Global biogeography of Acantheae and taxonomic revision of *Stenandrium* Nees (Acanthaceae) in Brazil

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Tese apresentada ao Programa de Pós-Graduação em Botânica
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ORIENTADORA:

CAROLYN ELINORE BARNES PROENÇA

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Ao papai, que sonhava que um

dia eu descobrisse uma espécie

nova muito antes de eu me

interessar em ser taxonomista.

E à tia Ciça, que adorava

plantinhas pequenas.

“Our imagination is struck only by what is great;

but the lover of natural philosophy should

reflect equally on little things.”

Alexander von Humboldt

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ÍNDICE

RESUMO

Acanthaceae é uma família ecologicamente importante nos ecossistemas tropicais. Possui alta diversidade funcional de flores e polinizadores generalistas e especialistas, incluindo abelhas, beija-flores, mariposas, morcegos e borboletas. Acantheae, a segunda maior tribo da família, apresenta padrões biogeográficos intrigantes que foram pouco explorados. *Stenandrium* Nees é um gênero pouco conhecido e taxonomicamente complexo de Acantheae, com altos níveis de endemismos no Neotrópico. Nós reconstruímos a história biogeográfica global de Acantheae, com uma filogenia molecular atualizada e datada dos principais gêneros da tribo. Contribuições para a taxonomia de *Stenandrium* são fornecidas, incluindo a descrição de duas novas espécies e uma revisão das espécies brasileiras, que formam um grupo monofilético de espécies. Nossos resultados indicam que alguns eventos e fatores influenciaram a diversificação da tribo ao longo do tempo. A maior diversificação de Acantheae foi na "Era das Ervas", no Mioceno tardio. Alguns eventos de dispersão a longa-distância foram cruciais para a diversificação da tribo, principalmente um a partir da África para a América, no Eoceno-Oligoceno. Encontramos fortes evidências que o surgimento dos beija-flores impulsionou a diversificação de gêneros do clado *Aphelandra*. Finalmente, devido aos intrigantes padrões biogeográficos e de diversidade, à beleza, à raridade e às propriedades medicinais potenciais dessa família, devemos concluir enfatizando a necessidade de pesquisas tanto básicas quanto avançadas com essas plantas.

ABSTRACT

Acanthaceae is an ecologically important family in tropical ecosystems. With high functional diversity of flowers, it has both generalist and specialist pollinators, including bees, hummingbirds, moths, bats and butterflies. Acantheae, the second largest tribe in the family, shows intriguing and unexplored biogeographic patterns. *Stenandrium* Nees is a poorly known and taxonomically complex genus of Acantheae, with high levels of endemism within the Neotropics. We reconstructed the global biogeographical history of Acantheae, using an updated dated molecular phylogeny of the main genera in the tribe. Contributions to the taxonomy of *Stenandrium* are provided herein, including a revision of the Brazilian species, which apparently form a monophyletic group of species. We also describe and illustrate two new species from a region of high endemism in southeastern Brazil. Our results indicate that certain events and factors influenced the diversification of the tribe along time. The greatest diversification of Acantheae was at the "Age of the Herbs", in late Miocene (11.6-5.3 Mya). Some long-distance dispersion events were crucial for the tribe's diversification, especially from Africa to the Americas at the Eocene-Oligocene, 33.6 Mya. We found strong evidence that the emergence of hummingbirds boosted the diversification of genera in the Aphelandra clade. Finally, due to the intriguing biogeographic and diversity patterns, the beauty, the rarity, and the potential medicinal properties of this family, we must conclude by emphasizing the necessity of either basic or advanced research with these plants.

INTRODUÇÃO GERAL

A família Acanthaceae Juss

Acanthaceae é uma família grande (~4,000 spp.) da ordem Lamiales, morfologicamente complexa, com uma alta diversidade de tipos de pólen, que é bem representada nos registros fósseis (Scotland and Vollesen 2000; Tripp and McDade 2014).

Os registros fósseis e moleculares atualmente disponíveis indicam que Acanthaceae se originou cerca de 81,9 milhões de anos atrás, no Cretáceo Tardio, mas a origem geográfica da família ainda é desconhecida. Apresenta padrões biogeográficos intrigantes, com muito eventos de disjunção (entre os hemisférios leste e oeste) e assimetrias na diversidade entre clados-irmãos. Além disso, existem fortes evidências que muitos eventos de dispersão a longa distância ocorreram do Velho Mundo para o Novo Mundo (Tripp and McDade, 2014).

É também uma das famílias ornamentais importantes devido à beleza de sua folhagem, enorme variabilidade floral e alta taxa de sucesso de reprodutivo por propagação vegetativa (Judd et al. 2009). São componentes importantes das comunidades tropicais e subtropicais em todo o mundo e são adaptadas a diversos tipos de habitats, desde ambientes extremamente xerófitos até bastante úmidos (McDade et al. 2008). Os principais centros de diversidade da família são o sudeste da Ásia, Malásia, Índia, África tropical, Madagascar, Brasil, região Andina, México e América Central (Daniel 2000). Os "acantos" (como são chamadas popularmente as Acanthaceae) Neotropicais ainda são insuficientemente conhecidos, e espera-se que muitas outras espécies serão descritas na região (McDade et al. 2008).

Estudos moleculares recentes (Hédren et al. 1995; McDade and Moody 1999; McDade et al. 2000; Scotland et al. 1995) revelaram que Nelsonioideae, Thunbergioideae e Mendonciodeae formam um grupo-irmão das Acanthoideae, confirmando Acanthaceae *sensu* Lindau (1895). Os estudos de Scotland et al. (1995) e McDade et al. (2000) indicam que Acanthaceae *sensu stricto* (Bremekamp 1965) forma um grupo monofilético e que, dentro deste grupo, podem ser distintos dois clados-irmãos. O primeiro clado é composto por gêneros que compartilham os seguintes caracteres: ausência de cistólitos, quatro estames com anteras monotecas e grãos de pólen colpados que caracterizam a subfamília Acanthoideae *sensu* Bremekamp (1965). O segundo clado é composto por gêneros com cistólitos e dois ou quatro estames, com anteras bitecas.

Scotland and Vollensen (2000), com base em uma seleção dos caracteres homólogos, estabeleceram uma nova classificação para a família Acanthaceae que representa uma síntese dos dados moleculares e morfológicos de 221 gêneros da família. A classificação divide Acanthaceae em três subfamílias: Nelsonioideae, Thunbergioideae (incluindo *Mendoncia*) e Acanthoideae. Acanthoideae (ou Acanthaceae *s.s.*) é uma linhagem com ca. de 3.200 espécies (em mais de 200 gêneros), que é marcada pela presença de retináculos nos frutos (McDade and Kiel 2006a), e é subdividida em duas tribos: Acantheae e Ruellieae.

A tribo Acantheae Dumort

A tribo Acantheae (*sensu* Scotland and Vollesen 2000) é composta por 20 gêneros que compreendem ca. 500 spp espécies no Velho e Novo Mundo (McDade et al. 2005). Estima-se que a tribo se originou há ~58.6 Ma no Velho Mundo e que se dispersou para o Novo Mundo, mas a história biogeográfica da tribo foi pouco explorada (Tripp and McDade 2014). Essa linhagem mostrou-se monofilética em alguns estudos moleculares (McDade and Moody 1999; McDade et al. 2000) e possui uma característica única dentre as Acanthaceae que são os estames com anteras monotecas. As Acantheae, em geral, também apresentam grãos de pólen colpados, sem as endoaberturas poradas presentes na maioria das outras Acanthaceae e Lamiales em geral. A tribo também pode ser diferenciada de seu grupo-irmão, Ruellieae, pela ausência de cistólitos.

Acantheae se dividide em duas linhagens principais, a unilabiada e a bilabiada (Capítulo 1, Figura 1). A linhagem unilabiada é exclusiva do Velho Mundo (principalmente África, Asia e Madagascar, mas também na Europa e Austrália). Os gêneros tradicionalmente reconhecidos na linhagem unilabiada (como *Acanthus* e *Crossandra*) são monofiléticos com bom suporte de dados moleculares e, na maioria dos casos, mostram sinapomorfias morfológicas evidentes.

Em contraste, a linhagem de corola bilabiada ocorre principalmente no Novo Mundo (especialmente na região Neotropical), com apenas um clado com espécies do Velho Mundo (clado *Stenandriopsis*). Não foram encontradas sinapomorfias morfológicas evidentes para os clados da linhagem bilabiada e não podem ser identificados os caracteres macromorfológicos para suportar a base da relação entre os clados (McDade et al. 2005).

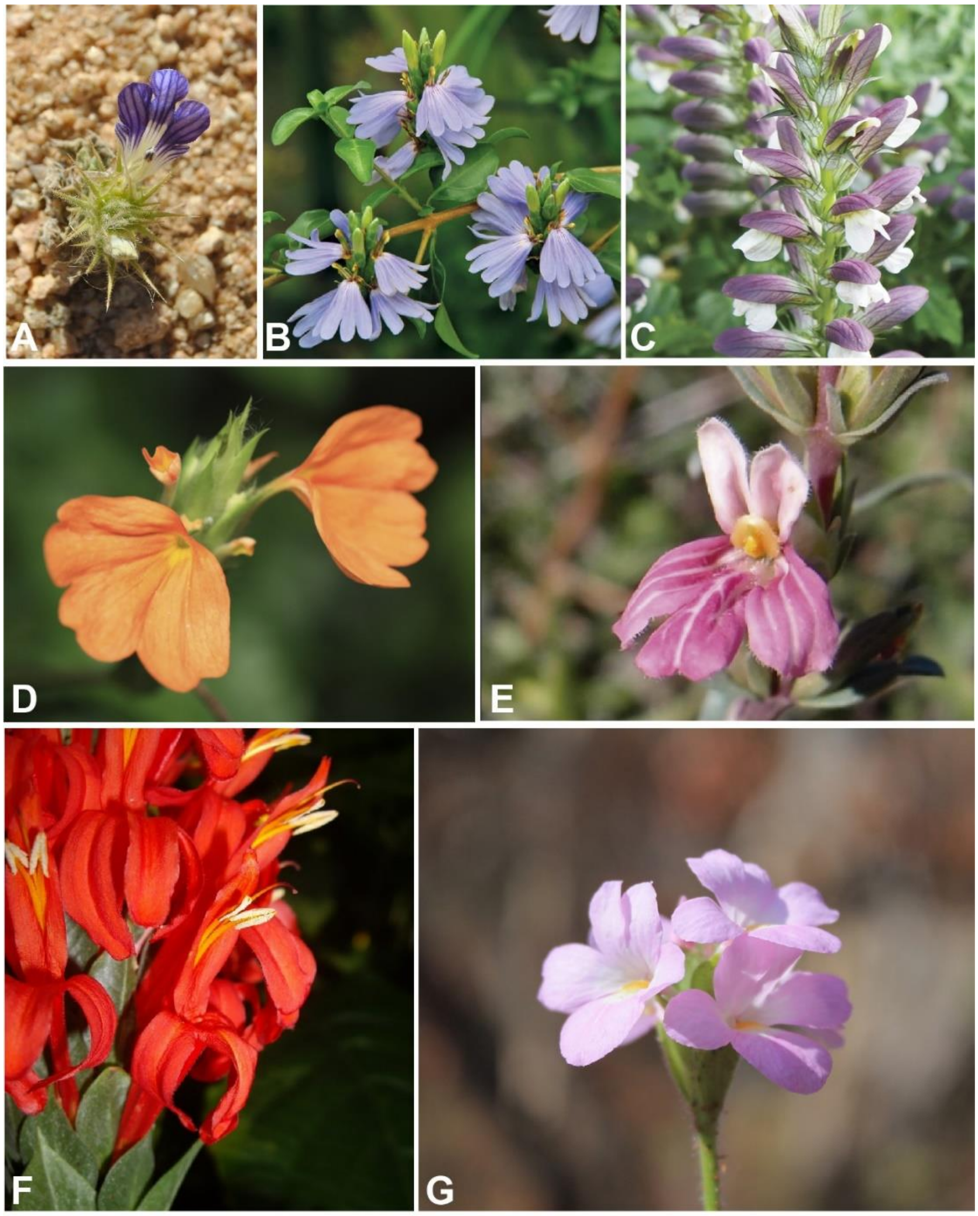

Figura 1. Inflorescências e flores de Acantheae. A-D: Linhagem unilabiada. A. *Acanthopsis disperma* Harv. (foto de A. Ivanov). B. *Sclerochiton harveyanus* Nees (foto de logees.com). C. *Acanthus mollis* L. (foto de mr-fothergills.co.uk). D. *Crossandra infundibuliformis* (L.) Nees (foto de J. Tanaka). E-G. Linhagem bilabiada. E. *Holographis virgata* (Harv. ex Benth. & Hook. f.) T.F. Daniel (foto de S. Carnaham). F. *Aphelandra aurantiaca* (Scheidw.) Lindl. (foto de R.M. Castro). G. *Stenandrium pohlii* Nees (foto de M.R. Zanatta).

O gênero *Stenandrium* Nees

Stenandrium é atualmente um gênero com cerca de 100 espécies e sua circunscrição atual é parafilética (inclui espécies paleotropicais do clado *Stenandriopsis,* ver Figura 2). De acordo com a análise molecular mais recente (McDade et al. 2005), *Stenandrium* faz parte de um grupo basal em Acantheae, o clado "*Stenandrium*", que está bem suportado e inclui os gêneros do Novo Mundo *Stenandrium* Ness e *Holographis* Nees, mais duas espécies jamaicanas, *Salpixantha coccinea* Hook (única espécie do gênero) e *Neriacanthus purdieanus* Benth. (uma das cinco espécies do gênero), e uma espécie de *Aphelandra, A. verticillata* Nees ex Hemsl. (que ficou separada de suas congêneres). A delimitação entre estes gêneros é baseada em caracteres pouco consistentes e necessita ser melhor investigada. Por exemplo, os caracteres que distinguem *Holographis* (inflorescências em espigas, brácteas estreitas, cálice 5-partido, corola zigomórfica, quatro estames de anteras monotecas e um pequeno estaminódio e cápsula elipsoide com quatro sementes; Daniel 1983) podem ser também encontrados em espécies de *Stenandrium* do grupo caulescente. *Aphelandra* é principalmente diferenciada pelas brácteas foliáceas e coloridas (Wasshausen 1975), menos proeminentes e inconspícuas nos outros gêneros, porém algumas espécies possuem brácteas verdes, como *A. verticillata*. Este clado é caracterizado por alta diversidade morfológica das corolas, o que está provavelmente associado com adaptações à diferentes polinizadores (McDade and Kiel 2006b).

Vollesen (1992), ao propor a sinonimização dos gêneros *Stenandrium* e *Stenandriopsis,* afirmou não ser possível distinguir esses dois gêneros morfologicamente e sugeriu que *Stenandrium s.l.* é mais intimamente relacionado às espécies de Acantheae do Velho Mundo do que às espécies do Novo Mundo, hipótese que foi refutada por McDade et al. (2005). Estes últimos autores argumentaram que as espécies paleotropicais de *Stenandrium* não compartilham caracteres apomórficos que conflitem com seus resultados; e que mantêm as condições plesiomórficas para os caracteres macromorfológicos de Acantheae. Portanto, é sugerido por McDade et al. (2005) reconhecer *Stenandrium s.s.* e *Stenandriopsis,* mas recomendam também que antes seja feito um estudo mais abrangente, com uma maior amostragem de espécies de *Stenandrium s.s.* e uma busca mais minuciosa de caracteres morfológicos relevantes para a separação desses gêneros.

Objetivos

O objetivo deste estudo foi investigar a história biogeográfica de Acantheae, estimando tempos de divergência, usando calibração fóssil e dados moleculares, combinados a reconstruções de áreas ancestrais; e contribuir para aumentar o conhecimento taxonômico de *Stenandrium*, revisando as espécies brasileiras do gênero.

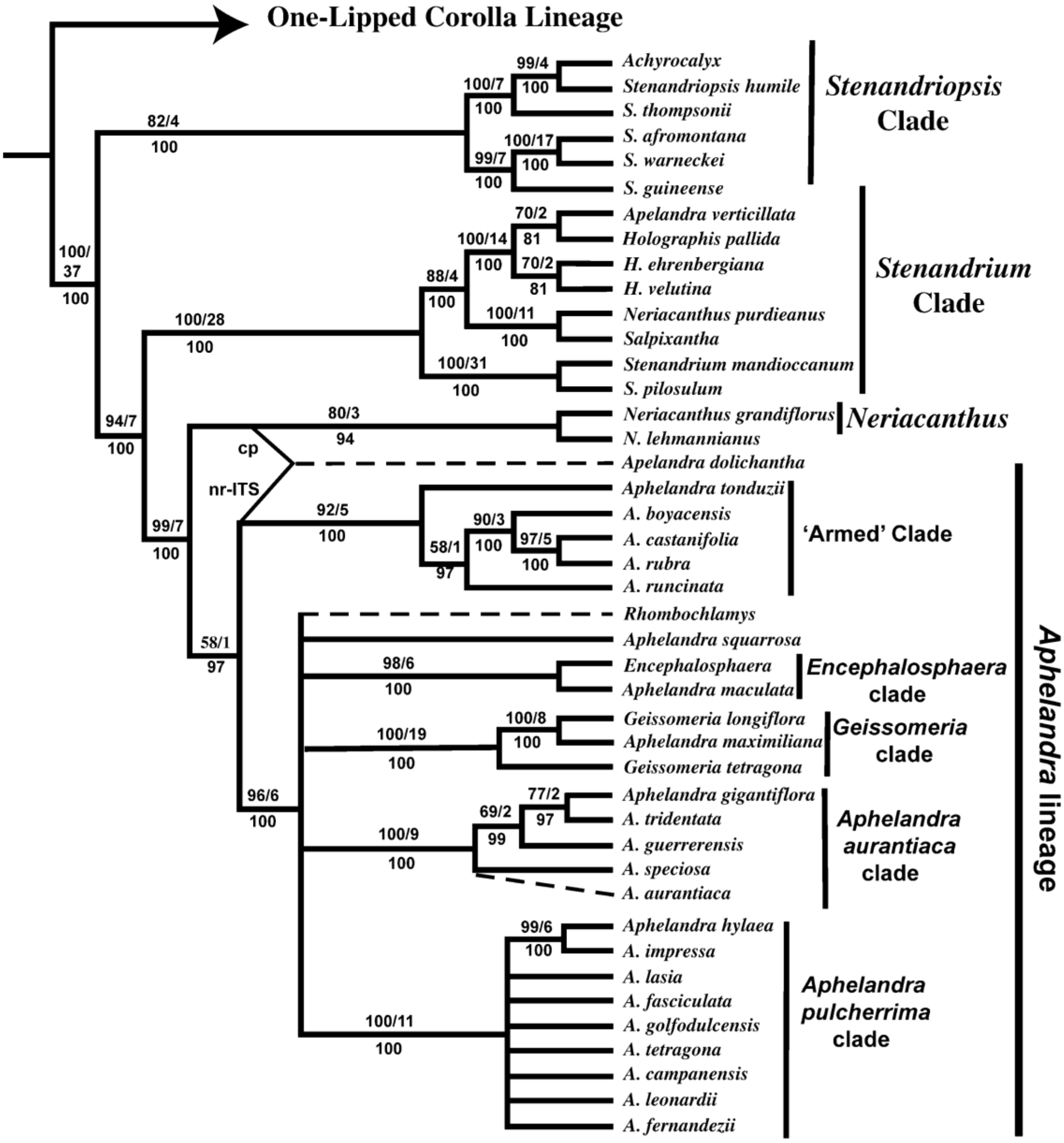

Figura 2. Cladograma da linhagem bilabiada de Acantheae. (Figure from McDade et al. 2005).

Organização da tese e plano de publicação

O Capítulo I apresenta uma história biogeográfica global de Acantheae, com uma filogenia molecular atualizada e datada dos principais gêneros da tribo. Os autores são: Maria Rosa V. Zanatta, Vanessa Rivera, Iain Darbyshire and Carolyn E. B. Proença. Será submetido para publicação na revista *Journal of Biogeography* (Qualis A1).

O Capítulo II apresenta algumas contribuições para a taxonomia de *Stenandrium*, com uma revisão taxonômica para as espécies do Brasil. O trabalho inclui lectotipificações, novos sinônimos, uma chave de identificação, pranchas ilustrativas e mapa de distribuição. Os autores são: Maria Rosa V. Zanatta, Thomas F. Daniel e Carolyn E. B. Proença. Será submetido para publicação na revista *Rodriguésia* (Qualis A3) ou *Phytotaxa* (Qualis A4).

O Capítulo III apresenta a descrição de duas novas espécies de *Stenandrium* de Centros de Endemismo da Serra do Espinhaço, Minas Gerais. Os autores são: Maria Rosa V. Zanatta, Thomas F. Daniel, Cíntia Kameyama & Carolyn E. B. Proença. O artigo já foi aceito para publicação na revista *Systematic Botany* (Qualis A3), com poucas revisões a serem feitas.

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CAPÍTULO I

Phylogenetic biogeography and insights on the evolution of Acantheae (Lamiales: Acanthaceae)

ABSTRACT

Reconstructing the history of organisms which are key actors in ecological relationships is important for the understanding of evolutionary processes and patterns. Dated phylogenies combined with biogeographical analyses might bring answers to key questions about global biodiversity patterns. We reconstructed the global biogeographic history of Acantheae, an ecologically important tribe in tropical ecosystems. A dated molecular phylogeny of the principal genera in the tribe was updated, including novel sequences for Neotropical species. We performed analyses in BioGeoBEARS to infer the distribution areas of ancestral nodes and indicate the direction and date of dispersion events. Our results indicate that the greatest diversification of clades occurred during the "Age of the Herbs", at late Miocene (11.6-5.3 Mya). Certain long-distance dispersions were crucial for the establishment of lineages in new continents, such as a trans-oceanic dispersion from Africa to Central America through the Equatorial Current, 33.6 Mya. The diversification of certain pollinator groups, such as bees and hummingbirds, seems to have played an important role in the diversification of Acantheae. The most diverse genus in the tribe, *Aphelandra*, emerged at the same time hummingbirds were diversifying in the Americas (~22Mya, Miocene). This is the earliest reported association between plants and hummingbirds to date.

RESUMO

Reconstruir a história dos organismos que são atores-chave nas relações ecológicas é importante para a compreensão de processos e padrões de evolução. Filogenias datadas aliadas a análises biogeográficas podem trazer respostas para perguntas-chave sobre os padrões de biodiversidade global. Nós reconstruímos a história biogeográfica global de Acantheae, uma tribo ecologicamente importante nos ecossistemas tropicais. Uma filogenia molecular datada dos principais gêneros da tribo foi atualizada incluindo sequências inéditas para espécies Neotropicais. Foram realizadas análises no BioGeoBEARS para inferir as áreas de distribuições dos nós ancestrais e indicar a direção e a data dos eventos de dispersão. Os resultados indicam que o período que ocorreu a maior diversificação dos clados foi na "Age of the Herbs", no Mioceno tardio (11.6-5.3 Ma). Algumas dispersões a longa-distância foram cruciais para o estabelecimento de linhagens em outros continentes, como a dispersão trans-oceânica a partir da África pela Corrente Equatorial para a América Central, há 33.6 Ma. A diversificação de alguns grupos de polinizadores, como abelhas e beija-flores, parece ter exercido importantes influências na diversificação de Acantheae. O gênero mais diverso da tribo, *Aphelandra,* surgiu na mesma data que a diversificação dos beija-flores nas Américas (~22 Mya, Miocene). Esta descoberta representa a associação mais antiga de plantas e beija-flores reportadas até o momento.

INTRODUCTION

Phylogenetic biogeography, the study of the history of monophyletic groups in time and space, was the first approach to use phylogenetic hypothesis of a group of organisms to infer its biogeographic history (Crisci et al. 2003). Phylogenetic biogeography studies of plants are essential to answer key questions of global biodiversity patterns, such as why South American tropical savanas and forest are more species-rich than African and Asian ones (Richards 1973 *apud* Raven and Axelrod 1974; Gentry 1993 *apud* Pennington et al. 2006).

These answers could be in the histories of the organisms in these environments as revealed by their phylogenies (Pennington et al. 2004). Some of the answers are already coming forward in studies that use dated phylogenies to infer the history of the world's most diverse biomes, such as Neotropical forests (Pennington et al. 2006) and South American savannas (Simon et al. 2009). One of the main emerging hypotheses is the predominance of dispersal (often across long distances) as the structuring mechanism of a highly diverse flora. Rates of immigration, speciation and extinction are very diferent between biomes, their histories were diferent and perhaps driven by ecological processes (Pennington et al. 2006), rather than tectonic history, as has been traditionally believed (Raven and Axelrod 1974). From this assumption and with sufficient phylogenetic biogeography studies, we would expect to find patterns in the timing and direction of transoceanic dispersal events since wind and water circulation systems are not distributed randomly in space and time (Renner 2004).

There is a growing consensus (Hughes et al. 2013, Tripp and McDade 2013) that a complete explanation of the high Neotropical diversity requires the synthesis of multiple ideas on the sources of diversity, including abiotic and biotic axis of speciation, as well as phylogenetic studies of lineages ocurring in the New World. Dating clades of organisms that are key participants in the main ecological relationships, such as pollinators and pollinized plants, is of great importance for the comprehension processes and patterns of evolution (Tripp and McDade 2013).

Acanthaceae Juss. is an ecologically important family especially in many tropical communities. It harbors high functional diversity of flower shape, colour and pollen morphology (Scotland and Vollesen 2000). It has both generalist and specialist pollinators, including bees, hummingbirds, moths, bats and butterflies (McDade and Weeks 2004; Muchhala et al. 2009; Tripp and McDade 2013; Matias and Consolaro, 2015). In *Ruellia* (Acanthaceae, Ruellieae) different pollination systems (hummingbirds, moths, and bats) evolved repeatedly during its evolutionary history, and bidirectional transitions between these systems ocurr (Tripp and Manos 2008). On the other hand, autochoric dispersal is predominant in the family; the fruits are small capsules provided with retinacula (a modified funiculus that is long, curved and hardened to promote explosive seed expulsion), a synapomorphy of Acanthaceae *sensu stricto* (Acanthoideae Link; Scotland and Vollesen 2000). Another factor that makes Acanthaceae an interesting group for phylogenetic and biogeographic studies is that the first reported case of a genus originated by hybridization between distantly related genera (Tripp et al. 2013) was recorded in this family. The family is also marked by the existence of many narrowly distributed species (Echternacht et al. 2011; Steyn and Van Wyk 2017; Chapter 3).

Currently available fossil and molecular records indicate that Acanthaceae originated about 81.9 million years ago, in the Late Cretaceous and that there have been at least 16 disjunction events in Acanthaceae *lato sensu* (between the Eastern and Western hemispheres). The majority of those happened from the Old World into the New World and one (*Staurogyne*, Nelsonioideae) happened from the New World into the Old World. Multiple reasons, including climate data, current taxon distribution, the lack of Asian-Neotropical sister groups and the scarcity of extratropical fossils support the hypothesis that long distance dispersal was the main mechanism for those multiple lineage translocations (Tripp and McDade 2014).

Long distance and transoceanic dispersions are apparently more common than was previoously thought. Advances in molecular biology, empirical and model-based oceanography and climate data, favor the inference of fast ocean crossing along specific ocean and wind currents, often followed by successful establishment of a lineage. Studies with many groups of plants and animals, including primates, small rodents and lizards (compiled by Renner 2004) also support transoceanic dispersions since the Cretaceous. Even on current days, tropical seeds are found on European coasts (Nelson 1978). It is known that organisms travel in floating debris, such as volcanic rocks, pumice, wood and more recently plastic (Barnes 2002). Due to their speed, equatorial currents are able to transport larger floating objects with surfaces exposed to Atlantic winds in less than two weeks, and this transport was probably faster in the narrower part of the Atlantic in the Early Tertiary (Renner 2004).

Investigating and reflecting about those questions contributed to the development of Darwin's ideas about evolution and distribution of living beings on the planet. Darwin was the first scientist to conduct experiments to investigate the survival and flotation of seed in high salinity water. He describes those studies in many books and papers (cited and discussed recently by Black 2009), concluding that seeds of many terrestrial plants are able to float and survive in high levels of salinity. Currently, halophytes are distinguished from glycophytes due to their capacity of remaining dormant in high salinity environments and germinate later when salinity is reduced (Ungar 1978).

The objective of this study is to investigate the biogeographical history of Acantheae Dumort, a tribe of Acanthaceae, using phylogenetic analysis dated using molecular and fossil data combined to ancestral area reconstructions.

MATERIAL AND METHODS

Study group

Acantheae is the second largest tribe of Acanthaceae, with 20 genera and approximatelly 578 species (theplantlist.org), distributed mainly in the Tropical and Subtropical regions (Figure 1). The presence of four stamens with monothecal anthers, a synapomorphy of the tribe, is an unique feature in Acanthaceae (Scotland and Vollesen 2000; McDade et al. 2005). The percentage of sampled species in each genus and their geographic distribution is presented in Table 1.

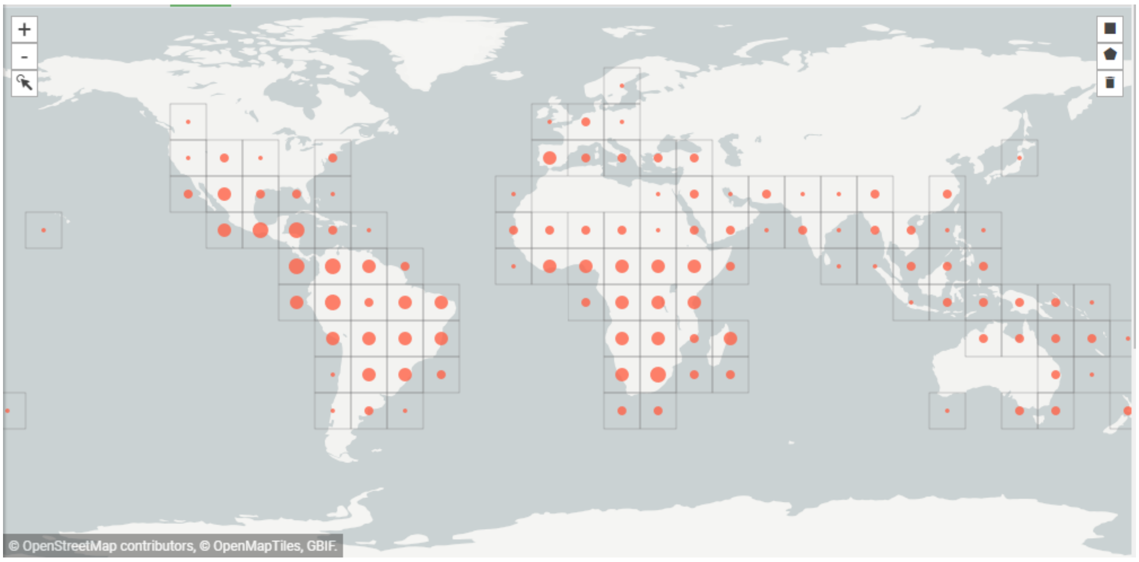

Figure 1. Acantheae map of occurrences generated in GBIF (gbif.org). Red circles sizes varying with number of occurrences.

Table 1. Acantheae genera with number and percentage of sampled species, pollination syndromes and geographic distribution (adapted from McDade et al. 2005). *Stenandrium* was divided into New World (NW) and Old World (OW) species.

	Species number	% sampled	Pollination	Geographic distribution
Old World				
Acanthopsis Harv.	8	37.5	?	Southern Africa
Acanthus L.	34	32.4	mel[1,2,3], nec[1]	Southern Europe, Africa, southern Asia, Malesia, Australasia
Achyrocalyx Benoist	4	25	?	Madagascar
Blepharis Juss.	126	25.4	mel[4]	Africa through Arabia, southern Asia, southeastern Asia
Crossandra Salisb.	61	11.5	psy[5], sph[6]	Africa, Madagascar, Arabia, India, Australia
Crossandrella C.B. Clarke	3	0	?	Tropical Africa
Cynarospermum Vollesen	1	0	?	India
Sclerochiton Harv.	16	6.3	?	Tropical and southern Africa
Stenandrium Nees	18	27.8	?	Africa, Madagascar
Streptosiphon Mildbr.	1	0	myr?[7]	Tanzania
New World				
Aphelandra R. Br.	207	12.1	tro[8,9], qui[9]	Central and South America
Cyphacanthus Leonard	1	0	?	Colombia
Encephalosphaera Lindau	3	33.3	?	Brazil, Colombia, Ecuador, Peru
Geissomeria Lindl.	17	11.8	tro[10]	Tropical America, mostly Brazil
Holographis Nees	16	25	?	Mexico
Neriacanthus Benth.	5	80	?	Panama, Colombia, Venezuela, Ecuador, Peru, Jamaica

Orophochilus Lindau	1	0	?	Peru
Rhombochlamys Lindau	1	0	?	Colombia
Stenandrium Nees	53	24.5	mel[11,12,13] syr[13]	Mainly Central and South America
Strobilacanthus Griseb.	1	0	?	Panama
Xantheranthemum Lindau	1	0	tro[5]	Peru
Totals	**578 spp.**	**18.9**		

List of pollination syndromes: mel - melitophily (bees); myr - myrmecophily (ants); nec – Nectariniidae (sunbirds); psy - psychophily (butterflies); qui - quiropterophily (bats); sph - sphingophily (hawk moths); syr - Syrphidae (flower flies); tro - trochilophily (hummingbirds).

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13. Personal observations (images and videos are available from M.R. Zanatta and C. Proença).

Taxon sampling

To establish the phylogenetic relations between the genera of Acantheae, we compiled all sequences available in GenBank for those genera. We also generated 49 new sequences of *Stenandrium, Holographis* and *Aphelandra,* resulting in 15 additional terminals to increase the taxonomic, geographic and morphological representation within the tribe. The final data matrix contains 381 sequences of 123 terminals (12 outgroups), representing 18.9% of the species, distributed in 12 genera, with all main genera included (Table 1). The list of taxa, regions, vouchers and distribution areas are in Appendix I.

DNA sampling, extraction, amplification and sequencing

Genomic DNA was extracted from material that was dried in silica-gel following the CTAB protocol (Doyle and Doyle 1987); for some herbarium samples we used *QuiaGen Mini Kit* with adaptations from Doyle and Doyle (1987), i.e., adding β-mercaptoetanol. After extraction, DNA was visualized in 1% agarose gel and frozen at -20^{o}C for posterior amplification.

The phylogenetic analysis were based on four DNA regions (Table 2): nuclear ribosomal spacer ITS (McDade et al. 2000) and three plastidial genome regions, rps16 (ACA5-3, Downie and Katz-Downie 1999), trnL-F (McDade and Moody 1999), and trnS-G (Hamilton 1999).

Tabela 2. Nucleotide sequences of the primers used for each DNA region

Primer	**Nucleotide sequence**
ITS4	TCCTCCGCTTATTGATATGC
ITS5	GGAAGTAAAAGTCGTAACAAGG
rps16 (ACA5)	GAGGACARRATCCGTTGTGGAT
rps16 (ACA3)	AGACGGCTCATTGGGATA
trnLe	GGTTCAAGTCCCTCTATCCC
trnFf	ATTTGAACTGGTGACACGAG
trnS	GCCGCTTTAGTCCACTCAGC
trnG	GAACGAATCACACTTTTACCAC

The DNA amplifications were made through *polymerase chain reaction* (PCR) at a final volume of 50 µL with the following reagent proportions: 0.5-2 µL of genomic DNA, 0.5 µL of Taq Polymerase (Invitrogen™), 2.5 µL of each primer (100 µM), 5 µL of Mg-Free Buffer, 5 µL of Mg, 2 µL of BSA, 4 µL of dNTP (250 µM) and 26.5-28 µL of ddH2O. PCR conditions were programed using anneling temperatures indicated at the primer labels and information obtained from McDade et al. (2005). All regions had been used in previous phylogenetic analysis of the family (McDade and Moody 1999; McDade et al. 2000) and for the tribe (McDade et al 2005).

Alignment and phylogenetic analyses

The new sequences obtained were initially visualized, edited and trimmed in Geneious v.11.1.5 (http://www.geneious.com, Kearse et al. 2012). The sequence alignment was made for each *locus* individually with MAFFT v.7 (Katoh et al.

2019) and later manually inspected in Geneious to minimize possible software errors. DNA substitution models were selected for each marker in jModeltest v.2.1.3 (Darriba et al. 2012), using the minimum values of corrected Akaike criteria (AICc). Specific values of proportions of bases and substitution rates for each model were applied to the analyses. Each dataset (plastidial and nuclear) was analyzed separately and then concatenated using maximum likelihood (ML) and Bayesian inference (BI). ML analyses were done using RAxML-HPC BlackBox (Stamatakis 2014) with default parameters, through the CIPRES Science Gateway (phylo.org/; Miller et al. 2010). BI analysis was done using Mr Bayes 3.2.6. (Ronquist et al. 2012) also through CIPRES. MrBayes analyses consisted of two independent runs of 10 million generations with sampling every 1000th generation. The initial thousand tree generations were discarded as 'burn-in' and the remaining were summarized in a consensus tree.

The divergence time estimates were made using a lognormal relaxed clock for Birth-Death speciation with 50 million generations using BEAUti v 2 (Bouckaert et al. 2019) and run in BEAST v2.6.1 (Bouckaert et al. 2019), also through CIPRES. To calibrate the analyses in time we used the date of an *Acanthus rugatus* fossil (images and further information in Appendix II and III, Reid and Chandler, 1926), with the same parameters used by Tripp and McDade (2014): Zero offset = 28.8, Log (SD) = 1.1, Mean = 1.5, 5% quantile = 28.9 e 95% quantile = 33.8. The fossil was positioned in the crown node of the *Acanthus* clade, which includes *Acanthus* + *Blepharis dhofarensis* (monophyletic group as observed in the IB and ML trees generated in the present study, see results), unlike Tripp and McDade (2014), who positioned it in *Acanthus* + *Acanthopsis.* Analysis convergence was tested using Tracer v. 1.6.0 (Rambaut & Drummond 2009), by ESS values (Effective Sample Size, >200) and then a tree was built with maximum credibility clades in TreeAnnotator v2.0.3 (Rambaut & Drummond 2007), with 20% burn-in. The final tree was visualized in FigTree v1.4.2 (Rambaut, 2014) and edited in Adobe Illustrator.

Distribution data and biogeographic analyses

The species distribution map was made in ArcMap (ESRI 2011) based on data extracted from the GBIF dataset. The data extracted from the plataform were curated and suspicious occurrence records were removed. We selected seven geographic areas (Figure 2; Buerki et al. 2011): A, Eurasia, from western Europe to Indochina; B, Africa; C, Madagascar, including the Comoro Islands and the Mascarene Islands; D, Southeast Asia, including India, the Malaysian Peninsula, the Philippines, Sumatra, Borneo and the Inner Banda Arc, as well as the Pacific Islands (e.g. Hawaii); E, Australia, including New Guinea, New Caledonia and New Zealand; F, North America; G, South America, including Central America and the West Indies.

For the inclusion of data in the BioGeoBEARS analysis (Matzke 2013, 2014), we first constructed a presence/absence table with the species and areas selected, using the ArcMap function "intersect". We made three types of biogeographic analysis: unconstrained, stratified and stratified with restrictions. Unconstrained analysis did not consider the distance between areas and allowed free dispersion between every combination of areas. In the stratified analysis we included a distance matrix between areas in selected time slices to restrict or facilitate dispersions between areas. We performed stratified analysis using the same distance matrices as Buerki et al. (2011), that considered oceanic currents to determine the distance values between areas. We also performed a second stratified analysis with more restrictive matrix than that used by Buerki et al. (2011), due to the particularities of the study group (low dispersal capacity, i.e., small plants with autochoric fruits). The more restrictive stratified analysis generated very similar results to those generated by the Buerki et al. (2011) matrix; therefore, its results will not be presented here. In all the analyses, the values of LnL (log-likelihood; Matzke 2013) were better for "star" analyses, which do not allow null range (Massana et al. 2015).

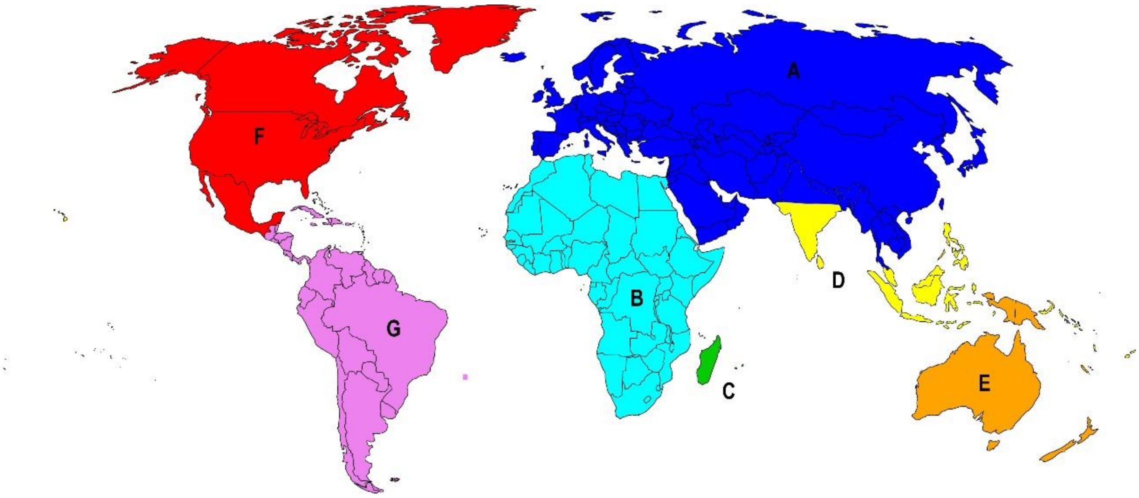


Figure 2. Biogeographic regions used in the study.

RESULTS

Phylogenetic analyses

The concatenated DNA matrix for all regions contains 4499 characters. The trees resulting from molecular data analysis (Appendix IV and V) in contrast with plastidial data (Appendix VI and VII), regardless of missing data, were congruent for larger clades, which presented well-supported nodes. The results described below refer to the ML (Figure 3) and BI (Figure 4) trees obtained from the data of the combined matrix (plastidial + nuclear).

Acantheae was monophyletic in all analysis with maximum support (bootstrap = 100 and PP= 1) as well as its main lineages: one-lipped and two-lipped. The one-lipped lineage is composed by the Old World (hencforth OW) genera that have corollas with five ventrally directly lobes (Figure 5: B-C). The two-lipped lineage has one OW clade (the *Stenandriopsis* clade) while all other clades occur in the NW, composed by species with corollas with a two-lobed superior lip and a three-lobed inferior lip (Figure 5: A, D-E).

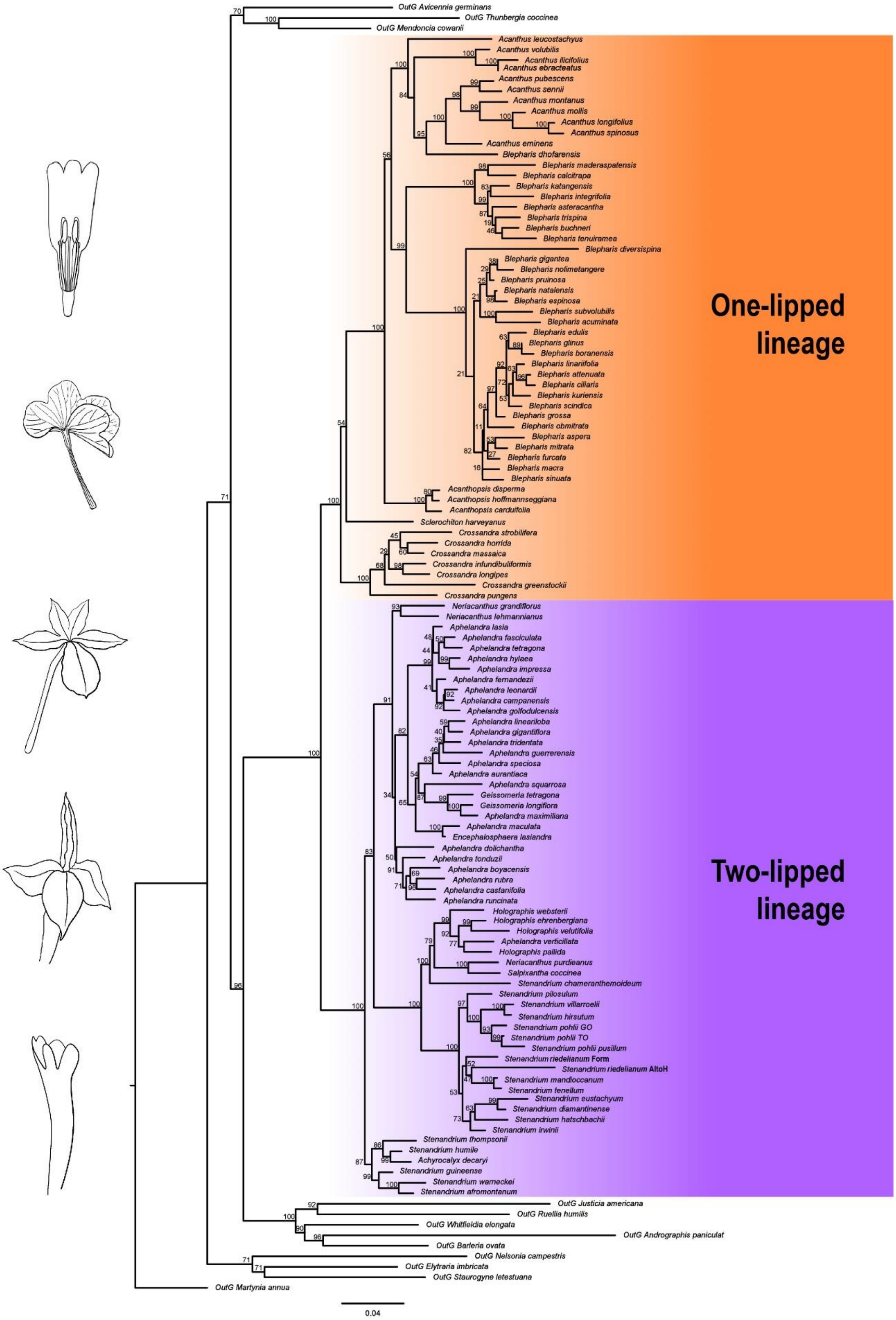


Figure 3. Acantheae phylogenetic tree obtained from the complete dataset (plastidial + nuclear markers) inferred from maximum likelihood reconstruction methods (RAxML) with bootstrap values at nodes. OutG - outgroup.

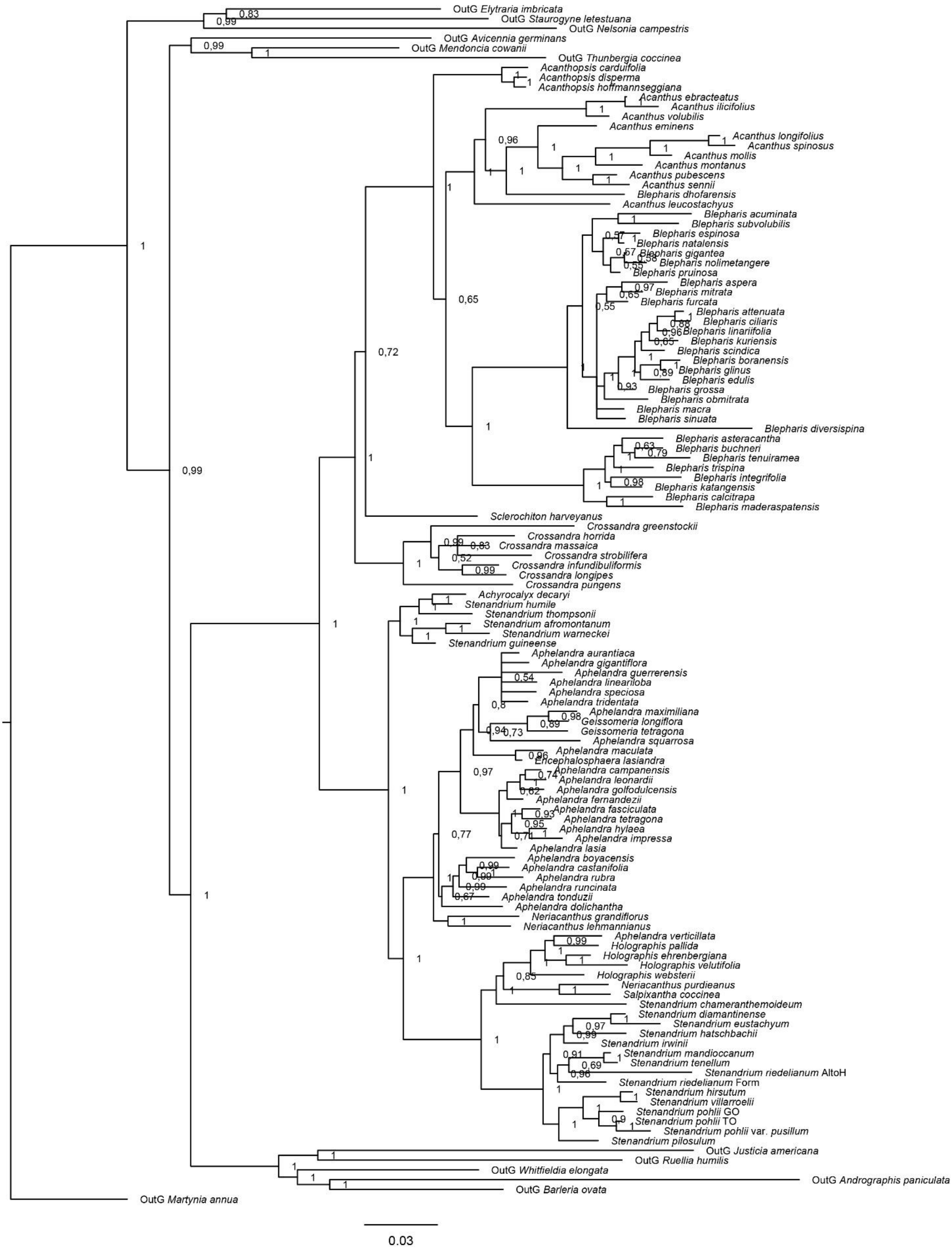


Figure 4. Acantheae phylogenetic tree obtained from the complete dataset (plastidial + nuclear markers) inferred from a Bayesian reconstruction method (MrBayes) with posterior probability values at nodes. OutG - outgroup.

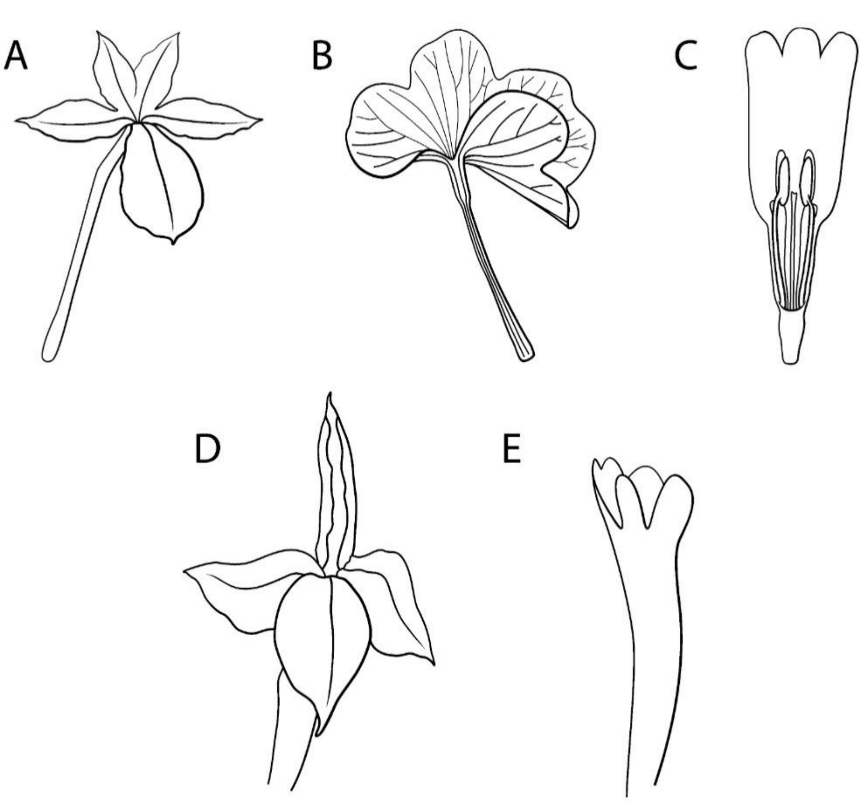


Figure 5. Corolla morphology in Acantheae adapted from McDade et al. (2005). A-C, corollas of Old World Acantheae. A. Two-lipped corolla of *Stenandrium thomense* (Milne-Redh.) Vollesen. B. One-lipped corolla of *Crossandra multidentata* Vollesen, dorsal view, note all five corolla lobes are directed ventrally. C. One-lipped corolla of *Cynarospermum asperrimum* (Nees) Vollesen, dorsal view, note three corolla lobes are directed ventrally and the upper corolla lip is extremely short. D, E. Corollas of New World Acantheae. D. *Aphelandra aurantiaca* (Scheidw.) Lindl., the upper lip is shallowly two-lobed and folded to form a sheath-like structure. E. *Geissomeria*, Edwards's Botanical Register [1827]).

There are four clades of maximum support in the one-lipped lineage (bootstrap >99 and PP = 1, Figures 3, 7): 1) the *Acanthopsis* clade (all *Acanthopsis* species); 2) the *Acanthus* clade (all *Acanthus* species + *Blepharis dhofarensis*); 3) the *Blepharis* clade (all other *Blepharis* species); and 4) the *Crossandra* clade (all *Crossandra* species).

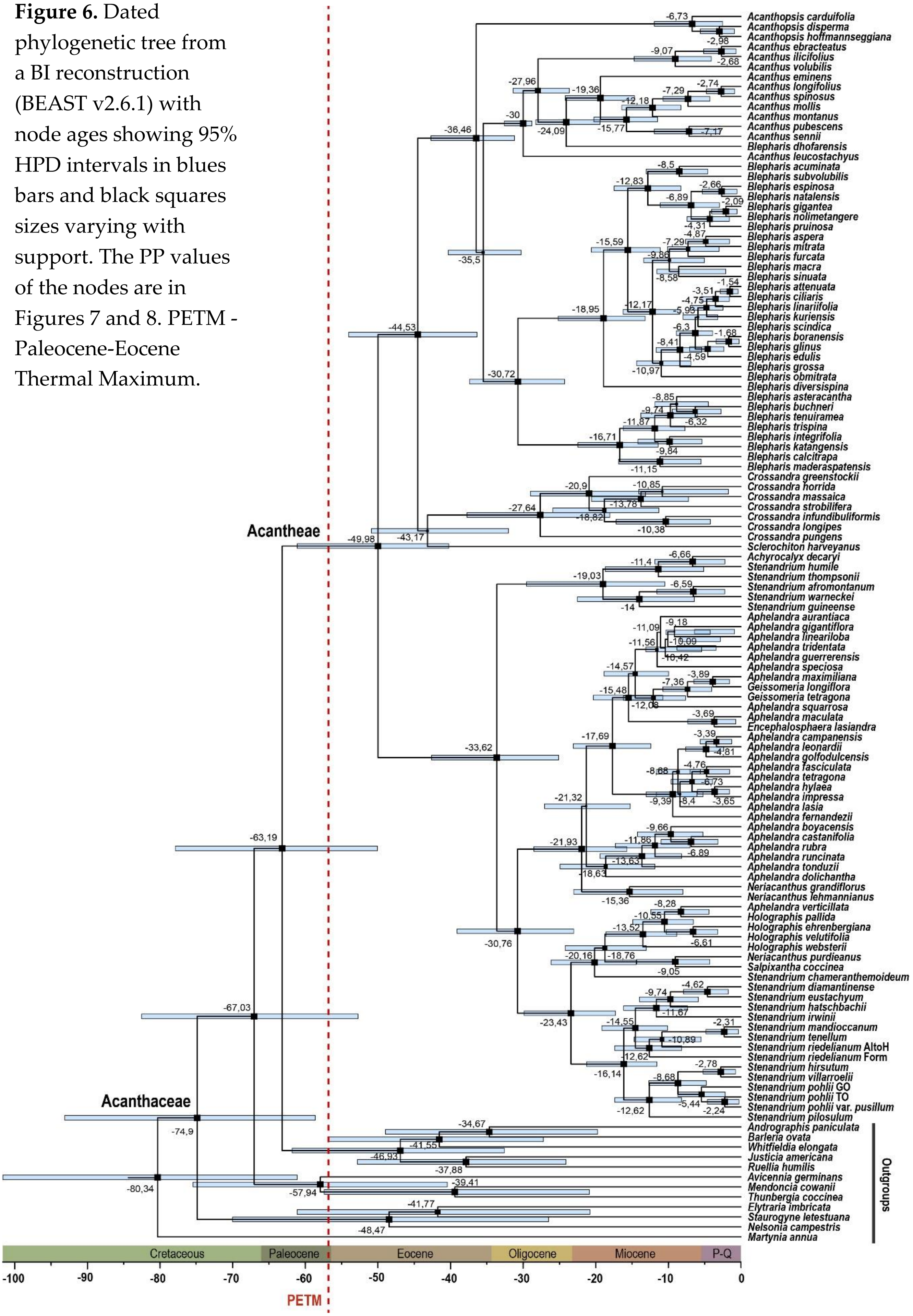
The position of *Sclerochiton* is uncertain, as it grouped with *Acanthopsis*, *Blepharis* and *Acanthus* in the nuclear analysis and with *Crossandra* in the plastidial analysis. Consequentialy, it has low support in the combined analysis. The position of *Acanthopsis* is also uncertain, emerging as sister group to a clade uniting *Acanthus* + *Blepharis* in the nuclear analysis and sister to *Acanthus* in the plastidial analysis. In the combined analysis it is positioned as sister group to the clade composed by *Acanthus* e *Blepharis*, with low support (PP = 0.53). *Blepharis* was shown to be a paraphyletic genus, as *Blepharis dhofarensis*is nested within *Acanthus* with very high support (PP=1).

The two-lipped lineage is composed of four highly supported clades (bootstrap >87 and PP=1, Figure 3, 8): 1) the *Stenandriopsis* clade (all OW *Stenandrium* species + *Achyrocalyx*); 2) the *Aphelandra* clade (all *Aphelandra* species [except *Aphelandra verticillata*], *Geissomeria, Encephalosphaera lasiandra, Neriacanthus grandiflorus* and *Neriacanthus lehmannianus*); 3) the *Holographis* clade (all *Holographis* species, *Aphelandra verticillata, Neriacanthus purdieanus, Salpixantha coccinea, Stenandrium chameranthemoideum*); and 4) the *Stenandrium* clade (all NW *Stenandrium* species, except *S. chameranthemoideum*). It can be observed that there are no monophiletic genera in this lineage under the current circunscriptions.

Dating analysis

The dating analysis results suggest that the Acantheae (Figure 6) arose between the Eocene and Paleocene, with a minimum age of 49.9 Mya for the crown node of the tribe. The analyses also suggest that the one-lipped lineage is older (44.5 Mya) then the two-lipped lineage (33.6 Mya). The diversification of all major clades in the tribe (*Acanthus* clade, *Blepharis* clade, *Crossandra* clade, *Stenandriopsis* clade, *Aphelandra* clade, *Holographis* clade and *Stenandrium* clade) happened between the Oligocene and the Miocene (30.7 to 16.1 Mya). The oldest to youngest crown nodes in this analysis were: *Blepharis* clade (30.7 My), *Acanthus* clade (30 Mya) and *Crossandra* clade (27.6 Mya). The most recent crown nodes found were the *Holographis* clade (20.1 Mya), the *Stenandriopsis* clade (19 Mya), and the *Stenandrium* clade (16.1 Mya).

Figure 6. Dated phylogenetic tree from a BI reconstruction (BEAST v2.6.1) with node ages showing 95% HPD intervals in blues bars and black squares sizes varying with support. The PP values of the nodes are in Figures 7 and 8. PETM - Paleocene-Eocene Thermal Maximum.



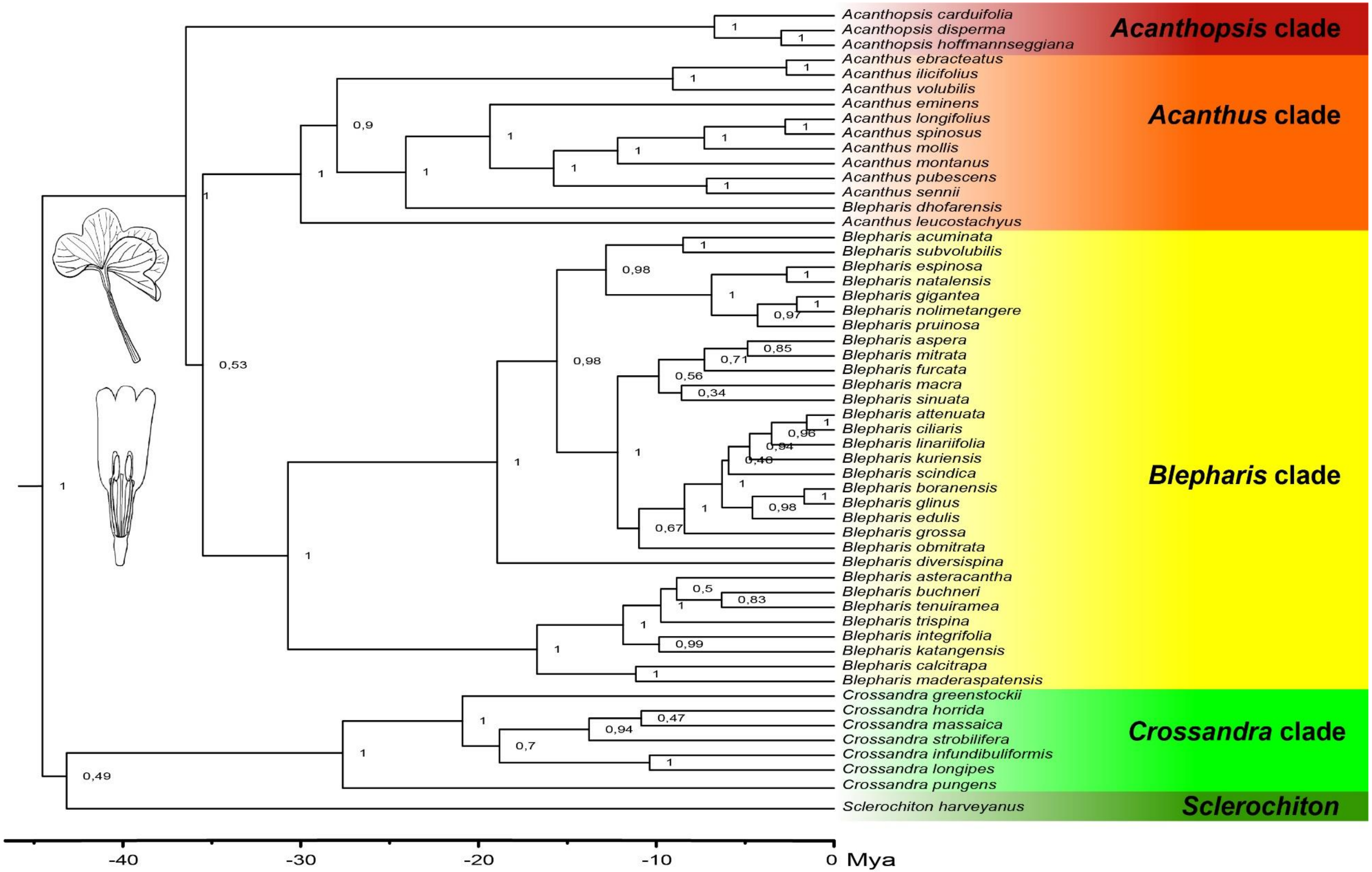


Figure 7. One-lipped corolla lineage dated phylogeny and the major clades with posterior probability values of the nodes.

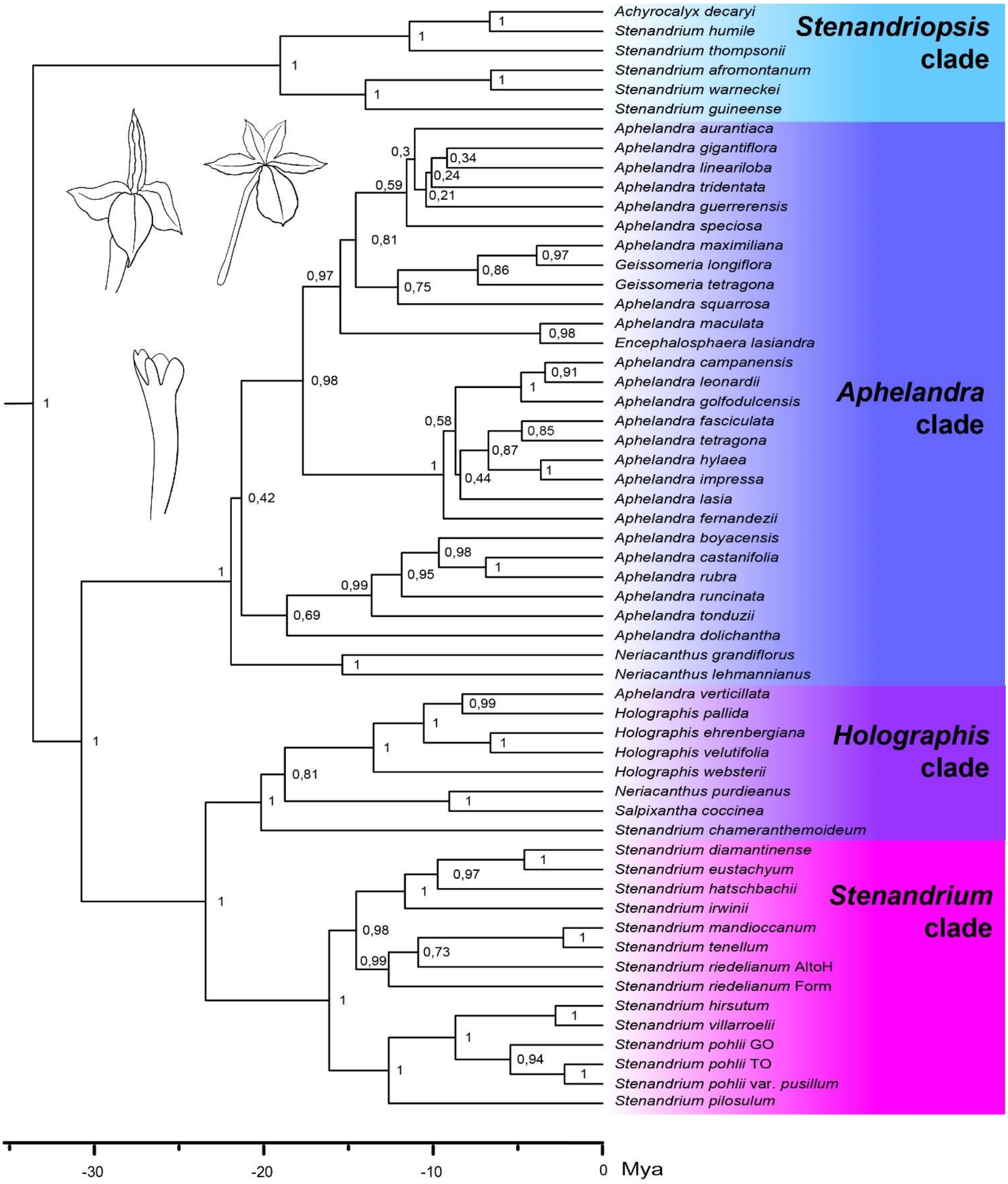


Figure 8. Two-lipped corolla lineage dated phylogeny and the major clades with posterior probability values of the nodes.

Biogeographic analysis

The BioGeoBEARS analysis results show high log likelihood values for the BAYAREALIKE and BAYAREALIKE+J models (both with LnL =-183.32). These models use dispersion, extinction, narrow sympatry and wide sympatry parameters. They do not allow speciation by vicariance. Regardless of the LnL values, all other models (DEC, DEC+J, DIVALIKE e DIVALIKE+J) indicate that the most likely ancestral area of the Acantheae stem and crown nodes is Africa (area B). The results described bellow refer to the model with highest LnL and smallest AICc value (BAYAREALIKE+J, Figure 9).

Dispersals from Africa to the other areas occurred at least nine times during a long period between the end of the Eocene and the Miocene, from 33.6 to at least 6.3 Mya and possibly later. In some cases, such as that of *Crossandra strobilifera* (to Madagascar), *Blepharis maderaspatensis* (to Southeast Asia), *Blepharis calcitrapa* (to Madagascar) and *Blepharis integrifolia* (to Southeast Asia) it is not possible to know when dispersal occurred (Figure 9). The first dispersal occurred at the crown node of the one-lipped lineage, 33.6 Mya to the Tropical America area (shift 1, Figure 9); the second occurred 30 Mya, at the *Acanthus* clade crown node, to the Eurasia (shift 2, Figure 9); the third to Madagascar, 11.4 Mya, at the *Stenandriopsis* clade (shift 3, Figure 9); the fourth at the *Crossandra* clade (shift 4, Figure 9), 10.4 Mya also to Madagascar; and the fifth was a dispersal of the *Blepharis* clade to Eurasia (shift 5, Figure 9), 6.3 Mya.

Three dispersal events occurred from Tropical America to North America, two in the Miocene and one at an uncertain period. The first occurred at the *Holographis* clade crown node, 20.1 Mya (shift 6, Figure 9), the second of a group within the *Aphelandra* clade, 11.5 Mya (shift 7, Figure 9) and the third is represented solely by *Stenandrium pilosulum* which occurred some time after 12.6 Mya.

The broad distributions of species such as *Acanthus mollis* (Eurasia + Africa + Australia), *Acanthus ilicifolius* and *Acanthus ebracteatus* (Eurasia + Southeast Asia + Australia), *Blepharis linarifolia* (Eurasia + Africa + Southeast Asia) and *Crossandra infundibuliformis* (Africa + Southeast Asia + Australia) increased the uncertainty of the distribution of ancestral nodes at the origin of the groups to which they belong. Therefore, the original ancestral areas probabilities are divided between two or more areas for these groups and it is not possible to know where the ancestors originated.

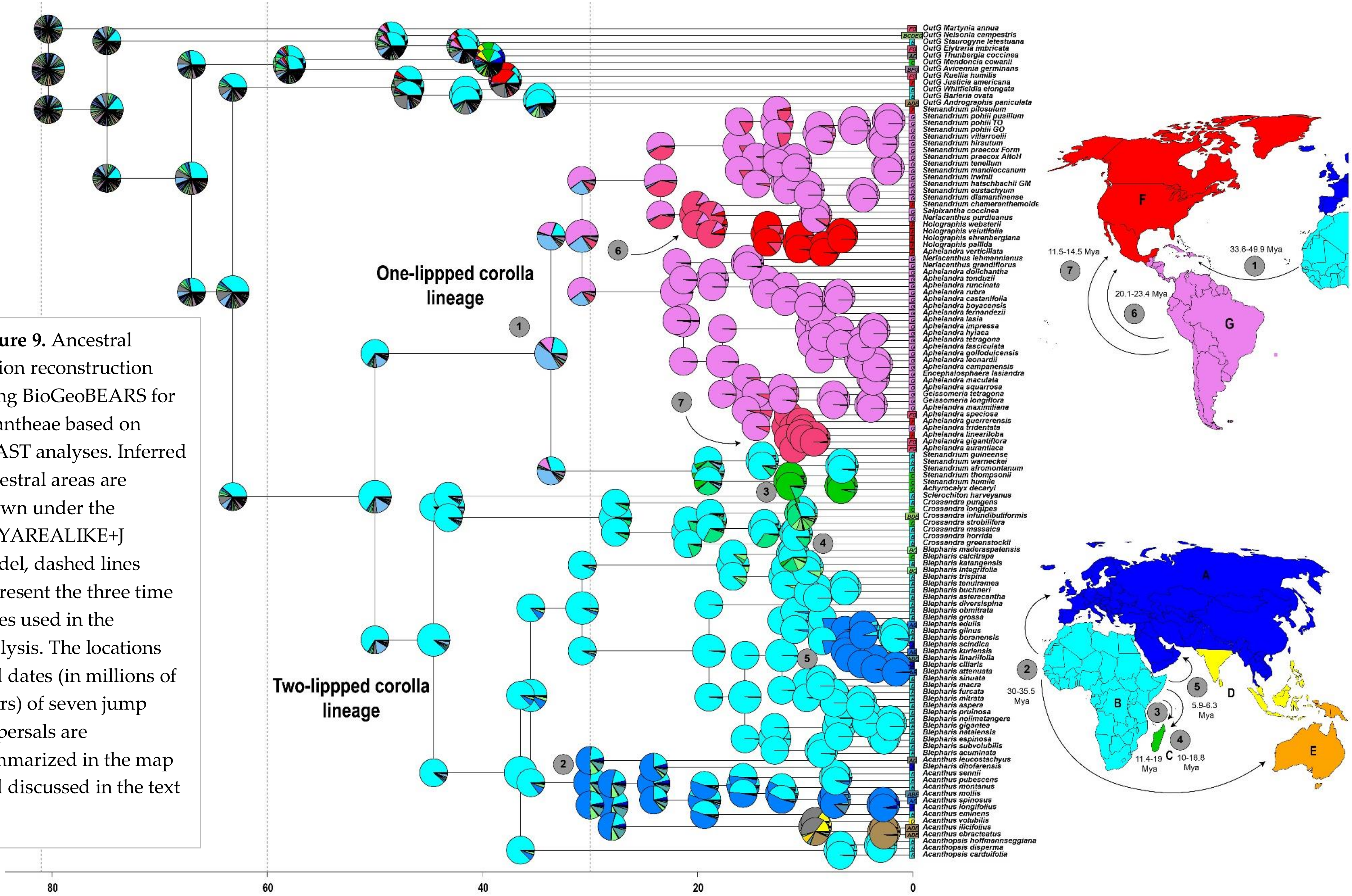


Figure 9. Ancestral region reconstruction using BioGeoBEARS for Acantheae based on BEAST analyses. Inferred ancestral areas are shown under the BAYAREALIKE+J model, dashed lines represent the three time slices used in the analysis. The locations and dates (in millions of years) of seven jump dispersals are summarized in the map and discussed in the text

DISCUSSION

Phylogeny

All analyses corroborate the monophyly of Acantheae and provide high support for the two main lineages found by McDade et al. (2005). The presence of one-lipped corollas can be considered a synapomorphy of the one-lipped lineage, and two-lipped corollas a synapomorphy of the two-lipped lineage.

The high resolution of the four major clades in the one-lipped lineage is in agreement with the estimates of McDade et al. (2005). The position of the *Acanthopsis* clade, of *Sclerochiton* (represented by a single species) and the relationship between *Acanthus* and *Blepharis* are poorly resolved due to incongruences between the nuclear and plastidial analyses, since their positions have high support in both analyses. The position of these groups inside the lineage was also uncertain in McDade et al. (2005) and data from more regions will be necessary to clarify the relationships of those groups within the one-lipped lineage.

Within the two-lipped lineage, the main clades were congruent with McDade et al. (2005), but we obtained a higher resolution between genera in the *Aphelandra* clade. However, the position of *Neriacanthus* (represented by two of its five species) in this clade is still poorly resolved, due to incongruence between the nuclear and plastidial analyses. We were able to definitely confirm the tentative suggestion made by McDade et al. (2005) that OW *Stenandrium* and NW

Stenandrium might not be closely related by including more NW *Stenandrium* species in the phylogeny, as suggested by those authors. Furthermore, NW *Stenandrium* is not a monophyletic group, with one species (*Stenandrium chameranthemoideum,* from Mexico) emerging in the *Holographis* clade. Future studies including more terminals and new regions for all the genera in this lineage are necessary to establish clear limits between these genera and possibly increasing the circumscription of some of them. The resurrection of *Stenandriopsis* (which previously contained the OW species of *Stenandrium*) is unquestionable, with the likely inclusion of *Achyrocalyx*.

Dating

The age of Acanthaceae *s.l.* in this analysis is ~7 My more recent than the estimated using a multiple calibration analysis (Tripp and McDade 2014). This might be due to the small quantity of terminals from other tribes of the family in our analyses. Since the objective of our study was to identify the origin, age and history of tribe Acantheae, we only included a few terminals from other tribes as outgroups; therefore, the age of the family age will not be discussed further.

The origin of tribe Acantheae is estimated at 49.9 Mya, that is ~8.7 My more recent than estimated by Tripp and McDade (2014). This period, between the Paleocene and the Eocene, was one of the warmest periods in the history of the Earth (Willis and McElwain 2014). Evidence from geological records and temperature reconstructions from terrestrial and oceanic sources suggests a general pattern of global warming in the Paleocene and that there was a temperature peak at ~56.3 Mya, known as the Paleocene-Eocene Thermal Maximum (PETM; Willis and McElwain 2014). In this period, it is estimated that temperatures increased very quickly, reaching ~31-34°C (Zachos et al. 2001). Fossil records of many types suggest that this was in fact one of the most diverse intervals in the history of the earth with the greatest global tropical forest coverage (Willis and McElwain 2014).

The transition from the Eocene to the Oligocene (~34 Mya) was a period of significant global climate cooling and increased aridity, with important changes in oceanic currents and the beginning of Antartic ice (Willis and McElwain 2014). In this period some of the major lineages in Acantheae tribe originated: the two-lipped lineage, which has many representatives from dry and savannic environments in its basal lineages (*Stenandrium* clade + *Holographis* clade); and the rise of arid environment genera in the OW: *Acanthus, Blepharis* and *Crossandra.* These irradiations in the two-lipped lineage are contemporary with the diversification of a dry forest clade of Bignoniaceae, the *Xylophragma* - *Fridericia* clade (34-27.2 Mya; tribe Bigonieae, Bignoniaceae), which might have shared pollinators with Acantheae in ancient South American Dry Areas (Lohmann et al. 2013).

In the Late Miocene (11.6-5.3 Mya) sea levels decreased, making the interior of continents drier and exposing large areas of coastline. These abiotic changes caused shifts in the global vegetation, including the expansions of grasslands and savannas, which clearly reflects the steep thermal gradients established from the equator to the poles (Willis and McElwain 2014). Some of those changes in vegetation were caused by the phenomenal success of herbs in this period, which has been described as the "Age of Herbs" (Briggs 1995). Not surprisingly, according to our results, this was the period of gratest diversification of Acantheae, which is composed predominantly by herbs, as is Acanthaceae and Lamiales in general (Stevens 2001 onwards).

These global climate changes towards cooler and drier conditions in the higher latitudes caused a series of change in the whole food chain and resulted in the evolution of the current terrestrial flora and fauna. The diversification of large herbivores increased considerably during the Miocene: proboscids and bovines in Africa, Equidae in North America and Cervidae arised for the first time in Europe, Asia and Africa (Briggs 1995).

Historical relationships with pollinators

The relationship between plant diversification and pollinator diversification can occur as a two-directional phylogenetic cascade, initiating at the lower trophic level (plants) followed by radiation at the next trophic level (pollinators); or in the opposite direction in which the pollinators diversify before the plants in response to other evolutionary pressures such as repetitive geographic isolation, for example (Wilson et al. 2013) leading to plant diversification as a response.

Tribe Acantheae is probably predominantly bee-pollinated although pollinators are unknown for several genera (Table 1); melitophilous type flowers are the most common. Hymenopterans (bees, wasps and ants, Peters et al. 2017) are an ancient group - they predate the Angiosperms - but the diversification of eusocial bees (Apidae) and the rise of important pollinator linegaes (Apinae and Xylocopinae) occured between ~65-40 Mya (Peters et al. 2017). This coincides with the origin of Tribe Acantheae (~50 Mya, Paleocene-Eocene) and it is likely that these pollinators co-evolved with the early lineages of Acantheae; the more basal genera (*Blepharis* and *Acanthus*) are bee-pollinated (see Table 1 for references). *Stenandrium* (personal observations) is visited by Euglossinii bees (27-42 Mya, Hymenoptera; Ramírez et al. 2010), a group that is highly diverse in the Neotropics; and by Syrphidae flies (31 Mya, Diptera, but with foraging behaviour that is similar to that of bees; Kumar et al. 2017).

Hummingbird pollination occurs in three closely related genera of Acantheae: *Aphelandra*, *Geissomeria* and *Encephalosphaera*; these formed a highly supported clade (see Figure 8). It also seems to have been a major influence in the diversification of the tribe’s largest genus, *Aphelandra*. Recent dated phylogenies for birds have indicated that the hummingbird lineage diverged from its nearest ancestor around 22.4 Mya, in the Early Miocene (McGuire et al. 2014) in the Americas. Hummingbirds are the main pollinators of *Aphelandra* (McDade, 1985), that is also the most diverse genus of Acantheae (~207 spp., all Neotropical). The divergence date recovered for *Aphelandra* (21.9 mya) is very close to that of the hummingbird divergence date (22.4). As far as we are aware, all other hummingbird pollinated lineages that have been dated so far (in families Bignoniaceae, Bromeliaceae, Gesneriaceae, and in other tribes of Acanthaceae, such as the Ruellieae), are much younger (Abrahamczyk and Renner 2016; Gervasi and Schiestl 2016; Givnish et al. 2014; Lagomarsino et al. 2016; Lohmann et al. 2013; Tripp and McDade 2013). Therefore, we tentatively suggest that co-evolution between proto-Trochilid birds and ancestral Acantheae might have played a key role in early hummingbird diversification. In the Gesneriaceae, rich in hummingbird-pollinated species, speciation rates are twice as high in hummingbird-pollinated lineages than in insect-pollinated lineages, suggesting that floral morphologies that easily adapt to hummingbird pollination may lead to speciation and rapid diversification (Gervasi and Schiestl 2017). Therefore, the existence of hummingbirds in the Americas are presumably a contributing factor to current Neotropical diversity.

Biogeography

This study corroborates the African origin of Acantheae found by Tripp and McDade (2014) and the hypothesis of long-distance dispersals as explanation for the disjunctions found on the tribe. According to the position of the continents at the times in which these dispersals occurred, considering the distances between areas and the types of environments occupied by current species, it is likely that they have occurred through two ways: oceanic and terrestrial.

The hypothesis of oceanic dispersal between Africa and Tropical America is supported by the fact that, in this period, both continents were isolated, with no terrestrial contact with other continents. In this period, the early Oligocene (34-23 Mya), there were changes in oceanic circulation, as mentioned above. The main east to west currents from Africa to the Americas are the South Equatorial Current (SEC) and North Equatorian Current (NEC, Figure 10). SEC bifurcates in Brazil and continues with NEC to Central America/Caribbean. NEC goes straight from Africa to Central America/Caribbean. It is not possible to infer which route this migration took place for two reasons: a) the resolution of this analysis does not reach specific regions in an area and b) this area change occurred in a deep branch of the tree. However, the dating analysis (Figure 6) shows that the oldest clade in the lineage that originated from this event was the *Holographis* clade, which has its current distribution in Mexico. Therefore, it is more likely that the dispersal route occurred from Africa to Central America, instead of to South America.

Another possible scenario is that Acantheae occupied Africa and Central/South America for tens of millions of years without forming fossils. It is known that tropical environments are less favorable to fossilization, therefore, this is a plausible hypothesis, but hard to be verified.

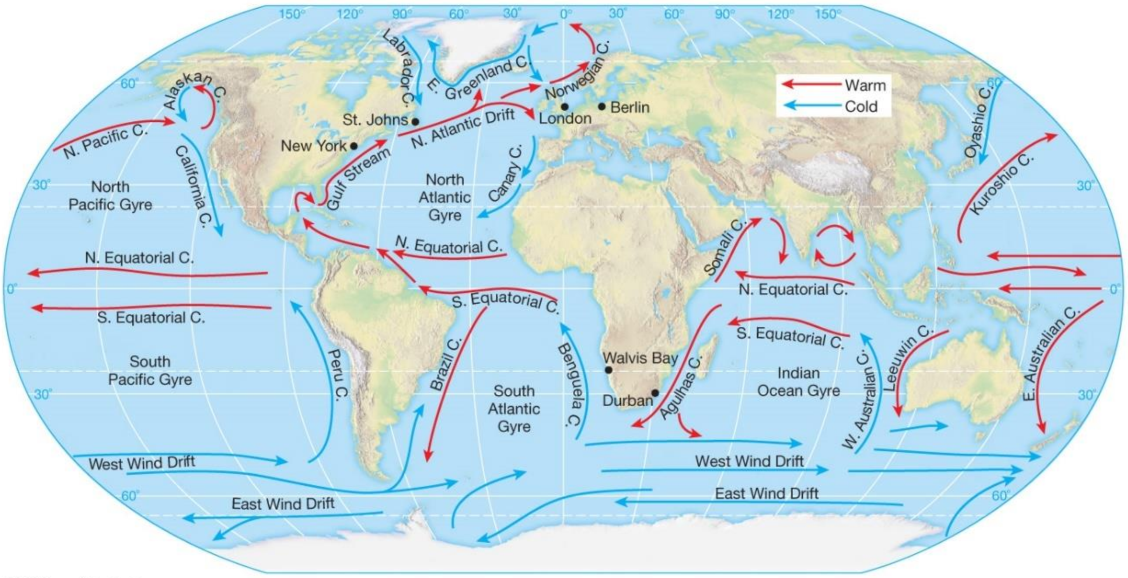


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Figure 10. Map of the main oceanic currents from Pearson Education.

Out of Africa dispersions

The dispersal of *Acanthus* from Africa to Eurasia, which was also maritime, occurred about 20 My before the emergence of the mangrove species (*Acanthus ebracteatus*, *A. ilicifolius* and *A. volubilis*). These mangrove species appeared in the

Miocene, most likely in Southeast Asia, according to fossil pollen records (Morley 1977), which coincides with our estimates (origin in Eurasia + India and Pacific Islands). Another interesting and coincident fact is the date and location of the *Acanthus rugosus* fossil used to calibrate this analysis; the fossil was found in England and dated at ~30 Mya and was positioned in this analysis exactly at the *Acanthus* clade crown node where the dispersal to Eurasia occurred. Since the fossil's distribution area was not included in BioGeoBEARS, this result increases its trustworthiness.

Another dispersal from Africa to Eurasia occurred in the *Blepharis* clade, in the Miocene, when Africa had a terrestrial connection to Eurasia through the Arabian Peninsula (Willis and McElwain 2014). This dispersal event has ocurred precisely in the group formed by species from the Arabic Peninsula, *B. attenuata, B. ciliaris, B. linariifolia, B. kuriensis,* and *B. scindica*. Since these plants are all low stature herbs, with small autochoric fruit and a putative low dispersal capacity, the question of how dispersal may have occurred arises. Herbivore mammals might have been eventual seed dispersors: there is recent molecular evidence (through DNA metabarcoding) of present-day consumption of several Acanthaceae species by large African mammals (Kartzinel et al. 2015) with their small fruits consumed along with the leaves, as hypthesized by Janzen (1984). Furthermore, these large herbivores were also diversifying in this period, as discussed previously.

Considering the proximity between Africa and Madagascar, it is interesting that only two dispersals occurred from Africa into Madagascar (see Results) in all the tribe's history. Madagascar is an island with exceptional high levels of diversity and endemism of many organisms (Gazhorn et al. 2001); over 90% of the flora is endemic (Schatz 2000). A possible explanation is that the Agulhas Current (Figure 10), which flows from north to south between the two areas might hinder the dispersal of seeds and other organisms from Africa to Madagascar. When Madagascar broke off from India c. 105 Mya, the rapid continental dispersal that prevailed during most of the late Cretaceous ensured it remained distant both from India and Africa until at least 75 Mya (Fielding et al. 2008). Nevertheless, phylogenetic reconstruction has provided recent evidence (Yoder and Nowark, 2006) that most of Madagascar's present biota descends from Cenozoic (from 66 mya until recent) dispersers of predominanatly African origin.

South America to North America dispersions

Of the three dispersal events from Central/South America to North America, two were maritime (*Aphelandra* clade and *Holographis* clade) since they occurred before the rising of the Panama ishtmus. The other event, *Stenandrium pilosulum* in Mexico, is interesting since its sister species *S. hirsutum, S. pohlii,* and *S. villarroelii* inhabits the savanna regions of central South America. The absence of terminals for Andean and Caribbean species from this clade in our phylogeny opens possibilities for this alternative dispersal route, which might include using the Caribbean islands as steppingstones over several million years. Besides, the date for this event is uncertain, occurring sometime after 12.6 Mya. The hypothesis of long-distance dispersal facilitated by herbivores is also plausible here, through the famous Great American Biotic Interchange (GABI), which occurred in the same period (~8-10 Ma). GABI was marked by the passage of many herbivores (proboscids, camelids, tayassuids, tapirids and procionids), mainly from North America to South America, but also in the opposite direction. There is evidence that one of the largest herbivores to inhabit the South American tropical savannas and forests migrated from South to North America through GABI: the giant sloth (Defler 2019).

CONCLUSIONS

The estimates of ages presented in this study, although using a single fossil, were very close to those obtained by Tripp and McDade (2014), who used multiple fossil calibrations. Acantheae arose ~50 Mya in Africa in a tropical forest environment, during the hottest period in the history of the earth, between the Paleocene and the Eocene. The most diverse clades of the tribe arose ~20 My later, in a period of significant global climate cooling and increased aridity between the Eocene and the Oligocene. The period in which the greater diversification of the groups occurred was in the "Age of the Herbs", in the late Miocene (11.6-5.3 Mya), when the current latitudinal thermal gradient was established.

The diversification of the main lineages of eusocial Hymenopteran pollinators (the main Acantheae pollinators), coincides with the origin and diversification of the Acantheae (from ~50 Mya, Paleocene-Eocene) suggesting coexistence and possibly coevolution and diversification between pollinating bees and the Acantheae since their origin. The evolution of hummingbird pollination also appears to have had major impact on the diversification of the tribe and of its largest genus, *Aphelandra.* Dates for the origin of hummingbirds and the origin of the *Aphelandra* clade are a close match (both ~22 Mya, Early Miocene) and this is the oldest date to be recovered for a hummingbird-pollinated lineage of plants.

In the biogeographic history of the tribe, some long-distance dispersals were crucial for the establishment of lineages in other continents. The two-lipped lineage diversified in Tropical America from a transoceanic dispersal from Africa mediated by the South Equatorial Current or the North Equatorial Current, most likely through Central America.

There are strong indications that large African herbivorous mammals, which also diversified intensily during the Miocene (Briggs 1995), had an important role in the terrestrial dispersals that happened during this period.

Ideally, phylogenetic biogeography studies should be based on DNA sequence data from many *loci* and many fossil calibrations, since this allows cross-validation of these factors (Pennington et al. 2004). Future studies including more terminals and new regions for some genera of the one-lipped lineage (*Acanthopsis* and *Sclerochiton*) and for all the genera of the two-lipped lineage will be necessary to refine the limits between these genera.

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APPENDIX I

Taxa, geographic distribution, vouchers and sources of the sequences used in this study.

Taxa	**Distribution**	**ITS**	**rps16**	**trnL-trnF**	**trnS-trnG**	**Source**
Acanthopsis carduifolia (L.f.) Schinz	Africa		Ward & Seely 10243 (K)	Ward & Seely 10243 (K)	Ward & Seely 10243 (K)	GenBank
Acanthopsis disperma Nees	Africa	Balkwill et al. 11780 (PH)	Balkwill et al. 11780 (PH)	Balkwill et al. 11780 (PH)	Balkwill et al. 11780 (PH)	GenBank
Acanthopsis hoffmannseggiana (Nees) C.B.Clarke	Africa	Balkwill et al. 11763 (J)	Balkwill et al. 11763 (J)	Balkwill et al. 11763 (J)	Balkwill et al. 11763 (J)	GenBank
Acanthus ebracteatus Vahl	Australia, Eurasia, Southeast Asia, Australia	Qiu 171 (SYS)		BKF188942		GenBank
Acanthus eminens C.B.Clarke	Africa		Friis et al. 9760 (C)	Friis et al. 9760 (C)	Friis et al. 9760 (C)	GenBank
Acanthus ilicifolius L.	Australia, Eurasia, Southeast Asia, Australia	Cultivated, Geneva, Accession No. 19700568/0 (G)	Cultivated, Geneva, Accession No. 19700568/0 (G)	Cultivated, Geneva, Accession No. 19700568/0 (G)	Cultivated, Geneva, Accession No. 19700568/0 (G)	GenBank
Acanthus leucostachyus Wall. ex Nees	Australia, Eurasia, Southeast Asia	GenBank KM652496 (no voucher)		GenBank KM652496 (no voucher)		GenBank
Acanthus longifolius Poir	Australia, Eurasia		Erixon & Bremer 44	Erixon & Bremer 44		GenBank
Acanthus mollis L.	Australia, Eurasia, Africa, Australia	Freeh & Johnson 94-029 (ARIZ)	Freeh & Johnson 94-029 (ARIZ)	Freeh & Johnson 94-029 (ARIZ)	Freeh & Johnson 94-029 (ARIZ)	GenBank
Acanthus montanus (Nees) T.Anderson	Africa	Cultivated, Duke University greenhouses,	Cultivated, Duke University greenhouses,	Cultivated, Duke University greenhouses,	Cultivated, Duke University greenhouses,	GenBank

		Accession No. 86-169	Accession No. 86-169	Accession No. 86-169	Accession No. 86-169	
Acanthus pubescens (Thomson ex Oliv.) Engl.	Africa	Cultivated, Royal Botanic Gardens Kew Accession No. 1986-2701 K	Cultivated, Royal Botanic Gardens Kew Accession No. 1986-2701 K	Cultivated, Royal Botanic Gardens Kew Accession No. 1986-2701 K	Cultivated, Royal Botanic Gardens Kew Accession No. 1986-2701 K	GenBank
Acanthus sennii Chiov.	Africa	Friis et al. 7006 (C)	Friis et al. 7006 (C)	Friis et al. 7006 (C)	Friis et al. 7006 (C)	GenBank
Acanthus spinosus L.	Australia, Eurasia, Africa	Anderson 3696 (CAS)	Anderson 3696 (CAS)	Anderson 3696 (CAS)	Anderson 3696 (CAS)	GenBank
Acanthus volubilis Wall.	Southeast Asia	GenBank KM652494 (no voucher)		GenBank KM888785 (no voucher)		GenBank
Achyrocalyx decaryi Benoist	Madagascar	Lorence 1947 (K)	Lorence 1947 (K)	Lorence 1947 (K)	Lorence 1947 (K)	GenBank
Andrographis paniculata (Burm.f.) Nees	Australia, Eurasia, Southeast Asia, Australia	YC0257MT02	Weaver 1806 (DUKE)	Weaver 1806 (DUKE)	Weaver 1806 (DUKE)	GenBank
Aphelandra aurantiaca (Scheidw.) Lindl.	North America, South America	Lundell & Contreras 19840 (PH)				GenBank
Aphelandra boyacensis Leonard	South America	McDade 989 (DUKE)	McDade 989 (DUKE)	McDade 989 (DUKE)	McDade 989 (DUKE)	GenBank
Aphelandra campanensis Durkee	South America	McDade 852 (DUKE)		McDade 852 (DUKE)		GenBank
Aphelandra castanifolia Britton ex Rusby	South America	Daniel 10175 (CAS)	Daniel 10175 (CAS)	Daniel 10175 (CAS)	Daniel 10175 (CAS)	GenBank
Aphelandra dolicantha Donn. Sm.	South America	McDade 243 (DUKE)	McDade 243 (DUKE)	McDade 243 (DUKE)	McDade 243 (DUKE)	GenBank
Aphelandra fasciculata Wassh.	South America	McDade 659 (DUKE)		McDade 659 (DUKE)		GenBank

Aphelandra fernandezii Leonard	South America	McDade 999 (DUKE)	McDade 999 (DUKE)		McDade 999 (DUKE)	GenBank
Aphelandra gigantiflora Lindau	South America	Daniel 8368 (CAS)	Daniel 8368 (CAS)	Daniel 8368 (CAS)	Daniel 8368 (CAS)	GenBank
Aphelandra golfodulcensis McDade	South America	McDade 251 (DUKE)	McDade 251 (DUKE)		McDade 251 (DUKE)	GenBank
Aphelandra guerrerensis Wassh.	North America	Daniel & Ton 6163 (CAS)		Daniel & Ton 6163 (CAS)	Daniel & Ton 6163 (CAS)	GenBank
Aphelandra hylaea Leonard	South America	McDade 1089 (DUKE)	McDade 1089 (DUKE)	McDade 1089 (DUKE)	McDade 1089 (DUKE)	GenBank
Aphelandra impressa Lindau	South America	McDade 911 (DUKE)			McDade 911 (DUKE)	GenBank
Aphelandra lasia Leonard	South America	McDade 1003 (DUKE)		McDade 1003 (DUKE)	McDade 1003 (DUKE)	GenBank
Aphelandra leonardii McDade	South America	McDade 310 (DUKE)	McDade 310 (DUKE)	McDade 310 (DUKE)	McDade 310 (DUKE)	GenBank
Aphelandra lineariloba Leonard	North America				Daniel 11912 (CAS)	Herbarium
Aphelandra maculata (Tafalla ex Nees) Voss	South America	Cultivated. National Botanic Garden of Belgium, Accession No. 19550109 BR	Cultivated. National Botanic Garden of Belgium, Accession No. 19550109 BR	Cultivated. National Botanic Garden of Belgium, Accession No. 19550109 BR	Cultivated. National Botanic Garden of Belgium, Accession No. 19550109 BR	GenBank
Aphelandra maximiliana (Nees) Benth. ex Lindau	South America	Wasshausen 2326 (US)	Wasshausen 2326 (US)	Wasshausen 2326 (US)	Wasshausen 2326 (US)	GenBank
Aphelandra rubra Wassh.	South America	Roca 332 (CAS)	Roca 332 (CAS)	Roca 332 (CAS)	Roca 332 (CAS)	GenBank
Aphelandra runcinata Klotzsch ex Nees	South America	McDade & Lundberg 1137 (DUKE)	McDade & Lundberg 1137 (DUKE)	McDade & Lundberg 1137 (DUKE)	McDade & Lundberg 1137 (DUKE)	GenBank

Aphelandra speciosa Brandegee	North America, South America	McDade 212 (DUKE)	McDade 212 (DUKE)		McDade 212 (DUKE)	GenBank
Aphelandra squarrosa Nees	South America	McDade 1173 (ARIZ)	McDade 1173 (ARIZ)	McDade 1173 (ARIZ)	McDade 1173 (ARIZ)	GenBank
Aphelandra tetragona (Vahl) Nees	South America	McDade et al. 642 (DUKE)	McDade et al. 642 (DUKE)	McDade et al. 642 (DUKE)	McDade et al. 642 (DUKE)	GenBank
Aphelandra tonduzii Leonard	South America	Daniel et al. 8105 (CAS)	Daniel et al. 8105 (CAS)	Daniel et al. 8105 (CAS)	Daniel et al. 8105 (CAS)	GenBank
Aphelandra tridentata Hemsl.	South America	Haber & Zuchowski 8680 (MO)	Haber & Zuchowski 8680 (MO)	Haber & Zuchowski 8680 (MO)	Haber & Zuchowski 8680 (MO)	GenBank
Aphelandra verticillata Nees ex Hemsl.	North America	Daniel et al. 3295 (CAS)	Daniel et al. 3295 (CAS)	Daniel et al. 3295 (CAS)	Daniel et al. 3295 (CAS)	GenBank
Avicennia germinans (L.) L.	Africa, North America, South America	Ricklefs 181 (KE)	Ricklefs 181 (KE)	Ricklefs 181 (KE)	U. Connecticut Acc #199900063	GenBank
Barleria ovata E.Mey. ex Nees	Africa	Bidgood 1988 (CAS)	Bidgood 1988 (CAS)	Bidgood 1988 (CAS)	Bidgood 1988 (CAS)	GenBank
Blepharis acuminata Oberm.	Africa	McDade et al. 1272 (J)	McDade et al. 1272 (J)	McDade et al. 1272 (J)	McDade et al. 1272 (J)	GenBank
Blepharis aspera Oberm.	Africa	West 7549 (K)				GenBank
Blepharis asteracantha C.B.Clarke	Africa	Faden et al. 96/204 (K)	Faden et al. 96/204 (K)		Faden et al. 96/204 (K)	GenBank
Blepharis attenuata Napper	Africa	Mudaidat 401				GenBank
Blepharis boranensis Vollesen	Africa	Gilbert & Demissew Sebsebe 8555				GenBank
Blepharis buchneri Lindau	Africa	Faden et al. 96/307 (K)	Faden et al. 96/307 (K)		Faden et al. 96/307 (K)	GenBank
Blepharis calcitrapa Benoist	Madagascar	Daniel et al. 10403 (CAS)	Daniel et al. 10403 (CAS)			GenBank

Blepharis ciliaris (L.) B.L.Burtt	Australia, Eurasia	Radcliffe-Smith 3897 (K)				GenBank
Blepharis dhofarensis A.G.Mill.	Australia, Eurasia	Thulin et al. 9715 (K)	Thulin et al. 9715 (K)	Thulin et al. 9715 (K)	Thulin et al. 9715 (K)	GenBank
Blepharis diversispina (Nees) C.B.Clarke	Africa	McDade et al. 1269 (J)		McDade et al. 1269 (J)	McDade et al. 1269 (J)	GenBank
Blepharis edulis (Forssk.) Pers.	Australia, Eurasia, Africa	Friis 6735 (K)	Friis 6735 (K)		Friis 6735 (K)	GenBank
Blepharis espinosa E.Phillips	Africa	Scheepers 1549 (S)				GenBank
Blepharis furcata (L.f.) Pers.	Africa	Werger 153 (K)				GenBank
Blepharis gigantea Oberm.	Africa	Kers 2646 (S)				GenBank
Blepharis glinus Fiori	Africa	Gillett et al. 22122 (K)				GenBank
Blepharis grossa T.Anderson	Africa	lken & Hardy 796 (K)				GenBank
Blepharis integrifolia (L.f.) E.Mey. ex Schinz	Africa, Southeast Asia	Balkwill et al. 11656 (J)	Balkwill et al. 11656 (J)	Balkwill et al. 11656 (J)	Balkwill et al. 11656 (J)	GenBank
Blepharis katangensis De Wild.	Africa	Bidgood et al. 3521 (K)	Bidgood et al. 3521 (K)		Bidgood et al. 3521 (K)	GenBank
Blepharis kuriensis Vierh.	Australia, Eurasia, Africa	Smith & Lavranos 702 (K)				GenBank
Blepharis linariifolia Pers.	Australia, Eurasia, Africa, Southeast Asia	Hedrn et al. 739 (UPS)				GenBank
Blepharis macra (Nees) Vollesen	Africa	van Breda 1347 (K)				GenBank
Blepharis maderaspatensis (L.) B.Heyne ex Roth	Africa, Southeast Asia	McDade et al. 1292 (PH)	McDade et al. 1292 (PH)	Balkwill et al. 11656 (J)	McDade et al. 1292 (PH)	GenBank
Blepharis mitrata C.B.Clarke	Africa	Seydel 3981 (M)				GenBank
Blepharis natalensis Oberm.	Africa	Balkwill et al. 11667 (J)	Balkwill et al. 11667 (J)	Balkwill et al. 11667 (J)	Balkwill et al. 11667 (J)	GenBank
Blepharis noli-me-tangere S.Moore	Africa	Henriques 454 (K)				GenBank

Blepharis obmitrata C.B.Clarke	Africa	Kami 4132 (K)				GenBank
Blepharis pruinose Engl.	Africa	Kers 622 (S)				GenBank
Blepharis scindica Stocks ex T.Anderson	Australia, Eurasia	Garser & Ghafoor 4153 (B)				GenBank
Blepharis sinuata (Nees) C.B.Clarke	Africa	McDade & Dold 1193 (PH)	McDade & Dold 1193 (PH)	McDade & Dold 1193 (PH)	McDade & Dold 1193 (PH)	GenBank
Blepharis subvolubilis C.B.Clarke	Africa	Balkwill et al. 10850 (J)	Balkwill et al. 10850 (J)	Balkwill et al. 10850 (J)	Balkwill et al. 10850 (J)	GenBank
Blepharis tenuiramea S.Moore	Africa	Bidgood et al. 3869 (K)	Bidgood et al. 3869 (K)		Bidgood et al. 3869 (K)	GenBank
Blepharis trispina Napper	Africa	Bidgood et al. 1102 (K)	Bidgood et al. 1102 (K)		Bidgood et al. 1102 (K)	GenBank
Crossandra greenstockii S.Moore	Africa	McDade & Balkwill 1241 (J)	McDade & Balkwill 1241 (J)	McDade & Balkwill 1241 (J)	McDade & Balkwill 1241 (J)	GenBank
Crossandra horrida Vollesen	Africa	Thulin et al. 8949 (K)	Thulin et al. 8949 (K)	Thulin et al. 8949 (K)	Thulin et al. 8949 (K)	GenBank
Crossandra infundibuliformis (L.) Nees	Africa, Southeast Asia, Australia	Cultivated McDade 1162 (ARIZ)	Cultivated McDade 1162 (ARIZ)	Cultivated McDade 1162 (ARIZ)	Cultivated McDade 1162 (ARIZ)	GenBank
Crossandra longipes S.Moore	Madagascar	Hearn Mad-62 (PH)	Hearn Mad-62 (PH)	Hearn Mad-62 (PH)	Hearn Mad-62 (PH)	GenBank
Crossandra massaica Mildbr.	Africa			DeFranco D6_K1214		GenBank
Crossandra pungens Lindau	Africa	Cultivated, Duke University greenhouses, Accession No. 91-036	Daniel s.n. (CAS)	Cultivated, Duke University greenhouses, Accession No. 91-036	Daniel s.n. (CAS)	GenBank
Crossandra strobilifera (Lam.) Benoist	Madagascar	Hearn Mad-49 (PH)	Hearn Mad-49 (PH)	Hearn Mad-49 (PH)	Hearn Mad-49 (PH)	GenBank

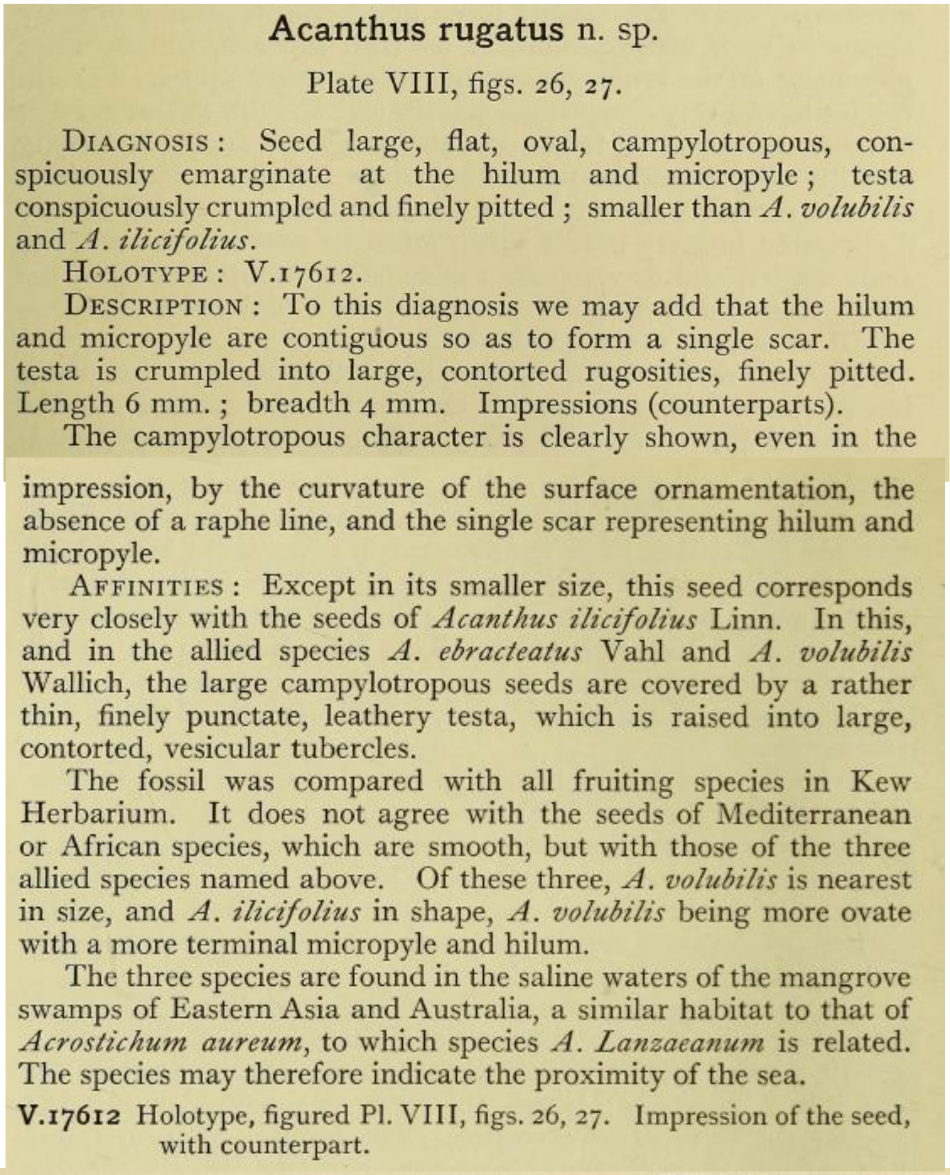
Elytraria imbricata (Vahl) Pers.	North America, South America	T.F. Daniel s.n. (CAS)	McDade & Jenkins 1155 (ARIZ)	McDade & Jenkins 1155 (ARIZ)	McDade & Jenkins 1155 (ARIZ)	GenBank
Encephalosphaera lasiandra Mildbr.	South America			Graham & Schunke Vigo 0958 (F)	Graham & Schunke Vigo 0958 (F)	GenBank
Geissomeria longiflora Lindl.	South America	Wasshausen 2337 (US)	Wasshausen 2337 (US)	Wasshausen 2337 (US)	Wasshausen 2337 (US)	GenBank
Geissomeria tetragona Lindau	South America	Daniel 10103 (CAS)	Daniel 10103 (CAS)	Daniel 10103 (CAS)	Daniel 10103 (CAS)	GenBank
Holographis ehrenbergiana Nees	North America	Daniel & Baker 3712 (CAS)	Daniel & Baker 3712 (CAS)	Daniel & Baker 3712 (CAS)	Daniel & Baker 3712 (CAS)	GenBank
Holographis pallida Leonard & Gentry	North America	Daniel et al. 8510 (CAS)	Daniel et al. 8510 (CAS)	Daniel et al. 8510 (CAS)	Daniel et al. 8510 (CAS)	GenBank
Holographis velutifolia (House) T.F.Daniel	North America	Salinas et al. 4779 (CAS)	Salinas et al. 4779 (CAS)	Salinas et al. 4779 (CAS)	Salinas et al. 4779 (CAS)	GenBank
Holographis websterii T.F.Daniel	North America		Daniel 10271 (CAS)	Daniel 10271 (CAS)	Daniel 10271 (CAS)	Campo
Justicia americana (L.) Vahl	North America	Daniel & Lott 10530 (CAS)	Daniel & Lott 10530 (CAS)	Daniel & Lott 10530 (CAS)	Daniel & Lott 10530 (CAS)	GenBank
Martynia annua L.	North America, South America	Damrel 1024 (ASU)	P. Jenkins 97-149 (ARIZ)	P. Jenkins 97-149 (ARIZ)	P. Jenkins 97-149 (ARIZ)	GenBank
Mendoncia cowanii (S.Moore) Benoist	Madagascar	Hearn Madagascar 3 (PH)	Hearn Madagascar 3 (PH)	Hearn Madagascar 3 (PH)	Hearn Madagascar 3 (PH)	GenBank
Nelsonia campestris R.Br.	Africa, Madagascar, Southeast Asia, Australia, South America	Daniel et al. 5452 (CAS)	Daniel et al. 5452 (CAS)	Daniel et al. 5452 (CAS)	Daniel et al. 5452 (CAS)	GenBank

Neriacanthus grandiflorus Leonard	South America	Daniel et al. 8152 (CAS)	Daniel et al. 8152 (CAS)	Daniel et al. 8152 (CAS)	Daniel et al. 8152 (CAS)	GenBank
Neriacanthus lehmannianus (Lindau) Lindau	South America	Wood 5379 (CAS)	Wood 5379 (CAS)	Wood 5379 (CAS)	Wood 5379 (CAS)	GenBank
Neriacanthus purdieanus Benth.	South America	Gillis 14978 (BM)	Gillis 14978 (BM)	Gillis 14978 (BM)	Gillis 14978 (BM)/Proctor 24475 (US)	GenBank
Ruellia humilis Nutt.	North America, South America	Tripp 14 (PH)	S. R. Hill 30665 (NY)	S. R. Hill 30665 (NY)	Tripp 14 (PH)	GenBank
Salpixantha coccinea Hook.	South America	Webster & Proctor 5635 (BM)	Webster & Proctor 5635 (BM)	Webster & Proctor 5635 (BM)	Webster & Proctor 5635 (BM)	GenBank
Sclerochiton harveyanus Nees	Africa	Balkwill 12274 (J)	Balkwill 12274 (J)	Balkwill 12274 (J)	Balkwill 12274 (J)	GenBank
Staurogyne letestuana Benoist	Africa	National Botanic Garden of Belgium 20000119-77 (BR)	National Botanic Garden of Belgium 20000119-77 (BR)	National Botanic Garden of Belgium 20000119-77 (BR)	National Botanic Garden of Belgium 20000119-77 (BR)	GenBank
Stenandrium afromontanum (Mildbr.) Vollesen	Africa	Lovett 274 (CAS)	Lovett 274 (CAS)	Lovett 274 (CAS)	Lovett 274 (CAS)	GenBank
Stenandrium chameranthemoideum Oerst.	North America		Daniel 11806 (CAS)	Daniel 11806 (CAS)	Daniel 11806 (CAS)	Field
Stenandrium diamantinense Zanatta & Kameyama	South America		Faria 7884	Faria 7884	Faria 7884	Field
Stenandrium eustachyum Zanatta & Proença	South America			Faria VER	Faria VER	Field
Stenandrium guineense (Nees) Vollesen	Africa	Cultivated, Palm House, Royal Botanic Gardens, Kew, Accession No. 1990-2299 K	Cultivated, Palm House, Royal Botanic Gardens, Kew, Accession No. 1990-2299 K	Cultivated, Palm House, Royal Botanic Gardens, Kew, Accession No. 1990-2299 K	Cultivated, Palm House, Royal Botanic Gardens, Kew, Accession No. 1990-2299 K	GenBank
Stenandrium hatschbachii Wassh.	South America		Zanatta 2508	Zanatta 2508	Zanatta 2508	Field

Stenandrium hirsutum Nees	South America		Faria 8445	Faria 8445	Faria 8445	Field
Stenandrium humile (Benoist) Vollesen	Madagascar		DuPuy et al. MB587 (CAS)	DuPuy et al. MB587 (CAS)	DuPuy et al. MB587 (CAS)	GenBank
Stenandrium irwinii Wassh.	South America	Zanatta 2387	Zanatta 2387/2398	Zanatta 2387	Zanatta 2387/2398	Field
Stenandrium mandioccanum Nees	South America	Wasshausen & Wood 2201 (CAS)	Wasshausen & Wood 2201 (CAS)	Wasshausen & Wood 2201 (CAS)	Wasshausen & Wood 2201 (CAS)	GenBank
Stenandrium pilosulum (S.F.Blake) T.F.Daniel	North America	Van Devender & Reina G. 97–434 (ARIZ)	Van Devender & Reina G. 97–434 (ARIZ)	Van Devender & Reina G. 97–434 (ARIZ)	Van Devender & Reina G. 97–434 (ARIZ)	GenBank
Stenandrium pohlii Nees	South America	Zanatta 2434 (GO)		Zanatta 2434 (GO)		Field
Stenandrium pohlii Nees	South America	Zanatta 2454 (TO)	Zanatta 2454 (TO)	Zanatta 2454 (TO)	Zanatta 2454 (TO)	Field
Stenandrium pohlii var. pusillum Nees	South America			Zanatta 2441		Field
Stenandrium riedelianum Nees	South America		Zanatta 2451 (Form)	Zanatta 2451 (Form)		Field
Stenandrium riedelianum Nees	South America	Faria 8364 (AltoH)	Faria 8364 (AltoH)			Field
Stenandrium tenellum Nees	South America		Proença & Harris 5220	Proença & Harris 5220	Proença & Harris 5220	Field
Stenandrium thompsonii S.Moore	Madagascar		Schatz 2714 (K)	Schatz 2714 (K)	Schatz 2714 (K)	GenBank
Stenandrium villarroelii J.R.I.Wood	South America		Wood & Villarroel 25742	Wood & Villarroel 25742	Wood & Villarroel 25742	Herbarium
Stenandrium warneckei (S.Moore) Vollesen	Africa	Steiner 403 (UPS)	Steiner 403 (UPS)	Steiner 403 (UPS)	Steiner 403 (UPS)	GenBank
Thunbergia coccinea Wall.	Australia, Eurasia, Southeast Asia	Schönenberger 144 (Z)	Schönenberger 144 (Z)	Wongakson 62	Schönenberger 144 (Z)	GenBank
Whitfieldia elongata (P.Beauv.) De Wild. & T.Durand	Africa	Manktelow 682 (UPS)	Manktelow 682 (UPS)	Manktelow 682 (UPS)	Manktelow 682 (UPS)	GenBank

APPENDIX II

Description and images of the fossil species *Acanthus rugatus* Reid & Chandler (1926, p. 131-132).



Acanthus rugatus n. sp.

Plate VIII, figs. 26, 27.

DIAGNOSIS: Seed large, flat, oval, campylotropous, conspicuously emarginate at the hilum and micropyle; testa conspicuously crumpled and finely pitted; smaller than *A. volubilis* and *A. ilicifolius*.

HOLOTYPE: V.17612.

DESCRIPTION: To this diagnosis we may add that the hilum and micropyle are contiguous so as to form a single scar. The testa is crumpled into large, contorted rugosities, finely pitted. Length 6 mm.; breadth 4 mm. Impressions (counterparts).

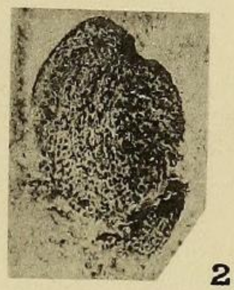
The campylotropous character is clearly shown, even in the impression, by the curvature of the surface ornamentation, the absence of a raphe line, and the single scar representing hilum and micropyle.

AFFINITIES: Except in its smaller size, this seed corresponds very closely with the seeds of *Acanthus ilicifolius* Linn. In this, and in the allied species *A. ebracteatus* Vahl and *A. volubilis* Wallich, the large campylotropous seeds are covered by a rather thin, finely punctate, leathery testa, which is raised into large, contorted, vesicular tubercles.

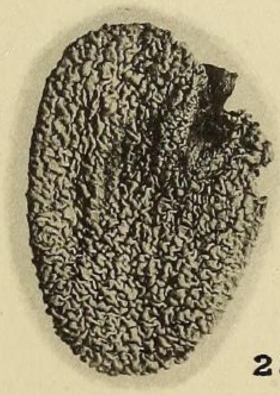
The fossil was compared with all fruiting species in Kew Herbarium. It does not agree with the seeds of Mediterranean or African species, which are smooth, but with those of the three allied species named above. Of these three, *A. volubilis* is nearest in size, and *A. ilicifolius* in shape, *A. volubilis* being more ovate with a more terminal micropyle and hilum.

The three species are found in the saline waters of the mangrove swamps of Eastern Asia and Australia, a similar habitat to that of *Acrostichum aureum*, to which species *A. Lanzaeanum* is related. The species may therefore indicate the proximity of the sea.

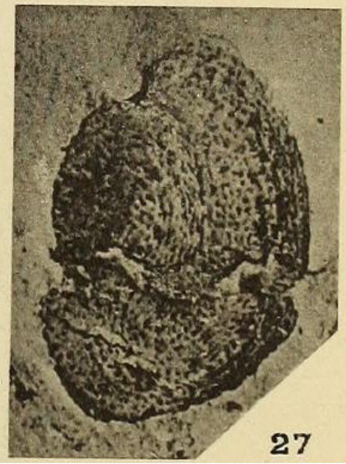
V.17612 Holotype, figured Pl. VIII, figs. 26, 27. Impression of the seed, with counterpart.



26



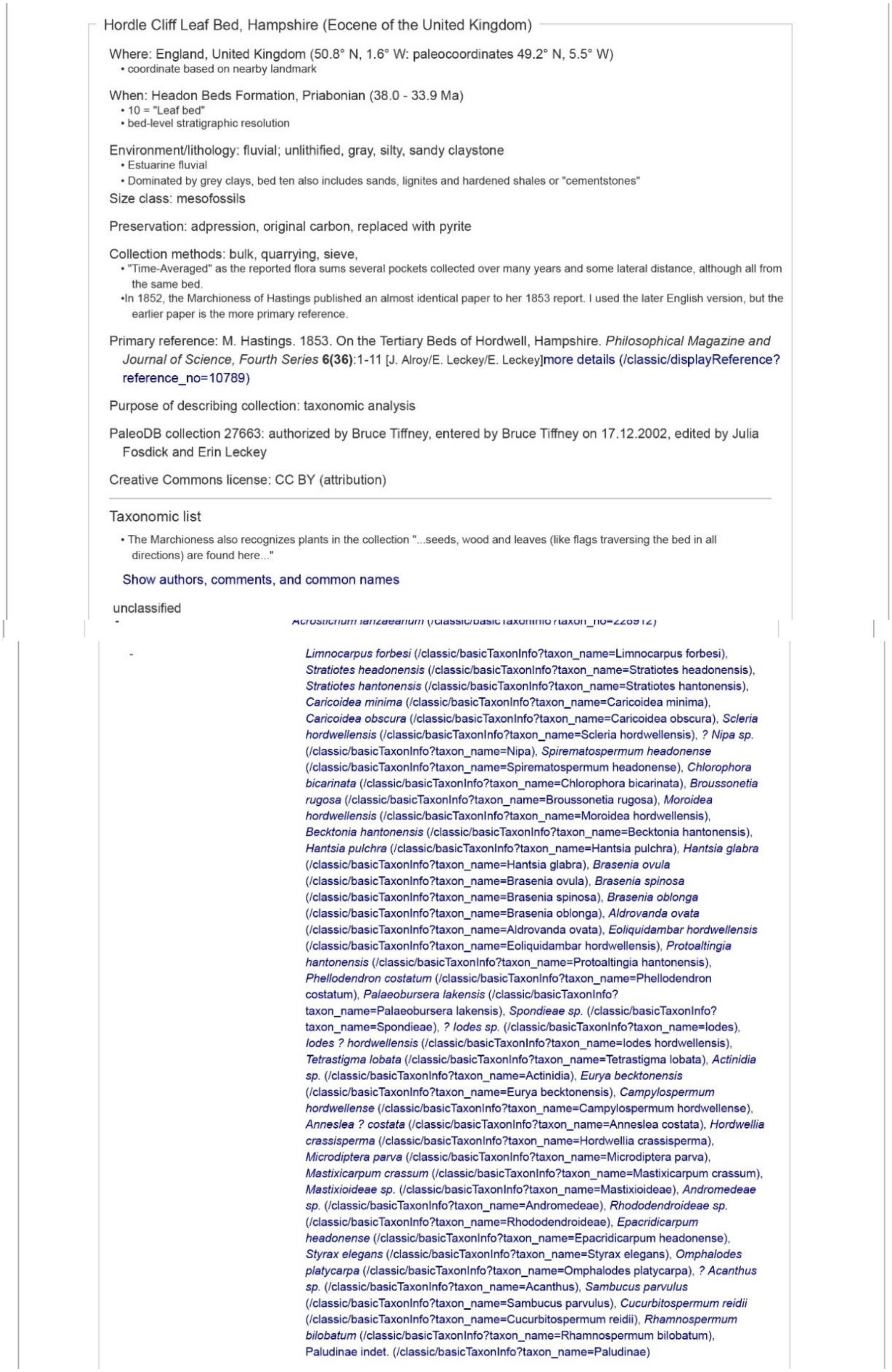
28



27

APPENDIX III

Information of the Hordle Cliff Leaf Bed, where the fossil *Acanthus rugosus* were found, available in PaleoBioDataBase (https://paleobiodb.org/).



Hordle Cliff Leaf Bed, Hampshire (Eocene of the United Kingdom)

Where: England, United Kingdom (50.8° N, 1.6° W: paleocoordinates 49.2° N, 5.5° W)
- coordinate based on nearby landmark

When: Headon Beds Formation, Priabonian (38.0 - 33.9 Ma)
- 10 = "Leaf bed"
- bed-level stratigraphic resolution

Environment/lithology: fluvial; unlithified, gray, silty, sandy claystone
- Estuarine fluvial
- Dominated by grey clays, bed ten also includes sands, lignites and hardened shales or "cementstones"

Size class: mesofossils

Preservation: adpression, original carbon, replaced with pyrite

Collection methods: bulk, quarrying, sieve,
- "Time-Averaged" as the reported flora sums several pockets collected over many years and some lateral distance, although all from the same bed.
- In 1852, the Marchioness of Hastings published an almost identical paper to her 1853 report. I used the later English version, but the earlier paper is the more primary reference.

Primary reference: M. Hastings. 1853. On the Tertiary Beds of Hordwell, Hampshire. *Philosophical Magazine and Journal of Science, Fourth Series* **6(36)**:1-11 [J. Alroy/E. Leckey/E. Leckey]more details (/classic/displayReference?reference_no=10789)

Purpose of describing collection: taxonomic analysis

PaleoDB collection 27663: authorized by Bruce Tiffney, entered by Bruce Tiffney on 17.12.2002, edited by Julia Fosdick and Erin Leckey

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Taxonomic list
- The Marchioness also recognizes plants in the collection "...seeds, wood and leaves (like flags traversing the bed in all directions) are found here..."

Show authors, comments, and common names

unclassified

\- *Acrostichum lanzaeanum* (/classic/basicTaxonInfo?taxon_no=228912)

\- *Limnocarpus forbesi* (/classic/basicTaxonInfo?taxon_name=Limnocarpus forbesi), *Stratiotes headonensis* (/classic/basicTaxonInfo?taxon_name=Stratiotes headonensis), *Stratiotes hantonensis* (/classic/basicTaxonInfo?taxon_name=Stratiotes hantonensis), *Caricoidea minima* (/classic/basicTaxonInfo?taxon_name=Caricoidea minima), *Caricoidea obscura* (/classic/basicTaxonInfo?taxon_name=Caricoidea obscura), *Scleria hordwellensis* (/classic/basicTaxonInfo?taxon_name=Scleria hordwellensis), *? Nipa sp.* (/classic/basicTaxonInfo?taxon_name=Nipa), *Spirematospermum headonense* (/classic/basicTaxonInfo?taxon_name=Spirematospermum headonense), *Chlorophora bicarinata* (/classic/basicTaxonInfo?taxon_name=Chlorophora bicarinata), *Broussonetia rugosa* (/classic/basicTaxonInfo?taxon_name=Broussonetia rugosa), *Moroidea hordwellensis* (/classic/basicTaxonInfo?taxon_name=Moroidea hordwellensis), *Becktonia hantonensis* (/classic/basicTaxonInfo?taxon_name=Becktonia hantonensis), *Hantsia pulchra* (/classic/basicTaxonInfo?taxon_name=Hantsia pulchra), *Hantsia glabra* (/classic/basicTaxonInfo?taxon_name=Hantsia glabra), *Brasenia ovula* (/classic/basicTaxonInfo?taxon_name=Brasenia ovula), *Brasenia spinosa* (/classic/basicTaxonInfo?taxon_name=Brasenia spinosa), *Brasenia oblonga* (/classic/basicTaxonInfo?taxon_name=Brasenia oblonga), *Aldrovanda ovata* (/classic/basicTaxonInfo?taxon_name=Aldrovanda ovata), *Eoliquidambar hordwellensis* (/classic/basicTaxonInfo?taxon_name=Eoliquidambar hordwellensis), *Protoaltingia hantonensis* (/classic/basicTaxonInfo?taxon_name=Protoaltingia hantonensis), *Phellodendron costatum* (/classic/basicTaxonInfo?taxon_name=Phellodendron costatum), *Palaeobursera lakensis* (/classic/basicTaxonInfo?taxon_name=Palaeobursera lakensis), *Spondieae sp.* (/classic/basicTaxonInfo?taxon_name=Spondieae), *? Iodes sp.* (/classic/basicTaxonInfo?taxon_name=Iodes), *Iodes ? hordwellensis* (/classic/basicTaxonInfo?taxon_name=Iodes hordwellensis), *Tetrastigma lobata* (/classic/basicTaxonInfo?taxon_name=Tetrastigma lobata), *Actinidia sp.* (/classic/basicTaxonInfo?taxon_name=Actinidia), *Eurya becktonensis* (/classic/basicTaxonInfo?taxon_name=Eurya becktonensis), *Campylospermum hordwellense* (/classic/basicTaxonInfo?taxon_name=Campylospermum hordwellense), *Anneslea ? costata* (/classic/basicTaxonInfo?taxon_name=Anneslea costata), *Hordwellia crassisperma* (/classic/basicTaxonInfo?taxon_name=Hordwellia crassisperma), *Microdiptera parva* (/classic/basicTaxonInfo?taxon_name=Microdiptera parva), *Mastixicarpum crassum* (/classic/basicTaxonInfo?taxon_name=Mastixicarpum crassum), *Mastixioideae sp.* (/classic/basicTaxonInfo?taxon_name=Mastixioideae), *Andromedeae sp.* (/classic/basicTaxonInfo?taxon_name=Andromedeae), *Rhododendroideae sp.* (/classic/basicTaxonInfo?taxon_name=Rhododendroideae), *Epacridicarpum headonense* (/classic/basicTaxonInfo?taxon_name=Epacridicarpum headonense), *Styrax elegans* (/classic/basicTaxonInfo?taxon_name=Styrax elegans), *Omphalodes platycarpa* (/classic/basicTaxonInfo?taxon_name=Omphalodes platycarpa), *? Acanthus sp.* (/classic/basicTaxonInfo?taxon_name=Acanthus), *Sambucus parvulus* (/classic/basicTaxonInfo?taxon_name=Sambucus parvulus), *Cucurbitospermum reidii* (/classic/basicTaxonInfo?taxon_name=Cucurbitospermum reidii), *Rhamnospermum bilobatum* (/classic/basicTaxonInfo?taxon_name=Rhamnospermum bilobatum), Paludinae indet. (/classic/basicTaxonInfo?taxon_name=Paludinae)

Dicotyledonae

Rhamnales - Rosidae	*Vitis uncinata* (/classic/basicTaxonInfo?taxon_no=157350), *Ampelopsis rotundata* (/classic/basicTaxonInfo?taxon_no=55498), *Parthenocissus hordwellensis* (/classic/basicTaxonInfo?taxon_no=264931)
Theales - Dilleniidae	*Diospyros headonensis* (/classic/basicTaxonInfo?taxon_no=54724), *Gordonia minima* (/classic/basicTaxonInfo?taxon_no=54708), *Gordonia truncata* (/classic/basicTaxonInfo?taxon_no=54708), *Cleyera ? stigmosa* (/classic/basicTaxonInfo?taxon_no=263334)
Scrophulariales - Asteridae	*Olea headonensis* (/classic/basicTaxonInfo?taxon_no=319869)
Juglandales - Hamamelidae	*Myrica boveyana* (/classic/basicTaxonInfo?taxon_no=55519)

Magnoliopsida

Magnoliales - Lauraceae	Lauraceae indet. (/classic/basicTaxonInfo?taxon_no=53510)
Fabales - Leguminosae	Leguminosae indet. (/classic/basicTaxonInfo?taxon_no=54652)
Myrtales - Lythraceae	Lythraceae indet. (/classic/basicTaxonInfo?taxon_no=55686)
Rosales - Rosaceae	*Rubus acutiformis* (/classic/basicTaxonInfo?taxon_no=264405)
Sapindales - Rutaceae	*Rutaspermum ornatum* (/classic/basicTaxonInfo?taxon_no=264324), *Zanthoxylum hordwellense* (/classic/basicTaxonInfo?taxon_no=319951), *Zanthoxylum compressum* (/classic/basicTaxonInfo?taxon_no=319951)
Cornales - Cornaceae	*Cornus quadrilocularis* (/classic/basicTaxonInfo?taxon_no=54721), "*Dunstania glandulosa*" = *Cornus glandulosa* (/classic/basicTaxonInfo?taxon_no=190852)
Cornales - Symplocaceae	*Symplocos headonensis* (/classic/basicTaxonInfo?taxon_no=319964), *Symplocos sp.* (/classic/basicTaxonInfo?taxon_no=319964)
Fagales - Betulaceae	*Carpinus boveyanus* (/classic/basicTaxonInfo?taxon_no=55066)
Ranunculales - Sabiaceae	*Meliosma sp.* (/classic/basicTaxonInfo?taxon_no=54697)
Ranunculales - Menispermaceae	*Palaeosinomenium obliquatum* (/classic/basicTaxonInfo?taxon_no=263038)
Celastrales - Icacinaceae	*Icacinicarya transversalis* (/classic/basicTaxonInfo?taxon_no=261760), *Icacinicarya becktonensis* (/classic/basicTaxonInfo?taxon_no=261760), *Natsiatum eocenicum* (/classic/basicTaxonInfo?taxon_no=261756)

Carpolithus

-	*Carpolithus fibrosus* (/classic/basicTaxonInfo?taxon_no=134692), *Carpolithus apocyniformis* (/classic/basicTaxonInfo?taxon_no=134692), *Carpolithus "sp. 8"* (/classic/basicTaxonInfo?taxon_no=134692), *Carpolithus "sp. 6"* (/classic/basicTaxonInfo?taxon_no=134692), *Carpolithus cf. gardneri* (/classic/basicTaxonInfo?taxon_no=134692), *Carpolithus "sp. 1"* (/classic/basicTaxonInfo?taxon_no=134692), *Carpolithus sp.* (/classic/basicTaxonInfo?taxon_no=134692)

Limnaea

-	*Limnaea longiscata* (/classic/basicTaxonInfo?taxon_no=91736)

Mastixiaceae

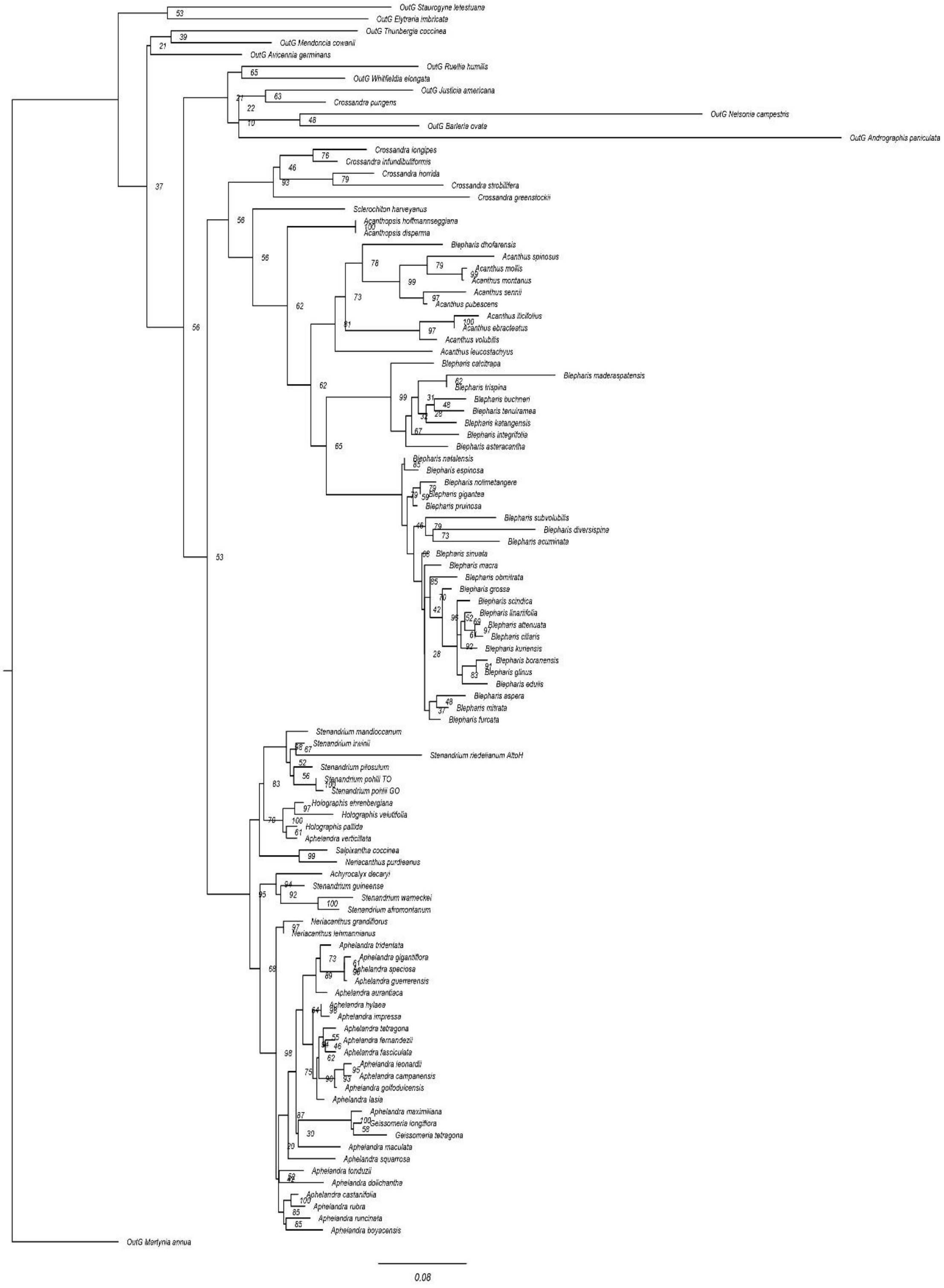
- Mastixiaceae	*Eomastixia rugosa* (/classic/basicTaxonInfo?taxon_no=260470)

Mastixia

- Mastixiaceae	*Mastixia ? glandulosa* (/classic/basicTaxonInfo?taxon_no=261812)

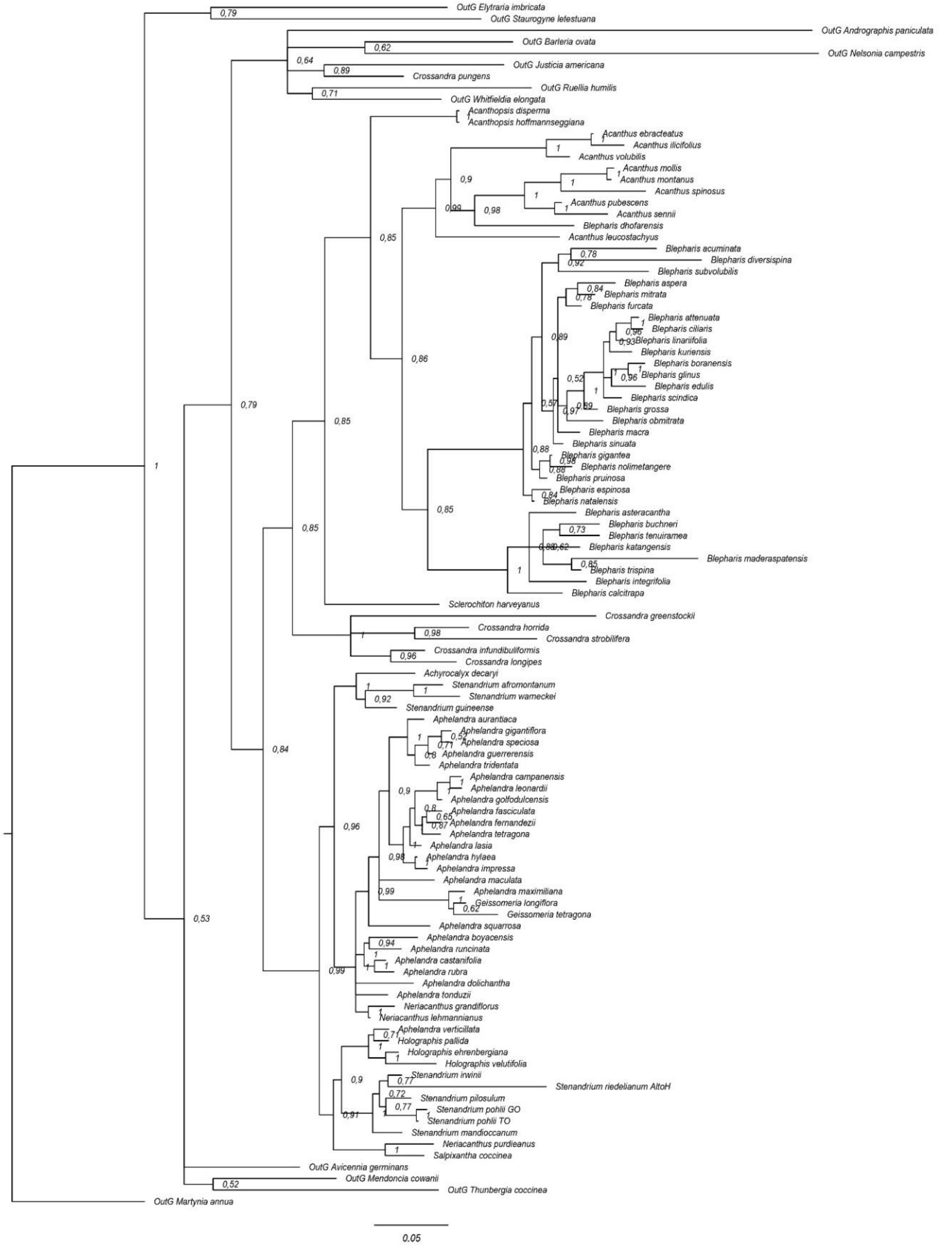
APPENDIX IV

Acantheae phylogenetic tree obtained from the ITS inferred from a maximum likelihood reconstruction method (RAxML) with bootstrap values at nodes. OutG - outgroup.



APPENDIX V

Acantheae phylogenetic tree obtained from the ITS inferred from a Bayesian reconstruction method (MrBayes) with posterior probability values at nodes. OutG: outgroup.

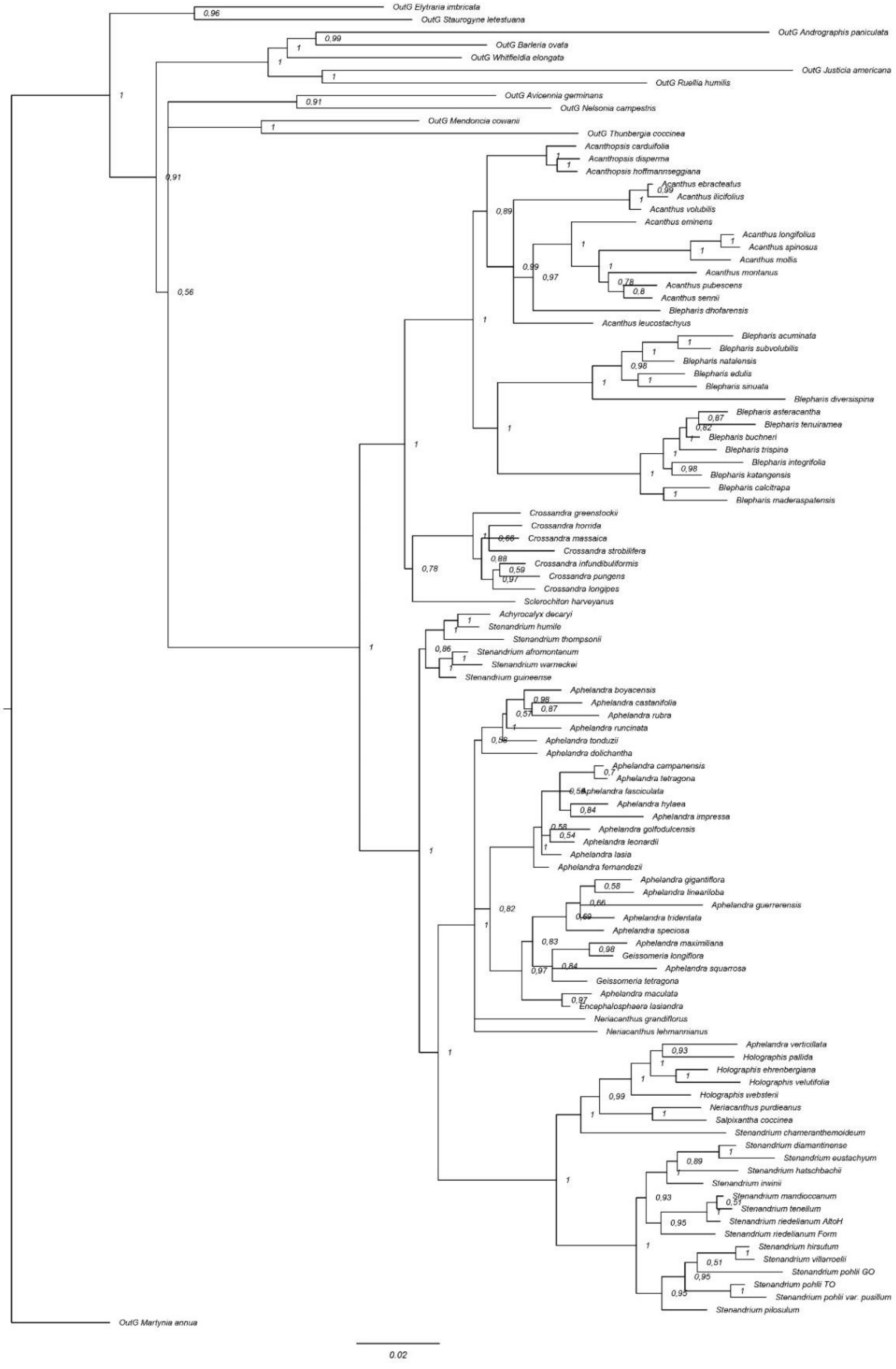


APPENDIX VI

Acantheae phylogenetic tree obtained from the combined plastidial matrix (rps16, trnL-F e trnS-G) inferred from a maximum likelihood reconstruction method (RAxML) with bootstrap values at nodes. OutG: outgroup.

APPENDIX VII

Acantheae phylogenetic tree obtained from the combined plastidial matrix (rps16, trnL-F e trnS-G) inferred from a Bayesian reconstruction method (MrBayes) with posterior probability values at nodes. OutG: outgroup.



CAPÍTULO II

Stenandrium Nees (Lamiales: Acanthaceae): taxonomic revision of the Brazilian species

ABSTRACT

Stenandrium Nees is a genus of ~100 species of herbs and subshrubs, potentially medicinal and ornamental, poorly known both to scientists and local people. Its currently circumscription is polyphyletic but the Brazilian species of *Stenandrium* form an apparently monophyletic group. Contributions to the taxonomy of the genus are provided herein, including a revision of the Brazilian species (almost half of the total Neotropical species of the genus), with morphological descriptions, images, and information on ecology and geographic distribution. An identification key is also provided, and species are evaluated as to extinction risk using IUCN criteria. The taxonomic uncertainties, intra-populational morphological variability, and difficulties in species circumscriptions found in this study show that a complete review of Neotropical species of *Stenandrium* is necessary.

RESUMO

Stenandrium Nees é um gênero de aproximadamente 100 espécies de ervas e subarbustos, potencialmente medicinais e ornamentais, pouco conhecido tanto pelos cientistas quanto pela população local. Atualmente, sua circunscrição é polifilética, mas as espécies brasileiras de *Stenandrium* formam um grupo aparentemente monofilético. Contribuições para a taxonomia do gênero são fornecidas aqui, incluindo uma revisão das espécies brasileiras (quase metade do total de espécies neotropicais do gênero), com descrições morfológicas, imagens e informações sobre ecologia e distribuição geográfica. Também é fornecida uma chave de identificação e as espécies são avaliadas quanto ao risco de extinção usando os critérios da IUCN. As incertezas taxonômicas, a variabilidade morfológica intra-populacional e as dificuldades nas circunscrições de espécies encontradas neste estudo mostram que é necessária uma revisão completa das espécies neotropicais de *Stenandrium*.

INTRODUCTION

Stenandrium Nees is a genus of ~100 species of herbs and subshrubs, that can be recognized by the presence of spikes, green bracts, and showy, zygomorphic or subactinomorphic flowers (Daniel 1984). In its current circumscription (*sensu* Vollesen 1992) it is found in tropical and subtropical regions of the Neotropics and Paleotropics (Africa and Madagascar). This circumscription was questioned by McDade et al. (2005) who found that the African and Madagascan species (previously recognized in *Stenandriopsis* S.Moore, a distinct genus) formed a distinct clade, basal to a strongly supported Neotropical clade in which all the Neotropical species of *Stenandrium* native to the Americas were included. Recent phylogenetic studies (Chapter 1) that included several new terminals of Neotropical *Stenandrium* species strongly support the recognition of *Stenandrium* and *Stenandriopsis* as separate genera. These studies have also found that not all Neotropical species of *Stenandrium* form a monophyletic group since Mexican *S. chameranthemoideum* appeared nested within the *Holographis* clade, which is sister clade to the *Stenandrium* clade. However, all other species of *Stenandrium* form a monophyletic group, including all the Brazilian species sampled.

In contrast to *Aphelandra* R. Br., a relatively well-known genus of the tribe Acantheae and in the same lineage, *Stenandrium* is poorly known and has hardly been studied at all in the Neotropics. This is probably due to its taxonomic complexity (high morphological intra-specific variability, particularly within the widely distributed species) and to its rarity (low frequency of occurence and high levels of endemism; Daniel 1984; Giulietti et al. 2009; Espinar and Ferrucci 1982 Martinelli and Moraes 2013; Chapter 3). After the publication of the *Prodromus* by Nees (1847b) no other inclusive taxonomic treatment was published for *Stenandrium*. Available literature are species lists for Mexico (Daniel 1984), Argentina (Espinar and Ferrucci 1982), Colombia (Leonard 1958) and Bolivia (Wasshausen and Wood 2004) and a few floras and regional checklists (Nees 1847a; Lindau 1895; Wasshausen and Smith 1969; Ezcurra 1993; Kameyama 2003; Vilar et al. 2010; Marchioretto et al. 2015; Flora do Brasil 2020 under construction). Also, many taxa described in *Stenandrium* are treated in online databases as unresolved (Tropicos.org 2019; The Plant List 2018).

Besides the ornamental potential of nearly all the species in the genus, *S. dulce* is used for medicinal purposes, due to the high concentrations of hydroxomic acids, molecules with a wide pharmacological spectrum, that includes antimicrobian, anticancer and antiinflamatory properties (Bravo et al. 2004).

Since the Brazilian species of *Stenandrium* form an apparently monophyletic group of species (McDade *et al.* 2005; see also Chapter 1) with several biologically interesting characteristics, and considering that this is a poorly known genus both to scientists and to local people, the main objective of this study is to contribute to improve taxonomic knowledge of this genus. Specific objetives were to review the Brazilian species of the genus, analysing and discussing the limits between them and to furnish an identification key.

MATERIAL AND METHODS

The protologues and relevant taxonomical literature were consulted. Herbarium sheets (ca. 400) were seen from BHCB, C, CAS, CEN, ESA, FURB, G, GB, HAS, HRB, HUEG, HTO, HUTO, IAC, INPA, IPA, K, MA, MIN, MO, NY, P, RB, SP, SPF, UEC, US, and VIES (abbreviations follow Thiers 2018) during visits or loaned to UB and to CAS. Digital images from SpeciesLink (2019), Reflora-Herbário Virtual (2019), and herbarium websites were also analyzed. We consulted original descriptions of all names applied to Neotropical material and all available type material (seen at K, P, SP, SPF, and UB) or digital images acquired from JSTOR Global Plants (plants.jstor.org) and herbarium websites. Indetermined materials from other families of Lamiales were also analyzed to find as many specimens as possible because some species of *Stenandrium* (the acaulescent group) are sometimes misidentified to family due to morphological similarities with Gesneriaceae and Verbenaceae, (e.g., small herbs with basal leaves and a long penduculate inflorescences).

When no holotype was cited in the original description of a species or infraspecific taxon, we selected a lectotype from among the syntypes. When the protologue cited a single specimen, even if it was not designated as holotype by the original descriptor, this specimen was accepted as the holotype. Species with a large number of specimens were cited as selected material. Selections were made from each county, then citing the most recent collections with the most information in the label from each locality. A complete list of examined exsiccatae is available in Appendix I. The study of herbarium specimens was complemented by fieldwork, mainly in the Cerrado Biome. Field expeditions were made during the Wet Season (October and November) between 2015 and 2017, to localities with previous records of rarely collected species, particularly within the Cerrado biome, where the genus is more diverse and variable. The following states and regions were visited:

1) Bahia: along the coast from Ilhéus to Trancoso in search of *S. serpens*, a poorly collected caulescent species from the Ombrophilous Atlantic Forest (October 2017 and January 2019, both unsucessful expeditions);

2) Distrito Federal: to Cerrado *sensu stricto* areas in the Jardim Botânico de Brasília (October 2016, unsucessful expedition; to the Reserva Ecológica do IBGE (October 2016, unsucessful expedition); to areas surrounding the Parque Ecológico Dom Bosco (sucessful expedition to accompany the flowering and fruiting of a

population of *Stenandrium pohlii* in October and November 2016); and to the Reserva Ecológica do Guará (November 2016, during which two populations of *S. pohlii* var. *pusillum* were found); and to a Dry Forest area within the Monumento Natural do Morro da Pedreira (October 2015, unsucessful expedition);

3) Goiás: five expeditions, three to the Chapada dos Veadeiros (August and October 2015 and October 2016), during which three populations of *S. irwinii*, endêmic to this region were found; one to Formosa (October 2016) to an area of Dry Forest where a population of *S. goiasense* was recorded, and one to Northern Goiás (October 2017, unsucessful expedition), in search of another populations of *S. goiasense*.

4) Minas Gerais: to the Northern Cadeia do Espinhaço, near Diamantina and Grão Mogol, where a population of *S. hatschbachii*, endemic to the Espinhaço Range was found but not *S. stenophyllum*, another endemic known only from the type and the same general region (November 2017).

5) Tocantins: to the Southern part of the state in the vicinity of Arraias, where a population of *S. pohlii* was found (November 2016).

Morphological measurements of the floral traits were made (according to Figure 1) from dried, rehydrated specimens using a light microscope micrometer.

Images of important morphological features are provided in Figures 3-8, while photos of individual species appear after the species information in Figures. 9-20.

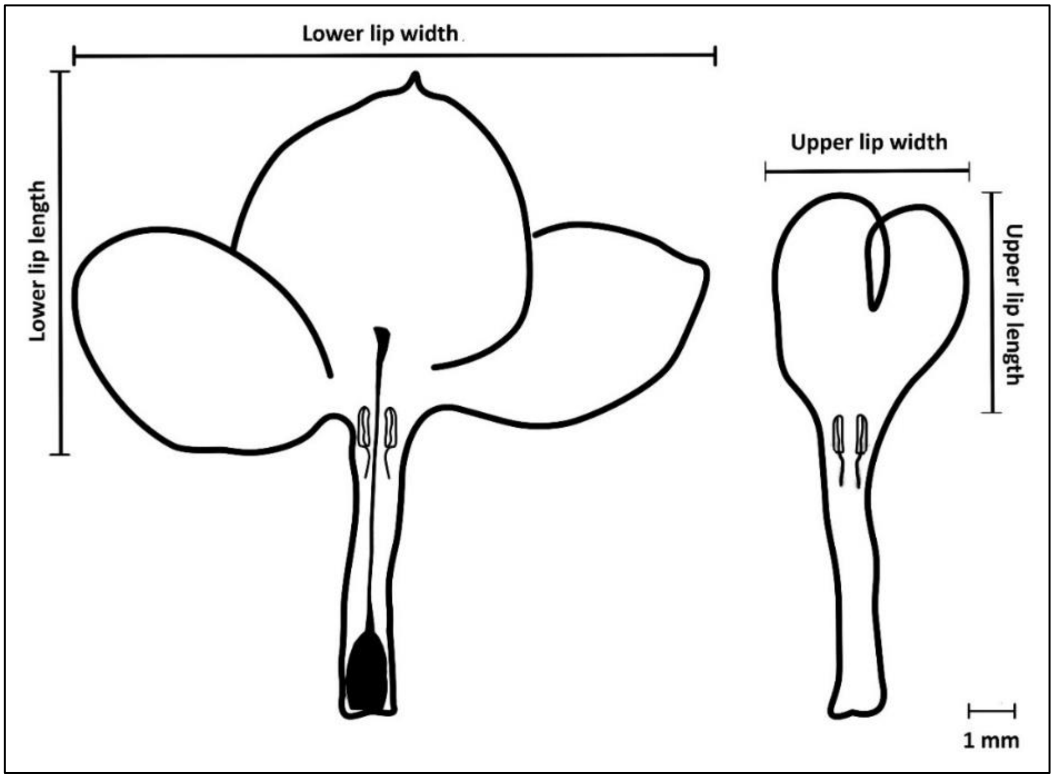


Figure 1. Pattern followed when taking floral measurements. Model species: *Stenandrium eustachyum.*

We also performed analyses of pollen morphology under scanning electron microscopy (SEM), following the method: the anthers (of dried plants) were placed on a double-sided adhesive tape stuck on a stub and the pollen grains (unacetolyzed) were gently removed from the anther to the tape, then sputter coated with gold and observed on SEM, at the "Laboratório de Microscopia e Microanálise" (UnB).

A distribution map (Figure 2) was prepared based on geographical coordinates given on specimen labels and sometimes inferred in GoogleEarth by the locality details. The map was produced in ArcGIS (ESRI 2011) from decimal coordinate files. The extent of occurence and area of occupancy numbers were calculated in RStudio (2015) using the ConR package (Dauby et al. 2017). All species were evaluated as to extinction risk according to the IUCN criteria (2017), based on geographical distribution data, and also some information obtained from fieldwork.

RESULTS

There are currently 18 names in *Stenandrium* for Brazil. After analysing the literature, type material, available specimens and images, 14 species are accepted, including two new species described during this study (Chapter 3). Homotypical synonyms are listed immediately following the accepted name, and heterotypical synonyms in chronological order.

Taxonomic history

Stenandrium was described by Nees (1836), based on *S. mandioccanum.* A few years later, Nees authored the taxonomic treatment for Acanthaceae in *Flora Brasiliensis* (Nees 1847a) which included 14 species of *Stenandrium* (some of which are now considered to be part of other genera, such as *Aphelandra*) and transferring *Ruellia dulcis* Cavanilles (1801) – the first species of the genus to be described - to *Stenandrium*. In the same year, in the *Prodromus*, De Candolle (1847), raised this number to 16 by including the Flora Brasiliensis species. Between 1855 and 1908, 13 other taxa from Brazil, Cuba, United States, Honduras, Mexico, Paraguay, Peru and Venezuela were described in several studies (Gray 1878; Grisebach 1866; Lindau 1895; Moore 1895; Oersted 1855). In the early XXth century, Urban published six new species and a new combination for the West Indies in the three volumes of Symb. Antill. (Urban 1911; 1912; 1923). Leonard (1936, 1938) and Daniel (1984) contributed to the knowledge of the species of Mexico, Guatemala and surrounding areas, publishing nine more taxa, including new combinations (transferring taxa from *Gerardia* and *Pseuderanthemum* to *Stenandrium*). Finally, in the latter half of the XXth Century, Wasshausen (1965; 1990; 1996) made important contributions to the taxonomy of the group, describing 11 new taxa and transfering species of *Aphelandra* and *Crossandra* to *Stenandrium*) based on specimens from Ecuador, Colombia and Brazil.

Generic circumscription

In its present circumscription (Vollesen 1992), that includes both Neotropical and Paleotropical species (originally described in *Stenandriopsis* S. Moore), *Stenandrium* is paraphyletic (McDade et al. 2005). Neotropical species of *Stenandrium* are phylogenetically closer to several other Neotropical genera (*Holographis, Neriacanthus, Salpixantha*, and *Aphelandra*) than to the *Stenandriopsis* clade (McDade et al. 2005; McDade et al. 2008; Chapter 1). To improve the circumscription of the Neotropical genera of Acantheae, including that of *Stenandrium*, additional molecular phylogenies should be built, improving the level of sampling within this Neotropical clade.

The closest morphological relative of *Stenandrium* that occurrs in Brazil is *Aphelandra*. Morphologically, *Stenandrium* can be distinguished from *Aphelandra* by the subactinomorphic or slightly zygomorphic corolla (strongly zygomorphic in *Aphelandra*) (Daniel 1984; Wasshausen 1996). Wasshausen (1975), in a review that included all known species of *Aphelandra*, wrote that *Stenandrium* could be distinguished from *Aphelandra* "in that is usually a subcaulescent or acaulescent herb, with short non imbricate bracts, a narrowly cylindric, rarely enlarging corolla tube, and a slightly two-lipped corolla with subequal, spreading lobes". Unfortunately, none of these characters can be applied to all the Brazilian species. *Stenandrium tenellum*, for example, shows none of these characters – it is caulescent, with imbricate bracts, and a large corolla tube – and emerged within the *Stenandrium* clade along with all the other Brazilian species in the genus (Chapter 1). Wasshausen himself, several years later, when reviewing *Aphelandra* and *Stenandrium* for Andean Venezuela, Colombia and Ecuador, noted that these characters are inconsistent and would have to be re-evaluated (Wasshausen, 1996).

Distribution and habitat

The Brazilian species of *Stenandrium* are distributed from the Northeast to the South (Figure 2) but do not ocurr in Amazonia. Brazil is the main center of diversity and endemism (14 spp.), followed by Mexico (10 spp.) and Cuba (9 spp.). The species grow in several diferent types of vegetation, from arid scrub (*Caatinga*), savana (*Cerrado, Pantanal*) and the very humid ombrophylous forests of the Coastal Atlantic Forest, as well as several types of fields and praries (in the South of Brazil).

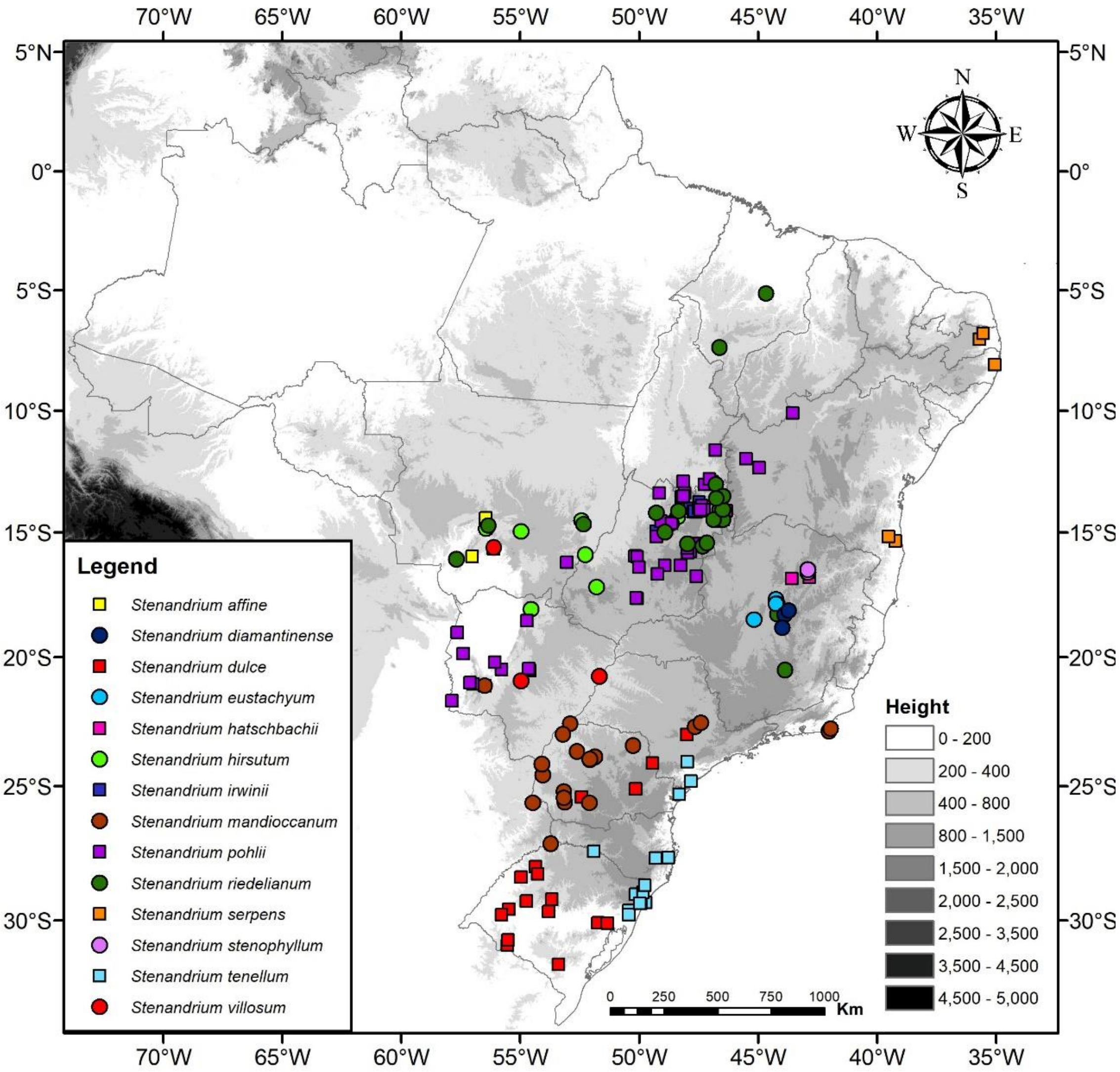


Figure 2. Distribution map of *Stenandrium* in Brazil.

Phenology

Most Brazilian species of *Stenandrium* flower and fruit simultaneously in the rainy season, mostly between the months of September and November, but some flowering collections from other periods are also found. In acaulescent species flowering is frequently stimulated by fire.

Morphology

HABIT

Regarding habit and life form, it is subdivided into two groups (Figure 3): the acaulescent species (perennial, rhizomatous herbs, with basal leaves grouped together that are lost in the dry season and a long pedunculate inflorescence, which blooms with new leaves in the rains, stimulated by fire) and the caulescent (erect or prostrate, branched or not, perennial, rarely rhizomatous subshrubs, of opposite or verticillate leaves distributed along the branches). When the above ground part of the plant burns, the inflorescence emerges with the new leaves that mature along with the fruit.

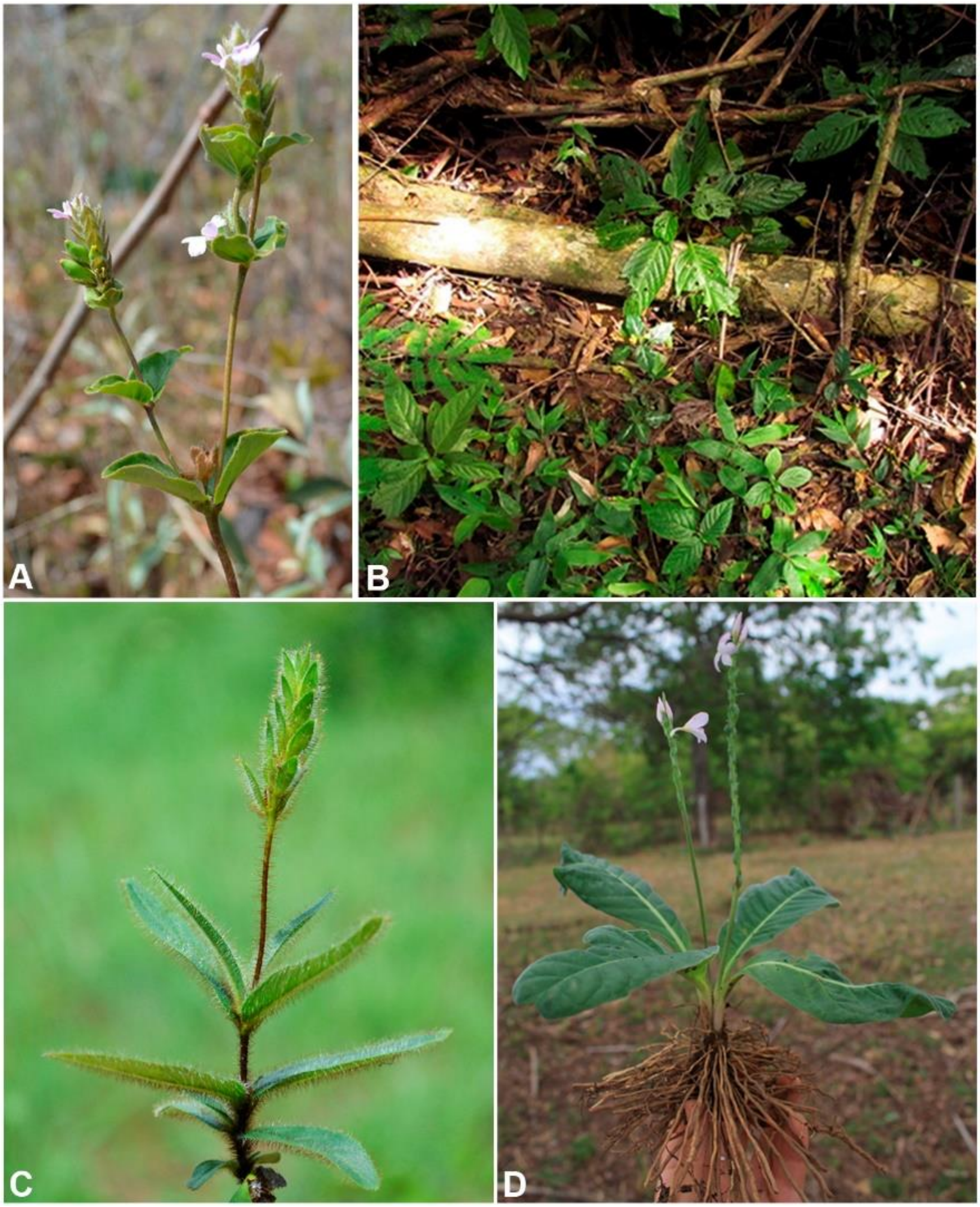


Figure 3. Different types of habits in *Stenandrium.* A. Caulescent branched. B. Caulescent prostrate. C. Caulescent unbranched. D. Acaulescent.

ROOT SYSTEM

In the acaulescent and caulescent unbranched species the roots (Figures 10C, 14D, 16B, and 17B) are born from a vertical rhizome and quite thick (up to 2 mm thick or more, when fresh). In the caulescent species observed the rhizome is vertical or horizontal (on prostrate ones) and the roots are thin (less than 1 mm thick).

INDUMENTUM

Trichomes are almost always present and are usually unicellular or multicellular eglandular trichomes (Figure 4) varying in size. The color can be white, yellowish or brownish, and two different colors can appear in the same plant (e.g. *S. hirsutum*). The length of the trichomes can vary in the same plant and organ (e.g., shorter on the central area of the bract and longer on the margins).

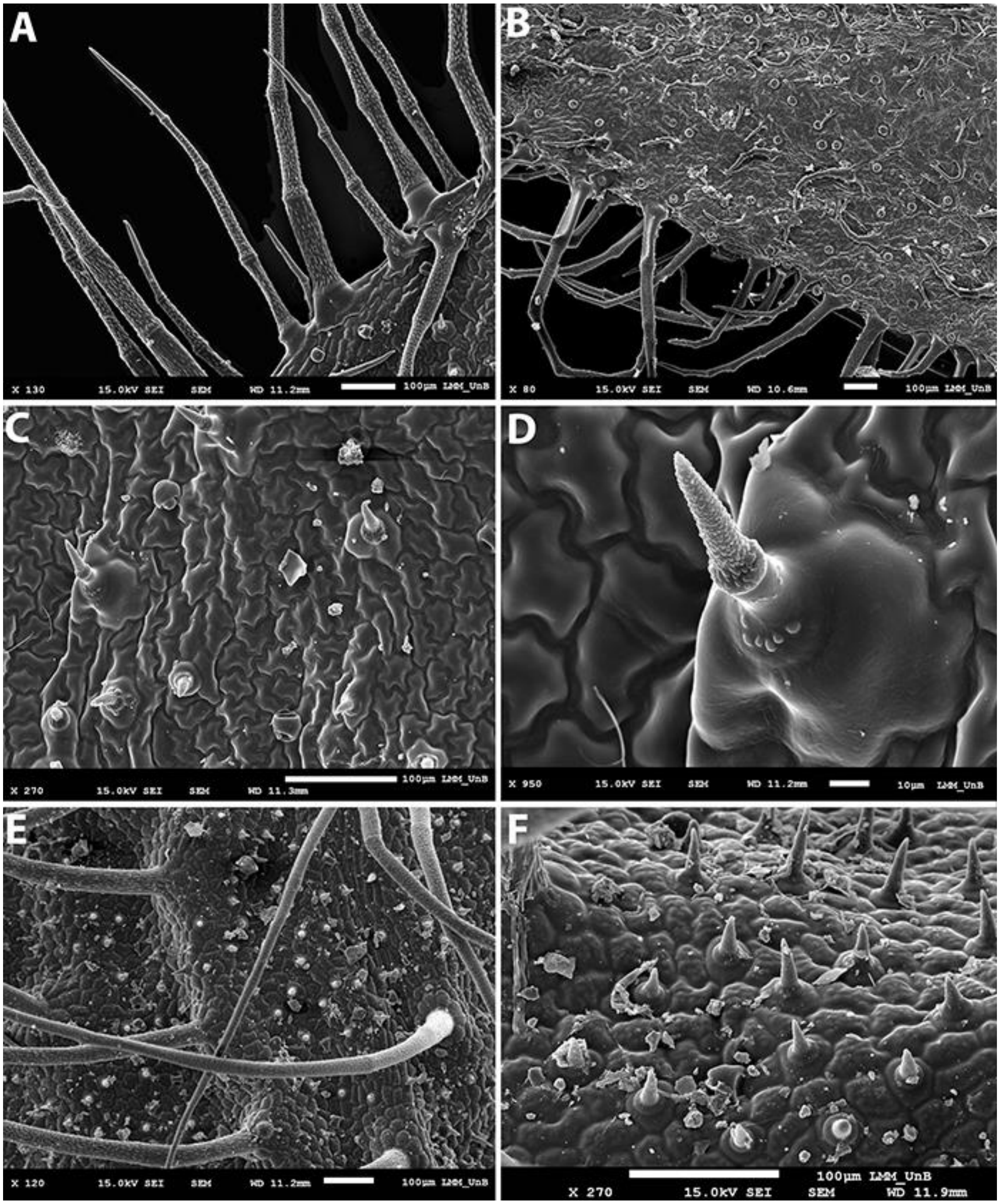


Figure 4. SEM images of *Stenandrium* trichomes. A-D. *S. eustachyum* (J.E.Q. Faria et al. 8828). A. Eglandular multicellular trichomes on the margin of the bract. B. Eglandular unicellular and multicellular trichomes, and glandular subsessile trichomes with globular 2-celled head on the bract. C. Eglandular unicellular papillose trichomes and glandular subsessile trichomes with a globular 2-celled head on the bract. D. Eglandular unicellular papillose trichomes on the bract. E-F. *S. diamantinense* (G. Antar & D.A. Chaves 1880). E. Eglandular unicellular conical and multicellular trichomes on the adaxial surface of the leaf. F. Eglandular unicellular conical trichomes on the abaxial surface of the leaf.

LEAVES

The leaves are usually opposite (caulescent) or clustered (acaulescent) and appear whorled, or rarely verticillate, subsessile or petiolate, sometimes with a decurrent leaf base. The texture is usually herbaceous or chartaceous, sometimes coriaceous or membranaceous, and rarely indurate. The blades are often ovate to obovate, sometimes oblanceolate or lanceolate.

INFLORESCENCE

The inflorescence is a terminal (in acaulescent species) or axillary (in caulescent species) spike. The flowers may be lax or congested in the spike and this character is usually conserved within a species. The size of the penduncule is very variable between species and within species, from almost sessile to more than four times longer than the spike.

BRACTS

The bracts (Figure 5) are very variable in size, shape, indumentum and venation, and are usually highly conserved in populations and species, making them a particularly useful character for species distinction.

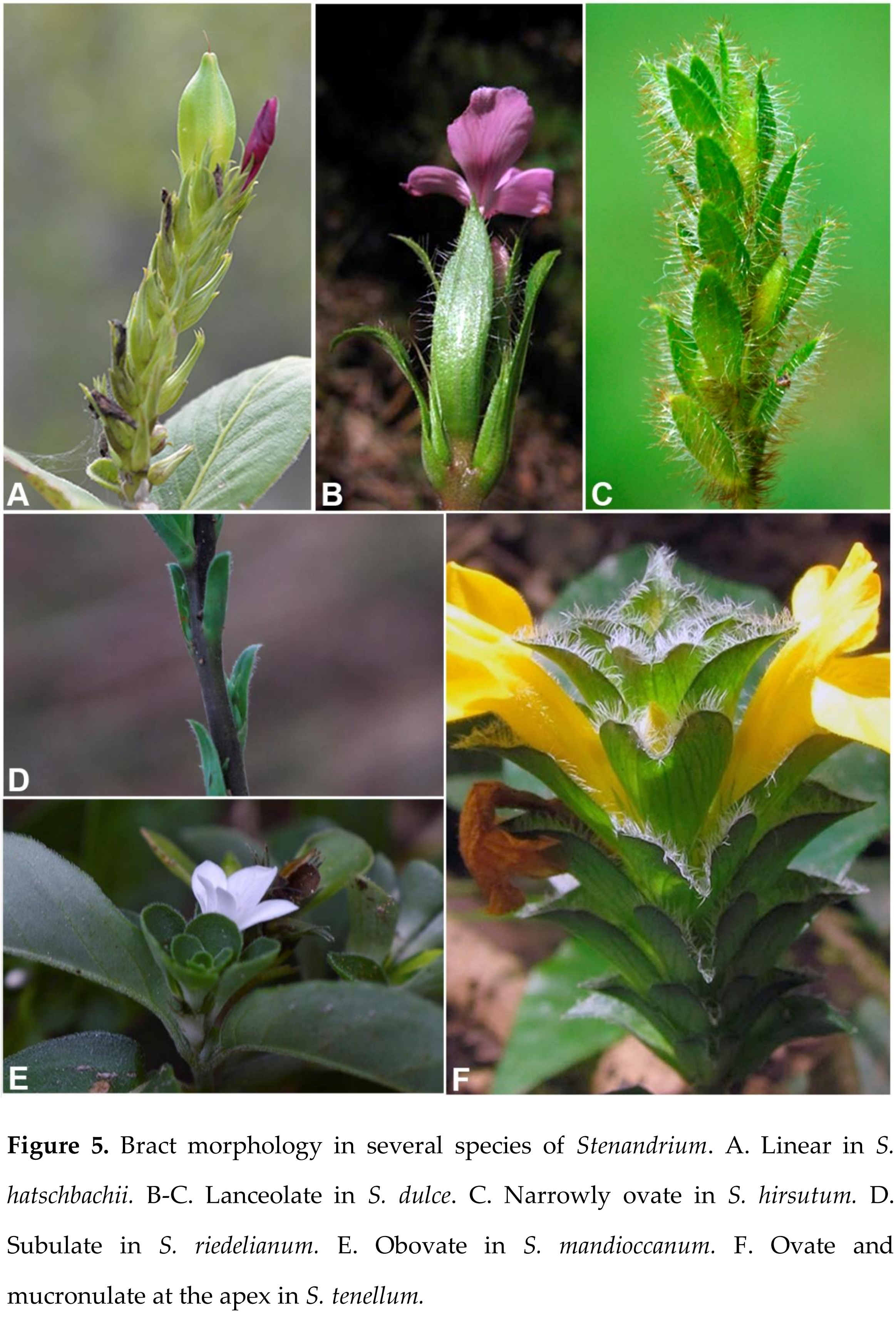


Figure 5. Bract morphology in several species of *Stenandrium*. A. Linear in *S. hatschbachii*. B-C. Lanceolate in *S. dulce*. C. Narrowly ovate in *S. hirsutum*. D. Subulate in *S. riedelianum*. E. Obovate in *S. mandioccanum*. F. Ovate and mucronulate at the apex in *S. tenellum*.

FLOWERS

The corolla (Figure 6) is usually pale rose, pink or purplish (rarely white or yellow), subactinomorphic in which case the corolla lobes are equal or subequal, or zigomorphic, in which case the upper lip (with two lobes) is much reduced in size in relation to the lower lip (with three lobes); the upper lip is deeply divided into two lobes close to its base and these two lobes are usually very prominent, and more or less obovate (Daniel 1984). The style is teret and the stigma is usually assymetrically, sometimes symmetrically funnelform. The stamens are included in the corolla tube, and the anthers are always four, monothecous with a small staminode present in several of the species.

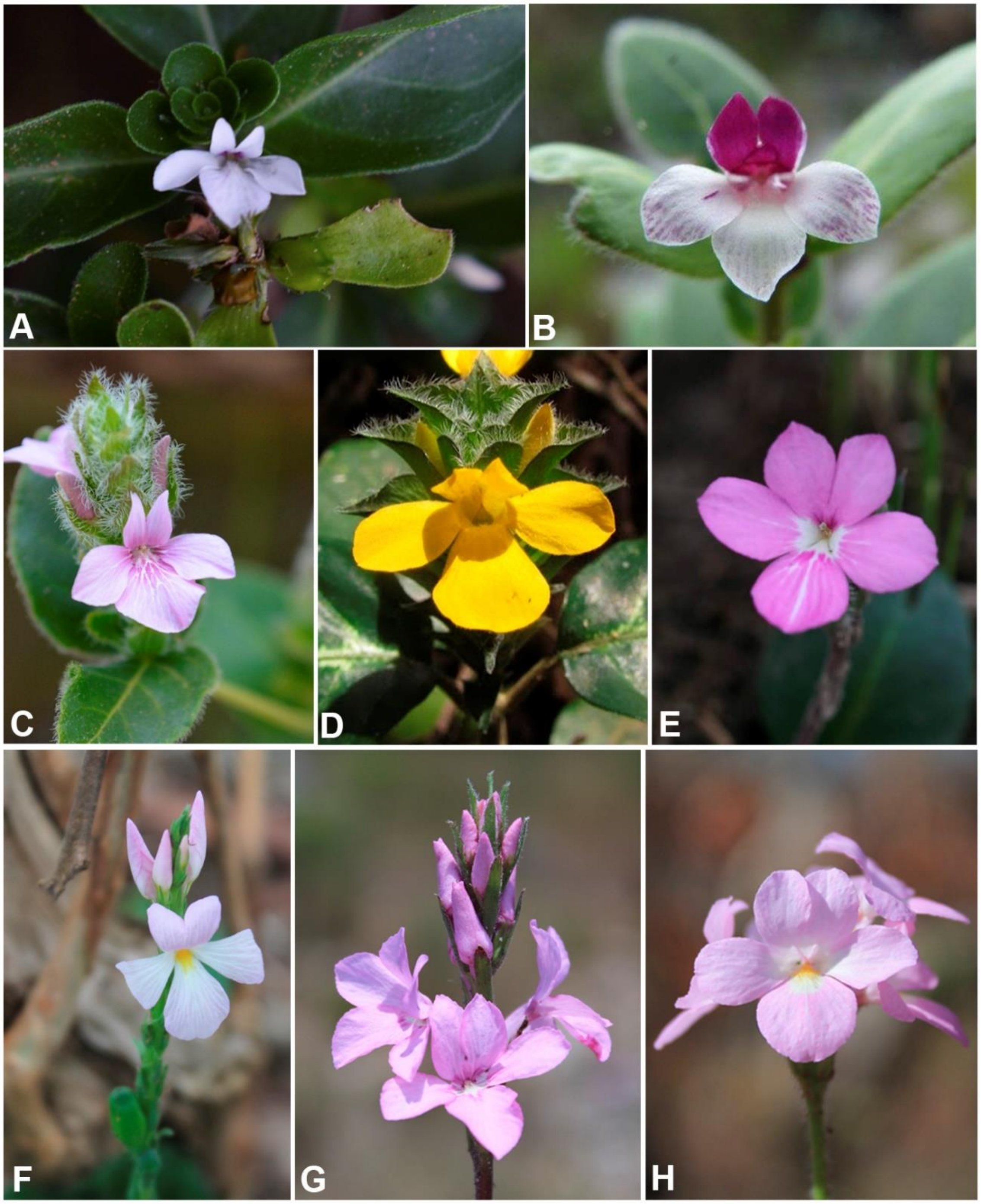


Figure 6. Variations in corolla color and symmetry in *Stenandrium.* A-D. Zygomorphic. E-F. Subactinomorphic.

POLLEN

Currently, two main types of pollen have been described in the Brazilian species: spheroid (Figure 7F) and tricolpate (Figure 7A-D). The tricolpate pollen is the most common type in the genus and also in the tribe. In this study we describe a new type of pollen, a pentacolpate pollen found in *Stenandrium hirsutum* (Figure 7C). In *Stenandrium dulce* these two types of pollen have been found in the same species (see the description and notes of *S. dulce*, p. 106-113), over all the distribution range of the species.

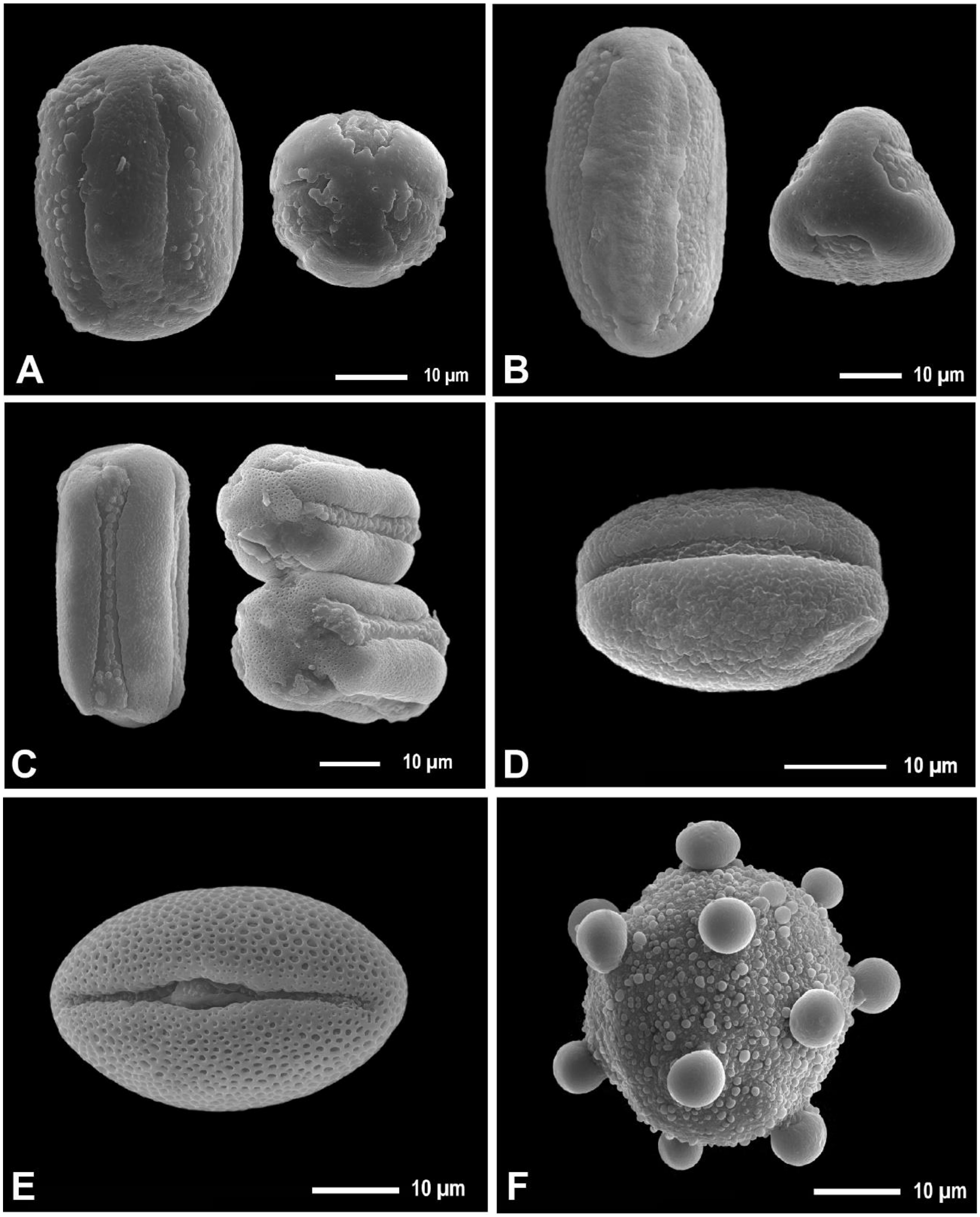


Figure 7. Pollen types of the Brazilian *Stenandrium* species under SEM. A. Tricolpate elliptic pollen of *S. riedelianum.* B. Tricolpate nearly triangular pollen of *S. irwinii*. C. Pentacolpate elliptic pollen of *S. hirsutum.* D. Tricolpate elliptic pollen of *S. tenellum.* E. Tricolpate elliptic of *S. pohlii.* F. Spheroid inaperturate pollen of *S. dulce.*

Fruits and Seeds

The fruits are relatively uniform in size and shape. They are usually more or less ellipsoid capsules, either glabrous or pubescent. The seeds are more variable but are always a maximum of four (frequently less, through abortion); they are laterally flattened, sub-ovoid and the surfaces are usually pubescent or tomentose with trichomes that frequently are barbed (Figure 8).

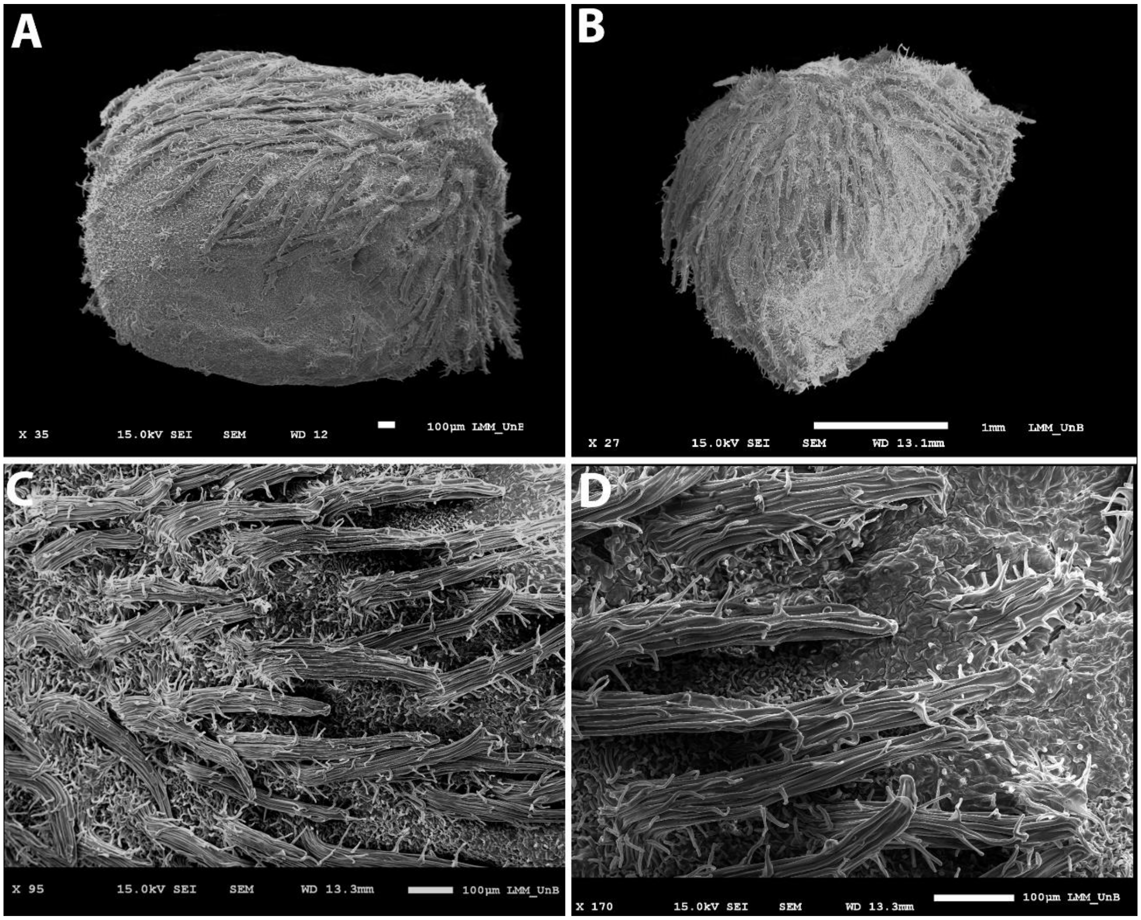


Figure 8. SEM images of *S. eustachyum* seeds. A-B. General view. C-D. Details of the barbed trichomes.

Stenandrium **Nees** in Lindl. Introd. Nat. Syst. Bot., 2nd edition, 444. 1836, *nom. cons.* Type: *Stenandrium mandioccanum* Nees.

= *Gerardia* L. Sp. P1. 610. 1753, *pro parte.*

Erect or prostrate, branched or unbranched, caulescent or acaulescent, perennial, 10-100 cm tall with whitish to ferrugineous (brownish?) trichomes varying in density and size on most vegetative structures; rhizome vertical or horizontal, 2-9 mm thick, roots up to 10 cm long. **Leaves** concolor or discolor, opposite or verticilate, subsesille or petiolate, petioles 0.5-2.5 mm long, glabrous to tomentose, blades linear, lanceolate, oblanceolate, ovate or obovate, 7.3- 53.1 mm long, 3.5-16.3 mm wide, 1-9 times longer than wide, membranaceous, herbaceous, chartaceous, coriaceous or indurate, attenuate, cordate or oblique at base, rounded to acute at apex, surfaces glabrous to tomentose, margins plane or revolute, entire or ciliate, rarely crenate. **Inflorescence** a sessil to pedunculate, terminal or axillary spike 10.5-80 mm long (including the peduncle, excluding the flowers) often concentrated at the subterminal nodes, peduncles 1-54.7 mm long, glabrous to tomentose. **Flowers** alternate to opposite along the rachis. **Bracts** linear, lanceolate, ovate, obovate, oblanceolate or subulate, 6.5-10.5- mm long, 1.3-3.8 mm wide, inconspicually or conspicually 3-7-veined, surfaces glabrous to tomentose, margins entire, rarely toothed, ciliate. **Bractlets** lanceolate 1.9-8 mm long, 0.3-0.7 mm wide, 1-veined, glabrous to puberulent, margins entire, ciliate or not. **Calyx** with subequal lanceolate lobes, lobes 5-nerved, 2.7-10.3 mm long, 0.8-1.4 mm wide, glabrous to puberulent, margins entire, ciliate or not. **Corolla** white, pale pink, pink, pale lilac, lilac, violet, purplish or red-wined, the lower lip often white striped or yellow to orange coloured atat the base of the central lobe, near the tube throat, subactinomorphic or slightly zygomorphic, tube 2.8-13.7 mm long, 1-2 mm wide, upper lip 6.4-9.7 mm long, 8.2-9.1 mm wide with equal lobes, lower lip 9-12.8 mm long, 17.7-18.2 mm wide with subequal lobes, lower-central lobe often tomentose at the base internally. **Stamens** 4, included, 1.1-2.8 mm long, anthers monothecate, 0.8-1.6 mm long, often tomentose; staminode 0.5-0.7 mm long; pollen grains prolate or sub-prolate, tricolpate, 25.4-32.3 × 15.7-18.5 μm, exine psilate or perforate, colpus with smooth to gemmate membrane. **Style** teret, 2.5-8.5 mm long, stigma asymmetrically or sometimes symmetrically funnelform. **Capsule** ellipsoid, glabrous to puberulent, 6.7-18 mm long, 2.5-6 mm wide. **Seeds** 4, ovoid, laterally flattened, 1.5-5 mm long, 1.2-3 mm wide, often tomentose or pubescent, with trichomes usually barbed, rarely simple.

KEY TO THE BRAZILIAN SPECIES OF *STENANDRIUM*

1. Caulescent ……………………………………………………………………….. 2

1. Acaulescent …………………………………………………………………….. 12

2. Branched stems ……………………..…………..………………………..… 3

2. Unbranched stems ……….……………………………………………………… 7

3. Leaves verticillate, linear-lanceolate to linear …………………… *S. stenophyllum*

3. Leaves opposite, broadly to narrowly ovate ………..…………………………4

4. Leaves congested, indurate, with strongly revolute to nearly conduplicate margins ………………………………..……………………….. *S. diamantinense*

4. Leaves not congested, membranaceous to coriaceous, with slightly revolute to plane margins ……………………………………………………………………… 5

5. Leaves membranaceous, bracts obovate to oblanceolate ……. *S. mandioccanum*

5. Leaves chartaceous to coriaceous, bracts linear to ovate …………….………… 6

6. Bracts linear to subulate; flowers zygomorphic with the upper lip wine-colored and the lower lip pale lilac to white ………………………………. *S. hatschbachii*

6. Bracts entire, ovate; flowers subactinomorphic and uniformly pink ………………………………………………………………………. *S. eustachyum*

7. Bracts toothed (often proximally entire with a few tooths near the apex and rarely lacking tooths) and mucronulate at apex; corollas yellow ……… *S. tenellum*

7. Bracts entire, rounded to acute at apex, white to purplish corollas …..………. 8

8. Rhizome vertical ……………………..……………………………………….. 9

8. Rhizome horizontal ……………………….……………………………… 11

9. Bracts widely ovate, conspicually 3-veind on the abaxial surface, corolla tube 7-8 mm long …………………………..……..…………..…………… *S. villosum*

9. Bracts lanceolate, sometimes narrowly ovate, inconspicually 3-veined on both surfaces, corolla tube up to 4.8 mm long ……………………………………… 10

10. Leaves concolorous, blades hirsute on both surfaces, margins plane, not cartilaginous, ciliate with trichomes up to 3 mm long, pollen grains pentacolpate ………………………………………………………...………..…… *S. hirsutum*

10. Leaves discolorous, blades glabrous on adaxial surface (sometimes sparsely hirsute), with trichomes confined to the veins, margins slightly revolute, cartilaginous, ciliate with trichomes up to 2 mm long, pollen grains tricolpate …………...…………………………………………...………………….. *S. affine*

11. Bracts lanceolate, 6-6.7 × 0.8-1.1 mm, 1-veined ……………………..…. *S. serpens*

11. Bracts obovate to oblanceolate, 7.5-11.5 × 2.7-6.7 mm, 3-veined ………………………………………………………………………. *S mandioccanum*

12. Spikes lax, with more than 3 mm (usually more than 5 mm) between the flowers (or the pair of flowers) ………………………………………………… 13

12. Spikes congested, with less than 2 mm between the flowers (or the pair of flowers) 14

13. Bracts 2.5-4.6 mm wide, corolla tube 8-12.3 mm long, pollen grains longer than 50.1 µm long ……………………………………………………….……… *S irwinii*

13. Bracts, 1.3-2 mm wide, corolla tube 4-7.5 mm long, pollen grains up to 49.1 µm long ....………………………………………………………… *S. riedelianum*

14. Bracts always lanceolate, 1.5-3 mm wide, 3-veined, corolla upper lip 5-5.2 mm long, 4.5-5.3 mm wide, lower lip 8.5-9 mm wide …………………….…… *S. dulce*

14. Bracts ovate sometimes lanceolate, 3.5-12.4 mm wide, 5-7-veined, corolla upper lip 7.2-13.3 mm long, 7.6-12 mm wide, lower lip 15-26.3 mm wide ………………………………………………………………………….. *S. pohlii*

1. ***Stenandrium affine*** **S.Moore** in Trans. Linn. Soc. London Bot. 4(3): 426. 1895. **Lectotype (designated here):** BRAZIL. Mato Grosso, 1891-1892, *S.L.M. Moore 588* (BM [photo!]; isolectotypes: K!, NY[photo!], P!, R [photo!]).

Erect, unbranched, caulescent herb to 20 cm tall with whitish to yellowish trichomes varying in density on most vegetative structures; stems hirsute with trichomes up to 3 mm long, rhizome vertical, 2-4.5 mm thick, roots up to 8 cm long. **Leaves** slightly discolorous, opposite, subsessile, petioles 1-2.5 mm long, hirsute with trichomes up to 3 mm long, blades lanceolate to oblanceolate, sometimes ovate 16.5-53.1 mm long, 5.8-16.3 mm wide, 1.3-6.3 times longer than wide, herbaceous to chartaceous, rounded to acute at apex, adaxial surface hirsute with trichomes up to 2 mm long, abaxial surface glabrous sometimes very sparsely hirsute, always with trichomes up to 2 mm long concentrated on the veins, margins slightly revolute, entire, cartilaginous, ciliate with trichomes up to 1.5 mm long. **Inflorescence** a long pedunculate, terminal or axillary spike 30-80.9 mm long (including peduncle, excluding flowers) often concentrated at the subterminal nodes, peduncles 18.1-54.7 mm long, hirsute with trichomes up to 2 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** lanceolate, 6.7-10.5 mm long, 1.7-3.8 mm wide, unsconspicually 3-veined, adaxial surface glabrous, abaxial surface hirsute to almost glabrous with trichomes up to 1.5, margins entire, ciliate with trichomes up to 2.5 mm long. **Bractlets** lanceolate, 1.9-2.1 mm long, 0.3-0.5

mm wide, 1-veined, glabrous, margins entire. **Calyx** with subequal lanceolate lobes, lobes 5-veined, 2.7-4 mm long, 0.8-1.1 mm wide, glabrous to puberulent with minute trichomes up to 0.1 mm long, margins entire. **Corolla** pink to lilac, the lower lip yellow to orange coloured atat the base of the central lobe, sub-actinomorphic, tube 2.8-4.6 mm long, 1-1.5 mm wide, upper lip 6.4-8.3 mm long, 8.2-8.3 mm wide with equal lobes, lower lip 7.5-9.8 mm long, 13.5-14.3 mm wide with subequal lobes, lower central lobe tomentose at the base internally. long. **Stamens** 1.1-1.63 mm long, anthers 0.8-1 mm long, tomentose with trichomes up to 0.2 mm long; staminode 0.5 mm long; pollen grains prolate, tricolpate, 25.4-32.3 × 15.7-18.5 µm, exine psilate or perforate, colpus with smooth to gemmate membrane. **Style** 2.5-2.7 mm long, with a tuft of trichomes up to 0.5 mm long near the stigma, stigma asymmetrically funnelform. **Capsule** glabrous to very sparsely puberulent with minute trichomes up to 0.1 mm long, 6,7-8.8 mm long, 2.5-4 mm wide. **Seeds** 1.5-1.8 mm long, 1.2-2 mm wide, the surface tomentose with barbed trichomes up to 0.8 mm. **Figure 9.**

Phenology. — Flowering and fruting specimens of *S. affine* were collected in October and November.

Distribution and habitat. — *Stenandrium affine* is endemic to Brazil and it is known from very few collections, all from the central region of Mato Grosso. It occurs in open grasslands and *cerrado* vegetation.

Conservation status. —Data is deficient (DD) to assess the conservation status of *S. affine*, since the two known georeferentiable collections of the species are not sufficient to calculate the Extent of occurrence (EOO). Using only the value of the Area of occupancy (AOO = 8 km^2), it would be assessed as EN (endangered), under the criterion B2a (IUCN 2017). This species is lacking in recent colletions (the last known collection was made 37 years ago) and, as far as we are aware, it has never been recorded in any protected area.

Notes. — *Stenandrium affine* is a frequently missapplied name. Several specimens from Mato Grosso do Sul and Paraguay that we recognize as *S. villosum* (based on the original description and analysis of type material) are determined in herbaria as *S. affine*. Our analyses of the type material of *S. affine* allied to many other collections in the genus, have led us to believe that it is a good species, occurring in campos and cerrados of Goiás and Mato Grosso, very similar to but distinct from *S. hirsutum* and *S. villosum.* According to Moore (1895), the original author, *S. affine* differs from other species of *Stenandrium* in the small size of the inflorescence, bracts, and corola, as well as reduced size of the plant. But the analysis of the specimens of *S. affine* and similar species, and the measurements of these structures, revealed variations in sizes of these structures, even in a single population (e.g. several collections of *S. hirsutum* by J.E.Q. Faria from the same locality). This intraspecificic variation was also observed in other *Stenandrium* species, specially the ones of the acaulescent group.

Specimens examined.— BRAZIL. Mato Grosso: **Diamantino**: Serragem, Linha do Diamantino, [14°24'31"S, 56°26'46"W], Oct 1914, *J.G. Kuhlmann 1402* (SP!); Km 99 da rodovia Cuiabá-Porto Velho, [15°56’96.8”S, 57°00’36”W], Nov 1982, *J.U. Santos & C.S. Rosário 473* (SPF!).

Figure 9. Images of *Stenandrium affine*. A. Specimen from NHM (S. Moore 588). B. Detail of leaves and spike. C. Pollen grain (J.U. Santos 473).

2. ***Stenandrium diamantinense*** **Zanatta & Kameyama**, sp. nov.

See Chapter 3.

3. ***Stenandrium dulce*** **(Cav.) Nees** in DC. Prodr. 11: 282. 1847 ≡ *Ruellia dulcis* Cav., Icon. Pl. 6: 62. 1801 ≡ *Gerardia dulcis* (Cav.) Blake (1917: Contr. Gray Herb. 52: 101). Holotype: CHILE. Concepción: near Talcahuano, *L. Née s.n.* (MA [photo!]).

= *Stenandrium diphyllum* Nees in Mart. Fl. Bras. 9: 75. 1847 – **Lectotype (designated here):** BRAZIL. São Paulo: Ad urbem S. Pauli et in campis siccis prope Itararé, Feb 1826, L. Riedel s.n. [281] (GZU [photo!]; isolectype: SI [photo!]). *syn. nov.*

= *Stenandrium neesianum* Lindau (1895: Nat. Pflanzenfam. 4: 321). **Lectotype (designated here):** BRAZIL. *F. Sellow s.n.* (†B [F, photo!]). *syn. nov.*

= *Stenandrium trinerve* Nees (1847: Fl. Bras. 9: 75). **Lectotype (designated here):** BRAZIL. Rio Grande do Sul: in campis ad Porto Alegre, *F. Sellow s.n.* (K!; isolectypes: BR [photo!], †B [F photo!], HAL [photo!]). *syn. nov.*

Acaulescent perennial herb to 7 cm tall, pubescent to tomentose with whitish trichomes on most vegetative structures; rhizome vertical, 1.3-5 mm thick, roots up to 8 cm long. **Leaves** slightly discolorous,, clustered and appearing whorled, petiolate, petioles (naked portion) 1.2-21 mm long, sparsely pubescent to glabrous with trichomes up to 1.5 mm long, blades ovate to obovate, sometimes oblanceolate or lanceolate, 5.3-40.3 mm long, 3.6-12.2 mm wide, 1.4-4 times longer than wide, herbaceous to chartaceous, gradually narrowed and attenuate at base, obtuse to rounded at apex, adaxial surface glabrous to pubescent with trichomes up to 1.5 mm long, abaxial surface glabrous to pubescent with trichomes up to 1.5 mm long and sometimes with the trichomes confined to the veins, margins plane to slightly convolute, entire, glabrous to ciliate with trichomes up to 1 mm long. **Inflorescence** a pedunculate floral scape, 26.3-63 mm long (including peduncle, excluding flowers), peduncles 18.1-49.5 mm long, glabrous to pubescent with trichomes up to 1.2 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** lanceolate, 6.8-13.7 mm long, 1.5-3 mm wide, 3-veined (the veins often inconspicuous on abaxial surface and conspicuous on adaxial surface, often occurs the opposite and sometimes they are insconspicuous on both surfaces), adaxial surface sparsely pubescent with trichomes up to 1.2 mm, abaxial surface almost glabrous with trichomes up to 1.2 mm, margins plane, entire, ciliate with trichomes up to 1.5 mm long. **Bractlets** lanceolatee, 4.6 mm long, 0.4-0.5 mm wide, 1-veined, glabrous to sparsely puberulent with trichomes up to 0.3 mm long, margins entire, gabrous to sparsely ciliate with trichomes up to 0.3 mm long. **Calyx** with subequal lanceolate lobes, lobes 5-7-veined, 5.4-6.2 mm long, 0.9-1.3 mm wide, glabrous to puberulent with minute trichomes up to 0.1 mm long, margins ciliate with minute trichomes up tp 0.1 mm long. **Corolla** pink, the lower lip yellow to orange coloured at the base of the central lobe, subactinomorphic, tube, 4.4-9.5 mm long, 1-2 mm wide, upper lip 5-5.2 mm long, 4.5-5.3 mm wide with equal lobes, lower lip 6.2-6.6 mm long, 8.5-9 mm wide with subequal lobes, lower-central lobe tomentose at the base internally with trichomes up to 0.5 mm long. **Stamens** 0.8-0.9 mm long, anthers 0.6-0.7 mm long, tomentose with trichomes up to 0.2 mm long; staminode 0.4 mm long; two pollen grains types (individuals are homomorphic), type I spheroid, isodiametric, inaperturate, 25.2-33.5 μm, exine gemmate, gemmae with two different sizes, type II ellipsoid, prolate, tricolpate, 35.2-48.7 × 17.6-23 μm, exine perforate sometimes fossulate perforate, colpus with smooth to gemmate membrane. **Style** 2.5-3.2 mm long, puberulent with trichomes up to 0.1 mm long, stigma symetrically funnelform. **Capsule** puberulent with minute trichomes up to 0.1 mm long, 6.6-6.9 mm long, 2.4-3 mm wide. **Seeds** 1.8-2.8 mm long, 1.4-2.4 mm wide, the surface pubescent to tomentose with barbed trichomes up to 1 mm long. **Figures 10, 11.**

Phenology.— Flowering and fruiting specimens were collected from October to February.

Distribution and habitat.— This is the most widely distributed species of the genus, occurring from Chile to Mexico, in areas of open vegetation and fields of several Neotropical biomes. In Brazil, it occurs only in the Southern and Southeast regions: Paraná, Rio Grande do Sul, and São Paulo states.

Conservation status.— This species is listed as EX (presumed extinct) on the regional Red List the São Paulo state (São Paulo, 2016). The EOO is 706,811 km^2 and the AOO is 76 km^2. Based on the geographic criteria B1a+B2a, *S. dulce* is provisionally assessed as least concearn (LC; IUCN 2017).

Notes.— *S. dulce* is very similar to *S. diphyllum* and their geographic distributions overlap as do the morphological characteristics used by former authors to separate these two species, their synonyms and varieties (Espinar and Ferrucci 1982; Ezcurra 1993; Nees von Esenbeck 1847a, 1847b). The most reliable character to distinguish them is the pollen that is prolate in *S. diphyllum* (Figure 11) as in most of its congeners and spheroid in *S. dulce* (Figure 11). The spheroid pollen of *S. dulce* is unique in *Stenandrium* and in fact in the Acantheae. Our study of the pollen of these two species covered a wide geographic range (Mexico, Ecuador, Peru, Bolivia, Brazil, Chile, Paraguay, and Argentina). Most specimens had spheroid pollen and a few had prolate pollen; both types were found in Brazil. Cromossome counts (Piovano and Bernardello 1991) for these two species have shown that *S. diphyllum* is diploid (2n=26, prolate pollen) and *S. dulce* is tetraploid (2n=52, spheroid pollen). A recent study of pollen morphology of polyploids have shown that polipolidy can change pollen grain shape and size (Kolarčik et al.

2018), while pollen dimorphism is sometimes associated to dioecy (Maurer 2013). These findings have led us to believe that possibly there is only one species, in which the diploid individuals have normal, prolate pollen and the tetraploid individuals have spheroid pollen. Another possibility, if the spheroid pollen is in fact inaperturate and sterile as we suspect, is that individuals with such pollen are functionally female, while individuals with normal pollen are presumably hermaphrodites or functional males. Thus, *Stenandrium dulce* could be gymno-dioecious or cryptic-dioecious. We did not observe morphological evidence of diminished female function in the flowers with normal pollen, however, we were struck by the fact that all the Brazilian specimens with prolate pollen observed lacked fruits. Due to spike morphology and presumably timing of floral anthesis, most specimens of *Stenandrium* have both flowers and fruits. The wide funnel-shape stigma would tend to maximize contact with the pollinator's body and the whole floral morphology is very similar to dioecious *Schlegelia parviflora*, one of the few dioecious species in the Lamiales (Maurer 2013). Thus, *S. diphyllum* is treated as a synonym of *S. dulce*. It is of course possible that *S. diphyllum* is a cryptic species. Detailed molecular and morphological studies and experimental crosses between the two pollen morphs would be necessary to elucidate this question.

***Selected specimens examined.*—** BRAZIL. Paraná: **Laranjeiras do Sul**: Km 127, [25°24'28.1"S, 52°24'58.0"W], 5 Dez 1969, *G. Hatschbach & R. Ravenna 23128* (K!, C!); **Sengés:** Rod. PR-11, próx. km 25, [24°06'46"W, 49°27'49"S], 18 Nov 1989, *G. Hatschbach, M. Hatschbach & V. Nicolack 53644* (GB!, MBM). Rio Grande do Sul: **Alegrete:** São Pedro, na Fazenda de Adão Soares, [29°46'59.2"S, 55°47'30.8"W], 24 Feb 1982, *B. Irgang & J. Vasconcellos 50485* (HAS!); **Pinheiro Machado:** Km 13 da Rodovia para Pelotas, 1 Dez 1983, J. Mattos & N. Silveira 25352 (HAS!); **Santa Maria**: Reserva Biológica do Ibicuí-Mirim, Barragem Saturnino, 8 Nov 1988, *N. Silveira 6034* (HAS!); Reserva Biológica do Ibicuí-Mirim, 8 Nov 1988, *M.L. Abruzzi 1615* (HAS!). São Paulo: **Conchas**: 14 Jun 1938, *F.C. Hoehne & A. Gehrt 39549* (SP!); Santo Amaro, Sem. Espírito Santo, 15 Out 1942, *L. Roth BP 5774* (SP!, IPA!).

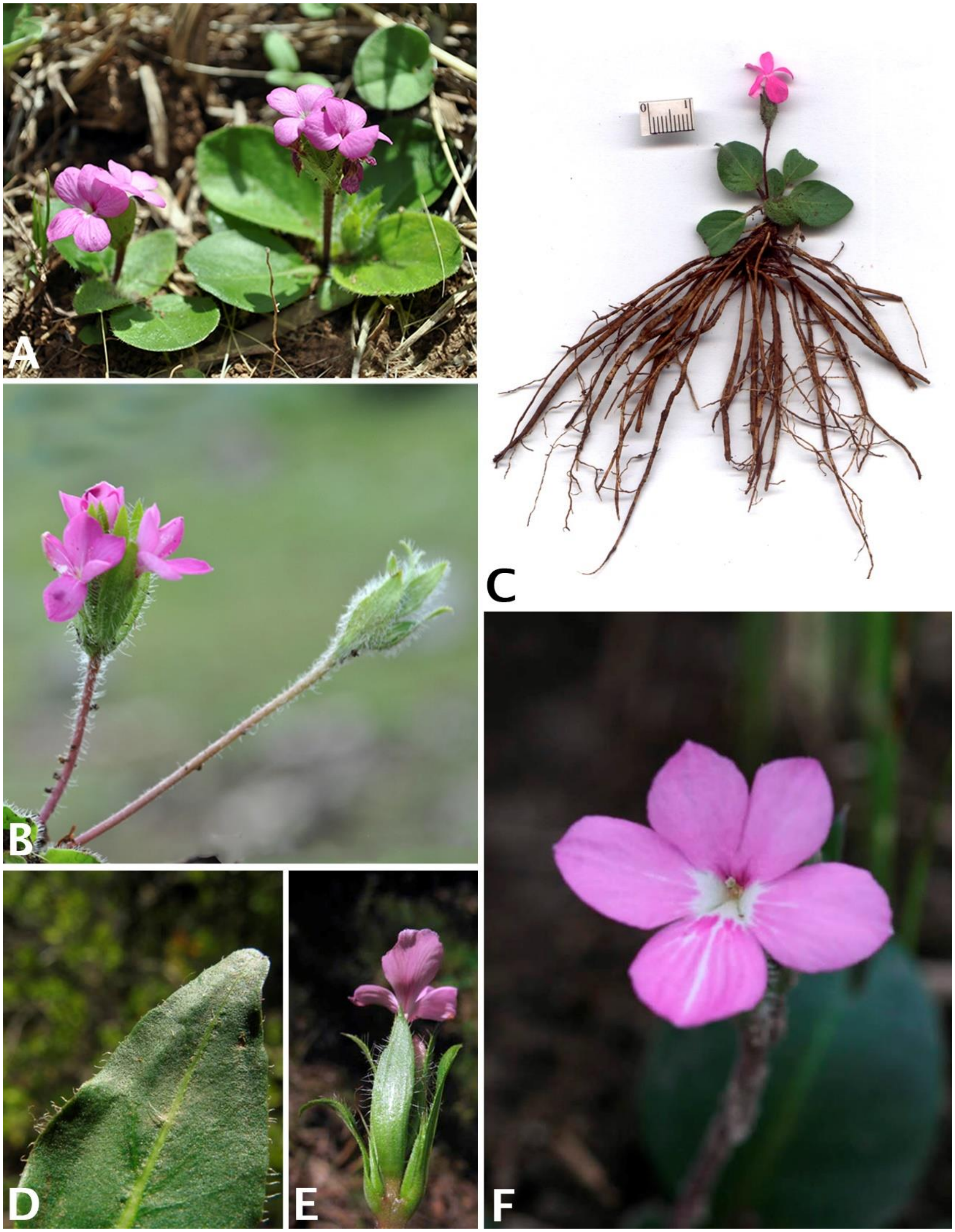


Figure 10. Field images of *Stenandrium dulce*. A. Habit and habitat (photo by C.V. Ely). B. Peduncles with spikes (photo by A.A. Schneider). C. Plant with roots (photo by J.A. Radins). D. Detail of leaf apex (photo by M. Belov). E. Detail of spike with bracts and flower (photo by M. Belov). F. Subactinomorphic corolla (photo by H. Hill).

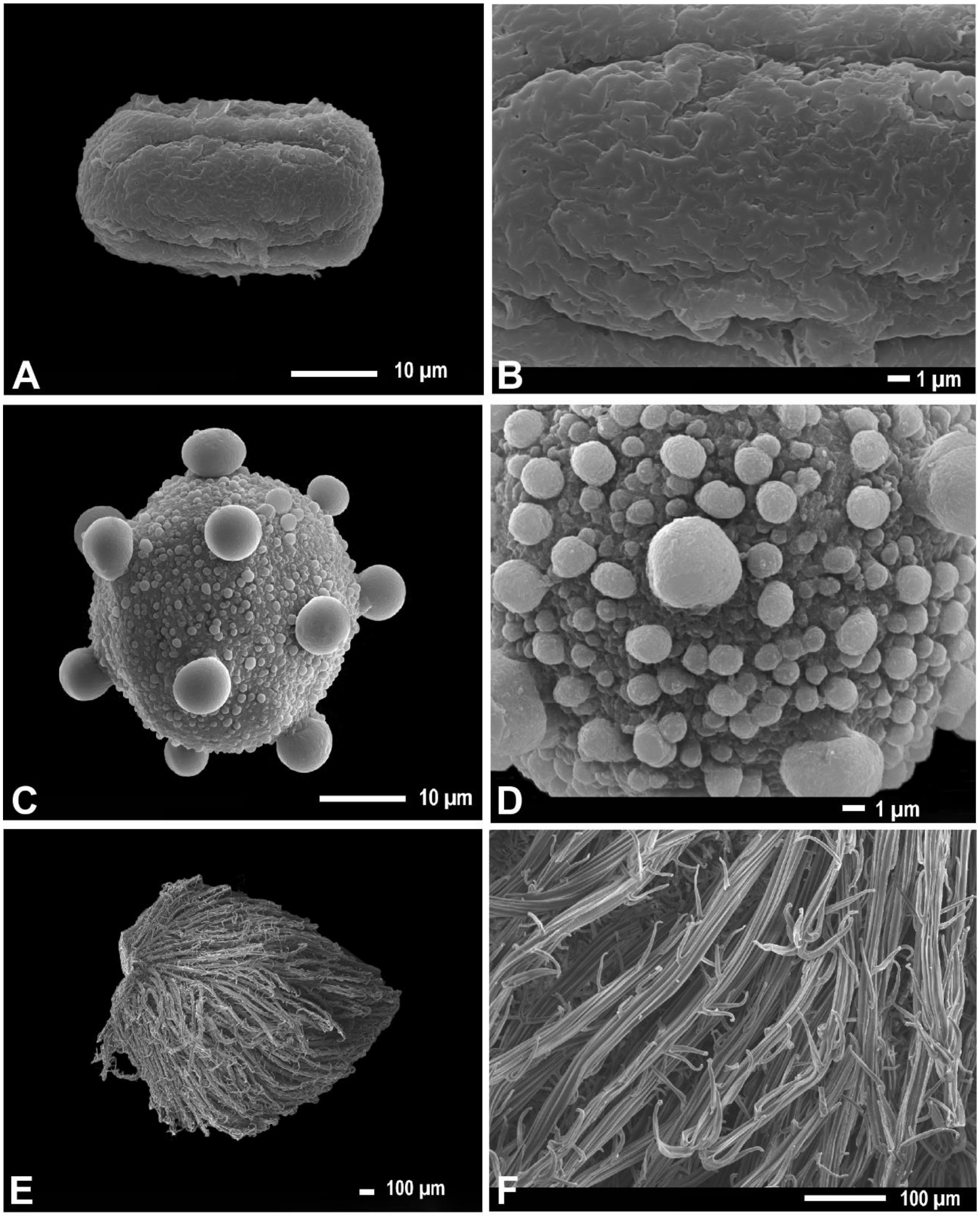


Figure 11. SEM images of *S. dulce.* A. Equatorial view of a ellipsoid tricolpate grain. B. Detail of the fossulate perforate exine surface. C. Spheroid grain. D. Detail of the gemmate exine with gemmae of two sizes. E. Seed general view. F. Details of the barbed trichomes.

4. ***Stenandrium eustachyum*** **Zanatta & Proença**, sp. nov.

See Chapter 3.

5. ***Stenandrium hatschbachii*** **Wassh.** in Brittonia 42: 1. 1990 – Holotype: BRAZIL. Minas Gerais, Vicinity of Grão Mogol, 24 Jul 1978, *G. Hatschbach 41518*. (MBM [photo!]; isotype: US [photo!]).

Erect caulescent branched perennial to 1 m tall, pubescent with trichomes whitish to yellowish varying in size on most vegetative structures; rhizome horizontal 5.5-7.2 mm thick, roots up to 3 cm long (a single short root from a single specimen); stems terete to subquadrate, glabrous to pubescent with trichomes up to 1.3 mm long, old stems glabrous. **Leaves** opposite, subsessile, petioles 1.4-2.1 mm long, pubescent to tomentose with trichomes up to 0.5 mm long, blades ovate to lanceolate 17.7-54 × 5.8-31.2 mm, 1.4-3 times longer than wide, chartaceous to coriaceous, cordate to oblique at base, acute to rounded at apex, adaxial surface glabrous to puberulent with trichomes up to 0.7 mm long, abaxial surface glabrous to pubescent with trichomes up to 0.7 mm long, margins revolute, cartilaginous, entire, ciliate with trichomes up to 0.7 mm long. **Inflorescence** a short-pedunculate to sessile terminal or axillary spike 10-56.5 mm long (including peduncles, excluding flowers) often concentrated at the subterminal nodes, peduncles dark purplish to dark red, 1.5-3 mm long, pubescent with trichomes up to 0.25 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** linear to subulate, 6-8 mm long, 0.9-1.2 mm wide, 1-veined, adaxial surface glabrous to puberulent with trichomes up to 0.2 mm long, abaxial surface pubescent with trichomes up to 0.8 mm long, margins ciliate with trichomes up to 0.8 mm long. **Bractlets** lanceolate, conduplicate, 2-5 mm long, 0.3-0.5 mm wide, inconspicuously 3-veined on the abaxial surface and conspicuously on the adaxial surface, puberulent with trichomes up to 0.1 mm long, margins ciliate also with trichomes up to 0.1 mm long. **Calyx** with subequal lanceolate lobes, glabrous to sparsely pubescent, lobes insconspicuously 3-veined, 4.3-4.9 mm long, 0.5-1.3 mm wide. **Corolla** often with the upper lip wine-colored and the lower lip pale lilac to white often wine-striated, zygomorphic, tube glabrous 5.7-8 mm long, 1.5-1.9 mm wide, upper lip 3.2-7 mm long, 3-5 mm wide with equal lobes, lower lip 5-10 mm long, 7-17 mm wide with subequal lobes, lower central lobe pubescent at the base internally with trichomes up to 0.5 mm long. **Stamens** 1.2-3 mm long, anthers 0.7-1.5 mm long, pubescent with trichomes up to 0.1 mm long; staminode not seen; pollen grains prolate, tricolpate, 44.4-45.5 × 25.4-29.9 µm, exine fossulate perforate, colpus with gemmate membrane. **Style** 5.3-6.7 mm long with a few minute trichomes at the tip up to 0.1 mm long, stigma asymmetrically funnelform. **Capsule** glabrous to puberulent with trichomes up to 0.1 mm long, 8.5-11.3 mm long, 4.2-4.4 mm wide. **Seeds** 2.6-

3 mm long, 1.5-2 mm wide, the surface tomentose with barbed trichomes up to 0.7 mm. **Figure 12.**

Phenology. – Flowering and fruiting specimens were often collected from September to January, and rarely on April and July.

Distribution and habitat.— *Stenandrium hatschbachii* is endemic to the rocky cerrados and rocky fields of the Northern Serra do Espinhaço, Minas Gerais, Brazil.

Conservation status.— *Stenandrium hatschbachii* was officially assessed as EN (endangered; IUCN 2017), based on its EOO (537.25 km^2) and AOO (16 km^2), using the geographic criteria B1ab(iii)+2ab(iii) (Martinelli and Moraes 2013). Therefore, it is currently included in the "Lista Oficial de Espécies da Flora Brasileira Ameaçadas de Extinção", the Brazilian official red list (MMA 2014). As far as we are concerned, there is no record of this species in any protected area. *Stenandrium hatschbachii* is also included as one of the rare species of Brazil by Giulietti et al. (2009).

Notes.— *Stenandrium hatschbachii* is most similar to *S. stenophyllum* but differs by its opposite leaves with ovate to lanceolate blades (vs. verticillate leaves with linear blades in *S. stenophyllum*). It is also similar to *S. diamantinense* but can be distinghished from the latter species by its zygomorphic corolla with the upper lip wine-colored and the lower lip pale lilac to white (corolla subactinomorphic, of a uniform pink in *S. diamantinense*).

Selected specimens examined. — BRAZIL. Minas Gerais: **Cristália:** Vale do Itacambiruçu, 19 Sep 2003, *A. Rapini 1094* (HUEFS [photo!]); Vale do Itacambiruçu, 16°36'27" S, 42°54'53" W, 21 Jan 2016, *A. V. Scatigna & J. M. Carmo 1051* (UEC). **Grão Mogol:** Vale do Rio Itacambiruçu, 4 Sep 1985, *T.B. Cavalcanti et al. CFCR 8366* (F!, UB!); Margem da estrada de Grão Mogol para Cristália, ca. 800 m depois da ponte do Rio Itacambiruçu, 16°35'38.1'S, 42°53'44.3"W, 18 Nov 2017, *M.R.V. Zanatta & J.E.Q. Faria 2506* (UB!); Grão Mogol-Cristália, 15 Apr 1981, *I. Cordeiro et al. CFCR 939* (HUEFS!); Córrego das Mortes, 3 Sep 1985, *D.C. Zappi et al. CFCR 8336* (NY!); Ribeirão dos Bois, 2 Sep 1986, *I. Cordeiro & R. Mello Silva CFCR 9993* (K!, NY!); Córrego das Mortes, 3 Sep 1985, *J.R. Pirani et al. CFCR 8322* (NY!); Trilha do Barão, 16°33'76"S, 42°53'42"W, *A.M. de Carvalho et al. 6520* (NY!); Contrafortes a leste da trilha da tropa, que vai da cidade até Periperi passando pelo alto da serra, nas encostas à esquerda do Ribeirão do Inferno, 1160 m, 27 Sep 1997, *R. Mello Silva et al. 1440* (RB!); Jambeiro à 7 km de Grão Mogol, 5 Sep 1985, *J.R. Pirani et al. CFCR 8498* (K!); Estrada Grão Mogol para Cristália, ponte sobre o Rio Itacambiruçu, 18 Jul 1998, *G. Hatschbach et al. 67995* (K!); **Juramento**: Estrada Itacambira-Juramento, 6 km antes de Juramento, 25 Jul 1985, *G. Martinelli 11294* (RB!).

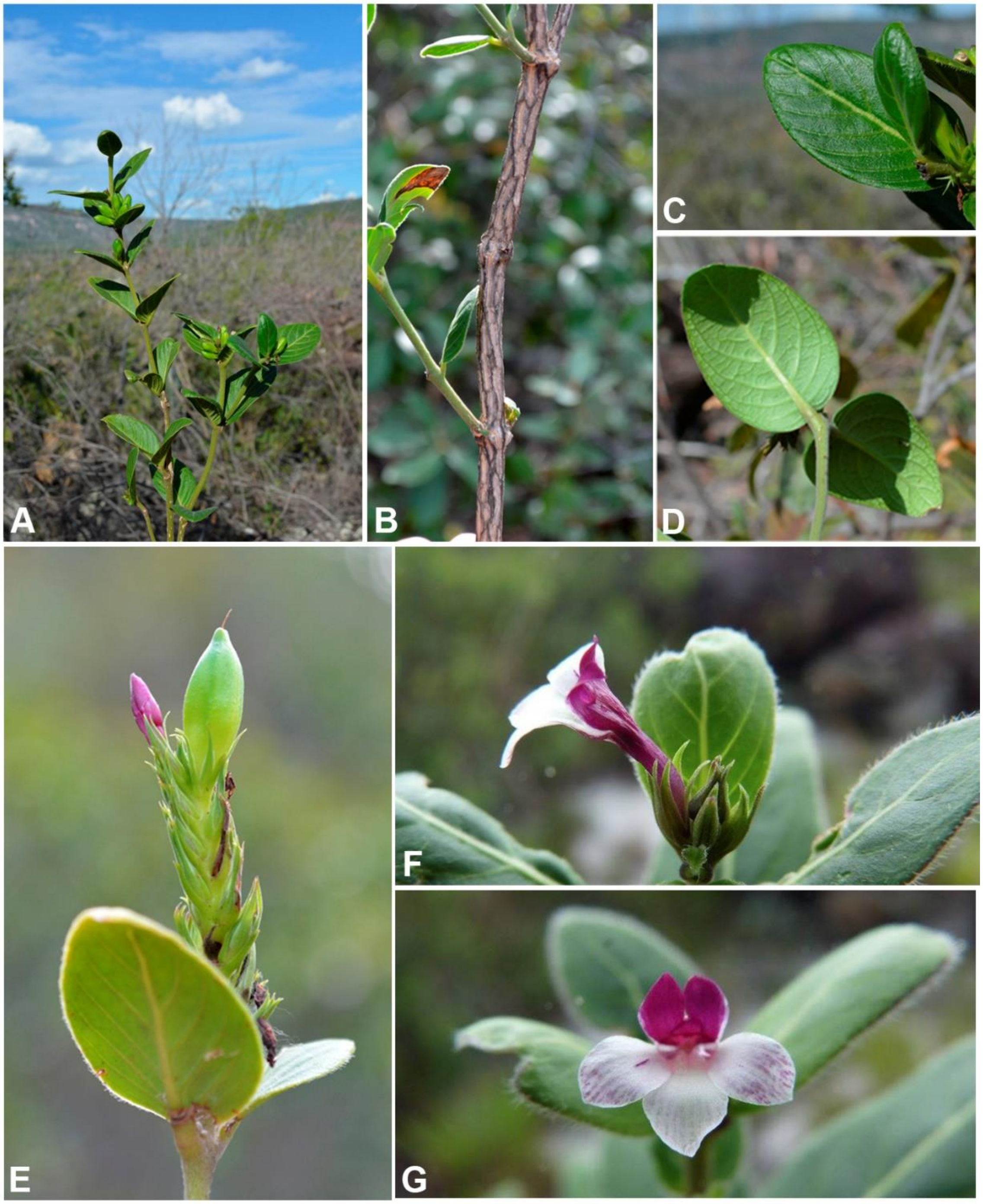


Figure 12. Field images of *Stenandrium hatschbachii*. A. Habit and habitat. B. Branch. C-D. Leaves. E. Terminal spike with bracts, bud, and fruit. F-G. Zygomorphic corolla. A-E photos by M.R.V. Zanatta, F-G photos by A.V. Scatigna.

6. ***Stenandrium hirsutum*** **Nees** in Mart. Fl. Bras. 9: 75. 1847 – **Lectotype (designated here):** BRAZIL. [Goiás] Ad S. Jozé, *J.B.E. Pohl s.n.* M [photo!], GZU [photo!], W).

Erect, caulescent perennial to 35 cm tall, hirsute with whitish, yellowish to ferruginous trichomes on most vegetative structures; stems hirsute with trichomes up to 3.5 mm long, old stems almost glabrous, rhizome vertical, 1.8-3.3 mm thick, roots up to 12.2 cm long. **Leaves** concolor, opposite, sub-sessile, petioles 1.5-2.5 mm long, hirsute with trichomes up to 3 mm long, blades lanceolate, 21.4-58.1 mm long, 4.8-13.1 mm wide, 3.2-9 times longer than wide, chartaceous, acute at apex, hirsute with trichomes up to 3.3 mm long on both surfaces, margins plane, entire, ciliate with trichomes up to 3 mm long. **Inflorescence** a long pedunculate, terminal or axillary spike 32.6-87.5 mm long (including peduncle, excluding flowers) often concentrated at the subterminal nodes, peduncles 23.4-72.8 mm long, hirsute with trichomes up to 2.5 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** lanceolate to ovate, 5.8-9.7 mm long, 2.3-4.5 mm wide, appearing 3-veined to the naked eye with two weak lateral veins visible under magnification, veins inconspicuous on both surfaces, adaxial surface almost glabrous, abaxial surface hirsute with trichomes up to 2.5 mm long, margins entire, ciliate with trichomes up to 2.5 mm long. **Bractlets** lanceolate, 1.8-2 mm long, 0.3 mm wide, 1-veined, glabrous, margins entire. **Calyx** with subequal lanceolate lobes, lobes unconspicually 3-veined, 3.8-4.4 mm long, 0.7-1 mm wide, glabrous to sparsely puberulent with minute trichomes up to 0.1 mm long, margins entire. **Corolla** light pink, the lower lip yellow to orange coloured at the base of the central lobe, subactinomorphic, tube glabrous 2.3-4.8 mm long, 1.3-1.5 mm wide, upper lip 5.5-6.5 mm long, 9-9.8 mm wide with equal lobes, lower lip 7.3-8.6 mm long, 12.7-17.6 mm wide with subequal lobes. **Stamens** 1.2-1.4- mm long, anthers 0.7-1.1 mm long, tomentose at tip and dorse with trichomes up to 0.3 mm long; staminode 0.3-0.7 mm long; pollen grains prolate, pentacolpate, 42.8-49.2 × 16.9-24.6 µm, exine perforate, colpus with gemmate membrane. **Style** 3 mm long, stigma asymmetrically funnelform. **Capsule**, 4.8-8.5 mm long, 2.5-4.8 mm wide. **Seeds** 2.8-3.3 mm long, 1.9-2.3 mm wide, the surface pubescent with barbed trichomes up to 0.6 mm. **Figure 13.**

Phenology. — Flowering and fruiting specimens were collected often from September to December and rarely in March.

Distribution and habitat. — *Stenandrium hirsutum* is a relatively widespread species, but rather uncommon, from areas of cerrado, grasslands and pastures on rocky and sandy soils in Goiás, Mato Grosso and Mato Grosso do Sul. There are no records from neighbouring countries.

Conservation status.— The EOO of *S. hirsutum* is 201,258 km^2 and the AOO is 40 km^2. Based on the geographic criteria B2a, *S. hirsutum* is provisionally assessed as vulnerable (VU; IUCN 2017). There is one record of this species near one protected area in Barra do Garças, Mato Grosso.

Notes. — *Stenandrium hirsutum* resembles *S. affine* but can be dintinguished from the latter species by its pentacolpate pollen grains (tricolpate in *S. affine*), concolorous leaves (slightly discolorous in *S. affine*), and non-cartilaginous leaf margins (cartilaginous in *S. affine*).

Specimens examined. — BRAZIL. Goiás: **Alto Horizonte:** Região da Sururuca, fazenda Cajás, 14°11′54″S, 49°17′32″W, 404 m, 2 Mar 2014, *J.E.Q. Faria 3880* (UB!); Região da Sururuca, fazenda Cajás, 14°11′54″S, 49°17′32″W, 404 m, 30 Dez 2014, *J.E.Q. Faria 4318* (UB!); **Caiapônia**: Ca. 35 km. E. of Caiapônia on road to Jataí, Serra do Caiapó, -17.22, -51.82, *H.S. Irwin & T.R. Soderstrom 7053* (SP!). **Niquelândia:** Estrada entre Niquelândia e a Companhia de Níquel Tocantins, 5 km da mina de níquel, 14°22′14″S, 48°23′15″W, 20 Oct 1996, *R.C. Mendonça et al. 2878* (K!); Serra do Caiapó, 17°12′S, 51°47′W, 19 Oct 1964, *H.S. Irwin & T.R. Soderstrom 7053* (NY!, UB!); Macêdo, ca. de 5 km após a mina de níquel CNT, 14°22′14″S, 48°23′15″W, *M.L. Fonseca et al. 1243* (SPF!); Mato Grosso: **Barra do Garças:** Pé da Serra, 4 Oct 1968, *Sidney & L. Onishi 1091* (UB!); **Cuiabá:** Rod. BR-070, Contorno Sul, 19 Nov 1996, *G. Hatschbach et al. 65668* (K!); **Nova Brasilândia:** Estrada entre Riolândia (Frieira) e Marzagão, ca 25km NNW (em linha reta) de

Nova Brasilândia, -14.75, -55.06, 7 Oct 1997, *V.C. Souza et al. 20171* (ESA!, MT, UEC!); Ca. 1km NE de Riolândia (Frieira) em direção a Planalto da Serra, -14.73, -54.96, 7 Oct 1997, *V.C. Souza et al. 20136* (ESA!, MT); **Nova Xavantina:** Estrada para projeto Cachoeira, Projeto Xavantina, à margem esquerda da estrada a 22 km de Nova Xavantina, 14°31′22.5″S, 52°25′28.7″W, 24 Sep 2001, *T.M.E.S. Pinto 19* (SP!); **Rosário Oeste**:, ca. 75km E de Rosário Oeste, estrada entre Riolândia (Frieira) e Marzagão, 14°43′S, 55°10′W, *V.C. Souza et al. 20217* (ESA!, MT). Mato Grosso do Sul: **Pedro Gomes:** Rod. BR-163, 12 Nov 1975, *Hatschbach 37426* (MBM! [photo!]).

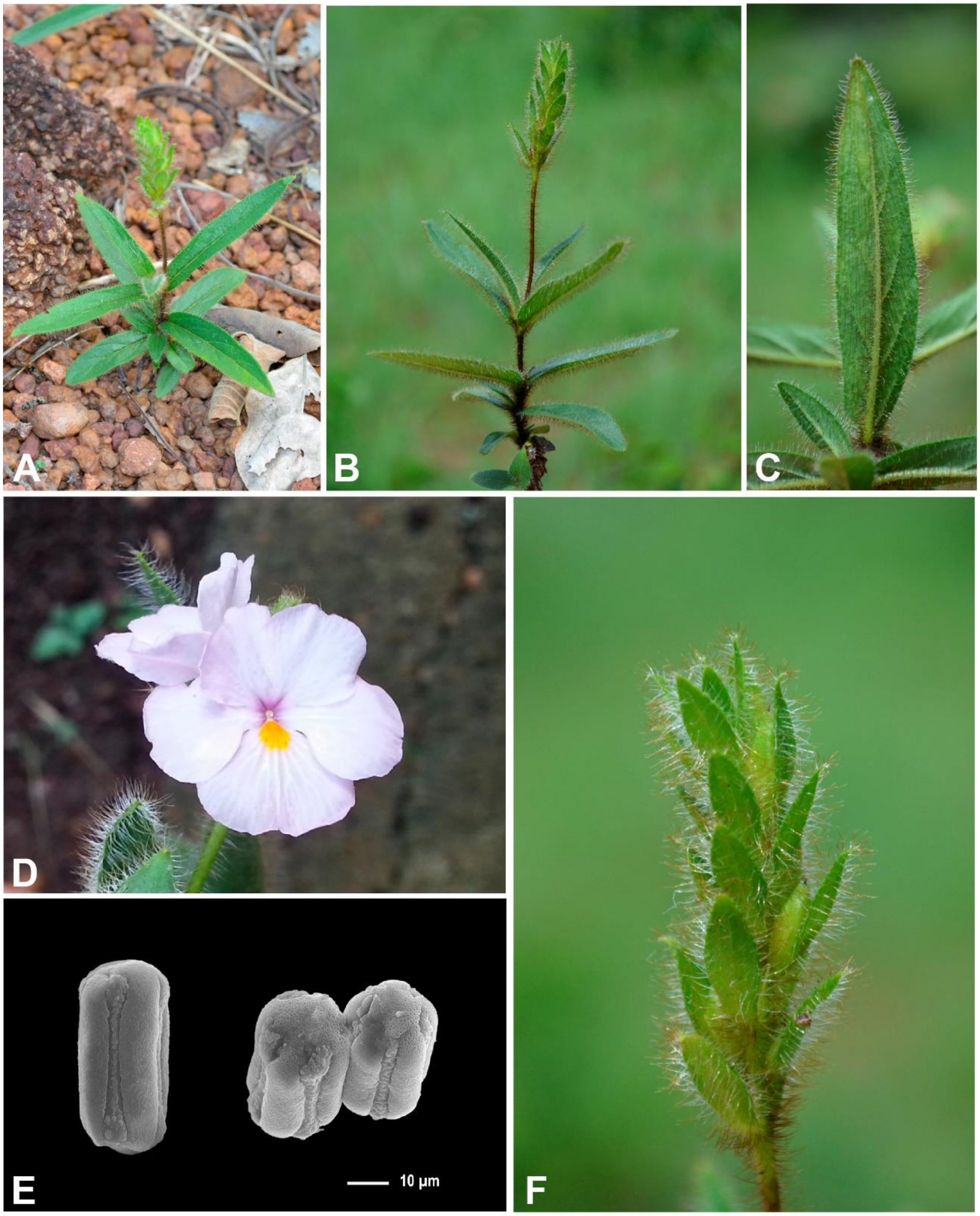


Figure 13. Field images of *Stenandrium hirsutum* (J.E.Q. Faria 4318). A. Habit and habitat. B. Leaves and terminal spike. C. Leaf. D. Subactinomorfic corolla. E. Pentacolpate pollen grain. F. Spike with bracts and fruits. A-D, and F photos by J.E.Q. Faria.

7. *Stenandrium irwinii* Wassh. in Brittonia 42: 4. 1990 – Holotype: BRAZIL. Goiás, Chapada dos Veadeiros, ca. 12 km NW of Veadeiros, 1200 m alt., 19 Oct 1965, *H. S. Irwin, R. Souza* & *R. Reis dos Santos 9272*. (UB!; isotypes: F [photo!], NY [photo!], US [photo!]).

Acaulescent perennial to 20 cm tall, pubescent to puberulent with whitish, yellowish to ferruginous trichomes on most vegetative structures; rhizome vertical, 3.8-6.7 mm thick, roots up to 9 cm long. **Leaves** concolorous, clustered and appearing whorled, petiolate, petioles (naked portion) 6.7-14.7 mm long, puberulent to tomentose with trichomes up to 0.7 mm long, blades lanceolate to ovate, sometimes oblanceolate, 20-37.2 mm long, 4-22.7 mm wide, 1-6 times longer than wide, herbaceous, often gradually narrowed and attenuate at base, acute to rounded at apex, adaxial surface glabrous to pubescent with trichomes up to 0.6 mm long, abaxial surface glabrous to pubescent with trichomes up to 0.8 mm long (confined mainly to the veins) and a glandular-punctate understory, margins plane, entire or sinuate, cartilaginous, ciliate with trichomes up to 0.8 mm long. **Inflorescence** a long pedunculate scape 105-188 mm long (including peduncle, excluding flowers), peduncles 28-99.8 mm long, glabrous to pubescent with trichomes up to 0.6 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** lanceolate to subulate, 7-14.5 mm long, 2.5-4.6 mm wide, insconscpicually or conspicually 3-veined, adaxial surface almost glabrous to puberulent with trichomes up to 0.3 mm, abaxial surface puberulent to tomentose with trichomes up to 1.5 mm long, margins plane, entire, ciliate with trichomes up to 1.5 mm long. **Bractlets** lanceolate, 4-8 mm long, 0.8-1.2 mm wide, appearing 1-veined to the naked eye with two lateral vein visible under magnification, glabrous to sparsely pubescent with trichomes up to 0.5 mm long, margins entire, ciliate with trichomes up to 0.5mm. **Calyx** with subequal lance-subulate lobes, lobes appearing 3-veined to the naked eye, with two latteral veins seen under magnification, 5-7 mm long, 0.6-1.3 mm wide, glabrous to puberulent (or sparsely pubescent at tip) with minute trichomes up to 0.2 mm long, margins entire, ciliate with trichomes up to 0.2 mm long. **Corolla** light pink to magenta, the lower lip yellow to orange coloured at the base of the central lobe, subactinomorphic to zygomorphic, tube 8-12.3 mm long, 1.7-4 mm wide, upper lip 8.5-12.3 mm long, 9.5-24.4 mm wide with equal lobes, lower lip 11-13 mm long, 20-22 mm wide with subequal lobes, lower-central lobe tomentose at the base internally with trichomes up to 0.7 mm long. **Stamens** 2.3-3 mm long, anthers 1.2-1.7 mm long, pubescent with trichomes up to 0.2 mm long; staminode 0.6 mm long; pollen grains prolate, tricolpate, 50.1-52.9 × 24-28.9 μm apocolpium psilate sometimes perforate, mesocolpium psilate, colpus with gemmate membrane. **Style** 8.5-9.8mm long, puberulent with minute trichomes up to 0.1 mm long, stigma asymmetrically funnelform. **Capsule** glabrous to puberulent with minute trichomes up to 0.1 mm long, 10.8 mm long, 4.3 mm wide. **Seeds** 2-5 mm long, 1.5-2.3 mm wide, the surface tomentose with barbed trichomes up to 1.2 mm long. **Figure 14.**

Phenology. – Flowering and fruiting specimens were often collected from September to November and rarely in May and August.

Distribution and habitat.— Recorded only in high-altitude rocky fields on sandy soils near Alto Paraíso de Goiás and Cavalcante, Goiás; the species is presumed to be endemic to the Chapada dos Veadeiros, in the Central Brazilian Plateau.

Conservation status.— *Stenandirum irwinii* was officially assessed as EN (endangered, IUCN 2017), based on its EOO (192.6 km^2) and AOO (12 km^2), using the geographic criteria B1ab(i,ii,iii,v)+2ab(i,ii,iii,v) by CNCFlora (2012). Despite this, it is not currently included in the "Lista Oficial de Espécies da Flora Brasileira Ameaçadas de Extinção", the Brazilian official red list (MMA 2014). Fortunately, it was recently recorded in the Parque Nacional da Chapada dos Veadeiros, a large protected area of the region. *Stenandrium irwinii* is also included as one of the rare species of Brazil by Giulietti et al. (2009).

Notes.— *Stenandrium irwinii* is morphologically most similar to *S. riedelianum,* but can be distinguished from the latter species by its cartilaginous leaf margins (non-cartilaginous in *S. riedelianum*), bracts 2.5-4.6 mm wide (1.3-2 mm wide in *S. riedelianum*), and corolla tube 8-12.3 mm long (4-7.5 mm long in *S. riedelianum*).

Selected specimens examined. — BRAZIL. Goiás: **Alto Paraíso de Goiás:**

GO-118, 15 km de Alto Paraíso para Teresina de Goiás, 14°05'19"S, 48°47'04"W, 5 Nov 2004, *Pastore 1084* (CEN!); Estrada para Teresina de Goiás, entrada da Fazenda Água Fria, 17 Oct 2006, *Paula-Souza et al. 8608* (SPF!); Parque Nacional da Chapada dos Veadeiros, ca. 5 km antes do alojamento do Parque, 15 Oct 2010, *Sartin et al. 121* (SP!, UFG); Parque Nacional Chapada dos Veadeiros, 23km de Alto Paraíso em direção a Teresina de Goiás, 12 Nov 1996, *Silva 3232* (RB!, UFG); Chapada dos Veadeiros, Rodovia GO-118, a ca. 12 km de Alto Paraíso, a ca. 200 m da estrada, 14°02'36"S, 48°28'14"W, 16 Sep 2014, *Siniscalchi et al. 563* (SPF!, UB!); Estrada para Campos Belos (GO-118), 15,5 km N de Alto Paraíso, 14°28'52"S, 48°23'33"W, 29 May 1994, *Splett 281* (UB!); Entrada do Morro da Baleia, entre Alto Paraíso e São Jorge, 14°27'41"S, 48°05'05"W, 15 Oct 2016, *Zanatta 2421* (UB!); Estrada entre Alto Paraíso e São Jorge, próximo ao Jardim de Maitréia, 14°23'25"S, 48°19'33"W, 15 Oct 2016, *Zanatta 2424* (UB!); Estrada de Alto Paraíso para Cavalcante, RPPN Cara Preta, 14°02'16"S, 48°27'37"W, 15 Oct 2016, *Zanatta 2433* (UB!); Chapada dos Veadeiros, about 30 km N of Alto Paraíso, [14°34'25"S, 48°46'10"W], 19 Aug 1971, *Gifford 253* (UB!); Chapada dos Veadeiros, ca. 12 km N.W. of Veadeiros, [13°55'00"S, 47°23'00"W], 19 Oct 1965, *Irwin & Santos R9272* (F! [photo!], NY!, RB!, UB!, US [photo!]); Chapada dos Veadeiros, [14°14'19"S, 47°52'39"W], 1 Oct 1972, *Rizzo 8400* (UFG!).

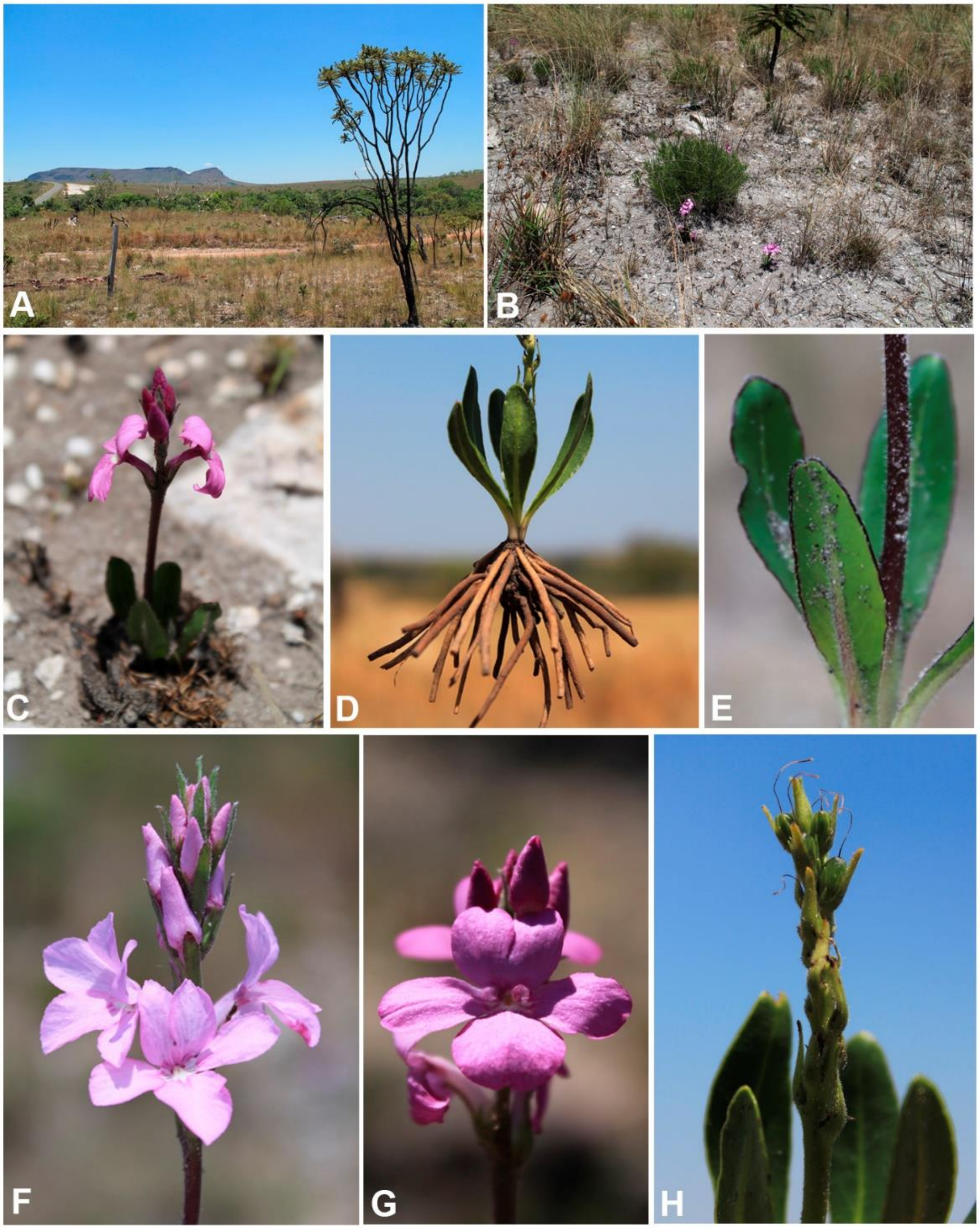


Figure 14. Field images of *Stenandrium irwinii* (M.R.V. Zanatta 2421). A. Habitat. B-C. Habit. D. Roots. E. Leaves. F-G. Spike with buds and flowers. H. Spike with bracts and imature fruits. Photos by M.R.V. Zanatta.

8. *Stenandrium mandioccanum* Nees in Lindl. Introd. Nat. Syst. Bot., 2nd edition, 444. 1836 – **Lectotype (designated here):** BRAZIL. [Rio de Janeiro, Serra dos Órgãos] prope Mandioccam in sylv[is] umbr[osis], 1822-1823, *H.K. Beyrich s.n.* (GZU [photo!]).

= *Stenandrium gracile* Rizzini in Dusenia 3: 195. 1952 – Holotype: BRAZIL. Paraná, Parque Nacional do Iguacu, 3 Jun 1949, *A. Duarte & E. Pereira 1944* (RB!).

= *Stenandrium spathulatum* S.Moore in Trans. Linn. Soc. London Bot. 4: 427. 1895 – **Lectotype (designated here):** BRAZIL. Mato Grosso: ad Corumbá, Jan 1891, S.M. Moore *958* (BM [photo!]).

Erect or prostrate caulescent herb to 35 cm tall; rhizome horizontal sometimes short and vertical, 1.3-2.5- mm thick, roots to 12 cm long; stems puberulent to pubescent with white trichomes up to 0.5 mm long. **Leaves** discolor, opposite, petiolate, petioles 2.7 – 20.9 mm long, puberulent to pubescent with trichomes up to 0.8 mm long, blades widely ovate to narrowly ovate, 9.2-84.4 mm long, 7.8-35.5 mm wide, 1.2-2.7 times longer than wide, membranaceous, gradually narrowed and attenuate at base, obtuse to acute at apex, adaxial surface glabrous to puberulent with minute trichomes up to 0.1 mm long and occasionally with a few trichomes up to 0.3 mm long, abaxial surface puberulent with minute trichomes up to 0.1 mm long, margins entire, ciliate with trichomes up to 0.5 mm long. **Inflorescence** a pedunculate, terminal or axillary reduced spike 14.8-22.7 mm long (including peduncle, excluding flowers) often concentrated at the subterminal nodes, peduncles 4-10.3 mm long, pubescent with trichomes up to 0.5 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** obovate to oblanceolate, 7.5-11.5 mm long, 2.7-6-7 mm wide, appearing 3-veined to the naked eye, with two other weak additional veins visible under magnification, puberulent to pubescent with trichomes up to 0.3 mm long on both surfaces, margins entire, ciliate with trichomes up to 0.5 mm long. **Bractlets** lanceolate, 3.5-4.2 mm long, 0.4-0.6 mm wide, appearing 1-veined to the naked eye, with two other weak additional veins visible under magnification on the adaxial surface, adaxial surface glabrous, abaxial surface pubescent with trichomes up to 0.2 mm, margins ciliate with trichomes up to 0.2 mm. **Calyx** with subequal lanceolate lobes, lobes 3-veined, veins conspicuous on the adaxial surface and insconspicuous on the abaxial surface, 4.7-6.2 mm long, 0.4-0.8 mm wide, adaxial surface glabrous, abaxial surface pubescent with trichomes up to 0.3 mm, margins ciliate with trichomes up to 0.2 mm. **Corolla** white, light pink, lilac or violet, lower central lobe sometimes white striated, subactinomorphic to zygomorphic, tube glabrous 3.3-3.8 mm long, 1-1.7 mm wide, upper lip 6.2-7.8 mm long, 4.6-7.6 mm wide with equal lobes, lower lip 7.3-10.8 mm long, 11.8-16.1 mm wide with subequal lobes, lower-central lobe tomentose at the base internally with trichomes up to 0.3 mm long. **Stamens** 1.1-

1.7 mm long, anthers 0.8-1.2 mm long, pubescent to puberulent with trichomes up to 0.2 mm long; staminode not seen; pollen grains prolate, tricolpate, 31.3-35.3 × 18.8-22.6 µm, exine psilate to fossulate, colpus with gemmate membrane. **Style** 2.8-4.4 mm long, stigma asymmetrically funnelform. **Capsule** glabrous to sparsely puberulent with minute trichomes up to 0.1 mm long, 6.4-9.4- mm long, 2.6-2.8 mm wide. **Seeds** 1.2-2 mm long, 1 mm wide, the surface pubescent with trichomes up to 0.3 mm long. **Figure 15.**

Phenology. — Flowering and fruiting specimens were often collected from August to January and rarely in April.

Distribution and habitat. — *Stenandrium mandioccanum* is widely distributed in South America: from northern Argentina to Ecuador, through Paraguay, southern and southeastern Brazil, Bolivia, and Peru (Wasshausen and Wood 2004). This species inhabits the ombrophilous forests but is also sporadic in dry forests, mainly in the Andes and around the Chaco region.

Conservation status. — The EOO of *S. mandioccanum* is 471,464 km^2 and the AOO is 76 km^2. Based on the geographic criteria B1a+B2a, *S. mandioccanum* is provisionally assessed as least concearn (LC; IUCN 2017). Besides, it was recorded in several protected áreas in Brazil: Área de Proteção Ambiental Pau Brasil (Rio de Janeiro), Estação Ecológica de Caiuá (Paraná), Estação Ecológica Municipal de

Fênix (Paraná), Parque Biológico Bueno de Miranda (São Paulo), Parque Estadual do Rio Guarani, Parque Nacional do Iguaçú (Paraná), and Reserva do Eco-Museu de Itaipu (Paraná).

Notes. — *Stenandrium mandioccanum* is morphologically most similar to *S. tenellum* but differs from the latter species by its whitish to purplish corollas (yellowish corollas in *S. tenellum*) and obovate to oblanceolate bracts with entire margins (ovate bracts with toothed margins in *S. tenellum*).

Selected specimens examined. –BRAZIL. In Brasilia tropica, *Riedel 63* (NY!); In Brasilia tropica, Sep 1832, *Riedel 788* (NY!); *Sellow s.n.* (F [photo!], K!); South Brazil, *Tweedie s.n.* (K!); South Brazil, Prov. St. Paul and Rio, 1861, *Weir s.n.* (K!). Mato Grosso do Sul: **Bonito:** Anastácio, Próximo da fazenda Serra Verde, 21°14'26"S, 56°49'23"W, 11 Oct 2003, *Hatschbach et al. 76155* (MBM [photo!]); Paraná: **Catanduvas:** Barra do Guarani, [25°20'49"S, 53°14'59"W], 16 Oct 1974, *Hatschbach & Pelanda 35120* (MBM [photo!], SP!); **Cianorte:** Rio Ivaí, [24°07'32"S, 53°00'31"W], 30 Apr 1966, *Hatschbach 14329* (MBM [photo!]); Barra São Tomé, Fazenda Lagoa, [24°06'27"S, 53°01'03"W], 24 Aug 1967, *Hatschbach 16966* (MBM [photo!]); **Cruzeiro do Iguaçu:** Área ecológica da Copel, [26°01'02"S, 53°12'37"W], 28 Aug 1999, *Silva et al. 3015* (BHCB!, C!, HUEFS [photo!], K!, MBM [photo!], SPF!); **Céu Azul**: Parque Nacional do Iguaçu, 25°39'29"S, 54°18'38"W, 22 Aug 2004, *Barbosa & Costa 980* (MBM [photo!]); **Diamante do Norte:** Estação Ecológica de

Caiuá, estrada da Conceição, 23°02'01''S, 53°49'26''W, 31 Oct 2011, *Beneli 35* (HUEM, UB!); **Fênix:** Estação Ecológica Municipal de Fênix, 25°04'18''S, 52°22'27''W, 27 Aug 2014, *Siqueira 1109* (HCF [photo!]); **Foz do Iguaçu:** Parque Nacional do Iguaçu, próximo às margens da BR-469, 25°37'43''S, 54°28'03''W, 14 Aug 2015, *Caxambu et al. 6766* (HCF!); Parque Nacional do Iguaçu, Trilha da Torre, 25°37'43''S, 54°28'03''W, 19 Oct 1991, *Cervi 3438* (MBM [photo!], SPF!, UPCB); Reserva do Eco-Museu de Itaipu, [25°37'43''S, 54°28'03''W], 22 Sep 1996, *Hatschbach et al. 65328* (ESA!, MBM [photo!]); Parque Nacional do Iguaçu, Trilha do Poço Preto, 25°37'43''S, 54°28'03''W, 3 Oct 2006, *Ribas et al. 7400* (HCF, MBM [photo!]); Parque Nacional do Iguaçu, Trilha das Bananeiras, 25°37'43''S, 54°28'03''W, 12 Oct 2009, *Temponi et al. 672* (IRAI, MBM [photo!], UNOP); **Jundiaí do Sul:** Fazenda das Acácias, Rio das Contas, 23°44'00''S, 50°26'48''W, 3 Jan 2002, *Carneiro 1262* (MBM [photo!]); **Marechal Cândido Rondon:** Corrego Alegre, [24°56'54''S, 54°04'55''W], 18 Sep 1981, *Buttura 674* (MBM [photo!]); **Santa Isabel do Ivaí:** [23°14'11''S, 53°26'11''W], 29 Aug 1967, *Hatschbach 17084* (MBM [photo!]); **São José da Boa Vista:** Terra Roxa, [24°31'57''S, 50°05'29''W], 19 Nov 1971, *Hatschbach & Guimarães 25543* (MBM [photo!]); **São Pedro do Ivaí:** Fazenda Barbacena, 24°27'12''S, 52°25'21''W, 15 Oct 2003, *Ribas 5509* (HUCS, MBM [photo!], RB!); **Três Barras:** Parque Estadual do Rio Guarani. Segunda Trilha, 25°26'24''S, 53°09'31''W, 2 Oct 2012, *Temponi 1195* (UNOP! [photo!]). Rio de Janeiro: **Armação dos Búzios:** Área de Proteção Ambiental Pau Brasil, Encosta acima do costão rochoso entre a Praia de José Gonçalves e a Praia de Caravelas, 22°48'36" S, 41°56'31" W, 24 Oct 2016, *Cordeiro et al. 3579* (SP!,UB!); **Cabo Frio:** Morro do Mico, Floresta Estacional, 22°51'26"S, 42°00'22"W, 7 Aug 2007, *Farney & Coelho 4795* (MBM [photo!], RB!, SPF!, UB!); Morro do Gavião, [22°51'26"S, 42°00'22"W], 13 Oct 1968, *Sucre 3924* (RB!,UB!); Rio de Janeiro: Sacopam, [22°57'58"S, 43°12'05"W], 6 Aug 1946, *Duarte 195* (RB!,UB!); Santa Catarina: **Itapiranga:** Steep slope by rio Uruguai, 3-4 km west of Itapiranga, [27°17'03"S, 54°15'52"W], 11 Nov 1964, *Smith & Klein 13153* (FLOR!, G [photo!], K!, MO, W [photo!]). São Paulo: **Limeira:** Mata da S.A.F.B, [22°58'42"S, 47°41'35"W], 1 Nov 1954, *Hoehne 15439* (SPF!); Parque Biológico Bueno de Miranda, [22°58'39"S, 47°41'37"W], 31 Oct 1951, *Kuhn 27* (SP!); **Piracicaba:** Mata da Pedreira, ESALQ/USP, Próximo a cachoeira do Piracicamirim, [23°11'23"S, 48°03'06"W], 22 Aug 1987, *Catharino 1110* (SP!).

Additional specimens examined. – BOLIVIA. Cochabamba: **Santa Cruz:** Chiquitos, on ascent to El Sancuario del Torre, Chochis from the west side, 18°07'S, 60°04'W, 19 Oct 2003, *Wood 19761* (UB!).

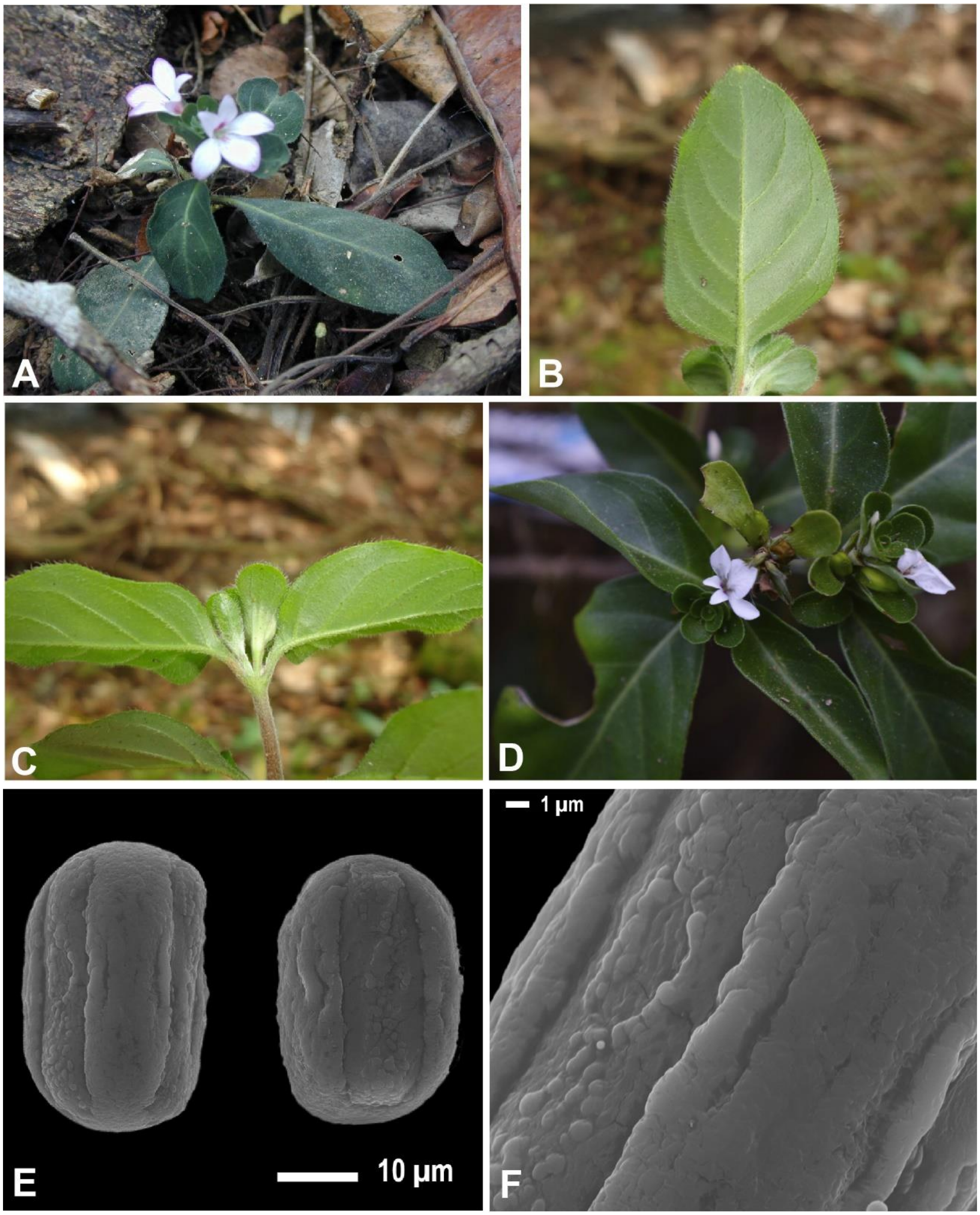


Figure 15. Field and SEM images of *Stenandrium mandioccanum.* A. Habit and habitat (photo by L. Cayola). B. Leaf (photo by D. Grasel). C. Terminal spike with obovate bracts (photo by D. Grasel). D. Spike with zygomorphic whitish corolla (photo by I. Gonçalves). E-F. SEM images of pollen grains (coleta). E. Equatorial view of tricolpate pollen grains. D. Detail of the pollen surface showing the fossulate exine and gemmate colpus.

9. ***Stenandrium pohlii*** **Nees** (1847: Fl. Bras. 9: 75). **Lectotype (designated here):** BRAZIL. Goiás, ad Corrego-Piau, prov. Goyazanae, *J.B.E. Pohl s.n.* (GZU [photo!]; isolectotypes: BR [photo!], M [photo!]).

= *Stenandrium pohlii* var. *pusillum* Nees in DC. Prodr. 11: 283. 1847 – **Lectotype (designated here):** Paraná ad fluvium, L. Riedel 49 (GZU [photo!]). *syn. nov.*

= *Stenandrium pohlii* var. *breviscapum* Nees in DC. Prodr. 11: 283. 1847 – **Lectotype (designated here):** Brazil. Piauí, 1836 G. Gardner 2291 (K!, isolectotypes: BM [photo!], NHM [photo!], NY[photo!], P! W[photo!]). *syn. nov.*

= *Caldenbachia elegans* Pohl ex Nees unpublished name

Acaulescent perennial to 22 cm tall with whitish, yellowish, ferrugineous to purplish trichomes on most vegetative structures; rhizome vertical, up to 7.8 mm thick, roots up to 16 cm long. **Leaves** concolorous to slightly discolorous, clustered and appearing whorled, sub-sessile to petiolate, petioles (naked portion) 2.3-29.5 mm long, puberulent to pubescent with trichomes up to 1 mm long, blades lanceolate, oblanceoate, elliptic or ovate, 25-225 mm long, 4-54 mm wide, 1.5-6 times longer than wide, herbaceous to chartaceous, gradually narrowed and attenuate at base, sometimes , obtuse to rounded at apex, surfaces glabrous to tomentose with trichomes up to 2 mm long, margins plane, entire or sinuate, ciliate with trichomes up to 1 mm long. **Inflorescence** a pedunculate floral scape 51-225 mm long (including peduncle, excluding flowers), peduncles 34-175 mm long, puberulent to pubescent with trichomes up to 2 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** ovate, sometimes lanceolate, 4-18.8 mm long, 3.5-12.4 mm wide, 5-7-veined, adaxial surface puberulent to pubescent with trichomes up to 1.8 mm long, abaxial surface glabrous to pubescent with trichomes up to 1.8 mm sometimes with a glandular-punctate understory, margins plane, entire, ciliate with whitish to purplish trichomes up to 2 mm long. **Bractlets** lanceolate, sometimes conduplicate, 1.5-3.5 mm long, 0.3-0.9 mm wide, 1-veined, glabrous to sparsely puberulent with minute trichomes up to 0.1 mm long, margins entire, ciliate with trichomes up to 0.1 mm long. **Calyx** with subequal lanceolate to subulate lobes, lobes 5-veined, 4.2-7.2 mm long, 0.5-1.2 mm wide, glabrous to sparsely puberulent with minute trichomes up to 0.1 mm long, margins entire, ciliate with tricomes up to 0.1 mm long. **Corolla** white, light pink, lilac, or magenta, the lower lip yellow to orange coloured at the base of the central lobe, subactinomorphic, tube 5.5-9.2 mm long, 1.5-2 mm wide, upper lip 7.2-13.3 mm long, 7.6-12 mm wide with equal lobes, lower lip 8-15 mm long, 15-26.3 mm wide with subequal lobes, lower-central lobe tomentose at the base internally with trichomes up to 1 mm long. **Stamens** 1.4-2.2 mm long, anthers 1-1.5 mm long, tomentose with trichomes up to 0.5 mm long; staminode 0.4-0.8 mm long; pollen grains prolate, tricolpate, 35.9-68.6 × 19.4-27.2 μm, apocolpium perforate sometimes psilate, mesocolpium psilate, perforate or foveolate, colpus with smooth sometimes gemmate membrane. **Style** 6.5-8.5 mm long, puberulent with trichomes up to 0.2 mm long at tip, stigma funnelform. **Capsule** glabrous to puberulent with trichomes up to 0.1 mm long and covered with minute, irregular shaped +/- flattened encrustations, 8.9-13.8 mm long, 3.2-5 mm wide. **Seeds** 2.5-3.7 mm long, 1.5-2.5 mm wide, the surface pubescent to tomentose with barbed trichomes up to 0.8 mm long. **Figure 16.**

Phenology. – Flowering and fruiting specimens were frequently collected from October to December and rarely from January to March and August. The blooming seems to increase in dry years (cpap.embrapa.br/plantas).

Distribution and habitat.— *Stenandrium pohlii* is the most widely distributed and most frequent species in the Bolivian and Brazilian cerrados on latossoils or sandy soils that are rarely or never flooded

Conservation status.— The EOO of *S. pohlii* is 715,563 km^2 and the AOO is 192 km^2. Based on the geographic criteria B1a+B2a, *S. pohlii* is provisionally assessed as least concearn (LC; IUCN 2017). Besides, it was recorded in several protected áreas in the Distrito Federal: Estação Ecológica do Jardim Botânico de Brasília, Parque do Guará Parque Nacional de Brasília, and Reserva Ecológica do IBGE.

Notes. *—* *Stenandrium pohlii* resembles *S. dulce* by the combination of congested spikes and acaulescent habit but can be distinguished from the latter species by its ovate (sometimes lanceolate) 3.5-12.4 mm wide, 5-7-veined bracts (always lanceolate, 1.5-3 mm wide, 3-veined bracts in *S. dulce*); and by the corolla upper lip 7.2-13.3 mm long, 7.6-12 mm wide, lower lip 15-26.3 mm wide (corolla upper lip 5-5.2 mm long, 4.5-5.3 mm wide, lower lip 8.5-9 mm wide in *S. dulce*). Its vernaculars names in Brazil are: "caiapiá" or "carapiá" in the Pantanal region (cpap.embrapa.br/plantas) and "berdoégua" in Piauí. It was recorded as an occasional forage plant, uselfull beekeepers with ornamental potential by the Embrapa/Pantanal (cpap.embrapa.br/plantas).

Selected specimens examined.— BRASIL. in prov. Minarum, *Vauthier s.n.* (W [photo!]); Bahia: **Barreiras:** Estrada Barreiras para Luis Eduardo Magalhães (BR-242), a 20 km do rio de Ondas, 14 Nov 2012, *Forzza 7454* (RB!). **São Desidério:** 13°17'36"S, 46°35'33"W, 12 Nov 2017, *Martins & Figueiredo 111* (HUEFS, RB!). Distrito Federal: **Brasília**: Oct 1964, *Barroso 666* (RB!); Fazenda Sucupira, 31 Oct 1995, *Assis 321* (CEN!); Burned-over cerrado between Brasilia and Sobradinho, 1 Jan 1965, *Irwin et al. 9168* (F, MO, NY!, RB!, UB!); Close to the campus of the University of Brasilia, 29 Sep 1976, *Ratter et al. 3690* (K!, MO, NY!, UB!, UEC!); BR próximo à cidade do Gama, 15°46'15"S, 48°37'07"W, 5 Oct 1999, *Calago 325* (CEN!); Reserva Ecológica do IBGE, 1 Jan 1996, *Freitas s.n.* (UB!); Catetinho, 6 Oct 1980, *Heringer 17901* (K!, MO, NY [photo!]); Confluence of Rio Torto with Lagoa Paranoá, 9 Oct 1965, *Irwin & Reis dos Santos 9082* (K!, NY, UB!); MSPW Q 17 Lote vazio ao lado do campo futebol da Vargem Bonita, 14 Sep 2000, *Lima 127* (UB!); Centro Olímpico da Universidade de Brasília, 1 Sep 2005, *Mendes 9* (UB!); EE do Jardim Botânico de Brasília, Cristo Redentor, 11 Sep 2003, *Oliveira & Milhomens 7* (HEPH!); Fazenda Água Limpa (University of Brasilia field station), near Vargem Bonita, c. 18 km SSW of Brasilia TV tower, 3 Nov 1978, *Ratter et al. R 4267* (K!, UB!); Parque Nacional de Brasília, 15°37'58" S, 49°59'29" W, 24 Oct 2011, *Zanatta & Bezerra 890* (UB!); Parque do Guará, EPTG, 15°48'38"S, 47°58'48.7"W, 25 Oct 2016, *Zanatta & Santos 2448* (UB!); Samambaia, Parque Boca da Mata, 20 Oct 1995, *Rezende 149* (CEN!, SPF!). Goiás: [Prov. Goyaz] Brasilia tropica, 1839, *G. Gardner 3415* (K!, NHM, BM!, BR!, K!, NY!, P!); [Prov. Goyaz], Brasilia tropica, May 1840, *G. Gardner 4341* (K!, NHM, W, BM!, BR!, K!, NY!, P!); 258 km de Anápolis para Belém, 8 Nov 1963, *Silva 57732* (K!, NY); **Abadiânia:** Serra dos Pirineus, ca. 14 km S. of Corumbá de Goiás, 30 Nov 1965, *Irwin 10749* (NY!); **Alto Paraíso de Goiás:** Rio das Almas, 47 km from Alto Paraíso de Goiás on the road to Teresina de Goiás, 14°34'25"S, 48°46'10"W, 12 Nov 1994, *Ratter et al. R 7425* (K!, UB!, UFG!); Estrada entre Alto Paraíso e Flores de Goiás, 14°04'58"S, 47°17'36"W, *Zanatta et al.* 2434 (UB!); **Alvorada do Norte**, Fazenda do Sr. Felipe, 3 Dec 2003, *Pereira-Silva 8047* (CEN!); Fazenda Itú, próximo a fábrica de calcário, 28 Aug 2003, *Sevilha 3180*

(CEN!, HUEFS); **Anápolis:** Campus UEG-Anápolis, 16°38'19"S, 49°34'52"W, 28 Oct 2004, *Faria 94* (HUEG!); **Campo Alegre de Goiás:** Conceição to Campo Alegre Goiás, Brazil, 17°32'21"S, 48°38'32"W, *Burchell 6394* (K!); **Campos Belos:** A 8km de Campos Belos para Taguatinga, 13°01'06" S, 47°09'32" W, 8 Oct 1972, *Rizzo 8442* (SPF!, UFG!); **Cavalcante:** Estrada saindo da balsa do "Porto dos Paulistas" (balsa sobre o rio Tocantins) para o Buracão, HU Curral de Pedra, À cerca de 2,8 km do rio Carmo, 14°09'27"S, 48°32'40"W, 8 Nov 2000, *Walter 4618* (CEN!, HUEFS); **Cristalina:** Serra dos Cristais, 5 km W. of Cristalina, 17°55'29"S, 48°00'00"W, 2 Nov 1965, *Irwin 9785* (NY!); **Edéia:** Fazenda Canadá (CSFCDC), 17°39'47"S, 50°06'03"W, 9 Oct 2012, *Projeto Biodiversidade BP et al. 175* (UB!); **Goiânia:** À margem direita da rodovia Goiânia São Paulo, Jardim Goiás, 4 Nov 1968, *Rizzo 2628* (SPF!, UFG!); **Goiás:** Rodovia BR-070, Goiás-Itaberaí. 1,2 km do primeiro trevo de acesso a Goiás, 4 Nov 2012, *Borges et al. 963* (SPF!, UB!); **Luziânia:** [16°56'33"S, 48°45'19"W], 27 Nov 1964, *Gomes 2453* (SP!); **Minaçu:** UHE Cana Brava-PA-SALV-CB, Sítio Acácia, margem esquerda do rio do Carmo, 14°17'37"S, 48°41'02"W, 11 Nov 1999, *Bucci 871* (UB!, UFG!); GO-060, Minaçu-Santa Tereza (em frente ao Motel), Área de influência indireta da UHE-Serra da Mesa, 13°33'50"S, 48°14'00"W, 9 Oct 1991, *Cavalcanti et al. 881* (CEN!, SPF!); Estrada Minaçu - balsa do "Porto dos Paulistas", à cerca de 6km de Minaçu (em referência ao Rio Bonito), 14°47'22"S, 48°45'57"W, 7 Nov 2000, *Walter 4522* (CEN!, HUEFS); **Niquelândia:** Fazenda Engenho, ca. 11 Km de Niquelandia em direção a Dois Irmãos, 14°22'14" S, 48°23'15" W, 2 Oct 1997, *Fonseca et al. 1613* (RB!); **Padre Bernardo:** Region of Fazenda Lagoa Santa, About 140 km N of Brasilia, 24 Sep 1972, *Ratter et al. 2516* (CAS!, UB!); **Posse:** Estrada de chão próximo ao Aeroporto, 14°30'41"S, 47°38'11"W, 16 Oct 2001, *Mendonça 4452* (CEN!); **São Domingos:** Saída da fazenda dos alagoanos, em direção à Estiva, beira da estrada, 13°38'53"S, 46°35'46"W, 12 Mar 2004, *Santos 2327* (CEN!); **Uruaçu:** Margem esquerda da rodovia 237, a 200 m da Polícia Militar Rodoviária, 14°31'53"S, 49°05'09"W, 20 Oct 1998, *Santos 315* (CEN!). Mato Grosso: **Barra do Garças:** Ca. 50 km. N. of Barra do Garças, on rd. to Xavantina, 16°12'25"S, 53°02'25"W, 14 Oct 1964, *Irwin 6867* (NY, SP, UB); **Cuiabá:** Bom Sucesso Road, near Cuiabá River, 16°10'11"S, 56°11'49"W, 26 Sep 1979, *Macedo et al. 1400* (NY!); **Lambari D'Oeste:** Fazenda Santana. Paiaguas, Pantanal, [18°21'10"S, 57°59'25"W], 23 Nov 1984, *Pott 1555* (CPAP, SPF!); Mato Grosso do Sul: **Aquidauana:** Distrito de Piraputanga, Pesqueiro do Serra, 28 Jan 1994, *Resende 914* (CGMS [photo!]); Fazenda Salina, Pantanal do Rio Negro, 19°30'S, 56°10'W, 23 Oct 1987, *Dubs 460* (G, MBM [photo!]); **Campo Grande:** 1969, *Nienstedt 190* (RB!); RPPN/UFMS, 21°44'17"S, 55°05'02"W, 29 Oct 2007, *Assunção & Romero 62* (CGMS [photo!]); **Corumbá:** Fazenda Nhumirim, Pantanal da Nhecolândia, 3 Oct 1979, *Allem 59* (UB!); Fazenda Ipanema, Nhecolândia, Pantanal, 3 Oct 1984, *Pott 1468* (CPAP, SPF!); **Coxim:** Reserva do Exército, 19 Sep 1996, Simon et al. 27 (E, UB!); **Porto**

Murtinho: MS-382, 63 km W de Bonito, 21°03'16"S, 56°58'45"W, 10 Nov 2002, *Pott et al. 10596* (CGMS,UB!); Rio Negro, Rodovia Transfrangeli, 17 Oct 1995, *Hatschbach et al. 63447* (MBM [photo!]). Minas Gerais: 1816, *Saint-Hilaire s.n.* (K!, MO, NY, P!); Piauí: **Oeiras:** Correntinho, próximo ao Rio Canidé, 16 Mar 2008, *F.C.S. Oliveira 364* (SP!, TEPB!). Tocantins: **Arraias:** Margem da estrada entre Conceição do Tocantins e Arraias, 10 Nov 2014, *Faria et al. 4307* (HUEG!, SP!, UB!); Estrada entre Arraias e Natividade, 12°49'09"S, 47°03'36"W, 6 Nov 2016, *M.R. Zanatta & G. V. Zanatta 2454* (UB!); **Dianópolis:** 12°32'04"S, 47°35'27"W, 30 Sep 2003, *Cavalcanti 3425* (CEN!, HUEFS); **Palmeirópolis:** Fazenda Mucambinho, prop. Sr. Edwardes do Nascimento Moura, 13°13'31"S, 49°05'39"W, 28 Feb 2008, *Pereira 126* (CEN!); **Paranã:** Canteiro da obra do UHE São Salvador, área de empréstimo, 14°04'17" S, 49°03'53" W, 21 October 2006, *Pereira-Silva 10985* (CEN!, HUEFS); Fazenda Petrolina, 14°06'03"S, 48°05'59"W, 14 Sep 2003, *Sevilha & Moreira 3570* (CEN!, HUEFS).

Additional specimens examined.— BOLIVIA. Cochabamba: **Santa Cruz:** San Ignacio-Conceicion, km 5, 18°07' S, 60°04' W, 30 October 1977, *C. Evrard, C.* 8415 (F [photo!], NY [photo!]).

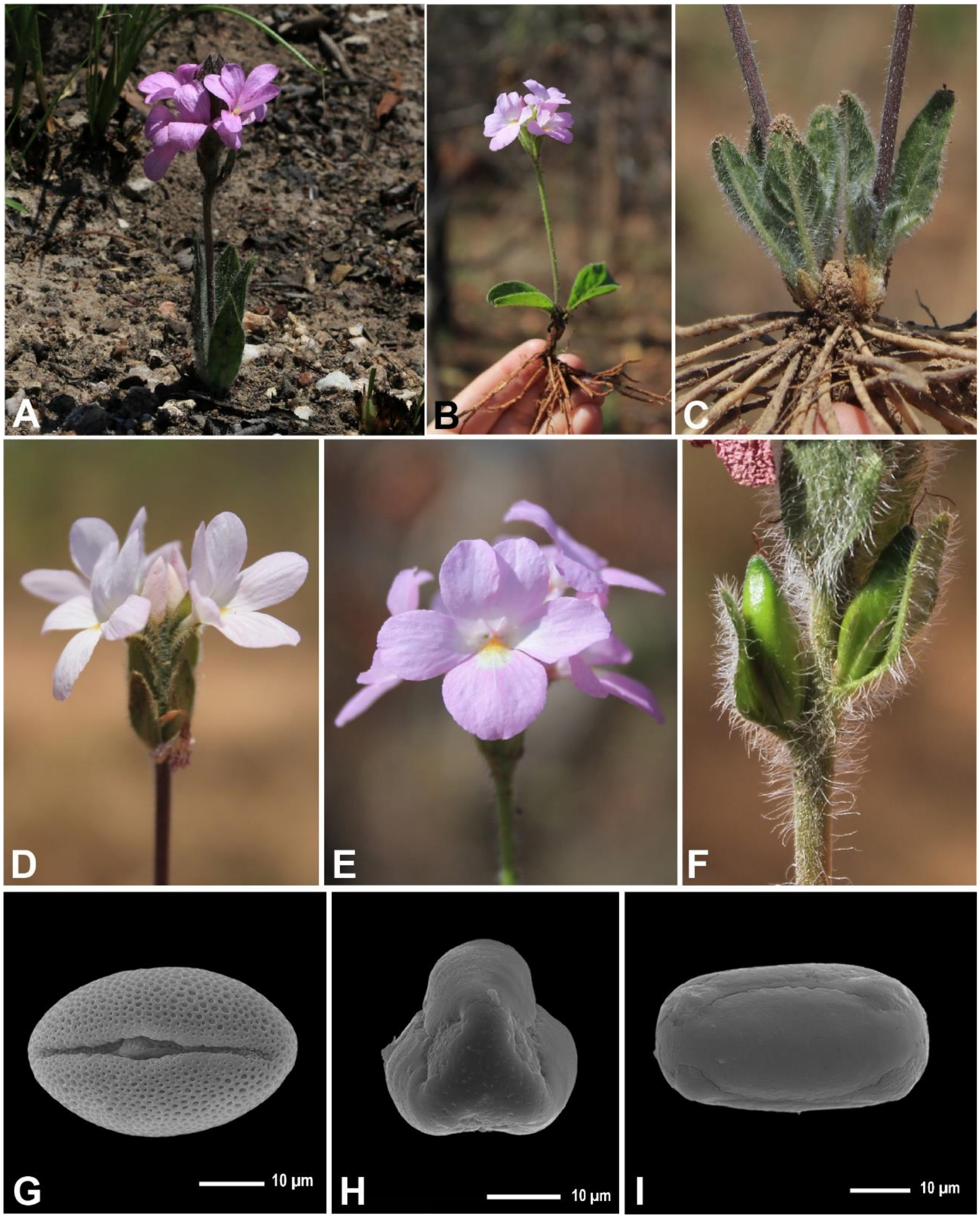


Figure 16. Field and SEM images of *Stenandrium pohlii.* A. Habit and habitat (photo by H. Moreira). B. Plant with roots. C. Clustered leaves. D. Spike with ovate bracts. E. Subactinomorphic corolla. F. Immature fruits. G-I. SEM images of tricolpate pollen grains.

10. *Stenandrium riedelianum* Nees in Mart. Fl. Bras. 9: 75. 1847 – **Lectotype (designated here):** BRAZIL. Mato Grosso, in umbrosis ad margines sylvarum montinum Serra da Chapada, Sep, *L. Riedel 58* (GZU [photo!]).

= *Stenandrium goiasense* Wassh. in Brittonia 42: 4. 1990 - Holotype: BRAZIL. Goiás, Rodovia BR-020, 10 km N of Alvorada do Norte, 8 Oct 1976, *G. Hatschbach 39009* (MBM [photo!]; isotypes: NY!, US [photo!]). *syn. nov.*

= *Stenandrium praecox* S.Moore in Trans. Linn. Soc. London Bot. 4: 426. 1895 – **Lectotype (designated here):** BRAZIL. Mato Grosso, in sylva juxta Santa Cruz, Sep, *S.M. Moore 293* (BM [photo!]). *syn. nov.*

Acaulescent perennial to 40 cm tall with whitish, yellowish to ferruginous trichomes on most vegetative structures; rhizome vertical, up to 8.5 mm thick, roots up to 22 cm long. **Leaves** concolor or slightly discolor, clustered and appearing whorled, subsessile to petiolate, petioles (naked portion) 4.2-25 mm long, puberulent to pubescent with trichomes up to 0.5 mm long, rarely with 2.5 mm long, blades oblanceolate, elliptic or obovate, 13.7-237 mm long, 11-73.3 mm wide, 1-3.5 times longer than wide, herbaceous to chartaceous, gradually narrowed and attenuate at base, obtuse to rounded at apex, surfaces glabrous to pubescent with trichomes up to 1.3 mm long, margins plane, entire to sinuate, rarely crenate, ciliate with trichomes up to 0.6 mm long. **Inflorescence** a long pedunculate floral scape 70.3-375 mm long (including peduncle, excluding flowers), peduncles 38.7-200 mm long, glabrous to pubescent with trichomes up to 1 mm long, rarely 2.5 mm long. **Flowers** lax,alternate to opposite along the rachis. **Bracts** lanceolate, subulate, or ovate, sometimes conduplicate, 2.2-9.5 mm long, 1.3-2 mm wide, conspicually to unsconspicually 3-veined, surfaces glabrous to tomentose with trichomes up to 1 mm long, rarely 2.5 mm long, margins plane, entire, ciliate with trichomes up to 1 mm long, rarely 2.5 mm long. **Bractlets** lanceolate to subulate, 0.5-1.9 mm long, 0.3-0.7 mm wide, 1-veined, pubescent with trichomes up to 0.3 mm long, margins entire, ciliate with trichomes up to 0.3 mm long. **Calyx** with subequal lanceolate to subulate lobes, lobes appearing 3-5-veined, 1.6-4.7 mm long, 0.6-1.1 mm wide, puberulent to pubescent with trichomes up to 0.2 mm long, margins entire, ciliate with trichomes up to 0.2 mm long. **Corolla** white, light pink to lilac, the lower lip yellow to orange coloured at the base of the central lobe, subactinomorphic, tube glabrous, 4-7.5 mm long, 1.5-1.9 mm wide, upper lip 7.5-11 mm long, 7.1-12 mm wide with equal lobes, lower lip 10.6-14 mm long, 14.4-22.5 mm wide with subequal lobes, lower-central lobe tomentose at the base internally with trichomes up to 0.6 mm long. **Stamens** 1.3-1.8 mm long, anthers 0.8-1.2 mm long, pubescent with trichomes up to 0.3 mm long; staminode 0.6 mm long; pollen grains prolate, tricolpate, 33.4-49.1 × 19.5-31.6 µm, exine psilate, fossulate or perforate, colpus with smooth to gemmate membrane. **Style** 4.7-7.2 mm long, pubescent with minutes trichomes up to 0.1 mm long, stigma asymmetrically or symmetrically funnelform. **Capsule** glabrous, 5-18 mm long, 3.4-6 mm wide. **Seeds** 2.3-3 mm long, 2-2.3 mm wide, the surface pubescent to tomentose with barbed trichomes up to 0.5 mm long. **Figure 17.**

Phenology. – Flowering and fruiting specimens were often collected from September to January.

Distribution and habitat.— *Stenandrium riedelianum* is a relatively widespread species, from dry forests and pastures in Goiás, Maranhão, Mato Grosso, and Minas Gerais, but it is also rarely found in the northern Distrito Federal. It is endemic to Brazil, there are no records from neighbouring countries.

Conservation status— One of the synonyms of *S. riedelianum*, *S. goiasense,* was not officially assessed by CNCFlora because is was deemed data deficient (DD; IUCN 2017). The synonymization proposed in this study increased the number of georreferentiable collections and consequently made possible the assessment of this species. The EOO of *S. riedelianum* is 1,268,101 km^2 and the AOO is 88 km^2. Therefore, based on both geographic criteria B1a+B2a, *S. riedelianum* is provisionally assessed as least concearn (LC; IUCN 2017). *Stenandrium goiasense* was included as one of the rare species of Brazil by Giulietti et al. (2009) but disappears when included within *S. riedelianum*. As far as we are aware, it has not been recorded in any protected area.

Notes— *Stenandrium riedelianum* is morphologically most similar to *S. irwinii* but differs from the latter species by its non-cartilaginous leaf margins (cartilaginous in *S. irwinii*), bracts 1.3-2 mm wide (2.5-4.6 mm wide in *S. irwinii*), and corolla tube 4-7.5 mm long (8-12.3 mm long in *S. irwinii*). It was not possible to define consistent limits between *S. riedelianum*, *S. goiasense* and *S. praecox*, so the two latter names are proposed as synonyms of the former, which is the oldest name for this species. Moore (1895) distinguished *S. praecox* from *S. riedelianum* by its larger flowers and bracts. Wasshausen (1990) described *S. goiasense* and distinguished it from *S. praecox* mainly by its subulate, conspicuously 3-nerved bracts (bracts were said to be lanceolate in *S. praecox*), and larger size of the corolla lobes. However, the wide range of material now available has shown that there is a gradient of floral and bract shape and dimensions that is neither correlated with geography nor with pollen morphology. The differences exist but seem to result from intraspecific polymorphism.

Specimens examined. – BRAZIL. Distrito Federal: **Brasilia:** APA da Cafuringa. Campo úmido inundado no período das chuvas, 15°27′27″S, 47°58′00″W, 11 Oct 2006, *A.C. Amaral & G. Pereira-Silva 34* (CEN!). Goiás: [Goyaz], Plantes recuiellies sur le plateau de la province de Goyaz, 1894-95, *A. Glaziou 21873* (G [photo!]); **Alto Horizonte:** Região da Sururuca, fazenda Cajás, proprietário senhor Jair Eustáquio, 14°34'38"S, 49°49'27"W, 3 Oct 2014, *Faria 4125* (HUEG!, K, R, RB!, SP!, UB!); Região da Sururuca, fazenda Cajás, proprietário senhor Jair Eustáquio, 14°34'38" S, 49°49'27" W, 23 Nov 2015, *Faria 5198* (H, HUE, HUEG!, RB!, SP!, SPF!, UB!); **Barro Alto:** Área da mineradora Anglo American Brasil, 15°06'05"S, 49°01'33"W, 28 Oct 2008, *Aquino et al. 465* (CEN!); Collected from the vicinity of the mine works about 22 km SSW of B.A. on the dirt road (or 30 km via the sealed higway), 15°28'52"S, 49°06'44"W, 20 Jan 2005, *Reeves 2847* (CEN!); **Caiapônia:** Ca. 35 km. S. of Caiapônia on rd. to Jataí, Serra do Caiapó, 18°02'25"S, 53°02'25"W, 29 Oct 1964, *Irwin 7562* (NY!); **Campos Belos:** 24 Oct 1965, *Duarte 9492* (RB!, UB!); **Guarani de Goiás:** Fazenda Forquilha, proprietário Sr. Virgilato Francisco Neto, 14°57'38"S, 47°47'59"W, 18 Oct 2001, *Mendonça 4481* (CEN!); **Iaciara:** Fazenda Sabonete, 14°03’55”S, 46°29’13”W, 17 Oct 2001, *M.L. Fonseca et al. 2919* (SPF!); Km 15 da estrada Posse/Iaciara, 14°10’S, 46°30’W, 20 Oct 1995, *B.A.S.Pereira & D. Alvarenga 2906* (CESJ!, UB!); **Formosa:** Rod. BR-020, prox. Rio Macacos, 7 Oct 1976, *G. Hatschbach 39005* (C!, HCF!, INPA! MBM [photo!], NY!); Fazenda Araras, 15°24’33.6”S, 47°09’07.8” W, 31 Oct 2016, *M.R.V. Zanatta & A.L.C. Moreira 2451* (UB!); **Niquelândia:** Mineradora Anglo American Brasil Ltda. Antiga CODEMIN, 40 Km norte da cidade, Localidade Cava Fruta de Lobo ca.6 Km da Sede, 14°38'37"S, 49°39'11"W, 14 Nov 2007, *Aparecida da Silva 6230* (RB!). Maranhão: **Balsas:** 14 Nov 2017, *Rômulo & Barbosa IFN 226* (UB!); **Tuntum:** Approx. 17 km N of Tuntum on MA 259, toward Barra do Corda, 5°7’S, 44°40’W, 1 Jan 1980, *D.C.*

Daly et al. D590 (HRB!, NY!). Mato Grosso: Ca. 90 km. N. of Xavantina, 12 Oct 1964, Irwin 6789 (NY!); **Cáceres:** Reserva da EMPA, Margens do Rio Paraná, 29 Sep 1988, *Nadruz 404* (RB!); **Nobres:** 5km S pela Rod. BR-163, 22 Oct 1995, *Hatschbach et al. 63991* (MBM [photo!]); **Nova Xavantina:** Olaria. Bar Village Ridge transect, 24 Sep 1967, *Argent et al. 6496* (MO, NY!, UB!). Minas Gerais: *Saint-Hilaire s.n.* (K!, P!); Fazenda da Piedade et Tamburil, 1814-1831, *F. Sellow 193* (BR [photo!], F [photo!], HAL [photo]!, K!); **Buenópolis:** 7 km ao sul da cidade, na estrada BR 135 para Curvelo, [19°32'07"S, 44°42'07"W], 11 Oct 1988, *Harley et al. 24834* (K!, NY!, SPF!); **Congonhas do Campo:** 5 Sep 1879, *Glaziou 13079* (C! ,G [photo!], K!, P!, R); **Santo Hipólito:** 5 km além de Santo Hipólito em direção a Monjolos, no antigo leito da estrada de ferro, [18°29'21"S, 44°18'50"W], 3 Oct 1997, *Mello-Silva et al. 1528* (MBM [photo!], NY!, SPF!, UB!).

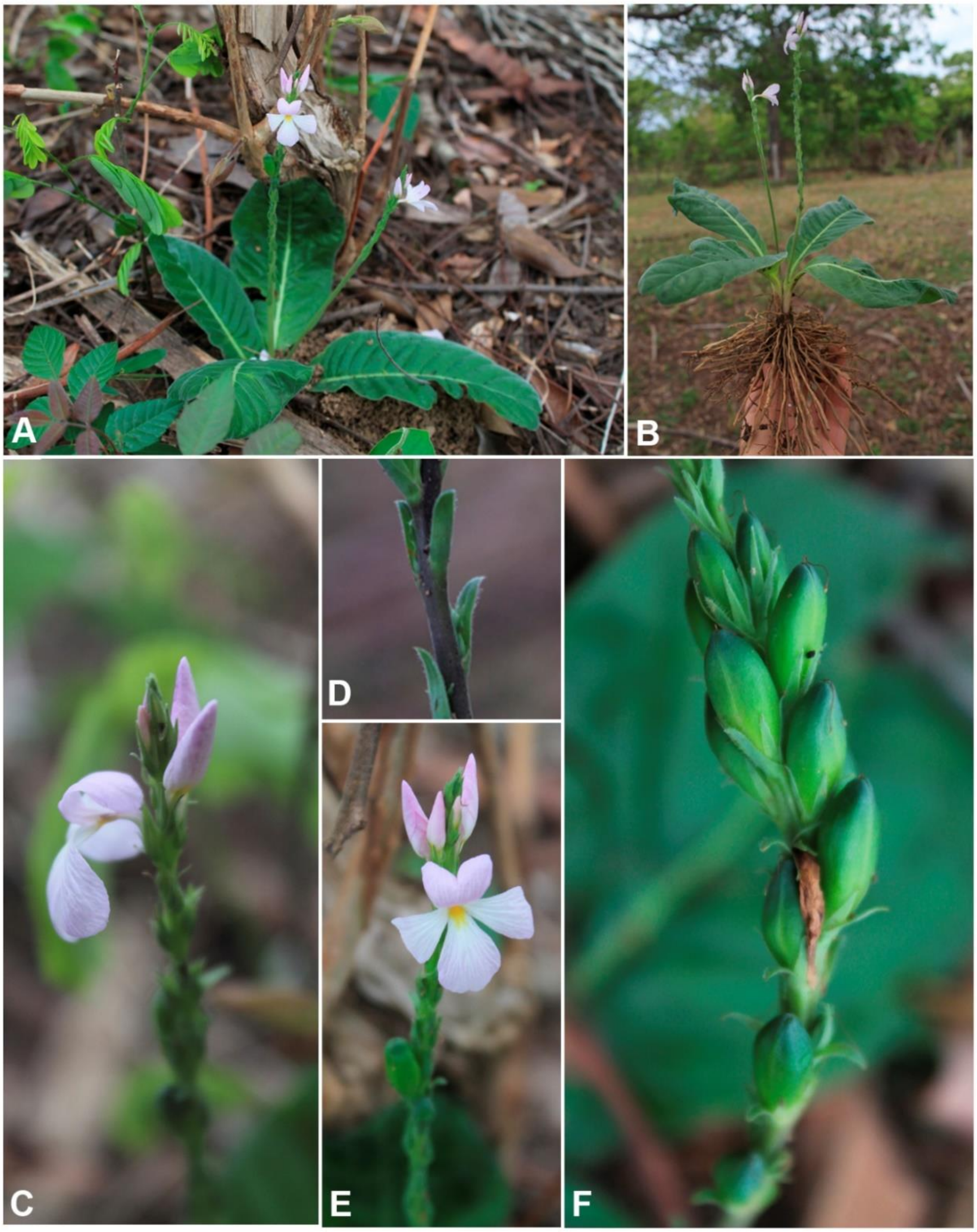


Figure 17. Field images of *Stenandrium riedelianum* (M.R.V. Zanatta & A.L.C. Moreira 2451). A. Habit and habitat. B. Plant with roots. C. Detail of the bud. D. Bracts. E. Subactinomorphic corolla. F. Spike with bracts and fruits. Photos by M.R.V. Zanatta.

11. ***Stenandrium serpens*** **Nees** in Mart. Fl. Bras. 9: 75. 1847 – **Lectotype (designated here):** BRAZIL. Bahia: In sylvis ad Porto Seguro, *M.P. Wied-Neuwied s.n.* (BR [photo!]; isolectotypes: GOET [photo!], GZU [photo!], MEL! [photo!]).

Prostrate, unbranched caulescent perennial to 30 cm tall glabrous to puberulent with trichomes varying in size on most vegetative structures; rhizome horizontal, 2.3-3.5- mm thick, roots to 8 cm long, stems glabrous to puberulent with minute trichomes up to 0.3 mm long. **Leaves** concolor, opposite, petiolate, petioles 1.8-5 mm long, puberulent to pubescent with trichomes up to 0.4 mm long, blades narrowly ovate to ovate, 28.6-86.7 mm long, 11.6-28.4 mm wide, 2-3 times longer than wide, chartaceous, obtuse to retuse at apex, adaxial surface glabrous to sparsely puberulent with trichomes up to 0.4 mm long, trichomes often confined to the veins, abaxial surface puberulent with trichomes up to 0.4 mm long, margins revolute, entire, glabrous to sparsely ciliate with trichomes up to 0.5 mm long. **Inflorescence** a sessile, terminal or axillary spike 17.2-67 mm long (excluding flowers), rachis quadrangular pubescent with trichomes up to 0,2 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** lanceolate, 6-6.7 mm long, 0.8-1.1 mm wide, 1-veined, sparsely pubescent with trichomes up to 0.6 mm long on both surfaces, margins entire, ciliate with trichomes up to 0.3 mm long. **Bractlets** lanceolate, 3.6-3.7 mm long, 0.5 mm wide, appearing 1-veined to the naked eye, with two other weak additional veins visible under magnification, glabrous to puberulent on both surfaces with trichomes up to 0.4 mm, margins entire. **Calyx** with subequal lanceolate lobes, lobes 3-veined, 4-4.6 mm long, 0.4-0.5 mm wide, glabrous to puberulent with trichomes up to 0.3 mm on both surfaces, margins entire. **Corolla** white, zygomorphic, tube glabrous 3.5 mm long, 1.1 mm wide, upper lip 4.2 mm long, 2 mm wide with equal lobes, lower lip 6 mm long, 11 mm wide with subequal lobes, lower-central lobe tomentose at the base internally with trichomes up to 0.3 mm long. **Stamens** 1-1.1 mm long, anthers 0.7 mm long, pubescent with trichomes up to 0.2 mm long; staminode not seen; pollen grains prolate, tricolpate, 29.9-33.8 × 17.5-20.5 µm, exine fossulate, colpus with gemmate membrane. **Style** 2.6 mm long, puberulent with trichomes up to 0.3 mm long, stigma asymmetrically funnelform. **Capsule** glabrous, 6.3 mm long, 2 mm wide. **Seeds** not seen. **Figure 18.**

***Phenology.*—** Flowering specimens were collected in July, September, October and December. There are no fruits within the analysed specimens.

***Distribution and habitat.*—** *Stenandrium serpens* is known from the northern Brazilian ombrophilous forests, in Bahia, Paraíba and Pernambuco states. During this study, two unsuccessfully field expeditions were carried out to the southern Bahia to collect this species.

Conservation status.— The EOO of *S. serpens* is 74,412 km² and the AOO is 20 km². Based on the geographic criteria B2a, *S. serpens* is provisionally assessed as endangered (EN; IUCN 2017). Fortunately, it was recently recorded in two protected areas: RPPN Serra do Teimoso (Bahia) and APA do Roncador (Paraíba).

Notes.— This species is morphologically most similar to *S. tenellum*, but differs mainly by the shape of the bracts and the collor of the corollas. The bracts of *S tenellum* are ovate with toothed apex and the corollas are yellow. The bracts of *S. mandioccanum* are obovate to oblanceolate with apex rounded to retuse and the corollas are white to violet.

Specimens examined: BRASIL. Bahia: **Ilhéus:** Feira de Santana - Ilhéus, Rodovia 001, Mata depois do Rio de Contas, 15°20'38"S, 39°15'12"W, 8 Oct 2010, *Côrtes et al. 270* (HUEFS [photo!]); **Jussari**: Rod. Jussari/Palmira, entrada ca. 7,5km de Jussari, Fazenda Teimoso, RPPN Serra do Teimoso, 15°09'29"S, 39°31'43"W, 20 Jul 1998, *Paixão et al. 54* (RB!). Paraíba: **Alagoa Nova:** Brejo Paraibano, Mata do Urucu, Beira da Estrada, 7°12'24"S, 36°33'09"W, 12 Dec 2011, *Melo 10851* (HUEFS [photo!]); **Bananeiras:** Brejo Paraibano, APA do Roncador, Cachoeira do Roncador, 6°46'48"S, 35°33'13"W, 11 Dec 2011, *Melo et al. 10844* (HUEFS [photo!]). Pernambuco: **São Lourenço da Mata:** Mata do Toró-Cuieira, 30 Sep 1980, *Andrade-Lima & Correia Lima 1* (IPA, SPF!).

25904 IPA — HERBARIUM

Fam. Acanthaceae
Sp. Stenandrium serpens.
Var.
N. V.
Habit. PE - São Lourenço da Mata. Mata do Toró-Cuieira. Na entrada da mata. Solo areno-argiloso.
Leg. Andrade-Lima et Correia Lima 01
30/09/1980
Det.
Obs. Erva rastejante. Flores alvas, pequenas.

Figure 18. Image of a herbarium specimen of *Stenandrium serpens* from SPF (Andrade-Lima & C. Lima 1).

12. ***Stenandrium stenophyllum*** **Kameyama** in Novon 6: 268. 1996 – Holotype: Brazil. Minas Gerais: Grão-Mogol, Estrada para Cristália a ca. 800 m da ponte sobre o rio Itacambiruçu, ca. 750 m alt., 16°36'S, 42°55'W, 14 Jun 1990, *A.A. Oliveira, D.C. Zappi, J.R. Pirani & R. Simão-Bianchini CFCR 12982* (SPF!; isotypes: K!, MBM [photo!], RB!, US barcode US01013651 [photo!], US00512895 [photo!]).

Erect, branched caulescent perennial to 40c m tall, glabrous to pubescent on all vegetative structures, trichomes varying in size; rhizome not seen, roots not seen; stems terete, pubescent with trichomes up to 0.6 mm long, old stems almost glabrous. **Leaves** verticillate, subsessile, petioles up to 0.7 mm long, pubescent with trichomes up to 0.5 mm long, blades linear to narrowly oblong 14-62.2 × 1.6-3.6 mm, 8-28 times longer than wide, coriaceous, rounded to emarginate at apex, adaxial surface glabrous to sparsely puberulent with minute trichomes up to 0.2 mm long, abaxial surface glabrous to pubescent with trichomes sometimes confined to the veins up to 0.2 mm long, margins strongly revolute. **Inflorescence** a short-pedunculate to sessile terminal or axillary spike 8.5-18.6 mm long (including peduncles, excluding flowers) often concentrated at the subterminal nodes, peduncles 1.3-2.9 mm long, puberulent to pubescent with trichomes up to 0.4 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** linear to lanceolate, 4.1-7.2 mm long, 0.6-1.1 mm wide, appearing 1-veined to the naked eye, with two other weak additional veins visible under magnification, the central vein darker than the others, puberulent with minute trichomes up to 0.1 mm long on both surfaces, margins entire, ciliate with minute trichomes up to 0.1 mm long. **Bractlets** lanceolate,conduplicate, 2.2 mm long, 0.6 mm wide, 1-veined, puberulent with trichomes up to 0.1 mm long, margins ciliate with trichomes up to 0.1 mm. **Calyx** with subequal lanceolate lobes, glabrous to sparsely pubescent, lobes 3-veined, 4-4.7 mm long, 0.9-1 mm wide. **Corolla** with the upper lip wine-colored and the lower lip pale lilac to white, zygomorphic, tube 4.4-7 mm long, 1.27-1.5 mm wide, upper lip 3.7 mm long, 2.1 mm wide with equal lobes, lower lip 4.2 mm long, 4.4 mm wide with subequal lobes, lower central lobe pubescent at the base internally with trichomes 0.3 mm. **Stamens** 1.4 mm long, anthers 0.9 mm long, tomentose at tip with trichomes up to 0.1 mm long, staminode 0.2 mm long; pollen grains subprolate, tricolpate, sometimes the exine irregularly covering the colpus, 33.6-38.3 × 27.7-29.8 μm, exine fossulate perforate, colpus with gemmate membrane. **Style** 4.6 mm long, sparsely puberulent with trichomes up to 0.1 mm, stigma asymmetrically funnelform. **Capsule** not seen. **Seeds** not seen. **Figure 19.**

Phenology. – Flowering and fruiting specimens were collected in June and December.

Distribution and habitat.— As far as we are aware, *S. stenophyllum* has only been collected twice and is presumed to be endemic to the Northern Serra do Espinhaço, Grão Mogol. It occurs in rocky *cerrado* areas, in the same type of vegetation and locality as *S. hatschbachii.*

Conservation status. — *Stenandrium stenophyllum* was officially assessed as EN (endangered, IUCN 2017), based on its EOO (427.4 km^2), using the geographic criteria B1ab(iii) (Martinelli & Moraes, 2013). Therefore, it is currently included in the "Lista Oficial de Espécies da Flora Brasileira Ameaçadas de Extinção", the Brazilian official red list (MMA 2014). The species is lacking recent colletions (the last known collection was made 29 years ago) and, as far as we are aware, it was not recorded in any protected area. *Stenandrium stenphyllum* is also included as one of the rare species of Brazil by Giulietti et al. (2009).

Notes.— *Stenandrium stenophyllum* can be distinguished from all other *Stenandrium* species by its verticillate leaves with linear blades. It is closely morphologically most similar to *S. hatschbachii* but the latter species presents opposite leaves with ovate to lanceolate blades.

Specimen examined. – BRAZIL. Minas Gerais: **Grão Mogol:** Rio Itacambiruçu, ao longo da estrada para Cristália, 16°30'S, 42°55'W, 10 Dec 1989, *Pirani et al. CFCR 12420* (SPF!).

Figure 19. Image of one of the isotypes of *Stenandrium stenophyllum* from K (A.A. Oliveira et al. 12982).

13. ***Stenandrium tenellum*** **Nees** in Mart. Fl. Bras. 9: 77. 1847 – **Lectotype (designated here):** BRAZIL. Rio Grande do Sul, Porto Alegre, Frequens in sylvis altis in Serra do S. Antonio, Oct 1814-1831, *F. Sellow s.n.* (GZU [photo!]; isolectotypes: K barcode K000484047!, K000484046!, BR [photo!], F [photo!], HAL [photo!], W No. 0056700 [photo!], W-Rchb. 1889-0095769 [photo!]).

Prostrate, rarely erect, unbranched caulescent to 20 cm tall with whitish to yellowish trichomes varying in density on most vegetative structures; stems tomentose with trichomes up to 1 mm long, rhizome horizontal, 1-2 mm thick, roots up to 8 cm long. **Leaves** discolor, opposite, petiolate, petioles 8.7-22.3 mm long, tomentose to pubescent with trichomes up to 0.7 mm long, blades widely ovate to narrowly ovate, rarely lanceolate to obovate 30-73.5 mm long, 14.4-40.1 mm wide, 1.5-3 times longer than wide, membranaceous to chartaceous, narrowed and attenuate at base, rounded to acute at apex, adaxial surface glabrous with very sparse trichomes up to 0.5 mm long and sometimes pubescent on the veins with trichomes up to 0.8 mm long, abaxial surface puberulent with sparse trichomes up to 0.5 mm long, more concentrated on the veins, margins entire, glabrous to ciliate with trichomes up to 0.8 mm long. **Inflorescence** a pedunculate, terminal reduced spike 14.6-32.5 mm long (including peduncle, excluding flowers), peduncles 2-5.6 mm long, tomentose with trichomes up to 1 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** ovate, 13.2-15.1 mm long, 8.4-9.7 mm wide, mucronulate, 5-veined, glabrous to sparsely puberulent with trichomes up to 0.3 mm long on both surfaces, margins toothed, often entire with a few tooths near the apex and rarely lacking tooths, ciliate with trichomes up to 1.5 mm long. **Bractlets** lanceolate, 5-6 mm long, 0.5-1.1 mm wide, appearing 1-veined to the naked eye, with two other weak additional veins visible under magnification, glabrous, margins entire. **Calyx** with subequal lanceolate lobes, lobes 3-veined, 7.1-7.6 mm long, 0.9-1.6 mm wide, glabrous, margins entire. **Corolla** yellow, zygomorphic, tube glabrous 12-13.7 mm long, 7-7.2 mm wide, upper lip 6.8 mm long, 8.6 mm wide with equal lobes, lower lip 9.4 mm long, 18.7 mm wide with subequal lobes. **Stamens** 4-5.9 mm long, anthers 1.6-1.7 mm long, glabrous; staminode absent; pollen grains prolate, tricolpate, 29.3-32.7 × 18.6-21.3 µm, exine fossulate perforate, colpus with granulate membrane. **Style** 6.7 mm long, with a tuft of trichomes up to 0.5 mm long near the stigma, stigma asymmetrically funnelform. **Capsule** not seen. **Seeds** not seen. **Figure 20.**

Phenology — Flowering specimens were collected often from September to January, and rarely on April. There are no fruits within the analysed specimens.

Distribution and habitat.— *Stenandrium tenellum* is known from the southern Brazilian ombrophilous forests, in Paraná, Santa Catarina, São Paulo and Rio Grande do Sul states.

Conservation status.— The EOO of *S. tenellum* is 110,987 km^2 and the AOO is 56 km^2. Based on the geographic criteria B1a+B2a *S. tenellum* is provisionally assessed as least concerned (LC; IUCN 2017). Besides, it was recorded in several protected areas: APA Municipal de Caraá (Rio Grande do Sul). Parque Estadual Carlos Botelho (São Paulo), Parque Nacional do Iguaçú (Paraná), Parque Nacional da Serra Geral (Santa Catarina), and Parque Nacional do Superagui (Paraná).

Notes— *Stenandrium tenellum* is easly distinguished from *S. mandioccanum*, the most similar species, by its yellow corollas (white to lilac in *S. mandioccanum*) and the toothed bracts (entire in *S. mandioccanum*). It is the only yellow flowered *Stenandrium* in Brazil and one of the few in the Neotropics. Its large corolla tube is also unique among the Brazilian *Stenandrium*. *Stenandrium tenellum* is one of the most beautiful *Stenandrium* species and for this reason it is highly recommended to be cultivated as ornamental plant.

Selected specimens examined. – BRASIL. Paraná: **Guaraqueçaba:** Rio do Cedro, [25°23'32"S, 48°31'53"W], 7 Jan 1970, *Hatschbach 23305* (MBM [photo!]); Rio Grande do Sul: **Caraá:** APA Municipal de Caraá, 30°19'35"S, 50°43'23"W, 3 Sep 2008, *Senna et al. 1457* (HAS!); **Marcelino Ramos:** Coxilha Seca, [27°53'05"S, 52°20'09"W], 12 Oct 1998, *Jarenkow 3850* (FLOR [photo!], ICN [photo!], MBM [photo!]); **Morrinhos do Sul**, Tajuva, [29°22'47"S, 49°58'39"W], Oct 1995, *Sobral & Miró 7939* (ICN [photo!], MBM [photo!]); Perdida, 29°22'47"S, 49°58'39"W, 8 Oct

2011, *Verdi & Durigon 6031* (FURB!); **Riozinho:** Conduto, 30°04'18"S, 50°45'59"W, 2 Oct 2003, *De Marchi 132* (ICN); **São Francisco de Paula**: Na estrada para Canela, perto da encruzilhada, 13 Dec 1978, *Mattos et al. 19107* (HAS!); **Torres:** Morro Azul, [29°36'39"S, 50°18'18"W], 23 Sep 1977, *Citadini 251* (ICN [photo!]); Tajuva, [29°36'22"S, 50°19'43"W], 18 Oct 1990, *Jarenkow & Waechter 1748* (FLOR [photo!], MBM [photo!]); Torres, Perdida, [29°36'10"S, 50°19'32"W], 1 Oct 1992, *Jarenkow 2144* (FLOR [photo!], MBM [photo!]); Santa Catarina: **Alfredo Wagner**: [28°09'59"S, 49°33'21"W], 16 Oct 2004, *Hatschbach et al. 78082* (MBM [photo!]); **Jacinto Machado:** Cará, 29°27'32"S, 51°35'36"W, 26 Sep 2009, *Verdi et al. 2757* (FURB!, RB!); **Morro Grande:** Três Barras, 28°42'36"S, 49°46'12"W, 23 Nov 2009, *Verdi et al. 3112* (FURB!); **Santo Amaro da Imperatriz:** Parque Estadual da Serra do Mar, Reserva Florestal dos Pilões, [28°18'55"S, 49°18'49"W], 30 Nov 1950, *Duarte & Falcão 3185* (RB!, UB!); **Uruguai**: Piratuba, Vila Rica, by Rio do Peixe, 24 Oct 1964, *Smith & Reitz 12926* (G, K!, NY, W). São Paulo: **Pariquera-Açu:** Propriedade do Sr. Flávio, 25°57'49"S, 49°10'10"W, 29 Apr 2012, *Caxambu 4060* (HCF, MBM [photo!]); **São Miguel Arcanjo:** Parque Estadual Carlos Botelho, Trilha para o Rio Taquaral, 24°03'27"S, 47°59'06"W, 13 Oct 2004, *Kinoshita et al. 224* (UEC!).

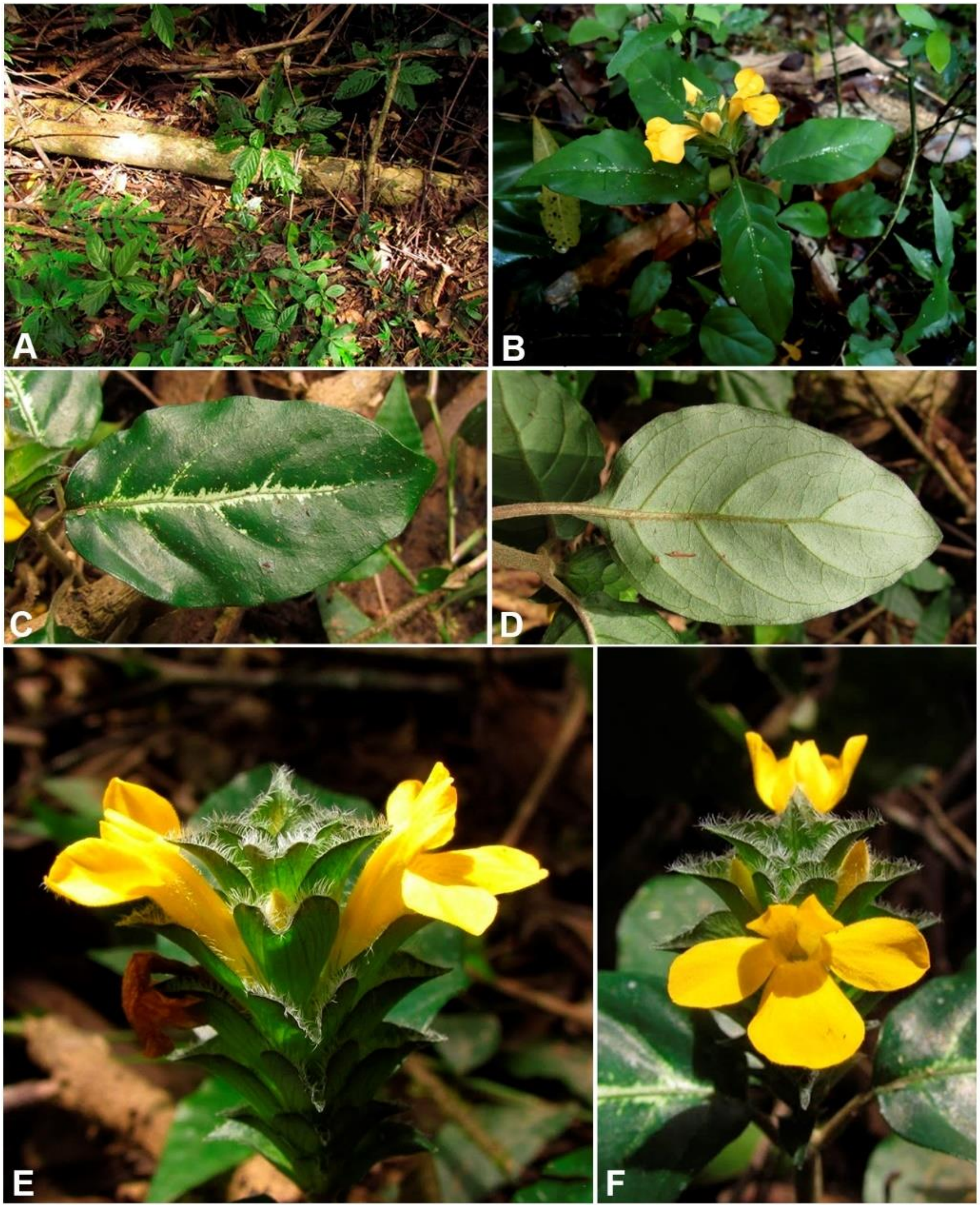


Figure 20. Field images of *Stenandrium tenellum.* A-B. Habit and habitat. C-D. Leaves. E. Terminal spike with bracts, bud, and flowers F. Zygomorphic corolla. Photos by M. Verdi.

14. ***Stenandrium villosum*** **Nees** in Mart. Fl. Bras. 9: 77. 1847 – **Lectotype (designated here):** BRAZIL. Mato Grosso, In siccis Cujabae, Sep, *L. Riedel s.n.* (GZU [photo!]) – **Epitype (here designated)**: PARAGUAY. In regione cursus superioris fluminis Apa., Nov 1901, *E. Hassler 7762* (C!; isoepitypes: NY!, P!, RB!, US [photo!]).

Erect, caulescent perennial to 43 cm tall, villous or hirsute with whitish, yellowish to ferruginous trichomes on most vegetative structures; stems villous with trichomes up to 2.5 mm long, old stems almost glabrous, rhizome and roots not seen. **Leaves** concolor, opposite, subsessile, petioles 1.6-2.6 mm long, villous with trichomes up to 2.5 mm long, blades lanceolate to obovate, 39.5-82.5 mm long, 11.2-25.2 mm wide, 2.3-4.2 times longer than wide, chartaceous, acute to rounded at apex, villous with trichomes up to 3 mm long on both surfaces, margins slightly revolute to plane, cartilaginous, entire, ciliate with trichomes up to 3 mm long. **Inflorescence** a long pedunculate, terminal or axillary spike 64-85.5 mm long (including peduncle, excluding flowers) often concentrated at the subterminal nodes, peduncles 31-65 mm long, hirsute with trichomes up to 3 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** ovate, 8.2-16.7 mm long, 4.4-8 mm wide, 3-veined (the veins conspicuous on abaxial surface, inconspicuous on adaxial surface), adaxial surface almost glabrous, abaxial surface hirsute to sparsely hirsute with trichomes up to 3 mm long, margins slightly revolute, entire, ciliate with trichomes up to 3 mm long. **Bractlets** lanceolate, 1.4-1.5 mm long, 0.3-

0.4 mm wide, 1-veined, glabrous, margins entire. **Calyx** with subequal lanceolate lobes, lobes appearing 3-veined to the naked eye, with two latteral veins visible under magnification, 3.4-4 mm long, 0.8-1 mm wide, glabrous to puberulent with minute trichomes up to 0.1 mm long, margins entire. **Corolla** pink to red purplish, sub-actinomorphic, tube glabrous 7-8 mm long, 1.8-2.1 mm wide, upper lip 8.2 mm long, 9.7 mm wide with equal lobes, lower lip 8.5 mm long, 13.6 mm wide with subequal lobes, lower-central lobe tomentose at the base internally with trichomes up to 0.5 mm long. **Stamens** 1.7-2.2 mm long, anthers 1-1.1 mm long, tomentose at tip and dorse with trichomes up to 0.5 mm long; staminode 1.1 mm long; pollen grains prolate, tricolpate, 39.5-41.7 × 18.7-20.1 μm, exine perforate, colpus with granulate membrane. **Style** 6.6 mm long, stigma asymmetrically funnelform. **Capsule** glabrous, 8.5-10.5 mm long, 3.5-5 mm wide. **Seeds** 1.9-3.5 mm long, 1.6-2.5 mm wide, the surface tomentose with barbed trichomes up to 1 mm. **Figure 21.**

Phenology.— Flowering and fruting specimens were collected on September and October.

Distribution and habitat.— *Stenandrium villosum* occurs in Mato Grosso, and Mato Grosso do Sul in Brazil, and there is one record in Paraguay. It is known from few and old collections on open cerrados.

Conservation status.— The EOO of *S. villosum* is 101,811 km^2 and the AOO is 12 km^2. Based on the geographic criteria B2a, *S. villosum* is provisionally assessed as endangered (EN; IUCN 2017).

Notes.— The holotype, that is a unicate, is a very incomplete specimen. It has a single, incomplete mature leaf, two imature leaves, a loose bract and a loose calyx. Nees himself made a note when describing the species: "*Specimen valde incompletum sive potius speciminum fragmenta". This species is very similar to S. affine* and to *S. hirsutum*; the most reliable characters that distinguish *S. villosum* from these last two species are the bracts that are widely ovate and conspicuously 3-veined on the abaxial surface (bracts lanceolate, sometimes narrowly ovate and inconspicually 3-veined on both surfaces in *S. affine* and *S. hirsutum)*, and the length of the corolla tube that is 7-8 mm (corolla tube up to 4.8 mm long in *S. affine* and *S. hirsutum*). These characters are either absent in the type (there is no corolla) or doubtful (the calyx and bract is loose) in the holotype. Therefore, designating an epitype seems in order. The specimen chosen as epitype (*Hassler 7762* from Paraguay), although not from the same locality as the holotype, is the only available sample of a robust plant with several inflorescences such as is the holotype , i.e., it is the closest match; it is also represented by several duplicates in different herbaria. All other specimens are of small, poorly developed plants with single inflorescences.

Specimens examined.— BRAZIL. Mato Grosso: **Sidrolândia:** Rod. BR 163, 27 Oct 1970, *G. Hatschbach 25267* (MBM [photo!], US [photo!]). Mato Grosso do Sul: **Campo Grande**: Estaca, 10 Sep 1936, *W. Archer & A. Ghert 161* (US [photo!]). **Três Lagoas:** Retiro de Telhas, margem direita do Rio Sucuruí, 22 Oct 1964, *J.C. Gomes 2392* (SP!). PARAGUAI. Sierra de Amambay, 1907, *Hassler 9877* (P!).

Figure 21. Images of *Stenandrium villosum*. A-C. Isolectotype from NY (Hassler 7762). A. Specimen. B. Detail of leaves C. Detail of spike and bracts. D-E. SEM images of the pollen grain (J.U. Santos 473). D. General view. E. Detail of sculpturing.

CONCLUSIONS

Although the Brazilian *Stenandrium* are part of a well supported clade (Chapter 1), their morphological and genetic similarity to the large genus *Aphelandra*, allied to the low level of sampling within this clade in the most recent phylogenetic studies in tribe Acantheae (McDade et al. 2005; McDade et al. 2008; Chapter 1), show the desirability of additional molecular studies. The taxonomic uncertainties, intra-populational morphological variability, and difficulties in species circumscriptions found in this study show how a complete review of Neotropical species of *Stenandrium* is necessary.

It would also be interesting to further investigate if the pollen dimorphism and polyploidy recorded in *Stenandrium dulce* has resulted in gymno-diocey or dioecy. Dioecy is extremely rare in the Lamiales (Maurer 2013) so a confirmed new record would be of great interest. There is some evidence that polyploidy disrupts self-incompatibility and leads to a proliferation of female plants in *Lycium* (Miller and Venable 2000). Detailed molecular and morphological studies as well as experimental crosses between the two pollen morphs would be necessary to resolve this question.

Finally, due to the rarity, beauty and potential medicinal properties of this genus we must conclude by emphasizing that a good understanding of the species is relevant to refine *Red Lists*, for studies of population genetics, programs of longterm population monitoring (particularly for the rare or endangered species), ethnobotanical and pharmaceutical studies, as well as multidisciplinary pratices to stimulate their cultivation.

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APPENDIX I

List of Exsiccatae. Ordered by collector name and number in alphabetical order; the number in parenthesis corresponds to the species number in the monograph.

Abruzzi, M.L. 1615 (3); Allem, A.C. 59 (9); Amaral, A.C. 34 (10); Amaral-Santos, A. 315, 2327 (9); Andrade-Lima 1 (11); Aparecida da Silva, M. 4548, 6230 (10); Aquino, F.G. 465 (10); Argent, G.C.G. 6496 (10); Assis, M.C. 321 (9); Assunção, V.A. 62 (9); Barbosa, E. 980 (8); Barroso, G.M. 666 (9); Beneli, T.M. 35 (8); Beyrich, H.C. s.n. (8); Boldrini, I. 1626 (3); Borges, L.M. 963 (9); Bucci, F. 871 (9); Burchell 6394, 7710, 7994, 8087 (9); Buttura, A. 674 (8); Cabral, E.L. 830 (3); Calago, K. 325 (9); Carneiro, J. 1262 (8); Carvalho, A.M.V. 6520 (5); Catharino, E.L.M. 1110, 1143 (8); Cavalcanti, T.B. 881, 3425 (9); 8366 (5); Caxambu, M.G. 4060 (13); 6766 (8); Cervi, A.C. 3029, 3438, 3944 (8); Citadini, V. 251 (13); Cordeiro, I. CFCR939 (5); 3579 (8); CFCR9993, 44458 (5); Cristóbal, C.L. 2270 (8); César, H.L. s.n. (9); Côrtes, A.L. 270 (11); Daly, D.C. 590 (10); De Marchi, T.C. 132 (13); Duarte, A.P. 195, 1944 (8); 3185 (13); 9492 (10); Dubs, B. 460 (9); Eiten, G. 190 (9); Evrard, C. 8415 (9); Faria, J.E.Q. 94, 94 (9); 3880 (6); 4125 (10); 4307 (9); 4318 (6); 5198 (10); Farney, C. 4795 (8); Ferreira, H.D. 4391 (9); Fonseca, M.L. 1243 (6); 1613 (9); 2919 (10); Forzza, R.C. 7454 (9); Freitas, E. s.n., 576 (3); Freitas, R.I.P. s.n. (9); Gardner, G. 2291, 3415, 4341 (9); Gifford, D.R. G253 (7); Glaziou, A.F.M. 13079, 21873 (10); 21877 (9); Gomes, J.C. 2392 (14); 2453

(9); Grings, M. 529 (13); Hagelund, K. 2831, 4751, 7431, 12254, 12730, 13758 (3); Harley, R.M. 24834 (10); Hassler, E. 7762, 9877 (14); Hatschbach, G. 9910, 14329, 16933, 16966, 17084 (8); 17415 (3); 20896 (13); 23128 (3); 23176 (8); 23305 (13); 25267 (14); 25543 (8); 27190 (3); 35120 (8); 37426 (6); 39005, 39009 (10); 41518, 41640 (5); 43994, 50632 (8); 53644 (3); 63447 (9); 63991 (10); 65328 (8); 65668 (6); 67995 (5); 74038 (9); 74115 (3); 76155 (8); 76257, 76547 (3); 78082 (13); s.n. (10); s.n. (5); Heringer, E.P. 8735, 16914, 17901 (9); Hoehne, F.C. 39549 (3); Hoehne, W. 15439 (8); IFN Rômulo & Barbosa 226 (10); Irgang, B.E. s.n., HAS50485 (3); Irwin, H.S. 6789 (10); 6867 (9); 7053 (6); 7562 (10); 9082, 9168, 9266 (9); 9272 (7); 9785, 10749 (9); Jarenkow, J.A. 1748, 2144, 3850 (13); Kalago, K. 325 (9); Kinoshita, L.S. 224 (13); Kozera, C. 2362 (3); Kuhlmann, J.G. 1402 (1); Kuhn, E. 27 (8); Lima, J.C.M. 127, 128, 129, 130, 131 (9); Lima, L.F. 245 (3); Lizanem s.n. (3); Luschnath, B. s.n. (8); Macedo, M. 1400 (9); Marino, F. 250 (5); Marquete, R. 2706 (6); Martinelli, G. 11294 (5); Martins, K. 111 (9); Mattos, J. 25352 (3); Mello-Silva, R. 1440 (5); 1528 (10); 3108 (8); Melo, E. 10844, 10851 (11); Mendes, H.V. 9 (9); Mendonça, R.C. 2878 (6); 4452 (9); 4481 (10); Ministério da Agricultura - Div. de Pedologia 18326 (9); Miranda, S.C. 1011 (9); Mondin, C. s.n. (13); Moore, S.M. 293 (10); 588 (1); 958 (8); Motta, J.T. 1902 (3); Moura, E.O. IFN1601780 (9); Múlgura de Romero, M.E. 3856 (8); Nadruz, M. 404 (10); Nienstedt, E.F. 190 (9); Oliveira, A.A. CFCR12982 (12); Oliveira, J.S. 7 (9); Pachêco, B. 9 (10); Paixão, J.L. 54 (11); Pastore, J.F.B. 1084 (7); 2312 (9); Paula-Souza,

J. 7405, 7744 (3); 8608 (7); Pereira, B.A.S. 2906 (10); Pereira, J.B. 126 (9); Pereira-Silva, G. 5691, 8047 (9); 8288 (10); 10985, 11026 (9); Pinto, T.M.E.S. 19 (6); Pirani, J.R. CFCR8322, CFCR8498 (5); CFCR12420 (12); Pohl, J.B.E. s.n. (6); s.n. (9); Pott, A. 1468, 1555, 1687, 6540, 10596, 10636 (9); Prance, G.T. 18870 (3); Proença, C.E.B. 5220 (13); Projeto Biodiversidade BP BP175, BP473 (9); Ramos, A.E. 2086 (9); Rapini, A. 1094 (5); Ratter, J.A. R2516, R3690, R4267, R7425 (9); Reeves, R.D. 2847 (10); Reitz, P. .R. 1212 (13); Resende, M.L.F. 2919 (10); Resende, U.M. 914 (9); Rezende, J.M. 149 (9); Ribas, O.S. 5509, 7400 (8); Riedel, L. s.n. (3); s.n., s.n. (9); s.n. (10); s.n. (6); s.n. (14); 63, 788 (8); Rizzo, J.A. 2628 (9); 8400 (7); 8442 (9); Robert, A. 716a (8); Rocha, L.C.F. 60, 61 (8); Roth, L. IPA10284 (3); Saint-Hilaire, A. s.n. (9); s.n. (10); Sano, S.M. 02 (9); Santos, A.A. 315, 2327 (9); Santos, J.U. 473 (1); Sartin, R.D. 121 (7); Scatigna, A.V. 1051 (5); Schinini, A. 23387 (3); Schlindwein, C. s.n. (8); Schneider, A.A. 1747 (3); Schvack 2908 (3); Sello s.n. (10); Sellow, F. s.n. (8); s.n. (3); s.n. (13); s.n., s.n. (3); s.n. (8); s.n. (3); s.n. (8); s.n. (13); s.n., s.n., 85 (3); 193 (10); Senna, R.M. 1457 (13); Setubal, R. 422, 450 (3); Sevilha, A.C. 1865 (10); 3180, 3570 (9); 4154 (10); Sidney 1091 (6); Silva, J.M. 3015 (8); Silva, M.A. 3232 (7); Silva, N.T. 57732 (9); Silva, S.P.C. 674 (9); Silva-Filho, P.J. .S. s.n. (13); Silveira, N. 6034 (3); Simon, M.F. 27 (9); Siniscalchi, C.M. 563 (7); Siqueira, E.L. 1109, 1263 (8); Sirk, H. 126 (6); Smith, L.B. 12926 (13); 13153 (8); Sobral, M. 2876, 7939 (13); Sosa, M.M. 184 (3); Souza, L.F. 2334, 2339, 2596 (3); Souza, V.C. 20136, 20171, 20217 (6); 22525 (4); 25719 (5); Splett,

S. s.n. (9); 281 (7); Sucre, D. 3924 (8); Temponi, L.G. 672, 1195 (8); Trevisan, R. 1014 (3); Tweedie, J. s.n. (3); s.n. (8); Vauthier, A.C. s.n. (9); Verdi, M. 2757, 3112, 6031 (13); Viana, L.C. s.n. (11); Walter, B.M.T. 4522, 4618 (9); Weir, J. s.n. (8); Wendel, C.F. s.n. (8); Wied-Neuwied, M.A.P. s.n. (8); s.n. (11); Wood, J.R.I. 19761 (8); Zanatta, M.R.V. 890 (9); 2421, 2424, 2433, (7); 2434, 2437, 2448 (9); 2451 (10); 2454 (9); 2506 (5).

CAPÍTULO III

Two new rare, endangered species of *Stenandrium* (Acanthaceae: Acantheae) reinforce proposed Centers of Endemism and Key Biodiversity Areas in the Serra do Espinhaço, Brazil

ABSTRACT

We describe and illustrate *Stenandrium diamantinense* and *Stenandrium eustachyum*, two new species from high-altitude rocky grasslands from a region of high endemism in southeastern Brazil. *Stenandrium diamantinense* is differentiated from other congeners by its unique indurate, small leaves with revolute margins. *Stenandrium eustachyum* is most similar to *Stenandrium villosum* Nees, the only congener with which it shares the combination of oval bracts and caulescent habit, it can be distinguished from the latter species by its ovate to widely ovate leaves (vs. lanceolate in *S. villosum*). It differs from other species of *Stenandrium* from the Serra do Espinhaço by its oval (vs. linear in all other regional species) bracts. Photographs of living plants in the field are supplemented by scanning electron micrographs of pollen, seeds, and trichomes. A distribution map and a key to the

Stenandrium species of the southern Serra do Espinhaço are also provided. S*tenandrium diamantinense* is provisionally assessed as endangered and *S. eustachyum* as critically endangered. We also found that the distribution of *Stenandrium* in the Serra do Espinhaço conforms to centers of endemism and Key Biodiversity Areas.

Keywords— Espinhaço Range, high-altitude rocky grassland, Minas Gerais, pollen, SEM.

RESUMO

Nós descrevemos e ilustramos *Stenandrium diamantinense* e *Stenandrium eustachyum*, duas novas espécies de campos rupestres de altitude de uma região de alto endemismo no sudeste do Brasil. *Stenandrium diamantinense* é facilmente diferenciada das outras congêneres por suas exclusivas folhas duras e pequenas, de margens revolutas. *Stenandrium eustachyum* é mais semelhante a *Stenandrium villosum*, a única congênere que compartilha a combinação de brácteas ovais e hábito caulescente, mas se diferencia da última espécie por suas folhas ovadas a amplamente ovadas (vs. lanceoladas em *S. villosum*). Se difere das outras espécies de *Stenandrium* da Serra do Espinhaço por suas brácteas ovais (vs. lineares em todas as outras espécies da região). As imagens das plantas vivas em campo são suplementadas por imagens de pólen, sementes e tricomas obtidas em microscópio eletrônico de varredura. Também fornecemos um mapa de distribuição e uma chave para as espécies de *Stenandrium* da Serra do Espinhaço Sul. *Stenandrium diamantinense* está provisoriamente avaliada como ameaçada de extinção e *S. eustachyum* como criticamente ameaçada. Também encontramos que a distribuição de *Stenandrium* na Serra do Espinhaço está de acordo com os centros de endemismo e Áreas-Chave para a Biodiversidade.

Palavras-chave — Campos rupestres de altitude, endemismo, MEV, Minas Gerais, pólen.

INTRODUCTION

The Serra do Espinhaço in southeastern Brazil is an old climatically buffered infertile landscape (Silveira et al. 2016), which harbors hundreds of endemic species of animals, mycorrhizal fungi and plants, many of the latter microendemics (Braga et al. 2016; Carvalho et al. 2012; Giulietti et al. 1987; Neves et al. 2018; Rapini et al. 2008; Vasconcelos et al. 2008). Its floristic composition accounts for approximately 15% of Brazilian vascular flora (Rapini et al. 2008; Silveira et al. 2016). The entire mountain range has an extent of approximately 1000 km north to south between the states of Bahia and Minas Gerais, with elevations ranging from ca. 800 to 2033m (Giulietti et al. 1987). It is subdivided into the northern Serra do Espinhaço (the northern subdivision of the range; sometimes also referred to as the *Planalto Setentrional*; Saadi 1995) and the southern Serra do Espinhaço (the southern subdivision of the range; sometimes also referred to as the *Planalto Meridional*; Saadi 1995).

The southern Serra do Espinhaço, where the new species that are the subject of this study occur, has a length of approximately 300 km and is located from 17°40′S to 19°40′S and from 43°15′W to 44°W. Climate is tropical humid, with precipitation higher than 1500 mm per year in the eastern part and tropical semi-humid climate with precipitation between 1200 and 1500 mm per year in the western part (Barreto et al. 2013). The vegetation includes forests, savannahs and high-altitude rocky grasslands, the latter of these being dominant (Oliveira and Marquis, 2002). In the southern Serra do Espinhaço, Echternacht et al. (2011) identified six major centers of endemism (grouped into areas 1-10 from north to south) and classified each as either a primary or secondary center of endemism, based on the relationship between those areas in a parsimony analysis of endemism: Grão Mogol (center 1), the Serra do Cabral (center 3), Conceição do Mato Dentro (center 8) and the Southern Mountains Complex (centers 9 and 10) were recognized as secondary centers of endemism; the Diamantina Plateau (centers 4 and 5) and the Serra do Cipó (centers 6 and 7) were considered primary centers of endemism. Their analysis was based on 178 endemic, recently reviewed species in 17 families of ferns and angiosperms, but this sample was phylogenetically biased in favor of the Monocotyledons (48% of the species they listed were in this clade). Among the Acanthaceae, only *Staurogyne* was included in this study. Giulietti et al. (2009) surveyed rare and endemic species in Brazil using broader sampling, including all vascular plant families. They identified 752 KBAs (Key Biodiversity Areas; Eken et al. 2004; Langhammer et al. 2007) in Brazil using specialist validated records. In the Serra do Espinhaço region, 56 KBAs were detected, with Grão Mogol, Serra do Cabral and Diamantina among them. In the Grão Mogol KBA, two species of *Stenandrium* were listed: *Stenandrium hatschbachii* Wassh. and *Stenandrium stenophyllum* Kameyama.

Stenandrium Nees (1836) is a genus of often rare, poorly-studied species of herbs and subshrubs in the tribe Acantheae of the Acanthaceae (Espinar and Ferrucci 1982; Daniel 1984; Wasshausen 1990; Giulietti et al. 2009). Daniel (1984) revised the Mexican species, and Franck and Daniel (2015) provided some taxonomic and nomenclatural notes on species in the West Indies. Vollesen (1992) reviewed the Paleotropical species (from Africa and Madagascar), which had previously been included in genus *Stenandriopsis* S.Moore (1906). Molecular phylogenetic studies have suggested that Neotropical and Paleotropical species of *Stenandrium* pertain to separate lineages (McDade et al. 2005, Tripp and McDade

2014).

During an ongoing revision of the genus in Brazil, two new caulescent species were detected. These species share morphological and environmental similarities with the two other caulescent species that occur in the southern Serra do Espinhaço*:* *S. hatschbachii* and *S. stenophyllum*.

These new species were collected decades ago but neither had been identified as new taxa nor formally described. The average lapse between first collection and species description for the Central Brazilian flora is c. 27 years, but varied from 83 years (for regional species) to 167 years for narrow-endemic species (Cavallin et al. 2016); standard deviation however increased steadily over time, going from 5 years (1775-1799) to 43 years (1975-1999). Small plant stature and small to medium flowers in few-flowered inflorescences are associated with longer than average delays (Cavallin et al. 2016). Thus, it is not surprising that these two species, both with low stature and small flowers, were first collected in 1934 (i.e., *S. diamantinense*; 84 years ago) and in 1988 (i.e., *S. eustachyum;* 31 years ago).

MATERIALS AND METHODS

Images of the new species were sent to researchers who were collecting in the Serra do Espinhaço so that hopefully new collections could be made and included in the study. Morphological descriptions of the new species were based on the analysis of eight specimens, four of *Stenandrium eustachyum* and four of *S. diamantinense*, using terminology previously associated with the genus and family (e.g., Daniel 1984; Ahmad 1978). Images of live flowers in the field were used to differentiate the shape, color and relative position of the upper and lower lips, as their interpretation in descriptions based exclusively on herbarium specimens is sometimes prone to error (e.g., Nees 1847; Wasshausen 1990). Pollen, trichomes and seeds were observed and photographed with a Jeol JSM-7000F Scanning Electron Microscope (SEM). Loans of *Stenandrium* and unidentified shrubby Acanthaceae were requested from the following herbaria: C, DIAM, FURB, G, GB, HAS, HRB, HUEG, IAC, IPA, MA, MIN, MO, NY, and US. Personal visits by the senior author were made to BHCB, CAS, CEN, ESA, HTO, HUTO, K, INPA, P, RB, SP, SPF, UEC, and VIES. Conservation assessments were done following the IUCN categories and criteria (IUCN 2017); geographic distributions and areas were calculated using GeoCAT (2019).

TAXONOMIC TREATMENT

Stenandrium diamantinense Zanatta & Kameyama, sp. nov. TYPE: BRAZIL. Minas Gerais: Diamantina, Margem da estrada para Conselheiro Mata, Campo rupestre de cascalho branco, 18°18′34″S, 43°53′23″W, 1284 m, 14 Jun 2017, fl., *J.E.Q. Faria 7884* (holotype: UB!; isotype: HDJF!).

Erect branched caulescent perennial 10-20 cm tall with unicellular and multicellular eglandular trichomes varying in size on most vegetative structures; rhizome 7-9 mm thick, roots not seen; old stems thickened, with corky bark. **Leaves** opposite, petiolate, petioles 0.5-1.5 mm long, pubescent with trichomes up to 0.3 mm long, blades ovate to narrowly ovate, sometimes almost conduplicate, 7.3-15 mm long, 3.5-6.8 mm wide, 1.7-2.5 times longer than wide, stiff, cordate at base, obtuse to acute at apex, adaxial surface puberulent with minute trichomes up to 0.1 mm and occasionally with a few trichomes 2.5-3.5 mm long, abaxial surface puberulent with minute trichomes up to 0.1 mm, margins revolute, entire, ciliate with trichomes 2.5-3.5 mm long. **Inflorescence** a pedunculate, terminal or axillary reduced spike 10.5-16.5 mm long (including peduncle, excluding flowers) often concentrated at the subterminal nodes, peduncles 1-2.5- mm long, pubescent with minute trichomes up to 0.1 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** linear-lanceolate to lanceolate, 6.5-8.8 mm long, 1.3-1.7 mm wide, 3-veined, puberulent with trichomes up to 0.1 mm long on abaxial surface, glabrous on the adaxial surface, margins entire, sparsely ciliate with trichomes like those of abaxial surface. **Bractlets** lanceolate, 4.9-5.8 mm long, 0.5-0.7 mm wide, 3-veined, glabrous to puberulent, margins ciliate with trichomes up to 0.1 mm long. **Calyx** with subequal lanceolate lobes, glabrous to puberulent, lobes 3-veined, 9-10.3 mm long, 0.8-1.4 mm wide. **Corolla** pink with white striate nectar guides in the middle of the central lobe of the lower lip, subactinomorphic, tube glabrous 9.8-11.7 mm long, 1.6-2 mm wide, upper lip 7.5-9.7 mm long, 8.5-9.1 mm wide with equal lobes, lower lip 9-12.8 mm long, 17.7-18.2 mm wide with subequal lobes, lower-central lobe tomentose at the base internally with trichomes 0.2-0.5 mm and puberulent externally with minute trichomes up to 0.05 mm. **Stamens** 2.5-2.8 mm long, anthers 1.5-1.6 mm long, pubescent along entire length, trichomes up to 0.3 mm long, sometimes concentrated at tip or extending to filaments; staminode 0.7 mm long; pollen grains prolate, tricolpate, 48.2-58.2 × 27.4-31.3 μm, apocolpium psilate and mesocolpium perforate, colpus with smooth membrane. **Style** 11.5-12.5 mm long, the stigma asymmetrically funnelform. **Capsule** not seen. **Seeds** not seen. Figures 1, 2, 6, 7 and 8.

Paratypes— **Brazil**. — MINAS GERAIS: Diamantina, Estrada entre Sopa e São João da Chapada. Campo rupestre. Solo arenoso-pedregoso seco, 18°09’04”S, 43°43’03”W, 1162m, 22 Sep 2017, fl., *G. Antar & D.A. Chaves 1880* (SPF!). Diamantina, Conselheiro Mata, Jun 1934, *A.C. Brade 13477* (RB!). Diamantina, Margem da estrada para Conselheiro Mata ca. de 26,5 km do asfalto, Campo rupestre de cascalho branco, 18°18’34”S, 43°53’23”W, 1284 m, 18 Jun 2017, fl., *J.E.Q. Faria 7910* (HDJF!, CAS!, UB!).

Phenology— Flowering specimens were collected in May, June and September. The fruiting period is not known, but flowers and fruits co-occurring is common in *Stenandrium*.

Distribution and Habitat— Recorded only in high-altitude fields on conglomeratic quartzites and rocky *cerrado,* near Diamantina; the species is presumed to be endemic to the Diamantina Plateau, in the southern *Serra do Espinhaço*.

Conservation Status— The extent of occurrence (EOO) of *S. diamantinense* is 107 km^2 and the area of occupancy (AOO; GeoCAT 2019) is 12 km^2. We identified a very impactful threat (1 location) affecting the whole population (>90%) of unknown severity: the legal and illegal quartzite mining activities reported historically and recently (Silva 2017). There is a continuing decline in extent of occurrence and area of occupancy inferred from the rate of habitat loss. None of the collection sites for this species occurs in a protected area. Therefore, based on both geographic criteria B1ab (ii, iii) and B2b (ii, iii) *S. diamantinense* is provisionally assessed as endangered (EN; IUCN 2017)

***Etymology*—** The specific epithet, *diamantinense,* refers to the city Diamantina, the type locality of the species, a Brazilian city built at the beginning of the 18th century that grew as a result of local diamond mining.

***Notes*—** This species can be distinguished from all other *Stenandrium* species by its stiff, small, congested leaves with revolute margins. It is morphologically most similar to *S. stenophyllum*, but differs by the characters noted in Table 1.

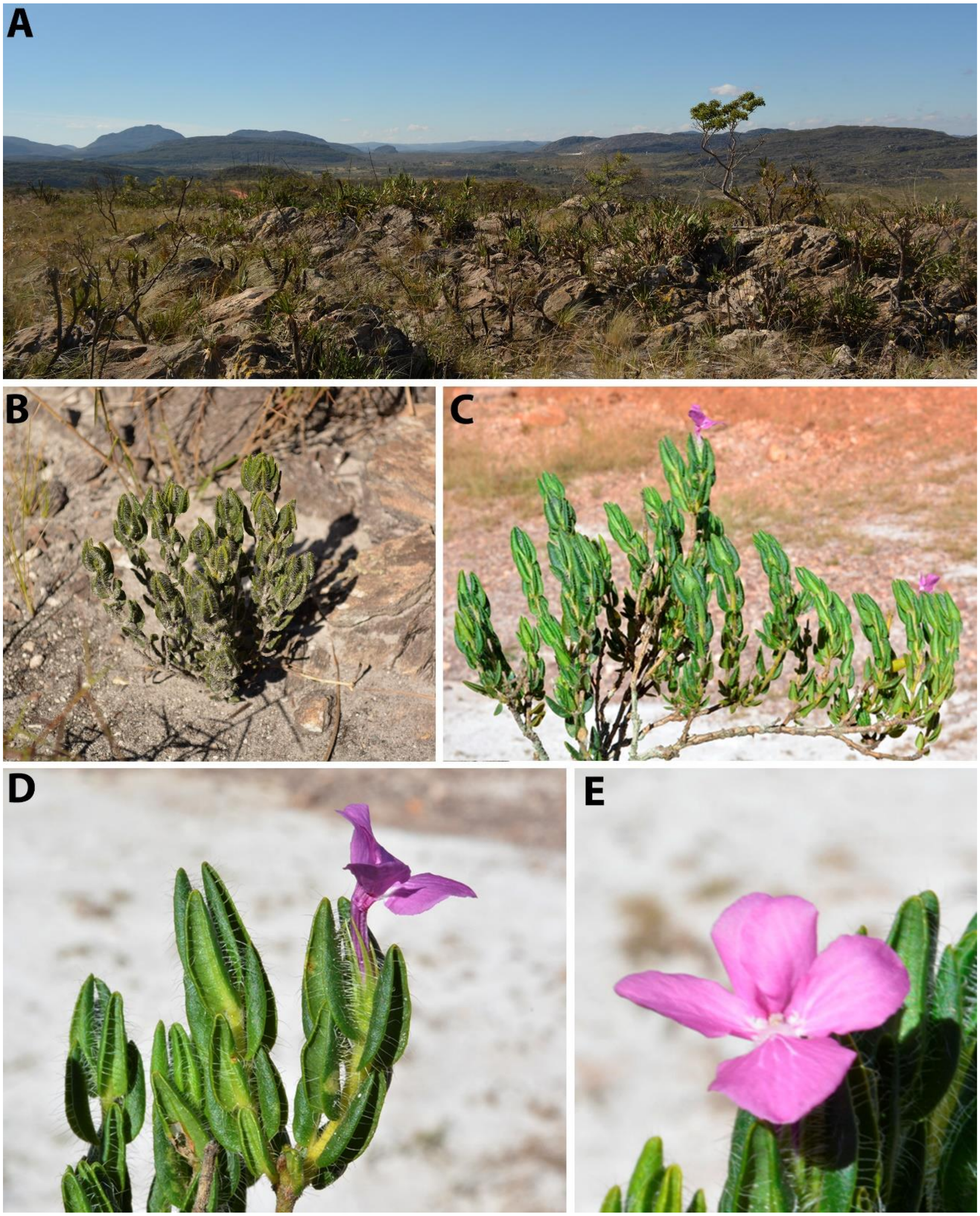

Figure 1. Field images of *Stenandrium diamantinense*. A. Habitat. B. Habit of a small individual. C. Branched stem. D. Leaves and terminal spike. E. Flower with sub-actinomorphic corolla (photos by J.E.Q Faria).

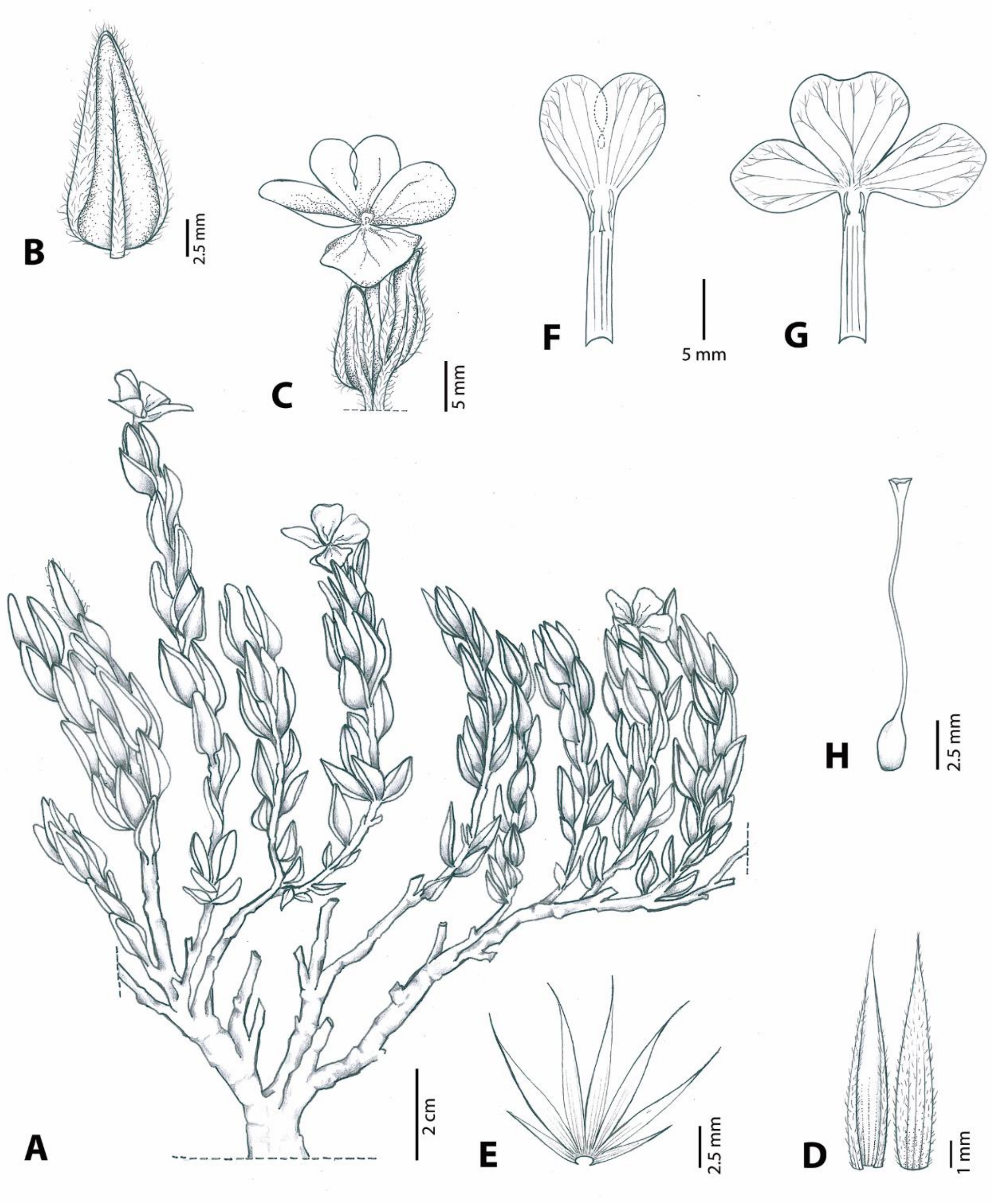

Figure 2. Illustration of *S. diamantinense*. A. Habit. B. Leaf. C. Terminal spike with subactinomorphic flower. D. Bract (left, adaxial surface; right, abaxial surface). E. Bractlets and sepals. F. Upper lip with two lobes. G. Lower lip with three lobes. H. Pistil. *G. Antar & D.A. Chaves 1880* and *J.E.Q. Faria 7910.*

Stenandrium eustachyum Zanatta & Proença, sp. nov. TYPE: BRAZIL. Minas Gerais: Joaquim Felício. Serra do Cabral, margem da estrada que atravessa a serra, saindo de Joaquim Felício, no topo da serra, Cerrado rupestre, 17°41′52″S, 44°15′22″W, 1176 m, 4 Sep 2018, fl., fr., *J.E.Q. Faria, Vasconcelos, T.N. & Fernandes, T. 8828* (holotype: UB!, isotypes: CAS!, HEPH!, HDJF!, K!, RB!, SPF!).

Erect caulescent perennial to 70 cm tall, pubescent on all vegetative structures, trichomes varying in size, eglandular (unicellular and papillose and multicellular but lacking papillae) and glandular (subsessile with globular 2-celled heads); rhizome 5-7 mm thick, roots 0.90-1.3 mm thick, 8-17 cm long. **Leaves** opposite, subsessile to petiolate, petioles 1-1.9 mm long, densely pubescent with trichomes 0.1-1.1 mm long, blades ovate to widely ovate 23.9-31.7 × 18.6-27.2 mm, 1.1-1.3 times longer than wide, chartaceous to coriaceous, cordate at base, obtuse to acute at apex, adaxial surface puberulent with trichomes 0.2-1 mm long, abaxial surface pubescent with trichomes concentrated on the veins and 0.1-1 mm long, margins plane, entire or sinuate, ciliate with trichomes 0.3-1 mm long. **Inflorescence** a pedunculate terminal or axillary spike 30-45.6 mm long (including peduncles, excluding flowers), peduncles 1.5-5.7 mm long, pubescent with trichomes 0.1-1.1 mm long. **Flowers** alternate to opposite along the rachis. **Bracts** ovate, 8-9.5 mm long, 4.6-5 mm wide, appearing 3-veined to the naked eye, with two other weak additional veins visible under magnification, pubescent with trichomes 0.15-0.5 mm long on both surfaces, margins densely ciliate with trichomes 1.5-2.1 mm long. **Bractlets** lanceolate, 4.4-5.7 mm long, 0.6-0.7 mm wide, conspicuously 3-veined on the adaxial surface, glabrous to sparsely pubescent, margins ciliate with trichomes 0.1-0.4 mm. **Calyx** with subequal lanceolate lobes, glabrous to sparsely pubescent, lobes 3-veined, 7-8.5 mm long, 0.8-1 mm wide. **Corolla** pink with white striate nectar guides on the middle of the central lobe of the lower lip, zygomorphic, tube glabrous 6.5-7 mm long, 0.7-1 mm wide, upper lip 5-5.8 mm long, 3-4 mm wide with equal lobes, lower lip 7.3-7.5 mm long, 11-13.5 mm wide with subequal lobes, lower central lobe tomentose at the base internally with trichomes 0.2-0.5 mm and puberulent externally with minute trichomes up to 0.05 mm. **Stamens** 1.4-1.8 mm long, anthers 0.8-0.95 mm long, pubescent along entire length, trichomes up to 0.3 mm long, sometimes concentrated at tip or extending to filaments; staminode 0.8 mm long; pollen grains prolate, tricolpate, 42-49 × 21.3-26.7 μm, exine psilate, colpus with smooth membrane. **Style** 6-7 mm long with a few trichomes at the tip up to 0.15 mm, the stigma asymmetrically funnelform. **Capsule** glabrous, 12-13 mm long, 3.5-4.5 mm wide. **Seeds** laterally flattened, 2.5-3.7 mm long, 1.8-2.3 mm wide, the surface pubescent with barbed trichomes up to 0.6 mm. Figures 3-8.

Paratypes— **Brazil**. — MINAS GERAIS: Joaquim Felício. Serra do Cabral, 17°41′55″S, 44°15′07″W, 16 May 1999, fl., *V.C. Souza, J.P. Souza, S.I. Elias, W. Forster & A.C.P. Oliveira 22525* (ESA!). Buenópolis, Serra do Cabral, a 10-15 km da cidade, na estrada para a Lapa Pintada, 17°53′S, 44°15′W, 1100-1200 m, 13 Oct 1988, fr., R.M. *Harley, A.M. Giulietti, B.L. Stannard, D.J.N. Hind, C. Kameyama, J. Prado, R. Rudall, R. Simão, N. Taylor & D. Zappi 24933* (SPF!). Minas Gerais: Joaquim Felício. Serra do Cabral, margem da estrada que atravessa a serra, saindo de Joaquim Felício, no topo da serra, Cerrado rupestre, 17°41′52″S, 44°15′22″W, 1176 m, 4 Sep 2018, fl., fr., *J.E.Q. Faria, Vasconcelos, T.N. & Fernandes, T. 8827* (HEPH!, BHCB!).

Distribution and Habitat— As far as we are aware, *S. eustachyum* has only been collected four times and is presumed to be endemic to the Serra do Cabral. It occurs in rocky *cerrado* areas.

Conservation Status— The extent of occurrence (EOO) of *S. eustachyum* is 66 km² and the area of occupancy (AOO) is 12 km² (GeoCAT 2019). There are two ongoing threats (2 locations) identified by personal communication with the collector affecting two subpopulations of unknown severity. One is a *Eucalyptus* plantation very close to one population and the other is an incipient mining activity very close to another subpopulation. Those threats indicate an ongoing habitat loss, and consequently an inferred continuing decline in extent of occurrence and area of occupancy. The three known subpopulations are protected in the *Parque Estadual da Serra do Cabral*, which is a conservation unit, but not fully protected because tourist activities are allowed. Based on the geographic criteria B1ab (ii, iii), *S. eustachyum* is provisionally assessed as critically endangered (CR; IUCN 2017).

Etymology— The specific epithet *eustachyum*, from the greek *eu* (good or true) and *stachys* (spike), refers to the beauty of its spikes and is also a tribute to botanist Jair Eustáquio Quintino de Faria, for his valuable contributions to the Brazilian flora. Jair Eustáquio has collected many new and rare species and enriched herbaria with specimens of many different groups of plants in a short period of time. This species had only been collected twice previously, with the most recent of those collections made 20 years ago; the collections and photographs of Faria et al. made at our request greatly enhanced the description of the species.

Notes— *Stenandrium eustachyum* resembles *S. villosum* by the combination of oval bracts and caulescent habit but can be distinguished from the latter species by its ovate to widely ovate, 2.4 to 3.2 cm long leaves (lanceolate, 4.8 to 8.4 cm long in *S. villosum*); flexuous, hyaline trichomes (bristly, sometimes ferrugineous trichomes in *S. villosum*); corolla with tube 6.5 to 7 mm long (6.7 to 8.5 m in *S. villosum*) and lower lip 7.3 to 7.5 mm long (8.6 to 9.3 mm long in *S. villosum*). In the Serra do Espinhaço, *S. eustachyum* is most similar to *S. hatschbachii* but differs by its oval bracts (narrow-lanceolate in *S. hatschbachii*) with trichomes more than 2 mm long (trichomes shorter than 2 mm in *S. hastchbachii*) and uniform pink corollas (corollas with the upper lip with two wine-colored lobes and the lower lip with three pale lilac to almost white lobes in *S. hatschbachii*).

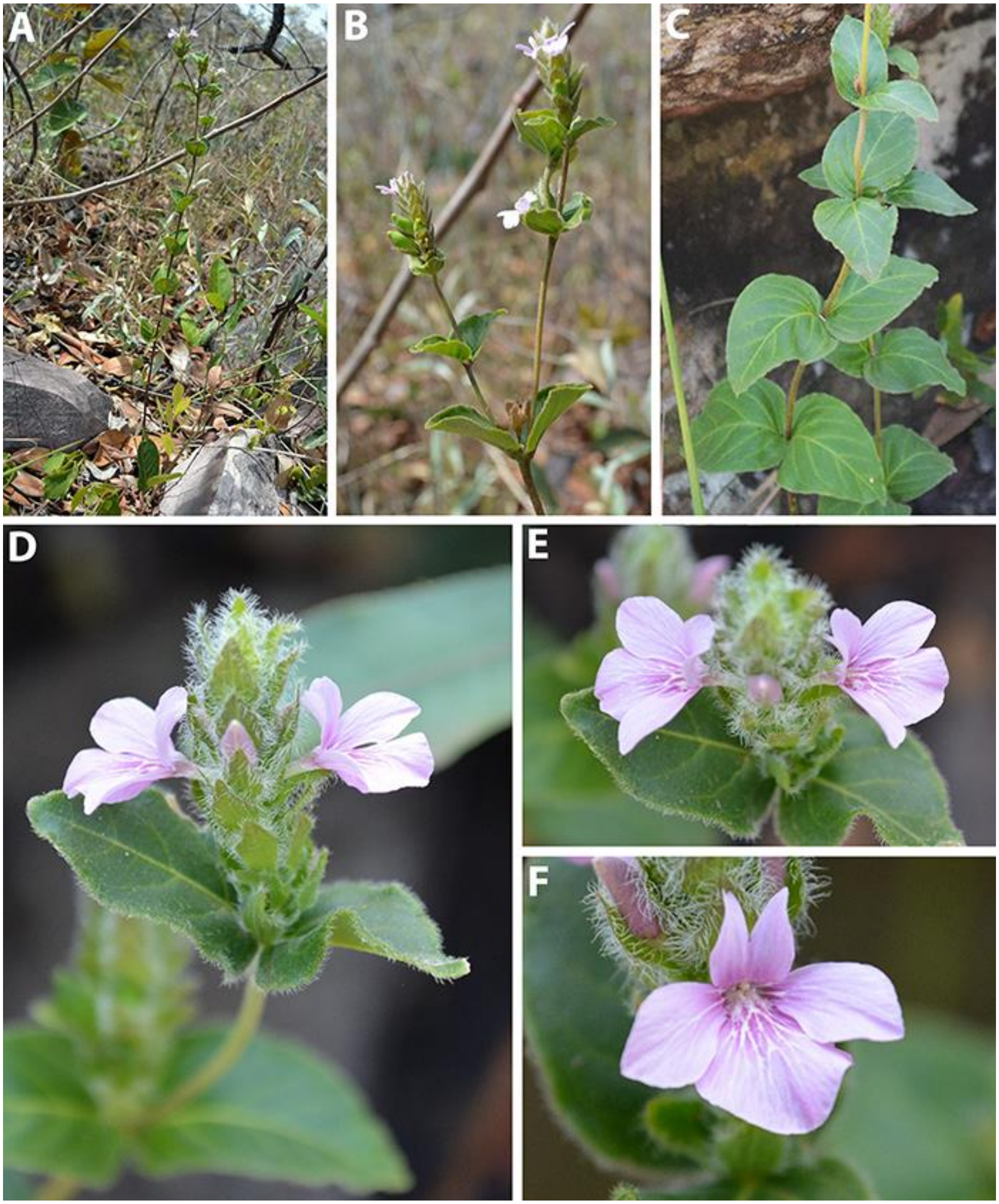

Figure 3. Field images of *Stenandrium eustachyum* (J.E.Q. Faria et al. 8828). A. Habit and habitat. B. Branches with spikes. C. Leaves. D-E. Spikes with flowers. F. Zygomorphic corolla (photos by J.E.Q. Faria).

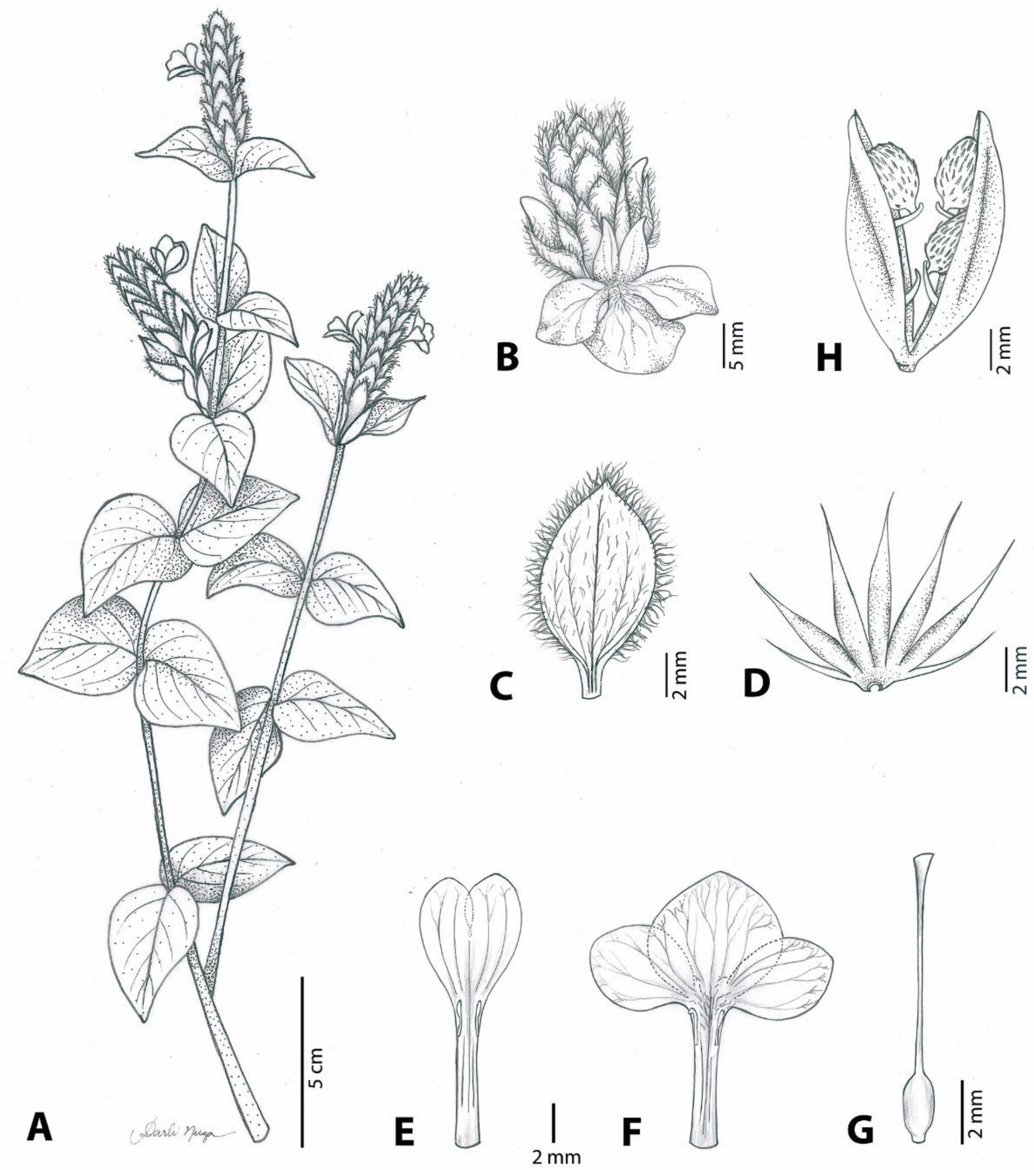

Figure 4. Illustration of *S. eustachyum*. A. Habit. B. Terminal spike with zygomorphic flower. C. Bract. D. Bractlets and sepals. E. Upper lip with two lobes. F. Lower lip with three lobes. G. Pistil. H. Fruit with seeds. V.C. Souza et al. 22525.

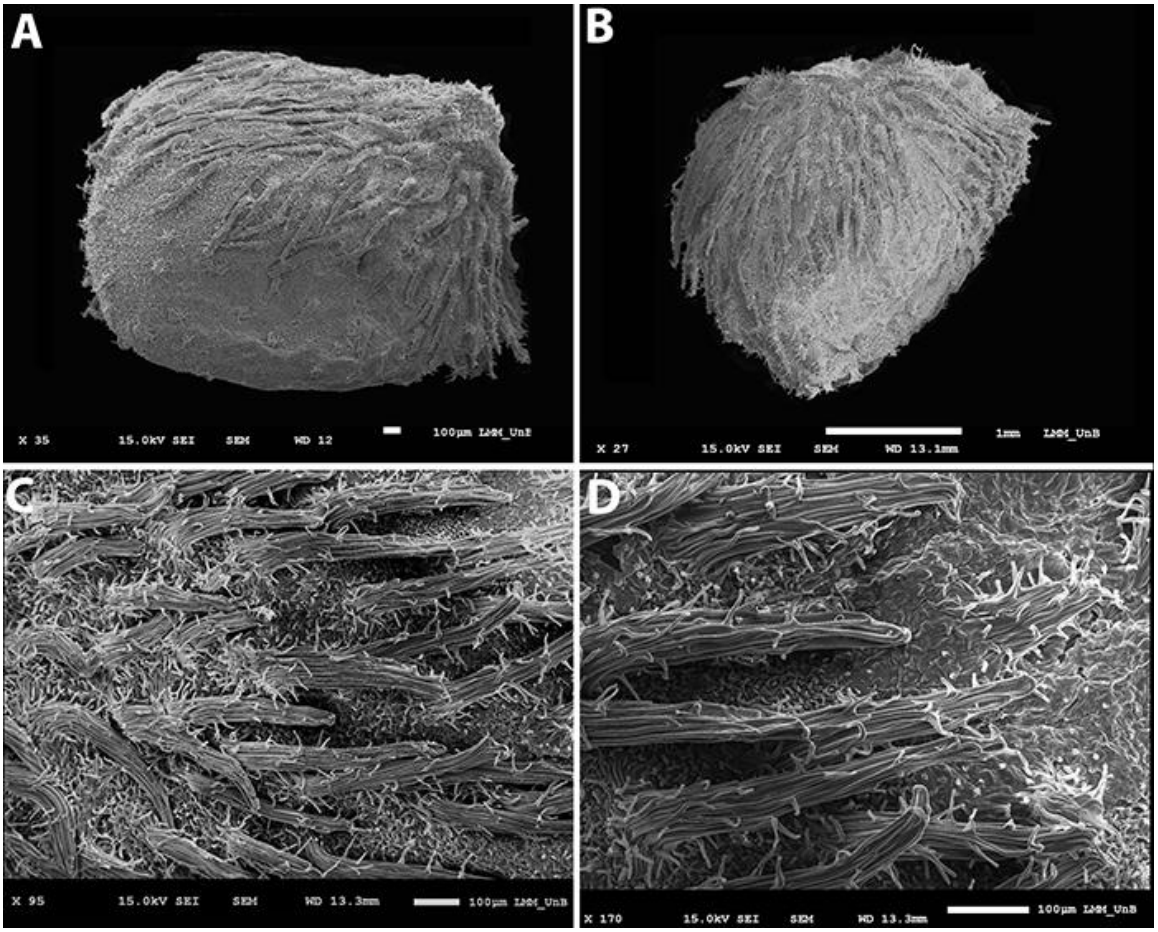

Figure 5. SEM images of *S. eustachyum* seeds. A-B. General view. C-D. Details of the barbed trichomes. J.E.Q. Faria et al. 8828.

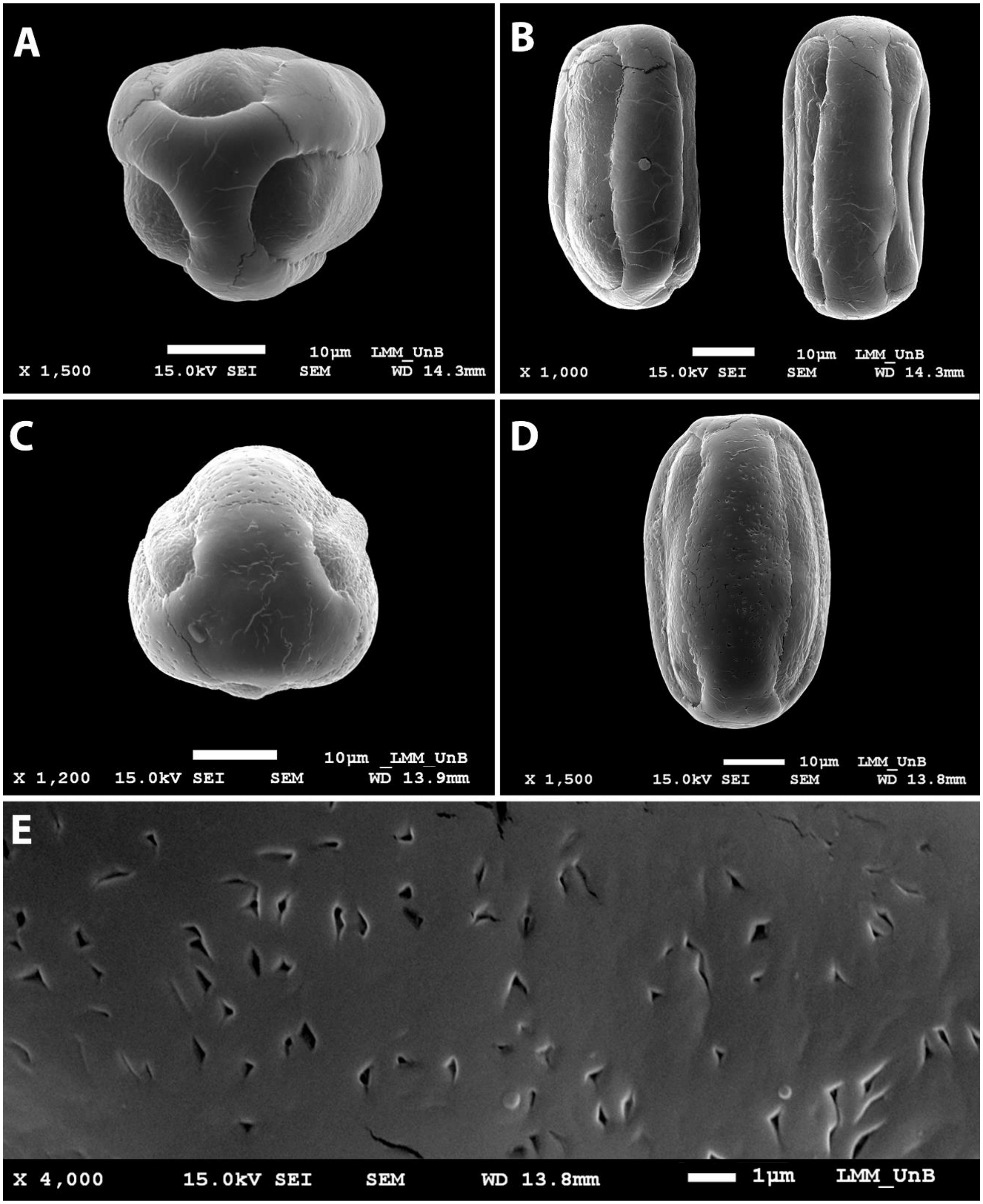

Figure 6. SEM images of *Stenandrium* pollen. A, B. *S. eustachyum* (J.E.Q. Faria et al. 8828). A. Polar view. B. Equatorial view. C-E. *S. diamantinense* (G. Antar & D.A. Chaves 1880). C. Polar view. D. Equatorial view. E. Detail of the sculpturing.

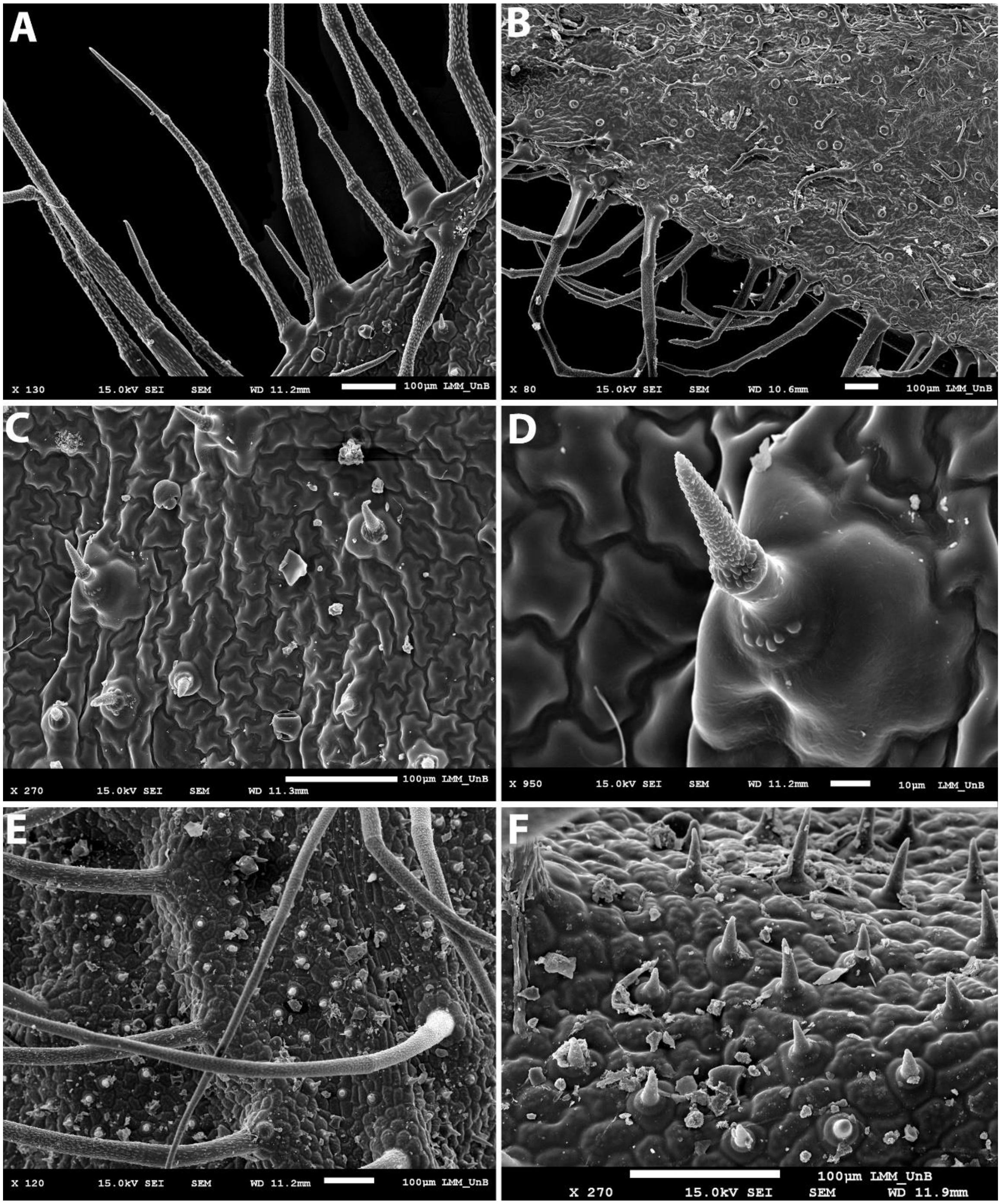

Figure 7. SEM images of *Stenandrium* trichomes. A-D. *S. eustachyum* (J.E.Q. Faria et al. 8828). A. Eglandular multicellular trichomes on the margin of the bract. B. Eglandular unicellular and multicellular trichomes, and glandular subsessile trichomes with globular 2-celled head on the bract. C. Eglandular unicellular papillose trichomes and glandular subsessile trichomes with globular 2-celled head on the bract. D. Eglandular unicellular papillose trichomes on the bract. E-F. *S. diamantinense* (G. Antar & D.A. Chaves 1880). E. Eglandular unicellular conical and multicellular trichomes on the adaxial surface of the leaf. F. Eglandular unicellular conical trichomes on the abaxial surface of the leaf.

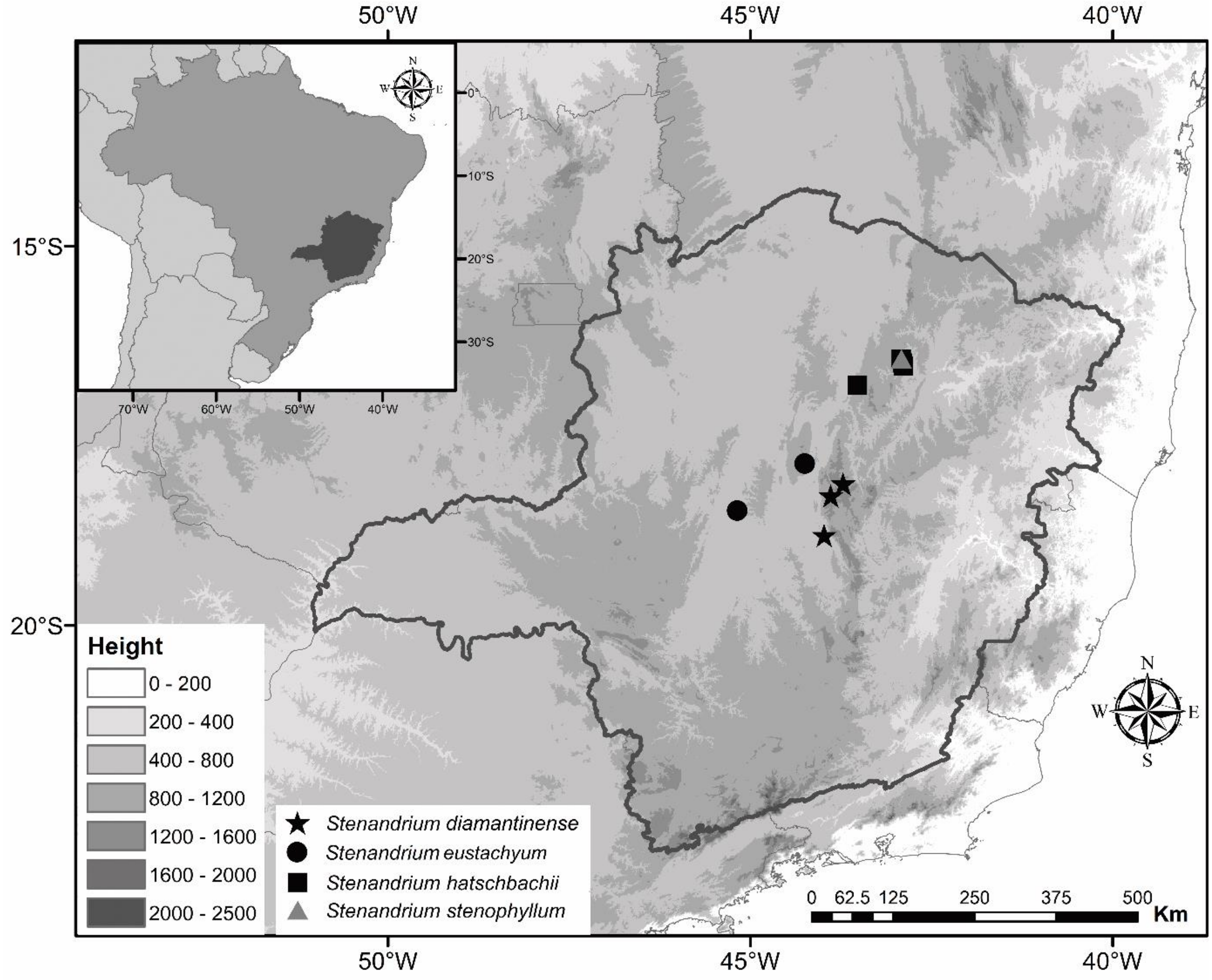

Figure 8. Distribution map of *Stenandrium* from the Serra do Espinhaço.

KEY TO THE SPECIES OF *STENANDRIUM* FROM THE SOUTHERN *SERRA DO ESPINHAÇO*, BRAZIL

1. Leaves verticillate, linear-lanceolate to linear ………………..…… *S. stenophyllum*

1. Leaves opposite, broadly to narrowly ovate ……………………………...…… 2

2. Leaves congested, indurate, with strongly revolute margins … *S. diamantinense*

2. Leaves not congested, chartaceous to coriaceous, with plane to nearly plane margins …………………………………………..…………………………………. 3

3. Bracts linear-lanceolate to lanceolate; flowers strongly zygomorphic with the upper lip wine-colored and the lower lip pale lilac to white (Figure 9) ……………………………………………………………………….. *S. hatschbachii*

3. Bracts ovate; flowers subactinomorphic and uniformly pink …...… *S. eustachyum*

Table 1. Comparison of the four species of *Stenandrium* known from the southern *Serra do Espinhaço*. *Based on Wasshausen (1990) and specimen observations. **Based on Kameyama (1996) and specimen observation.

Characters	*S. diamantinense, sp. nov.*	*S. eustachyum, sp. nov.*	*S. hatschbachii* *	*S. stenophyllum* **
Leaf arrangement	Opposite	Opposite	Opposite	Verticillate
Shape, size, texture, and margins of leaf blade	Ovate to narrowly ovate, 0.7-1.5 × 0.3-0.6 cm, stiff, revolute to almost conduplicate margins.	Ovate to widely ovate, 2.4-3.2 × 1.8-2.7 cm, chartaceous to coriaceous, plane margins.	Ovate to lanceolate, 0.8-2 × 1.2-4.1 cm, coriaceus, revolute to plane margins.	Linear-lanceolate to linear, 1.3-2 × 4.7-7.6 cm, coriaceous, margins revolute.
Shape, size and vestiture of bracts	Linear-lanceolate to lanceolate, 6.5-8.8 × 1.3-1.7 mm, puberulent to glabrous, with trichomes up to 0.1 mm long, sparsely ciliate.	Ovate, 8-9.5 × 4.6-5 mm, pubescent with trichomes 0.15-0.5 mm, densely ciliate with trichomes 1.5-2.1 mm.	Linear-lanceolate to lanceolate, 6-7 × 0.9-1.25 mm, pubescent with trichomes to 0.2 mm, ciliate with trichomes to 0.5 mm.	Linear-lanceolate to lanceolate, 4-6 × 0.5 mm, puberulent with trichomes to 0.1 mm, sparsely ciliate.

Flower type, color	Subactinomorphic, uniform pink	Zygomorphic, uniform pink	Zygomorphic, the upper lip wine-colored and the lower lip pale lilac to white	Zygomorphic, the upper lip wine-colored and the lower lip pale lilac to white
Pollen size	48.2-58.2 × 27.4-31.3 µm	42-49 × 21.3-26.7 µm	39 × 30 µm	49.4 × 33.5 µm

Figure 9. Field images of *Stenandrium hatschbachii*. A. Habit and habitat. B. Branch. C-D. Leaves. E. Terminal spike with bracts, bud, and fruit (photos by M.R.V. Zanatta). F-G. Zygomorphic corolla (photos by A.V. Scatigna).

DISCUSSION

This study increases to four the number of narrow endemic species of *Stenandrium* known from Serra do Espinhaço. All four conform to the centers of endemism (Echternacht et al 2011) and to the KBAs (Giulietti et al 2009): *S. stenophyllum* and *S. hatschbachii* are restricted to Grão Mogol (Northern Mountains Complex – Area 1), *S. eustachyum* is restricted to the Serra do Cabral (Area 3) and *S. diamantinense* is restricted to the Diamantina Plateau (Area 4). No other species of endemic Acanthaceae were found in the centers listed above, only in Areas 7, 9, and 10. Among the Acanthaceae, four species of *Staurogyne* endemic to Serra do Espinhaço were found: *Staurogyne elegans* (Nees) Kuntze (Conceição do Mato Dentro; Giulietti et al 2009; Area 7; Echternacht et al 2011), *Staurogyne hirsuta* (Nees) Kuntze (Area 7; Echternacht et al 2011), *Staurogyne minarum* (Nees) Kuntze (Catas Altas; Giulietti et al 2009), and *Staurogyne vauthieriana* (Nees) Kuntze (Ouro Preto; Giulietti et al 2009; Area 9 and 10; Echternacht et al. 2011). All of these species of *Staurogyne* occur in forest environments.

Species of *Stenandrium* endemic to Serra do Espinhaço share features with other endemic flowering plants in those areas (Echternacht et al., 2011 and Giulietti et al. 2009), including plant architecture, leaf shape, texture and arrangement, inflorescence type and arrangement, even though those plants are phylogenetically distant from Acanthaceae. The most closely related species found were *Lippia elliptica* Schauer and *Stachytarpheta monachinoi* Moldenke (Verbenaceae; Giulietti et al. 2009).

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