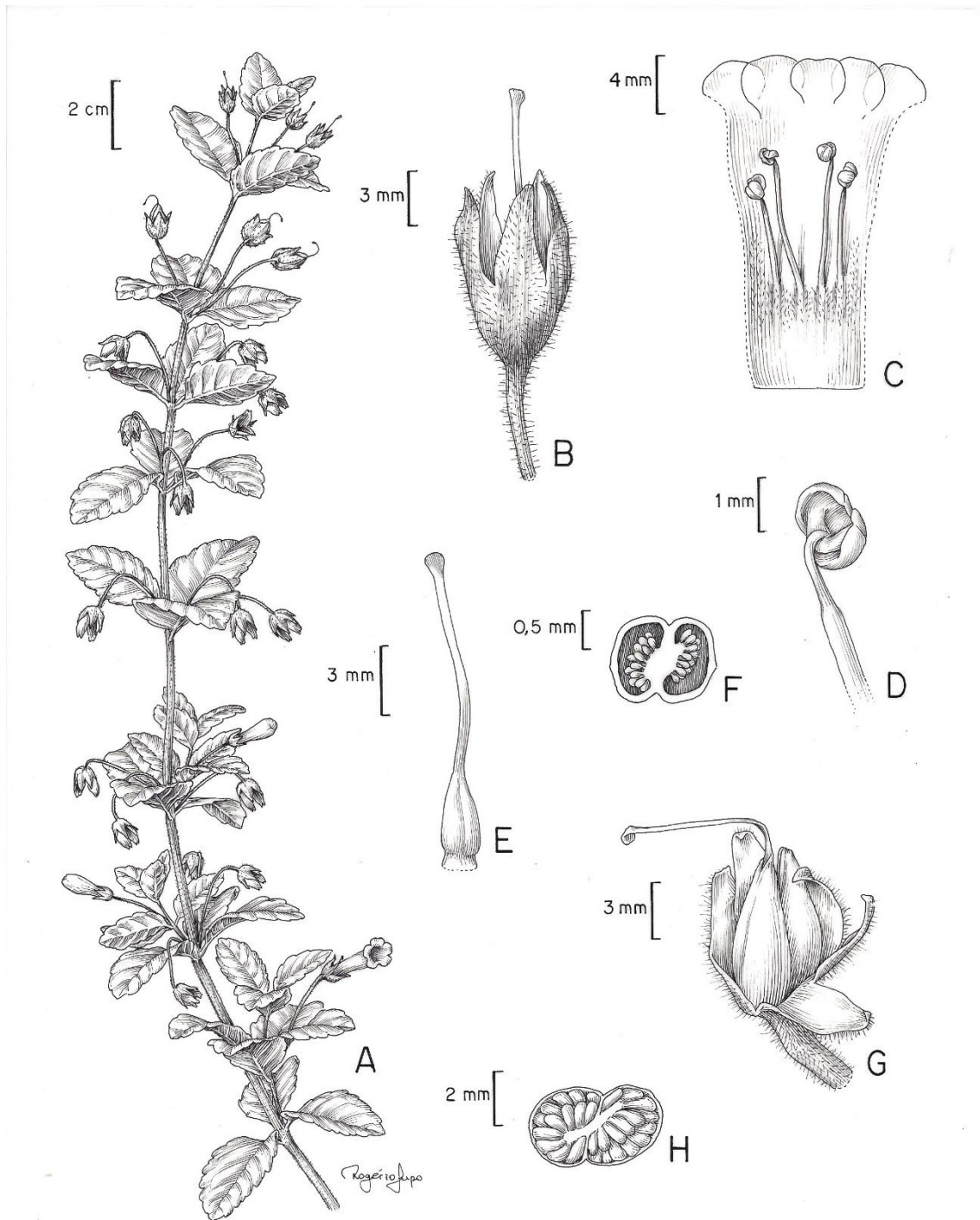


Figure 1. *Lapaea rubriflora*. A. Reproductive branch. B. Calyx with sepals connate at base and erect style. C. Dissected corolla tube and androecium. D. Stamen with arm-like connective separating thecae. E. Gynoecium. F. Cross section of ovary. G. Capsule and persistent calyx. H. Cross section of capsule. Drawn by Rogério Lupo after R.M. Harley *et al.* H51257 (ESA).

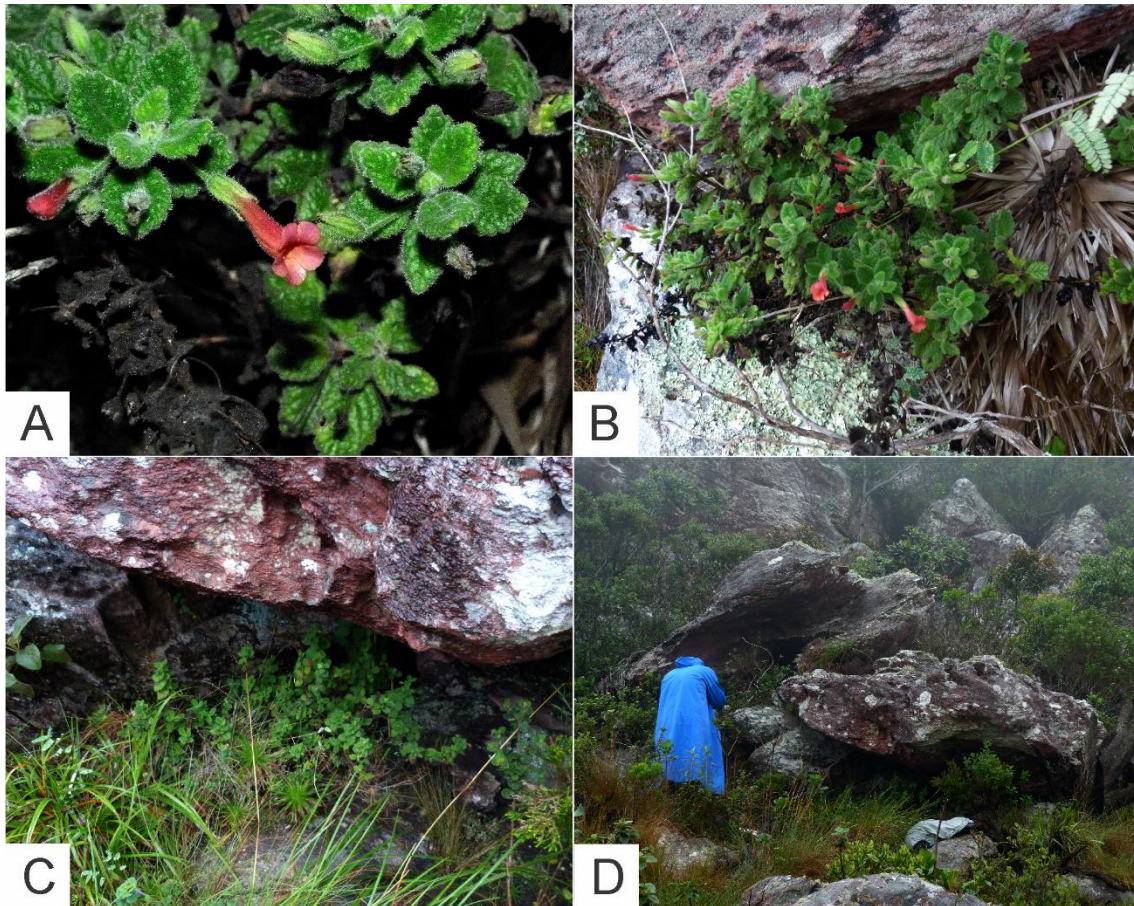


Figure 2. *Lapaea rubriflora*. A. Flowering branch. B. Habit. C. Specimen of *L. rubriflora* under a “lapa”. E. J.A.M.Carmo in the habitat of *L. rubriflora*.

Micromorphological study:—Of the six types of trichomes described by Scatigna et al. (2017) for the *Stemodia stellata* group, we observed the presence three types (types 1, 3 and 4), all of them glandular, on the surfaces of stems and leaves of *Lapaea rubriflora* (Fig. 3). We did not observe any type of eglandular trichomes on the studied samples. Type 1 has a broad base, a pedicel with two or three cells, and a rounded head, apparently unicellular (Fig. 3A₁); type 3 has a short unicellular (rarely two-celled) pedicel and a wide, multicellular, morular-shaped head (Fig. 3A₂); and type 4, which is a minutely capitate, filiform, flexible, and uniseriate trichome with variable length (200 – 1200 μm) (Fig. 3B). The three types of trichomes were more abundant on stem (Fig. 3C) and on abaxial surface of leaves (Fig. 3D), where type 1 was concentrated on blade and type 4 was concentrated on veins.

Notes:—*Lapaea rubriflora* is readily distinguished from any other species of *Lapaea* and *Stemodia* sensu lato due to its unique red flower (Figs 2A, 3E). In an account of the

vascular plants of the Catolés region, Bahia, Brazil (Zappi et al. 2003), specimens of *L. rubriflora* were identified as *Stemodia harleyi* (= *L. harleyi*), a similar species in the overall aspect; for comparison, see notes under the latter. Regarding the micromorphology, *L. harleyi* and *L. rubriflora* share type 4 trichomes, which are lacking in all other species of the genus; *L. harleyi* lacks type 1 trichomes, which are present in *L. rubriflora* and all remnant species of *Lapaea*.

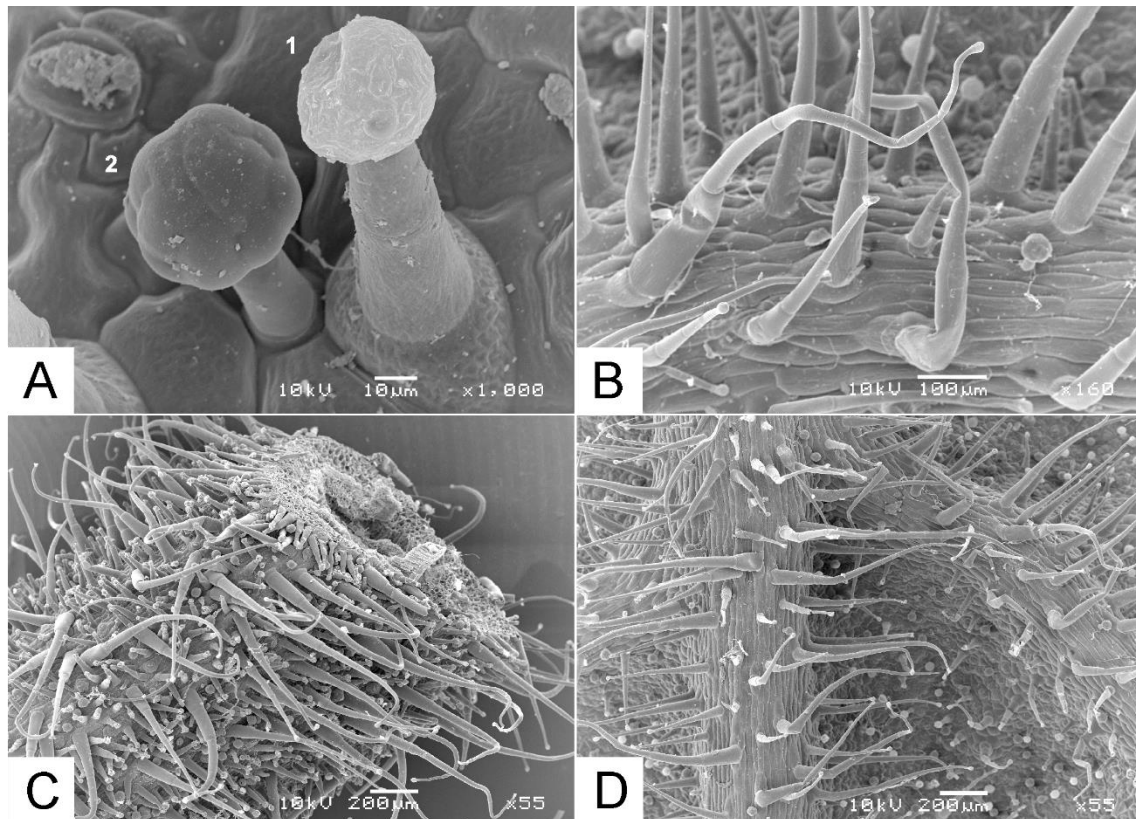


Figure 3. Scanning electron microscopy of vegetative parts of *Lapaea rubriflora*. A₁. Type 1 trichome. A₂. Type 3 trichome. B. Type 4 trichome. C. Stem. D. Abaxial surface of leaf. From *Scatigna & Carmo1035* (UEC).

Distribution, habitat and ecology:—*Lapaea rubriflora* is restricted to Bahia, being only known from the Catolés region, in the Chapada Diamantina. It grows on fractures of rocky blocks in areas of campo rupestre, at elevations of ca. 1500 – 2000 m a.s.l. Flowering and fruiting specimens were collected mainly between January and April, with one record in October. The unique red-colored corolla with a relatively long tube may indicate the first case of ornithophily in the Gratiolaceae, but studies in reproductive biology are crucial to confirm this hypothesis.

Etymology:—The epithet [*rubriflora*] refers to the red-colored corolla, a unique feature in the whole tribe Gratioleae.

Conservation status:—We assessed *Lapaea rubriflora* as CR (critically endangered) under criterion B1ab(iii). The species has the EOO equal to 13.006 km² and is known to occur in a unique location. Furthermore, there is observed continuing decline of area and quality of habitat due to due to historical mining activities and currently growing cattle grazing, in addition to invasion of exotic grasses facilitated by constant fire (Ganen and Viana 2006).

Lapaea stellata (B.L.Turner) Scatigna, **comb. nov.** *Stemodia stellata* B.L.Turner, *Phytologia* 75(4): 312. TYPE:—BRAZIL. Minas Gerais: Pico do Itambé. *W.R. Anderson et al.* 35828 (holotype: MO[digital image]!; isotypes: MBM!, NY[digital image]!, K[digital image]!, UB!). Fig. 4D.

Lapaea stellata is readily distinguished from any other known species of *Lapaea* and *Stemodia* s.l. by the presence of unique dendritic trichomes over the surfaces of vegetative parts. Souza and Giulietti (2009) asserted that *L. stellata* is similar to *S. microphylla* in the overall aspect, but the two species are readily differentiated in the 3-whorled leaves and ebracteolate, non-resupinate flowers in *L. stellata* vs. opposite leaves and bi-bracteolate and resupinate flowers in *S. microphylla* (Scatigna et al. 2017).

Representative specimens examined:—BRAZIL. Minas Gerais: Santo Antônio do Itambé, Parque Estadual do Pico do Itambé, [...] along the main trail., 43°20'53"W, 18°23'55"S, Alt. 1367 – 2038 m, 02 March 2009, *Almeda et al.* 9675 (UEC); *ibid.*, Parque Estadual do Pico do Itambé, trilha para o Pico do Itambé, 43°19'18"W, 18°24'01"S 07 July 2014, *Scatigna et al.* 477 (UEC); *ibid.*, Parque Estadual do Pico do Itambé, trilha para o Pico do Itambé, 43°19'25"W, 18°23'57"S, 07 July 2014, *Scatigna et al.* 478 (UEC); *ibid.*, Parque Estadual do Pico do Itambé, trilha para o Pico do Itambé, 43°19'44"W, 18°23'51"S, 07 July 2014, *Scatigna et al.* 479 (UEC); *ibid.*, Pico do Itambé 43°19'02"W, 18°24'05"S, Alt. 1500 m, 07 April 1998, V.C. Souza et al. 21126 (ESA); *ibid.*, Pico do Itambé, Subida para o pico, 43°20'54"W, 18°23'45"S, 08 April 2010, G.O. Romão et al. 2619 (ESA); *ibid.*, Pico do Itambé, 43°19'18"W, 18°24'02"S, 1551 m, 14 December 2007,

C.D.N. Rodrigues et al. 118, 119 (ESA); *ibid.*, Caminho para o Pico do Itambé, Alt. 1500 – 2000 m, 26 February 2002, V.C. Souza et al. 28474 (BHCB, ESA).

Distribution, habitat and ecology:—*Lapaea stellata* is restricted to the state of Minas Gerais and only known from the Pico do Itambé region, in the municipality of Santo Antonio do Itambé, which is encompassed by the Diamantina Plateau, a central-northern portion of the ERMG. Like the related species, it grows in “lapas” of large rocky blocks in areas of campo rupestre in elevations of ca. 1300 – 2000 m. Specimens were collected with flowers and fruits between December and April and in July.

Conservation status:—We assessed *Lapaea stellata* as CR (critically endangered) under criteria B1ab(iii) and B2ab(iii). The species has the AOO and EOO = 8 km² and is known from only one location. In addition, there is observed continuing decline of area and quality of habitat due to invasion of exotic grasses after fire (Versieux 2008).

Phylogenetic study

In addition to the sequences used by Scatigna et al. (2018a) and that are available on GenBank (www.ncbi.nlm.nih.gov/genbank/), our study included 45 newly generated sequences of 12 accessions representing the five currently known species of *Lapaea*. Trees from P and BI generated for each of the three cpDNA markers presented no significant incongruence; therefore, we felt confident to use the concatenated dataset (PC). Although both P and BI analysis of the ITS1 provided poorly resolved trees regarding the relationships among main clades, they showed a strong incongruence relatively to the PC dataset in one of them; therefore, the results from PC and ITS1 datasets are presented separately. In both PC and ITS1 datasets, the majority-rule consensus tree of the BI was completely resolved and presented more strongly supported nodes relatively to the strict consensus from the MP trees, but because the PC dataset resulted in a tree with higher support, it is the only one presented and discussed with detail (Fig. 5). The trees generated from the ITS dataset are provided as Supplementary Materials 1 – 3. Table 1 summarizes parsimony scores and nucleotide substitution models for each matrix.

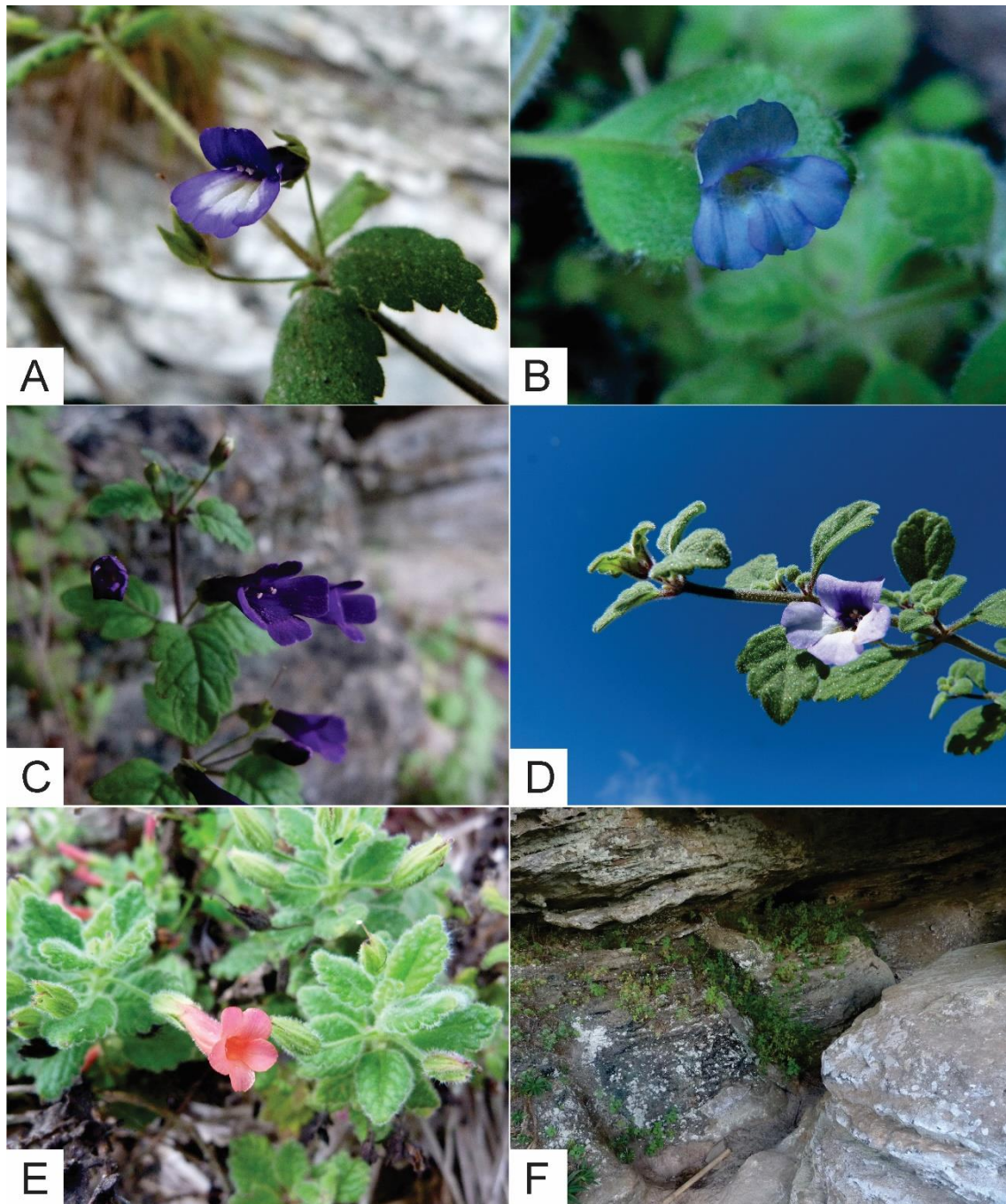


Figure 4. *Lapaea* spp. in natural habitat. A. *L. cipoensis*. B. *L. harleyi*. C. *L. lobata*. D. *L. stellata*. E. *L. rubriflora*. F. Specimens of *L. lobata* growing in a “lapa” in Ouro Preto, Minas Gerais.

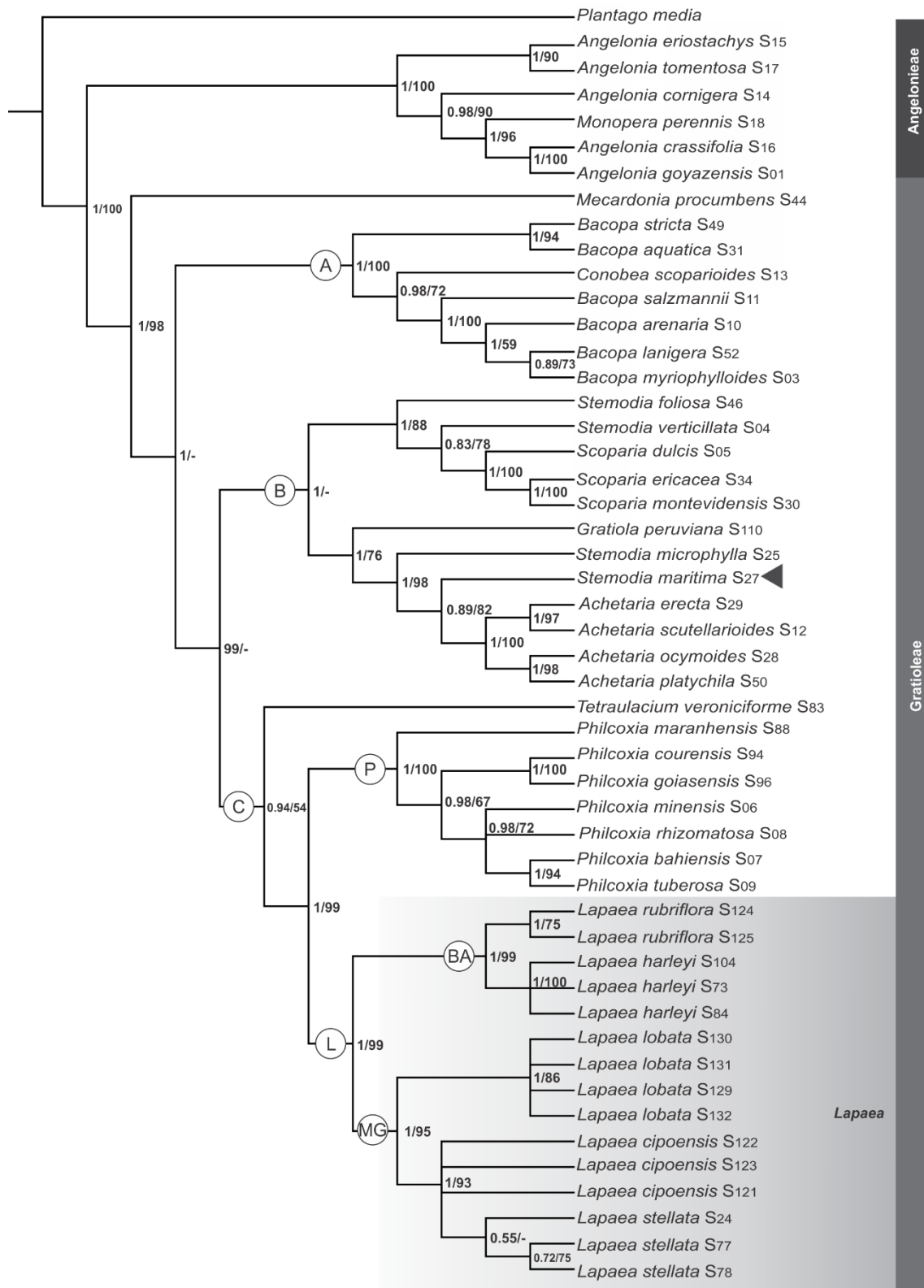


Figure 5. Majority-rule consensus tree generated by Bayesian inference from the Partial Combined dataset including sequences of the *rps16* and *trnL* introns and the *trnL-trnF* intergenic spacer of the cpDNA. Number next to nodes are Posterior Probability/Bootstrap values. Clades represented by letters are discussed in the text. Dark arrow points *Stemodia maritima*, type species of the genus.

PC dataset:—The PC dataset consisted of 50 terminals and 2301 aligned characters, of which 479 were parsimony-informative, 1531 were constant, and 291 were variable but uninformative. The nucleotide substitution model selected by the Bayesian Information Criterion (BIC) was TVM+G for *rps16*, TIM1+G for *trnL* and TPM3uf+G for *trnL-trnF*.

With *Plantago media* as rooting taxon, we recovered the Gratioleae in a well-supported clade (PP = 1.00; BS = 98) composed of *Mercadonia procumbens* and the same three main clades (clades A, B and C in Fig. 5) with the same composition and placement as in Scatigna et al. (2018a). All species of *Lapaea* formed a clade (subclade L; PP = 1.00; BS = 99) sister to *Philcoxia* (subclade P; PP = 1.00; BS = 100); these two subclades form a clade (PP = 1.00; BS = 98) sister to *Tetraulacium veroniciforme*, composing clade C (PP = 0.94; BS = 54). Within subclade L, *L. harleyi* and *L. rubriflora* form a subclade (subclade BA, PP = 1; BS = 99) sister to another subclade (subclade MG, PP = 1; BS = 95) formed by *S. lobata*, *S. cipoensis* and *S. stellata*. Within subclade BA, all accessions of *L. harleyi* formed a strongly supported clade (PP = 1.00; BS = 100) sister to the clade formed by the accessions of *L. rubriflora* (PP = 1.00; BS = 75). Within subclade MG, *L. cipoensis* and *L. stellata* form a clade (PP = 1.00; BS = 93) sister to *L. lobata*; the accessions of *L. stellata* formed a poorly supported clade (PP = 0.55) and the accessions of *L. cipoensis* were recovered in a polytomy.

ITS1 dataset:—The ITS1 dataset consisted of 49 terminals and 465 aligned characters, of which 176 were parsimony-informative, 235 were constant, and 54 were variable but uninformative. The nucleotide substitution model selected by the Bayesian Information Criterion (BIC) was GTR+G.

Sampled members of Gratioleae were also recovered in a strongly supported clade (PP = 0.98; BS = 80; Fig 5), but most of the clades within it had poor or no support, especially closer to the base. Of the seven clades and subclades described for the PC dataset, only clade A and subclades P, L, BA and MG were recovered with strong to maximum support values for the ITS1 dataset. Subclades P (PP = 1.00; BS = 100) and L (PP = 1.00; BS = 99) were not recovered in a polytomy. Subclade L was composed of all species of *Lapaea* in the same subclades BA (PP = 0.99; BS = 92) and MG (PP = 1.00; BS = 100) as in the PC dataset. Subclade BA, was composed of all representatives of *L. harleyi* forming a clade (PP = 1; BS = 99) sister to the clade formed by all *L. rubriflora* accessions (PP = 1.00; BS = 96). Subclade MG was formed by a subclade composed of all accessions of *Lapaea cipoensis* (PP = 0.92; BS = 81) sister to a subclade formed by *L.*

lobata and *L. stellata* (PP = 1.00; BS = 95); the accessions of *L. stellata* formed a clade (PP = 1.00; BS = 94), but the accessions of *L. lobata* were recovered in a polytomy.

Phylogeny:—Based of the PC dataset results, the general composition of the main clades recovered in our study and the relationships among and within them were generally consistent with those obtained by Scatigna et al. (2018a), even though we kept sequences of *rpl16* out of our analysis, which highlights the quality of information provided by the markers here implemented.

All species of the new genus *Lapaea* formed a well-supported monophyletic group, sister to *Philcoxia* and not related to *Stemodia maritima*. Our results corroborate the segregation of *Lapaea* from *Stemodia* s.s. and disagree with Souza and Giulietti (2009) regarding the relationship of *S. microphylla* with *L. stellata*. *Stemodia microphylla* is rather related to *S. veronicoides* with which it shares the resupinate, bibracteolate flowers (Souza and Giulietti 2009). Further phylogenetic studies associated with thorough morphological analysis may support the segregation of even more species from *Stemodia* s.s.

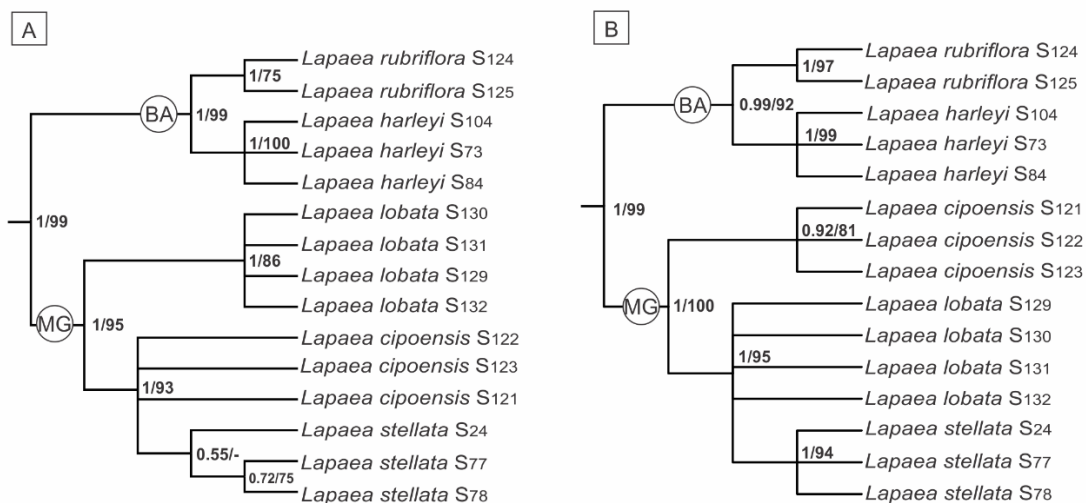


Figure 6. *Lapaea* clade extracted from the Majority-rule consensus tree generated by Bayesian inference from the Partial Combined dataset (A) and from ITS1 dataset (B). Numbers next to nodes are Posterior probability/Bootstrap values.

The *Lapaea* clade consists of two subgroups, the first comprising the two species restricted to the Chapada Diamantina, namely *L. harleyi* and *L. rubriflora*, and the second group including the three species restricted to the ERMG, *L. cipoensis*, *L. harleyi* and *L. stellata*. This phylogenetic pattern of a clade composed of species from the CD sister to

another formed by species from the ERMG is consistent with that found in other genera such as *Philcoxia* (Scatigna et al. 2018a), *Paepalanthus* (Eriocaulaceae; Trovó et al. 2013) and *Minaria* (Apocynaceae; Ribeiro et al. 2014).

Among the species from the ERMG, the relationships are still controversial. The cpDNA markers suggest that *S. stellata*, from de Diamantina Plateau, and *L. cipoensis*, from Serra do Cipó, are closer between each other than with *L. lobata*, from the Ouro Preto region, a relationship geographically consistent with the areas of endemism detected by Echternacht et al. (2011). However, according to the ITS1 dataset, *L. cipoensis* is sister to a clade formed by *L. lobata* and *L. stellata*.; incongruence between cpDNA (*trnL-trnL-trnF* and *rps16* regions) and nrDNA (ITS) was observed in tribe Veroniceae (Plantaginaceae) and attributed to biological causes such as hybridization and polyploidization (Albach and Chase 2004), but we do not have enough information to discuss this for *Lapaea*.

Although there is a high rate of endemism at the specific rank, only a few genera are exclusive to the *campo rupestre* (Alves and Kolbek 2010). This is the case of *Lapaea*, which is restricted to the *campo rupestre* of the ER, each of its five species having a narrow geographic distribution consistent with currently recognized biogeographic unities (Echternacht et al. 2011; Ribeiro et al. 2014). This genus is sister to *Philcoxia*, which in turn comprises a clade with species restricted to the *campo rupestre* of the ER. The geographically structured diversity found in the ER is recurrent in unrelated lineages (Echternacht et al. 2011; Silveira et al. 2016) and also occur in *Lapaea* and *Philcoxia*. The lineage comprising these two genera could be a good model to reconstruct biogeographic scenarios in a global hotspot with the highest rates of species/km² within the most megadiverse country (Alves and Kolbek 2010; Alves et al. 2014; Ribeiro et al. 2014).

Table 1. Parsimony statistics and evolutionary model for each dataset. PC = Partial Combined.

Dataset	<i>rps16</i>	<i>trnL</i>	<i>trnL-F</i>	PC	ITS1
Terminals	47	49	50	50	49
Missing taxa	6%	2%	0%	—	2%
Number of characters	1085	665	551	2301	465
Constant characters	685	506	340	1531	235
Variable, uninformative characters	146	63	82	291	54
Parsimony informative characters	254	96	129	479	176

% informative	23.4	14.4	23.4	20.8	37.8
Tree length	561	190	268	1043	740
Consistency index (CI)	0.66	0.72	0.66	0.65	0.47
Retention index (RI)	0.88	0.91	0.89	0.88	0.75
Evolution model (BIC)	TVM + G	TIM1 + G	TPM3uf + G	—	GTR + G

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Appendix 1. Voucher information. Accession numbers will be included for paper submission.

Taxon	DNA template	Voucher
<i>Achetaria erecta</i> (Spreng.) Wettst.	S29	Souza, V.C. 28935 (ESA)
<i>Achetaria ocymoides</i> Cham. & Schldl.	S28	Rodrigues, C.D.N. 103 (ESA)
<i>Achetaria platychila</i> (Radlk.) V.C.Souza	S50	Shimizu, G.H. s.n. (UEC)
<i>Achetaria scutellarioides</i> (Benth.) Wettst.	S12	Scatigna, A.V. 110 (UEC)
<i>Angelonia cornigera</i> Hook.f.	S14	Scatigna, A.V. 119 (UEC)
<i>Angelonia crassifolia</i> Benth.	S16	Scatigna, A.V. 73 (UEC)
<i>Angelonia eriostachys</i> Benth.	S15	Scatigna, A.V. 67 (UEC)
<i>Angelonia goyazensis</i> Benth.	S01	Scatigna, A.V. 48 (UEC)
<i>Angelonia tomentosa</i> Moric. ex Benth.	S17	Scatigna, A.V. 117 (UEC)
<i>Bacopa aquatica</i> Aubl.	S31	Souza, V.C. 28715 (ESA)
<i>Bacopa arenaria</i> (J.A.Schmidt) Edwall	S10	Scatigna, A.V. 272 (UEC)
<i>Bacopa lanigera</i> (Cham. & Schldl.) Wettst.	S52	Costa, I.G. 150 (GFJP)
<i>Bacopa myriophylloides</i> (Benth.) Wettst.	S03	Scatigna, A.V. 270 (UEC)
<i>Bacopa salzmännii</i> (Benth.) Wettst. ex Edwall	S11	Scatigna, A.V. 277 (UEC)
<i>Bacopa stricta</i> (Schrad.) Wettst. ex Edwall	S49	Scatigna, A.V. 404 (UEC)
<i>Conobea scoparioides</i> (Cham. & Schldl.) Benth.	S13	Scatigna, A.V. 99 (UEC)
<i>Gratiola peruviana</i> L.	S110	Scatigna, A.V. 666 (UEC)
<i>Lapaea cipoensis</i> (Scatigna) Scatigna	S121	Scatigna, A.V. 1116 (UEC)
<i>Lapaea cipoensis</i> (Scatigna) Scatigna	S122	Scatigna, A.V. 1119 (UEC)
<i>Lapaea cipoensis</i> (Scatigna) Scatigna	S123	Scatigna, A.V. 1120 (UEC)
<i>Lapaea harleyi</i> (B.L.Turner) Scatigna	S73	Scatigna, A.V. 651 (UEC)
<i>Lapaea harleyi</i> (B.L.Turner) Scatigna	S84	Scatigna, A.V. 653 (UEC)
<i>Lapaea harleyi</i> (B.L.Turner) Scatigna	S104	Scatigna, A.V. 652 (UEC)
<i>Lapaea lobata</i> (J.A.Schmidt) Scatigna	S129	Scatigna, A.V. 1142(UEC)
<i>Lapaea lobata</i> (J.A.Schmidt) Scatigna	S130	Scatigna, A.V. 1143 (UEC)
<i>Lapaea lobata</i> (J.A.Schmidt) Scatigna	S131	Scatigna, A.V. 1144 (UEC)
<i>Lapaea lobata</i> (J.A.Schmidt) Scatigna	S132	Scatigna, A.V. 1145 (UEC)
<i>Lapaea rubriflora</i> Scatigna & V.C.Souza	S124	Scatigna, A.V. 1035 (UEC)
<i>Lapaea rubriflora</i> Scatigna & V.C.Souza	S125	Scatigna, A.V. 1042 (UEC)
<i>Lapaea stellata</i> (B.L.Turner) Scatigna	S24	Souza, V. C. 28747 (ESA)
<i>Lapaea stellata</i> (B.L.Turner) Scatigna	S77	Scatigna, A.V. 477 (UEC)
<i>Lapaea stellata</i> (B.L.Turner) Scatigna	S78	Scatigna, A.V. 479 (UEC)
<i>Mecardonia procumbens</i> (Mill.) Small	S44	Mayer, F.S. 1356 (UEC)
<i>Monopera perennis</i> (Chodat & Hassl.) Barringer	S18	Farinaccio, M.A. 958 (UEC)
<i>Philcoxia bahiensis</i> V.C.Souza & Harley	S07	Scatigna, A.V. 107 (UEC)
<i>Philcoxia courensis</i> Scatigna	S94	Scatigna, A.V. 707 (UEC)
<i>Philcoxia goiasensis</i> P.Taylor	S96	Scatigna, A.V. 736 (UEC)
<i>Philcoxia maranhensis</i> Scatigna	S88	Barbosa, A.R. 1100 (HUEFS)
<i>Philcoxia minensis</i> V.C.Souza & Giul.	S06	Scatigna, A.V. 42 (UEC)
<i>Philcoxia rhizomatosa</i> Scatigna & V.C.Souza	S08	Scatigna, A.V. 319 (UEC)
<i>Philcoxia tuberosa</i> M.L.S.Carvalho & L.P.Queiroz	S09	Scatigna, A.V. 121 (UEC)

<i>Plantago media</i> L.	N.A.	no voucher*, UofC 55665 (UAC)**, Ronsted, N. 50 (C)***
<i>Scoparia dulcis</i> L.	S05	Scatigna, A.V. 102 (UEC)
<i>Scoparia ericacea</i> Cham. & Schltdl.	S34	Rodrigues, C.D.N. 102 (ESA)
<i>Scoparia montevidensis</i> (Spreng.) R.E.Fr.	S30	Souza, V.C. 32411 (ESA)
<i>Stemodia foliosa</i> Benth.	S46	Scatigna, A.V. 382 (UEC)
<i>Stemodia maritima</i> L.	S27	Souza, V. C. 28674 (ESA)
<i>Stemodia microphylla</i> J.A.Schmidt	S25	Souza, V. C. 28444 (ESA)
<i>Stemodia verticillata</i> (Mill.) Hassl.	S04	Scatigna, A.V. 81 (UEC)
<i>Tetraulacium veroniciforme</i> Turcz.	S83	Mota, N.F. 2787 (MG)

CAPÍTULO 2²

Systematics of Gratioleae (Plantaginaceae): Redefining *Stemodia* L.

André Vito Scatigna, Vinicius Castro Souza, Maria de las Mercedes Sosa and André Olmos Simões

Abstract: *Stemodia* sensu lato is one of the most diverse and heterogeneous genera in the Gratioleae (Plantaginaceae), traditionally defined by the calyx with equal to subequal sepals and four anthers with two, fertile thecae separated by the 2-brachiate connective. It has pantropical distribution and encompasses ca. 60 species. Recent phylogenetic studies in Gratioleae indicate that *Stemodia*, as currently circumscribed, is a polyphyletic genus. We generated new phylogenetic hypothesis, increasing the number of species sampled within both *Stemodia* s.l. and the rest of the tribe, and sequencing three regions from cpDNA (*rps16*, and *trnL* introns and the *trnL-trnF* intergenic spacer) and one from nrDNA (ITS1 spacer); we used both parsimony and Bayesian inference to reconstruct the phylogeny. We tested the monophyly of the genus and its placement with the tribe and, based on that, we proposed a new circumscription and morphological delimitation of a monophyletic group here called *Stemodia* sensu stricto. As herein proposed, *Stemodia* s.s. is characterized by the erect habit, clearly sessile leaves with usually clasping base, bi-bracteolate, non-ressupinate and usually short-pedicellate flowers that are often concentrated at the apex of flowering branches, calyx well-divided into five equal sepals, and androecium composed of four fertile stamens with two equal and glabrous separated thecae in each anther. It encompasses 26 species, 13 of them from the New World, one from Africa, one from Southeast Asia and Australia, and 11 exclusive from Australia. Gratioleae should be under taxonomic rearrangements at the generic level in order to accommodate the remnant species of *Stemodia* s.l. which were recovered in at least three main clades apart from *Stemodia* s.s.

Key words: Classification; Gratiolaceae; Lamiales; Molecular Phylogenetics; Polyphyly; Scrophulariaceae

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1. Introduction

Gratioleae is a mainly tropical tribe of the Plantaginaceae (Lamiales) comprising between 16 and 40 genera, depending on tribal and generic concepts, and over 300 species (Bentham 1846; Wettstein 1891; Fischer 2004; Albach et al. 2005; Estes and Small 2008). Studies on the systematics of this tribe are scarce and based on limited sampling, especially regarding Old World genera, which may reflect on its controversial circumscription (Albach et al. 2005). Nevertheless, molecular phylogenetic studies provided evidence for the non-monophyly of Neotropical and Pantropical genera such as *Gratiola* L., *Bacopa* Aubl., and *Stemodia* L. (Fritsch et al. 2007; Estes and Small 2008; Scatigna et al. 2018a).

Stemodia sensu lato is one of the most diverse and heterogeneous genera in the Gratioleae. It currently encompasses ca. 60 species distributed in the tropics, especially in the New World and Australia, usually occurring in open and wet areas (Turner and Cowan 1993a; Fischer 2004; Souza and Giulietti 2009). Despite a broad geographic distribution and a very diverse range in morphological characters (ie. crawling herbs to erect subshrubs, sessile to clearly petiolate leaves, sessile to long-pedicellate flowers, presence or absence of bracteoles, resupinate or non-resupinate flowers, among others), *Stemodia* s.l. has been widely defined by the possession of four fertile stamens with two thecae separated by a 2-brachiate connective in each anther, in addition to the calyx with equal to subequal sepals (Bentham 1846; Minod 1918; D'Arcy 1979; Turner and Cowan 1993a; Souza and Giulietti 2009). The genus was traditionally recognized as a member of the Gratioleae, first within subtribe Gratiolinae (“Eugratioleae” sensu Bentham 1846) then within subtribe Stemodinae (“Stemodieae” sensu Bentham and Hooker 1876); Wettstein (1891) also included *Stemodia* in the Gratioleae but did not use an infratribal classification. This placement was later corroborated by DNA sequence-based phylogenetic studies (Fritsch et al. 2007; Estes and Small 2008; Schäferhoff et al. 2010; Scatigna et al. 2018a).

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, being characterized by the bibrachiate filament. Subsequently Linnaeus (1759) published *Stemodia* L., based on Browne’s (1756) *Stemodiacra*, and *Stemodia maritima* L., based on *Stemodiacra maritima*; *Stemodia* was later conserved over *Stemodiacra*. During the following century, many species were described under *Stemodia* by several authors (ie. Kunth et al. 1818; Chamisso and

Schlechtendal 1828; Saint-Hilaire 1824; Bentham 1835). The first drastic change to the circumscription of this genus was made by Bentham (1846), who excluded 24 species from it, including several ones described earlier by himself (Bentham 1835), and transferring 13 taxa to *Limnophila* R.Br and the other 11 species into six different genera, namely *Conobea* Aubl., *Gratiola*, *Lindenbergia* Lehm., *Pterostigma* Benth., *Mazus* Lour. and *Vandellia* L. that also have anthers with separate thecae but exhibit differences in calyx and androecium morphology and/or in fruit dehiscence. Minod (1918) used morphological differences in habit, indument, style and fruits to segregate the following species into five monotypic genera: *Stemodiocris maritima* P.Browne nom. ileg. (= *Stemodia maritima* L.), *Lendneria humilis* (Sol.) Minod (= *S. verticillata* [Mill.] Hassl.), *Verena hassleriana* (Chodat) Minod (= *S. hassleriana* Chodat), *Valeria trifoliata* (Link) Minod (= *S. trifoliata* [Link] Reichb.) and *Chodaphyton ericifolium* (Kuntze) Minod (= *S. ericifolia* K.Schum.). This classification was not followed by subsequent authors, who adopted a broader concept of the genus (Barroso 1952; D’Arcy 1979; Turner and Cowan 1993a, 1993b; Fischer 2004; Souza and Giulietti 2009; Sosa and Dematteis 2013; Scatigna and Souza 2018; BFG 2018). Other currently accepted genera, *Darcya* B.L.Turner & C.Cowan, *Anamaria* V.C.Souza, and *Lindernia* All., comprise species previously treated under *Stemodia* (Turner and Cowan 1993b; Souza 2001; and Fischer et al. 2013, respectively). On the other hand, Barker (1990) argued that there were no significant differences between *Morgania* R.Br. and *Stemodia* and included all four species composing the former within the circumscription of the latter. Turner and Cowan (1993a) speculated that *Stemodia* s.l. consisted of a monophyletic assemblage, providing a list of highly inclusive morphological traits to delimit the genus such as “opposite subpinnate or pinnately veined leaves”, “flowers axillary or in terminal spikes” and “corollas mostly lavender to purple, zygomorphic with well-developed tubes”. Meanwhile, Souza and Giulietti (2009) recognized at least two distinct and morphological consistent assemblages, one characterized by the erect habit, sessile leaves with usually clasping base, short-pedicellate, bracteolate flowers frequently forming spiciform inflorescences, and the other characterized by the procumbent habit, clearly petiolate leaves and long-pedicellate, axillary flowers; the remaining species did not form a cohesive group. The emphasis on different morphological characters by different authors resulted in competing classifications and delimitations of *Stemodia*, which reinforces the need of thorough systematic studies in the genus to provide a stable generic classification for the tribe Gratioleae.

Previous DNA sequence-based phylogenetic studies in Gratioleae suggested the polyphyly of *Stemodia* in its current circumscription. Fritsch et al. (2007) used sequences both from the cpDNA (*rbcL* gene, 3' portion of the *ndhF* gene and the *trnL-trnF* and *matK/3'-trnK* intergenic spacers) and the nrDNA (ITS), and included five species of *Stemodia* in their analysis, recovering them in two main clades: the first in which *Stemodia verticillate* (Mill.) Hassl. and *S. suffruticosa* Kunth. formed a grade grouping with members of *Scoparia* L. and *Leucospora multifida* (Michx.) Nutt., and the second containing *Achetaria* Cham. & Schltdl. and *Otacanthus* Lindl. (= *Achetaria*) and in which the relationships of *S. durantifolia* (L.) Sw., *S. florulenta* Barker (identified as *S. glabra* Oerst.) and *S. schottii* Holz. were not resolved. Subsequently, Estes and Small (2008), based on sequences from the cpDNA (*ndhF* gene, *trnS-trnG* intergenic spacer and *trnG* intron), also included five species of *Stemodia*, replacing *S. durantifolia* for *S. maritima*, and found the same relationships. More recently, Scatigna et al. (2018a), based on sequences from both the cpDNA (*rpl16*, *rps16* and *trnL* introns and the *trnL-trnF* intergenic spacer) and the nrDNA (ITS1), sampled five species of *Stemodia*, three of them included for the first time in a phylogenetic study. These authors obtained similar results relatively to the previous studies except that they recovered one species of *Stemodia* in a third clade, not related to the two previously recovered clades: *S. foliosa* Benth. formed a grade with *S. verticillata* related to *Scoparia* spp.; *Stemodia microphylla* J.A.Schmidt formed a grade with *S. maritima*, related to *Achetaria* spp.; and *Stemodia stellata* B.L.Turner was recovered as sister group of *Philcoxia* P.Taylor & V.C.Souza and related to *Tetraulacium veroniciforme* Turcz. The sampling of these three studies in total represents less than 20% of the diversity of *Stemodia* and even less relatively to the whole tribe, which highlights the shortfall of knowledge regarding the phylogenetic relationships within and between genera of the tribe Gratioleae

One important step towards a stable generic classification is to provide a reliable and inclusive phylogeny. Based on a new DNA sequence-based phylogeny with improved taxa sampling in both the genus *Stemodia* and the tribe Gratioleae, we aim to 1) assess the circumscription of *Stemodia* sensu stricto and provide a new morphological delimitation of the genus; and 2) test the phylogenetic placement of the remaining members of *Stemodia* sensu lato and assess the taxonomic implications for their accommodation in other genera.

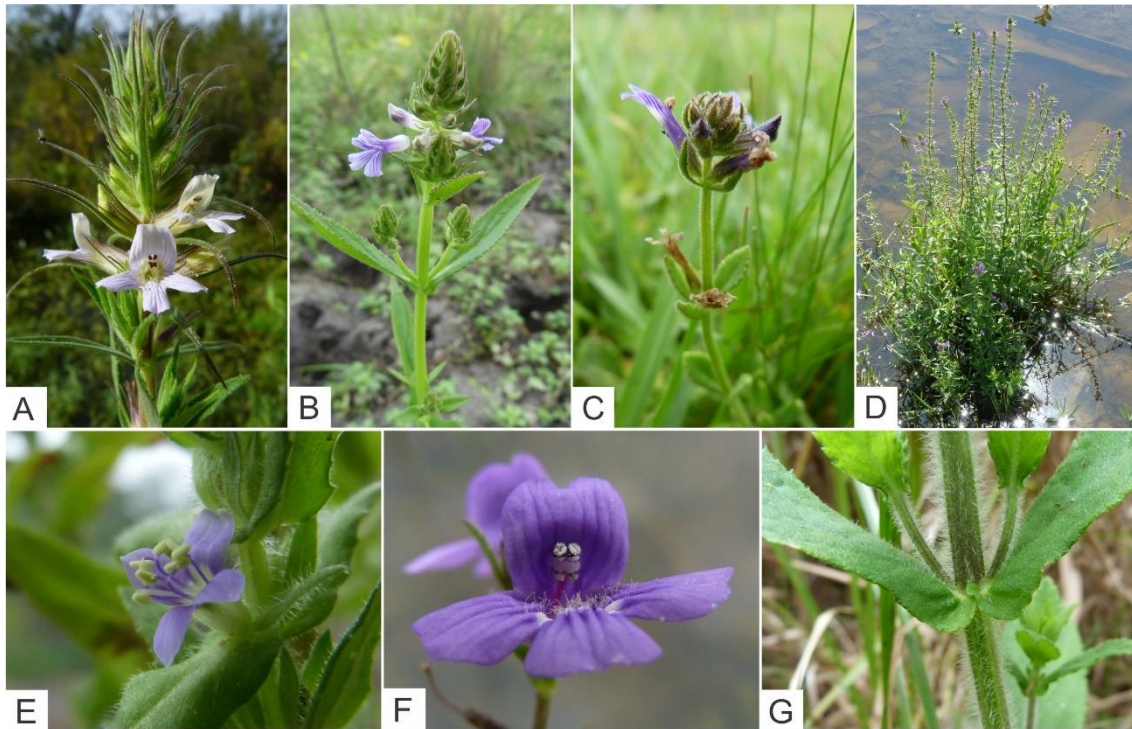


Figure 1. Representatives of *Stemodia* sensu stricto. A. Inflorescence of *S. lanceolata*. B. Inflorescence of *S. hyptoides*. C. Inflorescence of *S. stricta*. D. Habit of *S. lanceolata*. E. Flower of *S. maritima*. F. Flower of *S. palustris*. G. Sessile leaves with auriculate base of *S. hyptoides*.

2. Material and Methods

2.1. Taxonomy

For a better taxonomic understanding of the taxonomy of the study group, we consulted all Gratiioleae collections housed at the following herbaria: ALCB, BHCB, BHZB, CEN, CEPEC, CVRD, DIAM, EAC, ESA, HCJS, HRB, HRCB, HUEFS, IAC, IAN, ICN, MBM, MBML, MG, OUPR, R, RB, SP, SPF, UB, UEC, UFG and UPCB, in addition to the *Stemodia* s.l. collection from NY and S and digital images from G, K, MO, NY, and P databases; all abbreviations follow Thiers (2018).

2.2. Phylogenetic study

2.2.1. Taxon sampling

We improved upon the datasets published by Scatigna et al. (2018a) by including newly generated sequences and previously published sequences available at GenBank. We sampled 97 taxa, including 24 species of *Stemodia* s.l. and 67 species representing 16 other genera of tribe Gratiioleae. To test the monophyly of Gratiioleae, we included six species from two genera of its sister group, Angelonieae (Schäferhoff et al. 2010) were

included to test the monophyly of Gratioleae. *Plantago media* L., of tribe Plantagineae, was used as rooting taxon.

2.2.2. 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 markers were sequenced: the cpDNA *rps16*, and *trnL* introns and *trnL-trnF* intergenic spacer, and the nrDNA ITS1 intergenic spacer. Programs of polymerase chain reaction (PCR) amplification followed Scatigna et al. (2018a). Reactions were performed with the GoTaq Green Master Mix (Promega), with a final volume of 12.5 μ L composed of 6.25 μ L of the Master Mix, 0.75 μ L for both upstream and downstream primers at 5 μ M, 1 μ L template DNA, 1 μ L of bovine serum albumin (BSA) at 10 mg/mL, 2 μ L of dimethyl sulfoxide (DMSO) 5%, and Nuclease-Free water to 12.5 μ L. PCR products were generally purified with ExoSAP-IT PCR Product Cleaner (Thermo Fischer Scientific, Waltham, Massachusetts); 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 by using ca. 10 ng of DNA, 0.5 μ L of primer (the same used in PCRs) in a concentration of 5 pmol/ μ L, 2.0 μ L of Sequencing Buffer, 0.4 μ L of ABI Prism Big Dye Terminator vers. 3.1 (Applied Biosystems, Foster City, California), and distilled water in qsp for 10 μ L. Cleaning of sequence product was performed using 2.5 μ L of ethylenediamine tetra-acetic acid (EDTA) at 125mM and ethanol 100%, followed by a second cleaning with 30 μ L of ethanol 70% and further drying overnight at room temperature. Sequences were visualized on a 3500xL Genetic Analyzer (Applied Biosystems).

2.2.3. 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 improved with both newly generated and previously published (available at NCBI database) sequences, and then aligned with MAFFT version 7 (Kato and Standley 2013) using the *Auto* algorithm, followed by minor manual adjustments with Mesquite 3.10 (Maddison and Maddison 2016). The individual matrices were also combined into two datasets with Mesquite 3.10 (Maddison and Maddison 2016), one with the three cpDNA

regions (Partial Combined) and other with the ITS1 added to the Partial Combined (PC) dataset (Total Combined).

2.2.4. Phylogenetic analyses

Each DNA region separately, as well as both Partial and Total Combined datasets, were analyzed with two approaches: parsimony (MP) and Bayesian inference (BI). Best-fit nucleotide substitution models for each matrix were selected by using the Bayesian Information Criterion (BIC) in jModelTest2 (Guindon and Gascuel 2003; Darriba et al. 2012) on the CIPRES Science Gateway (Miller et al. 2010). Selected models were TVM + G for *rps16*, GTR + G for *trnL*, TVM + G for *trnL-trnF*, and GTR + I + G for ITS1. Bayesian inference was performed with MrBayes 3.2.6 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003), also on CIPRES; two parallel runs with four Markov Chain Monte Carlo (MCMC; Yang and 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 and Ronquist 2001), and clade posterior probabilities (PP) were assessed by a 50% majority-rule consensus tree generated in TreeAnnotator v1.5.4 (Rambaut and Drummond 2007) with the remaining trees.

Parsimony analyses were performed with PAUP* version 4.0 beta 10 (Swofford 2002). A heuristic search for most parsimonious trees (MPT) 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, and a 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 employing a full heuristic search with 1000 replicates, 250 RASR, three trees held at each step, TBR branch swapping with steepest descent, and MULTREES all in effect; 10 trees were saved for each RASR.

Tree files were opened and edited with FigTree v.1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

3. Results

3.1. Data matrices

In total, we generated 194 new DNA accessions from 52 specimens of 49 species. Our analyses included all the newly generated accessions and also 189 previously published sequences of representatives of Gratioleae, Angelonieae and *Plantago* available at GenBank. The Total Combined (TC) matrix consisted of 108 terminals and 2909 aligned characters, of which 875 (30%) were parsimony-informative, 1665 were constant, and 369 were variable but uninformative. Missing taxa in each matrix represented 10% (11/108) for *rps16*, 6% (7/108) for *trnL*, 7% (8/108) for *trnL-trnF*, and 21% (23/108) for ITS1. Detailed information of each dataset is presented in Table 1.

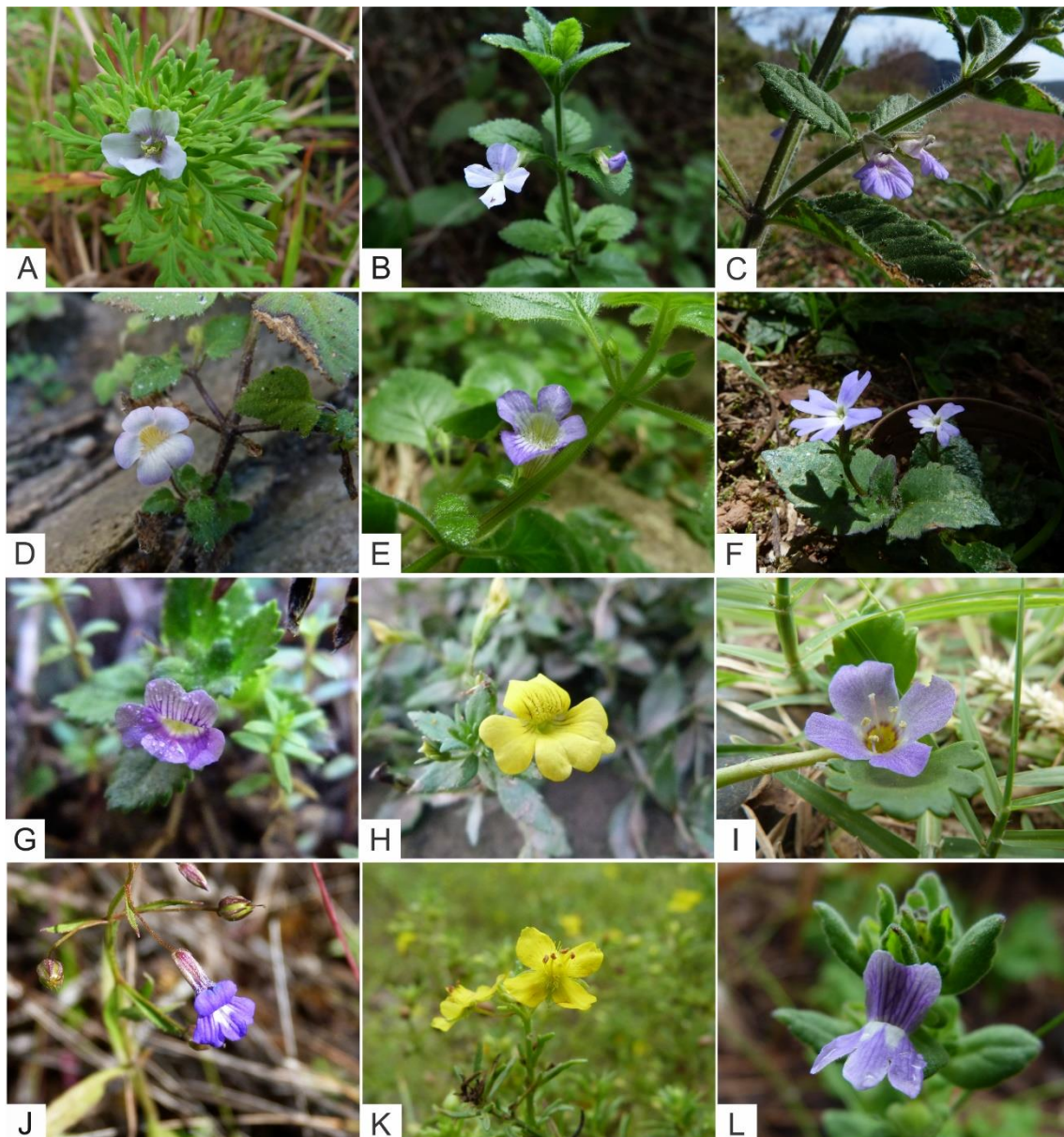


Figure 2. Representatives of *Stemodia* sensu lato and other genera of Gratioleae. A. *Stemodia hassleriana*. B. *S. trifoliata*. C. *S. foliosa*. D. *S. microphylla*. E. *S. veronicoides*. F. *S. vandellioides*. G. *S. verticillata*. H. *Mecardonia procumbens*. I. *Conobea glechomoides*. J. *C. scoparioides*. K. *Scoparia montevidensis*. L. *Achetaria erecta*.

3.2. Phylogenetic analyses

For each matrix, the BI approach resulted in completely resolved trees with generally higher support values relatively to the MP analyses. There were no significantly conflicting topologies (BS \geq 75% in the MP analysis, and PP \geq 0.95 in the Bayesian analysis) among trees generated by each approach in each matrix, except one incongruence within clade *Lapaea* in the ITS1 dataset, which has been discussed with detail by Scatigna et al. (in prep) and does not affect our discussion. The majority-rule consensus tree generated by the BI analysis of the Total Combined dataset was completely resolved and most nodes had strong support values (PP \geq 0.95); it is therefore the only one graphically presented and described below unless otherwise stated (Fig. 3).

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). Within the Gratioleae Clade, we identified six major clades (Clades 1 – 6 in Fig. 3).

Clade 1 (PP = 1.00; BS = 95) was composed of *Darcya*, *Mecardonia* Ruiz & Pav. and one species of *Stemodia*. It was formed by two subclades, one including the two sampled representatives of *Mecardonia* (PP = 0.9; BS = 89) and the other composed of *Stemodia vandellioides* (Benth.) V.C.Souza and *Darcya costaricensis* (B.L.Turner) B.L.Turner (PP = 0.92; BS = 89). This major clade was retrieved as sister to the clade (PP = 1.00; BS = 83) formed by Clades 2 to 6.

Clade 2 (PP = 1.00; BS = 100) included all representatives of *Bacopa* and part of *Conobea*. It was recovered as sister to a group (PP = 1.00; BS = 81) comprising the remaining clades of Gratioleae; it was composed by a subclade comprising two species of *Conobea* (PP = 1.00; BS = 95), including the type species *C. aquatica* Aubl., deeply nested within the sampled representatives of *Bacopa*.

Clade 3 (BS = 64) was formed by *Tetraulacium veroniciforme* as sister to a clade (PP = 1.00; BS = 97) composed of the sister clades *Lapaea* gen. ined. (PP = 0.95; 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*. It was formed by *Stemodia trifoliata* (Link) Rchb. and *S. foliosa* composing a grade related to a clade (PP = 0.98) comprising three subclades (A – C in Fig. 3). Subclade A (PP = 1.00; BS = 100) included *S. fruticosa* Lundell, *S. chiapensis* B.L.Turner and *S. angulata* Oerst. and was recovered as sister to

another clade (PP = 0.99; BS = 83) which in turn was formed by subclades B and C; the first (subclade B; PP = 1.00; BS = 100) was composed of all representatives of *Scoparia*, and the second (subclade C; PP = 1.00; BS = 72) was formed by clades C1 and C2. Clade C1 (PP = 1.00; BS = 98) included the two representatives of *Schistophragma* Benth. forming a grade, plus *Leucospora coahuilensis* Henrickson, *Stemodia jorullensis* Kunth and *S. palmeri* A.Gray whereas clade C2 (PP = 0.99; BS = 65) was formed by *Leucospora multifida*, *S. verticillata*, *S. hassleriana* Chodat and *Conobea glechomoides* (Spreng.) V.C.Souza

Clade 5 (PP = 1.00; BS = 93) included *Dopatrium*, *Gratiola*, *Hydrotriche* and *Limnophila*. It 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 (PP = 1.00; BS = 100) formed by *Dopatrium junceum* (Roxb.) Benth. and *Hydrotriche hottoniiflora* Zucc. as sister to a clade (PP = 1.00; BS = 98) composed of all sampled representatives of *Limnophila*.

Clade 6 (PP = 1.00; BS = 68) included *Dizygostemon* (Benth.) Wettst., *Achetaria* and *Stemodia*. It was formed by three subclades (D – F in Fig. 3). The first of them (subclade D; PP = 1.00; BS = 100) was composed of *Dizygostemon* as sister to the clade (PP = 1.00; BS = 100) formed by all sampled representatives of *Achetaria*; the second subclade (subclade E; PP = 1.00; BS = 100) was formed by two accessions of *Stemodia microphylla* in a clade (PP = 1.00; BS = 100) plus *S. veronicoides* J.A.Schmidt; and the third subclade (subclade F; PP = 1.00; BS = 100) was composed of *Stemodia maritima*, type species of the genus, as sister to a clade (PP = 0.96) comprising all remnant representatives of *Stemodia* recovered in subclade F. Subclade E was recovered either as sister to subclade D in the MP analysis (BS=66; not shown) or as sister of subclade F in the BI (PP=0.6). In the clade sister to *S. maritima* (within subclade F), all sampled representatives of *Stemodia* from Australia formed a well-supported monophyletic group (clade AUS; PP = 0.99; BS = 66) as well as the representatives of *Stemodia* from the New World (clade NW; PP = 1.00; BS = 75), apart from one accession of *S. hyptoides* Cham. & Schltdl., which placement was not clear and, therefore, obscured the relationship between clades AUS and NW. Relationships within clades AUS and NW were generally unresolved.

Table 1. Parsimony statistics and evolutionary model for each dataset.

Dataset	<i>rps16</i>	<i>trnL</i>	<i>trnLF</i>	<i>ITS1</i>	Partial Combined	Total Combined
Terminals	97	101	100	85	108	108
Missing taxa	10%	6%	7%	21%	—	—
Number of characters	1151	675	617	466	2443	2909
Constant characters	644	466	330	223	1440	1665
Variable, uninformative characters	157	74	91	49	322	369
Parsimony informative characters	350	135	196	194	681	875
% informative	0.30	0.20	0.32	0.42	0.28	0.30
Tree length	976	318	469	1296	1788	3131
Consistency index (CI)	0.565	0.657	0.642	0.330	0.595	0.476
Retention index (RI)	0.852	0.915	0.894	0.688	0.874	0.808
Evolutionary model (BIC)	TVM+ G	GTR+ G	TVM+ G	GTR+I+ G	—	—

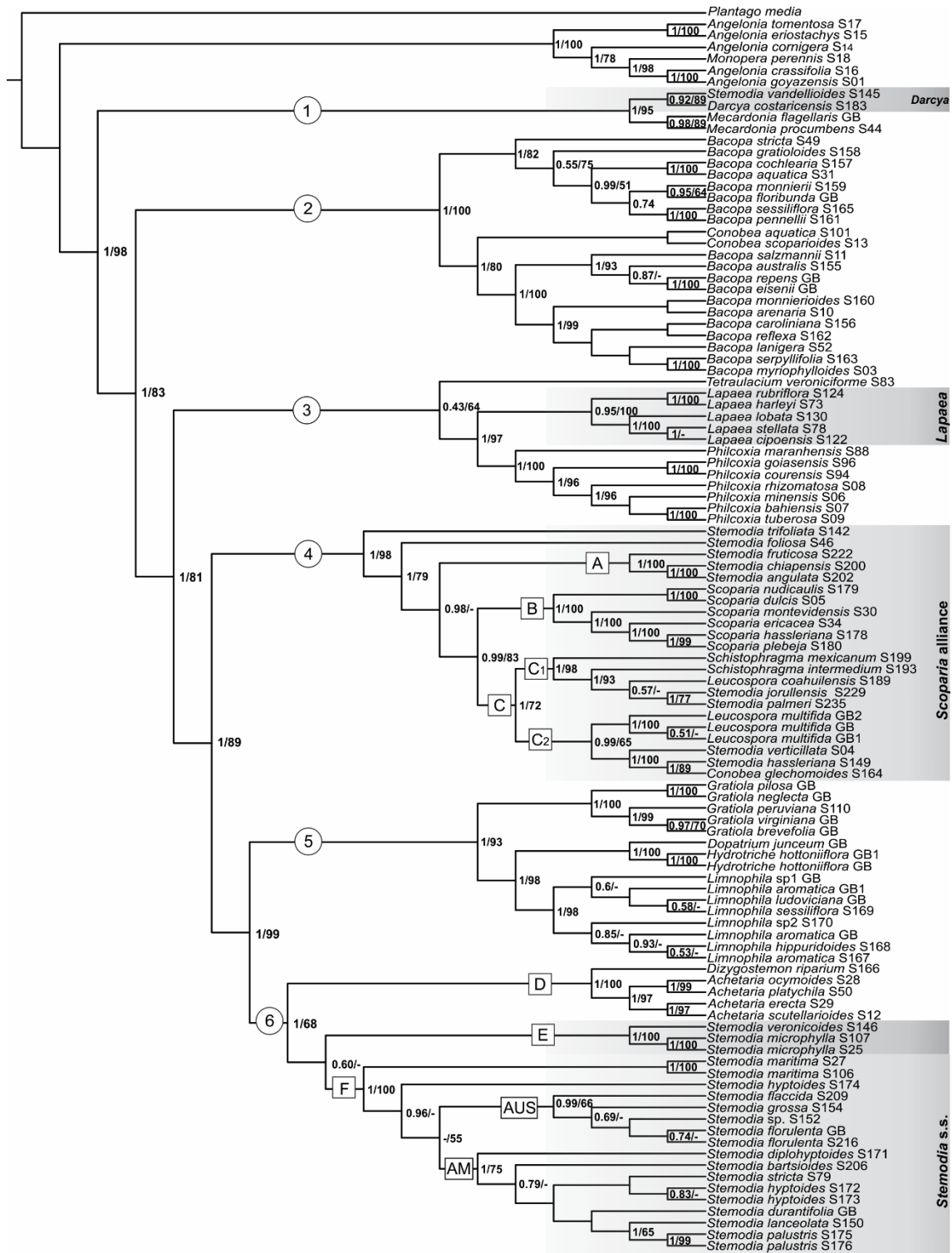


Figure 3. Majority-rule consensus tree generated by Bayesian inference based on the TC dataset, including sequences from the cpDNA (introns rps16 and trnL and intergenic spacer trnL-trnF) and the nrDNA (ITS1). Numbers next to nodes are posterior probability values/bootstrap support higher than 0.5 and 50% respectively. Clades denoted by numbers and letters are discussed in the text. Taxon names are followed by DNA template code.

4. Discussion

The circumscription of both the tribe Gratioleae and its genera have been long controversial due to the scarce phylogenetic studies published to date (Albach et al. 2005; Scatigna et al. 2018a). Our results corroborate the placement of sampled members of Gratioleae (sensu Albach et al. 2005) within this tribe, in accordance to previously published contributions (Fritsch et al. 2007, Estes and Small 2008, Scatigna et al. 2018a), and provide further evidence for critical reassessment in the circumscription of *Bacopa*, *Conobea*, *Leucospora* Nutt., *Scoparia* and *Stemodia*.

4.1. Polyphyly of *Stemodia* s.l.

We were able to include ca. 50% of the members *Stemodia* sensu lato in our analysis, including the type species *S. maritima* among other representatives of the genus from the New World and others from Australia, covering a wide range of both morphological variation and geographic distribution. Sampled representatives of *Stemodia* s.l. were recovered in three main clades, apart from that one containing the recently segregated genus *Lapaea* (Scatigna et al. in prep. [Cap. 1]). Here we characterize the main clades recovered in our study based on the TC dataset (Fig. 3) and discuss the taxonomic implications of our results.

4.2. Clade 6

4.2.1. Subclade F – *Stemodia* sensu stricto

Souza and Giulietti (2009) recognized a morphologically consistent group of Brazilian species of *Stemodia* characterized by the erect habit, sessile leaves with frequently clasping base, and bi-bracteolate, usually short-pedicellate and non-resupinate flowers. According to Sosa and 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*, *S. hyptoides*, *S. lanceolate* 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 F) along with other New World representatives (subclade AM) and all accessions from Australia (subclade AUS), which also exhibit a similar set of

morphological traits; this clade is referred here as *Stemodia* sensu stricto. Fritsch et al. (2007) obtained similar results, recovering *S. durantifolia*, *S. schottii* and *S. florulenta* (wrongly identified as *S. glabra*) in an unresolved topology, but related to *Achetaria* and *Otacanthus* (= *Achetaria*); *S. schottii* occurs in southern USA and northern Mexico and exhibits morphological traits consistent with *Stemodia* s.s., except for the unusual crawling habit. We examined the voucher of *S. glabra* and confirmed that it was in fact a specimen of *S. florulenta*, an exclusively Australian species that exhibits alternate leaves, a unique feature among the whole *Stemodia* s.l., but that was also retrieved within *Stemodia* s.s. in our analysis. Estes and Small (2008) also recovered *S. schotti* and *S. florulenta* (also mistakenly 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 southeastern 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., as herein proposed, is characterized by the erect habit, clearly sessile leaves with usually clasping base, bi-bracteolate, non-ressupinate and usually short-pedicellate flowers that are often concentrated at the apex of flowering branches, calyx well-divided into five equal sepals, and androecium composed of four fertile stamens with two equal and glabrous separated thecae in each anther. It encompasses part of the New World species of *Stemodia*, *S. serrata* from Africa, *S. viscosa* from Southeast Asia and Australia, and all exclusively Australian species but *S. debilis* Benth., of which overall aspect is closer to the concept of *Adenosma* Nees (petiolate leaves and dorsal sepal much larger than the others; Bentham 1846). A complete list of the accepted taxa assigned in our concept of *Stemodia* s.s. is presented in Table 2.

4.2.2. Subclade E – *Stemodia microphylla* and *S. veronicoides*

The second “natural group” recognized by Souza and Giulietti (2009) included all species transferred to *Lapaea* by Scatigna et al. (in prep) plus *Stemodia microphylla* and *S. veronicoides*, these described by Schmidt (1862). These last two species, according to Scatigna et al. (2017, in prep. [Cap. 1]), differ from *Lapaea* in the opposite leaves (vs. usually 3-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, in prep. [Cap. 1]) corroborate the segregation of *S. microphylla* from *Lapaea* and

its relationship with *Stemodia maritima* and *Achetaria* spp. In our contribution, we corroborate the close relationship between *S. microphylla* and *S. veronicoides* (Subclade E) and its exclusion from both *Lapaea* and *Stemodia* s.s. clades. According to our reconstruction, the inclusion of *S. microphylla* + *S. veronicoides* in our concept of *Stemodia* s.s. would not necessarily affect its monophylly but would drastically expand its morphological delimitation; these two species present crawling to ascending habit, petiolate leaves and long-pedicelate, resupinate flowers. Therefore, we believe that *S. microphylla* and *S. veronicoides* would be better included in a new genus segregated from *Stemodia* s.s. We are preparing a study with this taxonomic change.

4.2.3. Subclade D – *Achetaria* and *Dizygostemon*

Bentham (1846) considered *Achetaria* and *Dizygostemon* as two distinct sections of the genus *Beyrichia* Cham. & Schltdl. of the tribe Gratioleae, differing in the posterior pair of stamens being sterile in *Achetaria* and fertile in *Dizygostemon* and in the capsule dehiscence being 2-valved in *Achetaria* and 4-valved in *Dizygostemon*. Subsequently, Wettstein (1891), based on the differences presented by Bentham (1846), elevated *Dizygostemon* to the generic level. Souza and 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 and posterior bithecous). This putative phylogenetic relationship was corroborated by our results; the third subclade (subclade D) included in our clade 6 is formed by *Dizygostemon* sp. as sister to the clade composed of *Achetaria* spp.

The subtribe Stemodieae (sensu Bentham and Hooker 1876) was characterized by the anthers with separated thecae and comprised, besides *Beyrichia* (including *Achetaria* and *Dizygostemon*), seven other genera: *Adenosma*, *Hydrotriche* and *Limnophila* (recovered here in clade 5, along with *Gratiola*), *Lindenbergia* (currently in Orobanchaceae; Young 1999, Olmstead et al. 2001), *Morgania* (= *Stemodia* s.s.; Barker 1990), *Stemodia* s.l. (here recovered as polyphyletic), and *Tetraulacium* Turcz (recovered in clade 3 along with *Lapaea* and *Philcoxia*). *Adenosma*, with ca. 15 and distributed in the Old World (Fischer 2004), is the only genus of this subtribe that has never been

included in a molecular-based phylogenetic study. It has a wide variation in vegetative characters, but is characterized by the bi-bracteolate flowers, calyx with the posterior sepal much larger than the others, and androecium with four stamens with separated thecae and at least the anterior pair monothealous; this set of traits suggests a close relationship with *Achetaria* and *Dizygostemon*.

4.4. Clade 1 – *Darcya* and *Stemodia vandellioides*

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 within the tribe 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 separate thecae. More recently, Fischer et al. (2013), in a phylogenetic study of Linderniaceae, suggested that *S. vandellioides* should belong to *Lindernia* sensu stricto, 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 the tribe Gratioleae, but closely related to *Darcya costaricensis*, in our Clade 1, rather than within *Stemodia* s.s. (subclade F in Clade 6).

Darcya B.L.Turner & C.C.Cowan is a Central-American genus composed of three species originally described under *Stemodia* (D’Arcy 1979, Fernandez-Alonso 1987, Turner 1992, Turner and Cowan 1993c). According to Turner and 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. After a preliminary comparison between *Darcya* spp. and *Stemodia vandellioides*, we noticed that both taxa share angulate, sometimes sub-winged stems, leaves with 5-digitate main veins, absence of bracteoles, corolla salverform, and lower pair of stamens with reduced thecae. With a detailed study of herbarium material, we observed that the “3-lobed” lower lip of the corolla of *Darcya*, as described by D’Arcy (1979), Fernandez-Alonso (1987), and Turner (1992), is composed of two lateral lobes and a central lobule which in turn is formed by two fused corolla lobes, whereas the upper lip is formed by a single, emarginate corolla lobe; this means that, like *Stemodia vandellioides*, *Darcya* has resupinate flowers. Furthermore, the upper portion of the corolla tube, 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 of the upper stamen pair and the leaf-like (not differentiated) floral bracts. Unfortunately, we were able to generate sequence of only the *trnL* from a single accession of *Darcya* in our analysis, what may explain the low support for the clade *S. vandellioides* + *D. costaricensis*. Nevertheless, because of the morphological consistency observed, we suspect that *S. vandellioides* should be transferred to *Darcya*, broadening its circumscription.

4.3. Clade 4 – *Scoparia* alliance

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.

Scoparia is a cohesive and distinct assemblage of species which is 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 the tribe Sibthorpieae by Bentham (1846). Subsequently, Bentham and Hooker (1876) placed *Scoparia* in the 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 equaling that of the corolla lobes, and sagittate anthers (Souza and Giullietti 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 the tribe Gratiroleae. 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 2005, Fristch et al. 2007, Estes and Small 2008, Scatigna et al. 2018a). Our results support the phylogenetic placement of *Scoparia* within Gratiroleae and corroborate its monophyly.

Although Souza & Giullietti (2009) recognized part of Brazilian species of *Stemodia* into two morphological groups (see item 4.2), the remaining species (*S. foliosa*, *S. trifoliata*, *S. vandellioides* e *S. verticillata*) could not be assigned to a consistent assemblage. In our phylogeny, all these species (except *S. vandellioides*) in addition to *S. angulata*, *S. chiapensis*, *S. hassleriana*, *S. jorullensis* and *S. palmeri*, which were not treated by Souza & Giullietti (2009), were recovered within Clade 4, but not forming a

monophyletic group. Fritsch et al. (2007) and Estes and Small (2008) recovered *S. suffruticosa* in a clade with *S. verticillata*, *Leucospora multifida* and *Scoparia* spp. Part of these species of *Stemodia* were included by Bentham (1846) in the section *Diamoste*, 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 subgeneric division *Ebracteolatae*, being characterized by flowers lacking bracteoles.

Conobea sensu Wettstein (1891) was composed of three sections: 1) *Conobea* (“*Sphaerotheca*”), with globose capsules; 2) *Leucospora*, with ovoid capsules; and 3) *Schistophragma*, 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, indumenta, among others. The first group (sensu Wettstein 1891) is composed of *C. aquatica* (type of the genus), *C. scoparioides* (Cham. & Schldl.) Benth. and *C. punctata* Nees & Mart. and further discussed in item 4.4. as *Conobea* s.s.

Schistophragma and *Leucospora* are characterized by pinnatifid leaves, but the distinction between them is not clear (Turner and 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 similar to *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 is similar to *L. multifida*. The intermediate traits found in *L. coahuilensis* would be an indicative of the fragility of their generic boundaries. Dr. David Keil (pers. comm.) was preparing an account in which he would merge *Schistophragma* with *Leucospora*, making the appropriate combinations (Henrickson 1989, Turner and Cowan 1993), but the suspicion on more complex phylogenetic relationships led him not to proceed with these taxonomic changes. In our study, both genera were recovered as non-monophyletic groups, with *S. mexicanum*, *S. intermedia* and *L. coahuilensis* forming a grade related to *Stemodia jorulensis* and *S. palmeri* in a clade (subclade C₁) sister to another clade (subclade C₂) formed by *Leucospora multifida*, *Stemodia hassleriana* and *S. verticillata*.

Conobea glechomoides was first described under *Herpestis* C.F.Gaertn. by Sprengel (1827) and subsequently transferred by Chamisso and Schlechtendal (1828) to the monotypic genus *Geochorda* Cham. & Schltdl. under the illegitimate name *G. cuneata* Cham. & Schltdl. Kuntze (1898) finally provided the appropriate correct name *G. glechomoides* (Spreng.) Kuntze. Later, Souza and Giulietti (2009) claimed that there were no sufficient differences between *Geochorda* and *Conobea* and, therefore, created the combination *C. glechomoides*. However, these authors examined only one specimen and, probably because of that, they missed the nature of the anthers with two thecae separated by arm-like connectives (appropriately described and illustrated by Descole & Borsini 1954), and the lack of bracteoles beneath the calyx, being clearly divergent from *Conobea* as currently accepted, which is equivalent to *Conobea* section *Sphaerotheca* (sensu Bentham 1846); on the other hand, this set of features approximates it to *S. verticillata*. Furthermore, the shortly campanulate corolla is similar to that of *S. hassleriana*. In our study, *Conobea glechomoides* was recovered in a clade with *S. hassleriana*, *S. verticillata* and *Leucospora multifida*, relatively far-related to its congeners. In order to keep *Conobea* monophyletic, we propose the use of the name *Geochorda glechomoides* for this taxon until other taxonomic and nomenclatural changes (which are being prepared) are made.

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 older generic name in the group; this would imply in the loss of morphological cohesion and prediction, also principles of good systematics (Humphreys & Linder 2009), of a well-established and known genus. The alternative would be to retain *Scoparia* in its current concept and to recognize subclade C as a larger and more diverse *Leucospora*, including *Schistophragma*, *Conobea glechomoides* and part of *Stemodia* in its circumscription; in this case, we would have to recognize at least other three new genera, each one being represented by *Stemodia trifoliata*, *S. foliosa* and the subclade A, 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 taxonomic group.

The source of DNA of several taxa included in clade 4 was herbarium samples, and for most of them we were able to sequence only one or other marker and sometimes even incomplete sequences (ie. *rps16*). On the other hand, several species that could be assigned to this heterogeneous group (ie. *Stemodia peduncularis* Benth., *S. macrantha*

B.L.Rob., *S. pusilla* Benth., *S. tenuifolia* Minod, among others) were not sampled. An increased sampling and sequencing of additional regions (ie. complete ITS and *ndhF* gene) would help elucidating relationships within clade 4 that, associated with morphological support, would be crucial for making taxonomic changes that would be precipitated if made at this point.

4.4. Clade 2 – *Bacopa* and *Conobea* s.s.

Bacopa, in its current circumscription, is also one of the most diverse genera in the tribe Gratioleae, comprising ca. 50 – 60 spp. mainly distributed in the Tropics, especially in America (Sosa et al. 2018). The high morphological variation has led to the description of several genera now under the synonymy of *Bacopa* (Pennell 1946; D’Arcy 1979; Souza and Giulietti 2009; Sosa et al. 2018). Our results corroborate the inclusion of sampled species of *Bacopa* within this genus, but also point to its paraphyly due to the placement of members of *Conobea* embedded within it.

Bacopa is defined by its unequal sepals, the outer three being broader and inclosing the two, equally narrower inner sepals and by the anther thecae being closely sessile and converging at least at the apex (Pennell 1946; D’Arcy 1979; Sosa et al. 2018). Nevertheless, some species of *Bacopa* (i.e. *B. gratiolooides* [Cham.] Edwall, *B. depressa* [Benth.] Edwall, *B. reflexa* [Benth.] Edwall) have the sepals more or less alike (Souza and Giulietti 2009).

Conobea sect. *Conobea* (“*Sphaerotherca* sensu Wettstein 1891) includes the type species of the genus, *C. aquatica*, plus *C. punctata* and *C. scoparioides* and therefore should be referred to as *Conobea* sensu stricto. It is characterized by the sepals more or less alike, by the converging thecae and by the globose capsule (Souza and Giulietti 2009).

According to our results, the recognition of *Conobea* as a genus would imply 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 the tribe Gratioleae. Alternatively, merging *Conobea* and *Bacopa* in a single genus would not affect the morphological recognition of a currently heterogeneous group. We are preparing an account that deals with the transfer of members of *Conobea* s.s. into *Bacopa* (Scatigna et al. in prep.).

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 and Giulietti 2009; Scatigna and Mota 2017). Our results corroborate retaining *Bacopa reflexa* in the latter.

4.5. 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 within *Philcoxia* were discussed in detailed by Scatigna et al. 2018, whereas those of *Lapaea* were presented by Scatigna et al. (in prep). Although there is an incongruence between the ITS1 and PC datasets regarding the relationships among *L. cipoensis* comb. ined., *L. lobata* comb. ined. and *L. stellata* comb. ined., the composition of the clade is the same in both datasets and therefore we combined them in the TC dataset.

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

This clade encompasses four genera of mainly semi-aquatic or aquatic herbs with frequently very specialized features, such as leaf dimorphism and cleistogamy (Philcox 1970; Fischer 2004). The neotropical genus *Gratiola* was recovered here as a monophyletic group, in accordance with Estes and Small (2008), sister to a clade formed by the exclusively Old World genera *Dopatrium* Buch.-Ham. ex Benth., *Hydrotriche* and *Limnophila*. Our results suggest that *Dopatrium* and *Hydrotriche* are sister groups, but our limited sampling does not allow us to infer their monophyly. *Limnophila* was included by Bentham and Hooker (1876) within the tribe Stemodieae, along with *Stemodia* and other genera that have anthers with separated thecae. Our results corroborate a closer relationship with members of subtribe Dopatriineae (sensu Fischer 2004). Philcox (1970) used the capsule dehiscence to differentiate *Limnophila* from *Stemodia*, but we observed better diagnostic features such as flower resupination (at least in long-pedicellate species) and coherence of anthers present in *Limnophila* and absent in *Stemodia* s.s. Some species of *Limnophila* have leaf dimorphism, that is, submerged leaves that are pinnatisect in opposition to the aerial leaves that are entire; this dimorphism is also lacking in *Stemodia* s.s.

5. Conclusion

The taxonomic classification at the generic level, as a task of current systematics, seeks some basic principles such as 1) monophyly of taxa; 2) nomenclatural stability; 3) minimization of redundancy; 4) support for monophyly of groups; and 5) ease in groups recognition (Backlund & Bremer 1998; Stevens 2018). The traditional concept of *Stemodia* s.l. seems not to meet any of these principles. It is not a monophyletic group; nor has its nomenclature been stable; there are several species segregated from *Stemodia* s.s. that have been and could be assigned into monotypic genera; depending on the generic circumscription, its morphological delimitation may not be consistent.

According to Humphreys & Linder (2009), “‘good’ genera are predictive and stable”. We could define a monophyletic and morphological consistent *Stemodia* s.s. However, we feel that a more thorough taxonomic, morphological and nomenclatural investigation in the tribe Gratioleae is needed in order to support taxonomic changes aiming to accommodate the segregated species of *Stemodia* s.l. Only so we will be able to provide a new and more stable generic classification within Gratioleae, that could be informative and accessible to the general public, including not only systematists, but also ecologists and conservationists, among other professionals, and even lay enthusiasts of botanical identification.

Table 2. List of currently accepted species of *Stemodia* assigned to the *Stemodia* sensu stricto group.

<i>Stemodia bartsioides</i> Benth.	<i>Stemodia lobelioides</i> Lehm.
<i>Stemodia diplohyptoides</i> M.M.Sosa & Dematt.	<i>Stemodia lythrifolia</i> F.Muell ex Benth.
<i>Stemodia durantifolia</i> (L.) Sw.	<i>Stemodia maritima</i> L.
<i>Stemodia flaccida</i> W.Fitzg.	<i>Stemodia palustris</i> A.St.Hil.
<i>Stemodia florulenta</i> W.R.Barker	<i>Stemodia perfoliata</i> Scatigna & V.C.Souza
<i>Stemodia glabella</i> W.R.Barker	<i>Stemodia pubescens</i> (R.Br.) W.R.Barker
<i>Stemodia grossa</i> Benth.	<i>Stemodia schottii</i> Holz.
<i>Stemodia hyptoides</i> Cham. & Schltldl.	<i>Stemodia scoparioides</i> Hassl. ex Minod
<i>Stemodia kingii</i> F.Muell.	<i>Stemodia serrata</i> Benth.
<i>Stemodia lanata</i> Ruiz & Pav. ex Benth	<i>Stemodia</i> sp. ined.
<i>Stemodia lanceolata</i> Benth.	<i>Stemodia stricta</i> Cham. & Schltldl.
<i>Stemodia lathraia</i> W.R.Barker	<i>Stemodia tephropelina</i> W.R.Barker
<i>Stemodia linophylla</i> F.Muell.	<i>Stemodia viscosa</i> Roxb.

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Appendix 1. Voucher information (accession numbers will be included for paper submission). GB = GenBank.

Genus	species	code	Collector	Col. Number	Herbarium
<i>Achetaria</i>	<i>crenata</i>	50	G. H. Shimizu	s.n.	UEC
<i>Achetaria</i>	<i>erecta</i>	29	V. C. Souza	28935	ESA
<i>Achetaria</i>	<i>ocymoides</i>	28	C. D. N. Rodrigues	103	ESA
<i>Achetaria</i>	<i>scutellarioides</i>	12	A. V. Scatigna	110	UEC
<i>Angelonia</i>	<i>cornigera</i>	14	A. V. Scatigna	119	UEC
<i>Angelonia</i>	<i>crassifolia</i>	16	A. V. Scatigna	73	UEC
<i>Angelonia</i>	<i>eristachys</i>	15	A. V. Scatigna	67	UEC
<i>Angelonia</i>	<i>goyazensis</i>	1	A. V. Scatigna	48	UEC
<i>Angelonia</i>	<i>tomentosa</i>	17	A. V. Scatigna	117	UEC
<i>Bacopa</i>	<i>aquatica</i>	31	V. C. Souza	28715	ESA
<i>Bacopa</i>	<i>arenaria</i>	10	A. V. Scatigna	272	UEC
<i>Bacopa</i>	<i>australis</i>	155	A. V. Scatigna	1218	UEC
<i>Bacopa</i>	<i>caroliniana</i>	156	A. V. Scatigna	1234	UEC
<i>Bacopa</i>	<i>cochlearia</i>	157	A. V. Scatigna	1223	UEC
<i>Bacopa</i>	<i>floribunda</i>	GB			
<i>Bacopa</i>	<i>gratioloides</i>	158	A. V. Scatigna	533	UEC
<i>Bacopa</i>	<i>lanigera</i>	52	I. G. Costa	150	GFJP
<i>Bacopa</i>	<i>monnierii</i>	159	A. V. Scatigna	1149	UEC
<i>Bacopa</i>	<i>monnieroides</i>	160	N. F. O. Mota	3432	MG
<i>Bacopa</i>	<i>myriophylloides</i>	3	A. V. Scatigna	270	UEC
<i>Bacopa</i>	<i>pennellii</i>	161	N. F. O. Mota	3259	MG
<i>Bacopa</i>	<i>reflexa</i>	162	N. F. O. Mota	3373	MG
<i>Bacopa</i>	<i>repens</i>	GB			
<i>Bacopa</i>	<i>salzmanni</i>	11	A. V. Scatigna	277	UEC
<i>Bacopa</i>	<i>serpyllifolia</i>	163	A. V. Scatigna	797	UEC
<i>Bacopa</i>	<i>sessiliflora</i>	165	N. F. O. Mota	3254	MG
<i>Bacopa</i>	<i>stricta</i>	49	A. V. Scatigna	404	UEC
<i>Bacopa</i>	<i>eisenii</i>	GB			
<i>Conobea</i>	<i>aquatica</i>	101	N. F. O. Mota	794	UEC
<i>Conobea</i>	<i>glechomoides</i>	164	A. V. Scatigna	1159	CTES
<i>Conobea</i>	<i>scoparioides</i>	13	A. V. Scatigna	99	UEC
<i>Darcya</i>	<i>costaricensis</i>	183	F. Almeda	6919	NY
<i>Dizygostemon</i>	<i>sp</i>	166	R. Telles	s.n.	UEC
<i>Dopatrium</i>	<i>junceum</i>	GB			
<i>Gratiola</i>	<i>brevifolia</i>	GB			
<i>Gratiola</i>	<i>neglecta</i>	GB			
<i>Gratiola</i>	<i>peruviana</i>	110	A. V. Scatigna	666	UEC
<i>Gratiola</i>	<i>pilosa</i>	GB			
<i>Gratiola</i>	<i>virginiana</i>	GB			
<i>Hydrotriche</i>	<i>hottoniiflora</i>	GB			
<i>Hydrotriche</i>	<i>hottoniiflora</i>	GB1			
<i>Lapaea</i>	<i>cipoensis</i>	122	A. V. Scatigna	1119	UEC
<i>Lapaea</i>	<i>harleyi</i>	73	A. V. Scatigna	651	UEC

<i>Lapaea</i>	<i>lobata</i>	130	A. V. Scatigna	1143	UEC
<i>Lapaea</i>	<i>rubriflora</i>	124	A. V. Scatigna	1035	UEC
<i>Lapaea</i>	<i>stellata</i>	78	A. V. Scatigna	479	UEC
<i>Leucospora</i>	<i>coahuilensis</i>	189	Chiang	9631	NY
<i>Leucospora</i>	<i>multifida</i>	GB			
<i>Leucospora</i>	<i>multifida</i>	GB1			
<i>Leucospora</i>	<i>multifida</i>	GB2			
<i>Limnophila</i>	<i>aromatica</i>	167	A. V. Scatigna	1233	UEC
<i>Limnophila</i>	<i>aromatica</i>	GB			
<i>Limnophila</i>	<i>aromatica</i>	GB1			
<i>Limnophila</i>	<i>hippuridoides</i>	168	A. V. Scatigna	1232	UEC
<i>Limnophila</i>	<i>sessiliflora</i>	169	A. V. Scatigna	1235	UEC
<i>Limnophila</i>	<i>sp1</i>	GB			
<i>Limnophila</i>	<i>spv</i>	170	A. V. Scatigna	1236	UEC
<i>Limnophila</i>	<i>x ludoviciana</i>	GB			
<i>Mecardonia</i>	<i>flagellaris</i>	GB			
<i>Mecardonia</i>	<i>procumbens</i>	44	F. S. Mayer	1356	UEC
<i>Monopera</i>	<i>perennis</i>	18	M. A. Farinaccio	958	CGMS
<i>Philcoxia</i>	<i>bahiensis</i>	7	A. V. Scatigna	107	UEC
<i>Philcoxia</i>	<i>maranhensis</i>	88	A. R. Barbosa	1100	HUEFS
<i>Philcoxia</i>	<i>minensis</i>	6	A. V. Scatigna	42	UEC
<i>Philcoxia</i>	<i>rhizomatosa</i>	8	A. V. Scatigna	319	UEC
<i>Philcoxia</i>	<i>tuberosa</i>	9	A. V. Scatigna	121	UEC
<i>Philcoxia</i>	<i>courensis</i>	94	A. V. Scatigna	707	UEC
<i>Philcoxia</i>	<i>goiasensis</i>	96	A. V. Scatigna	736	UEC
<i>Plantago</i>	<i>media</i>		UofC 55665 (UAC)**, Ronsted, N. 50 (C)***		
<i>Schistophragma</i>	<i>intermedium</i>	193	Spellenberg	6829	NY
<i>Schistophragma</i>	<i>mexicanum</i>	199	Steinmann	1883b	NY
<i>Scoparia</i>	<i>dulcis</i>	5	A. V. Scatigna	102	UEC
<i>Scoparia</i>	<i>ericacea</i>	34	C. D. N. Rodrigues	102	ESA
<i>Scoparia</i>	<i>hassleriana</i>	178	A. V. Scatigna	1167	UEC
<i>Scoparia</i>	<i>montevidensis</i>	30	V. C. Souza	32411	ESA
<i>Scoparia</i>	<i>nudicaulis</i>	179	A. V. Scatigna	1207	UEC
<i>Scoparia</i>	<i>plebeja</i>	180	A. V. Scatigna	1174	CTES
<i>Stemodia</i>	<i>angulata</i>	202	Lugo	2130	NY
<i>Stemodia</i>	<i>bartsioides</i>	206	McVaugh	16935	NY
<i>Stemodia</i>	<i>chiapensis</i>	200	Breedlove	56329	NY
<i>Stemodia</i>	<i>diplohyptoides</i>	171	A. V. Scatigna	1175	CTES
<i>Stemodia</i>	<i>durantifolia</i>				
<i>Stemodia</i>	<i>flaccida</i>	209	Selling	S17-8347	S
<i>Stemodia</i>	<i>florulenta</i>	216	Hirst	84	S
<i>Stemodia</i>	<i>florulenta</i>	GB			S
<i>Stemodia</i>	<i>foliosa</i>	46	A. V. Scatigna	382	UEC
<i>Stemodia</i>	<i>fruticosa</i>	222	Stevens	17314	S
<i>Stemodia</i>	<i>grossa</i>	154			S
<i>Stemodia</i>	<i>hassleriana</i>	149	A. V. Scatigna	1217	UEC

<i>Stemodia</i>	<i>hyptoides</i>	172	A. V. Scatigna	1155	CTES
<i>Stemodia</i>	<i>hyptoides</i>	173	M. M. Sosa	s.n.	CTES
<i>Stemodia</i>	<i>hyptoides</i>	174	A. V. Scatigna	1201	UEC
<i>Stemodia</i>	<i>jurullensis</i>	229	Ripley	14823	NY
<i>Stemodia</i>	<i>lanceolata</i>	150	A. V. Scatigna	1212	UEC
<i>Stemodia</i>	<i>maritima</i>	106	I. G. Costa	667	RB
<i>Stemodia</i>	<i>maritima</i>	27	V. C. Souza	28674	ESA
<i>Stemodia</i>	<i>microphylla</i>	107	A. V. Scatigna	480	UEC
<i>Stemodia</i>	<i>microphylla</i>	25	V. C. Souza	28444	ESA
<i>Stemodia</i>	<i>palmeri</i>	235	Orcutt	6481	NY
<i>Stemodia</i>	<i>palustris</i>	175	A. V. Scatigna	1156	CTES
<i>Stemodia</i>	<i>palustris</i>	176	A. V. Scatigna	1158	CTES
<i>Stemodia</i>	<i>spn</i>	152	Nordenstam & Andenberg	237	S
<i>Stemodia</i>	<i>stricta</i>	79	A. V. Scatigna	674	UEC
<i>Stemodia</i>	<i>trifoliata</i>	142	A. V. Scatigna	509	UEC
<i>Stemodia</i>	<i>vandellioides</i>	145	A. V. Scatigna	571	UEC
<i>Stemodia</i>	<i>veronicoides</i>	146	A. V. Scatigna	1105	UEC
<i>Stemodia</i>	<i>verticillata</i>	4	A. V. Scatigna	81	UEC
<i>Tetraulacium</i>	<i>veroniciforme</i>	83	N. F. O. Mota	2787	MG

CAPÍTULO 3.

Produção científica relacionada à tese

Durante a execução deste projeto, realizamos diversas expedições de coleta, além de visitas a vários herbários para estudar espécimes da tribo Gratioleae. Estas atividades resultaram em algumas novidades taxonômicas para o gênero *Stemodia* sensu lato, entre elas duas novas espécies endêmicas do Brasil e uma nova ocorrência para o país. Neste capítulo, apresentamos os resumos dos trabalhos publicados, resultantes destas descobertas, e suas respectivas referências.

1. *Stemodia cipoensis* (Plantaginaceae): A new species from Serra do Cipó, Minas Gerais, Brazil.

Abstract:—The Serra do Cipó, part of the central portion of the Espinhaço Range in Minas Gerais, Southeast Brazil, is known to have been extensively investigated over the past three decades, especially because of the “Flora da Serra do Cipó” project. Nevertheless, some areas have been poorly collected as the efforts were largely concentrated near the roads. Various new species, in several families, have been recently discovered in the *campos rupestres* of the Serra do Cipó. Here we report the discovery of a new species of *Stemodia* (Plantaginaceae) from the northwestern slopes. We describe and illustrate the new species based on morphological and micro-morphological evidence, provide information on its taxonomy, habitat, geographical distribution, and conservation status, along with a key to distinguish it from the related species. *Stemodia cipoensis* is similar to *S. harleyi*, *S. lobata*, and *S. stellata*, being characterized by the presence of long, stiff, curved, eglandular trichomes. It has been assessed as CE (critically endangered) under criteria B1ab(iii) and B2ab(iii), following IUCN guidelines.

Scatigna A.V., Souza V.C. and Simões A.O. 2017. *Stemodia cipoensis* (Plantaginaceae): A new species from Serra do Cipó, Minas Gerais, Brazil. *Systematic Botany* 42: 371–377. <https://doi.org/10.1600/036364417X695583>

2. *Stemodia perfoliata* (Plantaginaceae): A 200 year old new species from the Caatinga of Minas Gerais, Brazil.

Abstract:—A new species of *Stemodia* (Plantaginaceae) from the Caatinga of northern Minas Gerais, Brazil, is described and illustrated. *Stemodia perfoliata* was first collected by Auguste de Saint-Hilaire, ca. 200 years ago, and remained unnamed until now. The new species is characterized by the connate-perfoliate leaves, a unique feature in the genus. We provide notes on morphology, geographic distribution and conservation status of *S. perfoliata*, along with a key to differentiate it from the similar species that occur in Brazil: *S. durantifolia*, *S. hyptoides*, *S. maritima*, *S. palustris* and *S. stricta*.

Scatigna A.V., Souza V.C. and Simões A.O. 2018a. *Stemodia perfoliata* (Plantaginaceae): A 200 year old new species from the Caatinga of Minas Gerais, Brazil. *Brittonia* 70: 252–256. <https://doi.org/10.1007/s12228-017-9518-9>

3. The identity of *Stemodia lanceolata* (Plantaginaceae) and its occurrence in Brazil.

Abstract:—In this contribution, we reassess the identity of *Stemodia lanceolata* (Plantaginaceae) and confirm its occurrence in Brazilian territory. We present a detailed and updated description, a fine illustration, and photographs of this species, along with comments on its distribution, habitat and phenology, and notes on taxonomic affinities. *Stemodia lanceolata* is characterized by its stiffy erect terminal inflorescence with filamentous aspect due to the long, linear-triangular, frequently out-curved floral bracts. Finally, we propose second-step lectotypifications for three names subordinated to *S. lanceolata*.

Scatigna A.V., Sosa M.M., Souza V.C. and Simões A.O. 2018b. The identity of *Stemodia lanceolata* (Plantaginaceae) and its occurrence in Brazil. *Phytotaxa* 375: 121–129 <https://doi.org/10.11646/phytotaxa.375.1.9>

CONCLUSÃO GERAL

Os resultados desta tese contribuem para um melhor esclarecimento do polifiletismo de *Stemodia* sensu lato e apontam para uma necessidade de se reverem circunscrições de outros gêneros de Gratioleae como *Bacopa*, *Conobea*, *Leucospora*, *Schistophragma* e *Scoparia*. Além disso, a amostragem de grupos do Velho Mundo como *Adenosma*, *Dopatrium*, *Hydrotriche* e *Limnophila* em filogenias moleculares ainda está bastante limitada, o que pode esconder ainda mais casos de não monofiletismo dentro da tribo.

O reconhecimento de *Lapaea* como um novo gênero que compreende cinco espécies com distribuição restrita a conhecidos centros de endemismo na Cadeia do Espinhaço levanta a possibilidade do uso de novos grupos modelo para estudos de biogeografia relacionados à origem e diversificação de plantas em campos rupestres e na própria Cadeia do Espinhaço.

Apesar de termos conseguido definir *Stemodia* sensu stricto como um grupo monofilético e morfológicamente bem delimitado, sentimos que, antes de tomarmos decisões sob uma perspectiva mais inclusiva (*lumper*) ou uma mais restritiva (*splitter*) acerca da redefinição de outros gêneros para a acomodação de espécies segregadas de *Stemodia*, (ex. *Stemodia vandellioides*; *S. microphylla* e *S. veronicoides*; *S. trifoliata*, *S. foliosa* e *S. hassleriana* entre outras), necessitamos realizar um estudo taxonômico, morfológico e nomenclatural mais aprofundado na tribo, de forma a gerar dados que sustentem uma nova classificação estável, informativa e acessível para o público em geral, que inclui não só sistematas, mas também ecólogos e conservacionistas, entre outros profissionais, e até mesmo entusiastas da identificação botânica.

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ANEXOS




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DECLARAÇÃO

Em observância ao **§5º do Artigo 1º da Informação CCPG-UNICAMP/001/15**, referente a Bioética e Biossegurança, declaro que o conteúdo de minha Tese de Doutorado, intitulada "**SYSTEMATICS OF GRATIOLEAE (PLANTAGINACEAE): REDEFINING STEMODIA L.**", desenvolvida no Programa de Pós-Graduação em Biologia Vegetal do Instituto de Biologia da Unicamp, não versa sobre pesquisa envolvendo seres humanos, animais ou temas afetos a Biossegurança.

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