UNIVERSIDADE FEDERAL RURAL DE PERNAMBUCO
DEPARTAMENTO DE BIOLOGIA
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Caracterização Palinológica, Citogenética e Morfológica de espécies do gênero *Calliandra* Benth. (Leguminosae - subfamília Caesalpinioideae e Clado Mimosoid) ocorrentes no Nordeste do Brasil.

ANTÔNIO DE PÁDUA DE OLIVEIRA PAULA

RECIFE-PE
2020

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Orientador:
Prof. Dr. Reginaldo de Carvalho

Coorientadoras:
Profa. Dra. Gardene Maria de Sousa
Profa. Dra. Ana Virginia Lima Leite

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Orientador:

_____

Prof. Dr. Reginaldo de Carvalho – UFRPE

Avaliadores:

_____

Dra. Margareth Ferreira de Sales – UFRPE (Titular)

_____

Dr. Leonardo Pessoa Felix - UFPB (Titular)

_____

Dra. Maria Betânia Melo de Oliveira - UFPE (Titular)

_____

Dr. Rodrigo César Gonçalves de Oliveira - UFPE (Titular)

_____

Dr. Felipe Nollet Medeiros de Assis – UFRPE (Suplente)

_____

Dra. Ana Christina Brasileiro Vidal - UFPE (Suplente)

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Dedicatória

A quem me deu a vida e me ensinou a conviver em harmonia com todos,
A quem sempre me deu apoio em todos os momentos da minha vida,
Sempre estarão em meus pensamentos.

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“E eu penso comigo...
Que mundo maravilhoso”

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Símbolos e Abreviações

CI	Intervalo de Confiança
x	Média
s_x	Desvio Padrão
V%	Coeficiente de Variabilidade
M	Mediana
CMA	Cromomicina–A3
DAPI	4´-6´-Diamidino-2-Fenilindol
AT	Adenina-Timina
GC	Guanina-Citosina
Sect.	Seção
HC	heterocromatina constitutiva
ca.	cerca
n	número cromossômico haplóide
x	número cromossômico básico

RESUMO

Calliandra Benth. é um gênero neotropical possuindo 139 espécies e distribuído em três principais regiões do continente americano. Uma destas regiões é o nordeste brasileiro, considerada um dos centros de diversidade do gênero, com 66 espécies, dentre estas vinte e seis são representantes da seção *Androcallis, quatro* da seção *Microcallis* e trinta e seis da seção *Monticola*, ocorrendo em áreas de formação vegetacional de caatinga, cerrado e ecótonos. O presente estudo teve como objetivo analisar unidades polínicas de espécies de *Calliandra* da seção *Androcallis* Barneby, seção *Microcallis* Barneby e seção *Monticola* ER Souza e LP Queiroz, ocorrentes no nordeste do Brasil, e fornecer análises citogenéticas comparativas quanto ao número cromossômico, nível de ploidia e distribuição das regiões heterocromáticas AT e CG em dez espécies de *Calliandra* pertencentes à seção *Androcallis*, usando a técnica CMA/DAPI, além de realizar um estudo taxonômico das espécies ocorrentes no estado do Piauí. Foram realizadas expedições para coleta de material botânico em diversos estados do Nordeste para estudos taxonômico, palinológico e citogenético. Foram realizadas análises palinológicas e citogenéticas comparativas na diferenciação das espécies estudadas por meio da técnica de acetólise para determinação dos padrões das unidades de dispersão polínicas (políades) e de bandeamento cromossômico com fluorocromos cromomicina A3 (CMA), para regiões cromossômicas ricas em pares de base guanina e citosina e o 4',6-diamidino-2-fenilindol (DAPI), para localização das regiões ricas em pares de base adenina e timina. Também foi utilizada a técnica de Citometria de Fluxo para mensurar o conteúdo de DNA existentes nas espécies estudadas. Os resultados observados nas análises palinológicas permitiram caracterizar e agrupar diferentes espécies, principalmente as pertencentes a seção *Monticola* em relação as suas respectivas características e dimensões de suas políades. Os dados coletados aumentam o número de informações para espécies ainda não estudadas e corrigi questões duvidosas em relação ao número de grãos por políades de algumas espécies do gênero. Foi verificada a existência de espécies diplóides ($2n = 2x = 16$) *C. macrocalyx*, *C. riparia*, *C. imperialis*, *C. ulei*, *C.spinosa* e *C. umbellifera*; de espécie tetraplóide ($2n = 4x = 32$) *C. fernandesii*; de espécie octoplóide ($2n = 8x = 64$) *C. sessilis* e de espécie decaplóide ($2n = 10x = 80$) *C. dysantha*, confirmando o proposto para o número básico do gênero de $x = 8$, caracterizados em seus diferentes níveis de ploidias. Os resultados encontrados para o conteúdo de DNA confirmam o que é proposto para as diferenças de tamanho cromossômico e de ploidia observadas nas espécies em estudo. Entre as espécies analisadas, não foi observado o evento de disploidia. A utilização da dupla coloração CMA/DAPI, revelou padrões de bandas que permitiu

identificar e diferenciar cada espécie. Quanto ao número de sinais CMA+ foi possível diferenciar três grupos, um com 6, outro com 8 e outro com 10 bandas para a seção *Androcallis*, sugerindo que os rearranjos cromossômicos e a poliploidia tenham contribuído para a evolução das espécies avaliadas. A utilização da técnica citogenética com fluorocromos CMA/DAPI possibilita a caracterização e diferenciação em estudos citotaxonômicos para este gênero. Os dados morfológicos observados contribuem para a taxonomia do gênero e a um melhor conhecimento na distribuição dos diferentes táxons encontrados no estado do Piauí, Brasil.

Palavras-chave: Leguminosae, Palinologia, Citotaxonomia, CMA/DAPI.

ABSTRACT

Calliandra Benth. is a neotropical genus with 139 species and distributed in three main regions of the American continent. One of these regions is northeastern Brazil, considered one of the centers of diversity of the genus, with 66 species; among these twenty-six are representatives of the *Androcallis* section, four of the *Microcallis* section and thirty-six of the *Monticola* section, occurring in areas of vegetation formation caatinga, cerrado and ecotones. The present study aimed to analyze pollinic units of *Calliandra* species in the *Androcallis* Barneby section, *Microcallis* Barneby section and *Monticola* ER Souza and LP Queiroz section, occurring in northeastern Brazil, and to provide comparative cytogenetic analyzes regarding chromosome number, ploidy level and distribution of heterochromatic regions AT and CG in ten species of *Calliandra* belonging to the *Androcallis* section, using the CMA/DAPI technique, in addition to conducting a taxonomic study of species occurring in the state of Piauí. Expeditions were carried out to collect botanical material in several states in the Northeast for taxonomic, palynological and cytogenetic studies. Comparative palynological and cytogenetic analyzes were carried out to differentiate the species studied using the acetolysis technique to determine the patterns of pollen dispersion units (polyads) and chromosomal banding with chromomycin A3 fluorochromes (CMA), for rich chromosomal regions in base pairs guanine and cytosine and 4',6-diamidino-2-phenylindol (DAPI), for localization of the regions rich in adenine and thymine base pairs. Flow Cytometry technique was also used to measure the DNA content of the species studied. The results observed in the palynological analyzes allowed to characterize and group different species, mainly those belonging to the *Monticola* section in relation to their respective characteristics and dimensions of their polyads. The data collected increases the number of

information for species not yet studied and corrects dubious questions in relation to the number of grains per polyad of some species of the genus. Diploid species ($2n = 2x = 16$) were found *C. macrocalyx*, *C. riparia*, *C. imperialis*, *C. ulei*, *C.spinosa* and *C. umbellifera*; tetraploid species ($2n = 4x = 32$) *C. fernandesii*; octoploid species ($2n = 8x = 64$) *C. sessilis* and decaploid species ($2n = 10x = 80$) *C. dysantha*, confirming the proposal for the basic number of the genus of $x = 8$, characterized in their different ploidy levels. The results found for the DNA content confirm what is proposed for the differences in chromosomal size and ploidy observed in the species under study. Among the analyzed species, the event of disploidy was not observed. The use of double CMA/DAPI staining revealed band patterns that allowed to identify and differentiate each species. As for the number of CMA+ signals, it was possible to differentiate three groups, one with 6, another with 8 and another with 10 bands for the *Androcallis* section, suggesting that chromosomal rearrangements and polyploidy have contributed to the evolution of the species evaluated. The use of the cytogenetic technique with CMA/DAPI fluorochromes enables the characterization and differentiation in cytotaxonomic studies for this genus. The observed morphological data contribute to the taxonomy of the genus and to a better knowledge in the distribution of the different taxa found in the state of Piauí, Brazil.

Keywords: Leguminosae, Palinology, Cytotaxonomy, CMA/DAPI

1. INTRODUÇÃO

Calliandra apresenta um total de 139 espécies, com distribuição restrita nas Américas, apresentando como os maiores centros de diversidade o Nordeste do Brasil, o Norte da Colômbia e da Venezuela e o Planalto Mexicano (Barneby, 1998; Sousa, 2001; 2007; Queiroz, 2009; Sousa *et al.*, 2013).

Distribuídas em regiões secas com forte sazonalidade como florestas estacionais semidecidual e decidual, as espécies deste gênero apresentam elasticidade fenotípica intraespecífica. Em alguns casos, a diferenciação entre espécies próximas que compõem este gênero geralmente consiste em poucos caracteres morfológicos, o que dificulta a identificação e determinação dos diferentes táxons (Queiroz, 2009).

Calliandra está atualmente circunscrito à tribo Ingeae, clado Mimosoide da subfamília Caesalpinioideae e família Leguminosae (LPWG, 2017). Este gênero é considerado monofilético a partir dos dados de filogenia molecular realizado para oito diferentes marcadores (Sousa *et al.*, 2013)

Calliandra foi inicialmente descrita por Bentham (1840) baseado em caracteres morfológicos, os quais abrangem a presença de androceu polistêmone e monadelfo, fruto legume com deiscência elástica do ápice para a base e valvas com margens espessadas. O gênero foi posteriormente revisado por Barneby (1998), que propôs classificação infragenérica reconhecendo cinco seções com distribuição exclusivamente neotropical. Mais recentemente são reconhecidas seis seções, a saber: seis seções (*Androcallis* (75 spp.), *Calliandra* (10 spp.), *Microcallis* (6 spp.), *Monticola* (37 spp.), *Septentrionales* (6 spp.) e *Tsugoideae* (4 spp.)) (Souza, 2013).

Diferenças morfológicas foram encontradas nas estruturas polínicas entre os grupos de espécies anteriormente consideradas como do gênero *Calliandra*, como as americanas, as asiáticas e as africanas, observadas por Guinet (1965), Desta forma foi possível determinar apenas para o primeiro grupo a presença 8 grãos de pólen na formação de sua unidade de dispersão, denominada de políade, com representantes exclusivamente das Américas, já as demais apresentam 16 grãos de pólen por unidade de dispersão e são representantes de zonas áridas do velho mundo (Guinet, 1965; 1981; Guinet & Hernández, 1989). Os resultados dos estudos até então realizados para a estrutura da unidade de dispersão descrevem exclusivamente a morfologia da políade das espécies deste gênero sem considerar sua importância sobre o aspecto taxonômico infragenérico, como observado por Van Campo &

Guinet, 1961; Guinet, 1965; 1981 Guinet & Hernández, 1989; Sousa, 2007, Santos & Romão, 2008; Buril et al., 2010; Leython & Ruiz-Zapata , 2014; Freitas Cruz et al.,2018.

Em estudos citogenéticos para *Calliandra*, os resultados obtidos foram realizados em poucas espécies deste gênero, cerca de 7% do total das espécies conhecidas (CCDB, 2019), revelando exclusivamente o número cromossômico. A ocorrência do número básico cromossômico $x = 8$ foi proposto por Goldblatt & Davidse (1977), posteriormente outros números básicos secundários foram sugeridos por Goldblatt (1981a); Goldblatt (1981b) citado por Hernández (1986); Santos et al. (2012) para $x = 11$ e 13. Eventos relacionados à evolução cromossômica para o gênero são raros e relatam como sendo a ocorrência de disploidia (Santos et al., 2012) e poliploidia (Shibata, K., (1962) citado no Chromosome Counts Database). Não há registros envolvendo estudos cariomorfológicos e de bandeamento cromossômico para as espécies deste gênero, neste caso existe a necessidade de análises mais aprimorada com o propósito de melhor responder ao processo evolutivo deste gênero.

O presente estudo objetivou analisar as unidades de pólen das espécies de Calliandra das seções Androcallis Barneby, Microcallis Barneby e ER Souza e LP Queiroz, ocorrendo no nordeste do Brasil, e fornecer análises citogenéticas comparativas quanto ao número cromossômico, nível de ploidia e distribuição das regiões heterocromáticas AT e CG em dez espécies de Calliandra pertencentes à seção Androcallis, usando a técnica CMA / DAPI , além de realizar um estudo taxonômico das espécies ocorrentes no estado do Piauí. Possibilitando a novos conhecimentos para os estudos taxonômicos e a um melhor entendimento dos processos evolutivos para o gênero.

2. REVISÃO BIBLIOGRÁFICA

2.1. Família Leguminosae Adans

Leguminosae é constituída atualmente por 727 gêneros e 19.325 espécies, considerada a terceira maior família das angiospermas, com distribuição cosmopolita (Lewis et al. 2005; 2013; LPWG, 2013a). Considerada monofilética com base em dados morfológicos e moleculares (Tucker & Douglas 1994; Chappil 1995; Kajita et al. 2001; Persson 2001; Wojciechowski et al. 2004), a família era tradicionalmente dividida em três subfamílias: Papilionoideae, Mimosoideae e Caesalpinioideae. No entanto, as subfamílias Papilionoideae e Mimosoideae (excluindo Dinizia Ducke) eram consideradas monofiléticas e Caesalpinioideae como parafilética (Bruneau et al 2000, 2001; Herendeen et al. 2003; Wojciechowski *et al*. 2004).

Recentemente estudos filogenéticos abrangentes com utilização de diversos resultados de marcadores moleculares, principalmente para o gene plastidial *mat*K, reconheceu seis subfamílias monofiléticas robustamente suportadas: Cercidoideae (12 gêneros com cerca de 335 spp.), Detarioideae (84 gêneros com cerca de 760 spp.), Duparquetioideae (1 gênero e 1 sp.), Dialioideae (17 gêneros com cerca de 85 spp.), Caesalpinioideae (148 gêneros com cerca de 4.400 spp.) e Papilionoideae (503 gêneros com cerca de 14.000 spp.), sendo que as espécies pertencentes a antiga subfamília Mimosoideae formam nesta nova circunscrição o clado Mimosoide e estão inclusas na atual subfamília Caesalpinioideae (LPWG, 2017).

No Brasil a família Leguminosae encontra-se distribuída em todas as formações vegetacionais, apresentando elementos com hábitos herbáceos, arbustivos, lianas e arbóreos, com cerca de 210 gêneros e 2.694 espécies (Forzza et al. 2010; Lima et al. 2010). Sua utilização econômica é bastante ampla e diversificada na alimentação humana e animal, contribuindo também na composição de fármacos, fabricação de bebidas e combustíveis, fornecendo madeira para a indústria, fabricação de moveis e construção de casas, sendo também utilizada em ornamentação e no melhoramento da qualidade do solo pelo processo de fixação de nitrogênio, sendo considerada a segunda maior família de importância econômica (Wojciechowski et al., 2004; Lewis et al. 2005; Lavin et al., 2005).

Para as atuais subfamílias de leguminosas a descrição morfopolinica demonstra existir uma variação nas estruturas das unidades de dispersão no formato de mônades para todas as subfamílias com exceção de Cercidoideae com mônades e raros casos de unidades em tétrades e Caesalpinioideae que apresenta mônades, tétrades, bitétrades e políades

(LPWG, 2017). Já em analises citogenéticas para estas subfamílias são observados números cromossômicos correlatos a múltiplos de $x = 7$, propondo ser o número ancestral comum para a família Leguminosae para as atuais espécies ou números derivados próximos a este, sugerindo também a possível ocorrência de eventos de poliploidia ou disploidia na evolução desta família (Poggio *et al*., 2008; LPWG, 2017; CCDB, 2018).

2.2. Clado Mimosoide

Com a nova classificação filogenética da família e a distribuição de seus táxons em seis subfamílias, a subfamília Mimosoideae tornou-se um clado, mesmo que informalmente pertencente à circunscrição da subfamília Caesalpinioideae (LPWG, 2017). Este clado é composto de gêneros anteriormente atribuídos a subfamília Mimosoideae incorporando também, o gênero *Chidlowia*, antes considerado membro da antiga Caesalpinioideae, (Manzanilla & Bruneau, 2012).

O clado Mimosoide está representado por cerca 83 gêneros e 3300 espécies distribuídos em quatro tribos: Mimoseae Bronn, Mimozygantheae Burkart, Acacieae Dumort e Ingeae Benth. (Lewis et al. 2005). Distribui-se principalmente nas regiões Pantropicais com características climáticas de áreas úmidas (maioria arbóreas) e secas (maioria herbácea e arbustiva). A morfologia das espécies deste clado é caracterizada por apresentar folhas bipinadas e na sua maioria com nectários extraflorais, flores agrupadas em inflorescência em glomérulo ou espiga, prefloração valvar (exceto em *Parkia*), cálice e corola geralmente unidos na base, estames numerosos (10 a mais de 100), pólen composto (tétrade, bitétrade ou políades) (Lewis et al. 2005; LPWG, 2017).

O clado Mimosoide está representado no Brasil por 35 gêneros e 818 espécies, distribuídas em todas as formações vegetacionais, tendo como os seus principais representantes os gêneros *Mimosa* L. (359 spp.) e *Inga* Mill. (131 spp.) (BFG 2018). A região Nordeste do Brasil apresenta uma grande diversidade em espécies para o clado Mimosoide com representantes em todos os Estados desta região, dentre os diversos estudos destaca-se o trabalho de Queiroz (2008) que identificou 22 gêneros e 95 espécies e Matos et al. (2019) que identificaram 9 gêneros e 14 espécies em uma área do semiárido do Estado de Pernambuco.

Em estudos morfopolínicos, Buril et al. (2010) identificou em uma área de caatinga no Estado de Pernambuco, 13 gêneros e 23 espécies do clado Mimosoide, destes 19 táxons foram caracterizados quanto a sua constituição polínica, sendo encontradas unidades de dispersão que vão desde mônades, tétrades e políades com 8, 16 e 32 grãos.

2.3. Tribo Ingeae Bentham

Baseado em caracteres morfológicos das leguminosas, Bentham (1875) classificou as espécies com numerosos estames monadelfos em 15 gêneros e 408 espécies no primeiro grupo descrito para a tribo Ingeae. Estudos filogenéticos da tribo Ingeae baseados em caracteres morfológicos para as espécies americanas foram realizados por Barneby & Grimes (1996; 1997), sendo proposto uma distribuição para os táxons desta tribo em 22 gêneros e cerca de 357 espécies. A mais recente classificação desta tribo foi realizada por Lewis & Rico-Arce (2005), os quais reorganizaram este grupo em 36 gêneros, sendo 24 exclusivamente neotropicais, com 966 espécies. As espécies da tribo Ingeae estão distribuídas em florestas da África, Ásia, América Central e principalmente na América do Sul. O maior centro de diversidade da tribo é o Brasil com 16 gêneros e 302 espécies (Morim, 2010a,b,c,d,e), sendo encontrada em todas as formações vegetacionais, com maior ocorrência na bacia amazônica e apresentando o gênero *Inga* com o maior número de representantes desta tribo (Ducke, 1943; Lima et al., 2010).

2.4. Estudos Palinológicos na Caracterização e Diferenciação de Espécies da Tribo Ingeae Bentham

A utilização de palinomorfos na caracterização e diferenciação entre gêneros e espécies para a tribo Ingeae é descrita em vários estudos como mais um instrumento diferenciador entre táxons. Estudos realizados por Taisma (2013) para espécies de *Inga* classificou 52 espécies distribuídas na Venezuela em 2 tipos e 2 subtipos com comprimento das políades que variou de 22 a 123µm.

Freitas Cruz et al. (2017) analisaram as estruturas polínicas de cinco gêneros e oito espécies de Ingeae ocorrentes na Mata Atlântica no Estado do Rio de Janeiro, Brasil. Estes autores conseguiram identificar a ocorrência de políades com 10, 16 e 32 grãos, possibilitando a diferenciação entre as espécies pela relação comprimento/largura e sua classificação.

Recentemente, Santos et al. (2019) descreveram a estrutura polínica de 13 gêneros e 60 espécies pertencentes a tribo Ingeae ocorrentes no Nordeste do Brasil, sendo confirmada a presença de diferentes aglomerados polínicos entre as espécies analisadas, contendo 8, 16, 20, 24, 28 e 32 grãos de pólen formando as diferentes unidades de dispersão. Constataram que unidades de dispersão apresentavam heterogeneidade tanto entre os gêneros quanto entre as espécies de um mesmo gênero, sendo possível caracterizar diferentes táxons mediante os dados quantitativos e qualitativos, pelo número de grãos de pólen por unidade de dispersão, forma, simetria e tamanho das políades.

2.5. Citogenética da Tribo Ingeae Bentham

Estudos citogenéticos em espécies da tribo Ingeae geralmente se restringem a contagem do número cromossômico dos diferentes táxons analisados, sem que haja uma melhor análise nos dados cariotípicos com utilização de descrições mais amplas da morfologia cromossômica e de métodos mais refinados de bandeamento cromossômico (Santos et al., 2019). Para a tribo Ingeae Goldblatt (1981a) designou como sendo o número básico $x = 13$ exceto para o *Calliandra sensu stricto* com $x = 8$, o que posteriormente também foi relatado por Poggio (2008) ao relatar ser o número diplóide para o gênero *Calliandra* atípico com $2n = 2x = 16$.

Figueiredo et al. (2014) analisaram treze espécies de seis seção pertencentes a *Inga* por meio da técnica citogenética convencional na determinação dos diferentes números cromossômicos e constataram haver variação nos padrões cariotípicos quanto ao nível de ploidia entre as espécies estudadas, com o número básico de $x = 13$ demonstrando haver uma série poliplóide na caracterização evolutiva do gênero com $2n = 2x = 26$ (espécies diplóides), $2n = 4x = 52$ (espécies tetraplóides) e $2n = 8x = 104$ (espécies octaplóide). O gênero *Albizia* também pertencente à tribo Ingeae é relatado por apresentar espécies com a mesma série poliplóide e o gênero *Samanea* com espécies constituídas dos mesmos números diplóides e tetraplóides encontrado em *Inga* (Santos et al., 2019).

2.6. Gênero *Calliandra* Bentham

O gênero *Calliandra* foi inicialmente descrito por Bentham (1840), o qual caracterizou 18 espécies neotropicais baseando-se em caracteres morfológicos tais como: flores com estames numerosos e frutos com deiscência elástica a partir do ápice. Posteriormente Bentham (1844; 1875) redefiniu o gênero em cinco séries (*Macrophyllae*, *Laetevirentes*, *Pedicellatae*, *Nitidae* e *Racemosae*) mediante a diferenciação de características foliares e de inflorescências, ampliando sua distribuição para o continente asiático. Espécies africanas e asiáticas foram também descritas para o gênero a partir dos trabalhos de Urban (1900); Harms (1921) e Thulin et al. (1981).

Barneby (1998) revisou o gênero *Calliandra* e redefiniu este em cinco seções (*Androcallis*, *Calliandra*, *Acroscias*, *Acistegia* e *Microcallis*) com distribuição exclusivamente nas Américas, baseado em caracteres morfológicos.

Calliandra é considerado monofilético e sua mais recente revisão foi realizada por Souza et al. (2013) a partir da filogenia molecular para 95 espécies baseado em oito tipos diferentes de marcadores moleculares. Esta atual circunscrição distribui as espécies do

gênero em seis seções: *Androcallis* (75 spp.), *Calliandra* (10 spp.), *Monticola* (37 spp.), *Tsugoideae* (4 spp.), *Septentrionales* (6 spp.) e *Microcallis* (6 spp.).

Diferentes estudos foram também realizados na caracterização das estruturas morfopolínicas para espécies do gênero *Calliandra* (Van Campo & Guinet, 1961; Guinet, 1965; 1981; Guinet & Hernández, 1989; Sousa, 2007, Santos & Romão, 2008; Buril et al. 2010; Leython & Ruiz-Zapata , 2014), definindo sua unidade de dispersão como uma políade formada por 8 grãos de pólen (dois centrais e seis periféricos), apresentando heteromorfismo polínico, ocorrência ou não de apêndice na região aguda do grão apical das políades, formato elipsóide, simetria bilateral, disposição uniplanar e políades calimadas. No entanto, tais características só foram utilizadas até o momento na diferenciação deste gênero em relação a outros, em estudos a nível infragenérico tais informações não são utilizadas na palinotaxonomia.

Os estudos citogenéticos para o gênero *Calliandra* contribuem atualmente apenas na contagem do número cromossômico, onde menos de 7% das espécies foram analisadas até o momento (CCDB, 2018). O número básico considerado para o gênero é $x = 8$ (Goldblatt & Davidse, 1977; Goldblatt, 1981a), é sugerido também à existência de um número básico secundário de $x = 11$ atribuído para cariótipos com ocorrência de eventos de disploidia (Goldblatt, 1981a; Goldblatt, 1981b citado por Hernández, 1986; Santos et al., 2012). A poliploidia e a disploidia são eventos citados como promotores da evolução para o gênero *Calliandra* (Santos et al., 2012).

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MANUSCRITO I

Palynological characterization of the *Androcallis*, *Microcallis*, and *Monticola* sections of the genus *Calliandra* Benth. (Leguminosae - Mimosoid Clade) present in northeastern Brazil

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Palynological characterization of the *Androcallis*, *Microcallis*, and *Monticola* sections of the genus *Calliandra* Benth. (Leguminosae - Mimosoid Clade) present in northeastern Brazil

Running title: Pollen characterization of *Calliandra*

ANTÔNIO DE PÁDUA DE OLIVEIRA PAULA[1], GENIALDO RAMOS DOS SANTOS[1], GARDENE MARIA DE SOUSA[2], ANA VIRGÍNIA LEITE[1,3], LUCIANO PAGANUCCI DE QUEIROZ[4] & REGINALDO DE CARVALHO[1,3]

[1]Programa de Pós-Graduação em Botânica, Universidade Federal Rural de Pernambuco, Recife, Brazil, [2]Departamento de Biologia, Centro de Ciências da Natureza, Universidade Federal do Piauí, Teresina, Brazil, 3Departamento de Biologia, Universidade Federal Rural de Pernambuco, Recife, Brazil, 4Programa de Pós-Graduação em Botânica – Universidade Estadual de Feira de Santana, Feira de Santana, Brazil.

Abstract

In Brazil, the genus *Calliandra* Benth. (Caesalpinioideae; Leguminosae) comprises 74 species, of which 59 are endemic; the northeastern region stands out as its largest diversity centre. *Calliandra* species display a pollen dispersal unit (polyad) formed by eight calymmate, ellipsoid pollen grains, with a uniplanar arrangement and bilateral symmetry. Pollen units of native and ornamental *Calliandra* species present in northeastern Brazil were analyzed to provide subsidies for studies on the ecology and taxonomy of the genus. Pollen morphometric data of twenty-five species and two varieties were used to characterize the genus into different *Calliandra* sections. The pollen material analyzed was acetolysed, then measured, described, and photomicrographed under a light microscope (LM). Quantitative data were submitted to descriptive statistical treatments. In the *Calliandra* sect. *Androcallis* and *C.* sect. *Monticola*, polyads tended to be larger in length and width than those in the *C.* sect. *Microcallis*. In the *C.* sect. *Monticola*, an appendage was present in the most acute region of the apical pollen grain. Quantitative data showed that the largest polyad occurred

Corresponding author: Ana Virgínia Leite, Departamento de Biologia, Programa de Pós-graduação em Botânica (PPGB), Universidade Federal Rural de Pernambuco (UFRPE) Av. Dom Manoel de Medeiros s/n, CEP 52171-900, Recife, PE, Brazil. E-mail: anavlleite@yahoo.com.br

in *C. macrocalyx* var. *macrocalyx*, with dimensions of 250 x 150 µm, and the smallest polyad occurred in *C. leptopoda*, with 117.5 x 65 µm. Based on the data obtained for polyad length, it was possible to differentiate the species into distinct groups within and between sections studied.

Keywords: caatinga, Fabaceae, palynology, taxonomy.

Introduction

Currently, *Calliandra* Benth. is classified as belonging to the Ingeae tribe, Mimosoid clade, subfamily Caesalpinioideae, family Leguminosae (LPWG 2017), and has 139 Neotropical species. Its distribution spreads from the southwestern United States to the northern and northeastern Argentina (Barneby 1998; Lewis 2005; Souza et al. 2013). Brazil has 74 species, of which 59 are endemic (Souza 2007). The northeastern region of the country stands out with the highest diversity: 66 species, distributed into several vegetation types, such as Caatinga, Cerrado, and transition areas (ecotones) (Santos & Romão 2008; Queiroz 2009).

The classification of *Calliandra* species is based on morphological characteristics, such as the number of pinnae and leaflets on leaves, leaflet size, size and shape of the calyx lacinia, flower color, and size of the legume and seed (Queiroz 2009). However, even using these characteristics, the classification is limiting and may be hindered by the occurrence of morphological polymorphisms (Leython & Jáuregui 2008). Thus, the analysis of pollen morphology is a valuable tool to complement or even define genus and species taxonomically (Souza 2007). Together with other characteristics, it can be used in phylogenetic and evolutionary analyses (Oswald et al. 2011).

Within the Ingeae tribe, palynological studies indicate possible changes in the calymmate character and reduction in the number of pollen grains per polyad as an evolutionary event for speciation. Taisma (2013) analyzed 52 species of *Inga* from Venezuela and observed a variation from 16 to 40 grains per polyad. Early studies on *Calliandra* showed that *C. parviflora* Benth. had the predominant type of polyad in the genus, composed of eight pollen grains (Salgado-Labouriau 1973). The polyads are usually calymmate, ellipsoid, with a uniplanar arrangement and bilateral symmetry (Van Campo & Guinet 1961; Guinet 1965; Souza 2007). They are spatially arranged as two central and six peripheral grains, forming a bitetrad polyad, and each of these polyads is composed of 4-5 pores. One of the peripheral pollen grains (apical pollen grain) stands out due to its usually acute shape at the extremity (Santos & Romão 2008).

Previous studies on the palynotaxonomy of *Calliandra* and other genera showed differences between continents and established the *Calliandra* genus as exclusive to the Americas (Souza 2007). Guinet (1965) defined the main bases for establishing differences between American, Asian, and African groups by comparing species of the *Calliandra* genus. In the first group, composed of only American species, eight pollen grains formed the

polyad, instead of 16 pollen grains found in other groups, whose species represented arid zones of the Old World (Guinet 1965, 1981; Guinet & Hernández 1989).

Santos & Romão (2008) studied the pollen morphology of 21 *Calliandra* species present in the Chapada Diamantina: six species of the *Calliandra* sect. *Androcallis*, one of the *C.* sect. *Microcallis*, and 14 of the *C.* sect. *Calliandra*. The following characteristics were assessed: total polyad length, appendage length, size of the central pollen grain of the polyad, characteristics of the polyad pores, presence or absence of appendage on the apical pollen grain of the polyad, ornamentation and exine thickness. It was possible to separate the species of the *C.* sect. *Androcallis* into five groups and those of the *C.* sect. *Calliandra* into 12 groups through the differentiation of the characteristics of appendage length, pollen pores, and exine ornamentation.

Calliandra exhibits evolutionary trends, such as those described before, as well as calymmate polyads (synapomorphic) and the lowest number of pollen grains per polyad among neotropical species of this tribe. Molecular analysis including this genus and its sister groups (*Zapoteca*, *Inga*, *Abarema*, *Albizia*, *Samanea*, *Enterolobium*) indicate remarkable differences in pollen morphology and recent diversification of the genus *Calliandra* (Souza et al. 2013).

The present study aimed to analyze pollen units of *Calliandra* species of *C.* sect. *Androcallis* Barneby, *C.* sect. *Microcallis* Barneby, and *C.* sect. *Monticola* E.R. Souza et L.P. Queiroz, both native and ornamental, occurring in northeastern Brazil, to provide subsidies for ecology and taxonomic studies of the genus.

Material and methods

Twelve species and two varieties of the *Calliandra* sect. *Androcallis* (46% of the total extant species), four of the *C.* sect. *Microcallis* (100% of the species), and nine of the *C.* sect. *Monticola* (25% of the species) were studied to obtain pollen morphometric data to characterize them into different sections present in northeastern Brazil. The species and varieties studied corresponded to 38% of the total number of species present in the northeastern region (Figure 1, Table I).

Pollen grains used in the present study were primarily collected from *in natura* flower buds. However, for *Calliandra subspicata* and *C. lanata*, materials from the PEUFR herbarium of the Federal Rural University of Pernambuco were used. At first, the hydration technique for flower buds was applied, with water and glycerin solution (95:1) (Santos & Romão 2008). For the light microscopy (LM) analysis, pollen samples were processed using

the acetolysis method (Erdtman 1952), and pollen grains were measured up to seven days after slide preparation (Salgado-Labouriau 1973). Twenty-five pollen grains were measured per sample, considering the length and width of each dispersal unit (polyad), length of the apical and central pollen grains of the polyad, presence or absence of the appendage on the apical pollen grain, and appendage length. Measurements were obtained using the Adobe Photoshop CS5 program, after calibration of the scales based on images of the polyads in general view. Pollen terminology follows Punt et al. (2007). The statistical analyses include the median (M), arithmetic mean (x), mean standard deviation (s_x), sample standard deviation (s), coefficient of variability (V%), and 95% confidence interval (CI). The data observed in the dispersal units (polyads) of the different species studied were run using the BioEstat software version 5.3 (Ayres et al. 2007).

Results

General Description

Quantitative and qualitative data (Tables II, III) obtained from the *Calliandra* species revealed that polyads are asymmetric, large to giant, formed by eight pollen grains (two central, six peripheral). Central pollen grains were morphologically different from peripheral pollen grains, rence of pollen heteromorphism (Figure 2). Polyads had an ellipsoid shape with an acute apex (Figures 2, 3), 4–5 porate, and were calymmate. Pollen grains arrangement in the polyad was uniplanar (Figures 4B, D, F; 5H-I; 7G-I).

Calliandra sect. *Androcallis* Barneby

Species in this section displayed very large to giant polyads. Some species showed a pore in the most acute region of the apical pollen grain. Polyads of this section showed the most significant variation in size (Table II) when compared to other sections studied. *Calliandra macrocalyx* var. *macrocalyx* (Table II) showed the largest polyad among all species, measuring 250 μm x 150 μm, and *C. harrisii* (Table II), the smallest polyad in this section, with 120 μm x 80 μm.

Based on polyad length, the 12 species and the two varieties studied were divided into three distinct groups. The first group included polyads with length ≥ 200 μm and comprised the species *Calliandra dysantha*, *C. macrocalyx* var. *aucta*, and *C. macrocalyx* var. *macrocalyx*. The second group included the polyads considered intermediate-sized in this

section, which showed length values < 200 μm and > 150 μm, and comprised the species *C. fernandesii, C. subspicata, C. imperialis, C. riparia, C. sessilis, C. ulei*, and *C. umbellifera* (Table II). The smallest polyads formed the third group, composed of the species *C. harrisii*, *C. spinosa*, and *C. blanchetti*, with length values < 150 μm. The largest apical pollen grain in length was recorded in *C. macrocalyx* var. *macrocalyx*, measuring 100 μm, and the smallest in *C. blanchettii* and *C. harrissi*, measuring 45 μm in both species. The presence of the pore in the acute region of apical pollen grains was observed only in *C. fernandesii*, *C. harrisii*, *C. macrocalyx* var. *aucta*, *C. sessilis*, *C. spinosa*, and *C. blanchetti* (Figure 3).

In all species of this section, we detected the presence of two pollen grains in the central region of the polyads (Figure 4A, C, E). Their size varied; the largest axis value was recorded in *Calliandra macrocalyx* var. *macrocalyx* (50 μm) and the lowest in *C. blanchetti*, *C. harrisii*, and *C. spinosa* (25 μm). All species in this section showed a uniplanar arrangement in the organization of their polyads (Figure 4B, D, F).

Calliandra sect. *Microcallis* Barneby

This section showed a slight variation in polyad length, with values < 150 μm and the smallest polyads of the present study (Table II). *Calliandra depauperata* showed the largest polyad of the section with a median value of 132.5 μm in length, whereas the mean values found for *C. aeschynomenoides*, *C. parviflora*, and *C. leptopoda* were 125 μm, 122.5 μm, and 117.5 μm, respectively (Table II). The values of the polyad width ranged from 62.5 μm to 75 μm and represented the smallest values found for this characteristic among the three sections studied (Table II).

The largest apical pollen grain occurred in *Calliandra depauperata*, with 52.5 μm in length, whereas the smallest values were found in *C. aeschynomenoides* and *C. leptopoda*, both measuring 47.5 μm (Table II). Width/length ratio values in this section varied from ≤ 0.60 μm to ≥ 0.50 μm; there was a higher variation in other sections (Table II). *Calliandra aeschynomenoides*, *C. leptopoda*, and *C. parviflora* showed a pore in the acute region of apical pollen grains (Figure 5, arrows in E-G). All species showed two pollen grains in the central region, with values of 25 μm in axis for *C. aeschynomenoides* and 22.5 μm for the other three species. All species showed a uniplanar polyad arrangement (Figure 5H-I).

Calliandra sect. *Monticola* E.R. Souza et L.P.Queiroz

In this section, polyads were large to giant, and the species showed an appendix located in the acute region of the apical pollen grain (Figure 6), without pores in this region (Figure 7A-C). The species were separated into two groups, according polyad length values:

Calliandra viscidula, C. hirtiflora, C. longipinna, C. calycina, C. hygrophila, C. asplenioides, and *C. lanata,* with values < 200 μm and > 150 μm (Figure 6A-B, E-I); and *C. bahiana* and *C. erubescens* with polyads > 200 μm (Figure 6C-D). The largest polyad length value was found in *C. erubescens* (205 μm), and the lowest, in *C. longipinna* (162.5 μm).

Polyad width varied within the section, with the lowest value found in *Calliandra hygrophila* (92.5 μm) and the largest in *C. viscidula* (130 μm; Figure 6A, G). The length of the apical pollen grain of the polyads also varied, ranging from 55 μm in *C. longipinna* to 70 μm in *C. viscidula* (Table II). The lowest length value of this structure was observed in *C. viscidula* (17.5 μm), and the largest, in *C. asplenioides* (37.5 μm; Figure 6A-B). *Calliandra longipinna* showed the smallest axis value (32.5 μm) for the two central pollen grains and the largest was observed in *C. viscidula* and *C. lanata* (both with 40 μm) (Figure 7D-F).

Discussion

Data from palynological analyses allowed us to characterize and group the different species into the three sections, *Androcallis, Microcallis,* and *Monticola,* proposed by Souza et al. (2013), and differentiate these taxa within each section regarding their respective dimensions. Our results added information about species not studied so far and clarified dubious issues in the literature about the number of pollen grains per polyads of some species of the genus (Souza 2007; Freitas Cruz et al. 2018). In the most recent phylogenetic study (Souza et al. 2013), *Calliandra* sect. *Monticola* emerged as the first lineage to diverge, whereas *C.* sect. *Microcallis* and *C.* sect. *Androcallis* appeared as sister groups, in a more derived position. Besides molecular evidence, these relationships are supported by pollen morphology and the presence of an appendage in the apical grain in the *C.* sect. *Monticola* (Santos & Romão 2008), which was lost in species of other sections.

All taxa studied showed calymmate polyads with eight pollen grains each; these characters are homogeneous among different species of the genus, corroborating the studies by Guinet (1965, 1981) and Guinet and Hernández (1989). Among the differences found in the three sections, the presence of the appendage in the acute region of the apical pollen grain stood out in the nine species of the *Calliandra* sect. *Monticola*, corroborating the study by Santos and Romão (2008), who analyzed 14 species of this section still classified in the circumscription of *Calliandra* sect. *Calliandra*. According to Leython and Ruiz-Zapata (2014), the main differences found in polyads characterize morphological variables to distinguish different groups at the infrageneric level. They highlighted the importance of the number of pollen grains, the shape of the polyad, and the presence or absence of the

appendage for taxonomic, ecological, and evolutionary studies of *Calliandra* species. Complementing the data obtained by Leython and Ruiz-Zapata (2014), our results showed the importance of also using morphological characteristics, including descriptive statistics data of the pollen in taxonomic studies to differentiate *Calliandra* species.

The appendage on apical pollen grains was found exclusively in species of the *Calliandra* sect. *Monticola*. Leython and Ruiz-Zapata (2014) observed the presence of a structure considered as a rudimentar appendage on specimens of *Calliandra coriacea* (Humb. et Bonpl. ex Willd.) and *C. cruegeri* Griseb. from Venezuela. However, the origin of this structure remains unclear and it was not possible to confirm whether it is homologous to the appendage found in *Calliandra* sect. *Monticola*. The presence of adhesive substances may suggest that this appendage could play a role in pollination, improving the adhesion of polyads to the stigma surface. Curiously, the apical pollen of species where this appendage is absent showed a pore for germination, as confirmed for species studied here. Investigation of this character may help in understanding the evolution of *Calliandra*. Our results corroborated the study by Santos and Romão (2008), who studied 21 species of the genus *Calliandra* present in the state of Bahia.

The most significant variation in polyad size found in the *Calliandra* sect. *Androcallis*, the smallest polyad size present in the *C.* sect. *Microcallis*, and the presence of the appendage in the *C.* sect. *Monticola* were characters that made the differentiation among sections possible and contributed to distinguishing species within sections. Polyad size and the presence or absence of the appendage on polyads were characters used as parameters to identify groups within the different sections of the genus *Calliandra*, as reported by Santos and Romão (2008), Buril et al. (2010), Souza et al. (2013), and Leython and Ruiz-Zapata (2014), and corroborated the present study.

There is heteromorphy among the pollen grains that form the polyads of *Calliandra* species; the two central pollen grains differ in size and shape from the six peripheral grains. Guinet (1965, 1981) described this character and observed it in all species of the three sections studied here. Leython and Ruiz-Zapata (2014) also described the presence of two central pollen grains in polyads of different *Calliandra* species while studying 13 species of the *Androcallis* and *Calliandra* sections present in Venezuela.

In the first palynological studies of this genus in Brazil, Barth and Yoneshigue (1966) observed eight pollen grains in polyads of the *Calliandra selloi* (Spreng.) J.F. Macbr. (synonym of *Calliandra seleri* Harms), the same pattern observed in the present study. However, Souza (2007) and Souza et al. (2013) reported only seven pollen grains in

polyads. Freitas Cruz et al. (2018), though, mentioned ten pollen grains in the polyads of *C. harrisii*. Such variations do not reflect the correct palynological pattern reported by Guinet (1965), which was corroborated in the present study by species in *C*. sect. *Microcallis* and *C*. sect. *Androcallis*.

The comparison between values of polyad measurements in the different sections corroborated the data reported by Santos and Romão (2008). The *Calliandra* sect. *Monticola* is differentiated by the presence of the apical appendage in all species. Leython and Ruiz-Zapata (2014) reported that the apical pollen grain of polyads was also a differentiating element in shape, size, and presence or absence of the appendage; thus, it is a structure that favors differentiating taxa and contributes significantly to the taxonomy of the genus.

Santos and Romão (2008) stated that the presence of the pore in the acute region of the apical pollen grain of polyads was exclusive to the *Calliandra* sect. *Androcallis*. However, after analyzing a more significant number of species in the *C*. sect. *Microcallis* in the present study, it became clear that the presence of this structure occurs in more than one section. This fact was also observed by Leython and Ruiz-Zapata (2014) in species of the *C*. sect. *Calliandra*.

Conclusion

Combined observation on pollen morphology and data of descriptive statistics analyses provided a better understanding of the pollen variation in each species studied. Polyad morphology data allowed us to distinguish species of the *Calliandra* sect. *Monticola* among all sections studied. For example, in *C*. sect. *Androcallis* and *C*. sect. *Monticola*, the polyads were larger in length and width than in the species studied in *C*. sect. *Microcallis*. In this last section, the remarkable character is the presence of the appendage in the most acute region of the apical pollen grain.

Palynotaxonomy is a promising botanical branch to check the variation at infrageneric levels in *Calliandra* and other genera, adding characteristics for the taxonomic treatment of different taxa.

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APÊNDICES

Tables

Table I. Studied species of *Calliandra* sections *Androcallis*, *Microcallis* and *Monticola* from north-eastern Brazil

Section	Species	Origin	Voucher Number	Municipality/State
Androcallis	*C. blanchetii* Benth.	S13°24'52.48" and W41°16'57.82"	HUEFS 239383	Ibicoara - Bahia
	C.dysantha Benth.	S09°51'57,60"and W45°13'02,01"	HUEFS 234497	Gilbués - Piauí
	C. fernandesii Barneby	S03°09'59,88"and W41°51'11,46"	HUEFS 221216	Buriti dos Lopes -Piauí
	C. harrisii (Lindl.) Benth.	S07°58'37,20" and W43°02'19,60"	HUEFS 221217	Canto do Buriti - Piauí
	C. imperialis Barneby	S08°12'58,30" and W42°46'40,50"	HUEFS 216093	Brejo do Piauí - Piauí
	C. macrocalyx Harms var. *aucta* Barneby	S08°10'35,95" and W43°16'07,53"	HUEFS 216092	Canto do Buriti -Piauí
	C. macrocalyx var. *macrocalyx* Harms	S08°15'23,30" and W42°25'09,40"	HUEFS 216089	São João do Piauí -Piauí
	C. riparia Pittier	S05°02'35,70"and W42°47'01,40"	HUEFS 231873	Teresina – Piauí
	C. sessilis Benth.	S08°00'32,10"and W42°56'42,40"	HUEFS 216095	Pajeú do Piauí - Piauí
	C. spinosa Ducke	S08°34'17,60" and W41°22'27,30"	HUEFS 231872	Queimada Nova - Piauí
	C. subspicata Benth.	S08°27'03.85" and W36°58'48,04"	PEUFR 661	Arcoverde - Pernambuco
	C. ulei Harms	S08°06'56,10"and W42°56'55,90"	HUEFS 216094	Canto do Buriti - Piauí
	C. umbellifera Benth.	S08°33'32,60" and W42°47'30,70"	HUEFS 216096	S. Raimundo Nonato - Piauí
Microcallis	*C. aeschynomenoides* Benth.	S08°31'38.94" and W37°15'13.55"	HUEFS 239384	Buique - Pernambuco
	C. depauperata Benth.	S09°02'33.50" and W42°40'37,70"	HUEFS 221221	S. Raimundo Nonato - Piauí
	C. leptopoda Benth.	S08°04'00,50" and W42°57'43,10"	HUEFS 231871	Canto do Buriti - Piauí
	C. parviflora Benth.	S09°49'54,0" and W45°20'38,0"	HUES 239387	Gilbués - Piauí
Monticola	*C. asplenioides* (Nees) Renvoize	S13°19'17.76" and W41°9'1.41"	HUEFS 239378	Ibicoara -Bahia
	C. bahiana Renvoize	S13°25'44.96" and W41°17'0.90"	HUEFS 234882	Ibicoara -Bahia
	C. calycina Benth.	S13°23'40.00" and W41°13'39,00"	HUEFS 239380	Ibicoara -Bahia
	C. erubescens Renvoize	S13°25'27.40" and W41°16'43.50"	HUEFS 234883	Ibicoara -Bahia
	C. hirtiflora Benth.	S13°26'56.72" and W41°17'6.75"	HUEFS 234880	Ibicoara -Bahia
	C. hygrophila Mackinder & G.P.Lewis	S13°19'17.76" and W41° 9'1.41"	HUEFS 239379	Ibicoara -Bahia
	C. lanata Benth.	S13°33'52,15" and W41°49'57,56"	PEUFR34422	Rio de Contas-Bahia
	C. longipinna Benth.	S13°25'27.40" and W41°16'43.50"	HUEFS 239381	Ibicoara -Bahia
	C. viscidula Benth.	S13°19'17,76" and W41° 9'1,41"	HUEFS 234879	Ibicoara -Bahia

Table II. Polyad sizes (μm) of north-eastern Brazilian species of *Calliandra* sections *Androcallis*, *Microcallis*, and *Monticola*. (-M = the lowest value found for the variable; M = median value for the variable; +M = highest value found for the variable).

Section	Species	Polyad						W/L (μm)	Apical pollen grain length (μm)			Appendage length (μm)			Axis of the central pollen grains (μm)			Axis of the central pollen grain/ width (μm)
		Length (L) (μm)			Width (W) (μm)													
		-M	M	+M	-M	M	+M		-M	M	+M	-M	M	+M	-M	M	+M	
Androcallis	*C. blanchetii* Benth.	107.5	(125)	137.5	72.5	(77.5)	87.5	0.62	40	(45)	47.5	---	---	---	22.5	(25)	27.5	0.32
	C. dysantha Benth.	205	(225)	245	125	(137.5)	150	0.61	67.5	(87.5)	107.5	---	---	---	35	(40)	47.5	0.29
	C. fernandesii Barneby	160	(175)	187.5	110	(122.5)	127.5	0.70	55	(65)	75	---	---	---	30	(37.5)	42.5	0.31
	C. harrisii (Lindl.) Benth.	110	(120)	125	75	(80)	87.5	0.67	42.5	(45)	50	---	---	---	20	(25)	30	0.31
	C. imperialis Barneby	172.5	(195)	210	112.5	(125)	132.5	0.64	70	(75)	87.5	---	---	---	37.5	(42.5)	50	0.34
	C. macrocalyx Harms var. *aucta* Barneby	200	(215)	225	130	(137.5)	145	0.64	72.5	(82.5)	95	---	---	---	40	(45)	50	0.33
	C. macrocalyx var. *macrocalyx* Harms	225	(250)	272.5	137.5	(150)	167.5	0.60	87.5	(100)	112.5	---	---	---	37.5	(50)	55	0.33
	C. riparia Pittier	160	(172.5)	190	80	(95)	112.5	0.55	50	(62.5)	72.5	---	---	---	25	(27.5)	35	0.29
	C. sessilis Benth.	142.5	(155)	167.5	90	(97.5)	122.5	0.61	50	(60)	70	---	---	---	25	(30)	35	0.31
	C. spinosa Ducke	130	(140)	147.5	72.5	(77.5)	85	0.55	45.5	(50)	57.5	---	---	---	22.5	(25)	27.5	0.32
	C. subspicata Benth.	172.5	(187.5)	197.5	112.5	(127.5)	142.5	0.68	57.5	(72.5)	85	---	---	---	37.5	(42.5)	50	0.33
	C. ulei Harms	175	(182.5)	192.5	107.5	(120)	125	0.66	62.5	(67.5)	77.5	---	---	---	27.5	(35)	37.5	0.29
	C. umbellifera Benth.	175	(185)	200	110	(120)	127.5	0.65	57.5	(65)	75	---	---	---	27.5	(37.5)	42.5	0.31
Microcallis	*C. aeschynomenoides* Benth.	112.5	(125)	132.5	65	(75)	80	0.60	42.5	(47.5)	55	---	---	---	25	(25)	27.5	0.33
	C. depauperata Benth.	122.5	(132.5)	145.0	57.5	(67.5)	72.5	0.51	45	(52.5)	72.5	---	---	---	20	(22.5)	25	0.33
	C. leptopoda Benth.	112.5	(117.5)	125	52.5	(65)	70	0.55	40	(47.5)	57.5	---	---	---	17.5	(22.5)	25	0.35
	C. parviflora Benth.	112.5	(122.5)	125	57.5	(62.5)	67.5	0.51	42.5	(50)	55	---	---	---	20	(22.5)	25	0.36
Monticola	*C. asplenioides* (Nees) Renvoize	172.5	(192.5)	200	100	(110)	120	0.57	55	(62.5)	75	30	(37.5)	42.5	32.5	(37.5)	40	0.34
	C. bahiana Renvoize	175	(200)	225	100	(112.5)	135	0.56	50	(57.5)	65	17.5	(25)	37.5	35	(37.5)	42.5	0.33
	C. calycina Benth.	150	(187.5)	197.5	100	(117.5)	125	0.63	50	(60)	62.5	30	(35)	37.5	35	(37.5)	40	0.32
	C. erubescens Renvoize	177.5	(205)	217.5	100	(110)	120	0.54	45	(62.5)	75	25	(35)	42.5	35	(37.5)	37.5	0.34
	C. hirtiflora Benth.	177.5	(192.5)	205	87.5	(97.5)	105	0.51	57.5	(62.5)	70	22.5	(35)	40	32.5	(35)	37.5	0.36
	C. hygrophila Mackinder & G.P.Lewis	162.5	(187.5)	202.5	82.5	(92.5)	117.5	0.49	45	(60)	72.5	25	(32.5)	37.5	32.5	(35)	40	0.38
	C. lanata Benth.	175	(195)	202.5	105	(117.5)	130	0.60	52.5	(60)	72.5	30	(32.5)	37.5	35	(40)	42.5	0.34
	C. longipinna Benth.	145	(162.5)	187.5	87.5	(100)	112.5	0.61	47.5	(55)	70	22.5	(30)	37.5	32.5	(32.5)	40	0.32
	C. viscidula Benth.	177.5	(190)	210	122.5	(130)	137.5	0.65	55	(70)	75	12.5	(17.5)	25	37.5	(40.0)	42.5	0.30

Table III. Measurements (µm) of *Calliandra* (polyad) in Length axis and Width axis using light microscopy.

Section	Species	Length axis					Width axis				
		CI –	($x \pm s_x$)	CI +	*s*	*V*%	CI –	($x \pm s_x$)	CI +	*s*	*V*%
Androcallis	*C. blanchetii* Benth.	120.87	(123.5 ± 2.63)	126.13	6.71	5.43	76.46	(78.0 ± 1.54)	79.54	3.94	5.05
	C. dysantha Benth.	219.36	(224 ± 4.64)	228.64	11.83	5.28	134.36	(137.2 ± 2.84)	140.04	7.26	5.29
	C. fernandesii Barneby	171.77	(174.4 ± 2.63)	177.03	6.72	3.85	119.24	(121.1 ± 1.86)	122.96	4.75	3.92
	C. harrisii (Lindl.) Benth.	117.56	(119.3 ± 1.74)	121.04	4.45	3.73	78.70	(80.1 ± 1.40)	81.50	3.57	4.45
	C. imperialis Barneby	190.01	(193.1 ± 3.09)	196.19	7.88	4.08	122.67	(124.6 ± 1.93)	126.53	4.93	3.95
	C. macrocalyx Harms var. *aucta* Barneby	210.69	(213.3 ± 2.61)	215.91	6.66	3.12	134.93	(136.5 ± 1.57)	138.07	4.00	2.93
	C. macrocalyx var. *macrocalyx* Harms	244.13	(248.2 ± 4.07)	252.27	10.38	4.18	148.02	(150.9 ± 2.88)	153.78	7.34	4.86
	C. riparia Pittier	170.39	(173.2 ± 2.81)	176.01	7.16	4.13	91.99	(94.3 ± 2.31)	96.61	5.90	6.25
	C. sessilis Benth.	153.86	(156.2 ± 2.34)	158.54	5.96	3.81	95.20	(97.5 ± 2.30)	99.80	5.87	6.02
	C. spinosa Ducke	136.84	(138.9 ± 2.06)	140.96	5.25	3.77	76.47	(77.9 ± 1.43)	79.33	3.65	4.68
	C. subspicata Benth.	183.68	(186.0 ± 2.32)	188.32	5.92	3.18	121.59	(124.8 ± 3.21)	128.01	8.18	6.55
	C. ulei Harms	180.04	(182.5 ± 2.46)	184.96	6.28	3.44	117.62	(119.3 ± 1.68)	120.98	4.27	3.57
	C. umbellifera Benth.	185.12	(187.7 ± 2.58)	190.28	6.59	3.51	116.84	(118.9 ± 2.06)	120.96	5.25	4.41
Microcallis	*C. aeschynomenoides* Benth.	120.16	(122.6 ± 2.44)	125.04	6.22	5.07	72.43	(73.9 ± 1.47)	75.37	3.75	5.07
	C. depauperata Benth.	128.94	(131.4 ± 2.46)	133.86	6.29	4.78	64.22	(65.7 ± 1.48)	67.18	3.78	5.75
	C. leptopoda Benth.	116.89	(118.3 ± 1.41)	119.71	3.59	3.03	62.38	(63.8 ± 1.42)	65.22	3.61	5.65
	C. parviflora Benth.	119.44	(121.1 ± 1.66)	122.76	4.25	3.50	61.69	(62.4 ± 0.71)	63.11	1.80	2.88
Monticola	*C. asplenioides* (Nees) Renvoize	188.02	(190.4 ± 2.38)	192.78	6.07	3.18	109.00	(110.7 ± 1.70)	112.40	4.33	3.91
	C. bahiana Renvoize	196.74	(201.3 ± 4.56)	205.86	11.64	5.78	110.83	(114.1 ± 3.27)	117.37	8.33	7.30
	C. calycina Benth.	180.17	(184.3 ± 4.13)	188.43	10.52	5.70	112.25	(114.8 ± 2.55)	117.35	6.52	5.67
	C. erubescens Renvoize	199.44	(203.5 ± 4.06)	207.56	10.37	5.09	107.25	(109.5 ± 2.25)	111.75	5.74	5.24
	C. hirtiflora Benth.	191.74	(194.5 ± 2.76)	197.26	7.04	3.61	96.09	(97.7 ± 1.61)	99.31	4.12	4.21
	C. hygrophila Mackinder & G.P.Lewis	183.55	(187.3 ± 3.75)	191.05	9.56	5.10	91.02	(93.9 ± 2.88)	96.78	7.35	7.82
	C. lanata Benth.	190.24	(193.0 ± 2.76)	195.76	7.04	3.64	114.22	(116.9 ± 2.68)	119.58	6.83	5.84
	C. longipinna Benth.	162.17	(166.0 ± 3.83)	169.83	9.77	5.88	97.73	(100.0 ± 2.27)	102.27	5.80	5.80
	C. viscidula Benth.	189.02	(191.6 ± 2.58)	194.18	6.59	3.43	113.67	(115.7 ± 2.03)	117.73	5.17	4.46

Note: Confidence interval (CI) at 95%, interval less (CI–) and interval larger (CI+), arithmetic mean (*x*), average standard deviation (s_x), sample standard deviation (*s*), coefficient of variability (*V*%).

Figure Captions

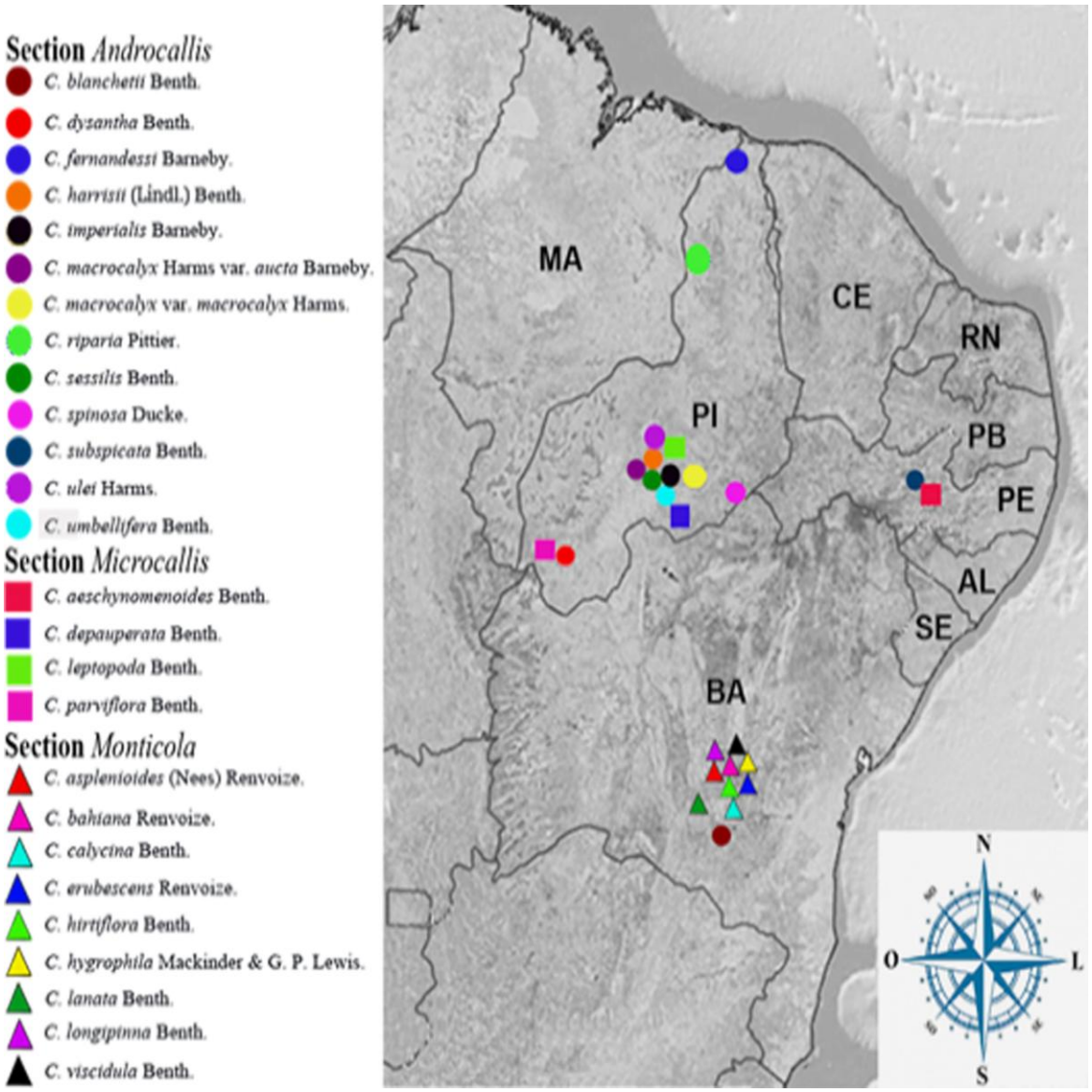

Figure 1. Distribution of species of *Calliandra* sect. *Androcallis*, *Calliandra* sect. *Microcallis*, and *Calliandra* sect. *Monticola* collected in northeastern Brazil.

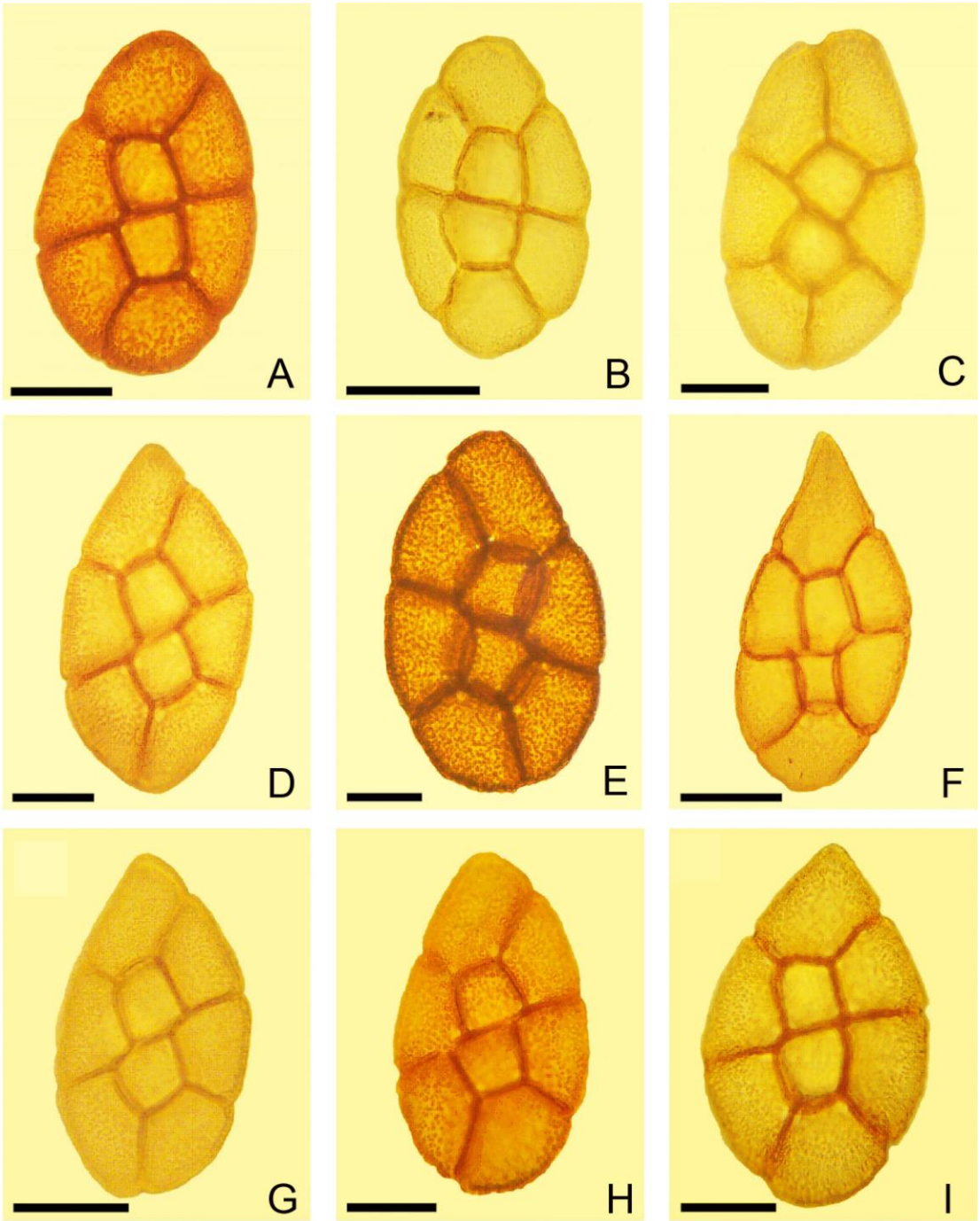

Figure 2. Polyads of species of *Calliandra* sect. *Androcallis*. **A**. *Calliandra fernandesii* Barneby, **B**. *Calliandra harrisii* (Lindl.) Benth., **C**. *Calliandra imperialis* Barneby, **D**. *Calliandra macrocalyx* Harms var. *aucta* Barneby, **E**. *Calliandra macrocalyx* var. *macrocalyx* Harms, **F**. *Calliandra riparia* Pittier, **G**. *Calliandra spinosa* Ducke, **H**. *Calliandra ulei* Harms, and **I**. *Calliandra umbellifera* Benth. Scale bars – 50 µm.

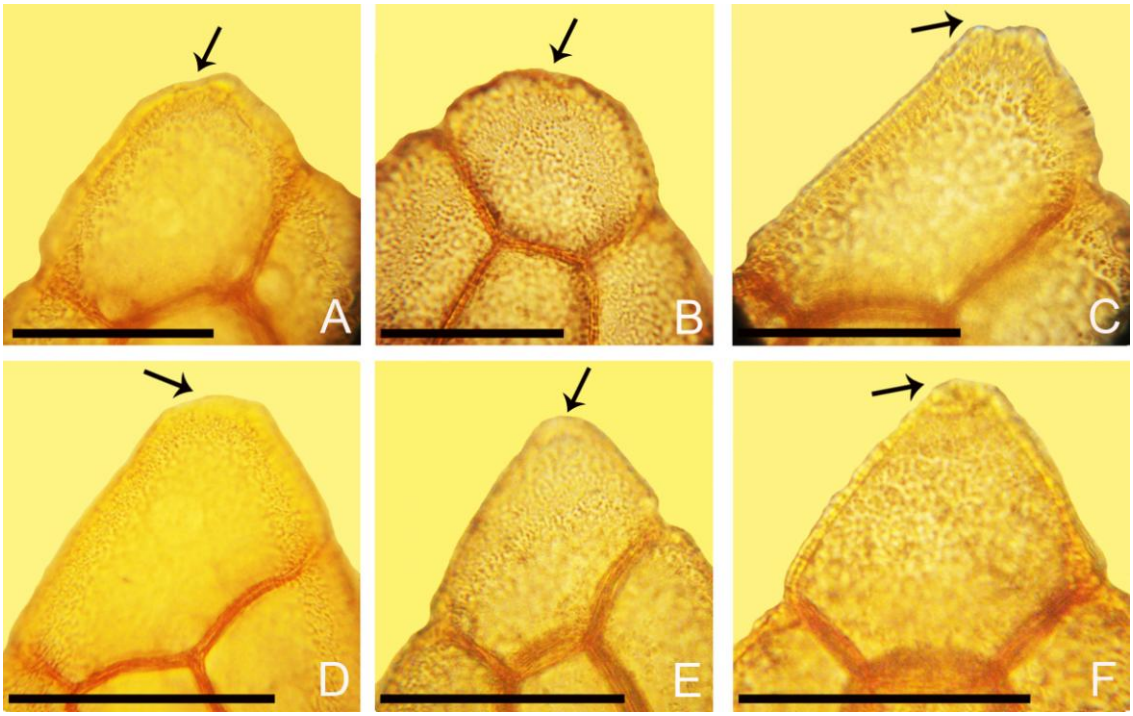

Figure 3. Photomicrographs of the apical pollen grain, showing the pore in the acute region of the polyads in species of *Calliandra* sect. *Androcallis*. **A**. *Calliandra fernandesii* Barneby, **B**. *Calliandra harrisii* (Lindl.) Benth., **C**. *Calliandra macrocalyx* Harms var. *aucta* Barneby, **D**. *Calliandra sessilis* Benth., **E**. *Calliandra spinosa* Ducke, and **F**. *Calliandra blanchetti* Benth. Note the pore in the acute region of apical pollen grains (arrows). Scale bars – 50 µm.

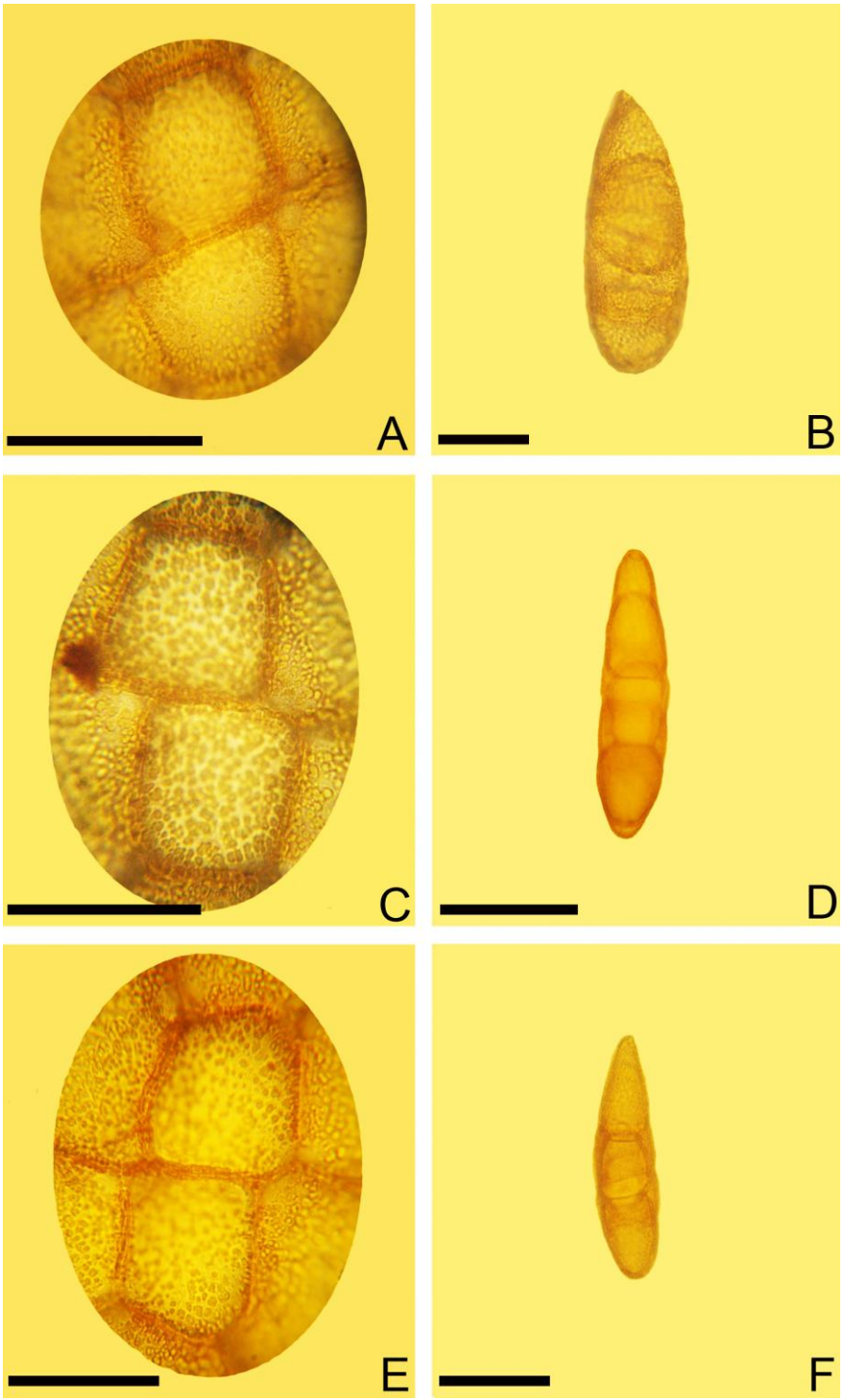

Figure 4. Photomicrographs of the central pollen grains and uniplanar polyad arrangement in species of *Calliandra* sect. *Androcallis*. Central pollen grains: **A**. *Calliandra fernandesii* Barneby, **C**. *Calliandra ulei* Harms, and **E**. *Calliandra umbellifera* Benth. Uniplanar arrangement: **B**. *Calliandra harrisii* (Lindl.) Benth., **D**. *Calliandra sessilis* Benth., and **F**. *Calliandra spinosa* Ducke. Scale bars – 50 µm.

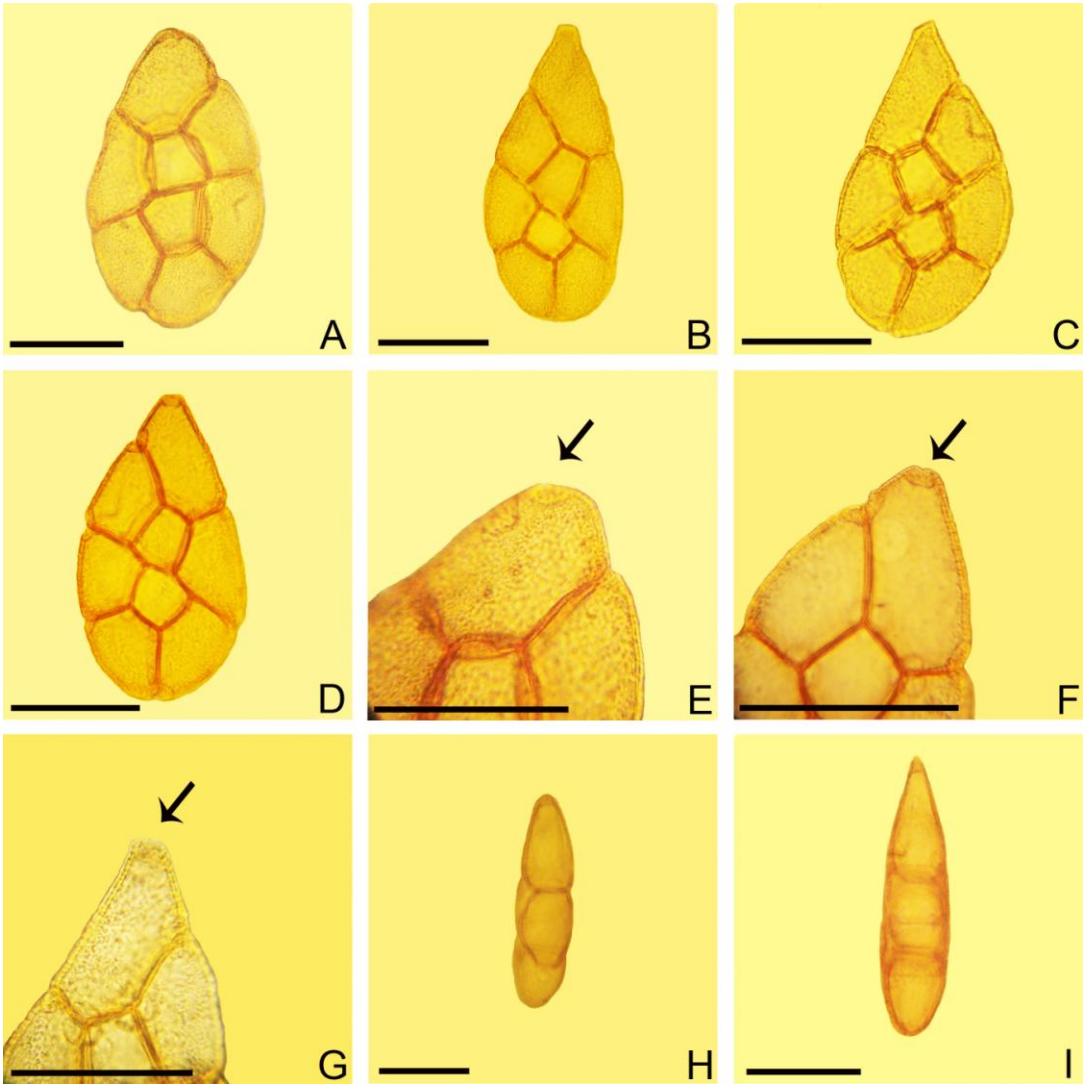

Figure 5. Photomicrographs of the dispersal unit in species of *Calliandra* sect. *Microcallis:* Polyads: **A**. *Calliandra aeschynomenoides* Benth., **B**. *Calliandra depauperata* Benth., **C**. *Calliandra parviflora* Benth., and **D**. *Calliandra leptopoda* Benth. Pore in the acute region of apical pollen grains (arrows): **E**. *Calliandra aeschynomenoides* Benth., **F**. *Calliandra leptopoda* Benth., and **G**. *Calliandra parviflora* Benth. Uniplanar arrangement of the polyad: **H**. *Calliandra leptopoda* Benth., and **I**. *Calliandra parviflora* Benth. Scale bars – 50 µm.

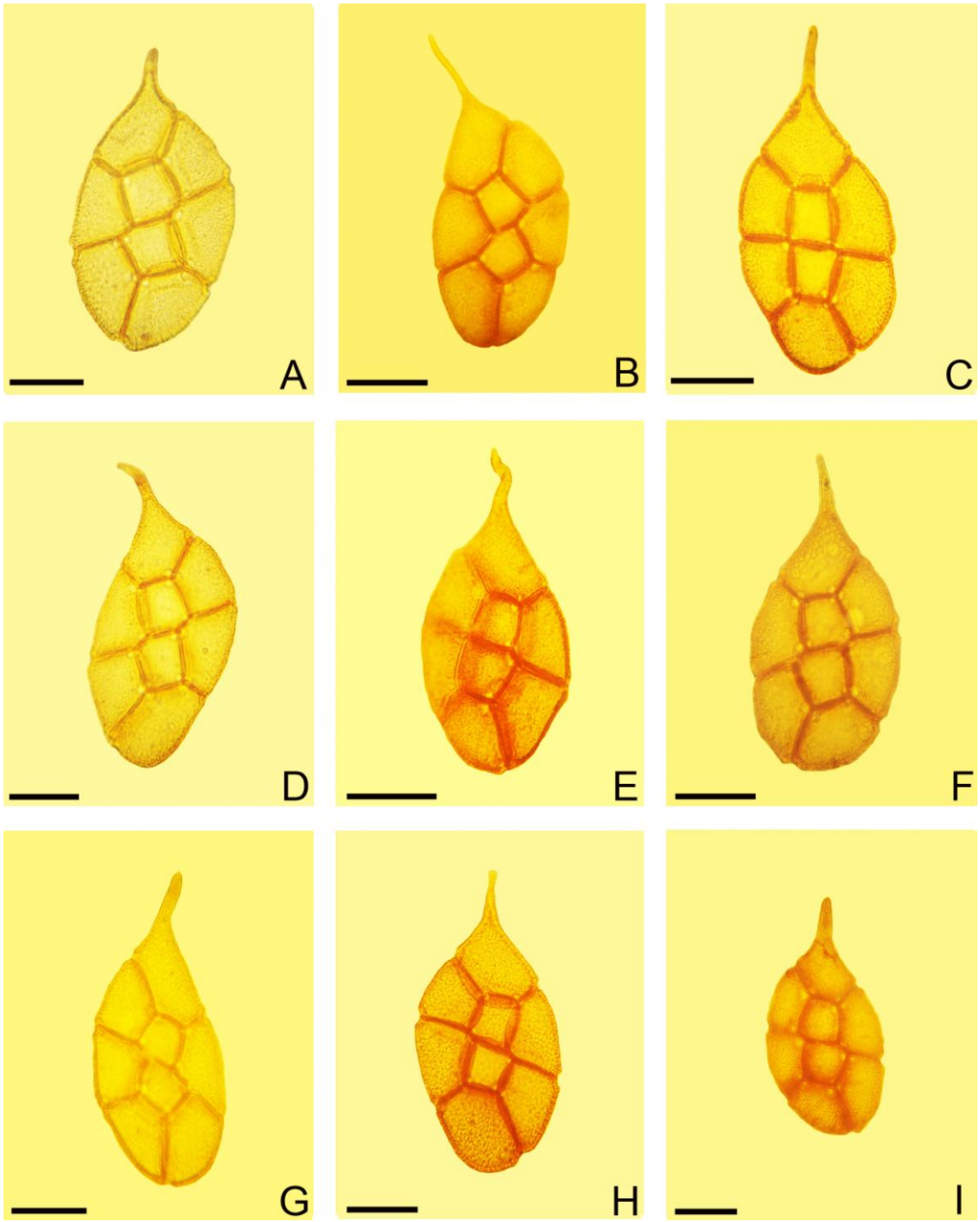

Figure 6. Photomicrographs of polyads in species of *Calliandra* sect. *Monticola*. **A**. *Calliandra viscidula* Benth., **B**. *Calliandra hirtiflora* Benth., **C**. *Calliandra bahiana* Renvoize, **D**. *Calliandra erubescens* Renvoize, **E**. *Calliandra longipinna* Benth., **F**. *Calliandra calycina* Benth., **G**. *Calliandra hygrophila* Mackinder et G.P. Lewis, **H**. *Calliandra asplenioides* (Nees) Renvoize, and **I**. *Calliandra lanata* Benth. Scale bars – 50 µm.

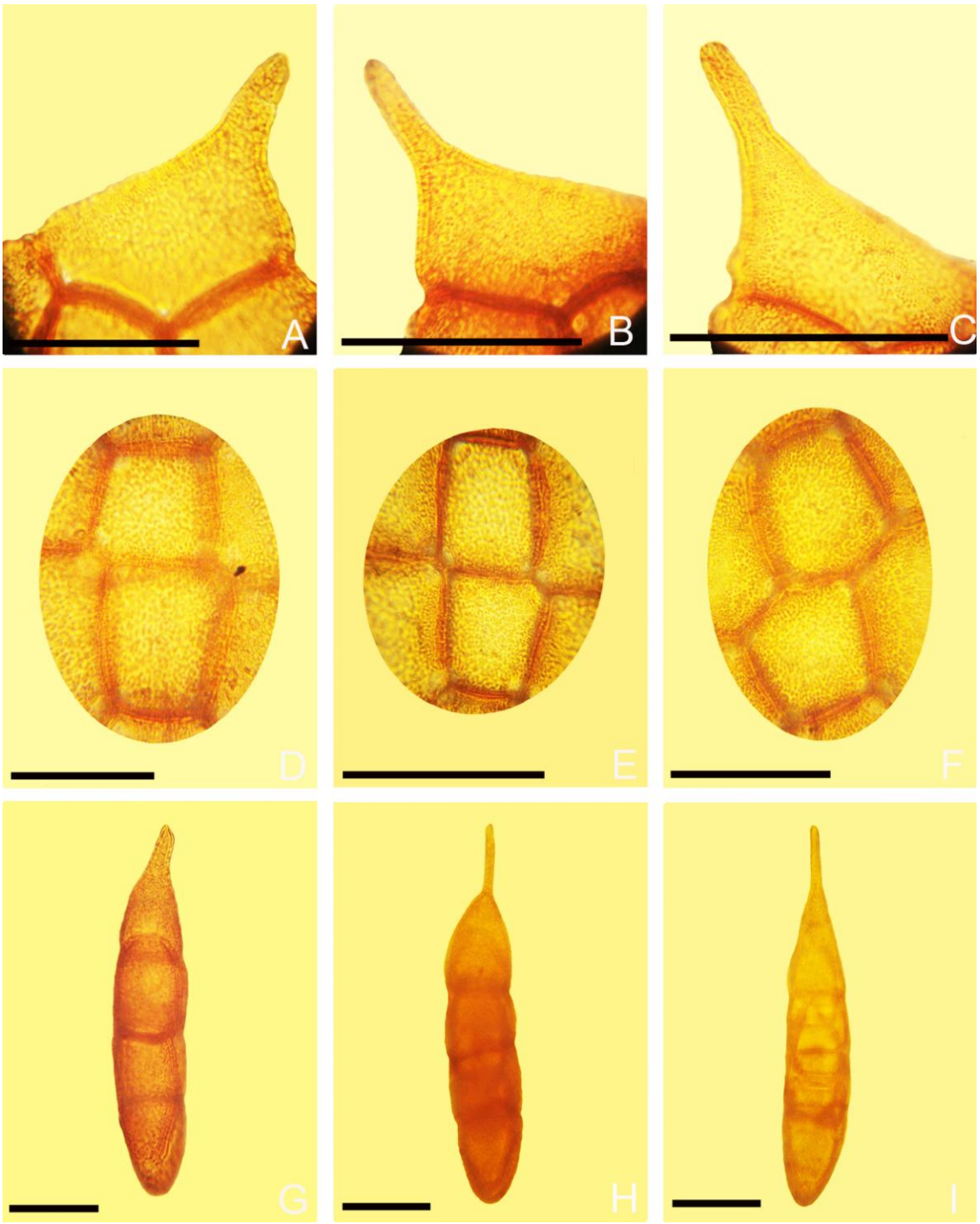

Figure 7. Photomicrographs of apical pollen grains with the presence of the appendage, central pollen grains, and uniplanar arrangement of the polyads in species of *Calliandra* sect. *Monticola*. Apical pollen grains with the presence of the appendage: **A**. *Calliandra viscidula* Benth., **B**. *Calliandra asplenioides* (Nees) Renvoize, and **C**. *Calliandra hygrophila* Mackinder et G.P. Lewis. Central pollen grains: **D**. *Calliandra viscidula* Benth., **E**. *Calliandra bahiana* Renvoize, and **F**. *Calliandra hygrophila* Mackinder et G.P. Lewis. Uniplanar arrangement: **G**. *Calliandra viscidula* Benth., **H**. *Calliandra asplenioides* (Nees) Renvoize, and **I**. *Calliandra hygrophila* Mackinder et G.P. Lewis. Scale bars – 50 µm.

MANUSCRITO II

KARYOTYPIC VARIABILITY IN *CALLIANDRA* BENTH. SECT. *ANDROCALLIS* (LEGUMINOSAE - CAESALPINIOIDEAE)

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Running head: Karyotypic variability in *Calliandra*

KARYOTYPIC VARIABILITY IN *CALLIANDRA* BENTH. SECT. *ANDROCALLIS* (LEGUMINOSAE - CAESALPINIOIDEAE)

ANTÔNIO DE PÁDUA DE OLIVEIRA PAULA[3], GENIALDO RAMOS DOS SANTOS[1], LUCAS COSTA[3], RAFAEL PESTANA[3], GUSTAVO SOUZA[3], GARDENE MARIA DE SOUSA[2], ANA VIRGÍNIA LEITE[1,4] & REGINALDO DE CARVALHO[1,4]

[1]Programa de Pós-Graduação em Botânica da Universidade Federal Rural de Pernambuco, Recife, PE. [2]Departamento de Biologia – Centro de Ciências da Natureza, Universidade Federal do Piauí, Teresina, PI. [3]Laboratório de Citogenética e Evolução Vegetal, Universidade Federal de Pernambuco, Brasil. [4]Departamento de Biologia da Universidade Federal Rural de Pernambuco.Brasil.

ABSTRACT

Calliandra Benth. is a neotropical genus composed of 139 species with distribution in North, Central and South America. In this work, a comparative cytogenetic analysis of ten species of the *Androcallis* section was performed by double staining with base-specific chromomycin A3 (CMA) and 4 ', 6-diamidino- 2-phenylindole (DAPI). The DNA content of the species was also estimated by Flow Cytometry. This study revealed that the species *C. macrocalyx*, *C. riparia*, *C. imperialis*, *C. ulei*, *C. spinosa* and *C. umbellifera* are diploid with $2n = 2x = 16$, *C. fernandesii* is tetraploid with $2n = 4x = 32$, *C. sessilis* is octaploid with $2n = 8x = 64$ and *C. dysantha* is decaploid with $2n = 10x = 80$. This result revealed that polyploidy is one of the recurrent cytogenetic events in the evolution of the genus *Calliandra*. As for the number of CMA + bands, it was possible characterize each species. No AT-rich DAPI band was located on the chromosomes of the species. The results suggest that the chromosomal rearrangements and the polyploidy contributed to the evolution of the *Calliandra* species, which was reported by the cytotaxonomic characterization in the genus.

Keywords: Leguminosae, Cytotaxonomy, Polyploidy, CMA and DAPI, Caatinga, Ecotone.

Correspondence: Antônio de Pádua de Oliveira Paula, Departamento de biologia, Programa de Pós-graduação em Botânica (PPGB), Universidade Federal Rural de Pernambuco (UFRPE) Av.Dom Manoel de Medeiros s/n, CEP 52171-900, Recife, PE, Brazil. Email: apo.paula@hotmail.com

INTRODUCTION

Leguminosae represents the third largest family of angiosperms, comprising 19,500 species distributed in 770 genera (Lewis et al. 2005; 2013; LPWG 2013a). Considered a cosmopolitan family, legumes are of fundamental ecological importance and contribute to the formation of many biomes, mainly in tropical regions (Schrire et al. 2005a; b; Yahara et al. 2013). Several species of this family are considered of high economic value as source of proteins and edible oils, besides being source of several substances used in the pharmaceutical industry (LPWG 2017).

The new classification of the Leguminosae family is proposed from phylogenetic data based on the plastid region *mat*K, including information from 698 genera out of a total of 765 and about 20% of total family species (LPWG 2017). Previous studies enable well-supported groups with phylogenetic analyzes using various plastids (*trnL-F*, *trnD-T*, *rbcL*, *rps*16, *rpl*16) and nuclear loci (rDNA ITS, SucS) (LPWG, 2013a). This classification suggests the redistribution of the three former subfamilies into six subfamilies (Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae, Duparquetioideae and Papilionoideae), so that the support of monophyleticism is robustly confirmed for the new grouping. In the recent classification there is the formation of the Mimosoid clade inserted in the subfamily Caesalpinioideae, with the inclusion of the members of the old subfamily Mimosoideae. This new phylogenetic classification, with increasing numbers of subfamilies and the creation of the Mimosoid clade helped to transform Caesalpinioideae, previously considered paraphyletic, in a monophyletic group.

Calliandra Benth. is a neotropical genus composed of 139 species, belonging to the subfamily Caesalpinioideae, on the Mimosoid clade (LPWG 2017). Its species are distributed in three main centers of diversity: Northern Colombia and Venezuela, Mexican Plateau and Northeast of Brazil (Barneby 1998; Souza and Queiroz 2004; Lewis et al. 2005; Souza et al. 2013). About 66 species are present at the Brazilian Northeast region, occurring mainly in the caatinga, cerrado and ecotonal transition areas (Souza 2001; Lemos 2004; Queiroz 2009).

This genus is one of the representatives of the Ingeae Benth tribe. Barneby (1998), based on morphological characters, divided the genus into five sections: *Androcallis*, *Acistegia*, *Acroscias*, *Calliandra* and *Microcallis*. A more recent review using nuclear (ITS) and plastidial (*trn*L-F) molecular markers, in addition to palynological characters, divided the genus into the following sections: *Androcallis* (76 spp.), *Calliandra* (10 spp.), *Monticola* (37

spp.), *Septentrionales* (6 ssp.), *Tsugoideae* (4 ssp.) and *Microcallis* (6 spp.), with the *Androcallis* section concentrating the largest number of species among the five sections (Souza et al. 2013).

The genus *Calliandra* presents an ancestral basic chromosome number of $x = 8$ (Goldblatt and Davidse 1977; Goldblatt 1981a). On the other hand, $2n = 22$ and 26 and the secondary basic number $x = 11$ was proposed for populations of *C. confusa* Sprague & L. Riley (=*C. houstoniana* var. *calothyrsus*) (Meissner) Barneby, *C. calothyrsus* Meisn. and *C. depauperata* Benth., attributing disploidy events to the occurrence of these karyotypes (Goldblatt 1981a; Goldblatt 1981b cited by Hernández 1986; Santos et al. 2012).

The genus *Mimosa* L., also part of Mimosoid clade, presents ancestral basic number $x = 7$ and two other secondary basic numbers $x = 13$ and $x = 14$. Studies report the existence of a polyploid series of $2n = 2x = 26$, $2n = 3x = 39$, $2n = 4x = 52$, $2n = 6x = 78$ and $2n = 8x = 104$ and also the occurrence of species presenting ascending disploidy ($2n = 2x = 28$) (Coleman and Demenezes 1980; Goldblatt 1981a; Alves and Carvalho-Custódio 1983; Goldblatt 1984; Goldblatt and Johnson 1998; Seijo 1993; 1999; Seijo and Fernández 2001; Dahmer et al. 2011).

Different analyzes are used to define informative patterns among taxa in order to better classify their components and propose evolutionary hypotheses for the group. For example, cytogenetic studies demonstrate that there is a great variability in the distribution of heterochromatin in different species of the same genus and among different genera in the angiosperms (Merita et al. 2015; Shamurailatpam et al. 2015). Heterochromatin can be distributed as tandem sequences, forming blocks or bands in the chromosomes. The location and quantification of heterochromatin along the chromosomes can be used as a source of important information about the structure of chromosomes, dynamics of gene expression and understanding of the various chromosomal rearrangements (Corradini et al. 2007). The emergence of chromosomal variations and consequent increase in karyotypic diversity may influence various biological adaptations and favor the emergence of new species (Van-Lume et al. 2017).

The staining of heterochromatic regions with fluorochromes cromomycin A3 (CMA, for chromosomal regions rich in pairs of base guanine and cytosine) and 4 ', 6-diamidino-2-phenylindole (DAPI, for localization of regions rich in base pairs adenine and thymine), has shown good results in the analysis of karyotypes and the determination of specific chromosomal characteristics (Schweizer 1976; Costa Silva et al. 2015; Souza et al. 2016).

Cytogenetic studies in Ingeae demonstrate that polyploidy and dysploidy are the main mechanisms in the formation of new species and secondary basic numbers in the tribe (Lewis et al. 2005; Poggio et al. 2008). Figueiredo et al. (2014) analyzed thirteen species from six different sections of the genus *Inga* and confirmed the existence of a polyploid series with basic number $x = 13$ and a polyploid sequence of $2n = 2x = 26$, $2n = 4x = 52$ and $2n = 8x = 104$.

In this work, a comparative cytogenetic analysis was performed concerning the chromosome number, ploidy level and distribution of the heterochromatic AT and CG regions in ten *Calliandra* species belonging to the *Androcallis* section, using the CMA / DAPI technique. Therefore, the characterization of the different species of Calliandra by means of cytogenetic analyzes with the use of molecular markers allows new knowledge for the taxonomic studies and a better understanding of the evolutionary processes for the genus.

MATERIALS AND METHODS

Plant material

The species of *Calliandra* sect. *Androcallis* analyzed here were *C. dysantha* Benth., *C. fernandesii* Barneby, *C. harrisii* (Lindl.) Benth., *C. imperialis* Barneby, *C. macrocalyx* Harms, *C. sessilis* Benth., *C. spinosa* Ducke, *C. riparia* Pittier, *C. ulei* Harms and *C. umbellifera* Benth., all from the Northeast region of Brazil. The studied species were identified and deposited in the Herbarium of the State University of Feira de Santana (Figure 1, Table 1).

Cytogenetic analysis

Root tips obtained from seeds were used for the cytogenetic analysis. The seeds were germinated in Petri dishes, and the roots were collected, pre-treated and fixed according to Guerra and Souza (2002). For pre-treatment, 8-hydroxyquinoline (8HQ) at 2mM concentration was used for 4 hours at 18 °C. The material was then fixed in a solution of methanol and glacial acetic acid (3: 1) for 24 hours at room temperature and stored at -20 °C in a freezer until further analysis.

For the preparation of the slides for CMA/DAPI technique, the root tips were removed from the fixative, washed twice in distilled water for 5 minutes each and digested in 2% cellulose/20% pectinase enzymatic solution at 37 °C for 3 hours (Guerra and Souza 2002),

followed by cell dissociation and drip technique (Carvalho and Saraiva 1993). After three days of preparation, the slides were stained with 10µL of CMA_3 (0.1 mg/ml) for 1 hour and then with 10µL of DAPI (2ug/ml) for half an hour. Before observation, the slides were stored for three days at room temperature in a darkroom for stabilization of the fluorochromes (Guerra and Souza 2002).

The cells at prometaphases or metaphases of each species were captured by a DC 345FX camera attached to a Leica DM2500 fluorescence microscope for chromosome number, morphology, and band pattern analysis. For the morphological analysis of the chromosomes, the following characters were observed: long arm and short arm length, total chromosome length and haploid set total length according to Levan et al. (1964). The brightness and contrast commands were improved in Adobe Photoshop CS3 program. For counting of the chromosomes and CMA + bands, at least five cells with good chromosome scattering and good image resolution were analyzed. The chromosome measurements for the construction of the ideograms were obtained using the software IDEOKAR 1.2 (Mirzaghaderia and Marzangib 2015).

Flow Cytometry

For DNA content estimation, young leaves of the 10 *Calliandra* sect. *Androcallis* species were stored with humid paper and sent from the collect location to the Laboratory of Plant Cytogenetics and Evolution at the Universidade Federal de Pernambuco (Recife-PE). Different reference standard species were used due to either the difference between target and DNA content [it should not exceed three times as per Pellicer and Leitch (2014)] or overlapping of peaks (Table 2). Sample preparation was done according to Loureiro et al. (2007) with small modifications. Approximately 25-50 mg of fresh leaf tissue from the target-species were co-chopped simultaneously with the chosen reference standard in a Petri dish (kept on ice) containing 2 mL of *Woody Plant Buffer* (WPB). The sample was then filtered through a 30 µm disposable mesh filter (CellTrics, SYSMEX, Norderstedt, Germany) with following addition of 50 µg/mL propidium iodide (from a stock of 1 mg/mL; Sigma-Aldrich) and 50 µg/mL RNase. For most species, at least six samples were analyzed with a minimum of 1000 particles per run. Due to the quality of the leaves, in the case of *C. dysantha, C. sessilis* and *C.* riparia only 3 samples were analyzed. Samples were measured at two different laboratories both using a CyFlow Space flow cytometer (SYSMEX, Norderstedt, Germany) equipped with a green laser (532 nm). For the determination of the

mean fluorescence channels of the corresponding peaks usually histograms with linear scale were used.

The histograms of relative fluorescence were obtained using the software Flomax v.2.3.0. (SYSMEX, Norderstedt, Germany). Mean fluorescence and coefficient of variation was assessed at half of the fluorescence peak. The DNA content (2C) of the target species was calculated using the equation "2C target (pg) = (G1 target/G1 standard) x 2C standard (pg)". "G1" refers to the mean fluorescence value emitted by nuclei in the G1 stage of interphase and "2C standard" refers to the DNA content from somatic cell nuclei of the reference standard used in the measurement. We used image editing software SCS Adobe Photoshop CS3 and CorelDraw X7 to edit the histograms and to prepare a simple graphic to illustrate the DNA content found on the different ploidy levels.

RESULTS

The present study was based on the direct staining of chromosomes with fluorochromes CMA and DAPI in 10 species of *Calliandra* Benth. sect. *Androcallis*. The species *C. spinosa*, *C. imperialis*, *C. umbellifera* and *C. ulei* are distributed in the vegetation formation of Caatinga, *C. dysantha* is distributed in the Cerrado formation, *C. macrocalyx*, *C. harrisii*, *C. riparia* and *C. sessilis* in the transition area between Caatinga/Cerrado and *C. fernandesii* in Caatinga/Amazonian transition area.

Cytogenetic analysis revealed variation in the size and number of chromosomes, ploidy level, karyotype formula and distribution of CMA bands between species (Table 2). The diploid numbers observed were: $2n = 2x = 16$ for *C. umbellifera*, *C. imperialis*, *C. riparia*, *C. spinosa*, *C. macrocalyx*, *C. ulei* and *C. harrisii* (Figures 2A; B; C; D; E; F; H); $2n = 4x = 32$ for the tetraploid, *C. fernandesii* (Figure 2G); $2n = 8x = 64$ for the octaploid *C. sessilis* (Figure 2I) and $2n = 10x = 80$ for the decaploid *C. dysantha* (Figure 2J). With this, we were able to differentiate the 10 species into four distinct ploidy groups (Figure 2). All the chromosome numbers described in the present work are unpublished for the genus *Calliandra*.

The chromosomes were metacentric and submetacentric with prometaphase sizes ranging from 1.63 μm in *C. riparia* to 8.70 μm in *C. macrocalyx*, with mean size of 2.63 μm (Table 2). The karyotypic formulas were $2n$ = 15M + 1SM for *C. imperialis*, *C. riparia* and *C. ulei*, $2n$ = 14M + 2SM for *C. spinosa*, *C. harrisii* and *C. umbellifera*, $2n$ = 10M + 6SM for *C.*

macrocalyx, 2*n* = 31M + 1SM for *C. fernandesii*, 2*n* = 56M + 8SM for *C. sessilis* and 2*n* = 70M + 10SM for *C. dysantha*.

DNA content of the analysed *Calliandra* species are presented on Table 2. The histograms yielded by the flow cytometer presented acceptable quality, with mean CVs (coefficient of variation) below 5%. However, some leaf samples had to be sent via mail to the institute where the cytometer was located. Because of this, some species like *C. fernandesii* yielded very few nuclei when compared to the internal standards (Figure 3), mainly because of the quality of the leaves. The C-values ranged from 2C = 1.25 ± 0.03 pg on *C. spinosa* (2*n* = 16) to 2C =7.19 ± 0.14 pg in *C. dysantha* (2*n* = 80) (Table 2). All diploid species presented similar DNA content (1.25 ~ 1.62 pg), except for *C. macrocalyx*, which showed 2C = 2.96 ± 0.14 pg in concordance with its bigger chromosomes. The polyploids showed DNA content crescent to its ploidy level as showed on the graphic of Figure 3, this fact suggests that there was an increase in the DNA content for the different ploidy levels correlating them to the polyploidy events, this is observed for the tetraploid, *C. fernandesii* presenting 2C = 3.63 ±0.13 pg, the octaploid *C. sessilis* presenting 2C = 5.99 ± 0.08 pg and the decaploid *C. dysantha* presenting 2C = 7.19 ± 0.14 pg.

The CMA + bands number vary from 6 to 10 bands. *C. spinosa*, *C. ulei*, *C. harrisii* and *C. riparia* presented 6 CMA bands in 3 pairs of chromosomes, which were distributed in the terminal regions of the long arm of pairs I, III and VII of *C. spinosa*, II, IV and V of *C. ulei*, I, II and IV of *C. harrisii* and I, III and IV of *C. riparia*. *C. imperialis* presented 6 CMA bands, 4 bands located in the terminal position of the long arm of the chromosomal pairs II and IV and 2 bands in the terminal position of the short arm of the chromosomal pair VII. For *C. umbellifera*, we also observed the occurrence of 6 CMA bands, with 4 bands located in the terminal position of the long arm of pairs I and VII, and 2 bands in pair IV, with one band on the terminal position of the short arm of the chromosome the other on the long arm of the homologous pair.

The species *C. macrocalyx*, *C. fernandesii* and *C. sessilis* presented 8 CMA bands. In *C. macrocalyx*, 6 bands were found in the terminal position of the short arm of the pairs II, III and VII and 2 bands were in the terminal position of the long arm of pair VIII. For the other two species, all 8 CMA bands were located in the terminal position of the long arm of pairs I, III, VI, XI for *C. fernandesii* and III, VI, XVIII and XXII for *C. sessilis*.

The highest number of CMA positive bands was observed in *C. dysantha* with a total of 10 bands. 8 bands were located in the terminal position of the long arm of pairs I, III, V and

VIII. 2 bands showed a polymorphism in pair XI, with one band at the terminal position of short arm of one of the chromosomes and the other on the long arm of the homologous pair.

The presence of chromosomal polymorphism was verified in relation to the size of the CMA bands, size of the homologous chromosomes and of bands position between the chromosome arms (Figure 4). For the diploid species, polymorphisms were observed in relation to the size of the CMA bands for *C. umbellifera*, *C. riparia*, *C. spinosa*, *C. macrocalyx* and *C. harrisii*, occurring at pair I in the first three species, pair II for the last, pair III for the second and fourth, pair IV for the last and in pair V for the fifth species.

Differences in the size of the chromosomes of diploid species was also noted on four pairs of *C. spinosa* (I, II, VII and VIII), *C. ulei* (V, VI, VII and VIII) and *C. harrisii* (II, IV, V and VI) and in three pairs of *C. umbellifera* (I, IV and VI), *C. imperialis* (I, V and VII) and *C. riparia* (I, II and III). Only two pairs of *C. macrocalyx* (III and IV) presented size polymorphisms. Among these species, only *C. umbellifera* presented a polymorphism regarding the position of the CMA bands, occurring in pair IV.

Regarding the size of CMA bands of the polyploid species, polymorphisms were detected on pairs I, III and VI of *C. fernandesii* and *C. dysantha* and on pair XXII of *C. sessilis*. Regarding chromosome size, polymorphisms were observed on pairs I, III, IV and VI of *C. fernandesii*, pairs I, V, VIII, IX, XIV and XXII of *C. sessilis* and pairs I, II, III, IV, V, VI, XI, XXIV, XXVII and XXXVII of *C. dysantha*. Regarding the position of CMA bands, it was only observed on pair XI of *C. dysantha*.

DISCUSSION

The occurrence of multiple numbers of the basic number $x = 8$ observed for all analysed sect. *Androcallis* species confirms the occurrence of this basic number proposed by other authors for the genus *Calliandra* (Goldblatt and Davidse 1977; Goldblatt 1981a; Poggio et al. 2008; Santos et al. 2012). However, the results obtained by Santos et al. (2012) also propose disploidy to be one of the events in the evolution of this group. In our sampling, no species with disploid numbers were found.

It was possible to detect the existence of a polyploid series among species, all multiple of $x = 8$, demonstrating the occurrence of speciation related to whole genome duplication in *Calliandra*. The event of polyploidy produces new cytotypes with different ploidy level, creating reproductive barriers among populations, favouring the speciation process. The chromosome number ranged from $2n = 2x = 16$ in most species to $2n = 10x = 80$ in *C.*

dysantha. Polyploid series were also observed in the genus *Mimosa* and *Inga,* both representative of the clade Mimosoid. Notably, the *Inga* genus belongs to the Ingeae tribe, same as *Calliandra* (Seijo and Fernández 2001; Dahmer et al. 2011; Figueiredo et al. 2014), suggesting that polyploidy may be the main mechanism of evolution for the tribe.

Lewke Bandara *et al.* (2013) analyzing the phylogeny of the genus *Onobrychis* Mill belonging to the Hedysareae tribe of the subfamily Papilionoideae described the existence of chromosomal variation of $x = 7$, 8 and 14 within the sect. *Onobrychis* found in two species belonging to this genus. For these authors, the variation suggests the occurrence of hybridization between cultivated species or the existence of cryptic species with morphological similarity. On the contrary, the species analyzed in this study for the genus *Calliandra* present their chromosomal numbers with $x = 8$ or multiples thereof according to the repetitions and the number of cells observed, suggesting that evolution occurred by duplicating the total genome (WGD) and subsequent rearrangements chromosomal, since the species considered here diploid and polyploid have consistent morphological differences (Barneby 1998, Queiroz 2009) and different ecological niches.

The standard distribution of the CMA+ bands among related species in *Androcallis* section allowed a characterization according to the number and position of these sites. Structural or numerical changes revealed from the chromosomal heterochromatin distribution of a genus make it possible to distinguish species and evaluate their evolution (Guerra 2000). The distribution of heterochromatin for taxonomic purposes is another possible use of this technique. This can be used in intraspecific studies, as was the case in populations of *Mimosa caesalpiniifolia,* which showed cytotypes with different ploidy (Sousa et al. 2012). Interspecific studies can also be helped by heterochromatin distribution evaluation, such as in the case of *Peltophorum,* which presented same chromosome number and were differentiated by CMA+ banding (Van-Lume and Souza 2018). In another study, ten genera of the Caesalpinia clade could be characterized in three distinct groups according to CMA+ standard banding (Van-Lume et al. 2017). In all ten species studied, CMA+ heterochromatin revealed band size and number polymorphisms, proving to be an important indicator for karyotypic variation and evolution. This was also observed for the genera *Abelmoschus* and *Vigna* (Merita et al. 2015; Shamurailatpam et al. 2015). Notably, all CMA+ bands observed in the *Calliandra* sect. *Androcallis* species analyzed were located in the terminal regions. Other genera of Leguminoseae have shown variability in regards to the position of the bands

such as *Vigna* with terminal and interstitial bands (Shamurailatpam et al. 2015) and *Arachis* sect. *Heteranthae* with proximal bands (Silva et al. 2010).

Barros e Silva and Guerra (2010) studied chromosomal banding using DAPI/CMA fluorochromes for three plant species. In the comparison with C and Fish bandings, there was no equality between the number of bands present and the staining intensity for the banding with direct application for DAPI and the banding of this fluorochrome after using the C and Fish banding proposing that there is no specificy DAPI fluorochrome staining only for AT sequences, but also for other types of sequences possibly existing in DNA. The heterochromatic blocks correlated with NOR and stained by CMA with specificity for sites rich in GC showed similarity in presence and size to those seen for C and Fish banding. For the results obtained in species of the genus *Calliandra* sect. *Androcallis* analyzed in this study, the heterochromatic blocks stained with fluorochrome CMA are brighter when compared to those stained by DAPI. Except for the different sites described for CMA, the rest of the chromosomal complement was stained with DAPI, in this case there was no possibility to differentiate the intensity of staining and the distribution of DAPI bands between the observed chromosomes. Therefore, it is demonstrated that there is a better response in banding by CMA to characterize the analyzed species.

The variation in chromosome number, ploidy level, karyotype formula, distribution of CMA+ bands and DNA content allowed to characterize and differentiate all studied species. Thus, such results contribute to a better cytotaxonomic understanding for the genus, as it has been shown that the few of morphological characters is one difficulty in the identification of *Calliandra* species (Queiroz 2009). These species often present huge phenotypic plasticity as a result of their distribution among different habitats of dry forests (Almeida, Carvalho and Guerra 2007). In this case, the cytogenetic polymorphisms identified in this work to the identification of *Calliandra* sect. *Androcallis* with new informative characteristcs.

In the studied species, the variation in DNA content does not exactly follow the addition of the number of chromosomes according to their ploidy level. For example, *Calliandra sessilis* has 5.99 pg of DNA content, with a total haploid length of 19.469 µm, with the number $2n = 64$ chromosomes in this species considered an octoploid and the occurrence of eight CMA+ sites, whereas *Calliandra dysantha* has 7.19 pg of DNA content, with total haploid length of 21.088 µm, with number $2n = 80$ chromosomes considered a decaploid and presence of ten sites of CMA+. This fact shows that there were changes in the chromosomal number with the increase in the level of ploidy; however the DNA content and

the number of CMA+ sites did not increase proportionally, demonstrating that there was a loss of repetitive sequences and chromosomal rearrangements after the polyploidy event in the evolution of these species.

The occurrence of polyploidy events due to whole genome duplication (WGD), segmental duplication and tandem duplication in angiosperms with subsequent diploidization process favored the emergence of new lineages and the speciation process (Wolfe 2001; Conant et al. 2014). The loss of duplicate genes over time and the return to meiotic condition of chromosomes to bivalent (diploidization) from tetravalent chromosomes enabled the emergence of varieties with inter and intrachromosomal rearrangements (Wolfe 2001).In the polyploids analyzed here, the heterochromatic bands number, size and DNA content variations tended to increase with increased ploidy level. In angiosperms, a tendency of genome downsizing after events of duplication is often observed (Leitch and Bennett 2004). This may indicate that some of the polyploids analyzed here (except *C. fernandesii,* which showed the same number of bands of one of the diploid species) did not undergo diploidization yet, which could point to a relatively recent increase in ploidy level (Ramsey and Schemske 2002; Costa et al. 2017). However, further studies about the meiotic behavior of the chromosomes of the analyzed species is essential to investigate if these could be classified as neopolyploids.

In conclusion, in *Calliandra* section *Androcallis* evolution probably occurred due to polyploidy events associated with chromosomal rearrangements and amplification/reduction of heterochromatic regions. That is, several duplications of chromosome number, loss of duplicate post-polyploid genes and the process of diploidization and their combinations favored the formation of new species within this genus. This enabled the emergence of restructured genomes and consequently the diversification of the characters found for this section of the genus.

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Tables

Table 1. Analyzed species from *Calliandra* sect. *Androcallis* with their respectives collect locations and voucher number.

Specie	Collect location	Voucher number
C. macrocalyx Harms	São João do Piauí S8°15'23,30" /W42°25'09,40"	HUEFS 216089
C. spinosa Ducke	Queimada Nova S8°34'17,60" / W41°22'27,30"	HUEFS 231872
C. imperialis Barneby	Brejo do Piauí S8°12'58,30" / W42°46'40,50"	HUEFS 216093
C. umbellífera Benth.	São Raimundo Nonato S8°33'32,60" / W42°47'30,70"	HUEFS 216096
C. ulei Harms	Canto do Buriti S8°06'56,10" / W42°56'55,90"	HUEFS 216094
C. harrisii *(Lindl.)* Benth.	Canto do Buriti S7°58'37,20" / W43°02'19,60"	HUEFS 221217
C. riparia Pittier	Teresina S5°02'35,70" / W42°47'01,40"	HUEFS 231873
C. fernandesii Barneby	Buriti dos Lopes S3°09'59,88"/ W41°51'11,46"	HUEFS 221216
C. sessilis Benth.	Pajeú do Piauí S8°00'32,10"/ W42°56'42,40"	HUEFS 216095
C. dysantha Benth.	Gilbués S9°51'57,60"/ W45°13'02,01"	HUEFS 234497

Table 2. Analyzed species from *Calliandra* sect. *Androcallis* with their respectives chromosome number, chromosome formula, chromosome size, CMA^+ band number, chromosome pairs with CMA^+, chromosome arms containing CMA^+ bands, DNA content and reference standard used for DNA content measurements.

Specie	Diploid number	Chromosome formula	Chromosome size (µm)	Number of CMA^+ bands	Chromosome pairs with CMA^+ bands	Chromosome arms containing bands	DNA content	Reference Standard for DNA content measurements
C. macrocalyx Harms	2n = 16	10M+6SM	6,77 (8,70) 10,75	8	II, III , VII and VIII	Short Long	2.96 ± 0.14	*Raphanus sativus* cv. Saxa (1.11 pg)
C. spinosa Ducke	2n = 16	14M+2SM	1,97 (2,72) 3,36	6	I, III and VII	Long	1.25 ± 0.03	*Solanum lycopersicon* cv. Stupicke (1.96 pg)
C. imperialis Barneby	2n = 16	15M+1SM	1,91 (3,06) 3,40	6	II , IV and VII	Long Short	1.62 ± 0.03	*Glycine max* cv. Polanka (2.50 pg)
C. umbellifera Benth.	2n = 16	14M+2SM	1,72 (2,09) 2,49	6	I, VII and IV	Long Short/Long	1.49 ± 0.06	*Solanum lycopersicon* cv. Stupicke (1.96 pg)
C. ulei Harms	2n = 16	15M+1SM	2,25 (3,04) 3,75	6	II, IV and V	Long	1.58 ± 0.03	*Glycine max* cv. Polanka (2.50 pg)
C. harrisii *(Lindl.)* Benth.	2n = 16	14M+2SM	1,96 (2,68) 3,84	6	I, II and IV	Long	1.57 ± 0.07	*Glycine max* cv. Polanka (2.50 pg)
C. riparia Pittier	2n = 16	15M+1SM	1,18 (1,63) 2,36	6	I, III and IV	Long	1.25 ± 0.05	*Solanum lycopersicon* cv. Stupicke (1.96 pg)
C. fernandesii Barneby	2n = 32	31M+1SM	2,73 (3,40) 6,15	8	I, III, VI and XI	Long	3.63 ± 0.13	*Solanum lycopersicon* cv. Stupicke (1.96 pg)
C. sessilis Benth.	2n = 64	56M+8SM	1,43 (2,43) 3,64	8	III, VI, XVIII and XXII	Long	5.99 ± 0.08	*Hordeum vulgare* cv. Sultan (11.12 pg)
C. dysantha Benth.	2n = 80	70M+10SM	1,56 (2,64) 4,72	10	I, III, V,VIII and XI	Long Short/Long	7.19 ± 0.14	*Hordeum vulgare* cv. Sultan (11.12 pg)

Figures

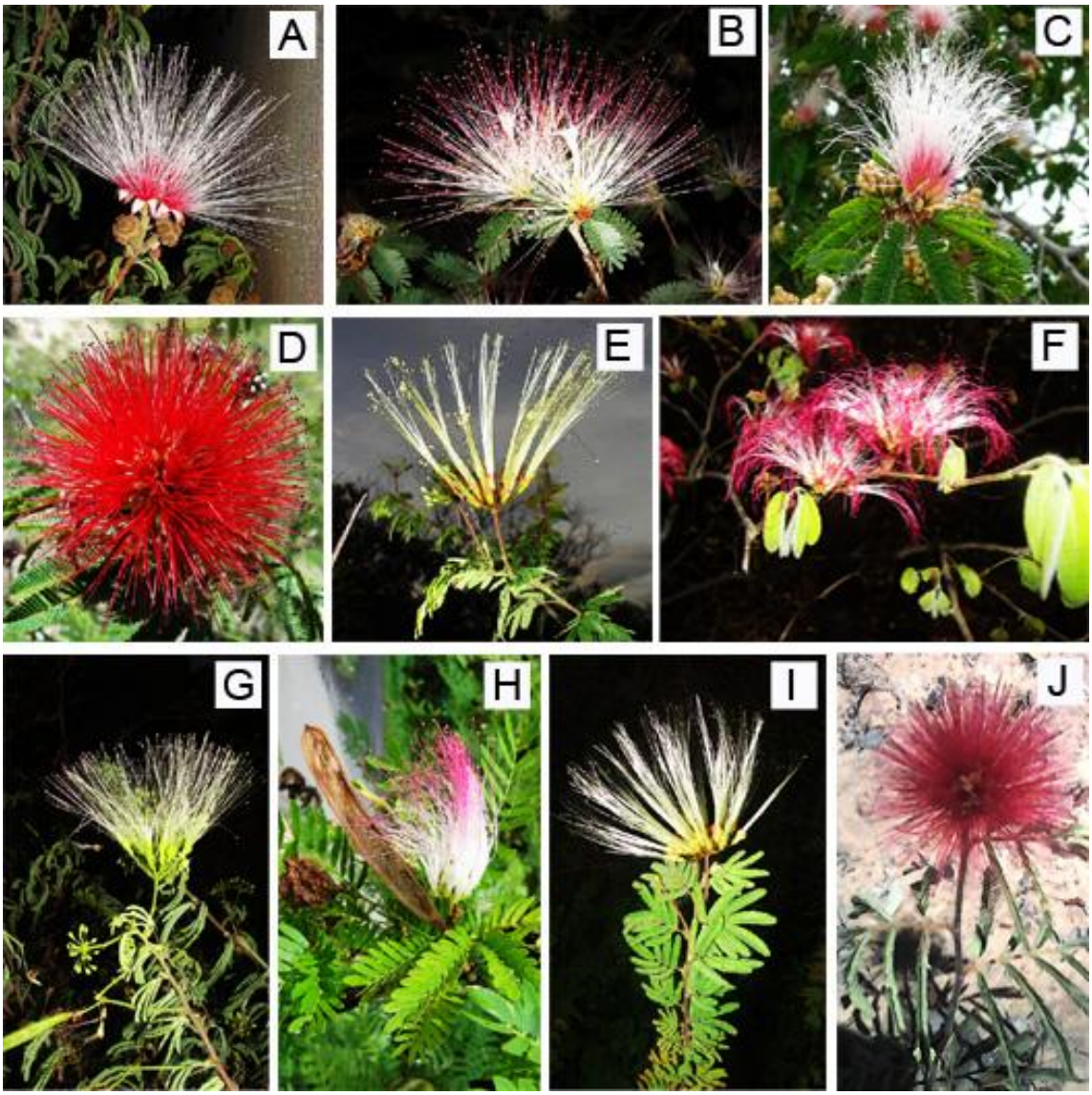

Figure 1. Species from *Calliandra* sect. *Androcallis* with distribution on Northeast Brazil*:* A: *Calliandra macrocalyx* Harms*;* B: *Calliandra sessilis* Benth.*;* C: *Calliandra spinosa* Ducke*;* D: *Calliandra fernandesii* Barneby*;* E: *Calliandra imperialis* Barneby*;* F: *Calliandra harrisii* (Lindl.) Benth.*;* G: *Calliandra umbellifera* Benth.*;* H: *Calliandra riparia* Pittier*;* I: *Calliandra ulei* Harms and J: *Calliandra dysantha* Benth..

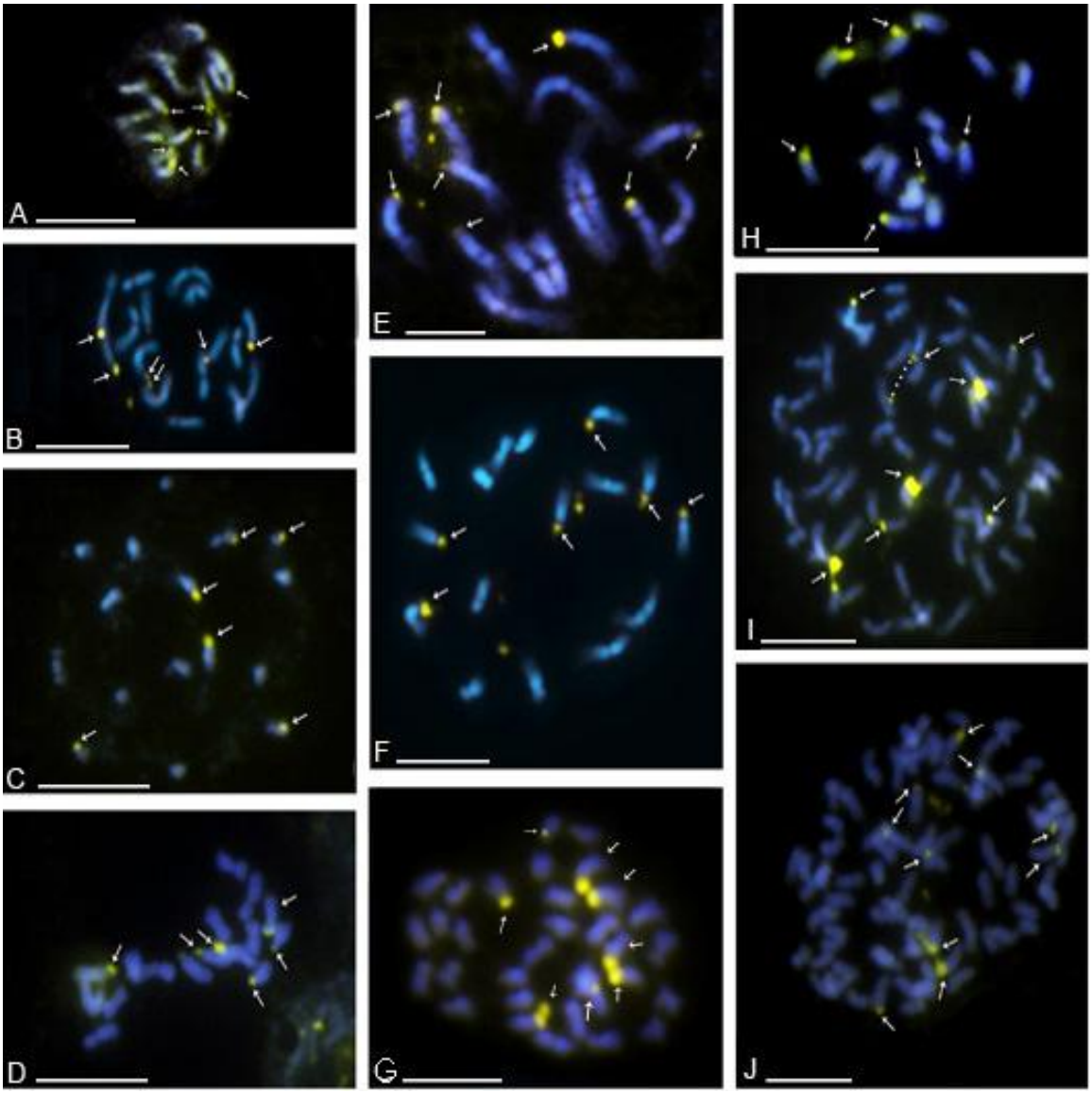

Figure 2. Chromosome number and CMA^+ bands in prometaphasis/metaphasis of species from *Calliandra* sect. *Androcallis*: A: Diploid species: *Calliandra umbellifera* Benth. 2*n* = 16 (6); B: *Calliandra imperialis* Barneby 2*n* = 16 (6); C: *Calliandra riparia* Pittier 2*n* = 16 (6); D: *Calliandra spinosa* Ducke 2*n* = 16 (6); E: *Calliandra macrocalyx* Harms 2*n* = 16 (8); F: *Calliandra ulei* Harms 2*n* = 16 (6) and H: *Calliandra harrisii* (Lindl.) Benth. 2*n* = 16 (6); Tetraploid species: G: *Calliandra fernandesii* Barneby 2*n* = 32 (8); Octaploid species: I: *Calliandra sessilis* Benth. 2*n* = 64 (8) and Decaploid species: J: *Calliandra dysantha* Benth. 2*n* = 80 (10). Scale bar =10µm

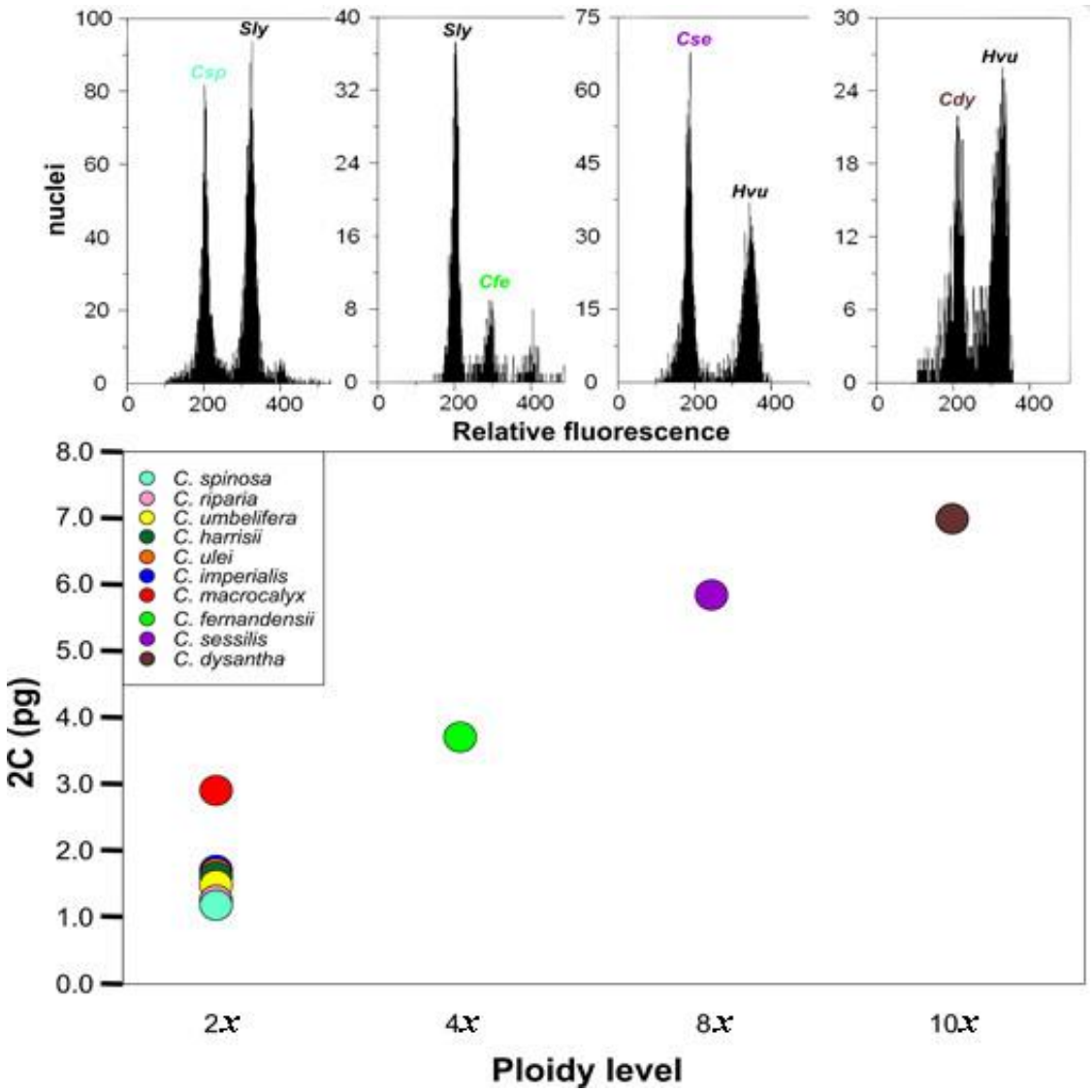

Figure 3. Histograms of relative fluorescence obtained in the Flow Cytometry analysis (upper part) for *Calliandra spinosa* (*Csp*) and *Calliandra fernandesii* (*Cfe*) using *S. lycopersicon* (*Sly*) as internal standard and for *Calliandra sessilis* (*Cse*) and *Calliandra dysantha* (*Cdy*) using *H. vulgare* (*Hvu*) as internal standard. In the lower part, a dot plot of genome size (2C) in picograms x ploidy level of analyzed species. Species are represented as circles in the dot plot, with colors explained in the legend on the upper right.

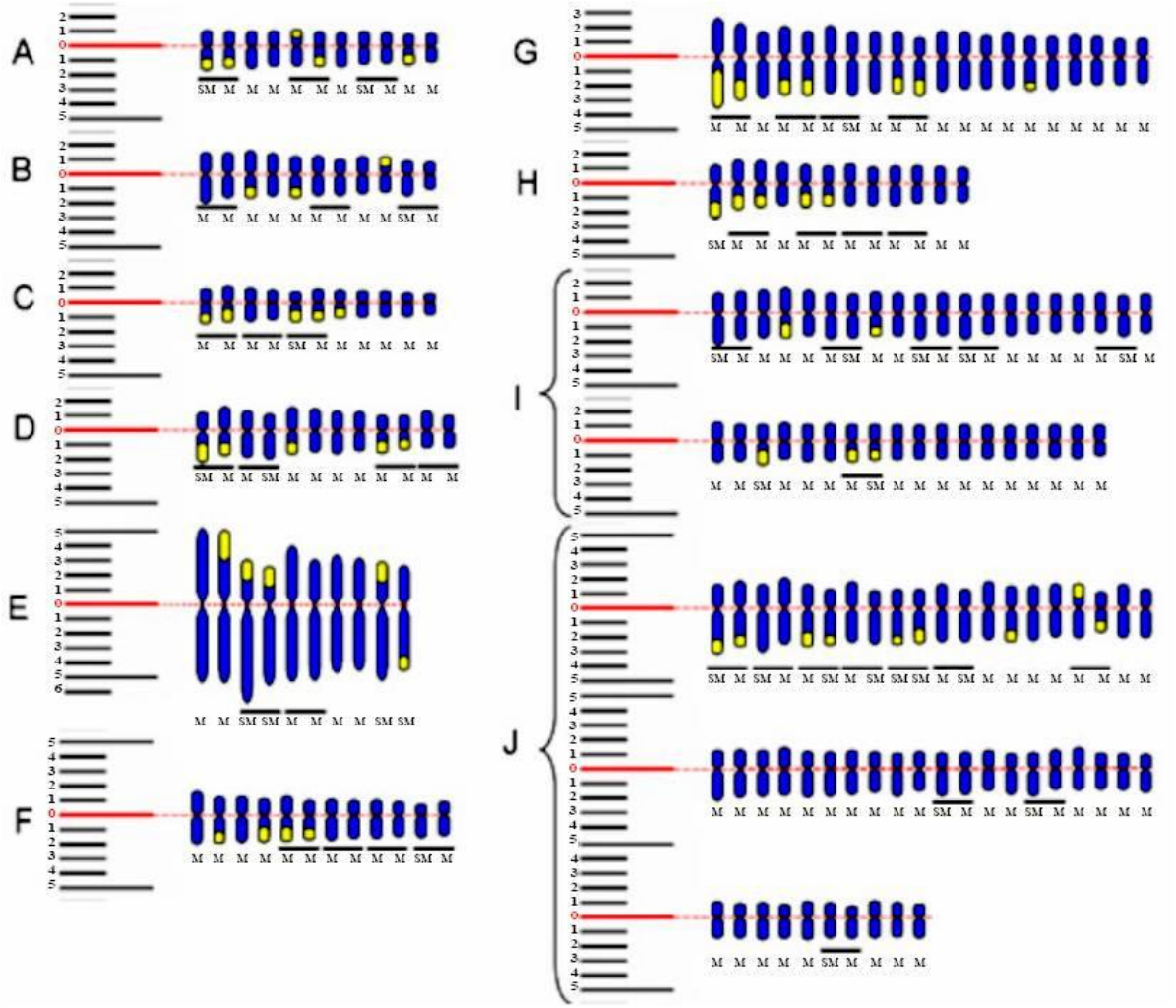

Figure 4. Idiograms representing the haploid complement with distribution and position of CMA^+ bands, chromosome polymorphisms, long arm and short arm length and total chromosome length in species from *Calliandra* sect. *Androcallis:* Diploid species: A: *Calliandra umbellifera* Benth.*;* B: *Calliandra imperialis* Barneby*;* C: *Calliandra riparia* Pittier*;* D: *Calliandra spinosa* Ducke*;* E: *Calliandra macrocalyx* Harms*;* F: *Calliandra ulei* Harms and H: *Calliandra harrisii* (Lindl.) Benth.; Tetraploid species: G: *Calliandra fernandesii* Barneby*;* Octaploid species: I: *Calliandra sessilis* Benth. and Decaploid species: J: *Calliandra dysantha* Benth. Scale in µm.

MANUSCRITO III

The genus *Calliandra* Benth. (Leguminosae – Caesalpinioideae subfamily) occurring in Piaui, Brazil

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The genus *Calliandra* Benth. (Leguminosae – Caesalpinioideae) in Piaui State, Brazil

O gênero *Calliandra* Benth. (Leguminosae – Caesalpinioideae) no Estado do Piauí, Brasil

Antônio de Pádua de Oliveira Paula[4], Genialdo Ramos dos Santos[1], Gardene Maria de Sousa[5], Ana Virgínia Leite[1,3], Luciano Paganucci de Queiroz[4] & Reginaldo de Carvalho[1,3]

[1]Programa de Pós-Graduação em Botânica da Universidade Federal Rural de Pernambuco, Av. Dom Manoel de Medeiros s/n, 52171-900, Recife, PE, Brazil.
[2]Departamento de Biologia – Centro de Ciências da Natureza, Universidade Federal do Piauí, R. Dirce Oliveira, 3397 – Ininga, 64048-550, Teresina, PI, Brazil.
[3]Departamento de Biologia da Universidade Federal Rural de Pernambuco, Av. Dom Manoel de Medeiros s/n, 52171-900, Recife, PE, Brazil.
[4]Programa de Pós-Graduação em Botânica, Universidade Estadual de Feira de Santana, Av. Transnordestina - Novo Horizonte, 44036-900, Feira de Santana, BA, Brazil.
[1]Corresponding author: apo.paula@hotmail.com

Abstract

The genus *Calliandra* belongs to the subfamily Caesalpinioideae and Mimosoid Clade, and comprises 139 species. Northeastern Brazil is considered the largest current center of diversity of the genus and its taxa are distributed in the various vegetation formations of Caatinga, Cerrado, and Atlantic Forest in that region, as well as in and Caatinga/Cerrado ecotones. The present survey sought to record the distribution of species of *Calliandra* occurring in Piauí State, Brazil, based on comparative analyses of the morphological characters of specimens deposited in herbaria and through field collections in different environments. It was possible to verify the occurrence of 15 species and 3 varieties within two sections: *Calliandra* sect. *Androcallis* (*C. blanchetii*, *C. dysantha* var. *dysantha*, *C. fernandesii*, *C. harrisii*, *C. imperialis*, *C. macrocalyx* var. *aucta*, *C. macrocalyx* var. *macrocalyx*, *C. riparia*, *C. sessilis*, *C. spinosa*, *C. ulei*, and *C. umbellifera*); and *Calliandra* sect. *Microcallis* (*C. depauperata*, *C. leptopoda* and *C. parviflora*).

Key words: Caatinga, Cerrado, Fabaceae, Mimosoid Clade, Taxonomy.

Resumo

Calliandra com 139 espécies, pertencente a subfamília Caesalpinioideae e Clado Mimosoid. A região nordeste do Brasil é considerada o maior centro atual de diversidade para o gênero. Estes taxons estão distribuidos nas formações vegetacionais de Caatinga, Cerrado e Floresta Atlântica desta região e em áreas ecotonais (ecótono Caatinga/Cerrado). O presente levantamento buscou registrar a distribuição de espécies do gênero ocorrentes no estado do Piauí, Brasil. Baseou-se em análise comparativa de caracteres morfológicos de espécimes depositados em herbários e por coletas botânicas em ambientes diversos. Constatou-se a ocorrência de 15 espécies e 3 variedades, posicionadas em duas seções: *Calliandra* sec. *Androcallis*. (*C. blanchetii*, *C. dysantha* var. *dysantha*, *C. fernandesii*, *C. harrisii*, *C. imperialis*, *C. macrocalyx* var. *aucta*, *C. macrocalyx* var. *macrocalyx*, *C. riparia*, *C. sessilis*, *C. spinosa*, *C. ulei* e *C. umbellifera*) e *Calliandra* sec. *Microcallis*. (*C. depauperata*, *C. leptopoda* e *C. parviflora*).

Palavras-chaves: Caatinga, Cerrado, Fabaceae, Clado Mimosoid, Taxonomia.

Introduction

Calliandra belongs to the Ingeae tribe of the former subfamily Mimosoideae, presently subfamily Caesalpinioideae, Mimosoid Clade and Leguminosae family (LPWG 2017). Prior to 1840, the species were referred to as belonging to the genera *Mimosa*, *Acacia,* or *Inga*. *Calliandra* was first described by Bentham (1840) based on such features as androecium united at the base (monaldephous), numerous, polyad pollen grains, elastic descent of the fruit from the apex, and valves with thickened margins. Bentham (1875) subdivided the genus into five series: *Macrophyllae* Benth., *Laetevirentes* Benth., *Pedicellatae* Benth., *Nitidae* Benth., and *Racemosae* Benth, supported by morphological characteristics such as: leaves (number of pinnae, size and consistency of the leaflets), flowers (sessile or pedicellate), and inflorescence (a glomerule or umbel).

The neotropical circumscription of the genus *Calliandra* was defined by Barneby (1998), being exclusively distributed in the territory of the Americas, excluding African and Asian taxa. That new classification was based on morphological criteria, such as: inflorescence architecture, stipule acquisition, and perianth and androecium shrinkage. A new classification was created from that circumscription to form five sections of the genus: *Androcallis* Barneby, *Acistegia* Barneby, *Acroscias* Barneby, *Calliandra,* and *Microcallis* Barneby.

The most recent review of the genus *Calliandra* was undertaken by Souza *et al*. (2013), who used nuclear (ITS) and plastidial (*trnL-F*) molecular markers as well as palynological characters to define the phylogeny of a monophyletic group for this genus. In that study, differentiated groups were diagnosed, which allowed the formation of six sections: *Androcallis* Barneby, *Calliandra*, *Monticola* E. Souza & L.P. Queiroz, *Tsugoideae* Barneby, *Septentrionales* E. Souza & L.P. Queiroz, and *Microcallis* Barneby.

The current classification of the species diagnosed for northeastern Brazil corresponds to 66 taxa distributed among three sections: *Microcallis* (4 *sp*.), *Androcallis* (26 *sp*.), and *Monticola* (36 *sp*.). The species of the latter section are concentrated in the Chapada Diamantina Mountains in southern-central Bahia State (Souza 2001). The other sections show more even distributions throughout all of the states in the region, although concentrating their greatest occurrences in areas of Caatinga (dryland) vegetation.

This work aimed to: diagnose and inventory species of the genus *Calliandra*, georeference their distributions and determine their occurrence in different vegetation formations or ecotone areas; elaborate a key to identify the sections and species occurring in Piauí state, Brazil; provide morphological descriptions, information concerning their flowering and fruiting phenologies, species comments, and illustrations.

Materials and Methods

Study area. Piauí State, Brazil comprises various interconnected vegetation formations (Figure 1) in a predominantly hot and dry climate with low annual rainfall rates. Ecotone areas are very extensive in Piauí (covering 48.8% of its area), followed by Caatinga vegetation (20.8%), Cerrado (19.8%), babaçu forests (8.6%), and coastal vegetation (2.0%) – for a total of 253,035 km2 (CEPRO 1992a; IBGE 1996; Castro 2007).

Characterized as the largest ecosystems in northeastern Brazil, Caatinga and Cerrado vegetations form extensive transitional areas called ecotones, with numerous species shared by both ecosystems, making it very difficult to precisely define the plant compositions of those areas (Farias & Castro 2004). As such, it is relevant to highlight the importance of those extensive ecotone areas as environments for the probable occurrence of new species (Smith *et al*. 1997).

The genus *Calliandra* is quite diverse in the semiarid region of Piauí, although its occurrence in Caatinga vegetations is restricted to environments with specific edaphoclimatic conditions, making its species difficult to locate in phytogeographic studies (Queiroz 2009). The genus generally occurs in distinct and specific environments in areas of Cerrado vegetation that harbor seasonally deciduous and semideciduous forests experiencing strong dry periods, poor rainfall distribution, and low relative humidity.

Taxonomic study. Field excursions were undertaken between 2016 and 2019 to observe natural populations and make botanical collections, following the methodologies described by Mori *et al*. (1989). Exsiccates from the following herbaria were also examined: ALCB, CEPEC, EAC, HUEFS, PEUFR (acronyms according to Thiers 2017). The identifications of the taxa were based mainly on Barneby (1998). The standardization of the vegetative and reproductive structural terminology was based on Hickey (1973) and Radford (1974). Species determinations were based on Souza

(2001) and Queiroz (2009). Specimen types were analyzed using the Global Plantson JSTOR (http://jstor.org/) and Reflora Virtual Herbarium (http://reflora.jbrj.gov.br) sites. Geographic distribution, habitat, and phenological data were based on field collections, information available in the literature, and exsiccate labels.

Maps with the geographic distributions of the species were prepared using QGIS 3.12 software, based on the geographic coordinates provided on herbarium labels, and the observations of specialists.

Identifications were made by consulting specialized bibliographies and by comparison with species deposited in herbariums. The specimens collected were photographed, georeferenced, and studied in their natural habitat.

The collected materials were dehydrated and herborized according to usual techniques (Fidalgo and Bononi 1989) and forwarded to the Feira de Santana State University herbarium (HUEFS) for identification. The morphological data are based on measurements of the vegetative organs of dehydrated materials; the reproductive organs were measured using fresh material.

Results and Discussion

Calliandra Benth., Journal of Botany (Hooker) 2(11):138-141. 1840.

Thickets, shrubs, or subshrubs. Stems persistent or deciduous, linear, lanceolate, deltoid, oval, and foliate; branches usually bent and slightly branched; trichomes present. Bipinnate leaves, extrafloral nectaries absent; pinnae 1 - 30 pairs; leaflets 1 - 92 pairs per pinnae; leaflets distal, usually asymmetrical, oblong, apex rounded, obtuse, acute to emarginate, base truncated to oblique; veins pinnate-palmate, palmate-dimidiate, and palmate. Inflorescences in glomerules or umbels, pedunculate (except *C. sessilis*), homomorphic or heteromorphic. Flowers sessile or pedicelate, actinomorphic, pentamers; calyx campanular to infundibuliform, lacinias deltoidal, acuminate or linear; Androecium monadelphous 10 – 158 stamens united at base; intrastaminal nectary present or absent; ovary usually superimposed, 5 - 10 ovules. Pods linear-oblanceolate, margins narrow or expanded, elastically dehiscent from apex to base along both margins, valves leathery to woody. Seeds ovoid to rhomboid, testa smooth, pleurograms U-shaped or absent.

Calliandra comprises approximately 139 species distributed exclusively in the Americas, from the United States to Argentina (Barneby 1998). The largest numbers of species of the genus occur in Brazil, a total of 74, with 59 endemic; the species

Calliandra imperialis and *Calliandra ulei* are endemic to Piauí State (Giulietti *et al*. 2009; Queiroz 2009). The northeastern region of Brazil is considered the largest center of dispersal for the genus (Barneby & Grimes 1996; Lewis *et al*. 2005). The species identified in Piaui have previously been recorded in various vegetation formations: Caatinga, Cerrado, and ecotone areas between the two. Species of the section *Androcallis* are the most widely distributed in vegetation formations and ecotone areas in Piauí.

Identification key for *Calliandra* species in Piaui State, Brazil

1. Inflorescences umbels.
    2. Leaves with more than one pair of pinnae; heteromorphic inflorescences; filaments totally white.
        3. Pinnae with up to 19 leaflets; presence of pedunculate glandular trichomes.....1.12. *C. umbellifera*
        3’. Pinnae with 20 to 32 leaflets; absence of pedunculate glandular trichomes.
            4. calyx lacinias acuminate.....1.11. *C. ulei*
            4’ calyx lacinias linear.....1.5. *C. imperialis*
    2’. Leaves with only 1 pair of pinnae; inflorescence homomorphic; flowers with bicolored or red filaments.
        5. Pinnae with 16–23 pairs of leaflets; flowers with bicolor filaments.....1.1. *C. blanchetii*
        5’. Pinnae with 3–5 pairs of leaflets; flowers with red filaments .....2.2. *C. leptopoda*
1’. Inflorescences glomerules.
    6. Heteromorphic glomerule.
        7. Leaves with only 1 pair of pinnae.
            8. Pinnae with 1 ½ pairs of leaflets.....1.4. *C. harrisii*
            8’. Pinnae with 8 - 24 pairs of leaflets.
                9. Androceu with 3 - 4 stemonozone tubes.....1.8. *C. riparia*
                9’. Androceu with only one stemonozone tube.
                    10. Central flowers with 13–20 stamens, filaments with vinous terminations.....1.9. *C. sessilis*

10’. Central flowers featuring 22–27 stamens, filaments with white terminations.....1.10. *C. spinosa*

7’. Leaves with 2 - 4 pairs of pinnae.

11. Central flowers with up to 71 stamens; pods 6.2 cm long.....1.6. *C. macrocalyx* var. *aucta*

11’. Central flowers with up to 158 stamens; pods 16.4 cm long.....1.7. *C. macrocalyx* var. *macrocalyx*

6’. Glomerules homomorphic.

12. Flowers with up to 12 stamens.

13. Leaves with 1-3 pairs of pinnae; 9-15 pairs of leaflets.....2.1. *C. depauperata*

13’. Leaves with 6-31 pairs of pinnae; 40-50 pairs of leaflets.....2.3. *C. parviflora*

12’. Flowers with more than 12 stamens.

14. Filaments fully red.....1.3. *C. fernandesii*

14’. Flowers densely arrayed with brown trichomes; androecium stamens with red filaments.....1.2. *C. dysantha* var. *dysantha*

Taxonomic Treatment

1. *Calliandra* section *Androcallis* Barneby, Memoirs of the New York Botanical Garden. 74 (3): 21. 1998. Type specie: *Calliandra laxa* (Willd.) Benth.

1.1. *Calliandra blanchetii* Benth., London Journal of Botany. 3:102. 1844. Type: BRAZIL. BAHIA: J.S. Blanchet *2584* (holotype in K!; isotype in G!, NY!, P!).

Subshrubs or shrubs, 1 - 2 m tall; branches sinuous and woody, most older branches smooth and leafy, youngest branches hairy with whitish and fine trichomes. Stipules lanceolate, 4.5 - 8 mm long. Leaves with 1 pair of pinnae and 16 - 23 pairs of leaflets, distich; median leaflets 3.1 - 4.4 x 1.2 - 1.5 mm, coriaceous, linear, apex acute to rounded, base truncated, asymmetric, glabrous, with margins sparsely ciliated, venation palmate-dimidiate, main vein eccentric; petiole 1.7 - 3.7 mm long; pinnae 17 - 26 mm long. Peduncles 13 - 22 mm long. Inflorescence a homomorphic umbel, 11 – 16 flowers, perianth yellowish green; pedicel 3 - 5 mm long. Flowers, calyx 2-4 mm long, campanulate; lacinias rounded; corolla 6 - 8 mm long, lacinias oblong. Stemonozone tube inclusive for all flowers 3.5 mm long, 16 - 40 stamens, filaments ¾ white with

vinaceous ends, 28 - 50 mm long; Pods 3.8– 6.5 x 0.6 – 0.7 cm, 5 seeds per pod; Seeds 0.6 x 0.4 cm.

Material examined: BRAZIL. PIAUI: Piripiri, W41°39’00”, S4°09’09”, 04.VIII.2011, fl., M.L. Guedes *et al. 22506* (ALCB).

Additional material examined: BRAZIL, BAHIA: Ibicoara, ao leste do município, W41°16’58”, S13°24’52”, 3.X.2017, fl., A.P.O. Paula *38* (HUEFS). Morro do Chapéu, Fazenda Formosa, nos arredores do Lajedo Bordado, W41°5’41”, S11°15’44”, 1.IX.2002, fl., A.M. Giulietti *2145* (HUEFS); Povoado Domingos Lopes, Cachoeira Domingos Lopes, W40°54’21”, S11°33’28”, 8.VI.2001, fl., E.R. de Souza *114* (HUEFS).

Distribution and habitat: This species is distributed in Brazil in the states of Bahia and Piauí, occurring only in Cerrado and Cerrado/Caatinga transition vegetation. The only specimen found in Piauí occurred in an ecotone area with Cerrado/Caatinga transition characteristics, between the municipality of Piripiri and Sete Cidades National Park. In this study, its occurrence was registered for the municipality of Ibicoara (S13°24'52.48" and W41°16'57.82"), in the southern-central region of Bahia State, growing at elevations of from 900 to 1,000 m a.s.l. on slopes, usually in stony soils.

Phenology: Flowering and fruiting from September to November.

Comments: Characterized by an umbel inflorescence, 1 pair of pinnae per leaf, leaflets smaller than those observed in *C. imperialis*, *C. ulei*, or *C. umbellifera*, which belong to the same group as the umbeliformes and *Androcallis* section.

1.2. *Calliandra dysantha* Benth. var. *dysantha*, Journal of Botany (Hooker). 2(11):138-139. 1840. Type: BRAZIL. PIAUÍ: G. Gardener *2556* (holotype in K!; isotype in K!).

Fig. 2a

Shrubs 1 – 2.5 m tall; Branches virgate, simple or branched, trichomes reddish; Stipules 5.1 - 8.7 mm long. Leaves 3 - 6 pairs of pinnae and 18 - 31 pairs of leaflets, median leaflets 10.3 – 14.9 x 3 – 5.7 mm, oblong, rarely lanceolate, base asymmetrically truncated or semi-cordate, apex acute or obtuse, venation palmate, main vein eccentric; petiole 1.7– 2.3 mm long; pinnae 6.5– 10.2 cm long; Peduncles 5 – 20 mm long; Inflorescence a glomerule, homomorphic, 13 - 15 flowers; Flowers subsessile; calyx 5-8 mm long, campanulate, densely sericeous; corolla campanulate, 12.5 – 14 mm long, densely sericeous; stamens 50 – 87, stemonozone tube 3 – 5.1 mm long, filaments red, 40 – 50 mm long; Pods 4.5 x 9 cm, 3 - 6 seeds per pod; Seeds 8 - 13 x 5 - 9 mm.

Material examined: BRAZIL, PIAUÍ: Gilbués, W45°13’2”, S9°51’58”, 4.VIII.2016, fl., A.P.O. Paula *26* (HUEFS).

Additional material examined: BRAZIL, BAHIA: Barreiras, Espigão Mestre, ca. 100 km sudoeste de Barreiras, W44°59’24”, S12°9’0”, 6.III.1972, fl., W.R. Anderson *36662.0* (NY). Correntina, 15 km SW cidade, rodovia para Goias, chapada Ocidental da Bahia, W44°43’0”, S13°29’0”, 25.IV.1980, fl., R.M. Harley *21778* (CEPEC). Cristópolis, Rodovia BR-242, W44°25’12”, S12°13’48”, 10.X.1981, fl., G.G. Hatschbach *44142.0* (NY). Ibotirama, Tabocas do Brejo Velho, ca. 101 km W de Ibotirama na BR-242, W43°58’48”, S12°15’0” 11.X.1994, fl., L.P. Queiroz & N.S. Nascimento *4076* (K). Igaporã, W42°39’0”, S13°49’0”, 13.III.1981, fl., S.B. da Silva *178* (CEPEC). São Desidério, Estrada para Sítio Grande, W42°2’0”, S12°33’0”, 4.IV.1984, fl., M.M. Fernandez & J.E.R. Collares *29* (RB).

Distribution and habitat: There are records for the states of Bahia, Goiás, Minas Gerais, Piauí, and Tocantins, in Cerrado vegetation formations and in rupestrian fields. In this study, specimens were recorded and collected in the municipality of Gilbués (S09°51'57.60" and W45°13'02.01"), in the southern region of Piauí, growing at elevations from 500 to 1,200 m a.s.l.

Phenology: Flowering and fruiting from January to June.

Comments: The numbers of pinnae pairs per leaf are similar to those seen in *Calliandra dysantha* var. *macrocephala*, it differs from that species, however, by having flowers with red stamens. The species also bears similarities to *Calliandra fernandesii* by its red glomerule and red stamens; it differs, however, from *Calliandra dysantha* var. *dysantha* by usually having larger numbers of pinnae pairs per leaf.

1.3. *Calliandra fernandesii* Barneby, Memoirs of the New York Botanical Garden. 74(3):67. 1998. Type: BRAZIL. PIAUÍ: A. Fernandes *et al.* (holotype in EAC 6839!).

Fig. 2b-c

Shrubs, 1 – 2 m tall; Stipules lanceolate 4.6 – 5.9 mm long; Branches virgate. Leaves with 4 - 10 pairs of pinnae and 32 - 45 pairs of leaflets, distich, median leaflets 3.3 – 8.8 x 0.9 – 1.9 mm, linear, apex obtuse, main vein eccentric; petiole 1.8 – 2.8 mm long; pinnae 38 – 53 mm long; peduncles 16.9 – 36 mm long; Inflorescence a glomerule, homomorphic, 18 - 25 flowers; Flowers, calyx campanulate 1 – 1.6 mm long; lacinias deltoidal, smaller than the tube, presence of sericeous trichomes; corolla ovate-

lanceolate 4.6 – 5.9 mm long; Stamens 21 - 37, filaments red, 19.6 – 24.4 mm long; Pods 7.3 x 0.7 cm, 6 seeds per pod; Seeds 5.4 x 3.3 mm.

Material examined: BRAZIL, PIAUÍ: Buriti dos Lopes, Aproximadamente 100 m na estrada de Buriti dos Lopes à Parnaíba, W41°51'11", S3°9'60", 5.VII.2013, fl., A.P.O. Paula *9* (HUEFS). Campo Maior, coletado a 18 km de Campo Maior, W42°10'7", S4°49'40", 12.VI.1995, fl., M.E. Alencar & M.S.B. Nascimento *1035* (K); Entre Altos e Campo Maior, W42º19'48" , S5º0'0", 29.VII.1979, fl., A. Fernandes *6837* (NY). Piracuruca, Sete Cidades, W41°42'0", S3°55'48", 26.X.1976, fl., A. Fernandes & Matos (K 205544); Parque Nacional de Sete Cidades (abrigo), W41°42'33", S3°55'42", 7.X.1995, fl., F. Medeiros *8.0* (NY).

Distribution and habitat: *Calliandra fernandesii* occurs in Brazil only in western Ceará and northern Piauí, most of its distribution within Piaui is in Cerrado/Caatinga ecotone vegetation. It was recorded in this study in the municipality of Buriti dos Lopes (S03°09'59.88" and W41°51'11.46"), in the northern region of Piauí. It grows at elevations of 50 to 700 m a.s.l. Its distribution is wider in northern Piauí, with records from the border between Piauí and Ceará, at Chapada da Ibiapaba, which is characterized as an ecotone between two vegetation formations.

Phenology: Flowering and fruiting from June to September.

Comments: Characterized by 4-10 pinnae pairs per leaf, 32-45 leaflets per pinnae, inflorescence a glomerule, stamen filaments red.

1.4. *Calliandra harrisii* (Lindl.) Benth., London Journal of Botany. 3:95. 1844. Type: BRAZIL. RIO DE JANEIRO: G. Casaretto *1477* (isotype in G!). Fig. 2d-e

Shrubs, 1.5 – 2.5 m tall; older branches smooth and thick, younger branches with fine whitish hairs; Stipules deltoidal, 2 – 2.9 mm long; Leaves with 1 pair of pinnae, 1½ leaflets per pinnae (3 leaflets), median leaflets 22 – 50 x 13.4 – 27 mm, elliptic or obovate, base asymmetrically obtuse, apex obtuse or rounded, main vein eccentric, high densities of thin, whitish trichomes on the adaxial and abaxial faces, petiole 17 – 33.9 mm long; Peduncles 5.3 – 16.7 mm long; Inflorescence a glomerule, heteromorphic, rosy green. Perianth 10 - 21 flowers; center flowers subsessile, calyx campanulate, 40 – 55 stamens, peripheral flowers subsessile, calyx cuneiform 1.2 – 2.1 mm, lacinias rounded; corolla subcylindrical, 6 – 7.7 mm long, lacinias oblong; stamens 20 – 33, stemonozone tube inclusive, 1.4 – 3.2 mm long, filaments ¾ white with vinaceous

terminations, 25 – 36.4 mm long; Pods 6.5 – 10.2 x 0.3 – 0.5 cm, 6 - 7 seeds per pod; Seeds 5 – 5.2 x 3 – 3.5 mm.

Material examined: BRAZIL, PIAUÍ: Canto do Buriti, Propriedade Tabuleiro I e II, W43°2'20" S7°58'37", 28.XI.2015, fl., A.P.O. Paula *10* (HUEFS).

Additional material examined: BRAZIL, BAHIA: Barra da Estiva, rodovia para Ituaçu, W41°19'12", S13°37'12", 18.IX.1984, fl., G.G. Hatschbach *48374.0* (NY). ESPÍRITO SANTO: Marilândia, Liberdade, propriedade Deoclécio Lorencini, W40°30'55", S19°21'15", 12.VI.2007, fl., V. Demuner *et al. 4218* (VIES). RIO DE JANEIRO: Rio de Janeiro, arboreto do Jardim Botânico do Rio de Janeiro, W43°13'37", S22°57'58", 27.XI.2008, fl., D. Cardoso *2357* (HUEFS); Copacabana, Morro São João, trilha entre Ladeira de Tabajaras e Vila Militar. Mata degradada, W43°11'16", S22°57'46", 16.V.2013, fl., C.L. Haroldo *et al. 7651* (RB).

Distribution and habitat: The species occurs in Bolivia, Paraguay (Chaco region), and Brazil, where there are records of its occurrence in the states of Bahia, Piauí, Minas Gerais, and Rio de Janeiro. In Piauí, it is found in Cerrado-Caatinga transition areas. Its occurrence in Piauí was expanded in this study, being recorded in the municipality of Canto do Buriti (S07°58'37.20" and W43°02'19.60"), in the southern-central region of that state. It grows at elevations from 400 to 500 m a.s.l.

Phenology: Flowering in October or November, usually in the second week after the first rains. Fruiting from February to May.

Comments: Is characterized by having one pinnae per leaf, with three leaflets, and filaments white and vinaceous.

1.5. *Calliandra imperialis* Barneby, Memoirs of the New York Botanical Garden. 74(3):70. 1998. Type: BRAZIL. PIAUÍ: B.E. Dahlgren *875*. (holotype in F). Fig. 2f-h Shrubs, 1.5 – 5 m tall; Stipules 2.6 – 5.6 mm long, lanceolate, pilose. Branches virgate, with short, white trichomes. Leaves with 3 - 4 pairs of pinnae, 20 - 23 pairs of leaflets, distich; median leaflets 3.4 – 4.2 x 1 – 1.3 mm, trichomes small and white, imbricate, apex oblong, obtuse, venation inconspicuous; petiole 1.3 – 1.9 mm long, pinnaes 20.7 – 29.7 mm long. Peduncles 21 - 30 mm long; Umbel heteromorphic, reddish green. Perianth with 19 - 31 flowers; Calyx campanulate, 1.5 – 2.8 mm long; lacinias linear; corolla tubular 5.5 – 7 mm long; Pedicel 10 - 18 mm long. Stamens 18 – 28, stemonozone tubes 5.3 – 6.8 mm long, filaments white, 48.5 – 57.7 mm long, short tube

included in the corolla, 2.1 – 2.9 mm long; Pods 8 x 1 cm, 7 seeds per pod; Seeds 7.5 x 4.4 mm.

Material examined: BRAZIL, PIAUÍ: Brejo do Piauí, W42°46’40”, S8°12’58”, 12.III.2015, fl., A.P.O. Paula *7* (HUEFS). Caracol, Parque Nacional da Serra das Confusões. Entre o Portal do Parque e a descida da Serra das Confusões, W43°29’20”, S9°13’18”, 20.II.2013, fl., G. Martinelli *et al. 18059* (RB). São Braz do Piauí, ca. de 40 km de São Braz do Piauí, na estrada para Caracol, W43°6’6”, S8°55’49”, 11.III.2005, fl., L.P. Queiroz *et al.* 10118 (ALCB). São João do Piauí, W42°35’18,28”, S8°12’06,68” , 18.II.2011, A.P.O. Paula *19* (HUEFS).

Distribution and habitat: *Calliandra imperialis* is endemic to Piauí State in northeastern Brazil. Occurs in Caatinga vegetation with high annual temperatures and low rainfall rates, in areas with the presence of cacti. The soils where it is found are usually sandy-clayey, with little organic material. In the present study, its occurrence was expanded to the municipality of Brejo do Piauí (S08°12'58.30" and W42°46'40.50") in the southern-central region of that state, at elevations of 200 to 600 m a.s.l. One population is in the Serra das Confusões National Park, in the municipality of Caracol.

Phenology: Flowering and fruiting from March to May.

Comments: *Calliandra imperialis* is similar in habit to *C. ulei* and *C. umbellifera*, mostly differing from those by having a calyx with linear lacinias and 20-24 leaflets per pinnae.

1.6. *Calliandra macrocalyx* Harms var. *aucta* Barneby, Memoirs of the New York Botanical Garden. 74(3):66. 1998. Type: BRAZIL. BAHIA: L.M.C. Gonçalves *209* (holotype in K!). Fig. 2i-k

Shrubs, 1 – 3 m tall; Branches long and woody. Stipules lanceolate, 4 – 5.8 mm long; Leaves with 2 - 4 pairs of pinnae, 14 - 20 pairs of leaflets, median leaflets 6.6 – 10.4 x 1.6 – 2.9 mm, oblong, slightly rhomboidal, apex acute, base asymmetric, cordate to truncated, main vein eccentric, petiole 2.6 – 5.9 mm long, pinnae 30 – 44.8 mm long; Peduncles 11 – 17.4 mm long; Inflorescence a glomerule, heteromorphic, 7 - 10 flowers; Calyx 6 – 9 mm long; lacinias acuminate, corolla lanceolate, 10-13 mm long; Stamens 47 – 71, stemonozone tube 5 – 8.8 mm long, filaments ¼ red at the base and white at the ends, 37.9 – 53 mm long, short tube included in the corolla, 2.2 – 3.3 mm long. Pods 6.2 x 1 cm, thickly coated with trichomes, 6 seeds per pod. Seeds 7.5 x 4.9 mm.

Material examined: BRAZIL, PIAUÍ: Canto do Buriti, W43°16’8”, S8°10’36”, 27.II.2011, fl., A.P.O. Paula *1* (HUEFS).

Additional material examined: BRAZIL, BAHIA: Casa Nova, Baixo Médio São Francisco, 2 km do entroncamento para cidade velha de Casa Nova, W41°13’50”, S9°18’45”, 1.IX.2002, fl., L.P. Queiroz *et al. 7415* (ALCB); Entrada ca. de 40 km de Casa Nova na estrada para Remanso para o Balneário das Dunas de Casa Nova, W41°8’56”, S9°25’6”, 18.IV.2004, fl., T.S. Nunes *1116* (HUEFS); Estrada para a Fazenda Santarém, W41°22’4”, S9°24’5”, 9.X.2004, fl., L.P. Queiroz *9624* (HUEFS).

Distribution and habitat: Endemic to northeastern Brazil, occurring in the states of Bahia and Piauí. Occurs on sandy soils and yellow latosols in Caatinga vegetation and Caatinga/Cerrado ecotones with low rainfall rates. Its occurrence is recorded here for the municipality of Canto do Buriti (S08°10'35.95" and W43°16'07.53") in southeastern Piauí. It grows at elevations of 400 to 600 m a.s.l.

Phenology: Flowering and fruiting from February to June.

Comments: *Calliandra macrocalyx* var. *aucta* differs from variety *macrocalyx* by having an inflorescence with larger numbers of flowers (7 – 10, versus 6 - 7 in *C.macrocalyx* var. *macrocalyx*) of smaller sizes (1 – 3 m), smaller and less numerous stamens (47 - 71), and smaller fruits and seeds. Little is known about its distribution in northeastern Brazil.

1.7. *Calliandra macrocalyx* var. *macrocalyx* Harms, Botanische Jahrbücher fur Systematik. 42:203. 1908. Type: BRAZIL. BAHIA: E.H.G. Ule *7586* (holotype in B – destroyed; isotype in K!). Fig. 2l; 3a-d

Shrub or thickets, 1 - 4 m tall; Stipules lanceolate, pilose, 3.2 – 5.4 mm long; branches long, straight, woody and pilose; Leaves with 2 to 3 pairs of pinnae, 15 - 22 pairs of leaflets, median leaflets 4.5 – 9 x 1.5 – 2.8 mm, oblong, slightly rhomboidal, apex acute; base asymmetric, cordate to truncated, main vein eccentric, petiole 2.6 – 3.4 mm long, pinnae 35 – 45 mm long, trichomes present. Peduncles 18 – 26.4 mm long, thickly layered with trichomes; Inflorescence a glomerule, heteromorphic, 6 - 7 flowers. Flowers: 1 central unisex flower, 6 – 7 peripheral hermaphroditic flowers; calyx 8 – 9.5 mm long, lacinias deltoidal. Stamens in the central flower numbering 93 – 158, stemonozone tube 11.9 – 15 mm long, filaments ¼ red at the base and white at their ends, 50 – 63.5 mm long; stamens in peripheral flowers numbering 52 – 79; short tube

included in the corolla, 7 – 10.19 mm long; Pods 16.4 x 1.4 cm, thickly covered with trichomes, 5 seeds per pod. Seeds 13.3 x 8.2 mm.

Material examined BRAZIL, PIAUÍ: São João do Piauí, W42°25'9", S8°15'23", 12.III.2015, fl., A.P.O. Paula *2* (HUEFS).:

Additional material examinated: BRAZIL, BAHIA: Carinhanha, rodovia para Cocos, 13 km a oeste da cidade, W43°52'1", S14°13'45", 16.IV.2001, fl., J.G. Jardim *3551.0* (NY). Urandi, Serra Geral, Cabeceira do Rio Raízes, W42°35'35", S14°46'38", 4.VIII.2009, fl., M.L. Guedes *et al. 15806* (ALCB).

Distribution and habitat: Occurs in the states of Bahia, Pernambuco, Piauí, and in northern Minas Gerais in Brazil. This study records its occurrence for the municipality of São João do Piauí (S08°15'23.30" and W42°25'09.40") in southeastern Piauí. It grows at elevations from 300 to 1,100 m a.s.l. in Caatinga vegetation and Caatinga/Cerrado ecotones.

Phenology: Flowering and fruiting from February to July.

Comments: *Calliandra macrocalyx* var. *macrocalyx* produces one of the largest inflorescences of the genus. Many of its structures are larger than those of other species, especially the seed pods and seeds, and it has greater numbers of stamens in the stemonozone. The variety considered here may show stamens with completely white filaments.

1.8. *Calliandra riparia* Pittier, Arboles y Arbustos Nuevos de Venezuela. 6-8:80. 1927. Type: VENEZUELA. ARAGUA: Pittier *12309* (holotype in VEN n. v.; isotype in G, NY). Fig. 3e

Shrub or thickets, 1 - 5 m tall; Stipules triangular-lanceolate, 4.2 – 5.7 mm long; Branches virgate and woody. Leaves with 1 pair of pinnae, 8 - 11 pairs of leaflets, distich, median leaflets 12.5 – 15.5 x 2.4 – 3.5 mm, linear to linear-elliptic, base semi-cordate, apex triangular-apiculate, venation palmate-pinnate, main vein eccentric; petiole 1.6 – 2.5 mm long, pinnae 38 – 43.9 mm long. Peduncles 15 – 22 mm long; Inflorescence a glomerule, heteromorphic, greenish, with 19 - 31 flowers. Calyx striated, 2 – 2.9 mm long; corolla tubular, 5.5 – 8 mm long; Presence of 3 - 4 stemonozone tubes 28 – 32 mm long, 11 - 13 stamens, filaments ½ white at the base and rose-colored at their ends, 13 – 16 mm long, 15 – 27 peripheral hermaphroditic flowers, 25 – 35 mm long, short tube included in the corolla, 2.5 – 3 mm long; Pods 6.9 - 8 x 0.7- 0.9 cm, 8 seeds per pod. Seeds 5.4 x 3.5 mm.

Material examined: BRAZIL, PIAUÍ: Teresina, planta ornamental encontrada no Centro Administrativo do Governo do Estado do Piauí, W42°47'1", S5°2'36", 18.II.2011, fl., A.P.O. Paula *18* (HUEFS).

Additional material examined: BRAZIL, BAHIA: Salvador, Ondina, campos da UFBA, W38°30'38", S12°58'16", 29.VII.1998, fl., J.A. Lombardi *2300.0* (NY). Ilhéus, área do CEPEC (Centro de Pesquisas do Cacau), km 22 da rodovia Ilhéus-Itabuna (BR-415), W39°13'12", S14°46'48", 29.XII.1981, fl., G.P. Lewis *998* (K). DISTRITO FEDERAL: Brasília, Estação Experimental de Biologia da UnB, W47°55'56", S15°46'47", 5.I.1982, fl., E.P. Heringer *18276.0* (NY). PERNAMBUCO: Recife, área da Universidade Federal Rural de Pernambuco, W34°57'3", S8°1'2", 15.III.2017, fl., G.R. Santos *20* (HUEFS).

Distribution and habitat: Its native occurrences are mainly recorded in Colombia and Venezuela, Central America, Bolivia, and the Brazilian Amazon. The species was introduced as an ornamental plant to northeastern and southeastern Brazil, easily adapting to environments with regular rainfall. Recorded in this study in the municipality of Teresina (S05°02'35.70" and W42°47'01.40") in northern Piauí. It grows at elevations up to 1,330 m a.s.l.

Phenology: Intermittent flowering and fruiting almost year-round, except under drought conditions.

Comments: It resembles *C. surinamensis* by the numbers of leaflets per pinnae, however, its leaves are linear and non-rhombic (similar to those of *C. riparia*), with one pair of pinnae per leaf, and three to four stemonozone tubes.

1.9. *Calliandra sessilis* Benth., Journal of Botany (Hooker). 2(11):141. 1840. Type: BRAZIL. BAHIA: J.S. Blanchet *2816* (holotype in K!; isotype in E!, G!, K!, NY!, P!).

Fig. 3f-i

Sub-shrub, 0.5 – 1.5 m tall; Stipules triangular, 2.3 – 3 mm long. Leaves with 1 pair of pinnae, 17 - 22 pairs of leaflets, distich; median leaflets 6 – 11 x 1.4 – 2 mm, coriaceous, oblong to lanceolate, slightly falcate, apex acute to rounded, base oblique, semicordate, margins setaceous, main vein eccentric; petiole long, 2 – 5.1 mm; pinnae long, 31 – 48.5 mm; peduncles 1.8 – 3.3 mm long; Inflorescence a glomerule, heteromorphic, 24 - 35 flowers; Flowers: 1 central flower, and 23-34 peripheral flowers; sessile, calyx campanulate, 1.2 – 1.9 mm long; lacinias acuminate; Stamens 13 - 20, filaments ½ white at base and vinaceous at their ends, 10 – 22.3 mm long in the central

flower, 24.2 – 31.8 mm long in peripheral flowers, short tube included in the corolla, 2 – 2.6 mm long; Pods 6.1 x 1 cm, 4 seeds per pod; Seeds 6.5 x 4.1 mm.

Material examined: BRAZIL, PIAUÍ: Caracol, Próximo ao estacionamento do complexo rochoso do Parque Nacional da Serra das Confusões, W43°29'06.70", S09°13'16.10", 6.II.2016, fl., A.P.O. Paula *15* (HUEFS). Floresta, estrada para Itanópolis, ca. 7 km sudoeste de Floresta, W41°43’60”, S7°28’33”, 12.III.2005, fl., L.P. Queiroz *et al. 10131* (ALCB). Pajeú do Piauí, W42°54’4”, S8°3’9”, 14.III.2015, fl., A.P.O. Paula *5* (HUEFS).

Additional material examined: BRAZIL, BAHIA, Abaíra, Boa Vista, W41°49’48”, S13°19’12”, 28.XI.1993, fl., W. Ganey *2600.0* (NY). Caetité, Serra Geral, ca. 3 km da cidade, estrada para Brejinho das Ametistas, W42°30’19”, S14°5’19”, 28.IV.2003, fl., N. Roque *et al. 658* (ALCB). Capitão da Volta, estrada para Jussiape, campo sujo de cerrado (campo geral), W41°24’23”, S13°26’39”, 18.V.1999, fl., V.C. Souza *et al. 22728* (ESA). Mucugê, Chapada Diamantina, a 1 km de Mucugê, W41°23’10”, S13°0’9”, 14.VI.2010, fl., M.L. Guedes *et al. 17153* (ALCB). PERNAMBUCO: Buique, Catimbau, Serra do Catimbau, W37°10’12”, S8°37’12”, 18.X.1994, fl., M.J.N. Rodal *436.0* (NY).

Distribution and habitat: Occurs in Brazil in the states of Bahia, Ceará, Maranhão, Minas Gerais, Pará, Pernambuco, Piauí, and Rio Grande do Norte. The present work increased its area of occurrence with records for the municipality of Pajeú do Piauí (S08°00'32.10" and W42°56'42.40") in southeastern Piauí. It grows at elevations from 70 to 1,400 m a.s.l. in Caatinga, Caatinga/Cerrado ecotones, and Cerrado vegetation formations, usually along forest borders and in rupestrian fields

Phenology: Flowering and fruiting from February to May.

Comments: It has the most developed stemonozone tubes (17.3 mm) among the species studied here, and its stamens are vinaceous colored at their ends. Additionally, it is a sub-shrub.

1.10. *Calliandra spinosa* Ducke, Anais Academia Brasileira de Ciências. 32(2):289. 1959. Type: BRAZIL. CEARÁ: A. Ducke *2117* (holotype in M G n. v.; isotype in G!, RB!, US!). Fig. 3j-m

Shrub or thickets, 1 - 4 m tall; Stipules linear-lanceolate, 3.3 – 5.7 mm long; Branches twisting, woody, whitish, becoming suberous with age; Leaves with 1 pair of pinnae and 17 - 24 pairs of leaflets, distich, median leaflets 6.3 – 7.8 x 1.4 – 1.7 mm,

coriaceous, linear, apex acute, base oblique, margins ciliated and pilose, main vein eccentric; petiole 2 – 4 mm long, pinnae 29.2 – 36.9 mm long; peduncles 5.3 – 10.6 mm long; Inflorescence a glomerule, heteromorphic, sessile, 27 - 30 flowers; Flowers: 1 central flower, 26-29 peripheral flowers; calyx campanulate, 2.8 – 4 mm long, corolla 5.5 - 7 mm long, lacinias acuminate; Stamens 22 - 27 in the central flower, filaments ¼ pink at the base, and white at their ends, 27.9 – 32.6 mm long, stamens 8 - 15 in peripheral flowers, short tube included in the corolla, 2.2 – 3.3 mm long; Pods 6.9 x 0.8 cm, 8 seeds per pod; Seeds 5.4 x 2.6 mm.

Material examined: BRAZIL. PIAUÍ: Queimada Nova, Próximo a cidade de Queimada Nova, a 5 km no sentido da rodovia que vai de Paulistana a Petrolina e ca. de 3 m da margem direita da rodovia estadual, W42°57’43”, S8°4’0”, 18.II.2011, fl., A.P.O. Paula *17* (HUEFS).

Additional material examined: BRAZIL, BAHIA: Barra do Mendes, Irecê, ca. 17,5 km do povoado de São Bento. Estrada para Torre, W42°11’17”, S11°48’25”, 17.XII.2009, fl., E. Melo *et al. 7648* (ALCB). Delfino, estrada Delfino-Mimoso de Minas, 20 km de Delfino, W41°20’35”, S10°29’31”, 8.III.1997, fl., A.M. Giulietti *et al. PCD6129* (ALCB). Santa Terezinha, ca. 5 km de Santa Terezinha na estrada para Elísio Medrado, W39°28’32”, S12°51’1”, 4.III.2001, fl., L.P. Queiroz *6519* (HUEFS). CEARÁ: Ibaretama, Conglomerado 177, sub. 3, árvore 6RN. Vegetação aberta com camada herbácea, arbustiva, arbórea alta. Solo profundo e arenoso, W38°41’60”, S4°51’36”, 27.II.2014, fl., J.M.D. Silveira *56* (EAC). Santa Quitéria, 40 km de Santa Quitéria em direção a Canindé, W39°49’0”, S4°15’0”, 18.III.2002, fl., V.C. Souza *et al. 28710* (ESA). RIO GRANDE DO NORTE: São Gonçalo do Amarante, Fazenda Arvoredo, ca. de 5 km a margem da área do Aeroporto de São Gonçalo do Amarante, em direção a comunidade Califórnia, W35°23’27”, S5°45’58”, 12.IX.2011, fl., J.L. Costa Lima *583.0* (NY).

Distribution and habitat: Occurs in the states of Bahia, Ceará, Pernambuco, Piaui, and Rio Grande do Norte in Brazil, being endemic to the Caatinga domain. The present work extends its occurrence to the municipality of Queimada Nova (S08°34'17.60" and W41°22'27.30") in eastern Piauí. It grows at elevations from 100 to 1,250 m a.s.l.

Phenology: Flowering and fruiting from February to March.

Comments: Characterized by having one pair of pinnae per leaf, inflorescence a glomerule, heteromorphic, sessile. The stamens of this specie have pink-colored bases

with white ends at the beginning of flowering, become totally pink after anthesis. Has large numbers of pods and seeds during its reproductive period, favored by seasonal rains.

1.11. *Calliandra ulei* Harms in Engler, Botanische Jahrbücher fur Systematik. 42:205. 1908. Type: BRAZIL. PIAUÍ: E.H.G. Ule *7440* (holotype in F; isotype in G!, K!).

Fig. 4a-e

Shrubs, 1 – 3 m tall; Stipules lanceolate, 6 – 8.6 mm long; Branches virgate, with denser leaf grouping at the apex. Leaves with 3 - 5 pairs of pinnae, 25 - 32 pairs of leaflets, distich; median leaflets 3.69 – 5.36 x 1.1 – 1.76 mm, imbricate, base oblong, apex rounded, main vein sub backspace-centric; petiole 2.1 – 3.2 mm long; pinnae 25 – 39.5 mm long. Peduncles 28 – 45 mm long; Umbel heteromorphic, reddish green. Perianth, 18 - 35 flowers; Flowers, calyx 2 – 2.8 mm long, corolla 8 – 9 mm long, lacinias acuminate. Stamens 21 – 34 in the central flower, with white filaments, 50.5 – 59.6 mm long, peripheral flowers hermaphroditic, pedicle 8 – 17.7 mm long, stamens 51.7 – 74 mm long, short tube included in the corolla, 2.3 – 4.9 mm long; Pods 8.8 x 0.9 cm, 5 seeds per pod; Seeds 7.7 x 4.7 mm.

Material examined: BRAZIL, PIAUÍ: Caracol, descida da ladeira das confusões, W43°28’0”, S9°13’0”, 22.III.2006, fl., G. Sousa *671* (HUEFS); Estrada a Sudoeste de Caracol para o Parque Nacional da Serra das Confusões, W43°25’47”, S9°14’45”, 24.V.2010, fl., L.P. Queiroz *14769* (HUEFS). Canto do Buriti, W42°56’56”, S8°6’56”, 26.XI.2013, fl., A.P.O. Paula *4* (HUEFS).

Distribution and habitat: *Calliandra ulei* is endemic to Piauí State in northeastern Brazil in Caatinga, being found in ecotone areas. The present work increases its occurrence to include the municipality of Canto do Buriti (S08°06'56.10” and W42°56'55.90”) at the ecotone between Caatinga and Cerrado vegetation areas. It grows at elevations from 240 to 700 m a.s.l.

Phenology: Flowering and fruiting from February to August.

Comments: Has morphological affinities with *Calliandra imperialis* and *Calliandra umbellifera*, differing from those by having acuminate lacinias, larger numbers of leaflets per pinnae (25-32), umbels, and heteromorphic inflorescences.

1.12. *Calliandra umbellifera* Benth., Journal of Botany (Hooker). 2:141. 1840. Type: BRAZIL. CEARÁ: G. Gardner *1581* (holotype in K!; isotype in P!, NY!, W!). Fig. 4f-j Shrubs, 1 – 3.5 m tall; Stipules lanceolate, 3.5 – 5.9 mm long; Branches virgate, long, with short white trichomes on younger branches, smooth when mature. Leaves with 1 - 3 pairs of pinnae, 15 - 19 pairs of leaflets, distich; median leaflets 5 – 6.6 x 2.3 – 3.4 mm, base oblong, apex rounded, main vein sub-centric, petiole 2 – 3.2 mm long, pinnae 37 – 48 mm long. Peduncles 33 – 39 mm long; Umbel heteromorphic, reddish green. Perianth with 15 - 21 flowers; Flowers with calyx campanulate, 1.9 – 2.7 mm long, corolla oval, 8.5 – 9.5 mm long, lacinias acuminate, presence of pedunculate glandular trichomes. Stamens 21 – 35 in the central flower, with white filaments, 42.8 – 60.7 mm long, peripheral flowers hermaphroditic, pedicle 10 - 15 mm long, stamens 20 - 28, 38.6 – 55.7 mm long, short tube included in the corolla, 3.7 – 6.4 mm long; Pods 11 x 1 cm, 6 seeds per pod; Seeds 8.4 x 4.5 mm.

Material examined: BRAZIL, PIAUÍ: Curimatá, Serra Vermelha, pista de pouso, W44°13’44”, S9°41’12”, 24.XII.2008, fl., A.S.F. Castro *2123* (EAC). Pio IX, W40°49’06,70”, S6°44’09,70”, 6.I.2016, fl., A.P.O. Paula *12* (HUEFS). São Raimundo Nonato, W42°47’31”, S8°33’33”, 14.III.2015, fl., A.P.O. Paula *6* (HUEFS).

Additional material examined: BRAZIL, CEARÁ: Aiuaba, Estação Ecológica de Aiuaba, W40°7’15”, S6°36’1”, 30.VI.2004, fl., J.R. Lemos & P. Matias *216* (EAC). Barbalha, 20 km de Crato, Sítio Barreira, W39°18’15”, S7°18’40” 22.VII.1964, fl., L. Duarte *468.0* (NY).

Distribution and habitat: *Calliandra umbellifera* occurs in three states in northeastern Brazil: Ceará, Pernambuco, and Piauí. The present work records a new occurrence for the municipality of São Raimundo Nonato (S08º33'32.60” and W42º47'30.70”) in southeastern Piauí, growing at elevations from 300 to 900 m a.s.l. in Caatinga vegetation or in Caatinga/Cerrado ecotones.

Phenology: Flowering and fruiting between January and June.

Comments: *Calliandra umbellifera* differs from *Calliandra imperialis* and *Calliandra ulei* by having the lowest numbers of leaflets per pinnae (15-19), umbels, heteromorphic inflorescences, and pedunculate glandular trichomes; those characters differ from other umbeliforms found in Piauí State, Brazil.

2. *Calliandra* sect. *Microcallis* Barneby, Memoirs of the New York Botanical Garden. 74(3):197. 1998. Type specie: *Calliandra parviflora* Benth.

2.1. *Calliandra depauperata* Benth., Transactions of the Linnean Society of London. 30:546. 1875. Type: BRAZIL. BAHIA: J.S. Blanchet *3900* (holotype in RB; isotype in G!). Fig. 5a-d

Sub-shrub to shrub, 0.2 – 1.5 m tall; branches tortuous, woody, with thorny apices, older branches smooth and thick, younger branches pilose with fine white hairs; Stipules linear-lanceolate, 1.9 – 2.2 mm long; Leaves with 1 - 3 pairs of pinnae, 9 - 15 pairs of leaflets, distich; median leaflets 2 – 3.2 x 0.5 – 0.7 mm, with white trichomes along their margins, linear-oblong, apex obtuse, main vein sub-centric, petiole 1 – 2.1 mm long; pinnae 8.2 – 14 mm long; Peduncles 1.5 – 2.5 mm long; Inflorescence a glomerule, homomorphic, greenish or reddish. Perianth with 2 - 4 flowers; Flowers sessile, calyx campanulate, 1.4 – 1.7 mm long, corolla tubular, 2.8 – 4 mm long, lacinias acuminate; Tube included in all flowers, 1.5 – 2 mm long, stamens 9 - 11, filaments red, 11 – 12.3 mm long; Pods 6.9 x 0.8 cm., 8 seeds per pod; Seeds 5.4 x 2.6 mm.

Material examined: BRAZIL, PIAUÍ: Paulistana, área próxima a cidade de Paulistana e aproximadamente 300 m da rodovia que vai para Simões, W41°7’55”, S8°7’45”, 2.II.2016, fl., A.P.O. Paula *13* (HUEFS). São Raimundo Nonato, 5 km além de Bom Jardim, na estrada São Raimundo Nonato, solo argiloso-piçarroso, W42°28’51”, S5°3’8”, 5.XII.1971, fl., D. Andrade-Lima *et al. 1315* (ASE); Km 0,5 da Fundação Ruralista (Sede) na estrada para Vitorino, a aproximadamente 220 km nordeste de Petrolina, W42°0’0”, S9°0’0”, 20.I.1982, fl., G.P. Lewis *1132.0* (NY); A 1500 m na saída de São Raimundo Nonato no sentido São Lourenço-Piauí, W42°40’38”, S9°2’34”, 6.II.2016, fl., A.P.O. Paula *14* (HUEFS).

Additional material examined: BRAZIL, BAHIA: Cruz das Almas, Recôncavo Sul, W39°6’0”, S12°40’0”, VI.1974, fl., G.C.P. Pinto *42327* (ALCB). Morro do Chapéu, Piemonte da Diamantina, caminho para o Morrão, W41°12’0”, S11°34’60”, 18.I.2014, fl., M.L. Guedes *et al. 21258* (ALCB). Rio de Contas, caminho para Lagoa Nova, W41°46’44”, S13°47’42”, 5.II.1997, fl., L. Passos *PCD5107* (HUEFS). PERNAMBUCO: Mirandiba, Fazenda Boa Esperança, 38°26’4” S8°4’17”, 10.III.2008, fl., E. Cordula *et al. 353* (ASE). Orocó, 14 km de Orocó, na estrada para Santa Maria da Boa Vista, W39°42’50”, S9°38’12”, 27.IV.2001, fl., R.M. Harley *et al. PFB54321*

(ALCB). Santa Maria da Boa Vista, 24,7 km Noroeste de Lagoa Grande, W40°12’0”, S8°48’0”, 7.III.1970, fl., G. Eiton *10863.0* (NY).

Distribution and habitat: Occurs in the Brazilian states of Bahia, Ceará, Pernambuco, Piaui, and Rio Grande do Norte, being endemic to the Caatinga domain. Recorded here for the municipality of São Raimundo Nonato (S09°02'33.50" and W42°40'37.70") in southeastern Piauí, growing at elevations from 100 to 1,250 m a.s.l.

Phenology: Flowering and fruiting from December to March.

Comments: *Calliandra depauperata* is characterized by having 1 - 3 pairs of pinnae per leaf, filaments red, and inflorescences small, usually with 4 flowers; that latter character is considered the main difference from the other taxa belonging to this section.

2.2. *Calliandra leptopoda* Benth., London Journal of Botany. 3:101. 1844. Type: BRAZIL. BAHIA: J.S. Blanchet *2833* (holotype in K; isotype in BM, K). Fig. 5e-h Shrubs, 0.5 – 1 m tall; branches slender, sometimes prostate, greenish in color; Stipules foliaceous, cordiform, persistent, 14 – 21 mm long; Leaves with 1 pair of pinnae, 3 - 5 pairs of leaflets, distich; median leaflets 19 – 27.4 x 11.9 – 18.5 mm, membranaceous, obovate to elliptic, apex rounded, base semicordate, main vein eccentric, petiole 17 – 24.7 mm long; pinnae 38 – 71 mm long; Peduncles green, 22.5 – 42.7 mm long; Umbel homomorphic, greenish. Perianth with 14 - 19 flowers; Flowers with pedicle 9 – 14 mm long, calyx campanulate, 0.4 – 0.6 mm long, lacinias deltoidal, corolla campanulate, 2.4 – 2.8 mm long; Presence of included tube, 1 – 1.2 mm long, stamens 27 - 30, filaments red, 5 – 7 mm long; Pods 5.4 x 1 cm, 4 -7 seeds per pod; Seeds 4 - 5 x 2.4 – 3.5 mm.

Material examined: BRAZIL, PIAUÍ: Canto do Buriti, W42°57’43”, S8°4’0”, 12.II.2011, fl., A.P.O. Paula *16* (HUEFS).

Additional material examined: BRAZIL, BAHIA: Livramento de Nossa Senhora, 17,5 km de Livramento na estrada para Lagoa Nova. Caatinga, W41°47’37”, S13°48’5”, 31.I.2005, fl., J. Paula-Souza *et al. 5167* (ESA). Morro do Chapéu, Fazenda São João Brejões. Rio Salitre, W41°5’42”, S11°15’29”, 14.IV.2007, fl., E. Melo *4726* (HUEFS). Oliveira dos Brejinhos, Canabrava, W42°53’45”, S12°19’1”, 16.III.1998, fl., G.G. Hatschbach *67788.0* (NY). Paramirim, caminho Catuarama para Mateus, W42°14’44”, S13°17’50”, 28.IV.2007, fl., A.A. Conceição *1900* (HUEFS). Remanso, caminho para Pau Ferro, W42°14’5”, S9°38’44”, 27.II.2000, fl., G. Cavalcanti *5.0* (NY); estrada para Pilão Arcado, entrada a direita, ca. 29 km da cidade, W42°18’10”,

S9°45’18”, 16.VI.2001, fl., T.S. Nunes *et al. BM PFB476* (ALCB); Baixo Médio São Francisco, ca. 5 km ao Norte de Remanso na estrada para São Raimundo Nonato (PI), W42°9’14”, S9°33’36”, 10.III.2005, fl., L.P. Queiroz *et al. 10059* (ALCB). Seabra, Queimada Nova, W41°46’13”, S12°25’7”, 13.I.1977, fl., G.G. Hatschbach *39533* (NY). Umburanas, Barra dos Alegres, caminho para Riacho da Barra, W41°26’31”, S10°37’4”, 28.I.2010, fl., J.G. Carvalho-Sobrinho *et al. 2618* (EAC).

Distribution and habitat: *Calliandra leptopoda* is restricted to Brazil, being recorded for the states of Bahia, Minas Gerais, Pernambuco, and Piauí, in the Caatinga domain. It usually occurs along the margins of non-perennial streams or at the edges of sandstone outcrops. Its occurrence in Piauí is expanded here to the municipality of Canto do Buriti (S08°04'00.50" and W42°57'43.10") in the southeastern region of Piaui. It grows at elevations from 200 to 800 m a.s.l.

Phenology: Flowering and fruiting from December to April.

Comments: *Calliandra leptopoda* is characterized by having one pair of pinnae per leaf, and a well-developed stipule, comparable only to that found in *Calliandra lanata* in the Montícola section; the latter has a high density of white trichomes on that structure, thus differing from *Calliandra leptopoda*. Inflorescence an umbel, usually with 11 - 19 flowers; the stamens of this species have red filaments. It is the only species of *Microcallis* sect. showing umbeliform inflorescences.

2.3. *Calliandra parviflora* Benth., London Journal of Botany. 3:112. 1844. Fig. 5i-l

Shrubs, 1- 2.5 m tall; older branches smooth and thick, younger branches pilose, with fine white or partially brown hairs; Stipules linear-lanceolate, 3.1 – 5.4 mm long; Leaves with 6 - 31 pairs of pinnae, 40 - 50 pairs of leaflets, distich; median leaflets 2 – 2.9 x 0.2 – 0.6 mm, lanceolate, with asymmetrical auricular bases, apex acute, main vein eccentric, petiole 2 – 3.3 mm long; pinnae 4.8 – 14 cm long; Peduncles 7.6 – 13.5 mm long; Inflorescence a glomerule, homomorphic, vinaceous green. Perianth with 9 - 12 flowers; Flowers, sessile, calyx turbinate-campanulate, 0.9 – 1.4 mm long, corolla oval, 1.8 – 2.6 mm long; tube included in all flowers, 0.9 – 1.4 mm long, stamens 9 - 12, filaments red, 4 – 5.3 mm long; Pods 6.7 – 9.6 x 0.4 – 0.8 cm, 5 - 8 seeds per pod; Seeds 4.5 x 3 mm.

Material examined: BRAZIL. PIAUÍ: Gilbués, rodovia Correntes-Bom Jesus, 2 km a Leste da cidade de Gilbués, W45°19’12”, S9°48’0”, 18.VI.1983, fl., R. Baker *et*

al. 5856 (K); Estrada que vai da cidade de Gilbués para a localidade Aroeira, W45°20’38”, S9°49’54”, 15.VI.2017, fl., A.P.O. Paula *27* (HUEFS).

Additional material examined: BRAZIL, BAHIA: Correntina, Rio Corrente, W44°37’48”, S13°20’24”, 21.I.1997, fl., G.G. Hatschbach *66048* (NY). MARANHÃO: Grajaú, MA 006, de Grajaú em direção a Buriticupu. A ca. de 22 km de Grajaú, W46°13’12”, S5°37”57”, 20.V.2012, fl., C. Snak *et al. 1005* (RB). Loreto, Ilha de Balsas, Fazenda Morros; Grota Grande, W45°4’7”, S7°21’44’, 17.V.2012, fl., C. Snak *et al. 961* (RB). São Raimundo das Mangabeiras, em direção a Balsas, W45°38’0”, S7°6’0” 19.III.1983, fl., C.A. Miranda & J.A. Ferreira *353* (RB).

Distribution and habitat: Occurs in northeastern Brazil in the states of Bahia, Ceará, Maranhão, Paraíba, Piauí, and Rio Grande do Norte. Shows wide distribution on the central Brazilian plateau, mainly in Cerrado vegetation. There are few reports of its occurrence in Caatinga and Atlantic Forest sites. The present work records its occurrence in the municipality of Gilbués (S09°49'54.0” and W45°20'38.0”) in southern Piauí. It grows at elevations from 170 to 800 m a.s.l.

Phenology: Flowering and fruiting from October to April.

Comments: Characterized by having large numbers of pinnae pairs per leaf (6 - 31), large numbers of leaflets per pinnae (40 - 50), and small flowers (less than 3 mm); the stamens of this species have red filaments, being the shortest among *Microcallis* section species occurring in Piauí State, Brazil.

Acknowledgments

We thank the Graduate Program in Botany and the Department of Biology of the Federal Rural University of Pernambuco (UFRPE) and the Federal University of Piauí (UFPI), Distance Education Center (CEAD) for allowing the use of the Biology Laboratory, Polo of Canto of Buriti for the respective analysis and at the Herbarium of Feira de Santana State University (HUEFS).

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Figures

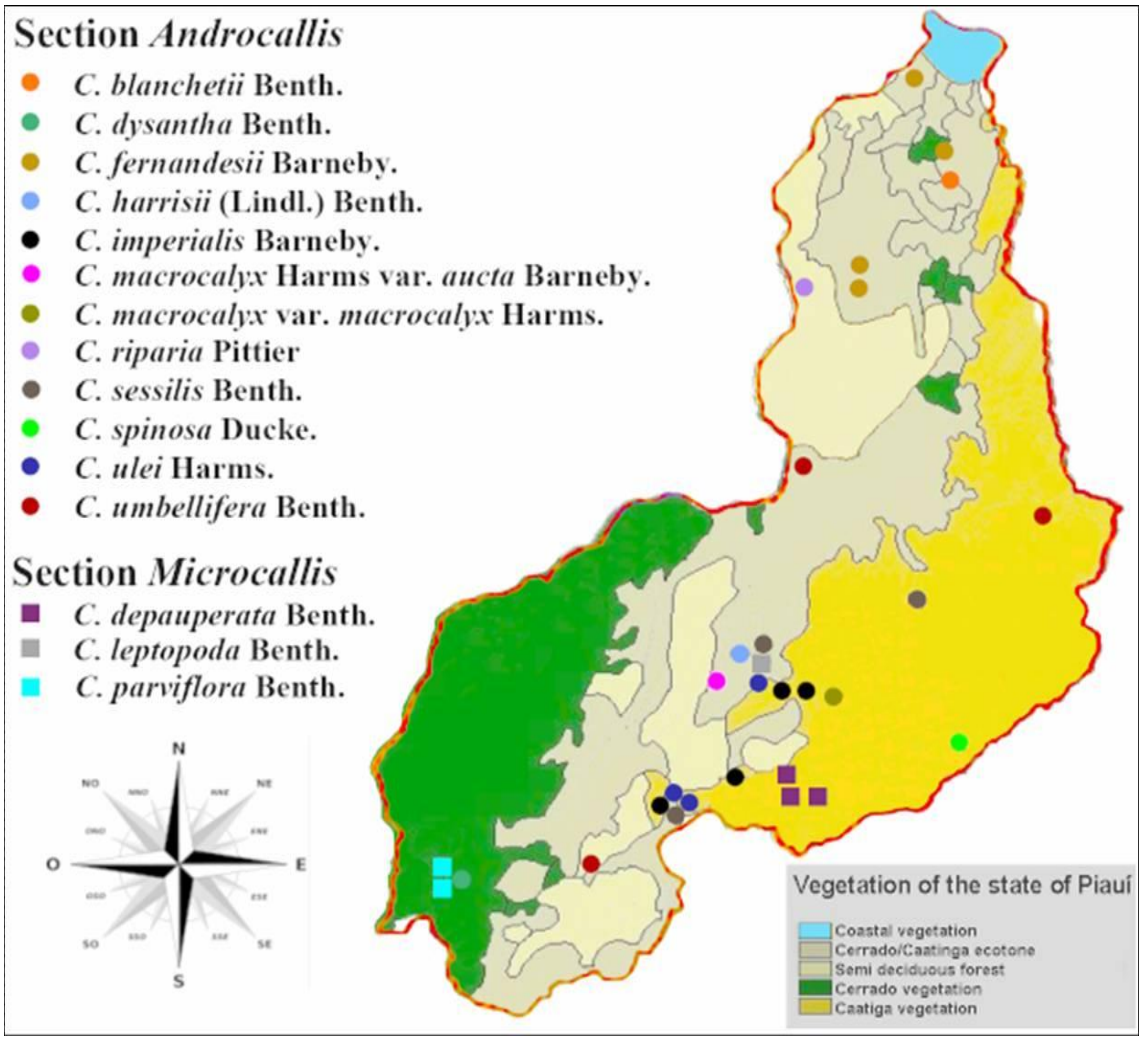

Figure 1. Phytogeographic domains of the vegetation formations of the State of Piauí, Brazil; Species distribution of the *Androcallis* sect. and species of *Microcallis* sect.

Figure 2. a. *Calliandra dysantha* var. *dysantha* Benth – inflorescence and glomeruli. b-c. *C. fernandesii* Barneby – b. inflorescence; c. calyx and corolla. d-e. *C. harrisii* (Lindl.) Benth. – d. pair of pinnae and leaflets; e. inflorescence. f-h. *C. imperialis* Barneby – f. pairs of pinnae and leaflets; g. inflorescence; h. calyx and corolla. i-k. *C. macrocalyx* Harms var. *aucta* Barneby – i. anthesis inflorescence; j. inflorescence; k. calyx and corolla. l. *C. macrocalyx* var. *macrocalyx* Harms - pairs of pinnae; leaflets and glomeruli.

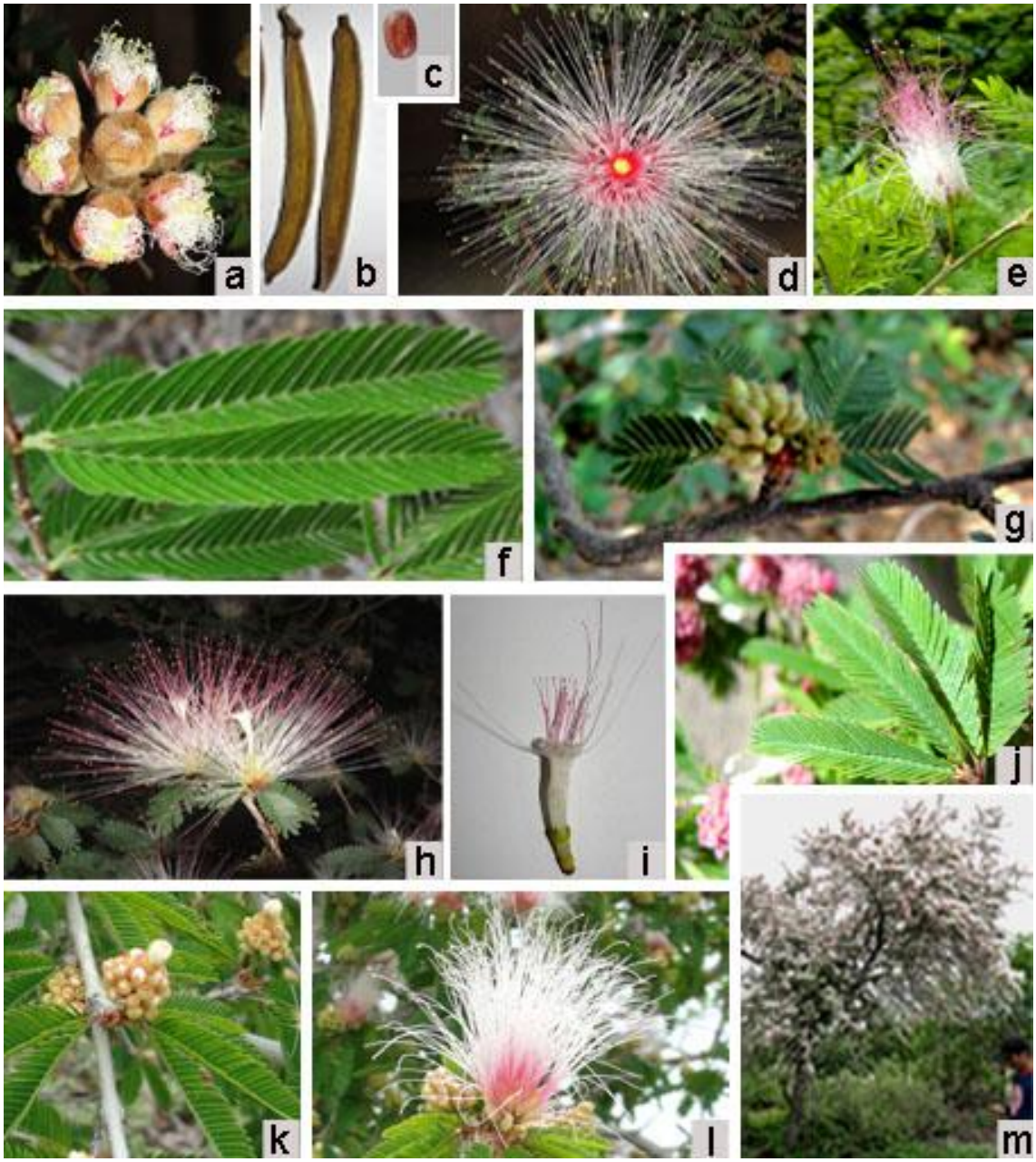

Figure 3. a-d. *C. macrocalyx* var. *macrocalyx* Harms - a. anthesis inflorescence; b. pods; c. seed; d. inflorescence. e. *C. riparia* Pitter – inflorescence. f-i. *Calliandra sessilis* Benth. – f. pairs of pinnae and leaflets; g. glomeruli; h. inflorescence; i. tube staminal. j-m. *C. spinosa* Ducke – j. pairs of pinnae and leaflets; k. anthesis inflorescence; l. inflorescence; m. thickets.

Figure 4. a-e. *C. ulei* Harms – a. pairs of pinnae and leaflets; b. stipule; c. umbel inflorescence; d. inflorescence; e. pod. f-j. *C. umbellifera* Benth. - f. pairs of pinnae and leaflets; g. anthesis inflorescence; h. inflorescence; i. umbel inflorescence; j. flower and presence of pedunculate glandular trichomes.

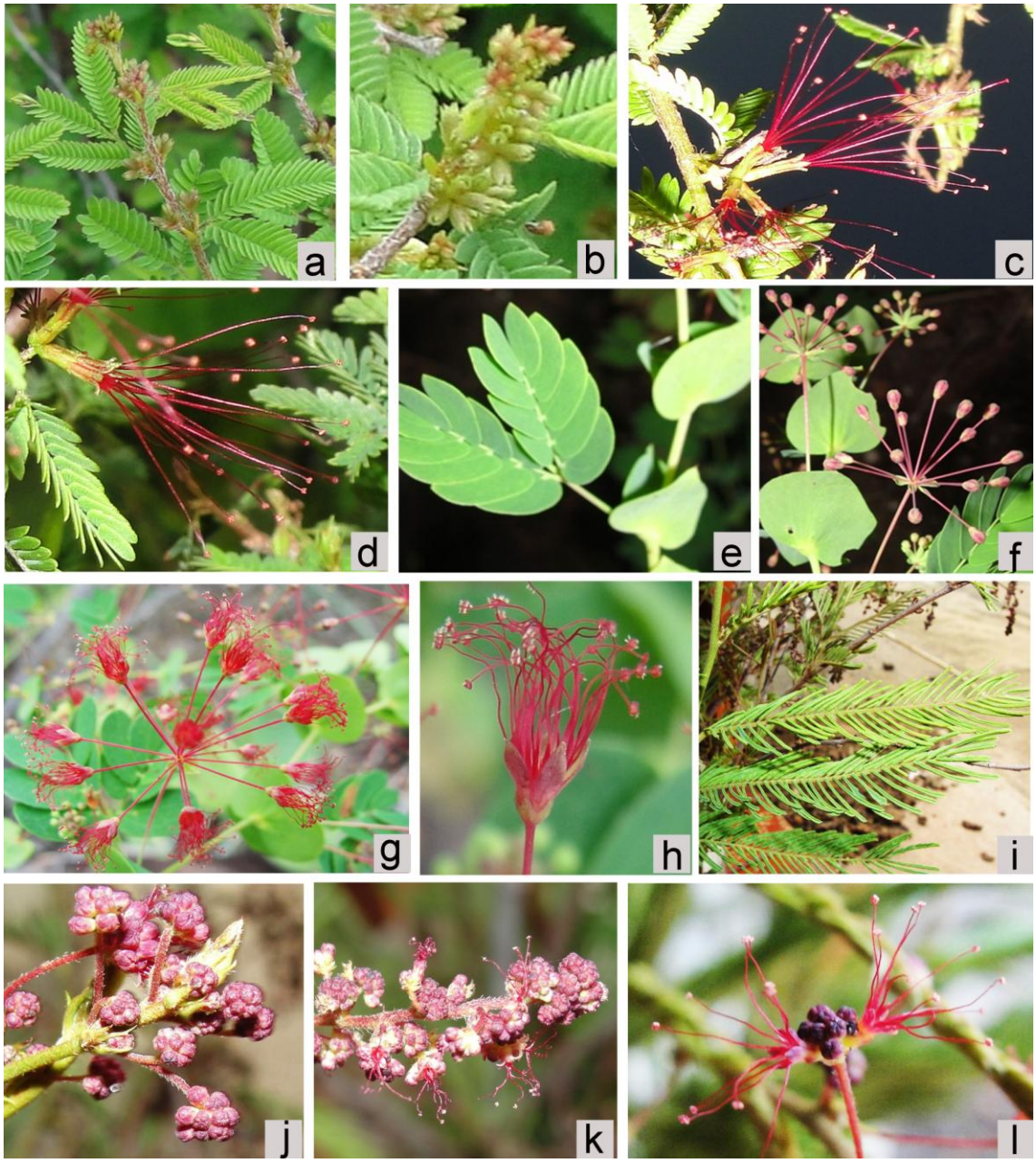

Figure 5. a-d. *Calliandra depauperata* Benth. – a. pairs of pinnae and leaflets; b. glomeruli; c. inflorescence; d. flower. e-h. *C. leptopoda* Benth. – e. pairs of pinnae and leaflets; f. anthesis inflorescence; g. umbel inflorescence; h. flower. i-l. *C. parviflora* Benth. – i. pairs of pinnae and leaflets; j. glomeruli inflorescence; k. inflorescence; l. flower.

CONSIDERAÇÕES FINAIS

1. A morfologia polínica das espécies pertencentes ao gênero *Calliandra*, seções *Androcallis*, *Microcallis* e *Monticola* ocorrentes na região Nordeste do Brasil e estudadas neste trabalho são formadas por uma unidade de dispersão (políade) constituída por oito grãos de pólen (seis grãos periféricos e dois grãos centrais), formato elipsóide.

2. As espécies do gênero *Calliandra* seção *Monticola* se diferencia da outras seções por apresentar um apêndice na região aguda do grão apical da políade, sendo esta característica um atributo favorável na diferenciação infragenérica possibilitando o uso da palinotaxonomia.

3. As maiores políades foram observadas para espécies das seções *Androcallis* e *Montícola*, já as menores foram encontradas para espécies da seção *Microcallis*.

4. O número cromossômico haplóide de $x = 8$ foi confirmado, assim como a existência de uma série poliplóide encontrada para as espécies aqui estudadas. A poliploidia foi o evento principal para a evolução do gênero.

5. O padrão de distribuição da heterocromatina com coloração CMA+ se mostrou bastante variável. Em seis espécies apresentando 6 bandas CMA em 3 pares de cromossomos, duas com 8 bandas em 4 pares de cromossomos e uma com 10 bandas em 5 pares de cromossomos. A distribuição das bandas CMA+ foram distintas para cada espécie em relação aos seus pares cromossômicos. O número de bandas CMA+ encontradas nas espécies poliplóides demonstra haver uma redução destas quando comparadas proporcionalmente ao número encontrado nas diplóides.

6. Os valores encontrados para o conteúdo de DNA presente nas espécies estudadas estão diretamente correlacionados com o tamanho do conjunto cromossômico e nível de ploidia das espécies.

7. A diversidade do gênero *Calliandra* está presente, na grande maioria, nos domínios das formações vegetacionais de Caatinga, Cerrado e áreas de transição Caatinga/Cerrado no Estado do Piauí, Brasil.

8. A grande maioria das espécies estudadas apresenta endemismo tanto para a formação vegetacional de Caatinga ou em áreas de transição (ecótonos). As espécies endêmicas da seção *Androcallis* para o Estado do Piauí são restritas a formação vegetacional da Caatinga.

APÊNDICES

SEÇÃO *ANDROCALLIS* BARNEBY

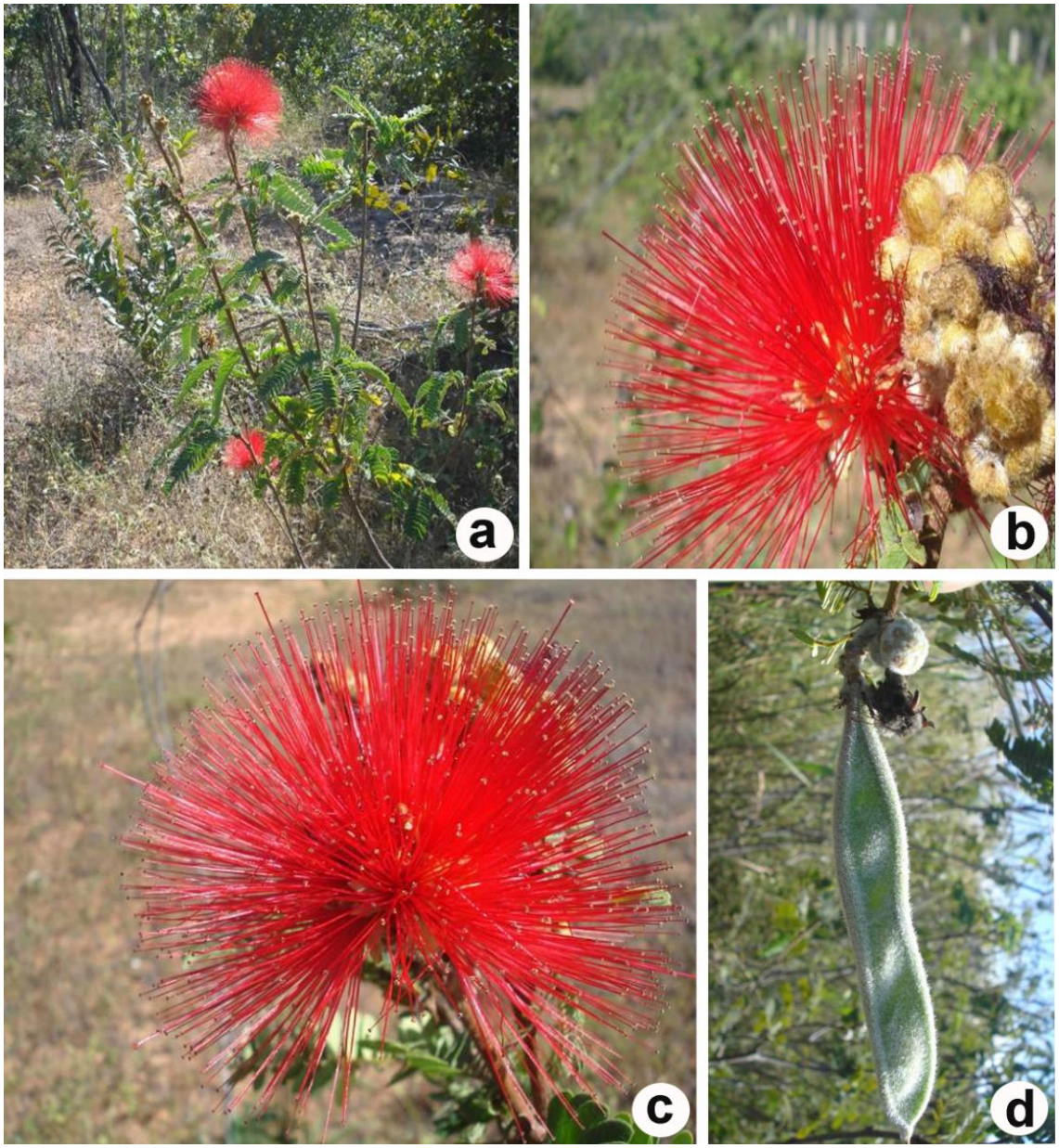

Figura 1. *Calliandra dysantha* Benth.. a. arbusto com folhas e flores. b. inflorescência em glomerulo em antese e com os estames abertos. c. inflorescência aberta. d. fruto legume.

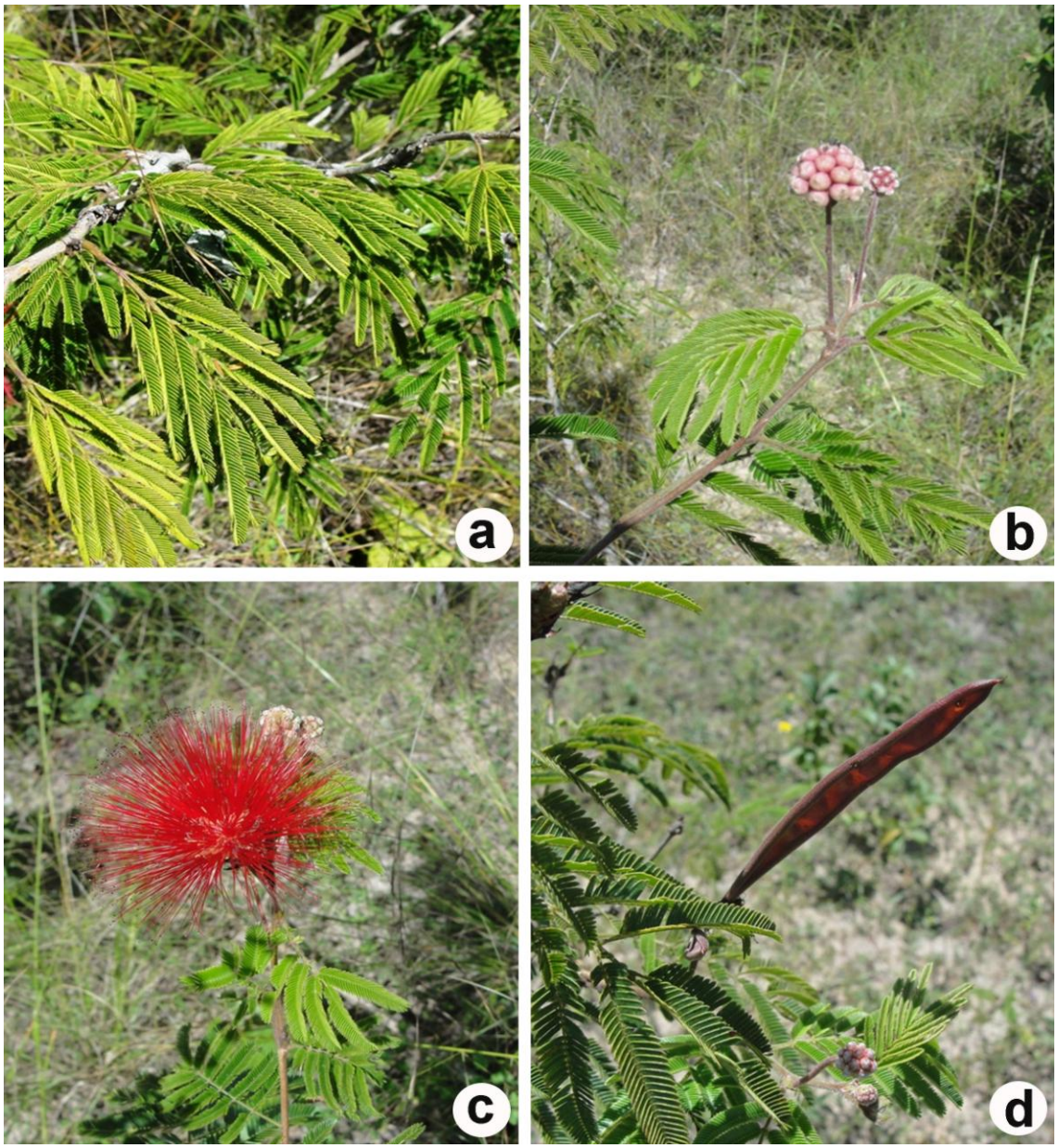

Figura 2. *Calliandra fernandesii* Barneby. a. arbusto com folhas e flores. b. inflorescência em glomerulo em antese. c. inflorescência aberta. d. fruto legume.

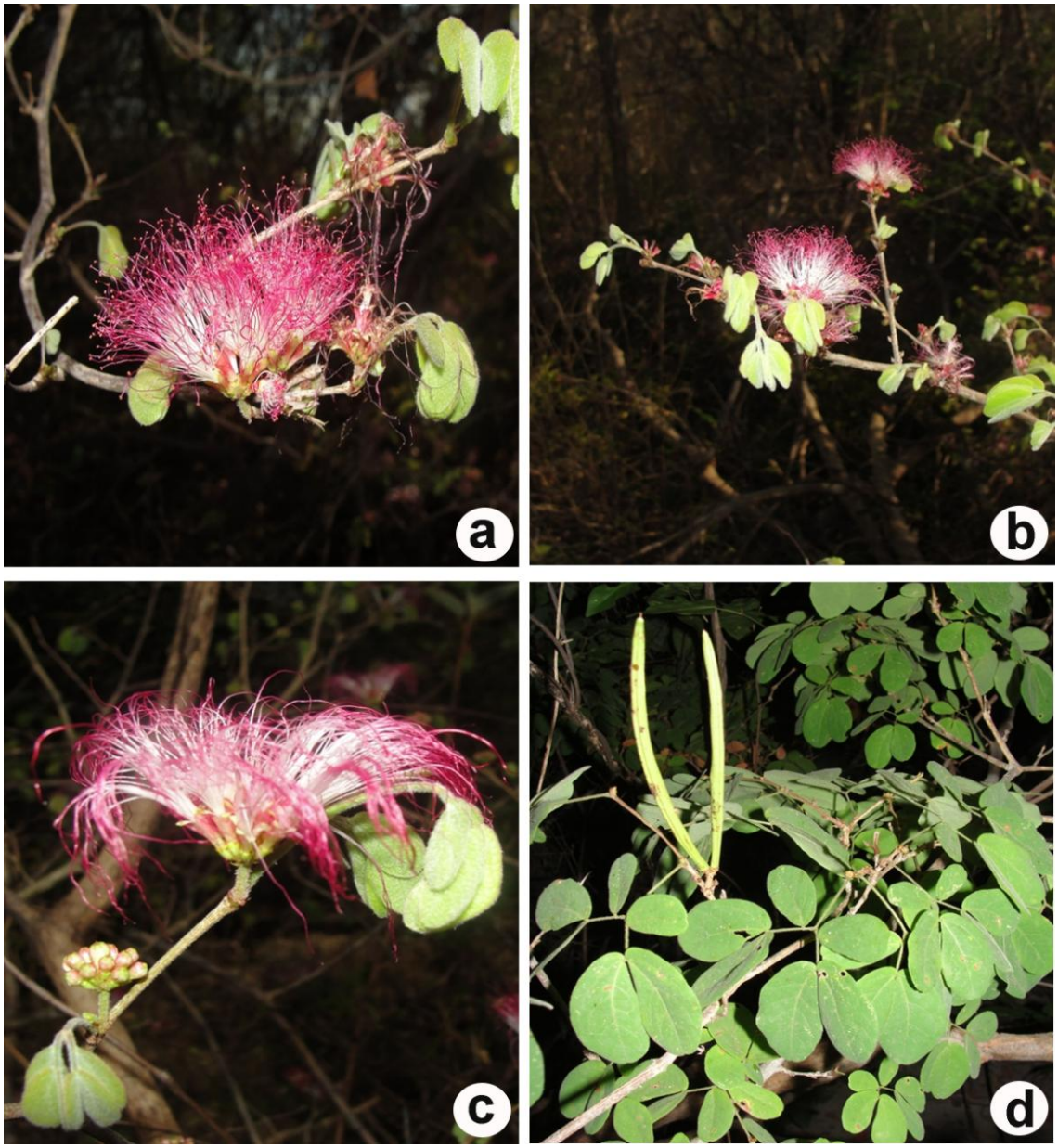

Figura 3. *Calliandra harrisii* (Lindl.) Benth.. a. arbusto com folhas e flores. b. inflorescência em glomerulo em antese e com os estames abertos. c. inflorescência aberta. d. fruto legume.

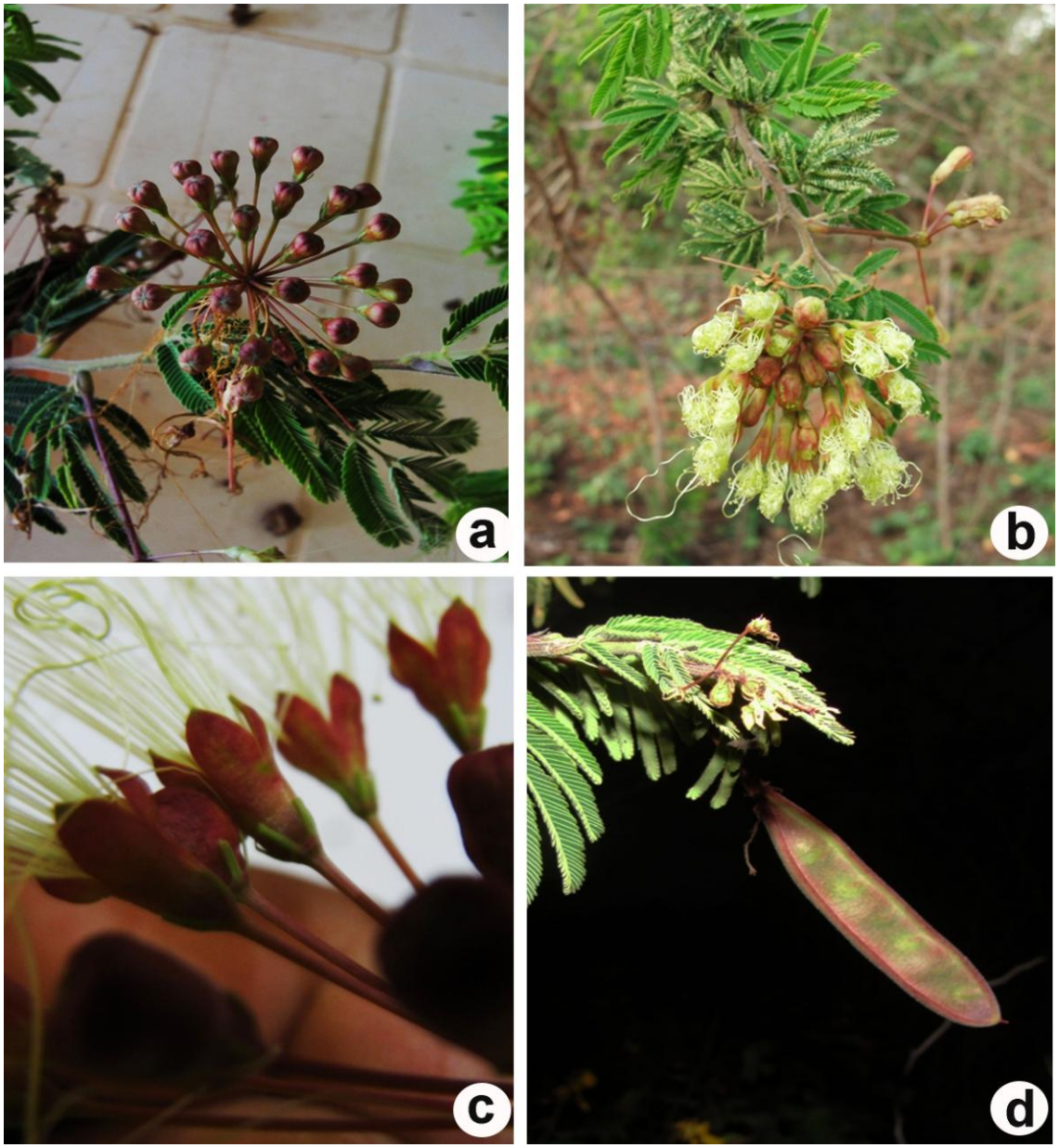

Figura 4. *Calliandra imperialis* Barneby. a - b. inflorescência em umbela em antese e com os estames abertos. c. flores com lacinias lineares. d. fruto legume.

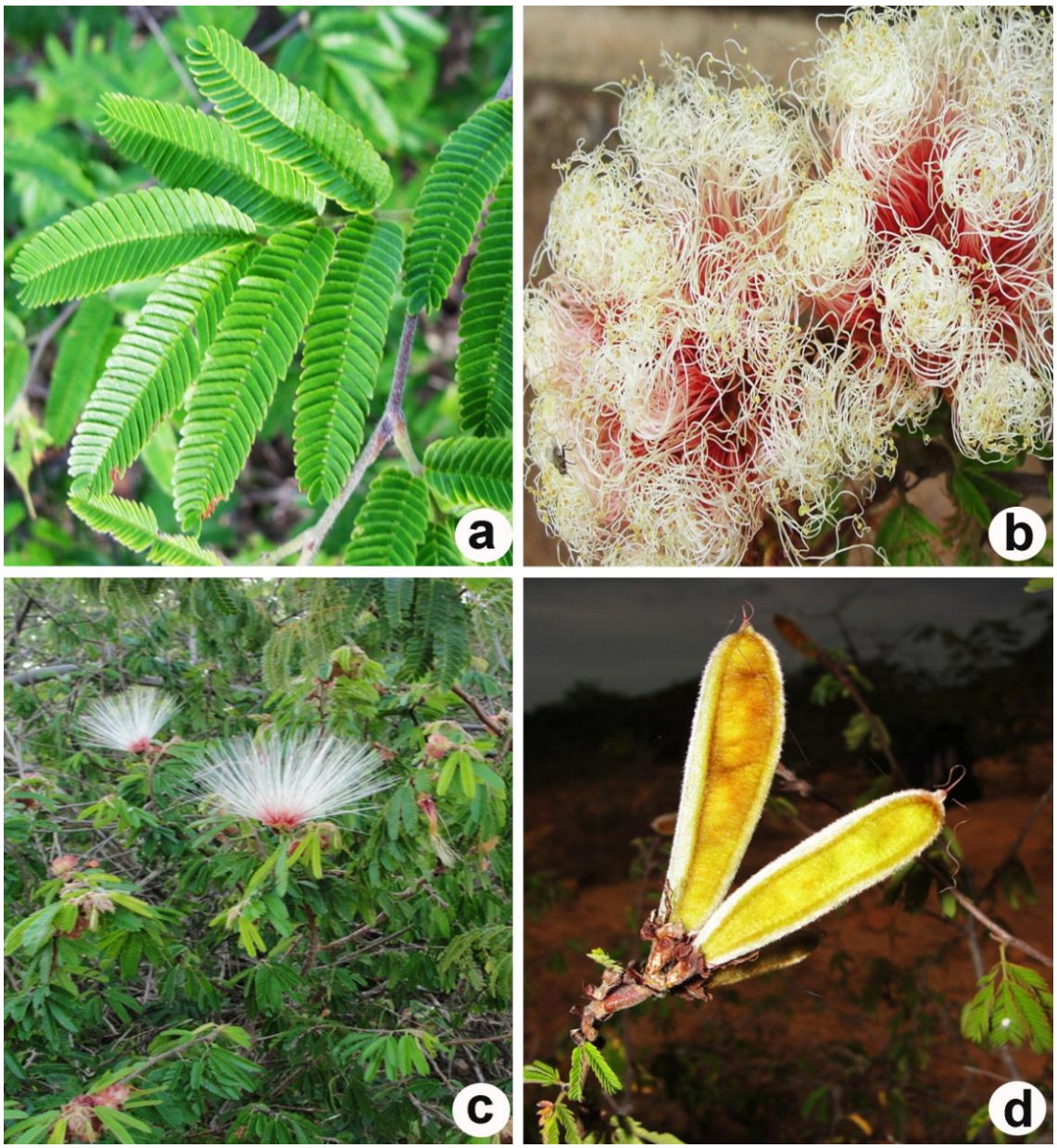

Figura 5. *Calliandra macrocalyx* Harms var. *aucta* Barneby. a. folhas com pinas. b. inflorescência em glomerulo em antese. c. arbusto com folhas e inflorescência aberta. d. fruto legume.

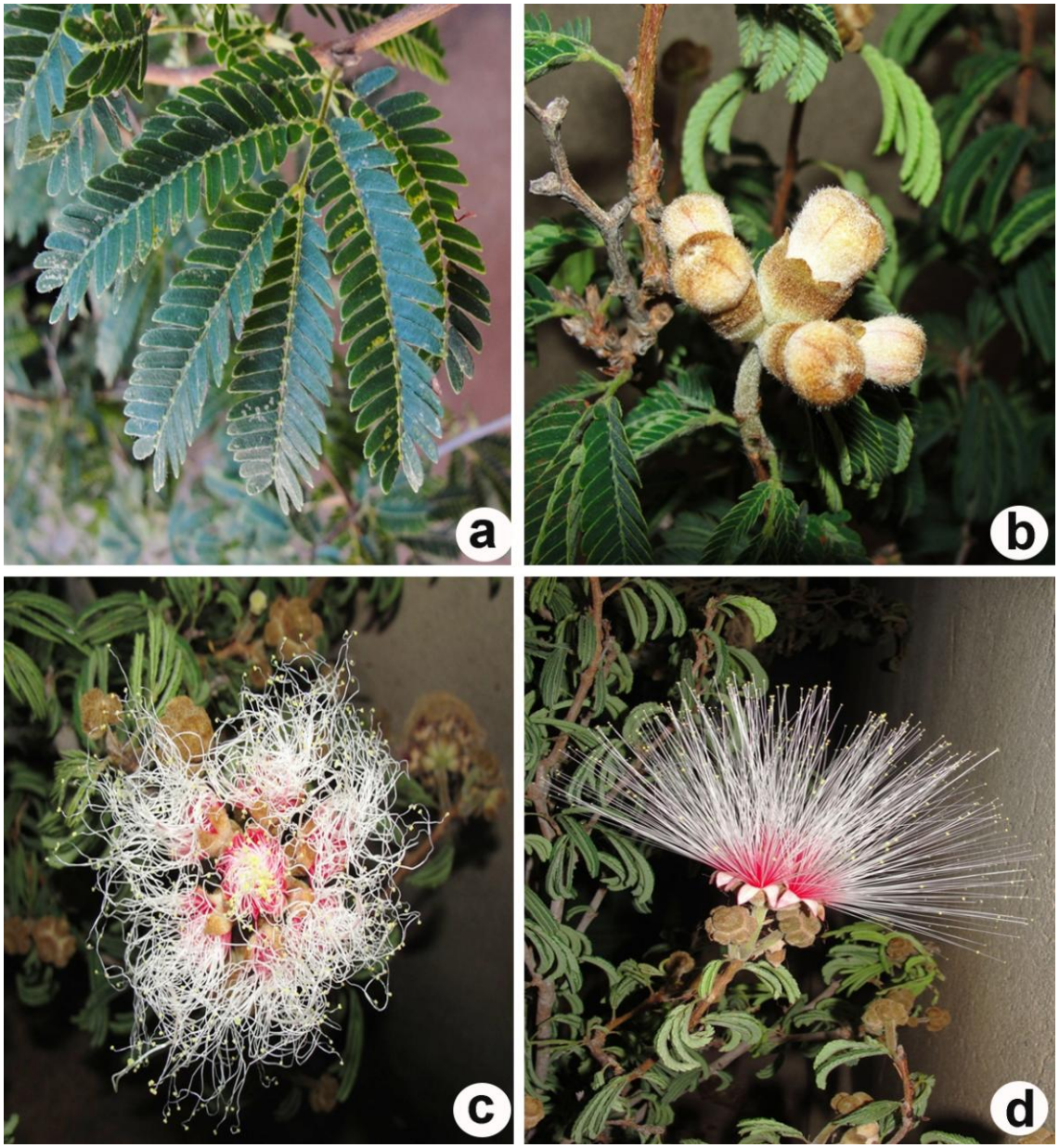

Figura 6. *Calliandra macrocalyx* var. *macrocalyx* Harms. a. folhas com pinas. b. inflorescência em glomerulo em antese. c. inflorescência em antese. d. arbusto com folhas e flores..

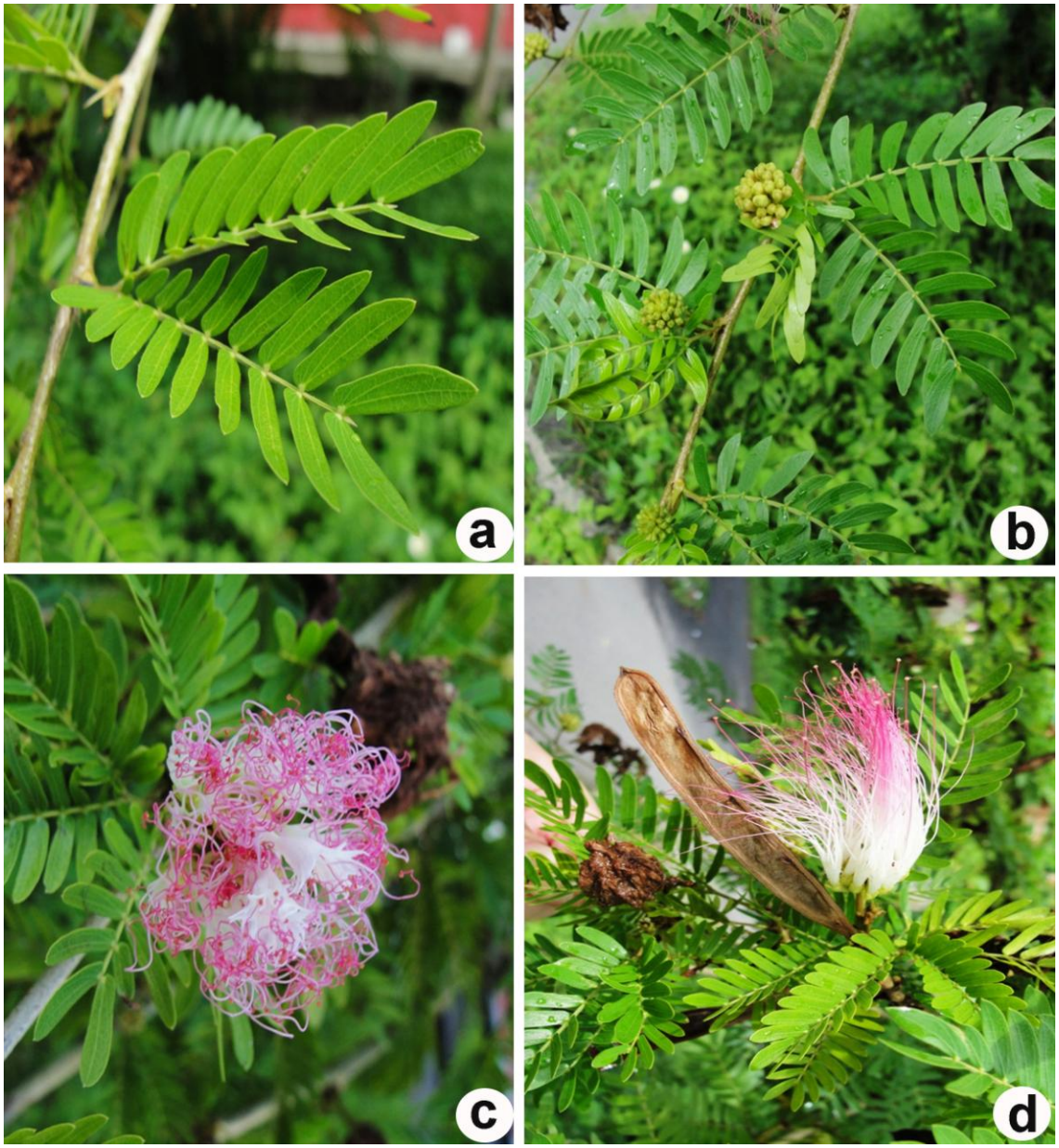

Figura 7. *Calliandra riparia* Pittier. a. folhas com pinas. b. inflorescência em glomerulo em antese. c. inflorescência em antese. d. fruto legume e inflorescência.

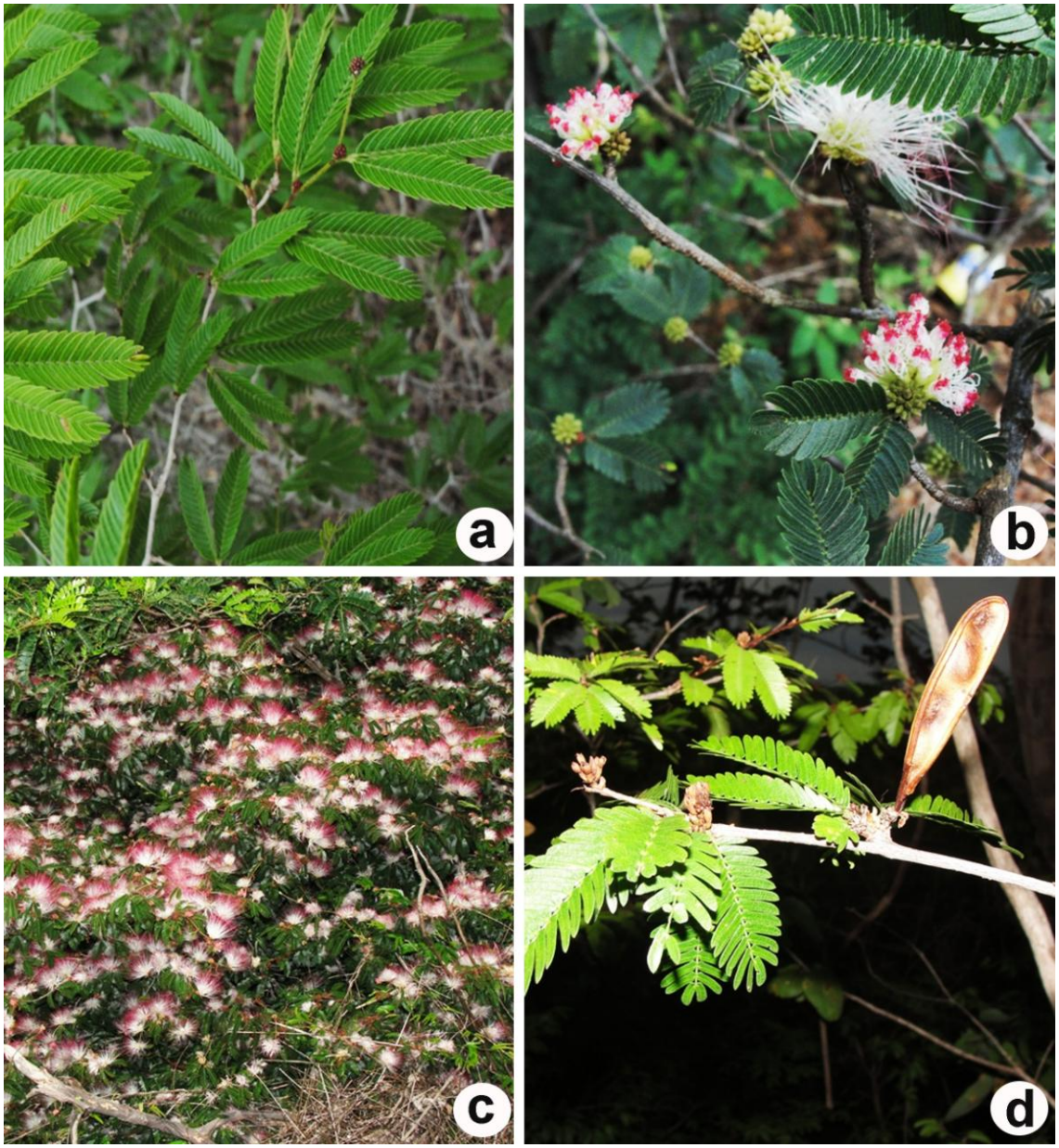

Figura 8. *Calliandra sessilis* Benth.. a. folhas com pinas. b. inflorescência em glomerulo em antese. c. inflorescências. d. fruto legume.

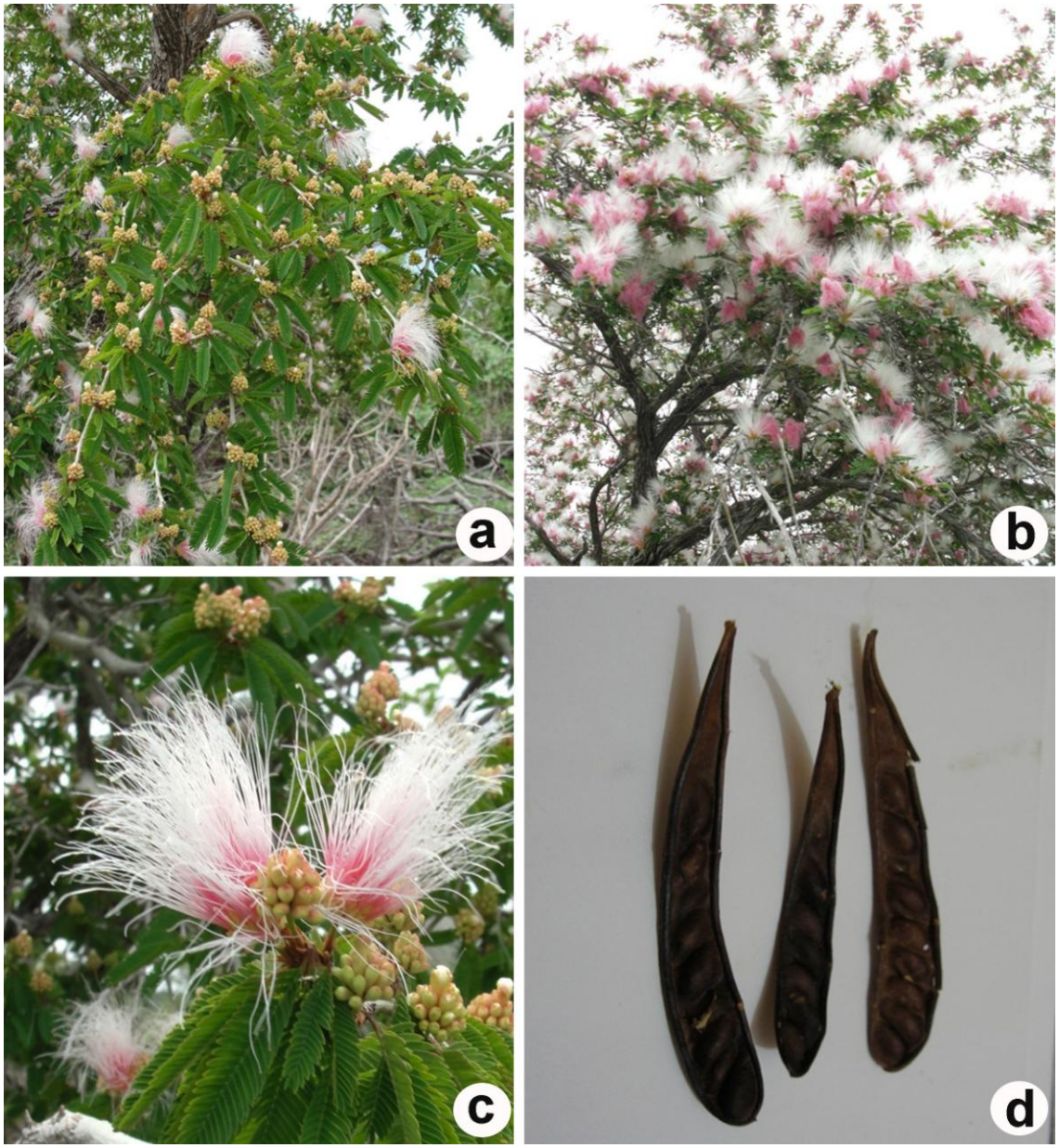

Figura 9. *Calliandra spinosa* Ducke. a. arbusto com folhas e flores. b. inflorescência em glomerulo em antese e com os estames abertos. c. inflorescência aberta. d. fruto legume.

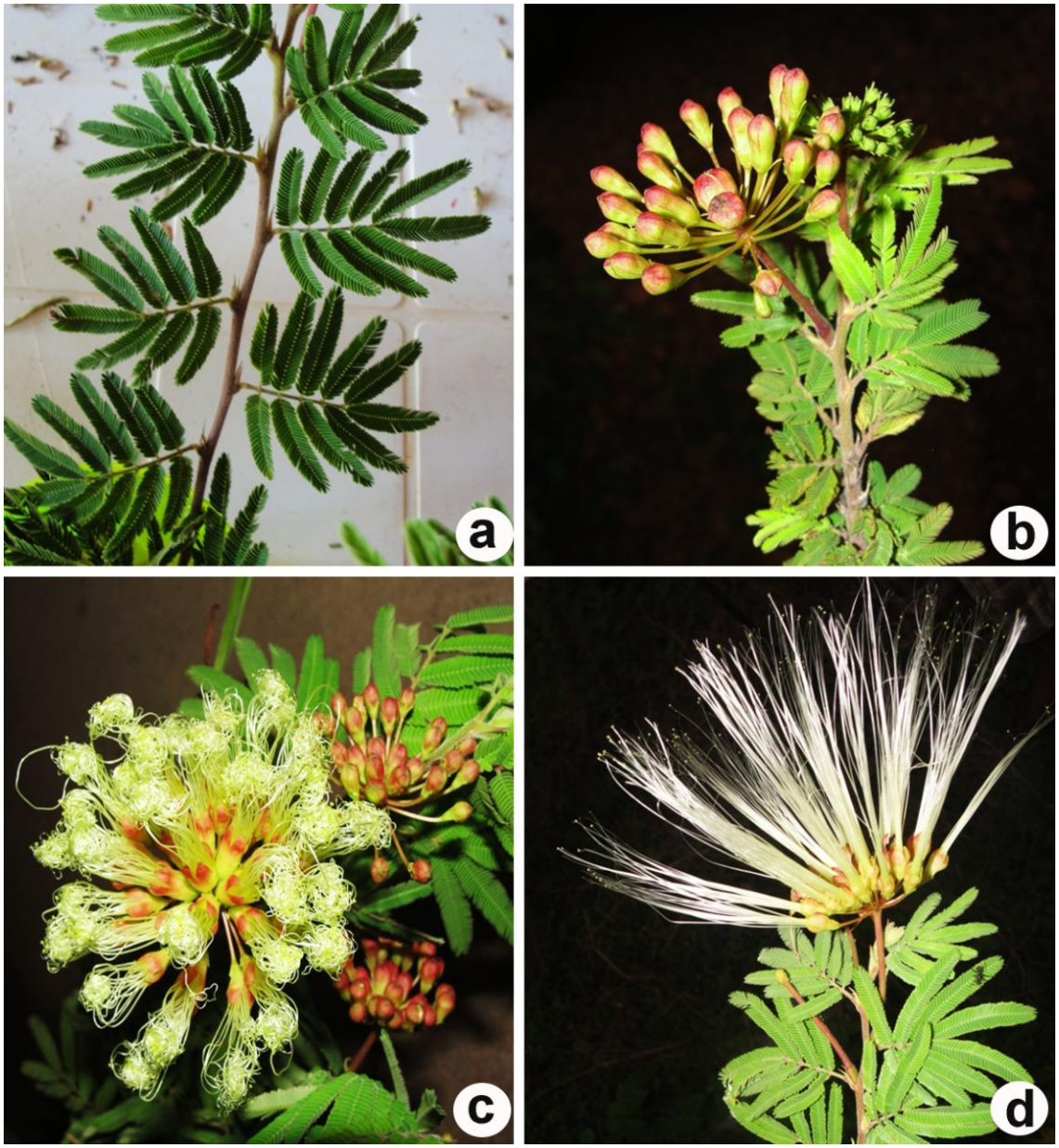

Figura 10. *Calliandra ulei* Harms. a. folhas com pinas e estipulas. b. inflorescência em umbela em antese. c. inflorescência em antese. d. inflorescência.

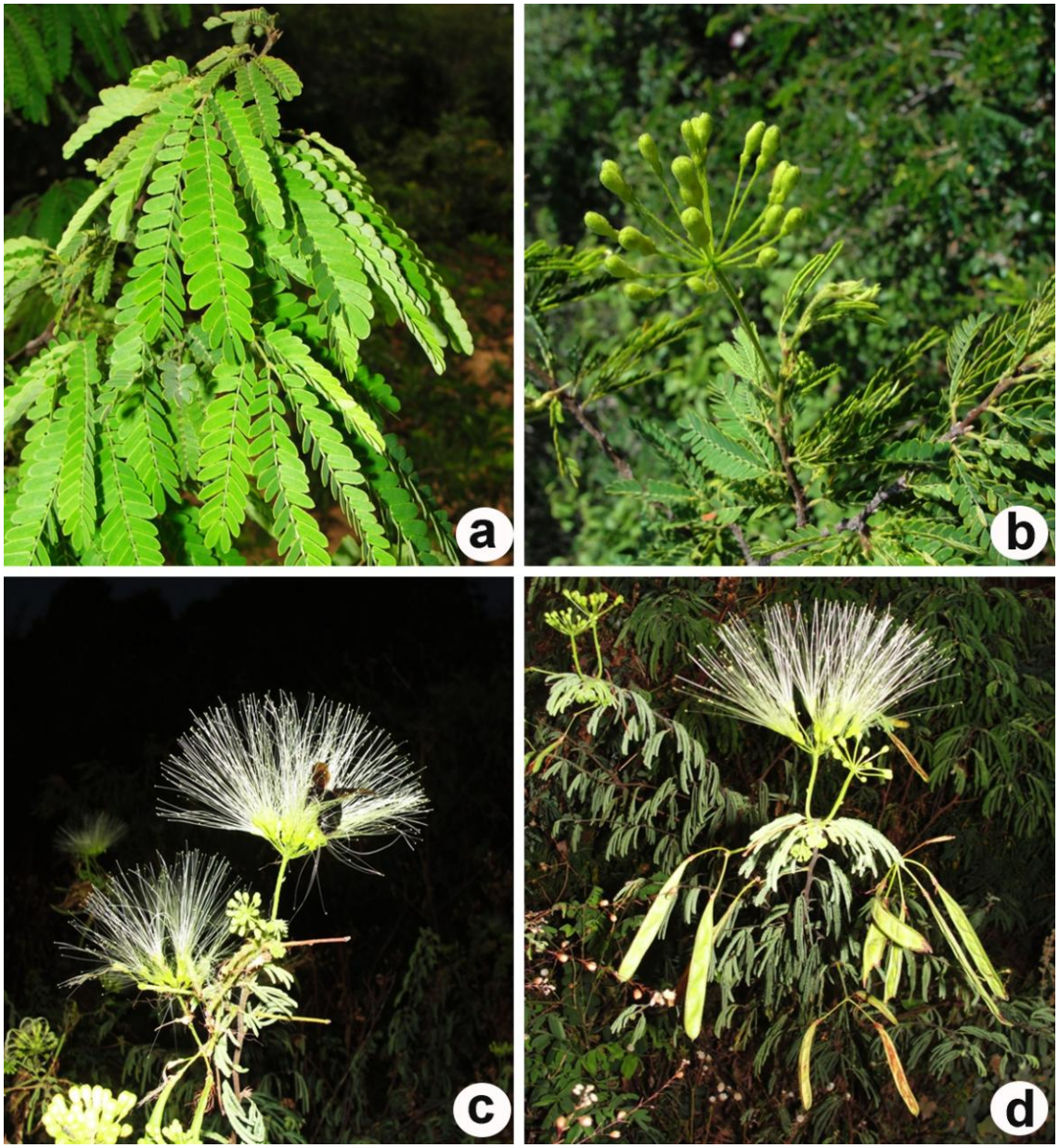

Figura 11. *Calliandra umbellifera* Benth.. a. folhas com pinas. b. inflorescência em umbela em antese. c. inflorescência aberta. d. fruto legume.

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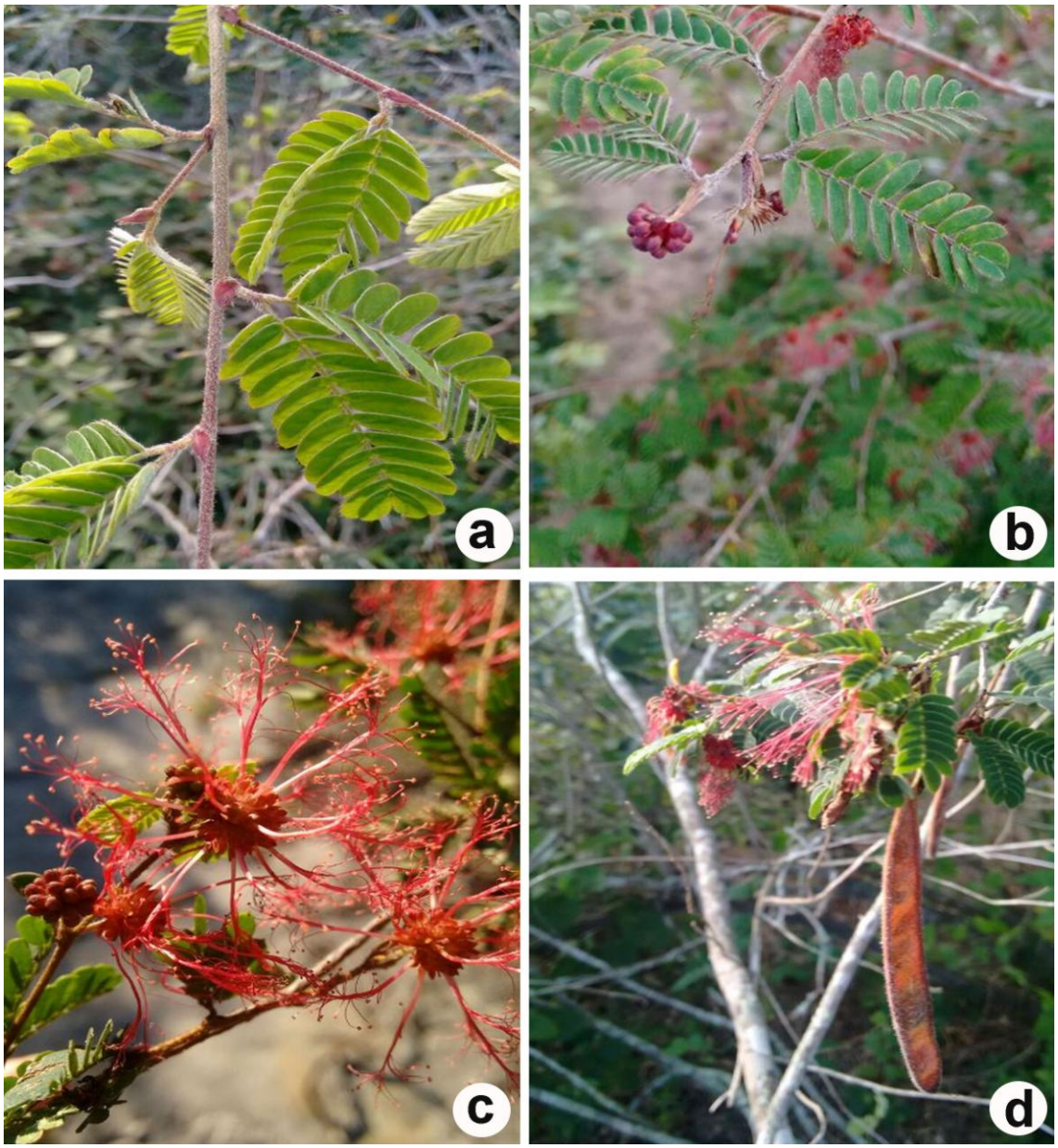

Figura 12. *Calliandra aeschynomenoides* Benth.. a. folhas com pinas. b. inflorescência em glomerulo em antese. c. inflorescência aberta. d. fruto legume.

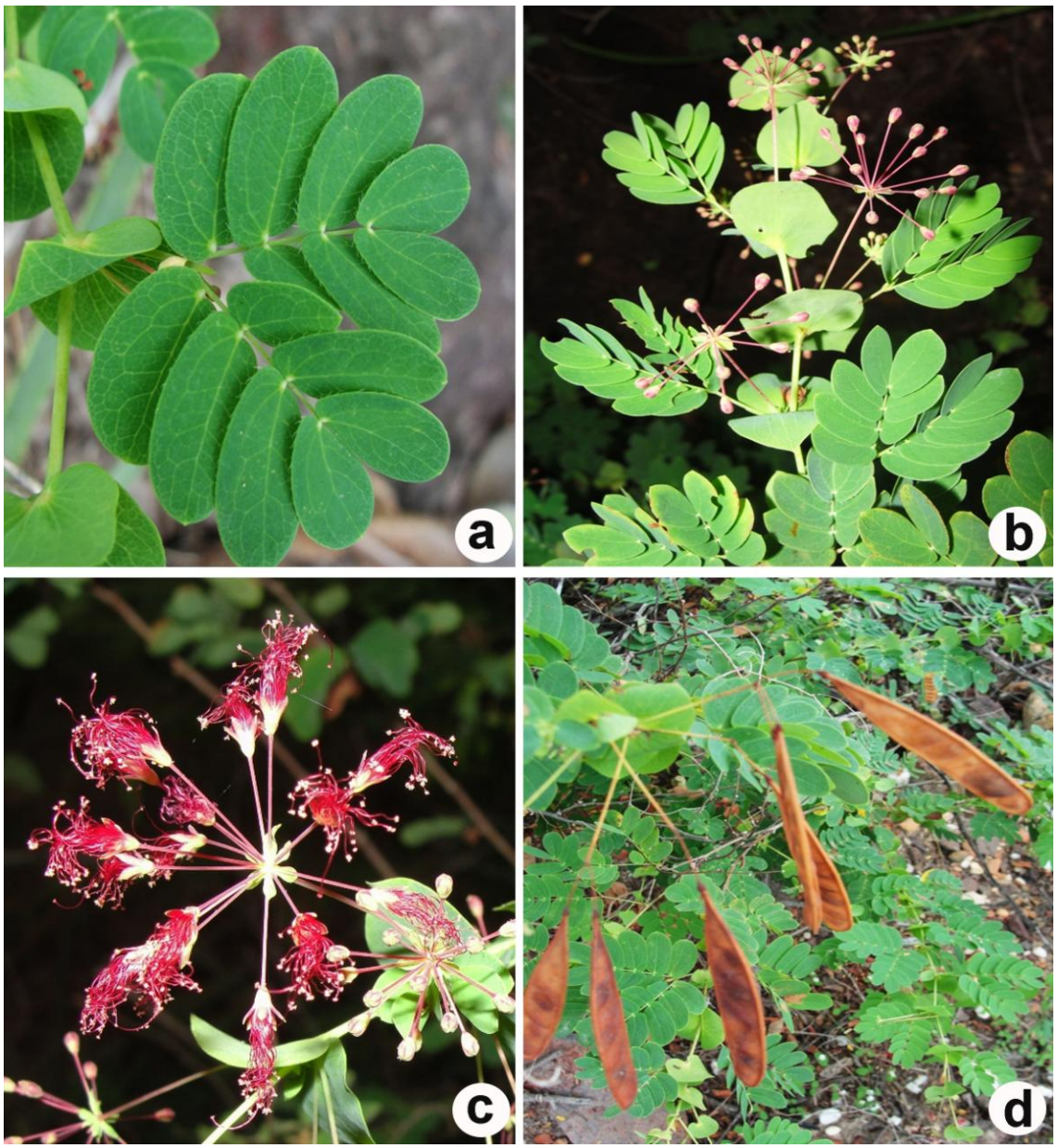

Figura 13. *Calliandra leptopoda* Benth.. a. folhas com pinas. b. inflorescência em umbela em antese. c. inflorescência aberta. d. fruto legume.

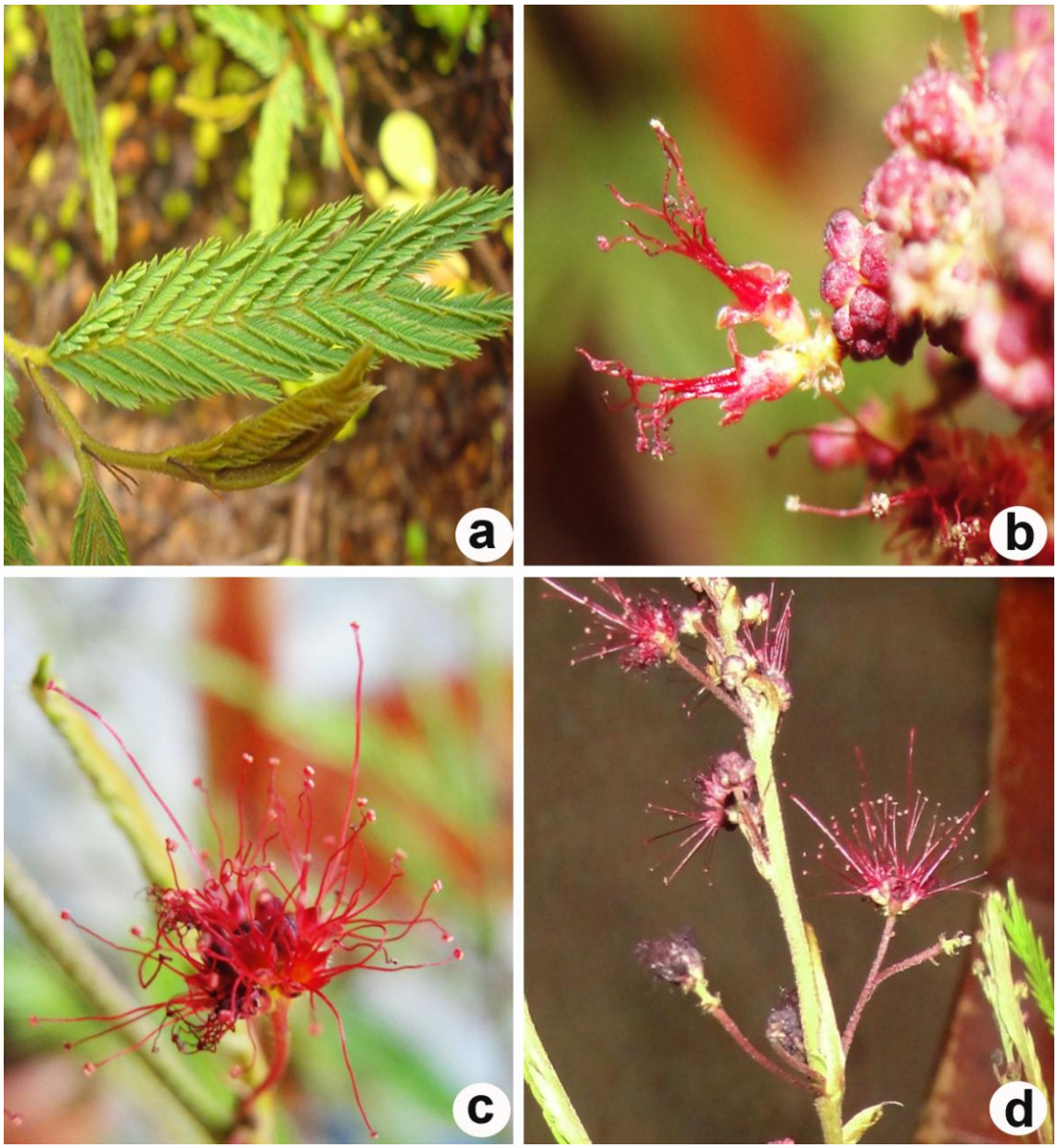

Figura 14. *Calliandra parviflora* Benth.. a. folhas com pinas. b. inflorescência em glomerulo em antese e com os estames abertos. c - d inflorescência aberta.