ADILVA DE SOUZA CONCEIÇÃO

FILOGENIA DO GÊNERO *CHAMAECRISTA* (LEGUMINOSAE-CAESALPINIOIDEAE) E TAXONOMIA DO GRUPO *BASEOPHYLLUM*

FEIRA DE SANTANA – BAHIA

2006

UNIVERSIDADE ESTADUAL DE FEIRA DE SANTANA
DEPARTAMENTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

FILOGENIA DO GÊNERO *CHAMAECRISTA* (LEGUMINOSAE-CAESALPINIOIDEAE) E TAXONOMIA DO GRUPO *BASEOPHYLLUM*

ADILVA DE SOUZA CONCEIÇÃO

Tese apresentada ao Programa de Pós-Graduação em Botânica da Universidade Estadual de Feira de Santana como parte dos requisitos para a obtenção do título de *Doutor em Botânica.*

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FEIRA DE SANTANA – BA
2006

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Feira de Santana – BA
2006

Ao meu Pai, exemplo de determinação, dedico.

“A vontade é a chave maravilhosa que abre todas as portas”.

A. M.

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RESUMO

Chamaecrista Moench é um dos maiores gêneros da subfamília Caesalpinioideae, com aproximadamente 330 espécies, distribuído amplamente nas América, África e Ásia. De acordo com a classificação de Irwin & Barneby, o gênero inclui seis seções. A seção *Absus* inclui quatro subseções, *Absus*, *Baseophyllum* e duas subseções monotípicas (*Otophyllum* e *Adenophyllum*). A subseção *Baseophyllum* inclui três espécies: *C. coriacea*, *C. cytisoides* e *C. depauperata.* A história taxonômica de subsect. *Baseophyllum* é bem confusa, a exemplo da espécie *C. cytisoides* que inclui sete variedades, tratadas por Bentham em diversos trabalhos como espécies distintas. Foram realizados estudos biossistemáticos e filogenéticos para gênero *Chamaecrista* com ênfase na subsect. *Baseophyllum.* Esses estudos tiveram o objetivo de fornecer hipóteses sobre os padrões de relacionamentos filogenéticos do gênero, delimitar o número e categoria dos táxons de *Chamaecrista* subsect *Baseophyllum*, revisar taxonomicamente a subseção, além de testar hipóteses de hibridação entre algumas espécies. Para atingir esses objetivos foram realizadas análises de parcimônia e bayesiana para o gênero usando dados moleculares das regiões ITS e *trnL-F*, além de análises multivariadas para a subsect. *Baseophyllum*, utilizando dados aloenzimáticos e morfométricos, usando os resultados dessas análises como base para a revisão taxonômica desse último grupo. Para as análises multivariadas foram coletados 660 indivíduos de 33 populações naturais de todos os táxons da subseção em 22 localidades nos estados do Espírito Santo, Minas Gerais, Bahia, Sergipe e Pernambuco. Essas análises foram baseadas em 15 loci aloenzimáticos (variabilidade genética) e 54 caracteres morfológicos florais e vegetativos (análise morfométrica). Para o

estudo filogenético foram seqüenciados 55 táxons para a região de ITS nuclear e 57 táxons para *trnL-F* de plastídio. O trabalho de revisão foi realizado com base em extensivo trabalho de campo, análises de material tipos, inventários de espécimes de herbários e, nos resultados obtidos nas análises filogenéticas e análises de variabilidade genética e morfológica. Todos os táxons apresentaram baixa variabilidade genética. Os dados genéticos e morfológicos confirmaram a circunscrição do status de espécie para *C. coriacea* e *C. depauperata*. No entanto, essas análises não sustentaram a delimitação de *C. cytisoides* com suas variedades. As análises mostraram que o táxon tipo do complexo cytisoides, *C. cytisoides* var. *cytisoides* é muito distante genética e morfologicamente de todas as variedades reconhecidas. Assim, estão sendo reconhecidas como distintas de *C. cytisoides* as seguintes espécies: *C. blanchetii*, *C. brachystachya*, *C. confertiformis*, *C. decora*, *C. unijuga*. Os resultados das análises aloenzimáticas e morfométricas confirmaram a ocorrência de hibridação natural entre *C. blanchetii* x *C. confertiformis* e *C. confertiformis* x *C. depauperata.* Nas análises filogenéticas o gênero *Chamaecrista* apareceu fortemente sustentado como um grupo monofilético, bem como as seções *Apoucouita* e *Xerocalyx*. A seção *Absus* apareceu como parafilética, com a subseção *Baseophyllym* monofilética mais relacionada com um clado herbáceo que inclui as seções *Caliciopsis*, *Chamaecrista* e *Xerocalyx* do que com a subseção *Absus* monofilética. Com base nos resultados das análises filogenéticas, genéticas e morfológicas estamos propondo elevar a subseção *Baseophyllum* para o nível de seção e reconhecendo oito espécies para o grupo.

SUMMARY

Chamaecrista Moench is one of the largest genera of Leguminosae subfamily Caesalpinioideae. It embraces about 330 species and is widely distributed in the Americas, Africa, and Asia. According to Irwin & Barneby's classification, this genus includes six sections. Section *Absus* is classified in four subsections: *Absus*, *Baseophyllum* and two monotypic subsections (*Adenophyllum* and *Otophyllum*). Subsection *Baseophyllum* embraces three species: *C. coriacea*, *C. cytisoides*, and *C. depauperata*. *Baseophyllum* has a confuse Taxonomic history mostly because of different circumscriptions for the taxa allied to *C. cytisoides*, treated as distinct species by Bentham in different works and as varieties of *C. cytisoides* by Irwin & Barneby. Phylogenetic and biosystematic studies were carried out in *Chamaecrista* emphasizing the subsect. *Baseophyllum*. These studies aimed to provide hypotheses about phylogenetic patterns in the genus and on number and category of taxa in subsect. *Baseophyllum*. To accomplish these goals, it a maximum parsimony and Bayesian analyses were carried out for the genus based on sequences of ITS and *trnL-F* regions, besides multivariate analyses for subsect. *Baseophyllum* using allozymatic and morphometric data, which provided the framework for a taxonomic revision of the subsection. A total of 660 individuals from 33 populations were sampled for the multivariate analyses, belonging to all taxa described for subsect. *Baseophyllum*, in Espírito Santo, Minas Gerais, Bahia, Sergipe, and Pernambuco states. These analyses were based in 15 allozymatic loci and 54 morphological characters. For the phylogenetic study, it 55 taxa were sampled for ITS and 57 for *trnL-F*. For taxonomic revision, extensive field work and

study of type collections and other herbarium sheets of the taxa included in subsect. *Baseophyllum* were carried out. All taxa presented low genetic variability. Genetic and morphological data were congruent in supporting the specific status of *C. coriacea* and *C. depauperata*. However, these analyses refuted the current circumscription for *C. cytisoides* with included varieties, favoring a hypotheses of six different species in the *C. cytisoides*-complex: *C. blanchetii*, *C. brachystachya*, *C. confertiformis*, *C. cytisoides*, *C. decora*, *C. unijuga*. Allozymatic and morphometric data confirmed hybridizing events among *C. blanchetii* x *C. confertiformis* and *C. confertiformis* x *C. depauperata*. *Chamaecrista* was supported as monophyletic in all analyses, as were sections *Apoucouita* and *Xerocalyx*. Section *Absus* appears to be paraphyletic, with the monophyletic subsect. *Baseophyllum* more related to an herbaceous clade including sections *Caliciopsis*, *Chamaecrista*, and *Xerocalyx* than to a monophyletic sect. *Absus*. Based on our results, we propose to raise subsect. *Baseophyllum* to sectional level and to recognize eight species for the group.

INTRODUÇÃO GERAL

Leguminosae é uma das maiores famílias vegetais com distribuição tropical e temperada, incluindo 727 gêneros e cerca de 19.325 espécies. A família inclui três subfamílias: Mimosoideae, Caesalpinioideae e Papilionoideae com 36 tribos (Lewis *et al.* 2005). Essas subfamílias, em alguns sistemas de classificação, como Cronquist (1981), Dalhgren (1983) e Hutchinson (1964), são tratadas como famílias independentes.

Estudos de filogenia apoiaram o monofiletismo das Leguminosae (Chase *et al.* 1993; Chapill 1994; Doyle 1995; Wojciechowski 2003; Wojciechowski *et al*. 2004). Esses estudos colocaram a família dentro do clado das Eurosidae I ou Fabídeas, próxima das Polygalaceae, Surianaceae e Quillajaceae as quais, em conjunto, constituem a ordem Fabales.

A subfamília Caesalpinioideae compreende quatro tribos: Cercideae, Detarieae, Cassieae e Caesalpinieae, com 172 gêneros e cerca de 2.250 espécies com ampla distribuição nas regiões tropicais e subtropicais do globo (Lewis *et al.* 2005). Essa subfamília aparece como parafilética (Doyle *et al.* 2000), sendo alguns gêneros mais próximos de Mimosoideae (p.e. *Tachigali, Sclerolobium* e *Dimorphandra*) e de Papilionoideae (p.e. *Duparquetia*).

A tribo Cassieae inclui cinco subtribos: Cassiinae, Ceratoniinae, Dialiinae, Duparquetiinae e Labicheinae. Cassiinae é a segunda maior subtribo das Cassieae com três gêneros: *Cassia, Chamaecrista* e *Senna*. Os estudos filogenéticos com *rbcL* mostraram que Cassiinae é monofilética (Kajita *et al*. 2001). Por outro lado, as análises baseadas em *trnL-F* e dados morfológicos não sustentaram o monofiletismo de

Cassiinae mostrando que há hipóteses conflitantes quanto aos relacionamentos dos três gêneros da subtribo (Doyle *et al.* 2000; Bruneau 2001; Herendeen *et al*. 2003).

O gênero *Chamaecrista* foi estabelecido por Moench (1794). Vogel (1837) propôs o táxon *Cassia* sect. *Lasiorhegma* que é coincidente com o conceito de *Chamaecrista* de Moench (1794). Bentham (1870) apresentou um estudo da tribo Cassieae no Brasil para a Flora Brasiliensis, reconhecendo três gêneros: *Martia, Dicorynia* e *Cassia*. Nesse estudo o gênero *Cassia* foi dividido em três subgêneros: *Fistula, Senna* e *Lasiorhegma*, este último incluindo as espécies hoje tratadas como *Chamaecrista.* No ano seguinte, Bentham (1871) realizou uma revisão de *Cassia*, subdividindo-o em três subgêneros e oito seções, onde foi mantida a mesma delimitação para subgênero *Lasiorhegma.*

Greene (1897) elevou *Cassia* subg. *Lasiorhegma* sect. *Chamaecrista* ao nível genérico, posição apoiada por Pollard (1902) e Pennel (1917). Britton & Rose (1930) também aceitaram o novo *status* do táxon e reconheceram 111 espécies de *Chamaecrista,* dividindo o gênero em 17 seções.

Irwin (1964) publicou a revisão de *Cassia* sect. *Xerocalyx*, não aceitando a proposta de Greene (1897) de tratar *Chamaecrista* como um grupo independente, reconhecendo o gênero *Cassia* no sentido proposto por Bentham (1871). Nesse trabalho a subseção *Xerocalyx* foi elevada para seção, onde foram incluídas 16 espécies. Quatro anos depois, Irwin & Rogers (1967) apresentaram a revisão de *Cassia* sect. *Apoucouita.*

Irwin & Barneby (1976) analisaram a história e o *status* de *Chamaecrista*, considerando o táxon como *Cassia* subg. *Lasiorhegma* sect. *Chamaecrista*, mantendo a classificação estabelecida por Irwin (1964). Três anos depois, os mesmos autores (Irwin & Barneby 1979) publicaram cinco espécies novas no gênero *Chamaecrista.* Porém,

apenas dois anos depois, Irwin e Barneby (1981) propuseram a divisão de *Cassia* em três gêneros: *Cassia, Chamaecrista* e *Senna.*

O gênero *Chamaecrista* Moench é um dos maiores da subfamília Caesalpinioideae. Segundo Irwin & Barneby (1982), o gênero inclui seis seções: *Chamaecrista* sect. *Apoucouita* (Benth.) H.S.Irwin & Barneby, *Chamaecrista* sect. *Absus* (Collad.) H.S.Irwin & Barneby, *Chamaecrista* sect. *Grimaldia* (Schrank) H.S.Irwin & Barneby, *Chamaecrista* sect. *Chamaecrista*, *Chamaecrista* sect. *Caliciopsis* H.S.Irwin & Barneby e *Chamaecrista* sect. *Xerocalyx* (Benth.) H.S.Irwin & Barneby. Essas seções estão representadas nas Américas, África e Ásia com cerca de 330 espécies (Lewis 2005). No Brasil, o gênero está representado por cerca de 232 espécies (Irwin & Barneby 1978, 1982).

Chamaecrista sect. *Absus* possui quatro subseções: *C.* sect. *Absus* subsect. *Baseophyllum* (Collad.) H.S.Irwin & Barneby, *C.* sect. *Absus* subsect. *Adenophyllum* (H.S.Irwin & Barneby) H.S.Irwin & Barneby, *C.* sect. *Absus* subsect. *Otophyllum* (H.S.Irwin & Barneby) H.S.Irwin & Barneby, *C.* sect. *Absus* subsect. *Absus* (Collad.) H.S.Irwin & Barneby.

Segundo Irwin & Barneby (1978), a subseção *Baseophyllum* ocorre nos estados de Pernambuco, Alagoas, Bahia e Minas Gerais. De acordo com a circunscrição adotada por Irwin & Barneby (1978, 1982), a subseção inclui duas espécies: *C. cytisoides* com sete variedades e *C. coriacea. Chamaecrista* subsect. *Baseophyllum* têm uma história taxonômica confusa por causa de diferentes visões sobre os táxons relacionados a *C. cytisoides*. Estes foram tratados por Bentham (1840, 1870, 1871) como espécies independentes e, posteriormente por Irwin & Barneby (1978, 1982) como variedades. Conceição *et al*. (2001), em um levantamento do gênero *Chamaecrista* para região de

Catolés (Chapada Diamantina, Bahia), reconheceram *C. depauperata* como uma nova espécie para a subseção *Baseophyllum.*

Os tratamentos taxonômicos tradicionais realizados por Bentham e Irwin & Barneby, não foram suficientes para elucidar os problemas de delimitação dentro do grupo. As dificuldades encontradas para delimitar táxons dentro da subseção *Baseophyllum* podem estar relacionadas com a sobreposição de caracteres morfológicos ou ocorrência de formação de híbridos naturais entre os táxons desse grupo.

Nesse trabalho é apresentado um estudo filogenético do gênero *Chamaecrista* e um estudo biossistemático para o grupo *Baseophyllum*, objetivando determinar padrões de relacionamento filogenético para o gênero, investigar o número e categorias taxonômicas existentes para o grupo *Baseophyllum*, testar hipóteses de hibridação entre espécies desse grupo e fornecer caracteres diagnósticos para os táxons. Para atingir esses objetivos foram realizadas análises filogenéticas com dados moleculares, para estabelecer hipóteses de relacionamentos filogenéticos dentro do gênero (Capítulo 1), análises multivariadas com dados genéticos e morfológicos para determinar a variabilidade genética e morfológica dos táxons que compõem o grupo *Baseophyllum* (Capítulo 2) e testar hipótese de ocorrência de hibridação natural entre espécies desse grupo (Capítulo 3). Uma revisão taxonômica do grupo *Baseophyllym* foi realizada baseada nos dados obtidos dessas análises, inventário de coleções e materiais-tipo e, extensivo trabalho de campo (Capítulo 4).

Capítulo 1

Phylogeny of *Chamaecrista* (Leguminosae-Caesalpinioideae) based on nuclear and chloroplast DNA regions and patterns in the evolution of selected morphological characters

Este capítulo está formatado para publicação na revista Sistematic Botany

RESUMO

O monofiletismo de *Chamaecrista*, um dos três gêneros da subrtribo Cassiinae, foi testado usando seqüências de DNA das regiões de ITS nuclear e *trnL-F* de plastídio. Foram seqüenciados 55 táxons para ITS e 57 para *trnL-F,* representando cinco das seis seções de *Chamaecrista*. Os dados foram analisados separadamente e combinados utilizando métodos de máxima parcimônia e bayesiana. As análises foram congruentes para os principais grupos com alto suporte de bootstrap e probabilidade posterior, exceto para um sub-clado do clado "*Chamaecrista*". As relações de grupo irmão de *Chamaecrista* com os gêneros *Cassia* e *Senna* não foram conclusivas. No entanto, o monofiletismo de *Chamaecrista* foi fortemente sustentado em todas as análises. Duas das seções reconhecidas por Irwin e Barneby, as seções *Apoucouita* e *Xerocalyx*, foram sustentadas como monofiléticas. A seção *Absus* aparece sustentada como parafilética, mas a subseção *Baseophyllum* aparece mais relacionada com um grupo herbáceo que inclui as seções *Caliciopsis, Chamaecrista* e *Xerocalyx* do que com a subseção *Absus*. A seção *Chamaecrista* aparece parafilética em relação às seções *Caliciopsis* e *Xerocalyx.* As análises sugerem que os padrões de diversificação em *Chamaecrista* ocorreram inicialmente pela mudança de árvores de florestas úmidas para um grupo mais diverso de arbustos de cerrado. Dentro desse último grupo, um clado de montanhas elevadas é definido pela perda de nectários extraflorais e pelo surgimento de tricomas glandulares viscosos sendo essa, provavelmente, uma inovação, parte da estratégia de defesa da planta. Outra linhagem bem diversa é marcada pela mudança para o hábito herbáceo, inflorescência fasciculada axilar e redução de números cromossômicos. Este último grupo é mais diverso em áreas de campo e inclui muitas ervas daninhas de áreas ruderais.

Phylogeny of *Chamaecrista* (Leguminosae-Caesalpinioideae) based on nuclear and chloroplast DNA regions and patterns in the evolution of selected morphological characters

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short title: Phlylogeny of *Chamaecrista*

ABSTRACT. The monophyly of *Chamaecrista*, one of three genera of the subtribe Cassiinae, was tested using sequence data from nuclear ITS and plastid *trnL-F* DNA regions. We sequenced 55 taxa for the ITS and 57 for *trnL-F* regions, representing five of the six sections of *Chamaecrista*. The data were analyzed separately and in combined analyses using maximum parsimony and Bayesian methods. The results were congruent in recovering the same major clades with high bootstrap and posterior supports, except for the subclades of a clade bringing together representatives of section *Caliciopsis*, *Chamaecrista* e *Xerocalyx*. Sister group relationships of *Chamaecrista* with *Cassia* or to a *Cassia-Senna* clade were not conclusive. However, the monophyly of *Chamaecrista* was highly supported in all analyses. Sections *Apoucouita* and *Xerocalyx* were supported as monophyletic. Section *Absus* appeared as paraphyletic with strongly supported subsections but subsect. *Baseophyllum* appearing more related to a herbaceous group than to subsect. *Absus*. Section *Chamaecrista* appeared paraphyletic with respect to sections *Caliciopsis* and *Xerocalyx*. Our analyses suggest that the diversification patterns in *Chamaecrista* occurred through an initial shift from rainforest trees to more successful clade of savannah shrubs. Within the latter group, a high mountain clade is marked by loss of extrafloral nectaries and the appearance of sticky glandular hairs, probably as a key innovation as part of the plant defense strategy. Another successful lineage is marked by a shift to herbaceous habit, axillary fascicled inflorescences and reduced chromosome numbers. This last group is more diverse in open grassland areas and includes many weeds of waste ground.

Chamaecrista Moench includes about 330 species and is most diverse in the Neotropics (Lewis 2005). This genus was segregated from *Cassia* L. and placed in subtribe Cassiinae of the Caesalpinioideae, together with *Cassia* and *Senna* P.Miller (Irwin and Barneby 1981).

Subtribe Cassiinae is defined by a syndrome of floral characters that could be interpreted as specializations for buzz pollination by large bees, such as absence of floral nectaries, poricidal anthers and many cases of enantiostyly (Irwin and Barneby 1981, 1982; Marazzi et al. 2006). It is a diverse group with about 700 species and Pantropical distribution with a Neotropical bias. Monophyly of the Cassinae was supported by *rbcL* data (Kajita et al. 2001). However, *trnL-F* and morphological data raised doubts about the monophyly of the Cassiinae and provided conflicting hypotheses about the relationship of *Chamaecrista* with *Senna* and *Cassia* (Bruneau et al. 2001, Herendeen et al. 2003).

In its modern concept, *Chamaecrista* includes shrubs, herbs or trees with pedicels bibracteolate near or above its middle, androecium actinomorphic with anthers pubescent along the sutures, pods elastically dehiscent and extrafloral nectaries, when present, disc- or cup-shaped. Irwin and Barneby (1978, 1982) recognized six sections (Table 1) mostly based on a combination of characters such as patterns of inflorescence and presence or absence of extrafloral nectaries and of sticky glandular hairs. Sections *Apoucouita* and *Absus* embrace the woody representatives of the genus, the first including mostly arboreal plants with cauliflorous racemes, the second mostly shrubs with terminal racemes. Sections *Grimaldia*, *Chamaecrista*, *Caliciopsis*, and *Xerocalyx* bring together mostly herbaceous to subshrubby species with inflorescence reduced to axillary fascicles. These sections are differentiated by a combination of patterns of sepal

venation, presence of extrafloral nectaries and secretory hairs. Sections *Apoucouita*, *Absus*, and *Chamaecrista* were further subdivided into infra-sectional taxa (Table 1).

Extrafloral nectaries (EFNs) are common in many plant families and occur on several plan organs (Buckley 1983; Koptur 1979). Amongst the Leguminosae, EFNs are common in subfamily Mimosoideae but rarely found in subfamily Caesalpinioideae. Within the latter, EFNs are found only in *Bauhinia*, *Chamaecrista* and *Senna*. Marazzi et al. (2006) found that EFNs may be taken as a key innovation of a rich species clade of *Senna* and the appearance of this feature could allow its diversification in areas subject to a climate with a marked dry season. This diversification may be related to the biological roles of the EFNs in establishing association with ants, which acts as a antiherbivore defense mutualistic system (Kawano et al 1999; Mondor and Addicott 2003; Katayama and Suzuki 2004).

Glandular trichomes in *Chamaecrista* are restricted to subsect. *Absus* (165 species) and *Grimaldia* (only one species). These trichomes present a very conserved structure with a bulbous dilated base continuing into a tube which exudes a sticky secretion (Irwin and Barneby 1978). An interesting feature associated with the presence of these glandular trichomes is that they are strongly correlated with absence of EFNs. Unfortunately we were unable to discover information about their biological role but was possible to test if they appeared only once or multiple times and, in the first case, if they replaced the EFNs in providing a protective role and allowing diversification of a monophyletic group.

Chromosome counts in Cassiinae are scattered (Fedorov 1969; Goldblatt 1981; Goldblatt and Johnson 1990, 1994, 2000; Souza and Benko-Iseppon 2004; Biondo et al. 2006). The available data for *Cassia* and *Senna* show that most of the species sampled have 2n = 28 and and Goldblatt (1981) hypothesized a basic chromosome number of x =

14 for these genera. *Chamaecrista* is chromosomally heterogeneous and distinct basic chromosome numbers were established for different sections: x = 14 for sect. *Absus*, x = 8 for sect. *Chamaecrista*, and x = 7 for sect. *Xerocalyx* (Goldblatt 1981). Irwin and Turner (1960) suggested that x = 7 is basic for *Chamaecrista* with n = 8 in sect. *Chamaecrista* derived from increasing aneuploidy, and n = 14 in sect. *Absus*, a result of polyploid increase. Goldblatt (1981) disputed this view based on the fact that the herbaceous sects. *Chamaecrista* and *Xerocalyx* seem to be specialized groups within genus *Chamaecrista*. Thus, there is no agreement about the question of chromosomal evolution in *Chamaecrista* and it could be benefited by an explicit phylogenetic hypotheses.

The main aim of the present work is to provide molecular phylogenetic hypotheses for *Chamaecrista* in order to evaluate current taxonomic framework (Irwin and Barneby 1982) and to provide hypotheses of morphological and chromosomal evolution in this genus. It also aims to answer the following questions: (1) Is *Chamaecrista* monophyletic? (2) What is the sister group of *Chamaecrista* (3) Are the current sections, subsections, and series monophyletic? (4) Are morphological and chromosomal evolution congruent with phylogenetic patterns?

MATERIALS AND METHODS

Taxon sampling. We sampled 57 taxa, of which 46 belong to *Chamaecrista* and nine to other Caesalpinioideae (Table 2). Complete sampling of all species for all DNA regions was not possible due to lack of material of *Senna pleurocarpa* and difficulties in sequencing *Chamaecrista chapadae* and *C. serpens*. However, representatives of five of all six sections of *Chamaecrista* were included. The monotypic section *Grimaldia* was

not included because we failed to obtain a sample of *C. absus* and were unable to amplify it from herbarium material. All samples were field collected except *C. calycioides*, which was obtained from herbarium material. The choice of nine outgroups was based on previously published phylogenies of Caesalpinioideae (Bruneau et al. 2001; Kajita et al. 2001).

DNA extraction, amplification, and sequencing. DNA was extracted mostly from fresh or silica-gel dried leaves using a modified version of the 2X CTAB procedure of Doyle and Doyle (1987). For amplification and sequencing of ITS, we used the primers 75 and 92 of Desfeaux (1996). The amplification was performed in a 50µl reaction containing with a final concentration of 1X reaction buffer, 2.5 Mm $MgCl_2$, 1Mm dNTPs, 0.5 µM of each primer, 1µg BSA, 1.0M betaine, 2% DMSO, 1.25 units Taq DNA polymerase and program consisting of 40 cycles of 94ºC denaturation for 1 min, 56ºC annealing for 1min, and 72ºC of extension for 1 min. For some samples we used the primers 17SE and 26SE (Sun et al. 1994) for amplification and sequencing of ITS, and PCR conditions were 28 cycles of 94ºC denaturation for 1 min, 50ºC annealing for 1min, and 72ºC of extension for 3 min. For amplification and sequencing of *trnL-F*, we used two universal primers (C, F) of Taberlet et al. (1991). The amplification was performed in a 50µl reaction with a final concentration of 1X reaction buffer, 2.5 Mm $MgCl_2$, 1Mm dNTPs, 0.5 µM of each primer, 1µg BSA, 1.25 units Taq DNA polymerase and program consisting of 40 cycles of 94ºC denaturation 1 min, 50-52ºC annealing for 1min, and 72ºC of extension for 2 min. All PCR reactions were performed using PCR GeneAmp 9700 thermocicler (Applied Biosystems). The PCR fragments were purified by enzimatic treatment with Exonuclease I (EXO) and Shrimp Alkaline Phosphatase (SAP) (Amersham Biosciences). The cycle-sequencing reactions

were performed with the Big Dye Terminator version 3.1 (Applied Biosystems). Samples were sequenced in both directions using the Spectrumedix SCI SCE9624 automated sequencer at Universidade Estadual de Feira de Santana (UEFS).

Phylogenetic Analyses. Eletropherograms were edited and assembled using Staden Package (Staden et al. 1998) and aligned using CLUSTAL X (Thompson et al. 1997), and the resulting alignment was corrected manually following the guidelines in Kelchner (2000). A manually coded binary gap matrix with all non-autopomorphic, unambiguous indels in the *trnL-F* gene data set was included. Gaps on the ITS dataset were not coded because of difficulties in the alignment and more ambiguous nature of the gaps. In the main alignment, gaps in both ITS and *trnL-F* data sets were considered missing data. In the *trnL-F,* we included one sequence (*Senna pleurocarpa*) from Genbank (Accesion number AF367007). Parsimony analyses were performed using PAUP* (Phylogenetic analysis using parsimony), version 4.0b10 (Swofford 2002) with Fitch parsimony (equal weights, unordered; Fitch 1971) as the optimality criterion. Initially we performed two separate searches for the ITS dataset alone and *trnL-F* data. A third analysis included the combined data from both DNA regions. Each search consisted of 2000 random taxon-addition replicates, with the tree-bisection-reconnection (TBR) algorithm, and limited swapping on 15 trees per replicate to prevent extensive swapping on islands with many trees. The resulting trees were then used as starting trees for TBR swapping with an upper limit of 40000 trees. Internal support was evaluated using 1000 replicates of character bootstrapping (Felsenstein 1985), with simple taxon-addition and TBR algorithm, saving 15 trees per replicate. The model-based analysis was performed with Bayesian inference (Larget and Simon 1999; Lewis 2001), using MRBAYES version 3.01 (Ronquist and Huelsenbeck 2003). The model

used for both analyses was GTR+I+G, as indicated by nested likehood-ratio tests using MRMODELTEST version 2.2 (Nylander 2004). MRBAYES was run for 1,500,000 generations, with four chains, sampling trees every 100 generations. The burn-in stage needed to reach stationarity was determined by plotting the likelihood scores against the number of generations. The trees sampled from within the burn-in stage were excluded (500-*trnL-F*, 120-*ITS* and 230-for combined analyses), and the remaining trees were assumed to be representative of the posterior probability distribution. The majority rule consensus tree was calculated in PAUP*version 4.0b10 (Swofford 2002), and the resulting group frequencies represent the posterior probabilities. Sequences will be submitted to Genbank.

In order to make discussion easier, major clades were named alphabetically using one of the most parsimonious trees of the combined analysis and the same names were showed on other trees for comparision.

RESULTS

Size and characteristics of the molecular data sets. General features of DNA data sets used are presented in Table 3. A total of 55 taxa were sequenced for the *trnL-F*. Sequence length varied from 642 base pairs (bp) in *Chamaecrista calycioides* to 1000 bp in *Copaifera coriacea*. Maximum parsimony analysis (MP) generated 27,900 trees (one of which randomly selected is shown in Fig. 1) with 225 steps, CI 0.844, RI 0.905. The ITS region was sampled for 57 taxa and length varied from 521 bp in *Chamaecrista nictitans* var. *disadena* to 802 bp in *C*. aff. *cavalcantina*. MP analysis resulted in 940 trees (one of which randomly selected is shown in Fig. 2) with of 1979 steps, CI 0.574,

RI 0.803. In the combined analyses the MP analysis generated 339 trees with 2277 steps, CI 0.606, RI 0.808.

Plastid trnL-F. In the parsimony analysis (Fig. 1) the strict consensus of all trees was well resolved for major groups (clades A-H) while the included subgroups were unresolved in a large polytomy. The genus *Chamaecrista* was supported as monophyletic with Bootstrap Support (BS) 99% and posterior probability (PP) 100%. Clade A includes species of sect. *Apoucouita* (BS 96%) and appears as sister of the remaining taxa of the genus (clade B). Clades C includes species of sect. *Absus* subsect. *Absus* (BS 94%, PP 100%). Clade D have low support and includes two main clades, E and F. Clade E (sect. *Absus* subsect. *Baseophyllum*) appear well-supported, but with internal relationships largely unresolved. Clade F (BS 81%, PP 100%) includes representatives of sections *Caliciopsis*, *Chamaecrista*, and *Xerocalyx* (clade G). Bayesian analysis was congruent with MP analysis in recovering the same major groups, with high posteriors, as it could be expected (Fig.1).

Nuclear ITS. Parsimony analysis of ITS data was congruent with the results of the *trnL-F* analysis regarding the major clades (clades A-H), except for the positions of clade H, of *Chamaecrista supplex* and the *C. pilosa-C. rotundifolia* clade (Fig. 2). Bayesian analysis also recovered the same major clades except clade F. However, relationships among the taxa included in clade D were different between MP and Bayesian analyses. Clade F had low support in MP analysis (BS < 50%) and was not recovered in Bayesian analysis. The other major disagreement was due to the position of *Chamaecrista supplex* which is supported as sister to the remainder of clade D in the MP trees and as part of clade H in the Bayesian analysis. Both analyses of ITS fragment provided better resolution within the major clades than the analyses carried out with the *trnL-F* (Fig. 2).

Combined analyses. No strongly supported incongruent patterns of relationship were detected in the individual analyses, which allowed us to carry out combined analyses. The topology observed in the parsimony analysis (Fig. 3) was very similar to the one found in the Bayesian analysis (Fig. 4), except by minor details. The sister group of *Chamaecrista* was pointed out as being *Cassia* (BS 66%) in the parsimony analysis while the Bayesian analysis supports a clade with representatives of *Senna* and *Cassia*. Both analyses support the genus *Chamaecrista* as a monophyletic entity as well as its subdivision in eight major clades (A-H) also differing in the relationships among the subclades of clade D (Figs. 3-4).

DISCUSSION

Molecular evolution- The levels of variation differ notably in the different fragments of DNA. As expected, ITS has the greatest number of variable sites associated with a fastest rate of change (Table 2). The CI and RI of these regions are clearly lower than *trnL-F,* due the higher rate of change per site in ITS, which is three times higher than *trnL-F*. The transition/transversion ratio (ts: tv) (Table 2) in ITS was 1.02, whereas in *trnL-F*, it is 1.08, similar to other studies with these regions (Whitten 2000; van den Berg 2005).

Comparison with Irwin and Barneby's classification and previous Caesalpinioideae phylogenetic analyses - All analyses support the monophyly of *Chamaecrista* as circumscribed by Irwin and Barneby (1981, 1982). Previous hypotheses of monophyly of this genus were provided by other studies using molecular data (Bruneau et al. 2001; Kajita et al. 2001), however using only a few species.

Putative synapomorphies of *Chamaecrista* are the presence of flat to concave extrafloral nectaries, pedicels bibracteolate near or above the middle, anthers with puberulent sutures, and fruits dehiscent with the valves becoming twisted. The MP analysis of the combined dataset (Fig. 3) indicated the genus *Cassia* as sister to *Chamaecrista*, but with low bootstrap value, whereas the Bayesian analysis (Fig. 4) of the same data supports *Chamaecrista* as sister to a *Senna*-*Cassia* clade. Thus, the present study did not elucidate the sister group of *Chamaecrista*, a question that came from other phylogenetic studies that sometimes suggested *Chamaecrista* as being more related to *Senna* (Doyle et al. 2000; Kajita et al. 2001; Herendeen et al. 2003) or in which *Senna* and *Cassia* appear as sisters groups while *Chamaecrista* appears as a more distant group (Bruneau et al. 2001; Marazzi et al. 2006).

At sectional level, only two of the six sections proposed by Irwin and Barneby (1982) were supported as monophyletic: *Apoucouita* and *Xerocalyx*. The monotypic section *Grimaldia* was not included in the present analyses. Section *Absus* appeared paraphyletic with subsection *Baseophyllum* as part of the clade D.

Section *Absus* includes four subsections according to Irwin and Barneby's (1982) classification. We were not able to include representatives of the monotypic subsections *Otophyllum* and *Adenophyllum*. Subsection *Absus* (clade C) was supported as monophyletic with high BS support (94-100%) and PP 100%. This is a very distinct group presenting as synapomorphies the loss of extrafloral nectaries and the presence of sticky glandular hairs. Our study did not include representative samples of the series included in subsect. *Absus* and most were represented only by one species. However, there is strong support for considering ser. *Rigidulae* as paraphyletic since all analyses recovered a closer relationship among *C. glaucofilix* (ser. *Rigidulae*) with *C. hispidula* (ser. *Absoideae*) than to *C. rupestrium* (Figs. 2-4). Series *Absoideae* appeared supported

as polyphyletic with their species scattered along the phylogeny of the subsect. *Absus* (Figs. 1-4). Subsection *Baseophyllum* (clade E) was also supported as monophyletic in all analyses with high support (BS 84-100%, PP 100 %) but as part of clade D, which brings together species of sections *Chamaecrista*, *Caliciopsis*, and *Xerocalyx* (Figs. 3, 4). Thus, these analyses support *Baseophyllum* as a distinct group within the genus *Chamaecrista* and that this group should not be considered as a subsection of section *Absus*.

Section *Chamaecrista* is also found to be paraphyletic in respect both to sections *Xerocalyx* and *Calyciopsis*. Within section *Chamaecrista*, our results refute the monophyly of series *Prostatae* and support the monophyly of the series *Chamaecrista* and *Coriaceae*, although with these conclusions should be accepted with caution due to limited taxon sampling. C*hamaecrista calycioides* is one of the two species of section *Caliciopsis*. In the present analyses, this species appeared embedded within a paraphyletic section *Chamaecrista*. Even not including all species of section *Caliciopsis*, it position nested within section *Chamaecrista* does not support the hypothesis that this section represents an isolated lineage within the genus *Chamaecrista*.

Irwin and Barneby (1982) recognized six series for section *Chamaecrista*. Series *Prostatae* appeared as polyphyletic while series *Coriaceae* and *Chamaecrista* were supported as monophyletic (Fig. 1-4). Series *Coriaceae* includes mostly subshrubs with underground xylopodia that enable them to sprout new branches after fire events (Irwin and Barneby 1982). This series is supported as sister to *C. flexuosa* (ser. *Flexuosae*) which presents similar vegetative features. Series *Chamaecrista* is defined by the supra-axillary position of the inflorescence (Irwin and Barneby 1982) and our results support this character as synapomorphic for this group. Species of this series are mostly weeds

with wide range and the species sampled for chromosome numbers present poliploidy with chromosome numbers 2n = 32 or 48, the only recorded polyploid number in sect. *Chamaecrista* (Fig. 5).

Phylogenetic relationships within Chamaecrista - Clade A – this clade appeared as sister to the rest of the genus *Chamaecrista* (BS 95%, PP 100%). All species of this clade belong to section *Apoucouita* (Irwin and Barneby 1982), a group of large trees from Neotropical rain forests, most diverse in the Amazon and Brazilian Atlantic forests (Irwin & Rogers 1967; Irwin and Barneby 1977). This group may be defined by the apomorphy presence of extrafloral nectaries on the inflorescence (Fig. 5) and anthers pubescent all over their surface. The latter is a rare character in Caesalpinioid legumes (Endress & Stump 1991, Tucker 1996) and not found in any other group of *Chamaecrista*. Other putative synapomorphies of section *Apoucouita* are the cauliflorous racemes (Fig. 5) and pendent fruits but a clearer view of sister group relationship of *Chamaecrista* is needed before better supported hypotheses about the evolution of these characters can be proposed. Similarly to the pattern found in section *Apoucouita*, the genus *Cassia* is most diverse in tropical rain forests and presents mostly cauliflorous racemes and pendent fruits. Thus, inflorescence position and fruit attitude in clade A could be interpreted either as a synapomorphy of this clade or as a synapomorphy of a larger *Cassia*-*Chamaecrista* clade.

Clade C – this clade includes species of subsection *Absus* (Irwin and Barneby 1982) and presents as unique synapomorphies the loss of extrafloral nectaries and the presence of sticky glandular hairs (Fig. 5). Subsection *Absus* includes c.165 species and is most diverse in Central Brazilian Plateau and in the Espinhaço mountain range, occurring mostly as shrubs or xylopodium bearing subshrubs in savanna-like habitats

(Cerrado and Campo Rupestre). These species also present a spiral phylotaxy but it is more properly interpreted as a symplesiomorphic condition. All the sampled species of this clade have a diploid chromosome number of 2n = 28 (Fig. 5) but this condition is also better interpreted as symplesiomorphic for *Chamaecrista* (see discussion on the evolution of chromosome numbers below).

Clade D – this clade combines representatives of sections *Chamaecrista*, *Caliciopsis*, and *Xerocalyx* (clade G), besides subsect. *Baseophyllum* of sect. *Absus* (clade E). A putative morphological synapomorphy for clade D is the distichous leaves.

Marazzi et al. (2006) stated that the presence of extrafloral nectaries (EFNs) increase fitness by establishing a plant protection system based on interactions with ants. This protection could increase survival, reprodution, dispersal, and potential for adaptation and could explain the fact the *Senna* clades with EFNs being more diverse than those without EFNs. Within *Chamaecrista*, we do not observe clade-size disparity, with clades C and E almost equally diverse. In the case of the subsection *Absus*, the protective role of the plant-ant interaction achieved due the presence of the EFNs seems to be transferred to the sticky glandular hairs. These hairs are denser on the shoot apex and near the inflorescences, have a bulbous secretory base and produces a glutinous secretion that covers the hair and young parts of the shoots. Due to the viscose to sticky consistency, these hairs could make it difficult for insects to walk on the soft parts of the plant deterring them from eating these plants.

Clade E – this clade includes taxa of the subsect. *Baseophyllum*. Irwin and Barneby (1982) classified this group as part of sect. *Absus* mainly because of the presence of terminal racemes (Fig. 5) and a generally shrubby habit. Our analyses refute

their taxonomic proposal and our results suggest that the characters mentioned are symplesiomorphies. On the other hand, subsection *Baseophyllum* is supported as part of clade D and the presence of distichous phylotaxy could be synapomorhic for superclade D.

Clade F – this clade includes plants with herbaceous to small subshrubby habit and racemes reduced to axillary fascicles (Fig. 5). Species of this clade occurs mainly in open vegetation forms as Cerrado and many are weedy of disturbed places.

Clade G – this clade in coincident with Sect. *Xerocalyx*. This section includes closely related taxa, sometimes treated as 16 different species (Irwin 1964), sometimes as just three species (Irwin and Barneby 1982). The main synapomorphies of this group are the resupinate flowers and the strongly graduate, parallel veined and striate sepals. Striate parallel veined sepals also occurs in section *Caliciopsis* but our results support this section as embedded in section *Chamaecrista* and therefore this character must have evolved independently in these two groups.

Evolution of selected morphological characters. The present results support the presence of EFNs as synapomorphic in *Chamaecrista*. These structures are absent in *Cassia* and common in *Senna* (Fig. 5). Marazzi et al. (2006) found that the most basal clades of *Senna* are EFNs-less and that these structures appeared only once, being synapomorphic for a species rich clade. EFNs found in *Senna* are morphologically distinct from those found in *Chamaecrista*, being mostly convex, while *Chamaecrista* have EFNs like a plate or a dish, thus presenting a concave secretory surface. The fact that EFNs are absent in *Cassia* and in the basal clades of *Senna* suggest that these

structures are not homologous between *Chamaecrista* and *Senna* but it is hard to explain why these are the only genera that present such structures in the entire Caesalpinioid subfamily.

In *Chamaecrista*, EFNs are absent in subsection *Absus* (clade C), in *C. absus* (sect. *Grimaldia*) and in *C. rotundifolia* (sect. *Chamaecrista* ser. *Bauhinianae*) (Fig. 5). Thus, the present phylogenetic hypotheses support a unique origin of the EFNs in *Chamaecrista* and secondary losses in these taxa. In subsection *Absus* (clade C), the protective role of the EFNs seems to be replaced by sticky glandular hairs. The presence of these hairs seems to be a key innovation of this group which allowed its diversification in high mountain habitats where it occurs.

The structure and position of the inflorescence were used by Irwin and Barneby (1982) as taxonomic characters at sectional level. Cauliflorous racemes appearing on the branches below the leaves are found only in section *Apoucouita*. This condition is also common in *Cassia* and may be taken as synapomorphic to the *Cassia*-*Chamaecrista* clade. The sister group of section *Apoucouita* (clade B) present terminal or axillary racemes in the distal leaves of the stem (Fig. 5). Again, the consideration of this feature as synapomorphic for clade B or the entire genus *Chamaecrista* depends on a better resolution of the sister group relationships among the genera of Cassiinae. On the other hand, our results refute Irwin and Barneby's (1978, 1982) proposition of using this feature as the diagnostic character of section *Absus*, since it appears here as a symplesiomorphy for clades C and E, the representatives of a paraphyletic section *Absus*. Most analyses support the idea that inflorescences reduced to axillary fascicles evolved only once and is supported as synapomorphic for clade F. A further elaboration of the inflorescence is the supra-axillary fascicles found in series *Chamaecrista* (clade H).

Habit evolution seems to be strongly correlated with habitat preference in *Chamaecrista*. Trees are found almost exclusively in section *Apoucouita* (clade A) and this group occurs almost exclusively in tropical rain forests of South America. This preference for wet forests is also found in *Cassia* whose species are large trees, too. Thus, it is probable that initial diversification of *Chamaecrista* occurred in tropical wet forests of the New World. The first cladogenetic event within *Chamaecrista* gave rise to section *Apoucouita* and the clade B. This last clade includes shrubs, subshrubs and herbs and occurs in Cerrado and other forms of open vegetation. This is a much richer clade in species number: 19 species for the section *Apoucouita* and 311 species for the groups included in clade B. Interestingly, a similar situation was found in *Senna* by Marazzi et al. (2006), with a relatively low diverse basal clade of forest species sister to a much richer clade of mostly shrubby plants from open and dry vegetation forms.

Evolution of chromosome numbers. Chromosome numbers show interesting evolutionary patterns in *Chamaecrista* and some shifts in numbers are congruent with some major cladogenetic events. Chromosome counts are unknown for section *Apoucouita* (clade A) and subsection *Baseophyllum* (clade E). Goldblatt (1981) highlighted that sections of *Chamaecrista* differ in chromosome number. The optimization of the known chromosome numbers on a cladogram (Fig. 5) supports the plesiomorphic number of 2n = 28 for *Chamaecrista*. This number is also found in most species of *Cassia* and *Senna* and is also found in all sampled species of subsect. *Absus* (clade C), although it is unknown in sect. *Apoucouita* (clade A). Within clade D, chromosome numbers are unknown for taxa of clade E. Taxa of sect. *Xerocalyx* (clade G) have 2n = 14, while the remaining taxa of sections *Chamaecrista* and *Caliciopsis*

(clade D) present chromosome numbers 2n = 16 (Fedorov 1969; Goldblatt 1981; Goldblatt and Johnson 1990, 1994, 2000).

A more robust hypothesis about chromosomal evolution within *Chamaecrista* depends upon data of chromosome numbers in sect. *Apoucouita* (clade A) and subsect. *Baseophyllum* (clade E) but the current data support that 2n = 28 may be a plesiomorphic condition for chromosome number of *Chamaecrista* while disploid or aneuploid diminished numbers are apomorphic. This view is supported by the fact that most of the sampled species of *Cassia* and *Senna* have 2n = 28 (Goldblatt 1981) as well as species belonging to clade C. Reduced chromosome numbers of 2n = 16 are recorded in *Chamaecrista*, only for the herbaceous representatives of the genus and may be interpreted either as a synapomorphy of an herbaceous clade (clade F) or a synapomorphy of the entire clade D. Further reduction to 2n = 14 is synapomorphic for sect. *Xerocalyx* (clade G). Within clade D, a secondary event of polyploid is recorded for *Chamaecrista nictitans*.

Final Remarks. Thus, our analyses suggest that the diversification patterns in *Chamaecrista* occurred through an initial shift from rain forest trees to a diverse savannah shrubby clade (clade B). Within this last group, a high mountain clade (clade C) is marked by the loss of extrafloral nectaries and the appearance of sticky glandular hairs, probably as part of the plant defense strategy. Another highly diverse group (clade F) is marked by a shift to herbaceous habit, axillary fascicled inflorescences and reduced chromosome numbers. This last group is more diverse in open field areas and includes many weeds of waste grounds.

Phylogenetic hypotheses obtained in the present work support the recognition of the sections *Apoucouita* and *Xerocalyx* in their current circumscription. Section *Absus*

must be redefined to exclude the subsection *Baseophyllum*, which may be taken as a different section. Section *Chamaecrista* appeared as paraphyletic and the relationship among its species as well as with respect to sections *Xerocalyx* and *Calyciopsis* need a wider sample to get more robust conclusions.

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TABLE 1. Classification of *Chamaecrista* used in the present study based on Irwin and Barneby (1982).

1. Section *Apoucouita* (19 spp.)		
		Serie *Pteridophyllae*
		Serie *Apoucouita*
2. Section *Absus* (c. 169 spp.)		
	Subsection *Baseophyllum*	
	Subsection *Adenophyllym*	
	Subsection *Otophyllum*	
	Subsection *Absus*	
		Serie *Catharticae*
		Serie *Trachycarpae*
		Serie *Oligospermae*
		Serie *Astroglandulosae*
		Serie *Incanae*
		Serie *Nigricantes*
		Serie *Bracteolate*
		Serie *Ursinae*
		Serie *Pinifoliae*
		Serie *Setosae*
		Serie *Geminatae*
		Serie *Andromedeae*
		Serie *Lomatopodae*
		Serie *Secundae*
		Serie *Microphyllae*
		Serie *Gracillimae*
		Serie *Rigidulae*
		Serie *Paniculate*
		Serie *Adenophyllae*
		Serie *Lucidae*
		Serie *Hassleranae*
		Serie *Hedysaroides*
		Serie *Ochnaceae*
		Serie *Confertae*
		Serie *Glutinosae*
		Serie *Incurvatae*
		Serie *Spinulosae*
		Serie *Unijugae*
		Serie *Strictifoliae*
		Serie *Ericifoliae*
		Serie *Absoideae*
3. Section *Grimaldia* (1 sp.)		
4. Section *Chamaecrista* (ca. 75 spp.)		
		Serie *Coriaceae*
		Serie *Flexuosae*
		Serie *Prostrataea*
		Serie *Bauhinianae*
		Serie *Greggianae*
		Serie *Chamaecrista*
5. Section *Caliciopsis* (2 spp.)		
6. Section *Xerocalyx* (3 spp.)		

TABLE 2. Taxa used in this study. See Table 1 for section and series names of *Chamaecrista* species. Vouchers deposited at the herbarium Universidade Estadual de Feira de Santana (HUEFS), excepted *S. pleurocarpa*. Acronyms for Brazilian States: BA, CE, ES, GO, MG, MT, PI, RN, SE.

Taxa	Voucher	Location	Fragment	GenBank accession numbers
Chamaecrista				
C. amorimii Barneby	Conceição 795	Itacaré, BA	ITS, *trnL-F*	
C. aff. *cavalcantina* (H.S.Irwin & Barneby) H.S.Irwin & Barneby	Queiroz 10265	Jaborandi, BA	ITS, *trnL-F*	
C. aff. *pascuorum* (Benth.) H.S.Irwin & Barneby	Queiroz 10569	Barra do Bugres, MT	ITS, *trnL-F*	
C. aff. *roncadorensis* (H.S.Irwin & Barneby) H.S.Irwin & Barneby	Queiroz 10279	Posse, GO	ITS, *trnL-F*	
C. anamariae Conc., L.P.Queiroz & G.P.Lewis	Conceição 787	Abaíra, BA	ITS, *trnL-F*	
C. belemii (H.S.Irwin & Barneby) H.S.Irwin & Barneby var. *belemii* H.S.Irwin & Barneby	Queiroz 9151	Casa Nova, BA	ITS, *trnL-F*	
C. blanchetii (Benth.) Conc., L.P.Queiroz & G.P.Lewis	Andrade 607	Morro do Chapéu, BA	ITS, *trnL-F*	
C. botryoides Conc., L.P.Queiroz & G.P.Lewis	Conceição 541	Abaíra, BA	ITS, *trnL-F*	
C. brachystachya (Benth.) Conc., L.P.Queiroz & G.P.Lewis	Conceição 713	Grão Mogol, MG	ITS, *trnL-F*	
C. calycioides (Collad.) Greene	Queiroz 11	Natal, RN	ITS, *trnL-F*	
C. campestris H.S.Irwin & Barneby	Queiroz 10440	General Cardoso, MT	ITS, *trnL-F*	
C. cathartica (Mart.) H.S.Irwin & Barneby	Conceição 789	Goveia, MG	ITS, *trnL-F*	
C. chapadae (H.S.Irwin & Barneby) H.S.Irwin & Barneby	Costa 129	Mucugê, BA	ITS	
C. choriophylla (Vogel) H.S.Irwin & Barneby	Conceição 733	Itacambira, MG	ITS, *trnL-F*	
C. confertiformis (H.S.Irwin & Barneby) Conc., L.P.Queiroz & G.P.Lewis	Costa 132	Mucugê, BA	ITS, *trnL-F*	
C. coriacea (Benth.) Conc. & L.P.Queiroz	Conceição 869	Conc. Mato Dentro, MG	ITS, *trnL-F*	
C. cytisoides (Collad.) H.S.Irwin & Barneby	Conceição 870	Sta. Bárbara do M. Verde, MG	ITS, *trnL-F*	
C. dalbergiifolia (Benth.) H.S.Irwin & Barneby	Queiroz 10318	Planaltina, GO	ITS, *trnL-F*	
C. decora (H.S.Irwin & Barneby) Conc., L.P.Queiroz & G.P.Lewis	Conceição 810	Grão Mogol, MG	ITS, *trnL-F*	
C. depauperata Conc., L.P.Queiroz & G.P.Lewis	Conceição 863	Mucugê, BA	ITS, *trnL-F*	

Taxa	Voucher	Location	Fragment	GenBank accession numbers
C. desvauxii (Collad.) Killip *desvauxii*	Queiroz 10453	Chapada dos Guimarães, MT	ITS, *trnL-F*	
C. desvauxii(Collad.) Killip *langsdorfii* (Kunth ex Vogel) H.S.Irwin & Barneby	Conceição 674	Abaíra, BA	ITS, *trnL-F*	
C. desvauxii (Collad.) Killip *latistipula* G.P.Lewis	Conceição 912	Guaraparí, ES	ITS, *trnL-F*	
C. desvauxii (Collad.) Killip *mollissima* (Benth.) H.S.Irwin & Barneby	Santos 356	Morro do Chapéu, BA	ITS, *trnL-F*	
C diphylla (L.) Greene	Queiroz 10269	Jaborandi, BA	ITS, *trnL-F*	
C. flexuosa (L.) Greene	Giulietti 2344	Santana do Riacho, BA	ITS, *trnL-F*	
C. glaucofilix (H.S.Irwin & Barneby) H.S.Irwin & Barneby	Conceição 861	Mucugê, BA	ITS, *trnL-F*	
C. hispidula (Vahl) H.S.Irwin & Barneby	Conceição 914	Feira de Santana, BA	ITS, *trnL-F*	
C. jacobinae (Benth.) H.S.Irwin & Barneby	Andrade 610	Morro do Chapéu, BA	ITS, *trnL-F*	
C. mucronata (Spreng.) H.S.Irwin & Barneby	Conceição 653	Abaíra, BA	ITS, *trnL-F*	
C. nictitans (L.) Moench subsp. *brachypoda* (Benth.) H.S.Irwin & Barneby	Queiroz 10335	Iporá, GO	ITS, *trnL-F*	
C. nictitans (L.) Moench var. subsp. *disadena* Steud.	Conceição 790	Junco de Minas, MG	ITS, *trnL-F*	
C. nictitans (L.) Moench var. *ramosa* (Vogel) H.S.Irwin & Barneby	Queiroz 10406	Barra do Garças, MT	ITS, *trnL-F*	
C. olesiphylla (Vogel) H.S.Irwin & Barneby	Nascimento 508	Grão Mogol, MG	ITS, *trnL-F*	
C. onusta H.S.Irwin & Barneby	Conceição 800	Itacaré, BA	ITS, *trnL-F*	
C. pascuorum (Benth.) H.S.Irwin & Barneby	Queiroz 9169	Iaçu, BA	ITS, *trnL-F*	
C. philippi (H.S.Irwin & Barneby) H.S.Irwin & Barneby	Giulietti 2245	Rio de Contas, BA	ITS, *trnL-F*	
C. pilosa (L.) Greene	Queiroz 10221	Barreiras, BA	ITS, *trnL-F*	
C. potentilla (Benth.) H.S.Irwin & Barneby	Queiroz 7606	Diamantina, MG	ITS, *trnL-F*	
C. rodundifolia (Pers.) Greene	Costa 128	Mucugê, BA	ITS, *trnL-F*	
C. rupestrium H.S.Irwin & Barneby	Santos 390	Rio de Contas, BA	ITS, *trnL-F*	
C. serpens (L.) Greene	Cardoso 980	Tucano, BA	*trnL-F*	
C. setosa (Vogel.) H.S.Irwin & Barneby	Queiroz 10460	Chapada dos Guimarães, MT	ITS, *trnL-F*	
C. speciosa Conc., L.P.Queiroz & G.P.Lewis	Conceição 546	Abaíra, BA	ITS, *trnL-F*	
C. supplex (Benth.) Britton & Rose ex Britton & Killip	Queiroz 10217	Barreiras, BA	ITS, *trnL-F*	
C. unijuga (Benth.) Conc., L.P.Queiroz & G.P.Lewis	Conceição 694	Sto. Amaro das Brotas, SE	ITS, *trnL-F*	

Taxa	Voucher	Location	Fragment	GenBank accession numbers
C. urophyllidia (H.S.Irwin & Barneby) H.S.Irwin & Barneby	Harley 54656	Rio de Contas, BA	ITS, *trnL-F*	
Outgroups			ITS, *trnL-F*	
Bauhinia ungulata L.	Araújo 1569	Crateús, CE	ITS, *trnL-F*	
Copaifera coriacea Mart.	Queiroz 7918	Casa Nova, BA	ITS, *trnL-F*	
***Cassia coccinea* Wall.**	Oliveira 610	Crateús, CE	ITS, *trnL-F*	
C. grandis L. f.	Queiroz 2878	Feira de Santana, BA	ITS, *trnL-F*	
C. javanica L.	Queiroz 11039	Feira de Santana, BA	ITS, *trnL-F*	
Hymenaea martiana Hayne	Queiroz 7833	Campo Alegre de Lurdes, BA	ITS, *trnL-F*	
Martiodendrum mediterraneum (Mart. ex Benth.) Koeppen	Queiroz 10162	Oeiras, PI	ITS, *trnL-F*	
Senna gardneri (Benth.) H.S.Irwin & Barneby	Queiroz 7860	Campo Alegre de Lurdes, BA	ITS, *trnL-F*	
Senna pleurocarpa (F. Muell.) Randell	Grimes & M-Grimes 3193	North, Australia	*trnL-F*	AF 367007

TABLE 3. Tree statistics for the parsimony analyses for all data sets.

DNA region	Aligned length	Number of variable sites	Number of potencially parsimony informartive sites	Number of changes/variable site	Fitch tree length	CI	RI	ts:tv
ITS analysis	988	604	466	3.27	1979	0.574	0.803	1.02
trnL-F analysis	973	169	85	1.33	225	0.844	0.905	1.08
Combined analyses	1961	801	569	2.84	2277	0.606	0.808	1.00

Note: CI, consistency index; RI, retention index; ts:tv, transition/transversion ratio.

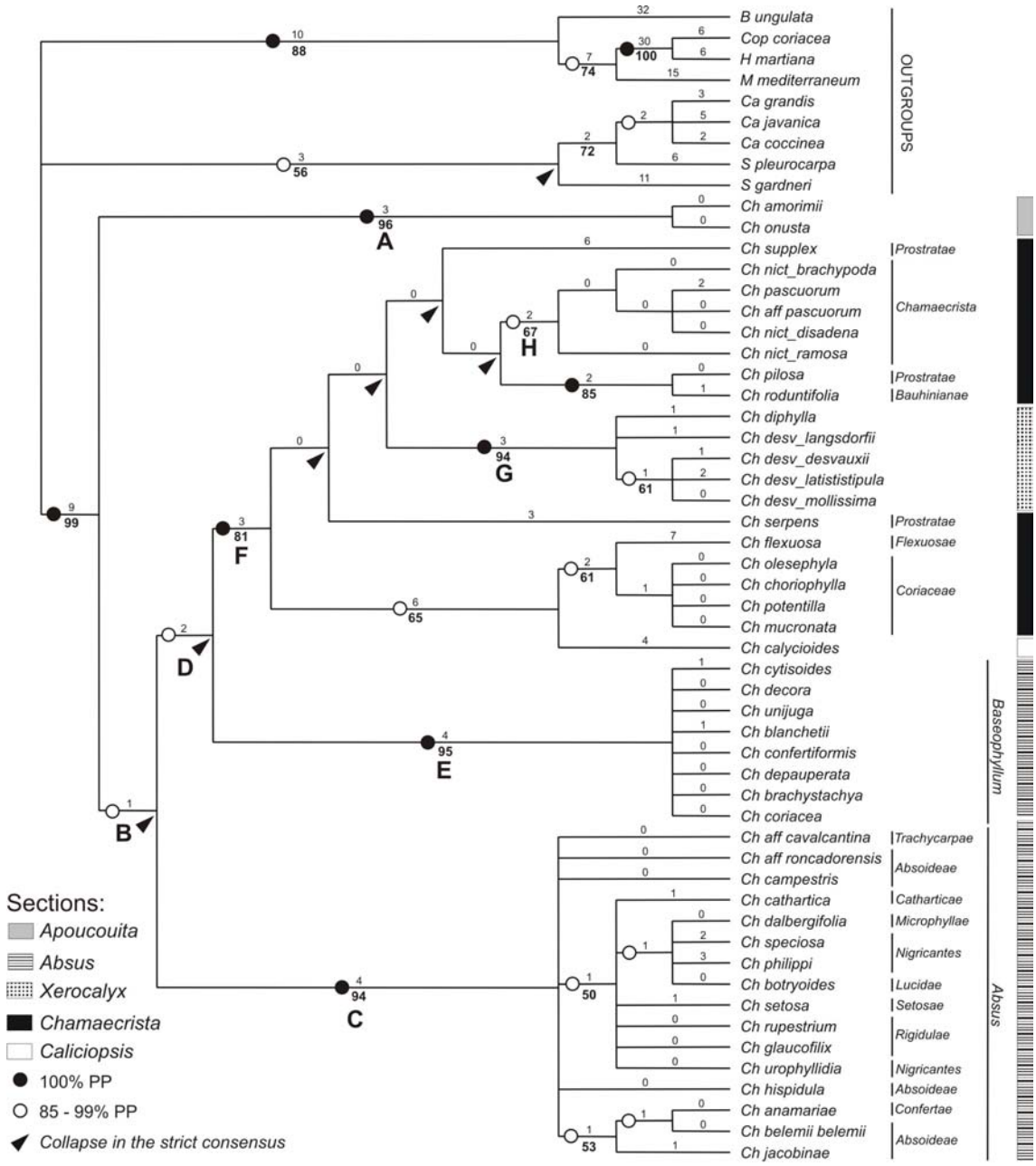

Figure 1. One of the most parsimonious trees for *trnL-F* region (L = 225, CI = 0.844, and RI = 0.905). The numbers above branches are Fitch branch lengths and bootstrap support percentages are indicated in bold below. Taxa with less than 50% of bootstrap are not indicated.

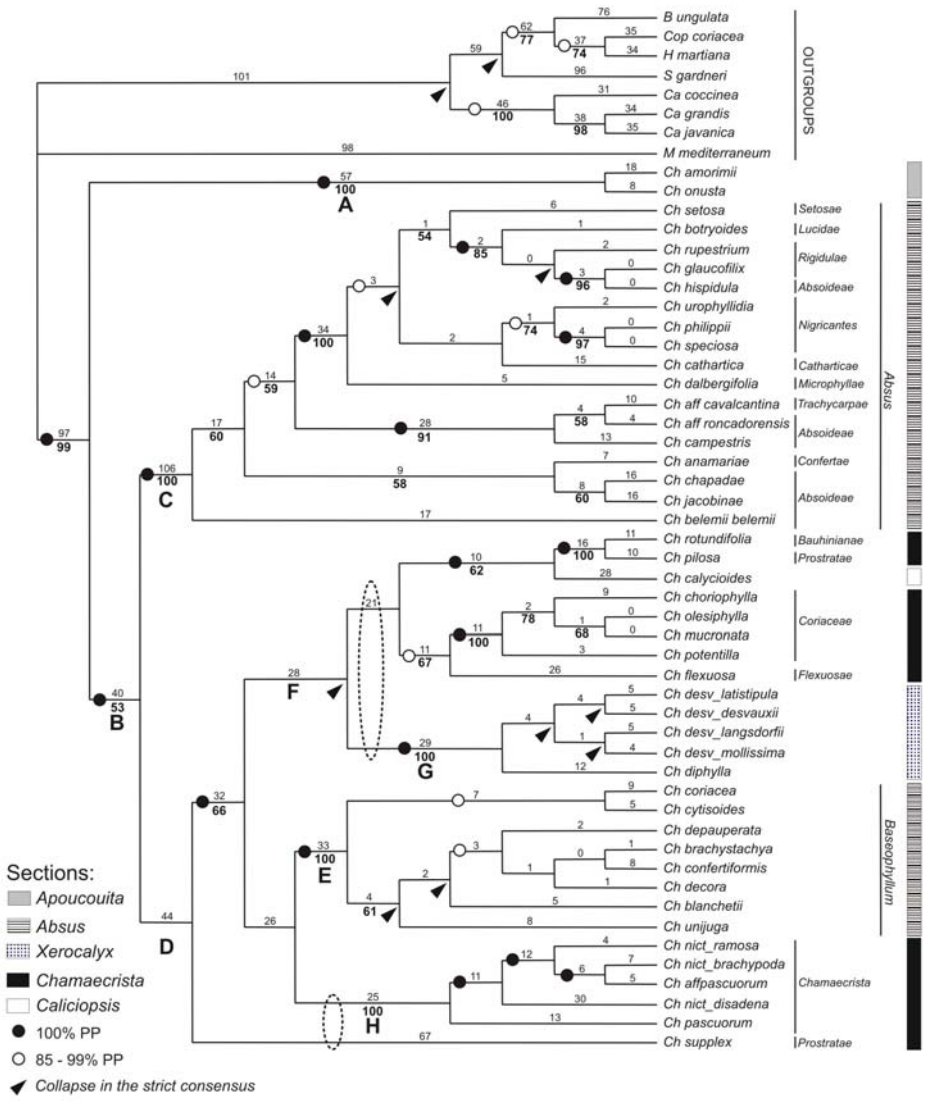

Figure 2. One of the most parsimonious trees for ITS region (L = 1979, CI = 0.574, and RI = 0.803). The numbers above branches are Fitch branch lengths and bootstrap support percentages are indicated in bold below. Taxa with less than 50% of bootstrap are not indicated. Dotted ellipses represent groups that form clades in other analyses.

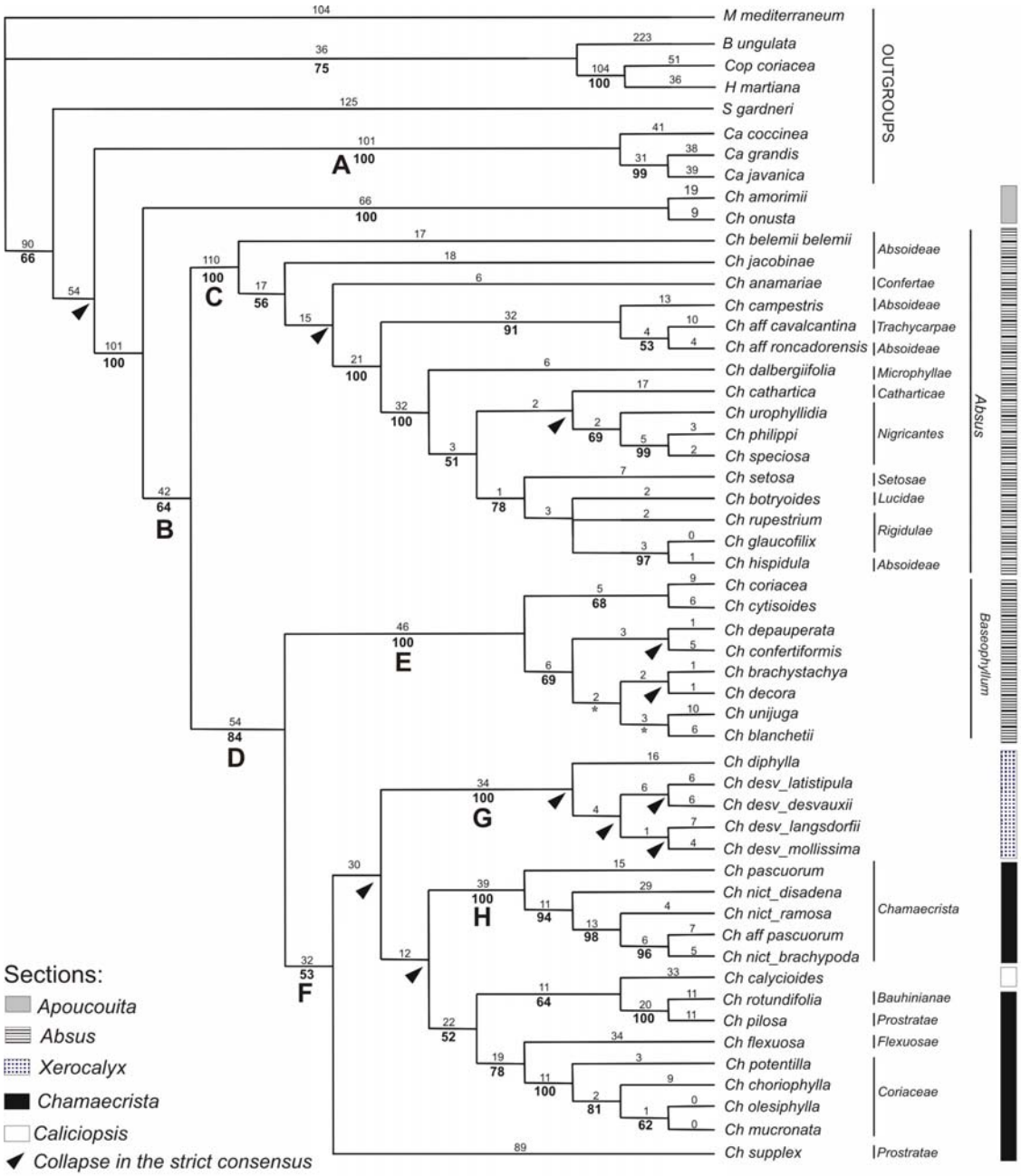

Figure 3. One of the most parsimonious trees in the combined analysis of *trnL-F* + ITS regions (L = 2277, CI = 0.606, and RI = 0.808). The numbers above branches are Fitch branch lengths and bootstrap support percentages are indicated in bold below. Taxa with less than 50% of bootstrap are not indicated.

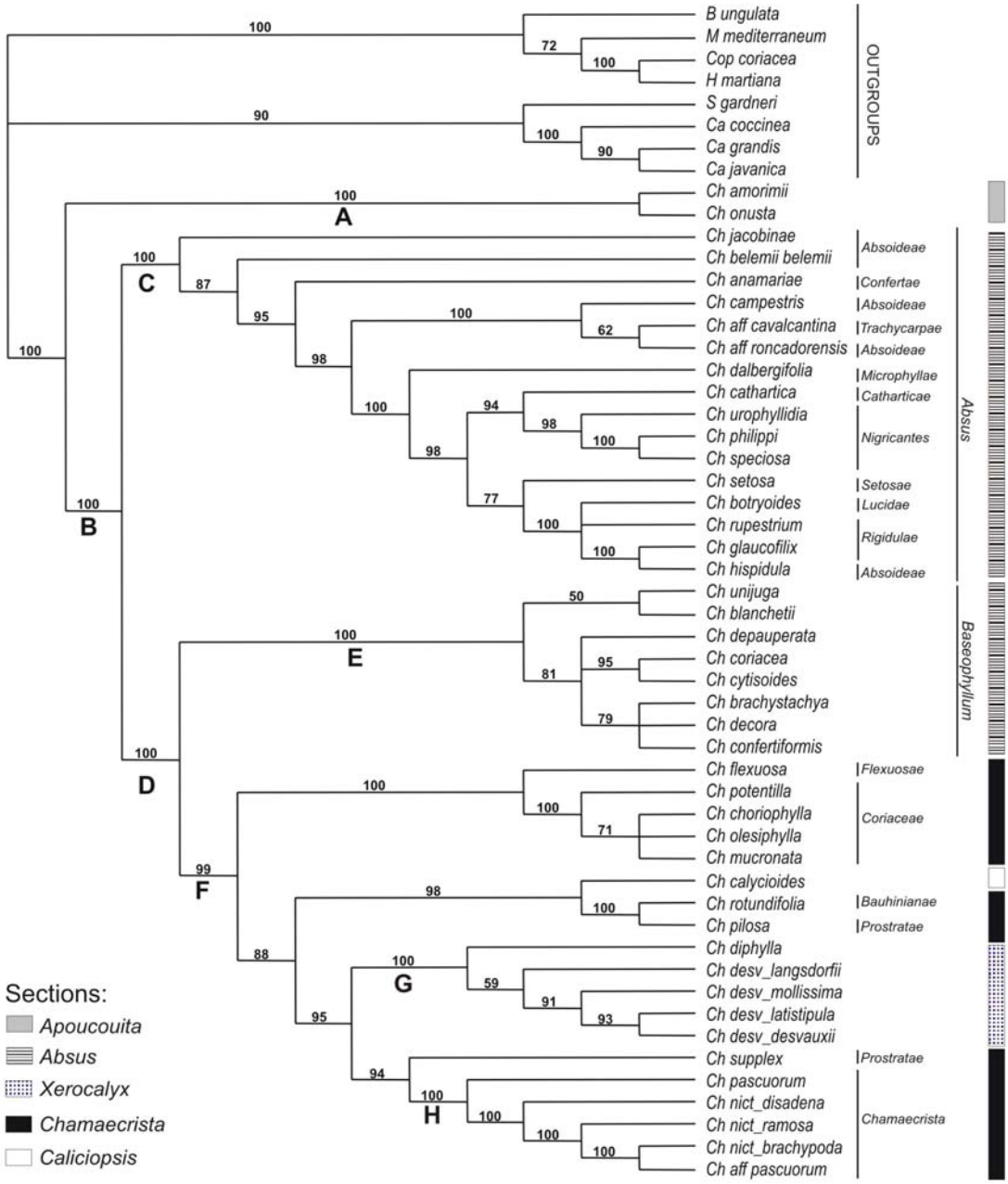

Figure 4. Majority-rule consensus of 14,770 trees obtained in the Bayesian analysis. Numbers above branches are posterior probabilities for clades.

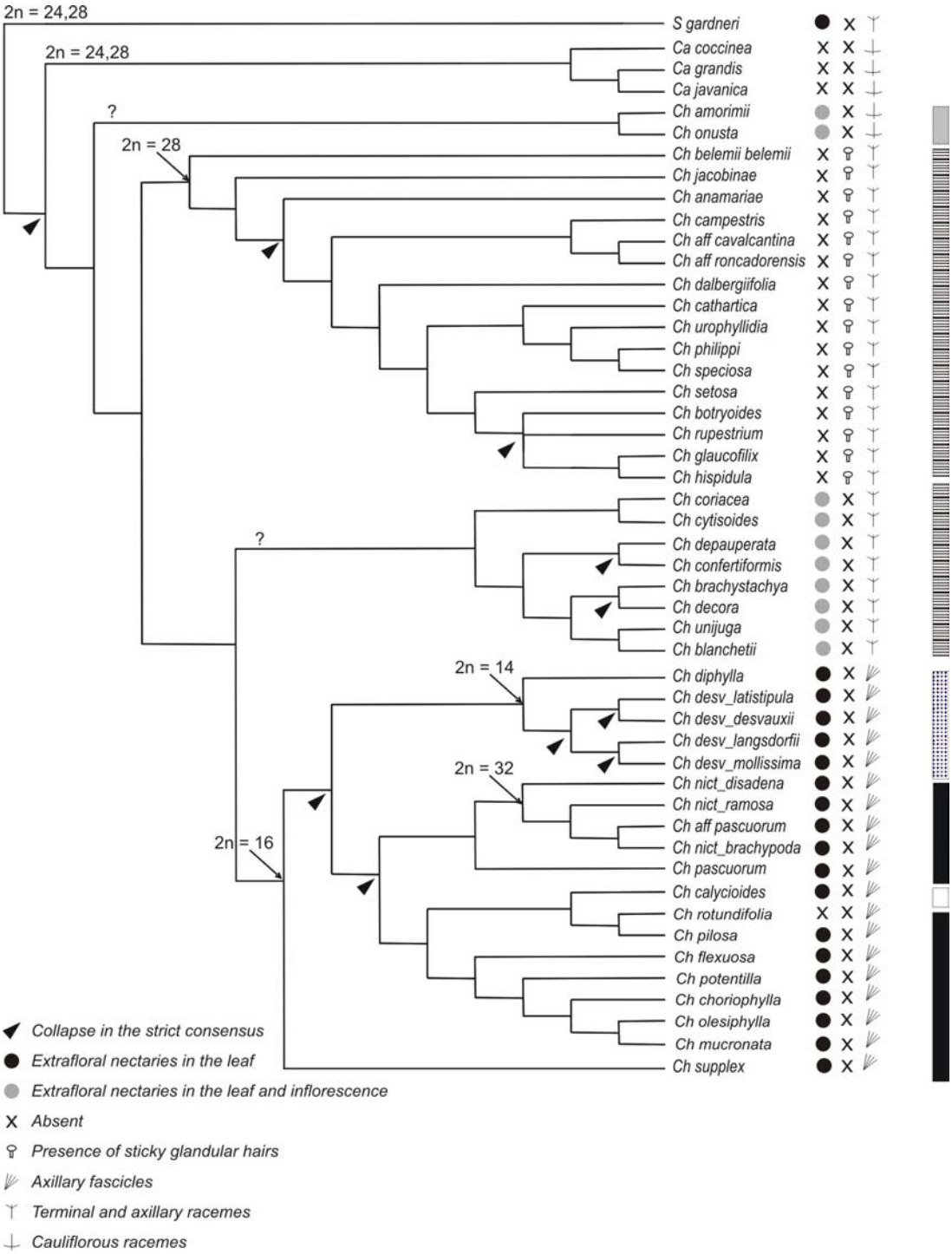

Figure 5. One of the most parsimonious trees in the combined analysis, with mapping key morphological characters.

Capítulo 2

Biosystematics of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* (Leguminosae-Caesalpinioideae) based on allozyme and morphometric analyses

Este capítulo está formatado para publicação na revista Annals of Botany

RESUMO

Chamaecrista Moench é um dos maiores gêneros da subfamília Caesalpinioideae, com aproximadamente 330 espécies amplamente distribuídas nas América, África e Ásia. De acordo com a classificação de Irwin & Barneby (1982), o gênero inclui seis seções. *Chamaecrista* sect. *Absus* é caracterizada principalmente pelo hábito subarbustivo com inflorescência terminal. *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* difere de todas as outras subseções de C. *Absus* por apresentar nectários extraflorais no pecíolo ou na raque, ausência de tricomas glandulares, folhas com um a quatro pares de folíolos, 3-9 nervuras palmadas e inflorescência racemoso-subcorimbosa (Irwin & Barneby, 1978). A subseção inclui três espécies: *C. coriacea*, *C. cytisoides* e *C. depauperata* (Irwin & Barneby, 1978, 1982; Conceição *et al.* 2001). A história taxonômica de *Baseophyllum* é bem confusa, a exemplo da espécie *C. cytisoides* que inclui sete variedades, tratadas pelo Bentham (1870, 1871) como espécies distintas. Esse trabalho teve o objetivo de investigar o número de categorias taxonômicas, status taxonômico e fornecer caracteres diagnósticos para os taxa de *Chamaecrista* sect. *Absus* subsect. *Baseophyllum*. Para atingir esses objetivos foram realizadas análises multivariadas com dados aloenzimáticos e morfológicos para determinar o nível de variabilidade genética e morfológica e a sub-estruturação dentro e entre populações co-específicas e intra-taxon, com 33 populações naturais de *C.* sect. *Absus* subset. *Baseophylllum*. Todos os táxons apresentaram baixa variabilidade genética (P = 0.0 – 33.3, A = 1.1 – 1.4, H_e = 0.010 – 0.067). Alto índice de fixação foi encontrado em apenas um táxon (F_{IS} = 0.48), que deve estar relacionado com a biologia e padrão de distribuição geográfico dos seus indivíduos. Elevada estruturação da variabilidade genética foi encontrada para dois táxons, devido à presença de populações disjuntas, um deles apresentou também alta estruturação morfológica. As análises estatísticas mostraram a existência de correlação entre a variabilidade genética e morfológica para os táxons desse grupo. Os resultados das análises

multivariadas com dados aloenzimáticos e morfológicos com todos os táxons de *C.* sect. *Absus* subset. *Baseophyllum* mostraram a existência de espécies distintas dentro do grupo. Os dados genéticos e morfológicos confirmaram a circunscrição do status de espécie para *C. coriacea* e *C. depauperata*. No entanto, essas análises não suportaram a delimitação de *C. cytisoides* var. *cytisoides* e suas variedades, mostrando claramente que o táxon tipo do complexo cytisoides, *C. cytisoides* var. *cytisoides* é muito distante geneticamente e morfologicamente de todas as outras seis variedades do complexo, sugerindo que o complexo deve ser rearranjado em seis espécies. Os dados genéticos e morfológicos suportaram o reconhecimento de oito espécies para a subseção *Baseophyllum*.

Biosystematics of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* (Leguminosae-Caesalpinioideae) based on allozyme and morphometric analyses

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running title: Biosystematics of *Chamaecrista* subsect. *Baseophyllum* (Leguminosae)

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- *Background and aims Chamaecrista* sect. *Absus* subsect. *Baseophyllum* (Leguminosae-Caesalpinioideae) is a group composed currently by three species, *C. cytisoides*, *C. coriacea* and *C. depauperata*, occurring in Northeastern and Southeastern Brazil. Taxonomy of the group has a controversial history, mainly due to the interpretation of *C. cytisoides*, currently arranged in seven varieties, some of them formerly described as species. We assessed genetic and morphological variation of these taxa, in order to provide hypotheses about the number of taxonomical entities and their taxonomic status in the group.
- *Methods* We surveyed genetic variability using allozymes (15 loci) and morphological variability using multivariate morphometric analyses (54 vegetative and floral characters) in 33 populations belonging to all nine taxa and one morphospecies of subsection *Baseophyllum* occurring in the Brazil.
- *Key Results* Genetic variability was low in all taxa (P = 0.0 – 33.3, A = 1.1 – 1.4, H_e = 0.010 – 0.067). High fixation index was found in only one taxon (F_{IS} = 0.48), which is correlated with its biology and pattern of geographic distribution of the individuals. High structuring of the genetic variability was found in two taxa, due to divergence in disjunct populations, one of these taxa presenting also high morphological structuring. Morphological and genetic data are consistent with the status of *C. depauperata* and *C. coriacea*. However, divergence between *C. cytisoides* var. *cytisoides* and all the other six conspecific varieties is higher than the divergence between it and the other two species.
- *Conclusions* Our data support the recognition of eight species in the subsection *Baseophyllum*. *Chamaecrista coriacea* and *C. depauperata* are supported in their original circumscription. However, the varieties ascribed to *C. cytisoides* by Irwin and Barneby are best treated as six different species, except for *C. cytisoides* var. *micrantha*, which is supported as a synonym of *C. cytisoides* var. *brachystachya.*

Key words: allozymes, Cadeia do Espinhaço, Caesalpinioideae, campos rupestres, *Chamaecrista*, Chapada Diamantina, Espinhaço range, genetic diversity, Leguminosae, morphological variability, morphometrics.

INTRODUCTION

Chamaecrista Moench is one of the largest genera of the subfamily Caesalpinioideae, includes about 330 species widely distributed in the tropical areas of America, Africa and Asia (Lewis, 2005). It includes six sections according to Irwin and Barneby (1982) classification, diagnosed by combining characters of the habit, inflorescence and indument. Section *Absus* is characterized by mostly shrubby habit with terminal racemes. Most of its subsections present glandular setose trichomes and lack extrafloral nectaries. Subsection *Baseophyllum* differs from all other subsections by the presence of extrafloral nectaries on the petiole or leaf rachis, the lack of glandular trichomes, the possession of leaflets in one to four pairs, each leaflet 3-9 palmately veined, and inflorescences racemose-subcorymbose (Irwin and Barneby, 1978).

Subsection *Baseophyllum* is mainly distributed in the Espinhaço mountain range in Bahia and Minas Gerais states, Brazil, usually in "campo rupestre" vegetation ('rocky fields') but sometimes occurring in "cerrado" (savanna-like vegetation). It also occurs disjunctly in coastal "restinga" vegetation (low forests or scrubs on salty and sandy soils) of Northeastern Brazil (Pernambuco to Bahia) and Espírito Santo state and at a spot of "caatinga" vegetation (seasonally dry thorny forest) in Pernambuco state (Irwin and Barneby, 1978; AS Conceição, unpubl.).

The taxonomy of the subsection *Baseophyllum* has a tortuous history mainly due to different interpretations of the species limits in the *Chamaecrista cytisoides* complex. Bentham (1870) recognized four independent species and two varieties (as *Cassia*): *Cassia blanchetii*, *Cassia brachystachya* var. *brachystachya*, *Cassia brachystachya* var. *unijuga*, *Cassia coriacea* and *Cassia cytisoides*. One year later the same author did not recognize the variety *unijuga* (Bentham, 1871). Irwin and Barneby (1978) treated all these taxa as varieties

of *Cassia cytisoides*, except for *Cassia coriacea*. Later, they transferred these taxa to varieties of *Chamaecrista cytisoides* (Irwin and Barneby, 1982). According to the circumscription of Irwin and Barneby (1982), the subsection *Baseophyllum* includes only two species: *Chamaecrista coriacea* (Benth.) H.S.Irwin & Barneby and *C. cytisoides* (Collad.) H.S.Irwin & Barneby, the later including seven varieties: *C. cytisoides* var. *blanchetii* (Benth.) H.S.Irwin & Barneby, *C. cytisoides* var. *brachystachya* (Benth.) H.S.Irwin & Barneby, *C. cytisoides* var. *confertiformis* (H.S.Irwin & Barneby) H.S.Irwin & Barneby, *C. cytisoides* var. *cytisoides, C. cytisoides* var. *decora* (H.S.Irwin & Barneby) H.S.Irwin & Barneby*, C. cytisoides* var. *micrantha* (H.S.Irwin & Barneby) H.S.Irwin & Barneby and *C. cytisoides* var. *unijuga* (Benth.) H.S.Irwin & Barneby.

According to Irwin and Barneby (1982), this wide circumscription of *C. cytiosoides* was supported by the conservative floral morphology amongst the putative varieties and the presence of intermediate morphs between the different taxa, suggesting a continuous variation pattern in vegetative traits. Some varieties of the *C. cytisoides* complex occur in sympatry and sometimes are syntopic, although they flower in different periods (according to data presented in Irwin and Barneby, 1978). Conceição (2000) recognized a "prostrata form" of *C. cytisoides* but did not ascribe a formal taxonomic rank since it was not possible to establish its relationships with other taxa of the complex. Later, Conceição *et al.* (2001) described *C. depauperata* Conc., L.P.Queiroz & G.P.Lewis, a species similar to the "prostrata" morph of *C. cytisoides* but with flowers smaller than those found in *C. cytisoides* and *C. coriacea*. A summary of the current taxonomic situation of the subsection *Baseophyllum* and diagnostic characters of their taxa is presented in Table 1 and Fig. 1-2.

The purposes of this work are to explore the number of taxonomical entities, their taxonomic status and to provide diagnostic characters for all taxa currently ascribed to *Chamaecrista* sect. *Absus* subsect. *Baseophyllym*. In order to achieve these objectives we

assessed the levels of genetic and morphological variation and sub-structuring within and between the species and populations of the taxa in subsect. *Baseophyllum* using allozyme markers and morphometric analyses, so as to expand the knowledge of intra and interspecific relationships within this group. We also investigated the correlation between genetic and morphologic variability. The use of genetic diagnostic markers, genetic distance/similarities, and multivariate and statistical analyses of morphometric data can be helpful in evaluating specific and infraspecific limits, mainly when the traditional taxonomic procedures are not satisfactorily conclusive (Crawford, 1989; van der Bank *et al*., 2001; Henderson, 2006). A similar approach has been successfully used in studies of other plant groups native to the Brazilian "campos rupestres" (Borba *et al.*, 2000, 2001, 2002; Lambert *et al.*, 2006a, 2006b).

MATERIALS AND METHODS

Populations sampled

We collected 660 individuals from 33 natural populations distributed in the nine taxa and one morphospecies of *Chamaecrista* subsection *Baseophyllum* (Table 2; Fig. 1-2) at 22 localities in Espírito Santo, Minas Gerais, Bahia, Sergipe and Pernambuco states: *C. coriacea* (one population), *C. depauperata* (two populations), *C. cytisoides* var. *blanchetii* (seven populations), *C. cytisoides* var. *brachystachya* (five populations), *C. cytisoides* var. *confertiformis* (five populations), *C. cytisoides* var. *cytisoides* (one population), *C. cytisoides* var. *decora* (three populations), *C. cytisoides* var. *micrantha* (four populations), and *C. cytisoides* var. *unijuga* (two populations). We also collected three populations of a supposed morphospecies for the subsection, called here *C. cytisoides* "f. prostrata". Vouchers for each

population are deposited in the herbarium of the Universidade Estadual de Feira de Santana (HUEFS).

Electrophoretic procedures

Small sections of leaf tissue were crushed in 0.5 mL of grinding buffer (Lambert *et al.*, 2006a). Extracts were absorbed in 1.0 x 0.3 cm Whatman number 3 paper wicks, which were loaded into 8.5% starch gels (Sigma hydrolyzed potato starch). For the electrodes and gels four buffer systems were used: 1) electrode: lithium hydroxide 0.05 mol L^{-1} boric acid 0.0935 mol L^{-1} EDTA 0.0059 mol L^{-1} pH 8.0; gel electrode solution diluted 1:10; modified from Ridgway *et al*. (1970); 2) electrode: citric acid 0.04M adjusted to pH 6.1 with N-(3-aminopropyl)-morpholine; gel: electrode solution diluted 1:20; modified from Clayton and Tretiak (1972); 3) electrode: Tris 0.17 mol/L, citric acid 0.04 mol/L, pH 8.6; gel: tris 0.02 mol/L, citric acid 0.0013 mol/L, pH 8.6; 4) electrode: boric acid 0.3 mol/L, NaOH 0.06 mol/L, pH 8.0; gel: Tris 0.01 mol/L, pH 8.5; modified from Shaw and Prasad (1970). Standard horizontal eletrophoresis was performed until the inner marker (bromophenol blue) reached 9 cm from the application site using the following running conditions: systems 1, 2 and 3 – 25 mA; system 4 – 15 mA. Nine enzymatic systems gave enough resolution for reading and were used. System 1 was used for malate dehydrogenase (MDH; EC 1.1.1.37), phosphoglucomutase (PGM; EC 2.7.5.1), esterase (EST; EC 3.1.1.1), shikimate dehydrogenase (SKDH; EC 1.1.1.25), and glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49); system 2 was used for isocitrate dehydrogenase (IDH; EC 1.1.1.42); system 3 was used for leucine aminopeptidase (LAP; AC 3.4.11.1); and system 4 was used for phosphoglucose isomerase (PGI; EC 5.3.1.9) and acid phosphatase (ACPH; EC 3.1.3.2). The staining procedures were similar to but slightly adjusted from Brune *et al*. (1998; ACPH, EST, LAP, SKDH, G6PDH), Corrias *et al*. (1991; IDH, PGI) and Soltis *et al*. (1983; PGM,

MDH). Modifications were mainly in the amounts of the components used; the exact recipes can be obtained on request. Enzymatic systems showing more than one locus were numbered in ascending order from the locus with lowest mobility. The alleles were numbered according their mobility relative to the allele with highest mobility of a standard individual present in all gels and designated as 100.

Analyses of allozyme data

The allelic frequencies were determined by manually counting the banding patterns of the homozygotes and heterozygotes stained in the gels. Genetic variability for each population was estimated by the following parameters: proportion of polymorphic loci (P; 0.95 criterion), mean number of alleles per locus (A), observed (H_o) and expected (H_e) mean heterozygosity per locus. Deviations from the expected mean heterozygosity under Hardy-Weinberg (HW) equilibrium were tested using χ^2 with a correction for small samples according to Levene (1949). Partitioning of genetic diversity among conspecific populations was estimated by F statistics (F_{IS}, the inbreeding coefficient measures the reduction in heterozygosity due to nonrandom mating within a population; $F_{ST,}$ measures the differentiation among populations; Wright, 1978).

Matrices of genetic distances (unbiased genetic distance; Nei, 1978) and genetic identities (unbiased genetic identity; Nei, 1978) were calculated for populations and species. Cluster analysis was performed with the genetic distance matrix of the populations using UPGMA (unweighted pair-group method using arithmetical averages) as grouping algorithm (Sneath and Sokal, 1973). We also accomplished analyses with other clustering algorithm (complete linkage, Ward, weighted pair-group) to test the consistence of the groups. All analyses were carried out using the BIOSYS 1.0 software package (Swofford and Selander,

1989), except for the cluster analysis that was performed using STATISTICA for Windows 6.0 version (StatSoft, 2000).

Morphometric analyses

The individuals sampled for the allozyme analysis were also used in analyses of morphological variability. We measured 54 quantitative morphological characters (except for variable #2, which is binary), with 38 floral and 16 vegetative characters (Table 3). All measurements of quantitative characters were taken with the aid of a vernier caliper. Based on morphology and position of the nectaries, we considered that the first pair of leaflets of bifoliolate leaves is homologous to the second pair of leaflets of tetrafoliolate leaves. We carried out the following analyses: 1) all populations, using only vegetative morphological characters; 2) all populations, using only vegetative morphological characters excluding variables #1 and #2 (both relative to the number of leaflets, which could force a separation of bi- and tetrafoliolate groups); 3) using only floral morphological characters (excluding CD2, CD3, CFP1, CB1, CC2, CC3, CC4, CC5, which were not found flowering); 4) only the populations which present two or three pairs of leaflets, using only vegetative morphological characters, and including for the first pair of leaflets the same variables used for the second pair (variable #3 to 9).

We performed canonical variate analysis (CVA) and cluster analysis using the software package STATISTICA 6.0. CVA was performed with population as the categorical variable (individuals were grouped according to the population to which they belong). The standardized coefficients for canonical variables obtained from discriminant analysis were used to identify the characteristics that most significantly contributed to the resulting patterns observed. The cluster analysis was carried out using the Mahalanobis Generalized Distance and UPGMA as the clustering algorithm (Sneath and Sokal, 1973). The morphological

variability of populations was obtained using the median of the Mahalanobis distances of individuals to the centroid of the group ($D2_m$) (Goldman *et al.*, 2004; Lambert *et al.*, 2006a, 2006b). We used the median of the squared Mahalanobis distances instead of an average of these distances because of the non-normal distribution of the data. A multi-response permutation procedure (MRPP) analysis made using PCOrd 4.10 program (McCune and Mefford, 1999) was used to calculate the chance-corrected within-group agreement (A_{MRPP}) among populations of every species, and A_{MRPP}-values were compared with the indexes of genetic differentiation among populations (F_{ST}) (Borba *et al.*, 2002; Lambert *et al.*, 2006a, 2006b). The average Euclidian distance among the individuals of each population (ED) resulting from de MRPP analysis was also used as a measure of morphological variability within populations (Borba *et al.*, 2002; Lambert *et al.*, 2006a, 2006b). The two indexes of morphological variability are essentially different, as $D2_m$ is more affected by form and ED is more affected by size of the characters (Lambert *et al.*, 2006a, 2006b). A Spearman rank correlation analysis between the morphological (ED and $D2_m$) and genetic (H_e) variability of populations was also carried out with STATISTICA 6.0.

RESULTS

Intra-populational variability

Using nine enzymatic systems 15 loci were obtained with good resolution and were used in the study. Three loci were monomorphic for all populations (ACPH, MDH and IDH-1). Five loci displayed low polymorphism, with only two or three alleles (PGI-2, EST-1, SKDH, G6PDH and LAP-2). The remaining loci present four to eight alleles per locus. PGM-1 was

the most polymorphic, with eight alleles (Table 4). Some alleles were exclusive to a single taxon: PGI-1 90, PGM-1 75, PGM-2 93, EST-1 75 and EST-3 94 to *C. coriacea*; PGM-1 120 to *C. cytisoides* var. *unijuga*; PGM-2 106, EST-2 90, EST-3 85, G6PDH 105 and LAP-1 89 to *C. cytisoides* var. *cytisoides*; PGM-2 81 and LAP-1 93 to *C. depauperata*. A few alleles were exclusive to one population: LAP-2 94 (CB2); PGI-1 115 and PGM-1 116 (CBR2); PGM-1 92 (CBR5) and LAP-1 90 (CM2) (Table 4).

The percentage of polymorphic loci (*P*; 0.95 criterion) ranged from 0.0 to 33.3%, the mean number of alleles per locus was 1.1 to 1.4, and mean heterozygosity (H_e) ranged from 0.010 to 0.067 (Table 5). Populations CCY1 (*C. cytisoides* var. *cytisoides*), D1 (*C. depauperata*), CBR1 (*C. cytisoides* var. *brachytachya*), CD3 (*C. cytisoides* var. *decora*) and CU1 (*C. cytisoides* var. *unijuga*) displayed the highest genetic variability. The majority of the populations (72.7%) were in HW equilibrium in all loci. The other populations presented significant deviations in only one locus each. Five loci were not in HW equilibrium in at least one population: EST-1 (C1), G6PDH (CCY1), LAP-1 (CM2) and IDH-2 (in all populations of CC) with deficit of heterozygotes, and LAP-2 (CD3) with excess of heterozygotes. The low values for F_{IS} reflects the HW equilibrium in most of the taxa, except for *C. cytisoides* var. *confertiformis* (CC) that presented very high F_{IS} (Table 6).

In both morphological analyses, the population with the highest variability was CBR1, a population which also presented one of the highest genetic variables (Table 5). The other populations presenting high morphological variability expressed as $D2_m$ were CM3, CBR5 and CU1, while the lowest variability was found in CC3 (Table 5). The most variable characters considering all taxa pooled were leaflet number (variable #1), number of main veins (#11), length of the leaflet (#3), width at 1/3 of the leaflet (#5), apex angle of the leaflet (#9), least distance from the margin to the main nervure, in the base of the leaflet (#7), length of the rachis (#12), and length of the petiole (#10). The least variable character was the

distance of the nectary in relation to the second pair of leaflets (#16). Spearman rank correlations between morphological and genetic variability resulted in a statistically significant positive high correlation between H_e and $D2_m$ (r= 0.917, p≤ 0.001) and between H_e and ED (r= 0.901, p≤ 0.001).

Structuring of the variability

Chamaecrista cytisoides var. *blanchetii* and *C. cytisoides* var. *brachystachya* displayed very high values of F_{ST}, interpreted as a very high level of genetic structuring. The other taxa showed moderate to low average values of F_{ST} (Table 6). By excluding populations CB1 and CB5 of *C. cytisoides* var. *blanchetii* and CBR2 of *C. cytisoides* var. *brachystachya* the average values of F_{ST} drop to 0.042 and 0.053, respectively. In *C. cytisoides* var. *blanchetii* this is due to an inversion in the frequency of the most frequent alleles of IDH-2 in these two populations. In *C. cytisoides* var. *brachystachya* this picture is due to an inversion in the frequency of the most frequent alleles of PGM-1 and PGI-1 in CBR2.

Chameacrista cytisoides var. *brachystachya* also presented the highest value of morfological struturing ($A_{MRPP=}$ 0.361), correlated with the highest values of genetic differentiation (F_{ST}). *C. cytisoides* var. *confertiformis* and *C. cytisoides* var. *micrantha* that presented high values of morphological differentiation had low values of genetic differentiation (Table 6).

Phenetic relationships - genetic data

Populations of *C. depauperata*, C. *cytisoides* var. *decora*, *C. cytisoides* var. *micrantha* and *C. cytisoides* var. *unijuga* did not present any intra-taxon genetic differentiation (Table 7). The populations of *C. cytisoides* var. *blanchetii*, *C. cytisoides* var. *confertiformis*, and *C. cytisoides* "f. prostrata" also presented very high values of genetic identity among intra-taxon

populations, ranging from 0.93 to 1.00. The lowest intra-taxon genetic identity (0.87) was found within *C. cytisoides* var. *brachystachya*, between the population CBR2 and the four remaining populations.

The UPGMA dendrogram obtained from the cluster analysis of Nei's (1978) unbiased genetic distances (Fig 3) reveals the formation of two main groups. The first contains the population of *C. cytisoides* var. *cytisoides* (CCY) and the two populations of C. *depauperata* (D). The high divergence of *C. cytisoides* var. *cytisoides* and *C. depauperata* is due to the presence of exclusive alleles fixed in two enzymatic systems (both in PGM-2 and LAP-1), besides the same fixed allele in PGI-1, similar for the two taxa (Table 4).The second and largest group may be subdivided in three subgroups: one formed by all of the populations of *C. cytisoides* var. *decora* (CD) and population (C) of *C. coriacea*; an other composed of the two populations of *C. unijuga* and population CBR2; the third formed by the populations of the remaining taxa. In this third subgroup only *C. cytisoides* var. *confertiformis* formed group containing exclusively all of their intrataxon populations. Populations of *C. cytisoides* var. *blanchetii*, *C. cytisoides* var. *brachystachya, C. cytisoides* var. *micrantha* and *C. cytisoides* "f. prostrata" formed mixed groups. Four populations of *C. cytisoides* var. *brachystachya* are nested in the group containing most of the populations of *C. cytisoides* var. *blanchetii*; the remaining population is very divergent (CBR2) due to the presence of two exclusive fixed alleles in two loci (Table 4).

Phenetic relationships - morphological data

The scatterplots of the scores of individuals on the first two CVA canonical axes of the morphological analyses are shown in Fig. 4. The first and second canonical axes explained respectively 56% and 16% of the variation in the analysis including only vegetative characters. On the first canonical axis there is a separation of three groups, one with *C.*

cytisoides var. *cytisoides*, one with the populations of *C. cytisoides* var. *confertiformis*, *C. coriacea* and *C. cytisoides* var. *brachystachya* (except for CBR5), and another with *C. unijuga*, *C. cytisoides* var. *decora*, *C. depauperata*, *C. cytisoides* "f. prostrata", *C. cytisoides* var. *blanchetii* and population CBR5 (Fig. 4A). The variables that more contributed to that separation in the first axis were the leaflets number (variable #1), length of the second pair of leaflets (#3) and number of main veins (#11). In the second axis *C. coriacea* was separated from the remaining taxa in the second group, and the third group was divided in two subgroups, one containing *C. unijuga*, *C. cytisoides* var. *decora* and *C. depauperata* and the other with the remaining taxa. The variables that contributed most to the separation on this second canonical axis were the length of the petiole (variable #10) and the least distance from the margin to the main nervure in the base of the leaflets (#7). This structure had no significant changes in the analysis in which the variables #1 and #2 (both relative to the number of leaflets) were excluded (data not shown). In the matrix of classification the percentage of correct classifications ranged from 38 to 100%. The incorrect classifications mostly occurred between populations of the same taxon, except for eight individuals of CBR1 which were classified in CU1 and CB7, eight individuals of CBR5 in CB7 and CB2, and one individual of CBR3 in CM4.

The first and second canonical axes explained 42% and 16%, respectively, of the variation in the analysis using only floral characters. In the first canonical axis the two populations of *C. depauperata* are separated from the other taxa (Fig. 4B). The variables that contributed most to this separation were maximum width of the fourth petal (variable #44), length of the anthers (#51), width at 2/3 of the fourth petal (#46) and number of ovules (#54). In the second axis occurred a slight separation of *C. cytisoides* var. *cytisoides* from the remaining taxa.

The same structure observed in the CVA was found in the UPGMA dendrogram obtained from the cluster analysis of Mahalanobis distance among the centroids of the populations. The analysis using vegetative morphological characters resulted in the separation of *C. cytisoides* var. *cytisoides*, the only taxon presenting predominantly three pairs of leaflets, and two other groups composed by the populations presenting one or two pair of leaflets (Fig 5A). Populations of the same taxon are mostly present only in one of these two last groups, except for *C. cytisoides* var. *brachystachya*, with three population in one group and two in the other. In the subgroup presenting predominantly two pairs of leaflets, the population CBR4 and *C. coriacea* displayed the greatest differentiation, and the remaining populations were divided into two subgroups, one including all the populations of *C. cytisoides* var. *confertiformis* and the other composed by all populations of *C. cytisoides* var. *micrantha* plus the populations CBR2 and CBR3. In the subgroup presenting predominantly one pair of leaflets, two subgroups were formed: one composed of all populations which do not have a petiole, intermingled (*C. cytisoides* var. *brachystachya*, *C. cytisoides* "f. prostrata" and *C. cytisoides* var. *blanchetii*), and an other of all populations that possess a petiole (*C. unijuga*, *C. cytisoides* var. *decora* and *C. depauperata*; the populations of each taxon grouping together in three subgroups).

The morphological cluster analysis carried out only with the taxa presenting two or three pairs of leaflets resulted in the same general structure of the analysis carried out with only the second pair of leaflets. In the analyses using other clustering algorithms, the same structuring was found. In the analyses which variables 1 and 2 were excluded, the same groups were maintained, except for the population CBR4 grouping with CCY1.

The UPGMA dendrogram obtained from the cluster analysis of morphological distance using only floral characters resulted in the formation of two main groups (Fig. 5B): a group containing only the populations of *C. depauperata*, and another larger group composed

by the populations of all remaining taxa. This larger group present high degree of chaining, with the populations of all taxa intermingled; no subgroups composed primarily by all populations of the same taxon were formed.

DISCUSSION

The levels of genetic variation found for all taxa of the subsection *Baseophyllum* are lower than the expected based on their ecological and life history characteristics. Hamrick and Godt (1992) revised the allozyme diversity in plants, finding higher values for plants of regional and endemic geographical distribution, respectively (P=36.4, A=1.55, H_e=0.118 and P=26.3, A=1.39, H_e=0.063). However, the variability levels found in this study were similar to the values found for other genera of Leguminosae (Schaal and Smith, 1980; Hoffman *et al*., 1986; Carulli and Fairbrothers, 1988; Baatout *et al*., 1991; Liston, 1992; Sonnante *et al*., 1997; Chamberlain, 1998; Jenczewski *et al*., 1999; Lange and Schifino-Wittmann, 2000; Casiva *et al*., 2002; Bi *et al*., 2003; Sotuyo *et al*., 2004).

The low variability presented within populations for all taxa of the subsection *Baseophyllum* may be associated with the pollinator behavior and with the geographical distribution pattern. These plants are self-compatible and pollinated by social bees, (CBN Costa, Universidade Estadual de Feira de Santana, Brazil, unpubl.), occurring in isolated populations in outcrops of "campo rupestre" vegetation along Espinhaço Range in Minas Gerais and Bahia states (interior of Brazil) with disjunctions in costal "restinga" areas of Northeastern and Southeastern Brazil. Some taxa are endemic of a single area, such as *C.*

coriacea, *C. depauperata, C. cytisoides* var. *confertiformis* and *C. cytisoides* var. *decora* (Irwin and Barneby, 1978; Conceição *et al*., 2001).

The populations of *C. cytisoides* var. *micrantha* and *C. depauperata* which had the highest diversity (CM3 and D1, respectively) are located in preserved areas, where human activity is restricted. Conversely, population CC2 of *C. cytisoides* var. *confertiformis* occurring in a site under anthropogenic action displayed the lowest genetic diversity of the taxon, in spite of its moderate morphological variability. The populations of *C. cytisoides* var. *blanchetii* with slightly higher genetic diversity are located in disjunct areas, CB1 in the restinga of Espírito Santo state, and CB5 in the savanna of Minas Gerais state, whilst the other populations occur in areas of "campos rupestres" in Bahia state. These two disjunct populations presented an exclusive fixed allelle in the locus IDH-2, indicating a restricted gene flow among these three major areas of occurrence of the taxon. Correlation between genetic and morphological variation has not been observed frequently in plants (Mitton 1978; Gilles 1984; Elisens *et al.* 1992; Avise 1994; Borba *et al.* 2001; Lambert *et al.*, 2006a, 2006b).

Chamaecrista cytisoides var. *confertiformis* was the only taxon presenting high values of F_{IS}, mainly due to the deficit of heterozygotes in the locus IDH-2, which could be the result of endogamy or sub-structuring of the populations. This taxon is endemic to the municipalities of Mucugê and Lençóis in the central area of Bahia state. This taxon displays a singular spatial distribution for subsection *Baseophyllum*, almost always occurring in valleys along the margins of rivers, forming dense linear populations. In addition to this spatial distribution, the self-compatibility of this taxon and the behavior of its pollinator (CBN Costa, unpubl.) favors endogamy and non-random mating, probably leading to genetic sub-structuring of the populations.

Studies of intra- and inter-populational multivariate morphological variability are apparently absent in *Chamaecrista* and scarce in Leguminosae (Riggins *et al*., 1977, Hoffman *et al*., 1988; Casiva *et al*., 2002; Leht and Jaaska, 2002; Boonkerd *et al*., 2005). The main characteristics that forced the separation of the three groups in the multivariate analyses were the number of leaflets, number of main veins and length of the leaflets. Because the same groups were maintained in the analysis excluding the variables 1 and 2, number of leaflets is not the only responsible for this structure, and length of the rachis, apex angle of the leaflet, length of the leaflets, and least distance from the margin to the main vein in the base of the leaflet sustained the structure of three groups. It is important to notice that these characters were not evidenced by Irwin and Barneby (1978) or by Bentham (1870, 1871) in the distinction of the taxa in the subsection *Baseophyllum*.

Chamaecrista coriacea was recognized (as *Cassia*) by Bentham (1840) as a singular species, presenting one or two pairs of palmately veined leaflets, and its status has never been objected (Irwin and Barneby, 1978, 1982). Both allozyme and morphological data supported its distinction at specific rank. *Chamaecrista coriacea* differs from the other taxa of the subsection *Baseophyllum* in at least one allozymic locus, with a genetic identity of 0.78 to *C. cytisoides* var. *cytisoides*. In the discriminant analysis *C. coriacea* differed from *C. cytisoides* var. *cytisoides* mainly is its leaves predominantly with two pairs of leaflets, smaller rachis and smaller leaflets.

The recently described *C. depauperata* was the only taxon with clearly distinctive flowers, as evidenced in the morphological analyses of floral characters, mainly due to the width of fourth petal, length of the anthers and fewer ovules. In the analyses using vegetative morphological data, *C. depauperata* also differed of the other taxa by presenting petiolate leaves with two pairs of smaller leaflets. Furthermore, this species is very distinctive genetically in at least three allozymic loci (two of them being diagnostic and the other similar

to *C. cytisoides* var. *cytisoides*). *Chamaecrista depauperata* and *C. cytisoides* var. *cytisoides* are quite different in both habit and habitat (prostrate sub-shrubs from "campo rupestre" vegetation vs. shrubs to treelets from "cerrado"), and in number and form of the leaflets (Table 1). Besides, *C. depauperata* occurs exclusively in the state of Bahia in the southern portion of the Chapada Diamantina, while *C. cytisoides* var. *cytisoides* is endemic to southern portion of Minas Gerais state (see Fig. 1).

The high similarity in floral traits has probably lead Irwin and Barneby (1978) to treat some of the former species of the subsection *Baseophyllum* as varieties of *C. cytisoides*. This picture was possibly reinforced by the occurrence of hybridization in some taxa, such as between *C. cytisoides* var. *confertiformis* and *C. cytisoides* var. *blanchetii* and between *C. cytisoides* var. *confertiformis* and *C. depauperata* (AS Conceição, EL Borba, LP Queiroz, unpubl.). These hybrids are rare in nature due to several isolation barriers between the sympatric species (CBN Costa, unpubl.), but may create confusion among taxonomists when they examine only herbarium collections.

The values of genetic identities found among populations of the *C. cytisoides* complex were similar to the values usually found between populations of distinct species (Thorpe, 1982; Crawford, 1989; van der Bank *et al*.; 2001). The average genetic identities between the population where the holotype of *C. cytisoides* var. *cytisoides* was collected and all other taxa in this complex are lower than the values found between conspecific populations, ranging from 0.64 to 0.78. Conversely, the genetic identity of that population with both *C. depauperata* and *C. coriacea* was higher. *Chamaecrista cytisoides* var. *cytisoides* was also the most divergent population in the morphological analyses based on vegetative characters, mainly due to its leaves with three pairs of leaflets. Both low values of genetic identity and high morphological differences of the type taxon for the complex with the other conspecific varieties reinforce the hypothesis of the existence of distinct species in the complex. Our

results points out that the population of *C. cytisoides* var. *cytisoides* studied should be the only population included in the species.

The populations of *C. cytisoides* var. *confertiformis*, *C. cytisoides* var. *decora* and *C. cytisoides* var. *unijuga* formed three very distinctive groups in both genetic and morphological analyses. These three taxa present different geographic distribution, *C. cytisoides* var. *confertiformis* being endemic to interior Bahia, *C. cytisoides* var. *decora* endemic to inland Minas Gerais, and *C. cytisoides* var. *unijuga* endemic to coastal areas of Sergipe and Alagoas states. Therefore, we propose they should be treated as distinct species, which may be easily recognized by morphological differences in their leaves: shortly petiolate leaves with two pairs of oblong to oblong-lanceolate leaflets in *C. confertiformis*, petiolate leaves with one pair of obliquely obovate leaflets in *C. decora*, and petiolate leaves with one pair of orbicular to reniform leaflets in *C. unijuga*.

The populations of *C. cystisoides* var. *blanchetii*, *C. cytisoides* var. *brachystachya*, *C. cystisoides* var. *micrantha* and *C. cytisoides* "f. prostrata" presented a reticulate pattern of grouping in both genetic and morphological analyses, but with different topology of phenetic relationships in each analysis. Almost all populations presented very high genetic identities, except for three disjunct populations of *C. cystisoides* var. *blanchetii* (CB1 and CB5) and *C. cytisoides* var. *brachystachya* (CBR2). The differences pointed out by Conceição (2000) in the populations named as *C. cytisoides* "f. prostrata" are not consistent with our analyses, and we suggest that these populations should not be treated as a distinct taxon.

Populations of *C. cytisoides* "f. prostrata" are morphologically very similar to *C. cytisoides* var. *blanchetii*, and the only genetic distinction was the absence of the loci EST-1 and EST-3 in *C. cytisoides* "f. prostrata" (but this may be due to a procedure artifact). Populations CBR2 (from which the holotype was collected) and CBR3 of *C. cytisoides* var. *brachystachya* are very similar morphologically to *C. cystisoides* var. *micrantha*, in spite of

the genetic differences of the former population. These two populations occur in the interior of Brazil, as also *C. cytisoides* var. *micrantha*, near the central (Pernambuco state, CBR3) and northern (center of Minas Gerais state, CBR2) extremes of distribution of the group (*C. cystisoides* var. *micrantha* occupies an intermediate distribution, occurring in Bahia and northern Minas Gerais states). The other three populations of *C. cytisoides* var. *brachystachya* are very similar morphologically to *C. cytisoides* var. *blanchetii*, differing by their occurrence near the coast (versus interior of Bahia in *C. cytisoides* var. *blanchetii*) and by the presence of two pairs of leaflets. These three populations have been identified as *C. cytisoides* var. *brachystachya* due to the presence of two pair of leaflets and probably by second pair being similar in morphology to that found in individuals of the population CBR2. However, the first pair of leaflets is very similar to the single pair found in *C. cytisoides* var. *blanchetii*.

Based on our present data, we suggest a broad circumscription of two polymorphic species in this group: (1) *C. brachystachya*, including the populations CBR2 and CBR3 (*C. cytisoides* var. *brachystachya s.s.*) and *C. cystisoides* var. *micrantha* populations, and (2) *C. blanchetii*, including the populations of *C. cytisoides* "f. prostrata" and populations CBR1, CBR4 and CBR5 formerly identified as *C. cytisoides* var. *brachystachya*. This decision is based mainly on morphology, but it is also supported by genetic data and by the occurrence of these two taxa in sympatry with no or low interbreeding (CBN Costa, unpubl.). In the multivariate morphological analyses the major characters which separated the populations of these two species were number of leaflets, width of leaflet at 1/3 of its length, angle of the apex of the leaflet, least distance from the margin to the main vein in the base of the leaflet, and length of the petiole.

Chamaecrista blanchetii is a variable species, with sessile leaves or bearing a minute petiole, with one to two (rarely three) pairs of coriaceous leaflets, the proximal pair reniform to sub-orbicular, base reniform to obtuse, frequently embracing the stem, and the distal pair

orbicular with base obtuse. *Chamaecrista brachystachya* is also a variable species, with petiolate leaves and two pairs of coriaceous to semi-succulent leaflets, the proximal pair orbicular to obovate with cordate to cuneate base, the distal pair oblong to suborbicular with cuneate base. The genetic differences presented by the population CBR2, as generally observed in allozyme differentiation, are probably a result of genetic drift or directional selection. The presence of two fixed exclusive alleles in this population can be interpreted as a result of its geographic isolation; gene flow between CBR2 and the remaining *C. brachystachya* populations is possibly very low or non-existent, and genetic drift could be responsible for this differentiation. This process can quickly lead to speciation in small, geographically isolated populations (Levin, 2000).

Taxonomic conclusions

The results of multivariate morphological and allozyme data in populations of all taxa of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* showed that several distinct species exist in the subsection. Genetics and morphology confirmed the circumscription and specific rank for *C. coriacea* and *C. depauperata*, corroborating the delimitation proposed by Bentham (1870, 1871) and Conceição *et al*. (2001), respectively.

However, the present analyses did not support the delimitation proposed by Irwin and Barneby (1978, 1982) for the *Chamaecrista cytisoides* complex. Our data showed that the type taxon of the complex, *C. cytisoides* var. *cytisoides*, is genetic and morphologically very distant from all the other recognized varieties, and thus the complex may be re-arranged in six species based on *C. cytisoides* var. *blanchetii, C. cytisoides* var. *brachystachya, C. cytisoides* var. *confertiformis, C. cytisoides* var. *cytisoides*, *C. cytisoides* var. *decora* and *C. cytisoides* var. *unijuga.* On the other hand, *C. cytisoides* var. *micrantha* is proposed as synonym of *C.*

cytisoides var. *brachystachya*. The taxonomic treatment of the group, including new combinations, descriptions, illustrations and identification keys, is being published elsewhere.

ACNOWLEDGEMENTS

We thank the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) for financial support (#245/04). ASC received a fellowship from the Universidade do Estado da Bahia (PAC-UNEB). ELB and LPQ are supported by a grant (PQ2) from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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TABLE 1. Diagnostic characters and distribution data of the taxa of *Chamaecrista* section *Absus* subsection *Baseophyllum* according to Irwin & Barneby (1982) classification with addition of *C. depauperata* by Conceição *et al.* (2001) and the unpublished form "prostrata" (Conceição, 2000). Distribution: C – center; N – Northern; S – Southern; ct – coastal. Acronyms for Brazilian States: AL – Alagoas; BA – Bahia; ES – Espírito Santo; MG – Minas Gerais; PE – Pernambuco; SE – Sergipe.

Taxa	Characters						Habitat	Distribution
	Habit	Petiole	Leaflet pairs	Leaflet shape	Leaflet color	Flower diameter cm		
C. coriacea	Prostate sub-shrub	Present	1-2	Suborbicular to oblanceolate	Green	1.5-1.8	campo rupestre	N-MG
C. depauperata	Prostate sub-shrub	Present	1	Orbicular to suborbicular	Green	0.5-1.0	campo rupestre	C-BA
C. cytisoides var. *blanchetii*	Shrub to treelet	Absent or short	1	Orbicular to suborbicular	Green	1.5-1.8	campo rupestre, cerrado, restinga	C-BA, N-MG, ct-ES
C. cytisoides var. *brachystachya*	Shrub to treelet	Absent or short	1-2 (-3)	Reniform to widely obovate	Green	1.5-2.0	campo rupestre, caatinga, restinga	N-MG, C-PE, ct-BA and SE
C. cytisoides var. *confertiformis*	Shrub to treelet	Short	2	Oblong to oblanceolate	Green	1.2-2.0	campo rupestre	C-BA

var. *cytisoides*	Shrub to treelet	Short	3-4	Ovate to oblanceolate	Green	1.5-2.0	cerrado	S-MG
var. *decora*	Shrub to treelet	Present	1	Obliquely obovate	Purple or Green	1.5-2.0	campo rupestre	N-MG
var. *micrantha*	Shrub to treelet	Absent or short	2	Obovate to oblanceolate	Green	1.5-1.7	campo rupestre	C-BA, N-MG
var. *unijuga*	Shrub to treelet	Present	1	Reniform to orbicular	Green	1.3-1.7	restinga	ct-PE, AL and SE
“f. prostrata”	Prostate sub-shrub	Absent or short	1	Suborbicular	Green	1.5-2.0	campo rupestre	C-BA

TABLE 2. Populations of *Chamaecrista coriacea*, *C. depauperata*, *C. cytisoides* var. *blanchetii*, *C. cytisoides* var. *brachystachya*, *C. cytisoides* var. *confertiformis*, *C. cytisoides* var. *cytisoides*, *C. cytisoides* var. *decora*, *C. cytisoides* var. *micrantha*, *C. cytisoides* "f. prostrata" and *C. cytisoides* var. *unijuga*, occurring in Northeastern and Southeastern Brazil, used in the study. Vouchers deposited at the herbarium of the Universidade Estadual de Feira de Santana (HUEFS). Acronyms for Brazilian States: AL – Alagoas; BA – Bahia; ES – Espírito Santo; MG – Minas Gerais; PE – Pernambuco; SE – Sergipe.

Population	Municipality	Location		Voucher
C. coriacea				
C1	Conceição do Mato Dentro-MG	Costa Sena-Guritiba Cima	18°43'31"S, 43°37'24"W	A.S.Conceição 869
C. depauperata				
D1	Abaíra-BA	Catolés-Virassaia	13°50'S, 41°22'W	A.S.Conceição 775
D2	Mucugê-BA	Paty-Gerais do Rio Preto	12°45'49.1"S, 41°30'05"W	A.S.Conceição 863
C. cytisoides var. *blanchetii*				
CB1	Guarapari-ES	P. Estad. Paulo César Vinha	20°35'7.8"S, 40°25'17.3"W	A.S.Conceição 826
CB2	Jacobina-BA	Serra do Tombador	11°12'S, 40°41'W	A.S.Conceição 772
CB3	Abaíra-BA	Catolés-Bicota	13°19'23.9"S, 41°50'58.7"W	A.S.Conceição 664

Population	Municipality	Location		Voucher
CB4	Mucugê-BA	Projeto Sempre Viva	12°59’30”S, 41°20’30”W	A.S.Conceição 759
CB5	Pedra Azul-MG	47 km ao Sul Pedra Azul	16°20’20.8”S, 41°05’23.3”W	A.S.Conceição 831
CB6	Mucugê-BA	Gobira	13°04’38”S, 41°22’31”W	E.L.Borba 1849
CB7	Indiaroba-SE	2 km da entrada de Pontal	11°29’40.4”S, 37°26’18.2”W	A.S.Conceição 693
C. cytisoides var. *brachystachya*				
CBR1	Esplanada-BA	Faz. Morro da Graça, Bú	12°03’38”S, 37°41’39”W	A.S.Conceição 687
CBR2	Diamantina-MG	7 km E de Diamantina	18°10’54”S, 43°33’45.5”W	A.S.Conceição 540
CBR3	Buíque-PE	Serra de Jerusalém	08°34’57”S, 37°14’24”W	A.S.Conceição 761
CBR4	Salvador-BA	APA Stella Mares	12°56’S, 38°21’W	A.S.Conceição 832
CBR5	Itabaiana-SE	Serra de Itabaiana	10°45’6.9”S, 42°43’38.3”W	A.S.Conceição 819
C. cytisoides var. *confertiformis*				
CC1	Mucugê-BA	Piabinha	12°59’33.5”S, 41°20’27.8”W	A.S.Conceição 755
CC2	Mucugê-BA	Campo Alegre	13°14’S, 41°23’W	A.S.Conceição 834
CC3	Mucugê-BA	Paty-Gerais do Rio Preto	12°46’15.7”S, 41°29’29.3”W	A.S.Conceição 864

Population	Municipality	Location		Voucher
CC4	Lençóis-BA	Foz do rio Capivara	12°33’S, 41°24’W	A.S.Conceição 833
CC5	Mucugê-BA	Morro Fervido	12°59’46.4”S, 41°20’17.7”W	A.S.Conceição 835
C. cytisoides var. *cytisoides*				
CCY1	Santa Bárbara M. Verde-MG	Três Cruzes-Serra Negra	21°58’0.3”S, 43°49’12.1”W	A.S.Conceição 870
C. cytisoides var. *decora*				
CD1	Grão Mogol-MG	Vau-Itacambiruçu	16°35’26.9”S, 42°54’8.1”W	A.S.Conceição 810
CD2	Diamantina-MG	Beriberi	18°08’53.2”S, 43°36’53.1”W	A.S.Conceição 828
CD3	Diamantina-MG	Cristais	18°10’0.08”S, 43°35’57.3”W	A.S.Conceição 827
C. cytisoides var. *micrantha*				
CM1	Saúde-BA	Serra de Saúde	10°56’37”S, 40°24’29”W	A.S.Conceição 774
CM2	Grão Mogol-MG	Cancela	16°25’7.1”S, 42°43’38.3”W	A.S.Conceição 818
CM3	Mucugê-BA	9 km de Igatu	12°56’40.5”S, 41°17’20.7”W	A.S.Conceição 754
CM4	Abaíra-BA	Catolés-Bicota	13°19’42.3”S, 41°51’09.9”W	A.S.Conceição 567
C. cytisoides “f. prostrata”				

Population	Municipality	Location		Voucher
CFP1	Ibicoara-BA	13.2 km de Ibicoara	13°26’37.8”S, 41°17’16”W	A.S.Conceição 757
CFP2	Abaíra-BA	Catolés-Virassaia	13°50’S, 41°22’W	A.S.Conceição 672
CFP3	Barra da Estiva-BA	7 km de Barra da Estiva	13°40’48.3”S, 41°18’40.2”S	A.S.Conceição 758
C. cytisoides var. *unijuga*				
CU1	Santo Amaro das Brotas-SE	Ca. 2 km Rio Pomonga	10°48’24”S, 36°57’50”W	A.S.Conceição 694
CU2	Pirambu-SE	8.2 km de Pirambu	10°40’56”S, 36°52’51”W	A.S.Conceição 820

TABLE 3. Characters used in the morphometric analyses of 33 populations of nine taxa and one morphospecies of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* occurring in Northeastern and Southeastern Brazil.

Characters
Vegetative characters
Leaves (second par of leaflets)
1- Number of leaflets
2- Occurrence of variation in the number of leaflets
3- Length of leaflet
4- Maximum width of leaflet
5- Width of leaflet at 1/3 of its length
6- Width of leaflet at 2/3
7- Least distance from the margin to the main vein, in the base of the leaflet
8- Maximum distances from the margin to the main vein, in the base of the leaflet
9- Apex angle of the leaflet
10- Length of the petiole
11- Number of main veins
12- Length of the rachis
13- Length of the nectary
14- Width of the nectary
15- Number of nectaries
16- Distances of the nectary to the second par of leaflets
Floral characters
Inflorescence

17- Length

18- Number of flowers per raceme

Sepal

19- Length of the first sepal

20- Width of the first sepal

21- Length of the second sepal

22- Width of the second sepal

23- Length of the third sepal

24- Width of the third sepal

25- Length of the fourth sepal

26- Width of the fourth sepal

27- Length of the fifth sepal

28- Width of the fifth sepal

Petal

29- Length of the first petal

30- Maximum width of the first petal

31- Width of the first petal at 1/3 of its length

32- Width of the first petal at 2/3

33- Maximum distance from the margin to the main vein, in the base of the first petal

34- Minor distances from the margin to the main nervure, in the base of the first petal

35- Length of the second petal

36- Maximum width of the second petal

37- Width of the second petal at 1/3

38- Width of the second petal at 2/3

39- Length of the third petal

40- Maximum width of the third petal

41- Width of the third petal at 1/3

42- Width of the third petal at 2/3

43- Length of the fourth petal

44- Maximum width of the fourth petal

45- Width of the fourth petal at 1/3

46- Width of the fourth petal at 2/3

47- Length of the fifth petal

48- Maximum width of the fifth petal

48- Width of the fifth petal at 1/3

50- Width of the fifth petal at 2/3

Anther

51- Length of the anthers

Style

52- Length of the style

Ovary

53- Length of the ovary

54- Number of ovules

TABLE 4. Allelic frequencies at 15 loci in populations of *Chamaecrista coriacea* (C), *C. cytisoides* var. *cytisoides* (CCY), *C. depauperata* (D), *C. cytisoides* var. *decora* (CD), *C. cytisoides* var. *unijuga* (CU), *C. cytisoides* "f. prostrata" (CFP), *C. cytisoides* var. *micrantha* (CM), *C. cytisoides* var. *blanchetii* (CB), *C. cytisoides* var. *brachystachya* (CBR) and *C. cytisoides* var. *confertiformis* (CC), occurring in Northeastern and Southeastern Brazil. *N* = sample size. See Table 2 for the name of the populations.

Locus	C1	CCY1	D1	D2	CD1	CD2	CD3	CU1	CU2	CFP1	CFP2	CFP3	CM1	CM2	CM3	CM4	CB1	CB2	CB3	CB4	CB5	CB6	CB7	CBR1	CBR2	CBR3	CBR4	CBR5	CC1	CC2	CC3	CC4	CC5
ACPH																																	
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(N)	*19*	*20*	*19*	*20*	*18*	*20*	*20*	*17*	*20*	*20*	*18*	*20*	*8*	*20*	*20*	*20*	*20*	*20*	*18*	*20*	*20*	*15*	*20*	*20*	*20*	*20*	*16*	*20*	*20*	*11*	*17*	*12*	*19*
PGI-1																																	
140	-	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00
115	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-	-	-
110	-	-	-	-	-	-	-	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	-	-	-	-	1.00	1.00	1.00	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	1.00	1.00	1.00	-	-	-	-	-
90	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(N)	*19*	*20*	*19*	*16*	*16*	*8*	*11*	*10*	*14*	*11*	*18*	*18*	*13*	*12*	*13*	*16*	*20*	*20*	*18*	*14*	*20*	*18*	*20*	*13*	*20*	*18*	*20*	*18*	*7*	*9*	*17*	*20*	*11*
PGI-2																																	
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.96	1.00	1.00	0.96	0.96
95	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	0.04	0.04
(N)	*19*	*20*	*19*	*20*	*17*	*18*	*11*	*14*	*14*	*15*	*19*	*20*	*13*	*18*	*15*	*17*	*18*	*20*	*19*	*14*	*20*	*18*	*14*	*20*	*16*	*16*	*19*	*12*	*13*	*8*	*17*	*13*	*14*

Locus	C1	CCY1	D1	D2	CD1	CD2	CD3	CU1	CU2	CFP1	CFP2	CFP3	CM1	CM2	CM3	CM4	CB1	CB2	CB3	CB4	CB5	CB6	CB7	CBR1	CBR2	CBR3	CBR4	CBR5	CC1	CC2	CC3	CC4	CC5
PGM-1																																	
120	-	-	-	-	-	-	-	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
116	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-	-	-	-	-	-	-	-
107	-	-	-	-	-	-	-	-	-	0.06	0.18	0.03	-	-	-	-	0.06	-	0.04	0.14	-	-	-	0.10	-	-	-	-	-	-	-	-	-
100	-	-	-	-	-	-	-	-	-	0.94	0.82	0.97	1.00	1.00	1.00	1.00	0.94	1.00	0.96	0.86	1.00	1.00	1.00	0.90	-	1.00	1.00	0.96	1.00	1.00	1.00	1.00	1.00
92	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-	-
83	0.83	1.00	0.83	0.88	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
75	0.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	-	-	0.17	0.13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(N)	*18*	*20*	*18*	*16*	*16*	*7*	*6*	*18*	*8*	*8*	*14*	*19*	*19*	*7*	*12*	*13*	*17*	*20*	*13*	*11*	*16*	*8*	*6*	*20*	*13*	*10*	*12*	*12*	*8*	*9*	*9*	*14*	*9*
PGM-2																																	
106	-	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	0.81	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
93	0.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
81	-	-	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(N)	*18*	*20*	*19*	*14*	*15*	*12*	*8*	*19*	*9*	*8*	*10*	*6*	*7*	*13*	*10*	*13*	*20*	*17*	*9*	*8*	*18*	*8*	*6*	*18*	*9*	*16*	*12*	*14*	*6*	*9*	*19*	*15*	*13*
MDH																																	
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(N)	*14*	*16*	*19*	*20*	*17*	*20*	*20*	*20*	*17*	*20*	*20*	*11*	*20*	*20*	*20*	*20*	*20*	*20*	*18*	*17*	*14*	*20*	*20*	*20*	*16*	*20*	*20*	*15*	*16*	*20*	*20*	*16*	*20*
EST-1																																	

Locus	C1	CCY1	D1	D2	CD1	CD2	CD3	CU1	CU2	CFP1	CFP2	CFP3	CM1	CM2	CM3	CM4	CB1	CB2	CB3	CB4	CB5	CB6	CB7	CBR1	CBR2	CBR3	CBR4	CBR5	CC1	CC2	CC3	CC4	CC5
106	-	-	-	-	-	-	-	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	0.80	1.00	1.00	1.00	-	-	-	0.94	1.00	-	-	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	1.00	1.00	-	-	-	-	-
75	0.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(N)	*5*	*19*	*19*	*16*	*20*	*20*	*20*	*8*	*6*	*20*	*20*	*20*	*20*	*20*	*20*	*20*	*12*	*13*	*6*	*11*	*7*	*6*	*6*	*8*	*5*	*20*	*6*	*9*	*20*	*20*	*20*	*20*	*20*
EST-2																																	
120	-	-	-	-	0.07	0.05	0.06	0.11	0.03	0.05	0.03	0.16	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-
105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-	0.17	-	-	-	-	-	-	-	-	-
100	0.82	0.83	0.84	0.91	0.86	0.83	0.81	0.87	0.93	0.96	0.98	0.84	1.00	1.00	0.8	1.00	1.00	1.00	0.92	1.00	0.79	1.00	0.85	0.81	0.98	1.00	1.00	1.00	0.92	0.89	0.90	0.90	0.92
95	0.18	-	0.16	0.09	0.07	0.12	0.12	0.03	0.05	-	-	-	-	-	0.15	-	-	-	0.08	-	0.21	-	0.12	0.03	0.02	-	-	-	0.08	0.11	0.10	0.10	0.08
90	-	0.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(N)	*19*	*20*	*19*	*17*	*14*	*20*	*16*	*19*	*20*	*11*	*20*	*19*	*20*	*10*	*20*	*11*	*20*	*13*	*13*	*11*	*7*	*8*	*20*	*18*	*20*	*19*	*16*	*18*	*12*	*13*	*10*	*10*	*13*
EST-3																																	
100	0.85	0.88	0.84	0.98	-	-	-	-	-	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	0.917
94	0.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
85	-	0.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	-	-	0.16	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.083
(N)	*17*	*16*	*16*	*20*	*20*	*20*	*20*	*20*	*20*	*20*	*20*	*20*	*6*	*5*	*8*	*8*	*20*	*9*	*9*	*8*	*7*	*6*	*10*	*6*	*5*	*20*	*5*	*10*	*11*	*10*	*8*	*7*	*12*
SKDH																																	
100	1.00	1.00	0.94	0.96	0.98	0.96	0.98	0.98	0.95	0.98	0.98	0.96	0.94	0.94	0.94	0.97	0.97	0.98	0.96	0.97	0.98	0.97	0.95	0.85	0.97	0.96	0.95	0.94	0.97	0.97	0.98	0.98	0.96
93	-	-	0.06	0.04	0.02	0.04	0.02	0.02	0.05	0.02	0.02	0.04	0.06	0.06	0.06	0.03	0.04	0.02	0.04	0.03	0.02	0.04	0.05	0.15	0.03	0.04	0.05	0.06	0.04	0.04	0.02	0.02	0.04

Locus	C1	CCY1	D1	D2	CD1	CD2	CD3	CU1	CU2	CFP1	CFP2	CFP3	CM1	CM2	CM3	CM4	CB1	CB2	CB3	CB4	CB5	CB6	CB7	CBR1	CBR2	CBR3	CBR4	CBR5	CC1	CC2	CC3	CC4	CC5
(N)	*15*	*20*	*9*	*14*	*20*	*14*	*20*	*20*	*19*	*20*	*20*	*13*	*16*	*17*	*18*	*19*	*15*	*20*	*13*	*18*	*20*	*15*	*20*	*17*	*18*	*14*	*20*	*16*	*15*	*15*	*20*	*20*	*11*
G6PD																																	
105	-	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	1.00	0.70	0.97	0.98	0.97	0.96	0.96	0.96	0.94	0.95	0.95	0.93	0.97	0.96	0.97	0.98	0.82	0.97	0.96	0.92	0.92	0.92	0.98	0.95	0.97	0.96	0.98	0.91	1.00	1.00	1.00	1.00	1.00
95	-	0.10	0.03	0.02	0.03	0.04	0.04	0.04	0.06	0.05	0.05	0.07	0.03	0.04	0.03	0.02	0.18	0.03	0.04	0.08	0.08	0.08	0.02	0.05	0.03	0.04	0.02	0.09	-	-	-	-	-
(N)	*12*	*20*	*18*	*20*	*15*	*14*	*11*	*13*	*16*	*20*	*20*	*20*	*17*	*13*	*19*	*20*	*14*	*18*	*13*	*12*	*13*	*13*	*20*	*19*	*15*	*14*	*20*	*16*	*11*	*9*	*13*	*9*	*10*
IDH-1																																	
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
(N)	*15*	*17*	*15*	*15*	*14*	*11*	*9*	*16*	*18*	*20*	*20*	*20*	*19*	*14*	*11*	*20*	*18*	*13*	*20*	*11*	*14*	*6*	*20*	*18*	*9*	*18*	*20*	*20*	*5*	*7*	*11*	*12*	*6*
IDH-2																																	
100	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	1.00	-	-	-	1.00	-	-	-	-	-	-	-	0.2	0.08	0.64	0.29	0.18
93	-	-	-	-	-	-	-	-	-	0.07	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-
90	-	-	-	-	-	-	-	1.00	1.00	0.93	1.00	1.00	1.00	1.00	1.00	0.95	-	1.00	1.00	1.00	-	1.00	1.00	1.00	1.00	1.00	1.00	0.95	0.8	0.92	0.36	0.71	0.82
(N)	*15*	*17*	*17*	*15*	*14*	*11*	*9*	*16*	*18*	*20*	*20*	*20*	*19*	*14*	*9*	*20*	*18*	*13*	*17*	*12*	*12*	*6*	*20*	*18*	*9*	*18*	*20*	*20*	*10*	*12*	*14*	*14*	*11*
LAP-1																																	
100	1.00	-	-	-	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
93	-	-	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
90	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89		1.00	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(N)	*10*	*17*	*19*	*12*	*9*	*8*	*7*	*14*	*10*	*12*	*8*	*10*	*19*	*20*	*10*	*16*	*6*	*13*	*16*	*10*	*9*	*9*	*20*	*11*	*11*	*16*	*5*	*6*	*16*	*13*	*11*	*11*	*9*

Locus	C1	CCY1	D1	D2	CD1	CD2	CD3	CU1	CU2	CFP1	CFP2	CFP3	CM1	CM2	CM3	CM4	CB1	CB2	CB3	CB4	CB5	CB6	CB7	CBR1	CBR2	CBR3	CBR4	CBR5	CC1	CC2	CC3	CC4	CC5
LAP-2																																	
117	-	-	-	-	0.17	0.17	0.25	0.19	0.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100	1.00	1.00	1.00	1.00	0.83	0.83	0.75	0.81	0.81	1.00	1.00	1.00	1.00	-	1.00	1.00	1.00	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
94	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
(N)	*19*	*17*	*19*	*20*	*12*	*20*	*12*	*16*	*16*	*12*	*20*	*10*	*16*	*20*	*15*	*16*	*12*	*15*	*14*	*7*	*7*	*9*	*20*	*12*	*16*	*16*	*10*	*16*	*16*	*13*	*10*	*11*	*15*

TABLE 5. Genetic variability at 15 allozymic loci and morfological variability ($D2_m$ and ED) based on the morphometric analyses of 54 characters in 33 populations of nine taxa and one morphospecies of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum*, occurring in Northeastern and Southeastern Brazil. *N*= mean sample size per locus; genetic parameters - *A* = mean number of alleles per locus, *P*= percentage of polymorphic loci, H_o= observed and H_e= expected mean heterozygosity per locus (Nei, 1978; unbiased estimate); morphological parameters - $D2_m$= median of the Mahalanobis Generalized Distance of the individuals to the centroid of the population, ED= mean of the Euclidean distance between the individuals of the population. Standard deviations in parentheses. A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95. See Table 2 for the name of the populations.

Population	*N*	*A*	*P*	H_o	H_e	$D2_m$	ED
C. coriacea							
C1	15.6 (1.1)	1.3 (0.1)	33.3	0.092 (0.041)	0.010 (0.039)	8.98	-
C. depauperata							
D1	17.6 (0.7)	1.3 (0.1)	26.7	0.075 (0.034)	0.067 (0.029)	5.88	12.26
D2	17.0 (0.7)	1.3 (0.1)	13.3	0.040 (0.020)	0.038 (0.018)	6.40	10.62
C. cytisoides var. *blanchetii*							
CB1	16.7 (1.1)	1.2 (0.1)	13.3	0.036 (0.025)	0.032 (0.021)	9.34	18.11
CB2	16.3 (1.0)	1.2 (0.1)	0.0	0.011 (0.006)	0.011 (0.006)	12.19	19.37
CB3	14.4 (1.1)	1.3 (0.1)	6.7	0.026 (0.012)	0.025 (0.012)	5.91	14.37
CB4	12.3 (1.0)	1.2 (0.1)	13.3	0.033 (0.021)	0.031 (0.019)	15.88	21.37
CB5	13.6 (1.4)	1.2 (0.1)	13.3	0.042 (0.030)	0.037 (0.025)	5.94	15.55
CB6	11.0 (1.3)	1.1 (0.1)	6.7	0.015 (0.011)	0.014 (0.011)	6.73	13.52
CB7	16.1 (1.5)	1.3 (0.2)	13.3	0.030 (0.021)	0.028 (0.019)	17.47	24.28

Population	*N*	*A*	*P*	H_o	H_e	$D2_m$	ED
C. cytisoides var. *brachystachya*							
CBR1	15.9 (1.2)	1.3 (0.2)	26.7	0.066 (0.033)	0.058 (0.028)	38.13	32.47
CBR2	13.5 (1.3)	1.2 (0.1)	0.0	0.011 (0.006)	0.011 (0.006)	7.17	18.02
CBR3	14.5 (1.6)	1.1 (0.1)	0.0	0.010 (0.006)	0.010 (0.006)	17.76	16.24
CBR4	14.7 (1.5)	1.1 (0.1)	6.7	0.010 (0.007)	0.010 (0.007)	14.34	19.55
CBR5	14.8 (1.1)	1.3 (0.1)	20.0	0.033 (0.016)	0.032 (0.015)	20.70	21.44
C. cytisoides var. *confertiformis*							
CC1	11.1 (1.3)	1.3 (0.1)	13.3	0.021 (0.012)	0.043 (0.024)	5.33	12.81
CC2	10.6 (1.1)	1.2 (0.1)	13.3	0.020 (0.016)	0.029 (0.017)	4.25	16.38
CC3	13.1 (1.4)	1.2 (0.1)	13.3	0.017 (0.014)	0.048 (0.033)	3.35	13.88
CC4	12.3 (1.2)	1.3 (0.1)	13.3	0.022 (0.014)	0.049 (0.030)	5.75	12.46
CC5	11.6 (1.2)	1.3 (0.1)	20.0	0.032 (0.015)	0.052 (0.024)	8.47	16.01
C. cytisoides var. *cytisoides*							
CCY1	18.6 (0.4)	1.3 (0.2)	20.0	0.053 (0.029)	0.066 (0.038)	7.12	-
C. cytisoides var. *decora*							
CD1	13.3 (1.4)	1.3 (0.2)	13.3	0.049 (0.028)	0.045 (0.025)	13.61	20.71
CD2	12.3 (1.7)	1.3 (0.2)	13.3	0.056 (0.031)	0.050 (0.027)	11.93	18.62
CD3	10.8 (1.6)	1.3 (0.2)	13.3	0.068 (0.048)	0.058 (0.033)	11.47	16.85
C. cytisoides var. *micrantha*							
CM1	14.2 (1.6)	1.1 (0.1)	6.7	0.012 (0.0009)	0.012 (0.009)	10.89	17.05
CM2	12.3 (1.7)	1.2 (0.1)	13.3	0.013 (0.0009)	0.019 (0.010)	4.55	9.10
CM3	13.4 (1.4)	1.3 (0.2)	13.3	0.031 (0.021)	0.034 (0.023)	20.90	19.64
CM4	15.3 (1.4)	1.2 (0.1)	6.7	0.014 (0.008)	0.013 (0.008)	5.57	14.10
C. cytisoides “f. prostrata”							

Population	*N*	*A*	*P*	H_o	H_e	$D2_m$	ED
CFP1	13.3 (1.8)	1.3 (0.1)	20.0	0.034 (0.014)	0.034 (0.014)	5.38	15.38
CFP2	15.3 (1.8)	1.3 (0.1)	13.3	0.037 (0.024)	0.033 (0.021)	8.52	19.90
CFP3	13.9 (1.8)	1.3 (0.1)	13.3	0.040 (0.023)	0.036 (0.020)	8.54	18.02
C. cytisoides var. *unijuga*							
CU1	14.7 (1.3)	1.4 (0.2)	20.0	0.059 (0.029)	0.054 (0.026)	20.47	23.48
CU2	13.7 (1.5)	1.3 (0.2)	26.7	0.050 (0.027)	0.046 (0.023)	15.94	23.22

TABLE 6. *F* statistics (Wright, 1978) at 15 loci and A_{MRPP} from morphometric analysis in populations of seven taxa and one morphospecies of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* occurring in Northeastern and Southeastern Brazil.

Taxon	No. of Pops	F_{IS}	F_{ST}	A_{MRPP}
C. depauperata	2	-0.141	0.016	0.128
C. cytisoides var. *blanchetii*	7	-0.122	0.539	0.182
C. cytisoides var. *brachystachya*	5	-0.105	0.648	0.361
C. cytisoides var. *confertiformis*	5	0.476	0.109	0.335
C. cytisoides var. *decora*	3	-0.177	0.005	0.055
C. cytisoides var. *micrantha*	4	0.084	0.049	0.321
C. cytisoides "f. prostrata"	3	-0.111	0.035	0.070
C. cytisoides var. *unijuga*	2	-0.141	0.007	0.080

TABLE 7. Matrix of mean genetic similarity ("unbiased genetic identity"; Nei, 1978) among populations of *Chamecrista coriacea* (C), *C. cytisoides* var. *cytisoides* (CCY), *C. depauperata* (D), *C. cytisoides* var. *decora* (CD), *C. cytisoides* var. *unijuga* (CU), *C. cytisoides* "f. prostrata" (CFP), *C. cytisoides* var. *micrantha* (CM), *C. cytisoides* var. *blanchetii* (CB), *C. cytisoides* var. *brachystachya* (CBR) and *C. cytisoides* var. *confertiformis* (CC), occurring in Northeastern and Southeastern Brazil. Minimum and maximum values are in parenthesis.

Taxon	No. of pops.	C	CCY	D	CD	CU	CFP	CM	CB	CBR	CC
C	1	-									
CCY	1	0.78	-								
D	2	0.79 (0.79-0.79)	0.85 (0.85-0.85)	1.00							
CD	3	0.92 (0.92-0.92)	0.78 (0.78-0.78)	0.79 (0.78-0.79)	1.00 (1.00-1.00)						
CU	2	0.79 (0.79-0.79)	0.64 (0.64-0.64)	0.66 (0.65-0.66)	0.79 (0.79-0.79)	1.00					
CFP	3	0.80 (0.79-0.80)	0.65 (0.65-0.65)	0.67 (0.66-0.67)	0.87 (0.86-0.87)	0.86 (0.86-0.87)	0.99 (0.99-1.00)				
CM	4	0.79 (0.79-0.80)	0.65 (0.64-0.65)	0.66 (0.66-0.67)	0.86 (0.86-0.86)	0.86 (0.86-0.86)	1.00 (1.00-1.00)	1.00 (1.00-1.000)			
CB	7	0.81 (0.79-0.86)	0.67 (0.64-0.72)	0.68 (0.66-0.73)	0.88 (0.86-0.93)	0.84 (0.79-0.87)	0.98 (0.93-1.00)	0.98 (0.93-1.00)	0.97 (0.93-1.00)		
CBR	5	0.79 (0.79-0.80)	0.65 (0.64-0.65)	0.66 (0.66-0.67)	0.85 (0.80-0.87)	0.86 (0.86-0.86)	0.97 (0.86-1.00)	0.97 (0.96-1.00)	0.95 (0.79-1.00)	0.95 (0.87-1.00)	
CC	5	0.82 (0.80-0.85)	0.67 (0.66-0.70)	0.69 (0.67-0.72)	0.82 (0.80-0.85)	0.85 (0.83-0.86)	0.92 (0.90-0.93)	0.92 (0.90-0.93)	0.91 (0.87-0.93)	0.91 (0.84-0.93)	1.00 (0.98-1.00)

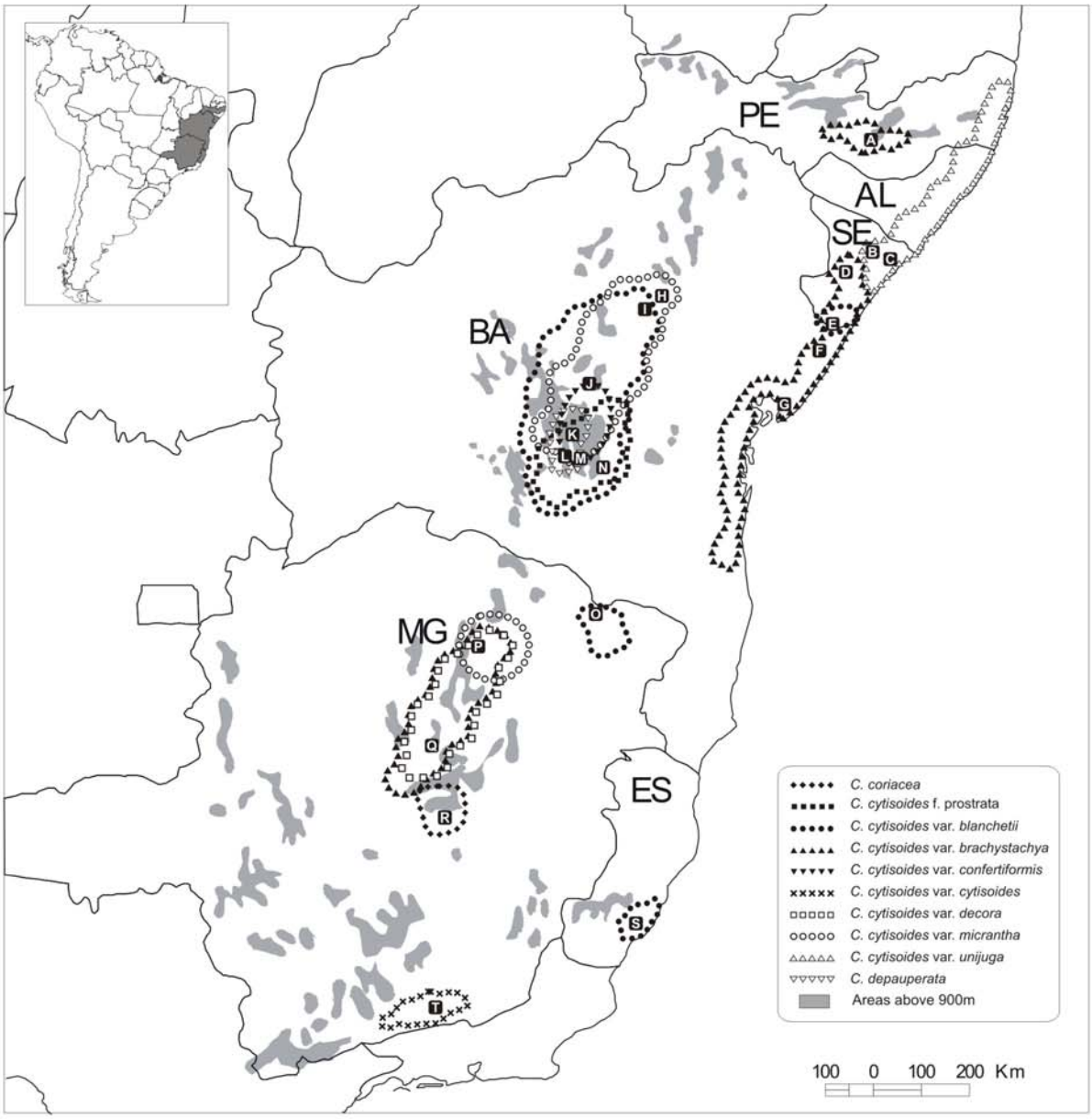

Fig. 1. Distribution map of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* based on field collection and herbarium material, and location of the populations studied: A, Buíque (CBR3); B, Santo Amaro das Brotas (CU1); C, Pirambu (CU2); D, Itabaiana (CBR5); E, Indiaroba (CB7); F, Esplanada (CBR1); G, Salvador (CBR4); H, Saúde (CM1); I, Jacobina (CB2); J, Lençóis (CC4); K, Mucugê (CB4, CB6, CC1, CC2, CC3, CC5, CM3, D2); L, Abaíra (CB3, CFP2, Cm4, D1); M, Ibicoara (CFP1); N, Barra da Estiva (CFP3); O, Pedra Azul (CB5); P, Grão Mogol (CD1, CM2); Q, Diamantina (CBR2, CD2, CD3); R, Conceição do Mato Dentro (C1); S, Guarapari (CB1); T, Sta. Bárbara M. Verde (CCY). See Table 2 for the names of the populations. Acronyms for Brazilian States: AL Alagoas; BA Bahia; ES Espírito Santo; MG Minas Gerais; PE Pernambuco; SE Sergipe.

Fig. 2. A, *Chamaecrista depauperata* (pop. D2); B, *C. coriacea* (C1); C, *C. cytisoides* var. *cytisoides* (CCY1); D, *C. cytisoides* var. *blanchetii* (CB2); E, *C. cytisoides* var. *brachystachya* (CBR5, population from coast); F, *C. cytisoides* var. *brachystachya* (CBR3, population from interior); G, *C. cytisoides* var. *confertiformis* (CC1); H, *C. cytisoides* var. *decora* (CD1); I, *C. cytisoides* var. *micrantha* (CM4); J, *C. cytisoides* var. *unijuga* (CU2); K, flower of *C. cytisoides* var. *blanchetii* (CB2); L, flower of *C. depauperata* (D2). See Table 2 for the names of the populations.

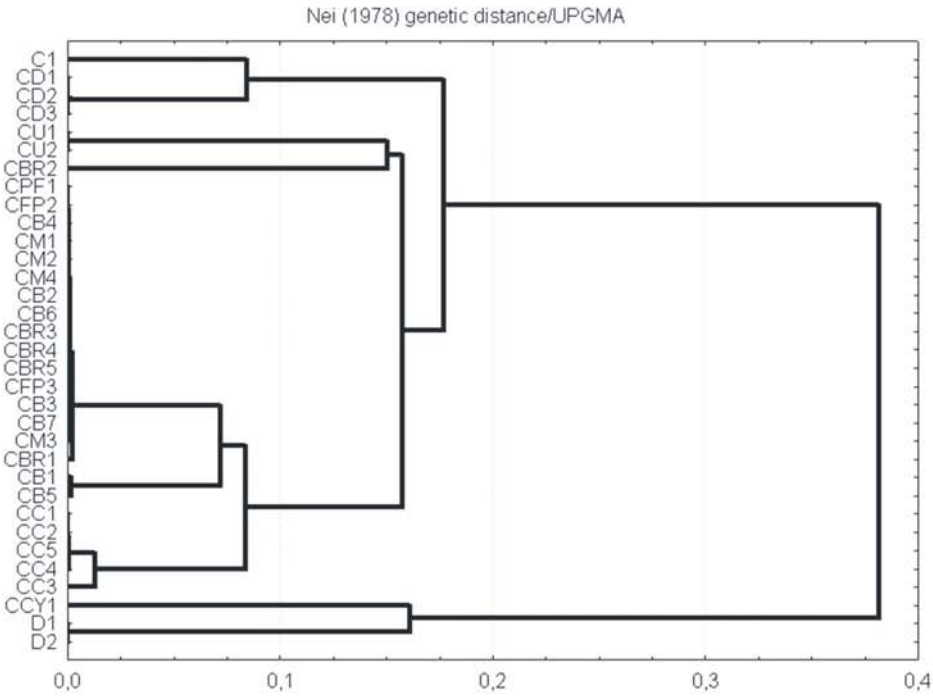

Fig. 3. Dendrogram showing the phenetic relationships among 33 populations of nine taxa and one morphospecies of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* occurring in Northeastern and Southeastern Brazil. Constructed using the matrix of genetic distances (Nei, 1978; unbiased estimate) based on 15 allozymic loci with UPGMA as clustering algorithm. See Table 2 for the names of the populations.

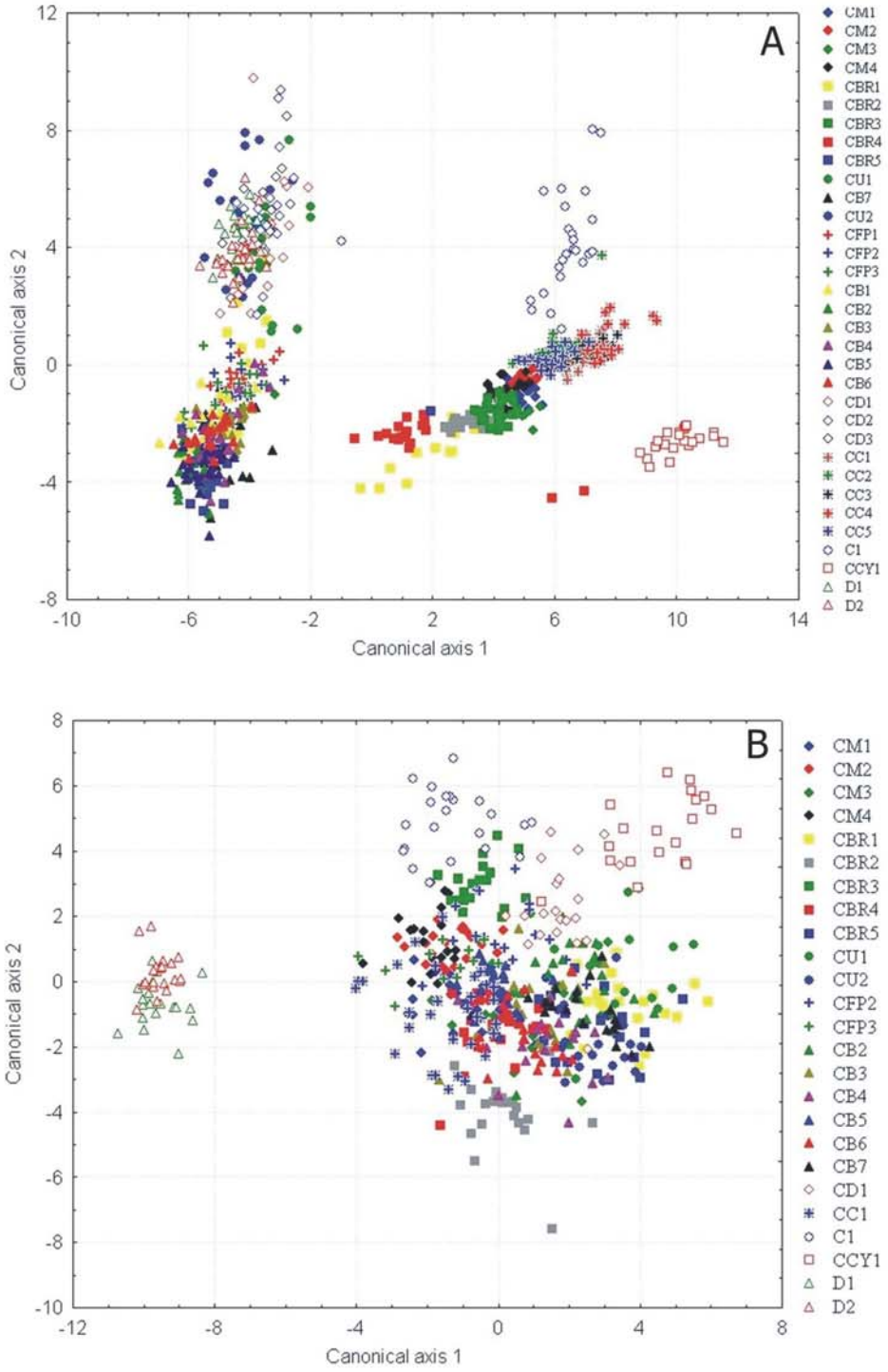

Fig. 4. Representation of the scores on the two first canonical axes of the CVA using 16 vegetative (A) and 38 floral (B) characters in 33 populations of nine taxa and one morphospecies of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* occurring in Northeastern and Southeastern Brazil. See Table 2 for the names of the populations.

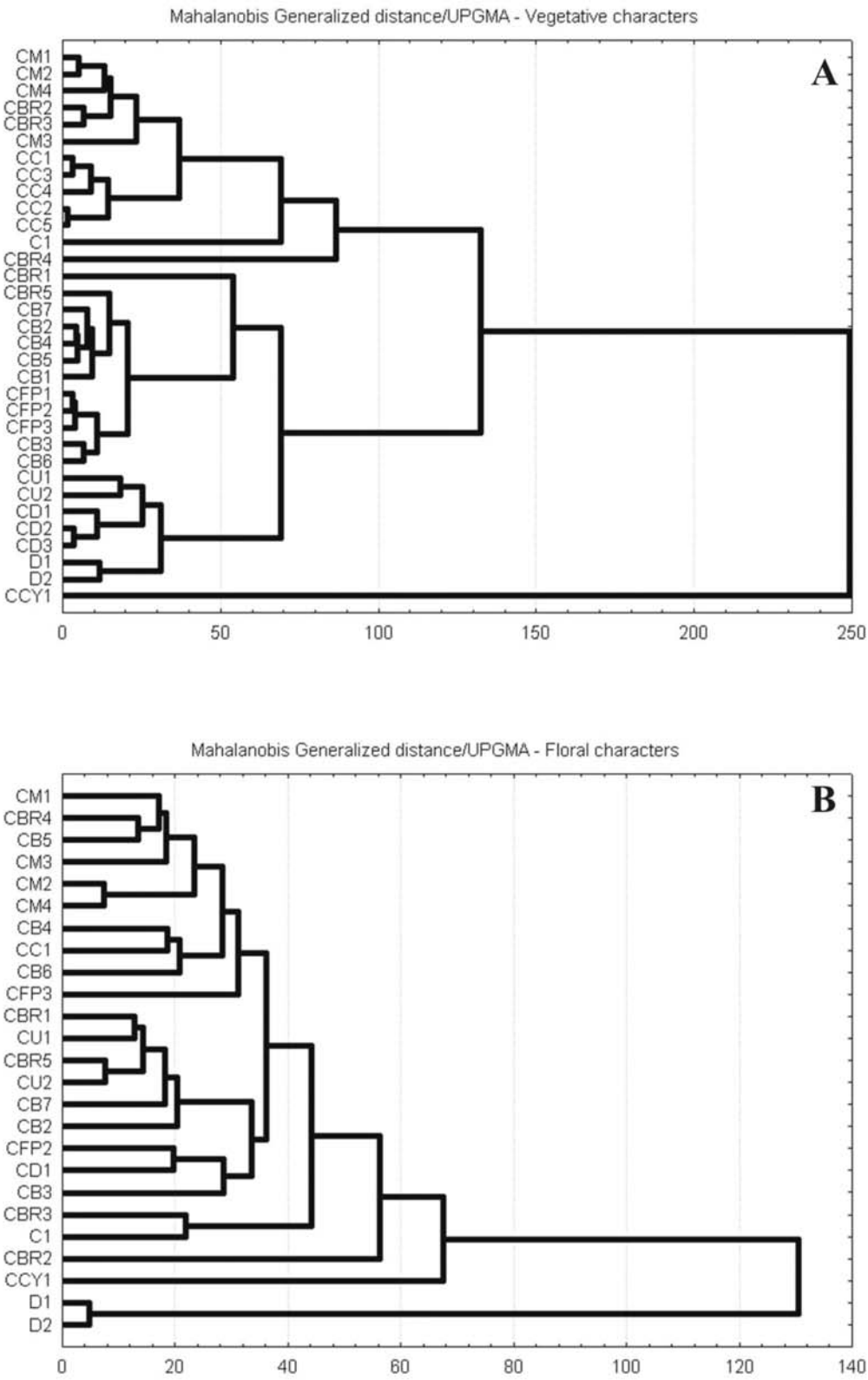

Fig. 5. Dendrogram showing the phenetic relationships among 33 populations of nine taxa and one morphospecies of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* occurring in Northeastern and Southeastern Brazil. Constructed using the matrix of Mahalanobis generalized distance based on 16 vegetative (A) and 38 floral (B) characters. See Table 2 for the names of the populations.

Capítulo 3

Natural hybrids in *Chamaecrista* sect. *Baseophyllum* (Leguminosae-Caesalpinioideae): genetic and morphological evidence

Este capítulo está formatado para publicação na revista Annals of Botany

RESUMO

Chamaecrista seção *Baseophyllum* (Leguminosae-Caesalpinioideae) inclui oito espécies com distribuição concentrada na Cadeia do Espinhaço nos estados da Bahia e Minas Gerais, leste do Brasil. A maioria das espécies ocorre em simpatria na Cadeia do Espinhaço, em áreas de campos rupestres. Durante o desenvolvimento da revisão taxonômica do grupo, foram encontrados indivíduos com morfologia intermediária entre três espécies simpátricas. Foram realizadas análises aloenzimática e morfométrica multivariada com o objetivo de avaliar a ocorrência de hibridação entre essas espécies, em quatro populações naturais e 14 supostos híbridos entre *C. confertiformis* e *C. depauperata* e entre *C. blanchetii* e *C. confertiformis*. Na análise de alozimas, todos os indivíduos apresentaram heterozigotos para alelos alternativos fixados em ambos supostos parentais (loci diagnósticos). Na análise de componentes principais os supostos híbridos ocuparam uma posição intermediária entre os supostos parentais, apresentando valores intermediários para quase todos os 11 caracteres analisados. Os resultados de ambas análises sustentaram a hipótese da ocorrência de hibridação natural entre as populações de *C. confertiformis* e *C. depauperata* e entre *C. blanchetii* e *C. confertiformis*. As análises indicaram que todos os indivíduos encontrados com morfologia intermediária entre as espécies são provavelmente híbridos F1. Os híbridos são formalmente descritos como *C. ×blanchetiformis* e *C. ×semprevivae.*

Natural hybrids in *Chamaecrista* sect. *Baseophyllum* (Leguminosae-Caesalpinioideae): genetic and morphological evidence

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Running title: Hybridization in *Chamaecrista* sect. *Baseophyllum* (Leguminosae)

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- *Background and aims Chamaecrista* sect. *Absus* subsect. *Baseophyllum* (Leguminosae-Caesalpinioideae) includes eight species with geographic distributions concentrated in the Cadeia do Espinhaço mountain range of the States of Bahia and Minas Gerais, in eastern Brazil. The majority of the species occur sympatrically in the Cadeia do Espinhaço, in areas of "campo rupestre" vegetation. During the taxonomic revision of this group, individuals were encountered with r with morphology intermediate between three sympatric species.
- *Methods* Allozyme and multivariate morphometric analyses were carried out to investigate the occurrence of hybridization between these species in four populations and 14 suspected hybrids between *C. confertiformis* and *C. depauperata* and between *C. blanchetii* and *C. confertiformis*. In the allozyme analysis all the individuals were heterozygous for the alleles fixed in the two suspected parents (the diagnostic loci). In the principal components analysis the suspected hybrids were intermediate between the suspected parents, and had intermediate values for almost all of the 11 characters analysed.
- *Key Results* The results of both analyses support the occurrence of natural hybridization between the studied populations of *C. confertiformis* and *C. depauperata* and between *C. blanchetii* and and *C. confertiformis*.
- *Conclusions* The analyses indicate that all the individuals with morphology intermediate between the species are probably F1 hybrids. The hybrids are formally described as *C. ×blanchetiformis* and *C. ×semprevivae.*

KEY-WORDS: allozymes, Cadeia do Espinhaço, Caesalpinioideae, campos rupestres, *Chamaecrista*, Chapada Diamantina, Espinhaço range, hybridization, Leguminosae, morphometrics.

INTRODUCTION

The process of hybridization is one of the most important phenomena in the evolution of plants because it stimulates genetic recombination and hence increases the level of variability within taxa (Grant, 1981; Rieseberg, 1997; Rieseberg and Carney, 1998; Barton, 2001). Rieseberg (1997) showed that natural hybridization occurs in about 34% of plant families, with 16% of genera recorded as having one or more records of natural hybrids. Hybridization in the family Leguminosae is recorded in genera from unrelated clades such as the Cercideae (*Bauhinia*, Lau *et al.,* 2005), Caesalpinieae (*Parkinsonia* e *Cercidium*, Hawkins *et al.,* 1999), Thermopsideae (*Baptisia*, Alson and Turner, 1963) and Vicieae (*Lathyrus*, Hammett *et al.,* 1994). However, for the genera of subtribe Cassiinae (*Cassia*, *Chamaecrista* e *Senna*) there is no previous record of natural hybrids, although the variation patterns observed in various species complexes suggest its existence (Irwin & Barneby 1982).

Chamaecrista Moench is one of the largest genera of subfamily Caesalpinioideae and includes about 330 espécies distributed widely in the Americas, Africa and Asia (Lewis, 2005). *Chamaecrista* sect. *Baseophyllum* includes eight species, geographically concentrated in the Cadeia do Espinhaço mountain range in the States of Bahia and Minas Gerais, usually in campo rupestre vegetation and occasionally in cerrado, restinga and caatinga. *Chamaecrista* sect. *Baseophyllum* has a confused taxonomic history due to the different delimitations proposed for taxa of the *C. cytisoides* complex. Bentham (1840, 1870, 1871) treated these taxa as independent species. Later, Irwin & Barneby (1978, 1982) considered these species as varieties of *C. cytisoides*. The majority of the species of sect. *Baseophyllum* occur sympatrically in the Cadeia do Espinhaço, in areas of campo rupestre. Some of them flower simultaneously for certain periods and share the same

pollinators and are occasionally inter-compatible, characteristics which favour the occurrence of natural hybridization (C.B.N.Costa *et al.,* unpubl.). Thus it may be hypothesized that the taxonomic difficulties in the delimitation of the taxa of the *C. cytisoides* complex are the consequence, at least in part, of natural hybridization between the species of this group (Conceição *et al.*, in press).

Conceição *et al.* (in press) carried out a taxonomic revision of sect. *Baseophyllum* using morphometric and allozyme analyses. In this study, individuals were found with morphology intermediate between sympatric species in the municipality of Mucugê, Bahia state, specifically between *C. confertiformis* (H.S.Irwin & Barneby) Conc. & L.P.Queiroz and *C. depauperata* Conc., L.P.Queiroz & G.P.Lewis and between *C. blanchetii* (Benth.) Conc. & L.P.Queiroz and *C. confertiformis* (H.S.Irwin & Barneby) Conc. & L.P.Queiroz, which led us to suspect them to be natural hybrids. Once the allozyme study had detected diagnostic loci between the suspected parental species, further allozyme studies and morphometric analyses were carried out with the specific objective of evaluating the suspected occurrence of hybridization between these species in this locality.

MATERIALS AND METHODS

Populations sampled

We collected 101 individuals from four natural populations of three species of *Chamaecrista* at two localities in Mucugê, Chapada Diamantina, Northeast Brazil and 14 supposed natural hybrids between those species (Table 1). *C. blanchetii* (one population, Fig. 1D), *C. confertiformis* (two populations, Fig. 1A,F), *C. depauperata* (one population,

Fig. 1C). Vouchers for each population are deposited in the herbarium of the Universidade Estadual de Feira de Santana (HUEFS).

Allozyme analysis

Small sections of leaf tissue were crushed in 0.5 mL of grinding buffer (100mL Tris-HCL 0.1 mol L^{-1} pH 7.0, 6.846 g sucrose, 0.6 g PVP [polyvinylpyrrolidine], 0.0292 g EDTA [ethylenediaminetetraacetic acid], 0.145 g BSA [bovine albumin], 0.13 g DIECA [sodium diethylcarbamate], 0.6 g borax, and 100 μL β- mercaptoethanol; modified from Sun and Ganders, 1990). Extracts were absorbed in 1.0 x 0.3 cm Whatman number 3 paper wicks, which were loaded into 8.5% starch gels (Sigma hydrolyzed potato starch).

For the electrodes and gels two buffer systems were used: 1) electrode: lithium hydroxide 0.05 mol L^{-1} boric acid 0.0935 mol L^{-1} EDTA 0.0059 mol L^{-1} pH 8.0; gel electrode solution diluted 1:10; modified from Ridgway *et al*. (1970); 2) electrode: boric acid 0.3 mol/L, NaOH 0.06 mol/L, pH 8.0; gel: Tris 0.01 mol/L, pH 8.5; modified from Shaw and Prasad (1970).

Standard horizontal electrophoresis was performed until the inner marker (bromophenol blue) reached 9 cm from the application site using the following running conditions: systems 1 – 25 mA; system 2 – 15 mA. Two enzyme systems were used which had been shown previously to be diagnostic for the species. System 1 was used for phosphoglucomutase (PGM; EC 2.7.5.1), diagnostic for *C. depauperata* and *C. confertiformis* and system 2 was used for phosphoglucose isomerase (PGI; EC 5.3.1.9) diagnostic for the previous species pair and for *C. blanchetii* e *C. confertiformis*. The staining procedures were similar to but slightly adjusted from Corrias *et al*. (1991; PGI) and Soltis *et al*. (1983; PGM). Modifications were mainly in the amounts of the components used; the exact recipes can be obtained on request. Enzymatic systems

showing more than one locus were numbered in ascending order from the locus with lowest mobility. The alleles were numbered according their mobility relative to the allele with highest mobility of a standard individual present in all gels and designated as 100.

Morphometric analysis

A multivariate morphometric analysis was carried out using the same individuals sampled for the allozyme analysis; twelve vegetative characters were measured with a caliper (Table 2). The leaflets pair in bifoliolate leaves was considered homologous to the second leaflet pair in tetrafoliolate leaves, based on the morphology and position of the foliar nectaries. A principal component analysis (PCA) was carried out based on the correlation matrix of the twelve variables using STATISTICA 6.0 (StatSoft, 2003).

RESULTS

Allozyme analysis

Population CC3 of *C. confertiformis* had fixed alleles at loci PGI-1 (130), PGM-1 (100) and PGM-2 (100), and population D2 of *C. depauperata* also had alternative fixed alleles at loci PGI-1 (140), PGM-1 (83) and PGM-2 (81), and thus these were loci diagnostic for these populations (Figure 2). The two indivduals to be hybrid between the two species, and occurring sympatrically with these populations, were shown to be heterozygous for the alleles in the three loci of the putative parent species.

Population CC1 of *C. confertiformis* had allele 130 fixed for locus PGI-1, while population CB4 of *C. blanchetii* had allele 100 fixed for this locus, thus comprising a locus

diagnostic for these populations (Figure 2). All twelve individuals to be hybrids between these two species, occurring sympatrically with the two populations, were heterozygous for the two alleles observed in the putative parental species.

Morphometric analysis

In a principal component analysis of population CC3 of *C. confertiformis*, population D2 of *C. depauperata* and the two suspected hybrid individuals, the three entities were separated on the first axis, with the putative hybrids occupying an intermediate position between the suspected parents (Figure 3A). Most of the variation was explained by the first axis (85.3%), and only 4.4 % by the second. All the variables showed a similarly high correlation with the first axis, variables #1, 10 and 11 being negatively correlated and the rest positively. On the second axis, variables #1 and 11 were the most important, and were negatively correlated with the axis. All morphological characters used can be considered diagnostic for the two populations, with the two putative hybrid individuals showing intermediate values in almost all characters (Table 2). Character overlap between the two species occurred only in leaflet length. The large difference in observed leaflet width reflects the distinctly different leaflet shapes of the two species, being as long as wide in *C. depauperata* and about four times longer than wide in *C. confertiformis*; the suspected hybrids had intermediate leaflet shape and size.

Similar results were obtained in the PCA of population CC1 of *C. confertiformis* and CB4 of *C. blanchetii* with 12 suspected hybrid individuals (Figure 2B). The first two axes explained 85.5% of total variation, of which 76.6% was concentrated in the first axis. The two taxa were separated on the first axis, with the hybrids in an intermediate position on the first axis and separated from the parent sepcies on the second. All the variables contributed to a similar degree in the partition of variation on the first axis, except variable

#1, which was mainly correlated positively with the second axis. Variables #8 and 10 correlated negatively with the first axis, and the rest positively. All the variables, except variable #1, were diagnostic for the two species and the hybrids had intermediate values in all cases except variables #10 and 11, for which they had values identical to those of *C. blanchetii*, which is due to the joint possession of a pair of leaflets (Table 2). As with *C. depauperata*, *C. blanchetii* also has leaflets as long as wide. As in the previous case, the hybrids also have intermediate leaflet shape, although some individuals have leaflets larger than those of either parent, which is the reason they are separated on the second PCA axis.

DISCUSSION

The results of the allozyme and morphometric analyses confirmed the occurrence of natural hybridization between the populations of *C. blanchetii* and *C. confertiformis* and between *C. confertiformis* and *C. depauperata*. The allozyme analysis indicated that all the individuals found with intermediate morphology are possibly F1 hybrids because they are also heterozygous for alleles which are fixed in the parents. Theses individuals also were shown to be intermediate in almost all morphological characters used, which strengthens the hypothesis that they are first generation hybrids, which usually show intermediate characters in respect of their parents (e.g., Rieseberg and Elstrand, 1993; Knyasev *et al.,* 2000; Stecconi *et al.,* 2004).

Chamaecrista confertiformis and *C. depauperata* occur syntopically in areas of campo rupestre vegetation. These species are morphologically very distinct and can be easily differentiated since *C. confertiformis* is a shrub or small tree up to 7 m, with shortly petiolate leaves having two pairs of semi-succulent, oblong to oblong-lanceolate leaflets,

with 3-5 palmate main veins and an extrafloral nectary situated below the distal leaflet pair, while C. *depauperata* is a procumbent to prostrate subshrub up to 20 cm, with petiolate leaves, a single pair of chartaceous orbicular to suborbicular leaflets, with 6-9 palmate main veins and an extrafloral nectary situated on the petiole. The natural hybrid of *C. confertiformis* and *C. depauperata* can be differentiated from the two parental species as a shrub up to 2.5 m, with shortly petiolate leaves with a single pair of semi-succulent, oblong-lanceolate leaflets with ca. 4 palmate main veins and a nectary located between the leaflet pair.

Chamaecrista confertiformis occurs sympatrically with *C. blanchetii* in the Municipal Park of Mucugê, in areas of campo rupestre vegetation. *Chamaecrista blanchetii* can be distinguished from *C. confertiformis* as a shrub or small tree to 3.5 m, with sessile to shortly petiolate leaves with a single pair of coriaceous, reniform to sub-orbicular leaflets, with 5-9 palmate main veins and an extrafloral nectary situated betweenthe leaflet pair. The natural hybrid of *C. confertiformis* and *C. blanchetii* differs from its parents in being a subshrub to 2 m, with sessile to shortly petiolate leaves, with one to two pairs of semi-succulent, sub-orbicular leaflets with 4-6 palmate main veins and an extrafloral nectary located on the rachis, varying in position below the distal leaflet pair, or between the leaflet pair when the leaves have only a single leaflet pair.

Hybridization can have various evolutionary consequences such as increase in interspecific genetic diversity, origin and transfer of genetic adaptations, and can lead to the formation of new species (Rieseberg, 1997). On the other hand, hybrization and introgression can inhibit the development of pure populations, leading to the extinction of species and compromising the continued existence of rare species (Levin *et al.*, 1996; Rhymer and Symberloff, 1996). Interspecific hybridization is very frequent between species which occur sympatrically (Rieseberg, 1997; Rieseberg and Carney, 1998; Grant,

1981), with the frequent formation and establishment of hybrids which vary considerably between families and genera (Whittemore and Schaal, 1991; Wolfe and Elisens, 1994; Milene *et al.,* 1999; Knyasev *et al.,* 2000; Church and Taylor, 2005; Houghton-Thompson *et al.,* 2005), recorded in Leguminosae by the formation of hybrids in various genera such as *Baptisia*, *Bauhinia* and *Lathyrus*, and even intergeneric, such as *Parkinsonia aculeata* × *Cercidium praecox* (Alson and Turner, 1963; Hammett *et al.,* 1994; Hawkins *et al.,* 1999; Lau *et al.,* 2005).

The hybrids found between the species studied here are rarely observed in nature. This rarity is probably due to the small overlap in flowering period or complete separation of flowering period in certain years (C.B.N. Costa *et al.*, unpubl.). Whatever the full explanation, this rarity of hybrids in comparison to their parents is not reflected in herbarium collections, which in most cases are limited to just a few specimens of each taxon, giving the false impression of continuity of characters between distinct taxonomic entities.

From the taxonomic point of view, the presence of rare hybrids within natural populations can cause problems for the delimitation of taxa. The taxonomic problems in section *Baseophyllum* could possibly be related to the occurrence of natural hybrids between species of this group and the very conserved floral morphology of these species (Conceição *et al.*, in press). These factors probably influenced the decision of Irwin & Barneby (1978, 1982) to treat the five species of section *Baseophyllum* as varieties of *C. cytisoides*: *C. blanchetii*, *C. brachystachya* (Benth.) Conc. & L.P.Queiroz, *C. decora* (H.S.Irwin & Barneby) Conc. & L.P.Queiroz, *C. confertiformis* (H.S.Irwin & Barneby) Conc. & L.P.Queiroz e *C. unijuga* (Benth.) Conc. & L.P.Queiroz (Conceição *et al.,* in prep.). With the object of avoiding future taxonomic problems, we formally describe here the natural hybrids found in these species of *Chamaecrista* sect. *Baseophyllum*.

Chamaecrista ×*blanchetiformis* Conc. & L.P.Queiroz [*C. blanchetii* (Benth.) Conc. & L.P.Queiroz × *C. confertiformis* (H.S.Irwin & Barneby) Conc. & L.P.Queiroz], nothosp. nov.

Type: Brasil, Bahia, Chapada Diamantina, município de Mucugê, Projeto Sempre Viva, 12°59'33,5"S; 41°20'27,8"W, 14 Nov. 2003, Conceição *et al*. 765 (holotype: HUEFS, isotype: K).

Diagnosis: planta inter *C. blanchetii* et *C. confertiformis* intermedia, foliis uni-bijugatis, foliolis 4-6-nervatis, suborbiculatis, folio nectario uno in rachidi diverse posito differt.

Description: Shrub to 2 m alt. Leaves1,8-3,8 cm long., petiole 0-2 mm, extrafloral nectary 1, located on the rachis, below the distal pair of leaflets, in varied positions or even between the leaflet pairs, veins 4-6, palmate, leaflets 1-2 pairs, semi-succulent, sub-orbicular, 16-36 x 13-23 mm, longest distance from margin to the main nervure, at leaflet base 3-8 mm, smallest distance from the margin to the main nervure, at leaflet base, 2-4 mm.

Chamaecrista ×*semprevivae* Conc. & L.P.Queiroz [*C. confertiformis* (H.S.Irwin & Barneby) Conc. & L.P.Queiroz × *C. depauperata* Conc., L.P.Queiroz & G.P.Lewis], nothosp. nov.

Type: Brasil, Bahia, Chapada Diamantina, município de Mucugê, Paty, Gerais do Rio Preto, 12°46'15,7"S; 41°29'29,3"W, 2. Feb. 2005, Conceição *et al*. 916 (holotype: HUEFS, isotype: K).

Diagnosis: planta inter *C. confertiformis* et *C. depauperata* intermedia quoad formam foliolorum cum *C. confertiformi* et quoad folia unijugata cum *C. depauperata* sat

congruens, ab hac foliolis oblongo-lanceolatis, frutice habito (non ut *C. depauperatae* foliolis orbicularis et habitu prostrato) et ab illa foliis unijugatis differt (in *C. confertiformi* foliis bijugatis).

Description: Shrub to 2.5 m tall. Leaves 2.5-2.7 cm long., petiole c. 1 mm, extrafloral nectary 1, located between the leaflet pair, one leaflet pair, with ca. 4 palmate veins, semi-succulent, oblong-lanceolate, 24-26 x 12-13 mm, longest distance from margin to the main nervure, at leaflet base 3 mm, smallest distance from the margin to the main vein, at leaflet base, c. 1 mm.

ACNOWLEDGEMENTS

We thank the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) for financial support (#245/04), Ana C. S. Pereira and Sabrina M. Lambert for helping in laboratory and Symon Mayo kindly translated the text to English. ASC received a fellowship from the Universidade do Estado da Bahia (PAC-UNEB). ELB and LPQ are supported by a grant (PQ2) from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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Table 1. Populations and number of individuals of *Chamaecrista depauperata*, *C. blanchetii*, *C. confertiformis* and suspected hybrids *C. depauperata* × *C. confertiformis* and *C. blanchetii* × *C. confertiformis* occurring in the municipality of Mucugê, Chapada Diamantina, Northeast Brazil, used in this study. Vouchers are deposited in the herbarium of the Universidade Estadual de Feira de Santana (HUEFS). The population names correspond to those of the populations used in Conceição *et al.* (in press).

Population	Localities		Individuals	Voucher
C. depauperata				
D2	Paty-Gerais do Rio Preto	12°45’49,1”S; 41°30’05”W	20	A.S.Conceição 863
C. blanchetii				
CB4	Projeto Sempre Viva	12°59’30”S; 41°20’30”W	26	A.S.Conceição 759
C. confertiformis				
CC1	Projeto Sempre Viva	12°59’33,5”S; 41°20’27,8”W	31	A.S.Conceição 755
CC3	Paty-Gerais do Rio Preto	12°46’15,7”S; 41°29’29,3”W	24	A.S.Conceição 864
supposed hydrid				
D2 × CC3	Paty-Gerais do Rio Preto	12°46’15,7”S; 41°29’29,3”W	2	A.S.Conceição 916
CB4 × CC1	Projeto Sempre Viva	12°59’33,5”S; 41°20’27,8”W	12	A.S.Conceição 765

Table 2. Characters used in the morphometric analysis of populations of *Chamaecrista depauperata* (D2), *C. blanchetii* (CB4), *C. confertiformis* (CC1 e CC3) and suspected hybrids *C. depauperata* × *C. confertiformis* (D2 × CC3) and *C. blanchetii* × *C. confertiformis* (CB4 × CC1) occurring in the municipality of Mucugê, Chapada Diamantina, Northeast Brazil.

Characteres	CC3	D2 × CC3	D2	CC1	CB4 × CC1	CB4
1- Length of leaflets	19-35	24-26	14-22	15-33	16-36	18-31
2- Maximum width of leaflet	4-9	12-13	15-25	4-10	13-23	20-44
3- Width of leaflet at 1/3 of its length	3-7	11-12	14-24	3-8	10-20	17-44
4- Width of leaflet at 2/3	4-9	12-13	12-23	4-10	12-23	19-41
5- Smallest distance from margin to the main nervure, at leaflet base	1-1,1	1-1	2-3	1-2	2-4	2-18
6- Longest distance from margin to the main nervure at leaflet base	2-2,1	3-3	5-11	1-3	3-8	7-25
7- Apex angle of the leaflet	82-114	121-124	138-161	72-115	116-155	138-175
8- Length of the petiole	2-3	1-1	6-15	1-3	0-2	1-1,1
9- Number of main veins	3-4	4-4	6-9	3-5	4-6	5-8
10- Length of the rachis	6-12	0-0	0-0	5-12	0-0	0-0
11- Distance of the nectary to the second par of leaflets	-1-0	0-0	-4- -2	-3- -1	0-0	0-0

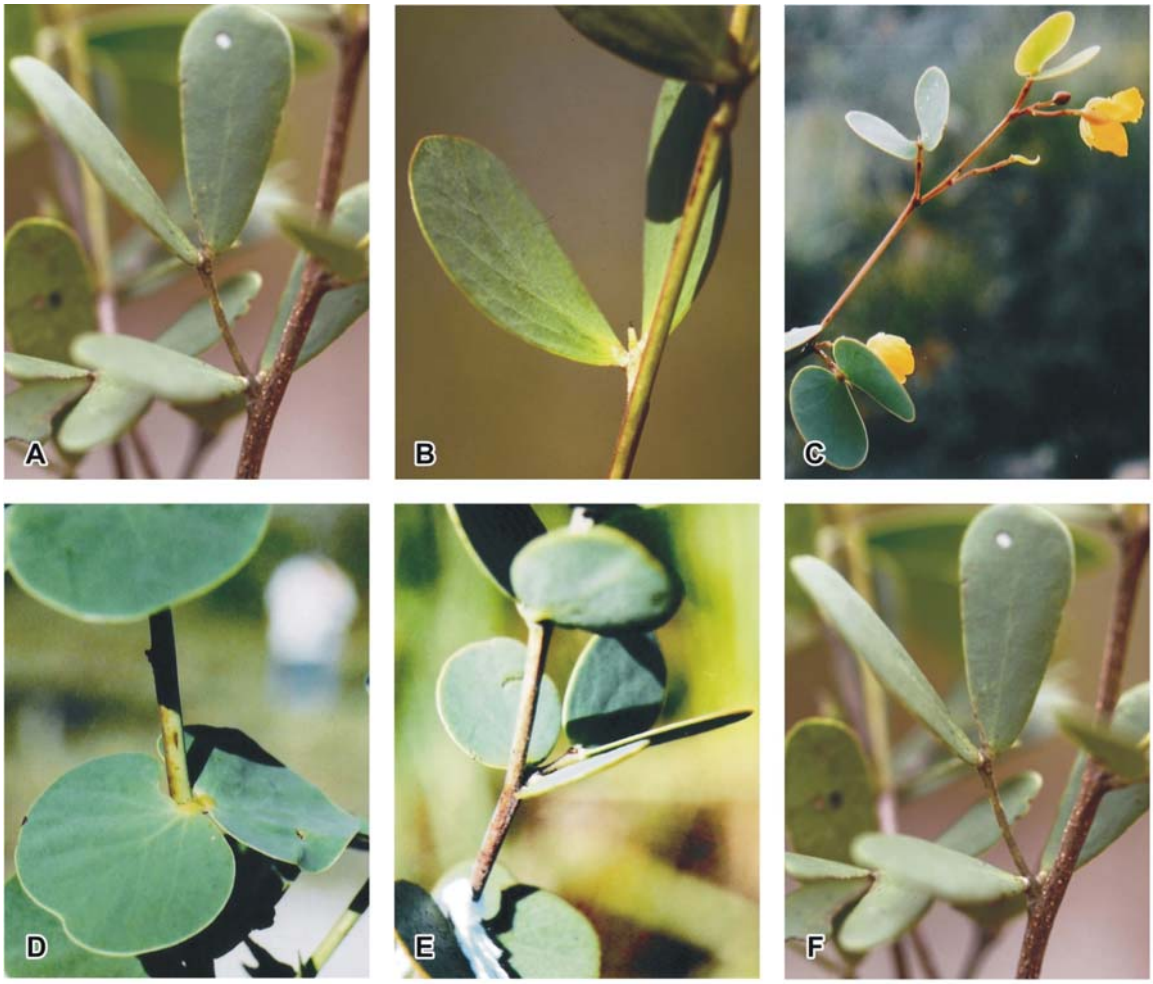

Fig. 1. A, F, *Chamaecrista confertiformis* (pop. CC3, CC1); B, *C.* ×*semprevivae* (D2 × CC3); C, *C. depauperata* (D2); D, *C. blanchetii* (CB2); E, *C.* ×*blanchetiformis* (CB4 × CC1).

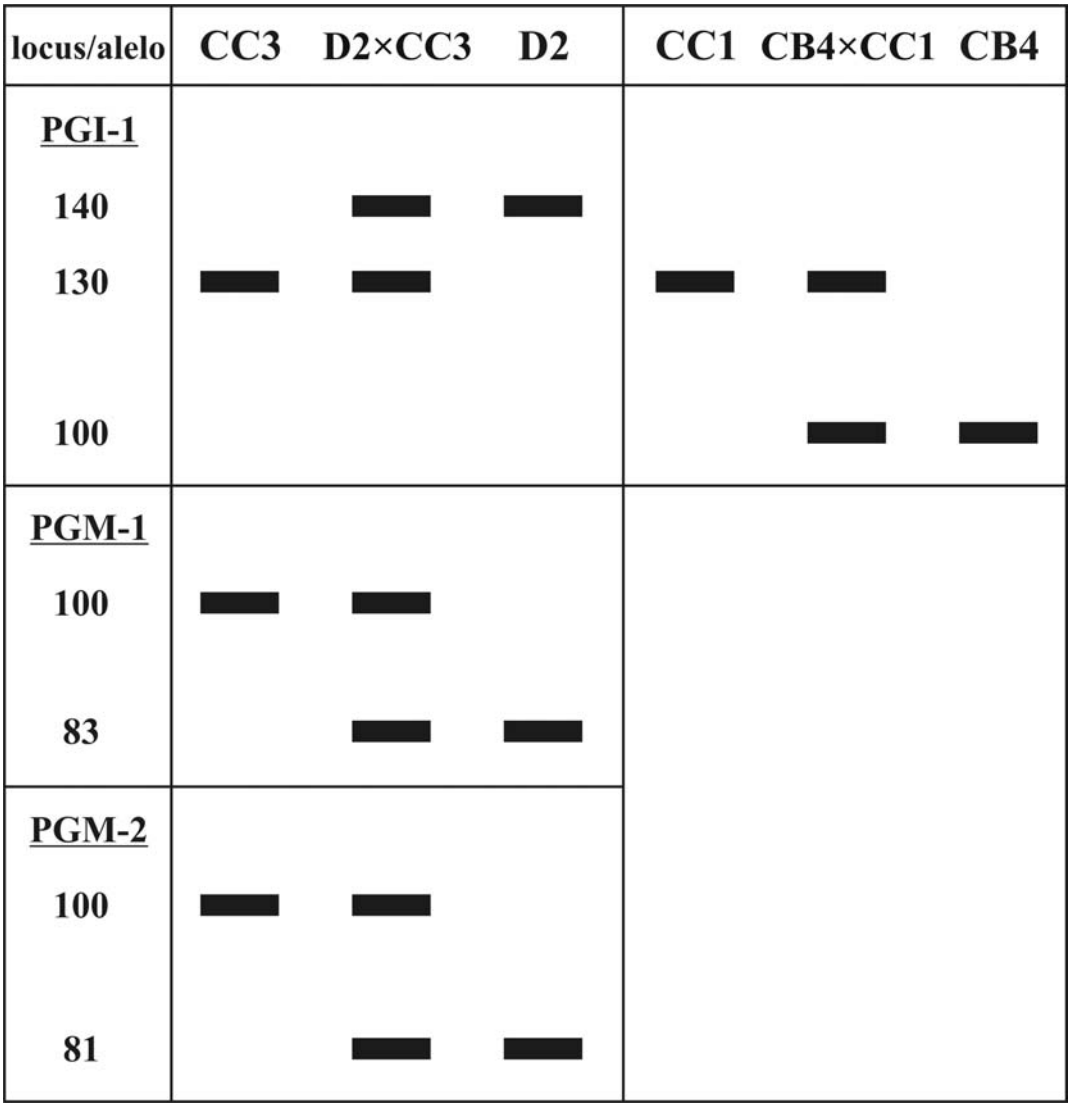

Fig. 2. Allozyme diagram of the alleles found in the enzymes phosphoglucose isomerase (PGI) and phosphoglucose mutase (PGM) in populations of *Chamaecrista depauperata* (D2), *C. blanchetii* (CB4), *C. confertiformis* (CC1 and CC3) and suspected hybrids *C. depauperata* × *C. confertiformis* (D2 × CC3) e *C. blanchetii* × *C. confertiformis* (CB4 × CC1) occurring in the municipality of Mucugê, Chapada Diamantina, Northeast Brazil.

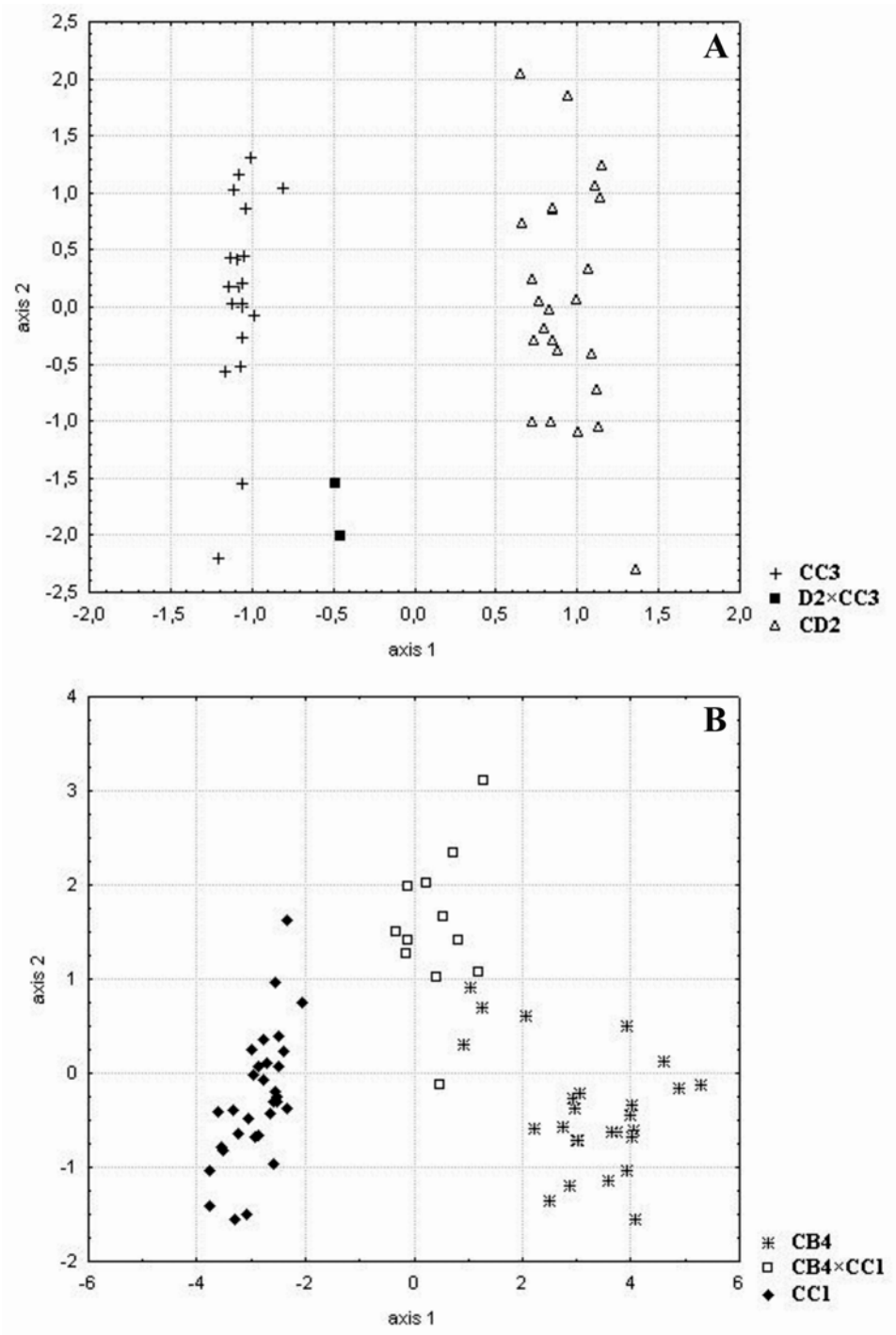

Fig. 3. Ordination scatterplot of the first two axes of a principal component analysis (PCA) based on the correlation matrix of 11 vegetative morphological characters of individuals of *Chamaecrista depauperata* (D2), *C. blanchetii* (CB4), *C. confertiformis* (CC1 and CC3) and suspected hybrids *C. depauperata* × *C. confertiformis* (D2 × CC3) and *C. blanchetii* × *C. confertiformis* (CB4 × CC1) occurring in the municipality of Mucugê, Chapada Diamantina, Northeast Brazil. Percentage variation explained by each axis: A = 89,8% (eixo 1 = 85,3%; eixo 2 = 4,4%); B = 85,5% (eixo 1 = 76,6%; eixo 2 = 8,9%).

Capítulo 4

Taxonomic re-evaluation *Chamaecrista* section *Baseophyllum* (Leguminosae-Casalpinioideae)

Este capítulo está formatado para publicação na revista Kew Bullletin

RESUMO

Chamaecrista Moench é um grupo segregado de *Cassia* L. e inclui espécies com pedicelo bi-bracteolado, androceu actinomórfico, frutos elasticamente deiscentes e nectários extraflorais, quando presente em forma de disco ou taça. O gênero inclui seis seções, com ca. 330 espécies distribuídas principalmente no Novo Mundo. *Chamaecrista* subseção *Baseophyllum* tem uma história taxonômica confusa, com alguns táxons sendo tratados com espécies independentes (Bentham 1840, 1870, 1871) e, posteriormente, como variedades (Irwin & Barneby 1978, 1982). O trabalho de revisão foi realizado com base em extensivo trabalho de campo, análises de materiais-tipo e de espécimes dos herbários onde se encontravam importantes coleções do grupo e em resultados de análises de variabilidade genética e morfológica. Com base nos dados das análises filogenéticas, estamos propondo elevar a subsect. *Baseophyllum* ao nível de seção. Estamos reconhecendo oito espécies para a seção, baseados em dados morfológicos e genéticos. São fornecidas chave de identificação, descrições para as espécies, dados de distribuição geográfica e discussão sobre caracteres diagnósticos.

Taxonomic re-evaluation *Chamaecrista* section *Baseophyllum* (Leguminosae-Casalpinioideae)

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Summary. A taxonomic survey of the *Baseophyllum* group is presented. Is reinstated the rank of section for *Baseophyllum* group. A total of eight species is recognised, five new combinations are proposed. Descriptions are presented. The work presents morphologic descriptions for *Baseophyllum* and their species. Illustrations and identification key are provided.

Key words. Caesalpinioideae, *Chamaecrista*, *Baseophyllum*, taxonomy.

Introduction

Chamaecrista Moench, in its modern concept, is a genus segregated from *Cassia* L. by Irwin & Barneby (1981), who widened circumscription to include species with pedicels bibracteolate near or above the middle, androecium actinomorphic, pods elastically dehiscent and extrafloral nectaries, when present, disc- or cup-shaped. The genus includes about 330 species mainly for the New World (Lewis 2005).

The current classification of *Chamaecrista* is based on the proposition of Irwin & Barneby (1982), who recognised six sections: *Chamaecrista* sect. *Apoucouita* (Benth.) H.S.Irwin & Barneby; *Chamaecrista* sect. *Absus* (Collad.) H.S.Irwin & Barneby; *Chamaecrista* sect. *Grimaldia* (Schrank) H.S.Irwin & Barneby; *Chamaecrista* sect. *Chamaecrista*; *Chamaecrista* sect. *Caliciopsis* H.S.Irwin & Barneby and *Chamaecrista* sect. *Xerocalyx* (Benth.) H.S.Irwin & Barneby. Section *Absus* groups species mostly shrubby with an expanded terminal raceme. Within this section, Irwin & Barneby (1978) recognized four subsections (under *Cassia*) one of which is the subsection *Baseophyllum*, characterized by the presence of scutellate glands on leaf stalks and inflorescence axis and leaflets strongly asymmetric at base.

Phylogenetic studies on *Chamaecrista* based on combined analyses of plastid and nuclear DNA sequences supported the monophyly of the *Baseophyllum* group but reject its inclusion in sect. *Absus*. In that analyses, the *Baseophyllum* group appears as sister to a clade bringing together species of the sections *Chamaecrista*, *Caliciopsis*, and *Xerocalyx* with 84 % of bootstrap support (parsimony analysis) and 100 % of posterior probability (Bayesian analysis; Conceição *et al*, *in prep.* a). For this reason, we are reinstated the rank of section for this group following the first proposition of Colladon (1816) under the genus *Cassia*.

Section *Baseophyllum* has a tortuous taxonomic history mostly because of distinct views about the treatment of the variation pattern found in the taxa related to *Chamaecrista cytisoides* (Collad.) H.S.Irwin & Barneby. Bentham (1840, 1870, 1871) recognized three independent species. Later, Irwin & Barneby (1978, 1982) treated three these species as varieties of *C. cytisoides* because of the high conserved floral morphology and a seemly continuous pattern of variation in vegetative characters among the taxa. On the other hand, Conceição *et al.* (*in press* b) demonstrated the occurrence of hybridization between some taxa which may contribute for Irwin & Barneby's (1978) view of a continuous variation pattern. Besides, phenetic analyses accomplished to access patterns of genetic variability using allozyme as well as morphological variability support the existence of different species inside the widened defined *Chamaecrista cytisoides* (Conceição *et al. in press* c). Here, we used these results to propose new circumscriptions for the species included in the sect. *Baseophyllum*. Thus, a taxonomic revision of this group is presented here, based on extensive field work sampling different populations along its distribution range and on specimens of the ALCB, BM, CEPEC, G, HRB, HUEFS, IPA, K, MBM, P, PEURF, RB, SP, SPF, UB, UEC, UPE herbaria (acronyms according to Holmgren & Holmgren, 2004).

History

The *Baseophyllum* group was recognised by Colladon (1816) as a section of *Cassia*, including only *Cassia cytisoides* DC. ex Collad. Vogel (1837), based on the inflorescence pattern and on the presence of a sub-filiform funiculus included this group in sect. *Lasiorhegma s*ubsect. *Absus* (Table 1).

Bentham (1840) recognized *Baseophyllum* as a section of *Cassia* and described four new species: *Cassia polystachya* Benth., *Cassia blanchetii* Benth., *Cassia brachystachya* Benth. and *Cassia coriacea* Benth. Later, the same author (Bentham, 1870; 1871) treated this group as a serie (*Baseophyllae*) of subg. *Lasiorhegma* sect. *Apoucouita*, includes *Cassia crenulata* Benth., *Cassia vauthieri* Benth. and *Cassia brachystachya* var. *unijuga* Benth., besides the species cited before (Table 1).

In their monography of *Cassia* sect. *Absus*, Irwin & Barneby (1978) treated the *Baseophyllum* group as a subsection of the sect. *Absus* based on the sharing of a mostly shrubby habit and racemose terminal inflorescences. These authors accepted only two species for this group (*Cassia coriacea* and *C. cytisoides*) and produced a radical change in the circumscription of *Cassia cytisoides*, sinking *Cassia blanchetti*, *Cassia brachystachya* and *Cassia brachystachya* var. *unijuga* to variety rank, and describing three new varieties of this species: *Cassia cytisoides* var. *confertiformis* Irwin & Barneby*, Cassia cytisoides* var. *decora* Irwin & Barneby and *Cassia cytisoides* var. *micrantha* Irwin & Barneby. Later, all these taxa were tranferred to *Chamaecrista* without changing their circumscription (Irwin & Barneby 1982). Besides, these authors removed *C. polystachya* to sect. *Apoucouita* and *C. crenulata* and *C. vauthieri* to sect. *Absus* subsect. *Absus* ser. *Ochnaceae* (Table 1).

The taxonomic composition of the *Baseophyllum* group remained unchanged until the description of *Chamaecrista depauperata* Conc., L.P.Queiroz & G.P.Lewis by Conceição *et al.* (2001). These authors also highlighted the complex morphological variation pattern found in this group and the need of a taxonomic reassessment of the species limits.

Distribution

Section *Baseophyllum* is restricted to Eastern Brazil, ranging from c. 08º34'S in the states of Pernambuco and Alagoas, to c. 22º40'S in the state of Minas Gerais. By and large, the species of sect. *Baseophyllum* are narrow endemics, occurring mostly in the states of Bahia and Minas Gerais, in campo rupestre vegetation of the Espinhaço range. Then, *Chamaecrista coriacea* is found only at Costa Sena (Northern Minas Gerais), *C. cytisoides* at Serra Negra (Southern Minas Gerais), *C. decora* to Diamantina plateau and Grão Mogol (Northern Minas Gerais) and *C. confertiformis* and *C. depauperata* to the Southern slopes of the Chapada Diamantina (Bahia).

Three other species have a wider range. *Chamaecrista brachystachya* is known of campos rupestres of Southern Bahia and Northern Minas Gerais and occurs disjunctly in a spot of caatinga in the Buíque region in Pernambuco state. *Chamaecrista blanchetii* occurs disjunctly between areas of campos rupestres of Southern Chapada Diamantina (Bahia), cerrados of Northern Minas Gerais and coastal restingas (coastal forests and scrubs on sandy soils) of the states of Sergipe, Bahia and Espírito Santo. *Chamaecrista unijuga* occurs exclusively in coastal restingas of Sergipe to Pernambuco states and, thus, is the only species of the sect. *Baseophyllum* that is not found on mountains.

Taxonomy

Chamaecrista sect. ***Baseophyllum*** (Collad.) Conc., L.P.Queiroz & G.P.Lewis, **comb. nov.**

Cassia sect. *Baseophyllum* DC. ex Collad., Hist. Cass.: 115 (1816).

Cassia sect. *Absus* ser. *Baseophyllae* (Collad.) Benth., Fl. Bras. (Martius) 15 (2): 138 (1870) [spelled as *Baseophylleae* by Bentham, Trans. Linn. Soc. London 27: 561(1871)].

Type species: *Chamaecrista cytisoides* (Collad.) H.S.Irwin & Barneby [*Cassia cytisoides* Collad.].

Shrubs to small trees 1-5 m, erect, profusely ramified from the base with a rounded to irregular canopy, subshrubs 0.1-0.2 m with xylopodia, prostate or procumbent; main stem brown, sometimes with white stains, branches green or purple, glabrous or glabrescent; indument, when present, of scattered and tiny trichomes, to c. 1 mm long, on the branches, stipules, inflorescence axis, bracts, bracteoles, sepal and ovary. **Stipules** erect, brown, deltate, ciliolate, persistent or tardily deciduous. **Leaves** ascending, patent, or deflexed, alternate, distichous; petiole green to glaucous or purple; pulvinus brown, wrinkled when dry; extrafloral nectaries 1-2, sessile, discoid to scutelate, located on the petiole or on the rachis, then on the interfoliolar segment of the rachis or between the leaflets; setae subulate, present between the last pair of leaflets; leaflets 1-3 (-4) pairs, chartaceous to coriaceous, somewhat succulent, green, glaucous or purple, oblanceolate to reniform, apex rounded, obtuse, rare acute, sometimes emarginate or apiculate, base asymmetrical, cuneate, rounded, cordate, with the outer side larger than the inner one, margin entire, concolor or discolor and then glaucous or

purple, main veins 3-9 palmately arranged, appearing from the base, main veins, secondary venation brochidodromous, raised on both surfaces when dry. **Inflorescence** subcorymbose raceme, peduncle provided with extrafloral nectaries similar to the found on the leaf axis, (1-) 2-12-flowered; bracts ciliolate, deltoid, subulate or oblanceolate; bracteoles resembling the bracts, alternating, placed in different positions of the pedicel; pedicel olivaceous to purple. **Flower** buds ovoid, apex rounded; sepals glabrous or ciliolate, oblanceolate, apex obtuse, reticulate, incurved; petals yellow, ascendent, somewhat incurved at the apex yieldind a campanulate to subglose corolla in profile, heteromorphic, two larger orbicular to oblong, two smaller orbicular to obovate and the cuculus latero-abaxial, falcate, bent around the stamens, or almost isomorphic, in this case with three larger petals obovate to oblong and two smaller suborbicular to obovate; stamens 10 homomorphic, anthers yellow, oblong, always larger than the filament, dehiscence for two apical rifts, lateral sutures puberulent; pistil opposed to set stamens-cucullus, ovary pale green, glabrous or glabrescent, 2-15-ovulate, style pale green, filiform, incurved at the apex, stigma chambered, stigmatic orifice pyriform, provided with a fringe of short hairs. **Legumes** elastically dehiscent, oblong, plan-compressed, apex apiculate, base rounded, margins straight; valves coriaceous, glabrous, shinning, brown to purple. **Seeds** compressed, rhomboid to pyriform, testa dark brown, pitted, pits aligned in vertical rows.

Key to species of sect. *Baseophyllum*

1. Petiole 5-24 mm.

    2. Leaflets widely oblanceolate to obovate, 1-2 (-3) pairs; Northern Minas Gerais at Costa Sena ..... 7. *C. coriacea*

2'. Leaflets obliquely obovate or suborbicular to reniform, 2 pairs; Chapada Diamantina (Bahia), Northern Minas Gerais and areas of Sergipe to Pernambuco states.

3. Leaflets 15-20 mm long, petals almost homomorphic, the cuculus not bent around the stamens ..... 8. *C. depauperata*

3'. Leaflets 26-55 mm long, petals heteromorphic, the cuculus falcately bent around the stamens.

4. Mature leaflets purple, obliquely obovate, base cuneate, smallest distance from margin to the main vein in the base 2-3(-4) mm; Diamantina plateau and Grão Mogol (Northern Minas Gerais)..... 5. *C. decora*

4'. Mature leaflets green, orbicular to reniform, base rounded to cordate, smallest distance from margin to the main vein in the base 4-20 mm, coastal restingas, areas of Sergipe to Pernambuco states ..... 6. *C. unijuga*

1'. Petiole 0-3 mm.

5. Leaves with 1-2 pairs of leaflet suborbicular to reniform, the proximal pair with base amplexicaul ..... 3. *C. blanchetii*

5'.Leaves with 2-4 pairs of leaflets oblanceolate to orbicular or oblong to suborbicular, the proximal with base not amplexicaul.

6. Leaflets 5-9 palmately nerved, distal pair oblong to suborbicular ..... 2. *C. brachystachya*

6'. Leaflets 3-6 palmately nerved, distal pair oblanceolate to obovate

7. Leaflets 2 pairs, semi-succulent, the proximal pair resembling the distal pair, both oblong to oblanceolate; Southern slopes of the Chapada Diamantina (Bahia)..... 4. *C. confertiformis*

7'. Leaflets 2-3(-4) pairs, chartaceous, the proximal pair suborbicular to orbicular to, distal pair oblanceolate to obovate; Southern Minas Gerais at Serra Negra .....1. *C. cytisoides*

1. ***Chamaecrista cytisoides*** (Collad.) H.S.Irwin & Barneby, Mem. New York Bot. Gard. 35: 647 (1982).

Cassia cytisoides DC. ex Collad., Hist. Cass.: 116 (1816). Type: "Hab. in Brasilia" (Holotype: G-DC!; probable isotype: P!).

Cassia glabra Collad., Hist. Cass. 131 (1816). *Cassia venosa* Desv., Journ. Bot. 3: 72 (1814), non *C. venosa* Castigl. ex Zuccagni in Roem., Collect. Rem Bot. Spectania 140 (1806). Type: "Hab. in Brasilia" (Holotype: P!).

Shrubs erect until 2.5 m high, little ramified at the base c. 90 cm above the ground, with a irregular canopy, main stem brown, branches green, glabrous, except by scattered and tiny trichomes, c. 1 mm long, present on the stipules, pulvinus, bracts and bracteoles. **Stipules** persistent, 0.7-1 mm long. **Leaves** ascending, 3.5-8.5 cm long; petiole green, 1-2 mm long, pulvinus 1-2 mm diam., rachis 9-35 mm long, interfoliolar segment 8-10 mm long; setae 1-2mm long; extrafloral nectary 1-2, placed in the interfoliolar segments of the rachis, 1-2 x c. 1 mm; leaflets 2-3 (-4) pairs, chartaceous, green to glaucous, margin concolor, glaucous, 5-6 palmately nerved; proximal par suborbicular to orbicular, 14-26 x 14-26 mm, apex and base rounded, longest distance from margin to the main nervure in the base 3-12 mm, smallest distance 2-9 mm; distal pairs oblanceolate to obovate, 19-36 x 12-24 mm, apex rounded, base cuneate, longest distance from margin to the main nervure in the base 3-6 mm, smallest distance from

margin to the main nervure in the base of the leaflets 2-4 mm. **Racemes** 3-7-flowered; inflorescence axis 55-65 mm; bracts deltoid, 1-2.5 mm long; bracteoles 0.5-1 mm long; pedicel olivaceous, 20-30 mm long. **Flower** 1.5-2 cm diam.; sepals purplish yellow, 11-15 x 5-10 mm; petals heteromorphic, two larger orbicular to oblong, 23-32 x 15-21 mm, two smaller orbicular to obovate, 18-25 x 10-16 mm and the cuculus falcate, bent around the stamens, 21-27 x 12-15 mm; stamens 6-9 mm long, filaments 1-3 mm long, anthers 5-6 mm long; ovary glabrous, 7-10 mm long, 8-10-ovulate; style 8-10 mm long. **Fruits** 45-80 x 5-12 mm. **Seeds** not seen. Fig. 1.

SELECTED EXAMINED SPECIMENS: BRAZIL. State of **Minas Gerais**, Santa Bárbara do Monte Verde, Distrito de Três Cruzes, Fazenda Cachoeira Alta, Sopé da Serra Negra, 20 Feb. 2005, *Conceição et al*. 870 (BM, CEPEC, HRB, HUEFS, K, P); 16 Sept. 2004, *Conceição et al*. 821 (HUEFS); entrada pela estrada de Conceição, c. 3 km de Três Cruzes, 25. Feb. 1999, *Mello-Silva et al.* 1640 (HUEFS, SP, SPF). Santa Rita da Jacutinga, 31 July 1970*, Krieger* 8895 (RB).

DISTRIBUTION & HABITAT. *Chamaecrista cytisoides* is known only from Southern Minas Gerais, between 21°58'S to 22°40'S, 43°49' to 44°30'W. It inhabits areas of cerrado (savanna like vegetation) on sandy gravelled soil, between 900 and 990 m. Map 1.

PHENOLOGY: It flowers from February to June and sets fruits between March and July.

Amongst the species of sect. *Baseophyllum*, *C. cytisoides* is very distinct because it presents of the leaves with 2-4 pairs of leaflets, with the proximal pair orbicular to suborbicular and the distal pars obovate to oblanceolate; longest distance from margin

to the main nervure in the base of the distal pair distal 3-8 mm. Besides, this species does not occur in sympatry with any taxa of the group.

Colladon (1816) described *Cassia cytisoides* and *Cassia glabra*. This author overlooked the older species *Cassia venosa*, described by Desvaux (1814). Desvaux (1826) widened the concept of *Cassia venosa* by treating the *Cassia cytisoides* as a synonym of *C. venosa* (Desvaux, 1826). In his treatment for the Flora Brasiliensis, Bentham (1871) recognised these taxa as conspecific but, adopted *Cassia cytiosoides* as the correct name since *Cassia venosa* is a later homonym for another species described by Zuccagni (1806).

Irwin & Barneby (1978, 1982) adopted a wide circumscription for *Chamaecrista cytisoides*, embracing almost all the variation found in sect. *Baseophyllum*. However, morphometric studies carried out at populational level support a much narrower concept of *C. cytisoides*, restricting it to populations of plants with leaves with 2-4 pairs of leaflets distributed in areas of cerrado of Southern Minas Gerais (Conceição *et al., in press* a). Besides, it is a very divergent species since it presents five exclusive alleles in the PGM-2, EST-2, EST-3, G6PDH, and LAP-1 (*Conceição et al*., in *press* a).

2. ***Chamaecrista brachystachya*** (Benth.) Conc., L.P.Queiroz & G.P.Lewis, **comb. nov.** *Cassia brachystachya* Benth. J. Bot. (Hooker) 2: 78 (1840). *Cassia cytisoides* var. *brachystachya* (Benth.) H.S.Irwin & Barneby, Mem. New York Bot. Gard. 30: 13 (1978). *Chamaecrista cytisoides* var. *brachystachya* (Benth.) H.S.Irwin & Barneby, Mem. New York Bot. Gard. 35: 647 (1982). Lectotype (designated among the syntypes by Irwin & Barneby, 1978): "Tejuco, Herb, Acad. Petrop." sd., *Riedel* 566 (Lectotype: K-Benth.!; isolectotype: LE!).

Cassia cytisoides var. *micrantha* H.S.Irwin & Barneby, Mem. New York Bot. Gard. 30: 12 (1978). *Chamaecrista cytisoides* var. *micrantha* (H.S.Irwin & Barneby) H.S.Irwin & Barneby, Mem. New York Bot. Gard. 35: 647 (1982). Type: Brazil, Bahia, Chapada Diamantina, Chique-Chique (Xique-Xique), mun. Andarahy, April 1938, *Bondar* 2610 (Holotype: SP!).

Shrubs until 2.5 m high, profusely ramified from the base, c. 10 cm above the ground; canopy rounded; main stem brown, branches green, glabrescent, with scattered and tiny trichomes, c. 1 mm long, present on the branches, stipules, inflorescence axis, bracts, bracteoles, sepals and ovary. **Stipules** persistent, 0.8-1 mm long. **Leaves** ascending, 3-6.5 cm long, sessile to subsessile, petiole green, 0-3 mm long, pulvinus 1-2 mm diam., rachis 6-15 mm long; setae 1-2mm long; extrafloral nectary 1(-2), placed on the rachis or between the proximal pair, 1-5 x 1-3 mm; leaflets 2 pairs, coriaceous to semi succulent, purple when young, green to glaucous when mature, margin concolor, glaucous, 5-7 palmately nerved; proximal par obovate to orbicular, 19-60 x 9-29, apex rounded, base cordate to cuneate, longest distance from margin to the main vein in the base 2-9 mm, smallest distance 1-4 mm; distal pairs oblong to suborbicular, 19-59 x 9-30 mm, apex rounded, base cuneate, longest distance from margin to the main vein in the base 2-10 mm, smallest distance 1-5 mm. **Racemes** 3-12-flowered; inflorescence axis 35-75 mm; bracts deltoid, 1-2.2 mm long; bracteoles 0.7-1 mm long; pedicel olivaceous, 20-30 mm long. **Flower** 1-2 cm diam.; sepals ciliolate, purplish yellow, 7-13 x 2-8 mm; petals heteromorphic, two larger orbicular to oblong, 11-25 x 7-19 mm, two smaller orbicular to obovate, 10-20 x 6-15 mm and the cuculus falcate, bent around the stamens, 15-26 x 7-11 mm; stamens 5-8 mm long, filaments 1-2 mm long, anthers

4-7 mm long; ovary glabrous to glabrescent, 5-13 mm long, 6-13-ovulate; style 3-9 mm long. **Fruits** 50-70 x 1-13 mm. **Seeds** 5-8 x 3-5 mm. Fig. 2.

SELECTED EXAMINED SPECIMENS: BRAZIL. State of **Bahia**, Abaíra, Catolés, subida do Bicota, 30 May 2003, *Conceição et al.* 567 (ALCB, HRB, HUEFS, RB, SPF); Catolés de Cima-Barbado, subida da Serra, 26 Oct. 1992, *Ganev* 1351 (K, HUEFS). Andaraí, South Andaraí 16 km along road to Mucugê near small town of Xique-Xique, 16 Feb. 1977, *Harley et al.* 18665 (K, P). Delfino, Campo Formoso, 25 Oct. 1971, *Ramalho* 44 (RB). Ibicoara, Campo Redondo, 6 April 2005, *Santos et al.* 288 (HUEFS). Itaberaba, Fazenda Monte Verde, Serra do Orobó, 13 Nov. 1983, *Bautista et al*. 1230 (HRB, HUEFS, MBM). Jussiape, c. 10 km de Jussiape em direção a Barra da Estiva, 16 June 2002*, Queiroz et al. 7108* (HUEFS). Lençóis, 11 Aug. 1973, *Costa* 223 (ALCB, HUEFS); Serra da Chapadinha, 30 June 1995, *Guedes et al.* PCD 2086 (ALCB, SPF). Minas do Mimoso, 22 km North-west de Lagoinha, 6 March 1974, *Harley et al*. 16878 (K). Mucugê, estrada de Guiné para Palmeiras, 20 June 2005, *Cardoso & Conceição* 586 (HUEFS); 30 km na estrada Andaraí para Mucugê, 19 March 1990, *Carvalho & Saunders* 2924 (CEPEC, HUEFS, MBM); no rio Combuca, c. 3 km S de Mucugê, 04 Feb. 1974, *Harley et al*. 15936 (K); estrada Andaraí-Mucugê, 9 km saindo de Igatú, sentido Andaraí, 21 Oct. 2003, *Conceição et al*. 754 (HRB, HUEFS, MBM, RB, SPF). Palmeiras, km 9 da rodovia para Mucugê, 9 April 1992*, Hatschbach* 56901 (CEPEC, HUEFS, MBM). Pindobaçu, Carnaíba, entrada Santa Terezinha-Carnaiba, vôo da morte, 25 Oct. 1993, *Ganev* 2351 (K, HUEFS). Rio de Contas, arredores, 16 May 1983, *Hatschbach* 46480 (K, MBM, RB); estrada do Fraga, c. 2 km SE Rio de Contas, 13 July 1985, *Kral et al.* 72726 (SP, SPF); 9-11 km ao N de Rio de Contas, na estrada para povoado de Mato Grosso, 20 June 1979, *Mori et al*. 12323 (K). Rui Barbosa, Serra de

Rui Barbosa, 8 Feb. 1991, *Tylon et al*. 1601 (CEPEC, K). Saúde, Serra de Saúde, c. 1.9 km saindo do centro cultural, 4 Dec. 2003, *Conceição et al*. 774 (HRB, HUEFS, MBM, SPF). Seabra, c. 2 km SW de Lagoa da Boa Vista, 22 July 1993, *Queiroz & Nascimento* 3369 (HUEFS, K, MBM). Umburanas, Serra do Curral Feio, Cachoeirinha, 11 April 1999, *Queiroz et al*. 5364 (HUEFS, MBM, SPF). **Minas Gerais**, Botumirim, contrafortes orientais da Serra da Canastra, 29 Sept. 1997, *Kawasaki et al*. 1037 (HUEFS, SP, SPF). Buenópolis, Curimatai arredores, 19 June 2001, *Hatschbach et al*. 72249 (MBM). Datas, estrada Datas-Serro, c. 2 km de Datas, 24 Nov. 1985, *Pirani et al*. CFCR 8686 (HUEFS, K, SPF). Diamantina, Tejuco, Dec. 1824, *Riedel* 1225 (BM, P); 7 km E de Diamantina, na estrada para Couto de Magalhães, 10 Jan. 2003, *Conceição et al.* 540 (MBM, HUEFS, RB, SPF); c. 12 km NE of Diamantina, road Medanha, 27 Jan. 1969, *Irwin et al* 22714 (K, UB). Gouveia, 13 Nov. 1971, *Hatschbach & Pelanda* 27896 (MBM). Grão Mogol, c. 15 km West of Grão Mogol, 19 Feb. 1969, *Irwin et al*. 23538 (UB); c. 30 km de Grão Mogol, saída pela fazenda Cancela, 14 April 2004, *Conceição et al*. 818 (ALCB, CEPEC, HRB, HUEFS, SPF); Jambeiro, à 7 km de Grão Mogol, 5 Sept. 1985, *Zappi et al*. CFCR 8515 (HUEFS, K, SPF); Nascente do córrego das Taiobeiras, 2 Sept. 1986, *Cordeiro & Mello-Silva* CFCR 10031 (HUEFS, K, SPF). Itacambira, estrada Juramento-Itacambira, no alto da Serra de Itacambira, 1 Oct. 1997, *Mello-Silva* 1500 (HUEFS, SP, SPF); Serra de Itacambira, 10 Sept. 2003, *Conceição et al.* 728 (HUEFS). Mato Verde, estrada para Montezuma, 25 Sept. 1997, *Mello-Silva 1415* (HUEFS, SP, SPF,). Monte Azul, alto da Serra do Espinhaço, 18 Sept. 1996, *Hatschbach et al.* 65020 (HUEFS, MBM). **Pernambuco**, Buíque, Parque Nacional do Catimbau, vale do Catimbau, 23 Sept. 2004, *Miranda et al.* 4468 (HUEFS, PEUFR); Serra do Catimbau, 14 Nov. 1999, *Schlindwein* 994 (UFP); Serra de Jerusalém, 10 Nov. 2003, *Conceição et al*. 761 (CEPEC, HRB, HUEFS, SP, SPF).

DISTRIBUTION & HABITAT. *Chamaecrista brachystachya* occur disjunctly in areas of campo rupestre vegetation of Northern slopes of the Serra do Espinhaço (Minas Gerais) and Southern slopes of the Chapada Diamantina (Bahia) and in an area of caatinga in the region of Buíque (Pernambuco). In this last area, this species is found on sandy soil in elevations around 682 m while in the areas of campo rupestre it is found on shallow soils among quartizite rocks between 910 to 1250 m high. Map 1.

PHENOLOGY: It flowers from January to June and sets fruits between March and October.

Chamaecrista brachystachya was described by Bentham (1870, 1871) as *Cassia brachystachya*. Irwin & Barneby (1978) reduced it to a variety of their widely circumscribed *C. cytisoides*. Results of both morphometric and genetic studies support its distinctiveness as a different species. These studies also demonstrated that *C. brachystachya* and *C. cytisoides* var. *micrantha* present high morphological and genetical similarities (Conceição *et al., in press* c). The main morphological characters that join these taxa are the leaves with two pairs of leaflets, the distal pair oblong to suborbicular.

Some morphs *C. brachystachya* are similar to *C. confertiformis* but they can be differentiated by oblong-obovate to suborbicular (vs. oblong to oblanceolate in *C. confertiformis*), 5-7-nerved leaflets (vs. 3-5-nerved in *C. confertiformis*). It occurs in sympatry with *C. decora* and *C. blanchetii* in campos rupestres, but can be differentiated by the leaves with two pairs of leaflets (vs. 1 pair in *C. decora* and *C. blanchetii*).

3. ***Chamaecrista blanchetii*** (Benth.) Conc., L.P.Queiroz & G.P.Lewis, **comb. nov.**

Cassia blanchetii Benth. J. Bot. (Hooker) 2: 78 (1840). *Cassia cytisoides* var. *blanchetii* (Benth.) H.S.Irwin & Barneby, Mem. New York Bot. Gard. 30: 13 (1978). *Chamaecrista cytisoides* var. *blanchetii* (Benth.) H.S.Irwin & Barneby, Mem. New York Bot. Gard. 35: 647 (1982). Type: "Serra de Jacobina", *Blanchet*, 2649 s.d. (Holotype: K! [photo: HUEFS!]; isotypes [numbered 2549] †B, BM!, C, F, G!, K! LE, MG, MO, NY!, P, SP!, [photo: HUEFS!]).

Shrubs to small tree erect until 3.5 m, very ramified the base, c. 15 cm above the ground; canopy rounded, subshrubs with xylopodia, procumbent 0.1-0.2 m, profusely ramified from the base to; main stem brown, branches green, glabrescent, with scattered and tiny trichomes, c. 1 mm long, present on the blanches, stipules, inflorescence axis bracts, bracteoles, sepals and ovary. **Stipules** persistent, 0.9-1 mm long. **Leaves** ascending, 2.5-8.0 cm long; sessile or subsessile, petiole green, 0-2 mm long, pulvinus 1-2 mm diam., rachis, when present, 19-40 mm long, interfoliolar segment, when present, 10-12 mm; setae 2-6 mm long; extrafloral nectary 1, placed between the leaflets or on the rachis, 1-2 x 1-2 mm; leaflets mostly 1 pair, 2(-3) pairs in coastal areas, coriaceous, purple when young, glaucous when mature, margin concolor, glaucous, 5-9 palmately nerved; proximal par suborbicular to reniform, 14-46 x 16-70 mm, apex rounded, base amplexicaul, reniform to obtuse, longest distance from margin to the main vein in the base 4-31 mm, smallest distance 2-26 mm; distal pairs, when present, orbicular, 14-50 x 16-50 mm, apex rounded, base obtuse, longest distance from margin to the main nervure in the base 2-33 mm, smallest distance 1-25 mm. **Racemes** 3-12-flowered; inflorescence axis 35-85 mm; bracts deltoid, 0.7-1 mm long; bracteoles 0.9-1 mm long; pedicel olivaceous, 17-40 mm long. **Flower** 1.4-2 cm diam.; sepals ciliolate,

purplish to yellow, 8-13 x 4-10 mm; petals heteromorphic, two larger orbicular to oblong, 12-33 x 9-19 mm, two smaller orbicular to obovate, 11-26 x 6-15 mm and the cuculus falcate, bent around the stamens, 13-28 x 6-16 mm; stamens 5-8 mm long, filaments 1-2 mm long, anthers 4-7 mm long; ovary glabrous to glabrescent, 4-12 mm long, 8-12-ovulate; style 8-15 mm long. **Fruits** 60-100 x 10-15 mm. **Seeds** 6-8 x 4-5 mm. Figs. 3-4.

SELECTED EXAMINED SPECIMENS: BRAZIL. State of **Bahia**, Abaíra, Catolés, campo do Virassaia, 23 July 2003, *Conceição et al.* 672 (ALCB, HRB, HUEFS, MBM, SP, SPF); campo do Virassaia, 31 May 2003, *Conceição et al.* 611 (HUEFS); Boa Vista, 28 Nov. 1993, *Ganev* 2595 (HUEFS, K). Andaraí, Serra da Cotinguiba, 25 Aug. 1986, *Orlandi et al*. 773 (HRB, HUEFS); 8 km South of Andaraí, road Mucugê, 13 Feb. 1977, *Harley* 18607 (K, P, RB). Barra da Estiva, 7 km saindo da entrada de Barra da Estiva, sentido Ituaçu, 22 Oct. 2003, *Conceição et al*. 758 (CEPEC, HUEFS, MBM, SPF); c. 6 km N. of Barra da Estiva, 28 Jan. 1974, *Harley* 15526 (P). Belmonte, 1 Feb. 1967*, Belém & Pinheiro* 3249 (UB). Cairú, Garapuá, 8 Aug. 2003, Rigueira s.n (ALCB). Camaçari, 6 April 1991, *Ferreira* 354 (HRB); BA-099 estrada do cocô, entre Arempebe e Monte Gordo, 14 July 1983, *Bautista & Pinto* 813 (HRB, K). Canavieiras, rodovia Canavieiras-Santa Luzia, km 20-30, 18 Oct. 1998, *Hatschbach et al*. 68640 (CEPEC, HUEFS, MBM, UB). Costa do Sauípe, Mata de São João, 17 Oct. 2003, *Loureiro et al.* 717 (ALCB). Entre Rios, Subaúma, 8 Dec. 1982, *Bautista et al*. 679 (HRB). Esplanada, linha verde, km 123, 5. 3 km depois da fazenda Morro da Graça, 18 Aug. 2003, *Conceição et al*. 687 (CEPEC, HRB, HUEFS, SPF). Ibicoara, estrada Mucugê-Ibicoara, c. 13.2 km da entrada para Ibicoara, 22 Oct. 2003, *Conceição et al.* 757 (CEPEC, HRB, HUEFS, SP, SPF). Ilhéus, s.d, *Blanchet* 1836 (syntype of *C.*

cytisoides var. *brachystachya*: BM, K, P [photo HUEFS]). Itacaré, estrada Serra Grande-Itacaré, c. de 13 km de Serra Grande, 1 Sept. 1993, *Amorim et al.* 1323 (CEPEC, RB, UB). Jacobina, Serra do Cruzeiro, 7 April 2001, *Jesus et al.* 1325 (ALCB, HUEFS); Oeste de Jacobina, Serra do Tombador, estrada para Lagoa Grande, 23 Dec. 1984, *Lewis et al*. 7509 (K, SPF); estrada para Lagoa Grande, c. 3.8 km da entrada Jacobina-Serra do Tombador, 3 Dec. 2003, *Conceição et al*. 772 (HUEFS). Lauro de Freitas, praia de Ipitanga, 14 June 1987, *Santos* s.n (ALCB). Lençóis, Serra da Chapadinha, 30 June 1995, *Guedes et al*. PCD 2086 (ALCB, RB). Licínio de Almeida, c. 12 km da cidade em direção a Brejinho das Ametistas, 12 March 1994, *Roque* CFCR 15021 (HUEFS, K, SPF). Morro do Chapéu, Morro das Pedras Duas Irmãs, 2 May 1999, *França et al*. 2847 (ALCB, HUEFS, MBM); BA-052, 2 km SE de Morro do Chapéu, 19 Nov. 1986, *Queiroz et al*. 1253 (HUEFS); Cachoeira do Rio Ferro Doido, 5 March 1997, *Silva et al.* PCD 6035 (ALCB, CEPEC, HUEFS, SPF); BA 052 road Mundo Novo, 2 March 1977, *Harley* 19231 (CEPEC, K, P). Mucugê, estrada Mucugê-Ibicoara c. 13.2 km da entrada para Ibicoara, 22 Oct. 2003, *Conceição et al.* 757 (HUEFS); Projeto Sempre Viva, trilha do Tiburtino, 23 Oct. 2003, *Conceição et al*. 759 (CEPEC, HRB, HUEFS, MBM). Mundo Novo, 19 Feb. 1982, *Orlandi* 262 (CEPEC). Piatã, Morro três Morros, 19 Jan. 2006, *Conceição et al.* 1656 (HUEFS); Serra do Atalho, próximo ao garimpo da cravada, 11 June 1992, *Ganev* 473 (HUEFS, K, SPF). Rio de Contas, Água Quente, Pico das Almas, vale ao NW do pico, 26 Nov. 1988, *Harley et al*. 26698 (CEPEC); Ladeira do Toucinho, caminho Catolés-Arapiranga, 30 Aug. 1993, *Ganev* 2171 (HUEFS, K); Estrada para Mato Grosso, 21 Jan. 1984, *Hatschbach 47373* (HUEFS, MBM); c. 6 km of the town of Rio de Contas on road to Abaíra, 16 Jan. 1974, *Harley* 15091 (K, P). Salvador, dunas de Itapuã, próximo ao condomínio Alameda da Praia, 30 Oct. 1991, *Queiroz 2491* (ALCB, HUEFS, MBM,

UB); Oeste do Aeroporto, 12 Nov. 1983, *Callejas* 1748 (CEPEC, K, SPF, RB); dunas de Stella Mares, área do aeroporto, 2 Oct. 2004, *Conceição et al*. 832 (CEPEC, HRB, HUEFS, MBM, SPF). Seabra, Serra do Bebedor, c. de 4 km W de Lagoa da Boa Vista na entrada para Gado Bravo, 22 June 1993, Queiroz & Nascimento 3350 (HUEFS). Umburanas, Serra do Curral Feio, Cachoeirinha, 11 April 1999, *Queiroz et al*. 5357 (HUEFS, K, MBM). State of **Espírito Santo**, Guarapari, Lagoa do milho, 8 April 1980, *Weinberg* 386 (CEPEC); rod. do Sol, km 36 em frente as duas torres, 18 Sept. 2004, *Conceição et al*. 826 (CEPEC, HRB, HUEFS, MBM, RB, SPF). State of **Minas Gerais,** Pedra Azul, Serra da Sapucaia, 3 Sept. 1959, Magalhães 15577 (RB, UB); Serra de Areia, c. 47 km ao sul de Pedra Azul, na estrada para Jequitinhonha, 20 Oct. 1988, *Harley et al.* 23234 (K, SPF); estrada Pedra Azul- Jequitinhonha, c. 47 km ao sul de Pedra Azul, em frente ao poste de eletricidade, 21 Sept. 2004*, Conceição et al*. 831 (HUEFS). State of **Sergipe**, Areia Branca, Serra de Itabaiana, área da Sede do IBAMA, 4 Sept. 2004, *Conceição et al.* 819 (CEPEC, HRB, HUEFS, MBM, SPF). Estância, rodovia Estância/Abais, 15 June 1994*, Silva et al*. 2985 (CEPEC, HUEFS). Indiaroba, c. de 2 km depois da entrada para Pontal, 19 Aug. 2003, *Conceição et al*. 693 (ALCB, CEPEC, HUEFS, MBM, SPF).

DISTRIBUTION & HABITAT. *Chamaecrista blanchetii* occurs in three disjunct areas: in campos rupestres of the Eastern slopes of the Chapada Diamantina (Bahia) between 800-1450 m elevation, in cerrado vegetation of Northern Minas Gerais, between 1000-1246 m elevation, and in coastal restingas of Northeastern Brazil and Espírito Santo, between 18 and 20 m elevation. Map 2.

PHENOLOGY: It flowers from July to November and sets fruits between September and January.

Chamaecrista blanchetii was first recognised by Bentham (1840) as *Cassia blanchetii,* based on specimens collected by J.S.Blanchet in Serra de Jacobina, Bahia, Brazil. Unfortunately, there is some confusion with type material of this taxon. In the protologue, Bentham (1840) refered to the specimen "Blanchet 2649". However, we found only one material labelled as "Blanchet 2649", deposited in K. In this same sheet, there is a piece of paper, probably a field label, identified as "2549" besides another plant labeled as "Blanchet 2549". In other herbaria, there are 12 duplicates of "Blanchet 2549". We found in K, another plant labelled as "Blanchet 2649"but it is a specimen of *Evolvulus speciosus* Moric. (Convolvulaceae). Thus, it is probable that the number "2649" was a copy mistake and we are talking the specimens labelled "2549" as isotypes in agreement of similar decision by Irwin & Barneby (1978). Theses same authors considered *C. blanchetii* as a variety of *Cassia cytisoides*, later renamed *Chamaecrista cytisoides* var. *blanchetii* (Irwin & Barneby, 1982). It is here considered as a distinct species but with a wider circumscription, including some populations from coastal areas of Northeastern Brazil that were treated by Irwin & Barneby (1978, 1982) as part of the variation of *C. cytisoides* var. *brachystachya*. These authors highlighted that *C. brachystachya* (as *Cassia cytisoides* var. *brachystachya*) 'probably embraces two distinct geographical races, one native to the crest of the Serra do Espinhaço above 900 m, the other to the Atlantic lowlands' (Irwin & Barneby 1978: 13). Morphometric and genetic studies carried out by Conceição *et al.* (*in press* c) showed that coastal populations previously ascribed to *C. brachystachya* are close related to *C. blanchetii* and they are here considered as conspecific. According to these results, the populations growing on mountain areas present one pair of leaflets while the coastal populations are variably with one to two (rarely three) pairs of leaflets. In other features these populations are identical.

The mountain morphs of *Chamaecrista blanchetii* can be differentiated from *C. brachystachya s.s* by the presence of only one pair of leaflets (vs. two pairs in *C. brachystachya*), leaflets reniform to suborbicular with amplexicaul base, (vs. orbicular to obovate, base not amplexicaul in *C. brachystachya*). The costal morph of *C. blanchetii* occurs in sympatry with *C. unijuga*, but they can be differentiated by the sessile leaves, with no or short (to 2 mm long) petiole in *C. blanchetii* (vs. petiole 9-24 mm long in *C. unijuga*) and by the number of leaflets: 2(-3) pairs in coastal populations of *C. blanchetii* and one pair in *C. unijuga*.

4. ***Chamaecrista confertiformis*** (H.S.Irwin & Barneby) Conc., L.P.Queiroz & G.P.Lewis, **comb. et stat. nov.**

Cassia cytisoides var. *confertiformis* H.S.Irwin & Barneby, Mem. New York Bot. Gard. 30: 12 (1978). *Chamaecrista cytisoides* var. *confertiformis* (H.S.Irwin & Barneby) H.S.Irwin & Barneby, Mem. New York Bot. Gard. 35: 647 (1982). Type : Brazil, Bahia, ±3km S. of Mucugê, among sandstone rocks along Rio Cumbuca, *R.M.Harley* 15936 (Holotype: NY!, isotypes: K!, P!).

Shrubs to small trees erect until 7 m, profusely ramified the base, c. 15 cm above the ground; canopy irregular to rounded; main stem brown, branches green or purple, almost glabrous, with scattered and tiny trichomes, c. 1 mm long, present on the stipules, bracts, bracteoles. **Stipules** late caducous, 0.8-1 mm long. **Leaves** ascending, 2-5 cm long; petiole green, 1-5 mm long; pulvinus 0.8-1mm diam.; rachis 7-15 mm, setae 0.3-1 mm long; extrafloral nectary 1, placed in the rachis below the distal par, 1-2 x 1-2 mm; leaflets 2 pairs, semi succulent, green to purple, margin green to purple, the

proximal par resembling the distal par, oblanceolate to oblong, 15-51 x 4-17 mm, apex acute to rounded, base cuneate, 3-5 palmately nerved, longest distance from margin to the main vein in the base 2-3 mm, smallest distance 1-3 mm. **Raceme** 3-8-flowered, inflorescence axis 35-50 mm; bracts deltoid, 0.9-1 mm long; bracteoles 0.9-1 mm long; pedicel olivaceous to purple, 2-3.5 mm long. **Flower** 1.2-2. cm diam.; sepals yellowish green to purple, 8-12 x 4-6 mm; petals heteromorphic, two larger orbicular to oblong, 13-25 x 8-16 mm, two smaller orbicular to obovate, 12-23 x 6-14 mm and the cuculus falcate, bent around the stamens, 18-25 x 8-10 mm; stamens 5-10 mm long, filaments 1-2 mm long, anthers 4-5 mm long; ovary glabrous, 5-8 mm long, 6-12-ovulate; style 6-10 mm long. **Fruits** 45-65 x 9-11 mm. **Seeds** 6-7 x 3-4 mm. Fig. 5.

SELECTED EXAMINED SPECIMENS: BRAZIL. State of **Bahia**, Mucugê, 22 June 1984, *Hatschbach* 47531 (MBM, RB); c. de 3 km N de Mucugê, 5 Feb. 1974, *Harley* 16019 (CEPEC, K, P); 2 km Andaraí, 25 Jan. 1980, Harley *et al*. 20628 (CEPEC, K, SPF); 3 km Mucugê, na estrada que vai para Jussiape, 22 Dec. 1979, *Mori & Benthon* s.n (K); Guiné, trilha para o Pati, 27 Oct. 1997, *Ferreira & Veronese* 1217 (HRB, HUEFS,); Projeto Sempre Viva, beira do rio Tomboro, 24 Oct. 2000, *Souza et al*. 83 (HUEFS); Parque Nacional da Chapada Diamantina, Gerais do rio Preto, 19 June 2005, *Cardoso & Conceição* 601 (HUEFS); c. 4 km N de Mucugê na Chapada para Andaraí, caminho da mata acima do Tiburtino, sede do projeto Sempre Viva, 23 Jan. 2000, *Queiroz et al*. 5626 (HUEFS); 1 km N de Mucugê, 10 Oct. 1987, *Queiroz et al*. 1831 (HUEFS); Parque Municipal de Mucugê, próx. ao rio Combuca, 12 April 2003, *Costa* 110 (HUEFS); Cachoeira do rio Piabinha, 22 Oct. 2003, *Conceição et al.* 755 (ALCB, CEPEC, HRB, HUEFS, MBM, SPF,); margem do rio Cumbuca, 30 Sept. 2004, *Ambrósio & Silva* 8 (HUEFS); trilha do Garimpeiro, 7 Jan. 1998, *Stradmanm et al.* 715

(ALCB); Campo Alegre, trilha para Riachão até o Rumo, 29 Dec. 2004, *Conceição et al*. 834 (CEPEC, MBM, HUEFS, RB); Serra do Sincorá, c. 15 km of Mucugê on the road to Guiné & Palmeiras, 26 March 1980, *Harley* 20994 (RB); Projeto Sempre Viva, Morro Fervido, base do Morro, 1 Feb. 2005, *Conceição et al.* 835 (CEPEC, HRB, HUEFS, BMB); Serra do Sincorá, S.W. of Mucugê on the Cascavel, 27 March 1980, *Harley* 21033 (K, RB); aprox. 2-3 km on the road to Cascavel, 12 Feb. 1977, *Harley* 18792 (CEPEC, K, P, RB); Guiné, Paty de Fora, Gerais do Rio Preto, próximo a margem do Rio Preto, 2 Feb. 2005, *Conceição et al*. 864 (ALCB, HUEFS, RB, SPF,); by rio Cumbuca, c. 3 km of Mucugê, 4 Feb. 1974, *Harley* 15959 (CEPEC, K, P, RB); rio Cumbuca, about 35 km N of Mucugê of the to Andaraí, 15 Feb. 1977, *Harley* 18697 (CEPEC, K, P, RB, UEC); rod. para Andaraí entre km 5-15, 15 Sept. 1984, *Hatschbach* 48258 (CEPEC, MBM, RB, SPF); rio Mucugê, 16 June 1984, *Hatschbach* 47964 (CEPEC, MBM, SPF); Serra de São Pedro, 12 Dec. 1984, *Lewis et al*. CFCR 7038 (K, SPF). Lençóis, foz do rio Capivara, 8 March 2000, *Stradmanm et al.* 627 (ALCB, CEPEC); foz do rio Capivara, 27 Dec. 2004, Conceição *et al*. 833 (CEPEC, HRB, HUEFS, MBM, SPF).

DISTRIBUTION & HABITAT. *Chamaecrista confertiformis* is endemic of the Serra do Sincorá, in the Eastern slopes of the Chapada Diamantina, state of Bahia, at the municipalities of Mucugê and Lençóis. Its occurs in campos rupestres vegetation with clay soils with rocks along the river valleys, in elevations between 500 and 945 m. Map 3.

PHENOLOGY: It flowes from September to December and sets fruits between October and January.

Chamaecrista confertiformis was described by Irwin & Barneby (1978) as a variety of their widely defined *Cassia cytisoides*. However, it is a quite distinct taxon, presenting leaves with two pairs of oblong to oblanceolate, 3-5 palmately nerved leaflets. Besides, it is genetically distinct since it shows inverted frequencies of PGI-1, PGM-2 and LAP-1 alleles in relation to *C. cytisoides* (*Conceição et al*., *in press* c).

It occurs in sympatry with *C. depauperata* and *C. blanchetii*, but it is easily differentiated by the present of two pairs of leaflets (vs. one pair in the mountain morphs of *C. blanchetii* and in *C. depauperata*) and much narrower leaflets ranging from oblanceolate to obovate (vs. suborbicular in *C. blanchetii* and *C. depauperata*).

5. ***Chamaecrista decora*** (H.S.Irwin & Barneby) Conc., L.P.Queiroz & G.P.Lewis, **comb. et stat. nov.**

Cassia cytisoides var. *decora* H.S.Irwin & Barneby, Mem. New York Bot. Gard. 30: 14 (1978). *Chamaecrista cytisoides* var. *decora* **(**H.S.Irwin & Barneby) H.S.Irwin & Barneby, Mem. New York Bot. Gard. 35: 647 (1982). Type: Brazil, Minas Gerais, Serra do Espinhaço, ±18 km E of Diamantina, 19. March.1970, *Irwin, Fonseca, Souza, Reis dos Santos & Ramos 27894* (Holotype: UB; isotypes: C, F, GH, K!, NY!, RB!, UC, US).

Shrub to small tree until 3 m high, very ramified c. 50 cm above the ground; canopy rounded; main stem brown, branches green to purple, almost glabrous, with scattered and tiny trichomes, c. 1 mm long presents on the stipules, bracts and bracteoles. **Stipules** late caducous, 0.5-1 mm long. **Leaves** deflexed, 2-7.5 cm long; petiole purple to glaucous, 5-24 mm long, pulvinus 1-3 mm diam, rachis absent, setae 0.5-1 mm long;

extrafloral nectary 1, placed mostly near the middle of the petiole, 1-2 x 1-2 mm; leaflets 1 pair, semi-succulent, green when young, purple when mature, margin purple to glaucous, obliquely obovate, 26-55 x 23-55 mm, apex obtuse, base cuneate, 5-8 palmately nerved, longest distance from margin to the main vein in the base 5-29 mm, smallest distance 2-3(-4) mm. **Racemes** 3-7 flowered, inflorescence axis 40-65 mm; bracts deltoid 1-1.2 mm long; bracteoles 0.8-1 mm long; pedicel purple to glaucous, 25-40 mm long. **Flower** 1.5-2 cm diam; sepals purple tinged with yellow, 9-12 x 5-7 mm; petals heteromorphic, two larger oblong to obovate, 15-28 x 11-21, two smaller obovate to orbicular, 11-19 x 7-15 and the cuculus falcate, bent around the stamens, 20-26 x 9-15 mm; stamens 8-10 mm long, filaments 1-3 mm long, anthers 5-7 mm long; ovary glabrous, 8-10 mm long, 8-14-ovulate, style 4-6 mm long. **Fruits** 55-90 x 10-15 mm. **Seeds** 7-8 x 4-5 mm. Fig. 6.

SELECTED EXAMINED SPECIMENS: BRAZIL. State of **Minas Gerais**: Diamantina, entre Rio Manso e Medanha, 28 Nov. 1973, *Mello Barreto* 10054 (SP); vale do Biribiri, 25 Feb. 1975, *Anderson* 11574 (MBM); estrada de Biribiri, próximo ao Rio sobre as pedras, 6 April 2004, *Andrade et al*. 546 (HUEFS, MBM, SPF); 25 km by road NE of Diamantina, 2 km W of rio Jequití, 9 April 1973, *Anderson* 8371 (UB); Cristais, morro do lado direito, 12 Sept. 2003, *Conceição et al*. 740 (CEPEC, HRB, HUEFS, SPF); vale do Biribiri, Serra acima do rio Biribiri, 12 Sept. 2003, *Conceição et al*. 738 (CEPEC, HRB, HUEFS, MBM); Biribiri, 24 March 1872 s.c (P); ca 8 km of Diamantina, road to Extração, 16 March 1970, Irwin *et al*. 27645 (UB). Grão Mogol, Rio Itacambiruçu, 21 April 1978, *Hatschbach* 41265 (MBM, SPF); arredores, 22 July 1978, *Hatschbach* 41479 (MBM); serra do Barão, 30 July 1980, *Faria* 97 (HRB, RB); estrada Grão Mogol-Cristália, 14 April 1981, *Pirani et al*. CFCR 911 (HUEFS, K,

SPF); vale do rio Itacambiruçu, 04 Sept. 1985, *Mello-Silva et al*. CFCR 8354 (HUEFS, K, SPF); próximo da Ponte sobre o rio das Mortes, beira do rio, 23 May 1982, *Giulietti et al.* CFCR 3557 (HUEFS, K, SPF); 12 April 1981, *Furlan et al*. CFCR 763 (K, SPF); margem do córrego à saída da cidade, na estrada para o rio Ventania, 25 Feb. 1986, *Menezes* CFCR 9626 (HUEFS, SPF); vale do rio Itacambiruçu, saindo de Grão Mogol c. 300 km depois da ponte do rio Itacambiruçu, 9 Sept. 2003, *Conceição et al*. 716 (CEPEC, HUEFS, RB, SPF, SP); c. 5 km North of Grão Mogol, 18 Feb. 1969, Irwin *et al*. 23493 (K, UB); estrada entre Grão Mogol e Cristália, 2 km da ponte sobre o rio Congonhas, 2 April 2005, *Souza et al.* 1107 (HUEFS).

DISTRIBUTION & HABITAT. This species is restricted to the Northern portion of the Espinhaço moutain range in the State of Minas Gerais at the municipalities of Grão Mogol and Diamantina. It occurs in campo rupestre vegetation on sandy gravelled soil, between 682 and 1250 m elevation. Map 3.

PHENOLOGY: It flowers from February to July and sets fruits from March to September.

Chamaecrista decora was described by Irwin & Barneby (1978) as a variety of *Cassia cytisoides*, later transferred to *Chamaecrista cytisoides* (Irwin & Barneby, 1982). However, it is here considered as a distinct species diagnosed by the petiolate leaves with one pair of obliquely obovate leaflets. Besides, genetic studies showed that *C. decora* presents inverted frequencies of EST-2, LAP-1, PGI-1 and PGM-2 alleles in relation to *C. cytisoides* (Conceição *et al., in press* c).

It is more similar to *Chamaecrista unijuga*, both sharing unijugate, petiolate leaves, with one extrafloral nectary placed on the petiole. *Chamaecrista decora* can be differentiated from this species by deflexed leaves (vs. ascending leaves in *C. unijuga*),

leaflets purple when mature (vs. leaflets green), obliquely obovate (vs. orbicular to reniform), and smallest distance from margin to the main vein at the base of the leaflets 2-3 (-4) mm (vs. 4-20 mm). Besides, *C. decora* is endemic to the campos rupestres of Northern Minas Gerais while *C. unijuga* is exclusive of coastal Restinga of the States of Pernambuco, Alagoas and Sergipes in Northeastern Brazil. Genetically, *C. decora* presents inverted frequencies of PGI-1 and PGM-1 alleles in relation to *C. unijuga* (*Conceição et al*., *in press* c).

6. ***Chamaecrista unijuga*** (Benth.) Conc., L.P.Queiroz & G.P.Lewis, **comb. et stat. nov.**

Cassia brachystachya var. *unijuga* Benth., Fl. Bras. (Martius) 15 (2): 139 (1870). *Cassia cytisoides* var. *unijuga* (Benth.) H.S.Irwin & Barneby, Mem. New York Bot. Gard. 30: 14 (1978). *Chamaecrista cytisoides* var. *unijuga* (Benth.) H.S.Irwin & Barneby, Mem. New York Bot. Gard. 35: 647 (1982). Lectotype (designated among the syntypes by Irwin & Barneby, 1978): "in maritimis prope Maceio prov. Alagoas", April 1848, *Gardner* 1282 (Lectotype: K!; isolectotype: BM!).

Shrub to small tree until 5 m high, profusely ramified from the base, c. 5 cm above the ground, with a rounded canopy, main stem brown with white stains, branches green, glabrous, with scattered and tiny trichomes, c. 1 mm long, present on the stipules, bracts and bracteoles. **Stipules** late caducous, 0.9-1 mm long. **Leaves** ascending, 3-8 cm long; petiole green, 9-24 mm long; pulvinus 1-3 mm diam, rachis absent, setae 0.8-1 mm long; extrafloral nectary 1, placed in the petiole, 1-5 x 1-2 mm; leaflets 1 pair, coriaceous, green, margin green, orbicular to reniform, 28-48 x 28-53 mm, apex obtuse,

base rounded to cordate, 5-9 palmately nerved, longest distance from margin to the main nervure in the base 8-25 mm, smallest distance 4-20 mm. **Raceme** 5-12-flowered, subcorymbose, inflorescence axis 45-80 mm; bracts deltoid to subulate, 1-1.5 mm long; bracteoles 0.8-1 mm long; pedicel green, 15-35 mm long. **Flower** 1.3-1.7 cm diam; sepals yellowish green, 9-12 x 5-7 mm; petals heteromorphic, two larger oblong to obovate, 16-26 x 12-18 mm, two smaller obovate to orbicular 15-19 x 9-14 mm and the cuculus falcate, bent around the stamens, 20-23 x 8-12 mm; stamens 5-8 mm long, filaments 1-2 mm long, anthers 5-6 mm long; ovary glabrous, 7-12 mm long, 8-13-ovulate; style 5-10 mm long. **Fruits** 50-80 x 5-15 mm. **Seeds** immature. Fig. 7.

SELECTED EXAMINED SPECIMENS. **BRAZIL.** State of **Pernambuco**: Prazeres, s.d., *Pickel* s.n. (IPA); Piedade, 29 April 1950, *Andrade-Lima* 484 (IPA); rio Formoso, praia de Guadalupe, 1986, *Coelho* s.n. (IPA). State of **Sergipe**: Pirambu, 13 Aug. 1974, *Fonseca* 01 (HRB, RB); estrada Pirambu-São José da Caatinga, c. 8.2 km saindo de Pirambu, 5 Sept. 2004, *Conceição et al*. 820 (CEPEC, HUEFS, MBM, SPF, SP). Santo Amaro das Brotas, rodovia SE 226, próximo ao rio Pomonga, 16 Nov. 1991, *Farney & Bezumord* 2833 (RB); restingas ao lado do rio Pomonga, 5 April 2004, *Ribeiro & Moraes* 504 (HUEFS); c. 2 km da ponte do rio Pomonga, sentido Pirambu, 19. Aug. 2003, *Conceição et al*. 694 (CEPEC, HRB, HUEFS, MBM).

DISTRIBUTION & HABITAT. *Chamaecrista unijuga* is restrict to coastal areas of Northeastern Brazil, in the states of Sergipe and Pernambuco. It occurs in restinga scrub and forest vegetations near the shore line, on sandy soil, in elevations close to sea level. Map 3.

PHENOLOGY: It flowers from September to December and set fruits between October and January.

Chamaecrista unijuga was described as a variety of *Cassia brachystachya* by Bentham (1870). Later, Irwin & Barneby (1978) included it in the variation of their widened defined *Cassia cytisoides*. It is considered here as a distinct species based on petiolate leaves with leaflets 1 pair, orbicular to reniform, 28-48 x 28-53 mm, apex obtuse, base rounded to cordate. Besides, it is well differentiated from a genetic standpoint showing exclusive alleles in PGM-2 and EST-2 loci and absence of the locus EST-3 (*Conceição et al., in press* c).

It is more similar to *Chamaecrista decora* but they are widely allopatric and present distinct vegetative and genetic characters as previously mentioned in the discussion of *Chamaecrista decora*.

7. ***Chamaecrista coriacea*** (Benth.) H. S.Irwin & Barneby, Mem. New York Bot. Gard. 35: 647 (1982).

Cassia coriacea Bong. ex Benth., J. Bot. (Hooker) 2: 78 (1840). Type: "Inter Serra del frio et Cachoeira, Herb, Acad. Petrop.", *Riedel* 563, 01 Jan. 1825. (Holotype: LE [photo K!]; isotypes: A, K! [photos: HUEFS!, IPA!]).

Subshrub procumbent 0.1-0.2 m, profusely ramified from the base, branches assurging form an underground xylopodium; main branches brown, younger branches purple, almost glabrous, with scattered and tiny trichomes, c. 1 mm long, present on the stipules, bracts and bracteoles. **Stipules** persistent, 1-2.5 mm long. **Leaves** ascending, 1-5.5 cm long; petiole green, 5-20 mm long, pulvinus 1-2 mm diam., rachis, when present, 6-20 mm long, interfoliolar segment, when present, 8-9 mm long, setae 1-2 mm long; extrafloral nectary 1, placed near the middle of the petiole, 1-2 extrafloral nectaries on

the rachis of the 2-3-jugate leaves, 1-3 x 0.9-1mm; leaflets 1-2 (-3) pairs, coriaceous, green, margin olivaceous to purple, the proximal par resembling the distal par, both widely oblanceolate to obovate, 14-30 x 9-16 mm, apex rounded, sometimes apiculate, base rounded to cuneate, 4-5-palmately nerved, longest distance from margin to the main vein in the base 2-5 mm, smallest distance 1-2 mm, margin raised. **Raceme** 1-3 flowered, inflorescence axis 40-65 mm; bracts subulate to oblanceolate, 1-1.2 mm long; bracteoles 2-3 mm long; pedicel olivaceous, 10-30 mm long. **Flower** 1.5-1.8 cm diam; sepals yellow tinged purple at the middle, 9-13 x 4-7 mm; petals heteromorfic, two larger orbicular to suborbicular, 13-26 x 9-18 mm, two smaller orbicular to obovate, 10-21 x 7-15 mm and the cuculus falcate, bent around the stamens, 12-22 x 8-13 mm; stamens 5-9 mm long, filaments 1-3 mm long, anthers yellow, 4-5 mm long; ovary glabrous, 6-8 mm long, 4-9-ovulate, style 5-7 mm long. **Fruits** 35-55 x 5-6 mm. **Seeds** immature. Fig. 8.

SELECTED EXAMINED SPECIMENS: BRAZIL. State of **Minas Gerais**, Conceição do Mato Dentro, Costa Sena, Guritiba de Cima, c. 10.6 km saindo de Costa Sena, sentido a fazenda Cachoeira, 18 Feb. 2005, *Conceição et al*. 869 (BM, CEPEC, HUEFS, K, MBM, MO, NY, P, RB, SP, SPF).

DISTRIBUTION & HABITAT. This species has a very narrow range in Northern portion of the Espinhaço range in Minas Gerais, near the municipalities of Diamantina. It occurs only in campos rupestres vegetation with gravelled sandy soil, c. 1187 m elevation. Map 4.

PHENOLOGY: It flowers from December to February and sets fruits between January and March.

Chamaecrista coriacea can be recognised by habit procumbent 0.1-0.2 m and leaves with 1-2 (-3) pairs of leaflets widely obovate to oblanceolate, with sometimes apiculate.

Within its range, it could be confused with *C. depauperata* a species of the Southern Chapada Diamantina in Bahia from which *C. coriacea* differs by leaflets widely obovate to oblanceolate (vs. leaflets orbicular to suborbicular in *C. depauperata*), larger flowers (1.5-1.8 vs. 0.5-1.0 cm diam.) or still with semiprostrate morphs with unijugate leaves of *C. blanchetii*, from which *C. coriacea* differs by petiolate leaves (vs. sessile or short petiole leaves).

It is a rare plant and is known only by the type, collected by Riedel in 1825, and a second collection made 180 years later by one of us (ASC) probably at the same area. It shows a high genetic divergence from other species of the *Baseophyllum* group, presenting five exclusive alleles in PGI-1, PGM-1, PGM-2, EST-1 e EST-3 (Conceição *et al., in press* c).

8. ***Chamaecrista depauperata*** Conc., L.P.Queiroz & G.P.Lewis, Sitientibus Sér. Ci. Biol. 1(2): 114 (2001). Type: Brazil, Bahia, Município de Abaíra, distrito de Catolés, Campo do Virassaia, 30 Dec 1993, *W. Ganev* 2722 (Holotype: HUEFS!; isotypes: K!, SPF!).

Subshrub procumbent to prostate 0.1-0.2 m high, with few branches radiating from an underground xylopodium; main stem brown, branches purple, almost glabrous, with scattered and tiny trichomes, c. 1 mm long, present on the stipules, bracts and bracteoles. **Stipules** persistent, 1-3 mm long. **Leaves** ascending, 2.5-4 cm long; petiole purple, 10-15 mm long, pulvinus 1-3 mm diam., rachis absent, setae 1.5-4 mm;

extrafloral nectary 1, placed on the petiole, 1-3 x 1-1.3 mm; leaflets 1 pair, chartaceous, purple when young, green when mature with purple margin, orbicular to suborbicular, 15-30 x 10-30 mm, apex obtuse, base rounded, 6-9 palmately nerved, longest distance from the margin to main vein in the base 5-10 mm, smallest distance 2-5 mm. **Raceme** (1-) 2-4-flowered, inflorescence axis 19-35 mm long; bracts deltoid, 1-1.5 mm long; bracteoles 1-2 mm long; pedicel purple, 12-15 mm long. **Flower** 0.5-1.0 cm diam; sepals yellow tinged with purple at the middle, 6-7 x 2-3 mm; petals almost isomorphic, with three larger obovate to oblong 6-14 x 4-8 mm and two smaller suborbicular to obovate, 6-13 x 3-7 mm; stamens 4-7 mm long, filaments 1-3 mm long, anthers 3-4 mm long; ovary glabrous, 2-6-ovulate, 3-5 mm long, style 3-4 mm long. **Fruits** 25-40 x 4-9 mm. **Seeds** 4-6 x 4-5 mm. Fig. 9.

SELECTED EXAMINED SPECIMENS: BRAZIL. State of **Bahia**: Abaíra, Riacho da Taquara, 3 Feb. 1992, *Stannard et al.* H 51142 (paratype: K); Catolés, Virassaia, 16 Dec. 2003, *Conceição et al.* 775 (HUEFS). Mucugê, Paty de Fora, Parque Nacional da Chapada Diamantina, 6 Feb. 1997, *Conceição et al.* 372 (paratypes: HUEFS, SPF); Paty de fora, Gerais do Rio Preto, 2 Feb. 2005, *Conceição* 863 (HUEFS); Serra do Esbarrancado, 16 March 2005, *Cardoso & Conceição* 433 (HUEFS).

DISTRIBUTION & HABITAT. *Chamaecrista depauperata* is recorded only from the Southern portion of the Chapada Diamantina mountain range, at the municipalities of Abaíra and Mucugê. It occurs in campos rupestres with gravelled sandy soil, in elevations between 1500 to 1530 m. Map 4.

PHENOLOGY: It flowers from December to February and sets fruits between January and March.

Chamaecrista depauperata differs from others taxa of sect. *Baseophyllum* by its smaller flowers, with almost isomorphic petals and ovary 2-6-ovulate. Regarding the habit, *C. depauperata* is similar to *C. coriacea* and semiprostrate morphs with unijugate leaves of *C. blanchetii,* for both displayed habit prostrate to procumbent. However, *C. depauperata* does not happen in sympatry with *C. coriacea* and can be differentiated from the cited taxa by ovary with fewer ovules (2-6 vs. 8-12 ovules) and several morphological characters and genetic, mentioned in the discussion of *C. coriacea.*

Studies of genetic variability in the *Baseophyllum* group demonstrated that *C. depauperata* is very different from all others taxa of this group since it presents two exclusive alleles in PGM-1 and LAP-1 (Conceição *et al*., *in press* c).

Acknowledgements

Thanks are due to the Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) for financial support (#245/04). ASC received fellowships from the Universidade do Estado da Bahia (PAC-UNEB) and Margaret Mee Fellowship programme at the Royal Botanic Gardens, Kew. LPQ was supported by a grant (PQ2) from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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Table 1. Synopsis of the taxonomic history of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum*.

Colladon (1816)	Bentham (1840)	Bentham (1870, 1871)	Irwin & Barneby (1978)	Irwin & Barneby (1982)
Sect. *Baseophyllum*	Sect. *Baseophyllum*	Sect. *Apoucouita*	Sect. *Absus*	Sect. *Absus*
		Ser. *Baseophyllae*	Subsect. *Baseophyllum*	Subsect. *Baseophyllum*
	Cassia coriacea	*Cassia coriacea*	*Cassia coriacea*	***Chamaecrista coriacea***
Cassia cytisoides	*Cassia cytisoides*	*Cassia cytisoides*	***Cassia cytisoides***	***Chamaecrista cytisoides***
			var. *cytisoides*	var. *cytisoides*
	Cassia blanchetii	***Cassia blanchetii***	var. *blanchetii*	var. *blanchetii*
	Cassia brachystachya	***Cassia brachystachya***	var. *brachystachya*	var. *brachystachya*
		Cassia brachystachya var. *unijuga*	var. *unijuga*	var. *unijuga*
			var. *confertiformis*	var. *confertiformis*
			var. *decora*	var. *decora*
			var. *micrantha*	var. *micrantha*
			Sect. *Absus*	Sect. *Absus*
			Subsect. *Absus*	Subsect. *Absus*
		Cassia crenulata	***Cassia crenulata***	***Chamaecrista crenulata***
		Cassia vauthieri	***Cassia vauthieri***	***Chamaecrista vauthieri***
			Sect. *Apoucouita*	Sect. *Apoucouita*
	Cassia polystachya	***Cassia polystachya***	***Cassia polystachya***	***Chamaecrista polystachya***

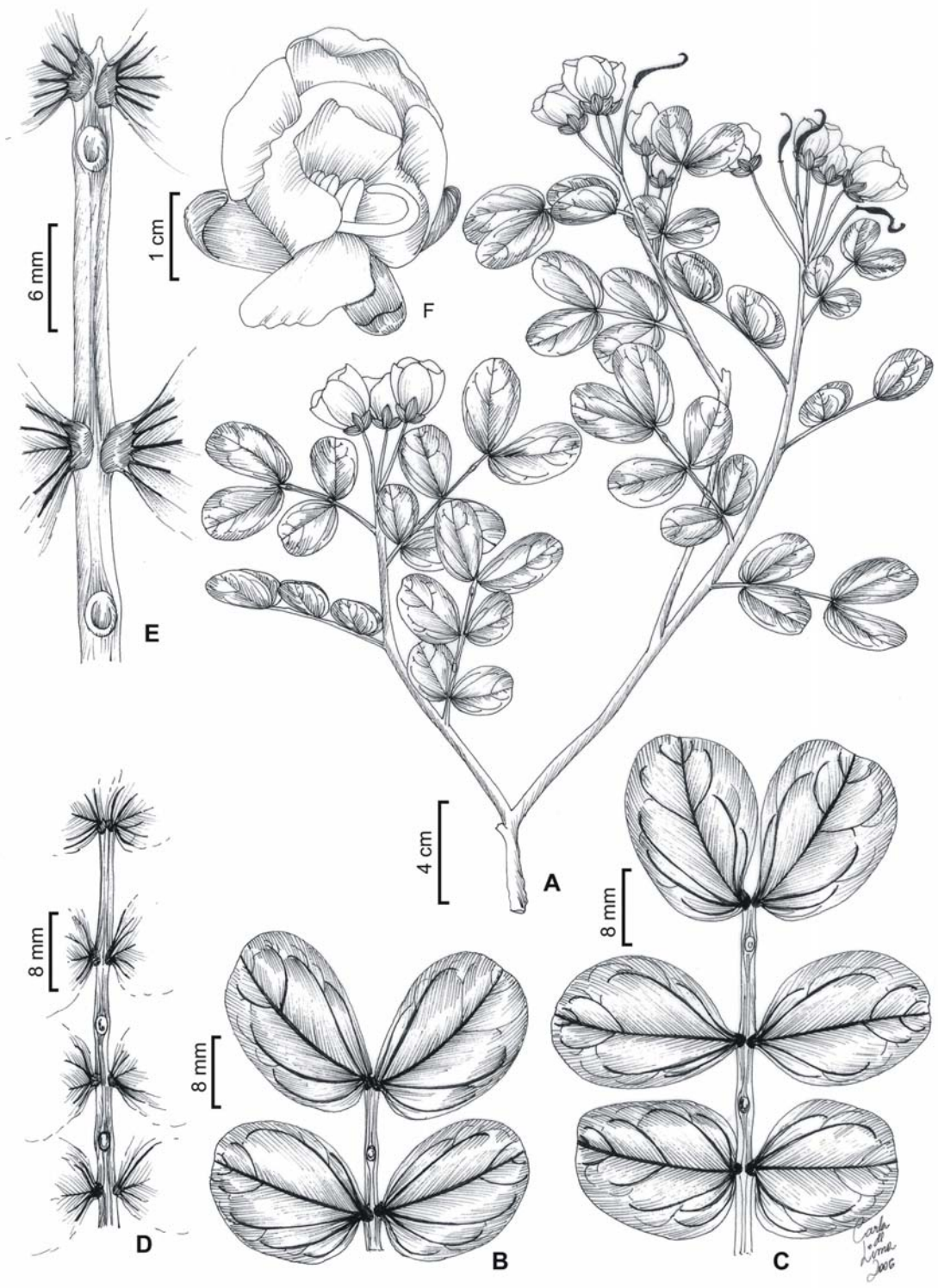


Fig. 1. *C. cytisoides*. **A** habit; **B-C** leaves; **D-E** details of extrafloral nectaries; **F** flower from A.S. Conceição *et al.* 870.

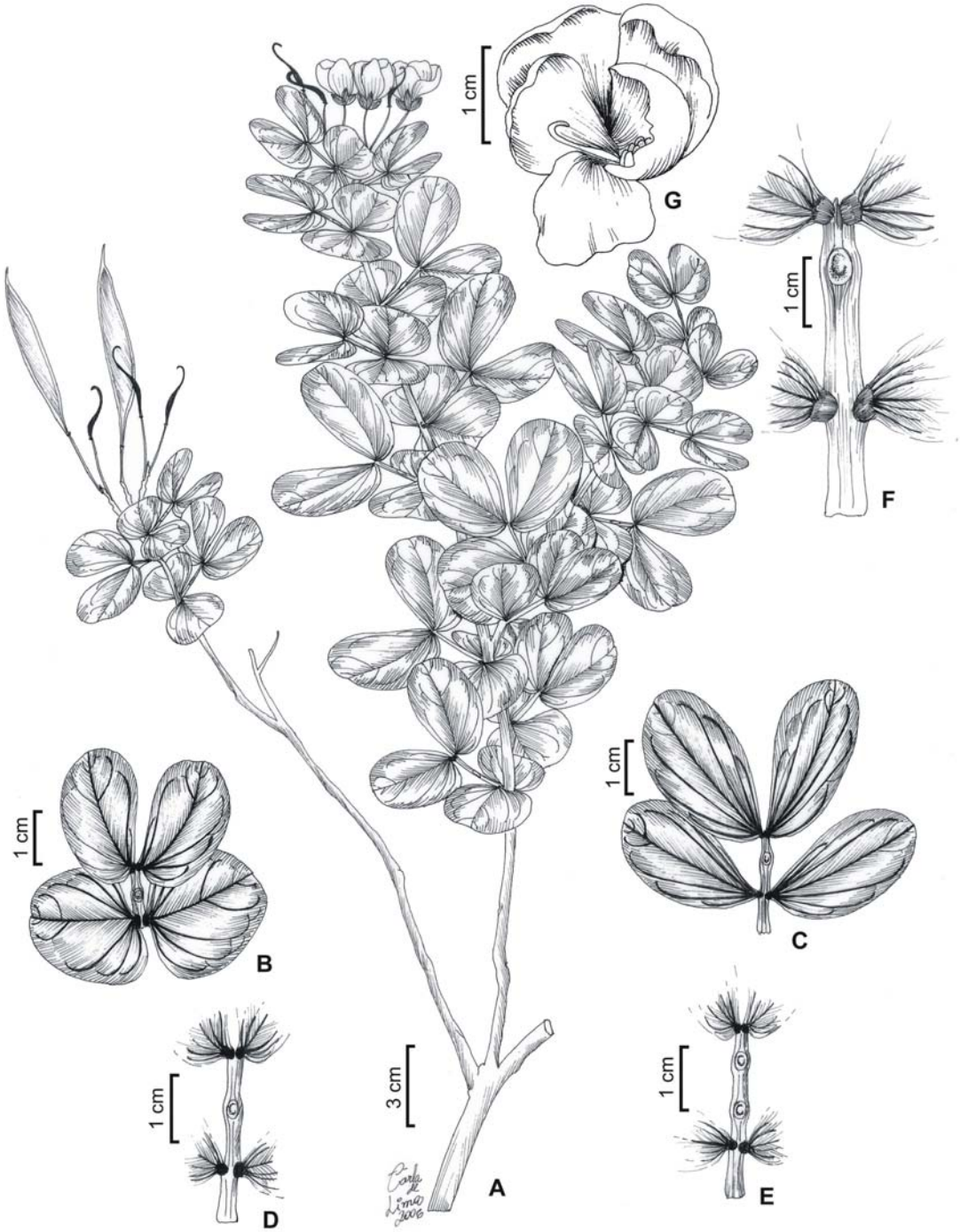


Fig. 2. *C. brachystachya*. **A** habit; **B-C** leaves; **D-F** details of extrafloral nectaries; **G** flower from A.S. Conceição *et al.* 540.

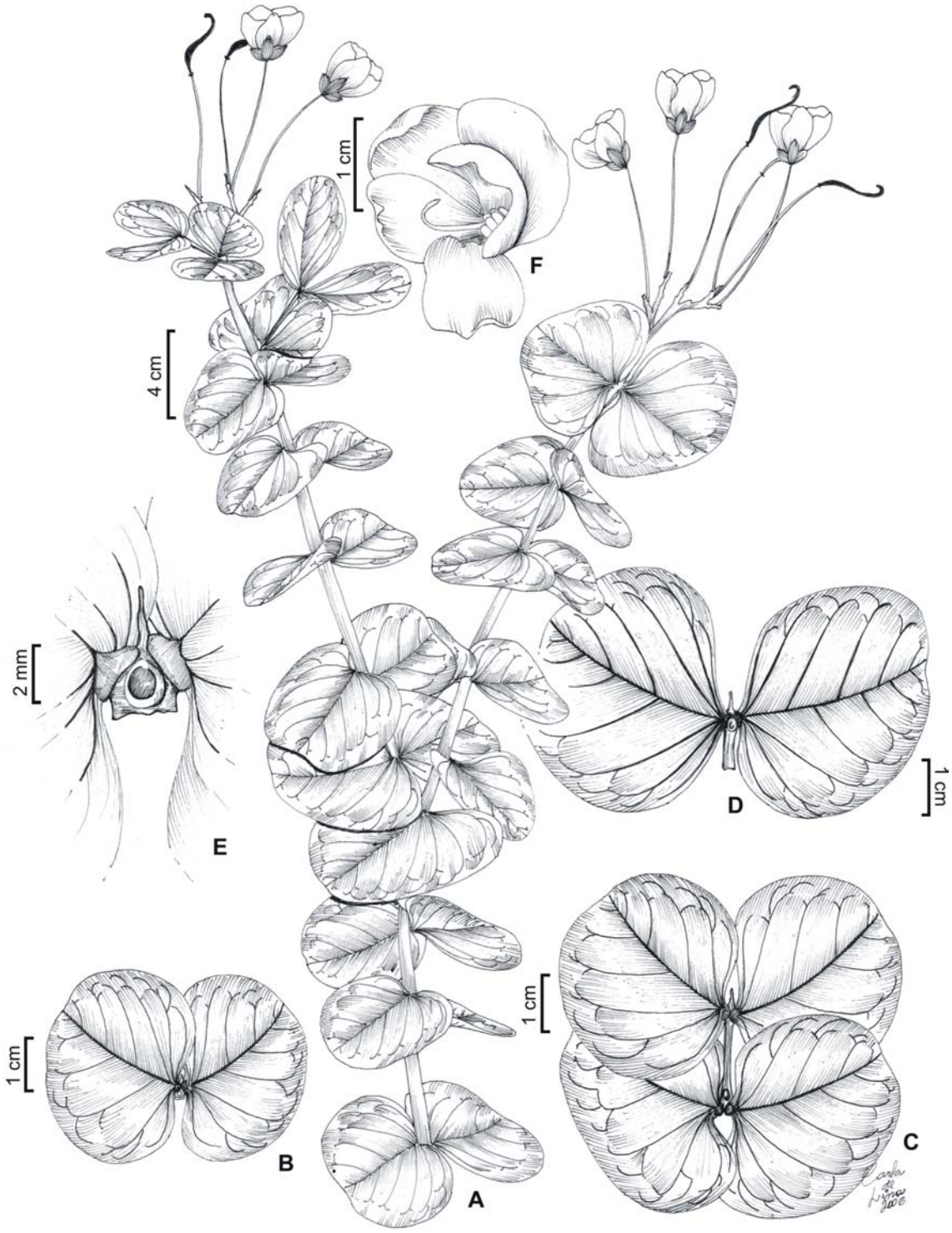


Fig. 3. *C. blanchetii*. **A** habit; **B-D** leaves; **E** detail of extrafloral nectary; **F** flower from A.S. Conceição *et al.* 672.

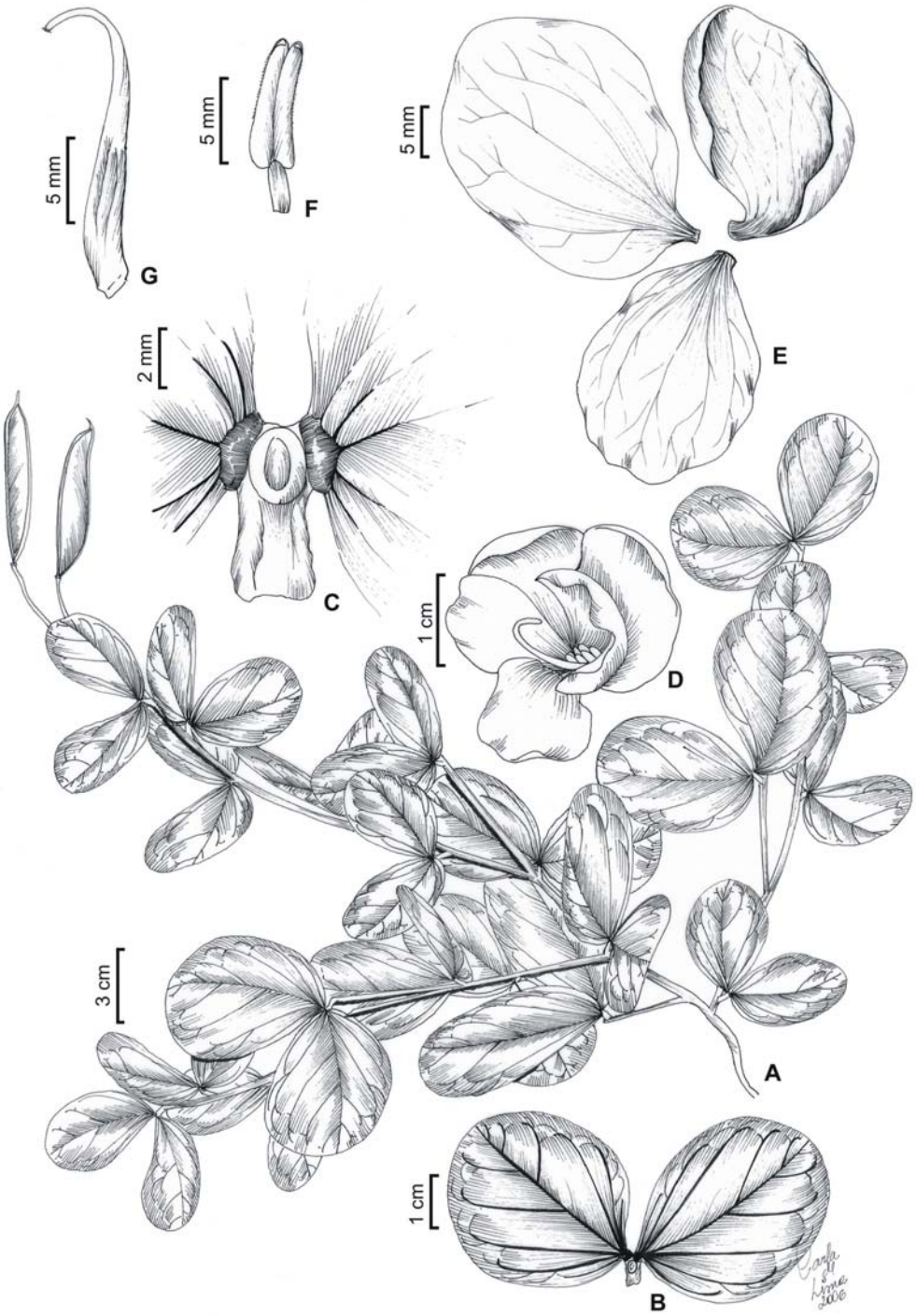


Fig. 4. *C. blanchetii*. **A** habit procumbent; **B** leaf; **C** detail of extrafloral nectary; **D** flower; **E** petals; **F** stamen; **G** pistil from A.S. Conceição *et al.* 611.

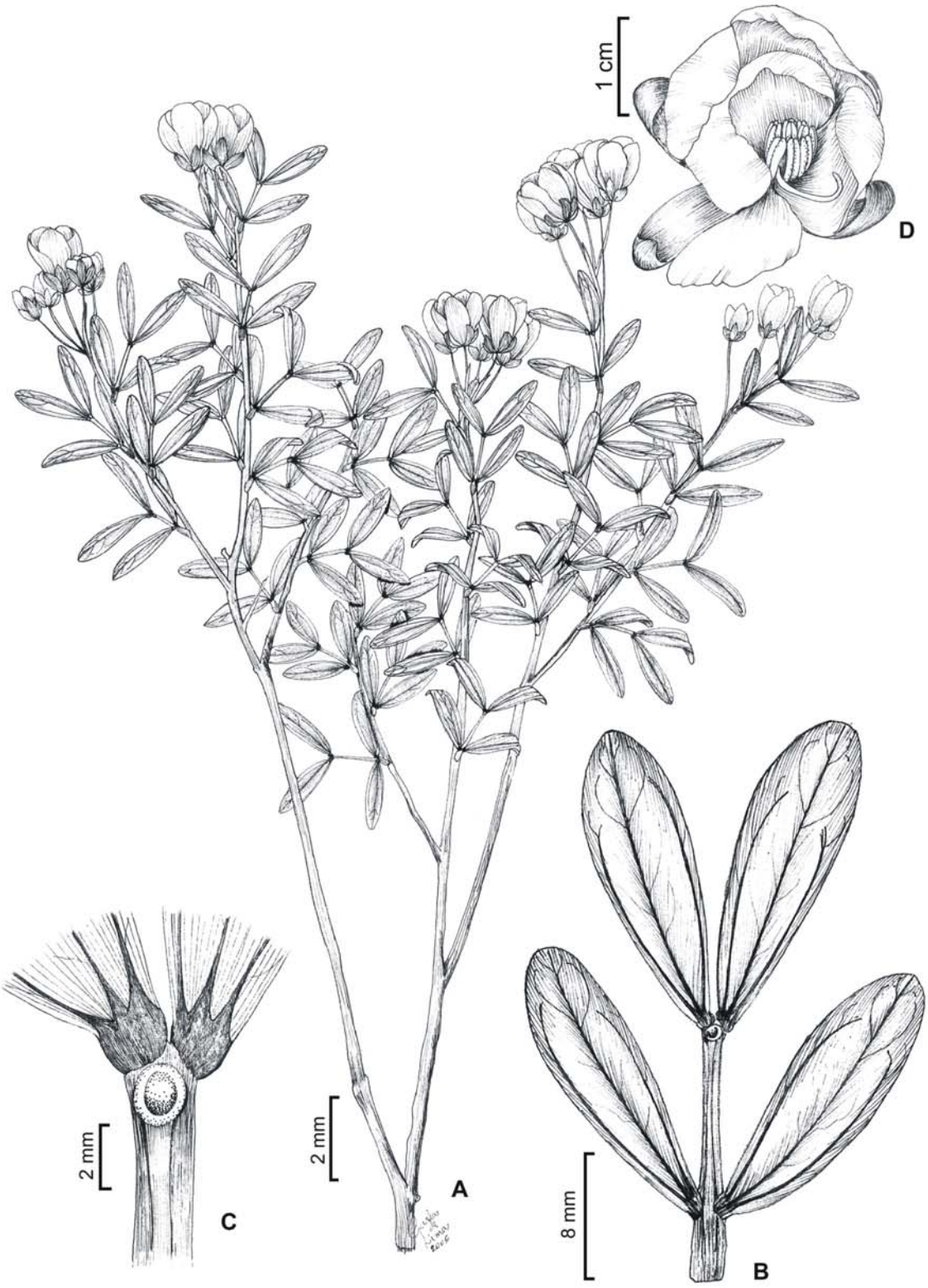


Fig. 5. *C. confertiformis*. **A** habit; **B** leaf; **C** detail of extrafloral nectary; **D** flower from A.S. Conceição *et al.* 775.

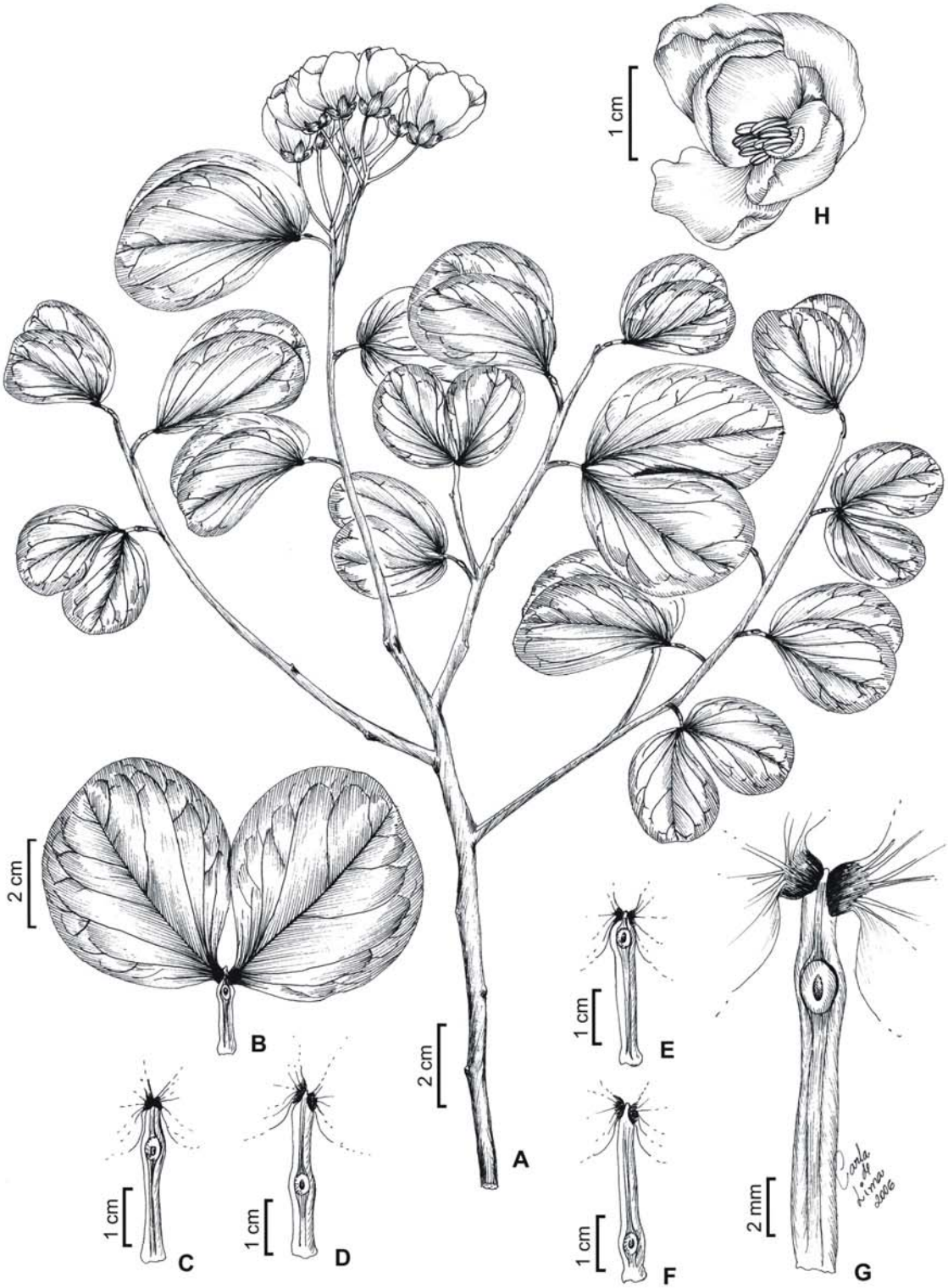


Fig. 6. *C. decora*. **A** habit; **B** leaf; **C-G** details of extrafloral nectaries; **H** flower from A.S. Conceição *et al.* 740.

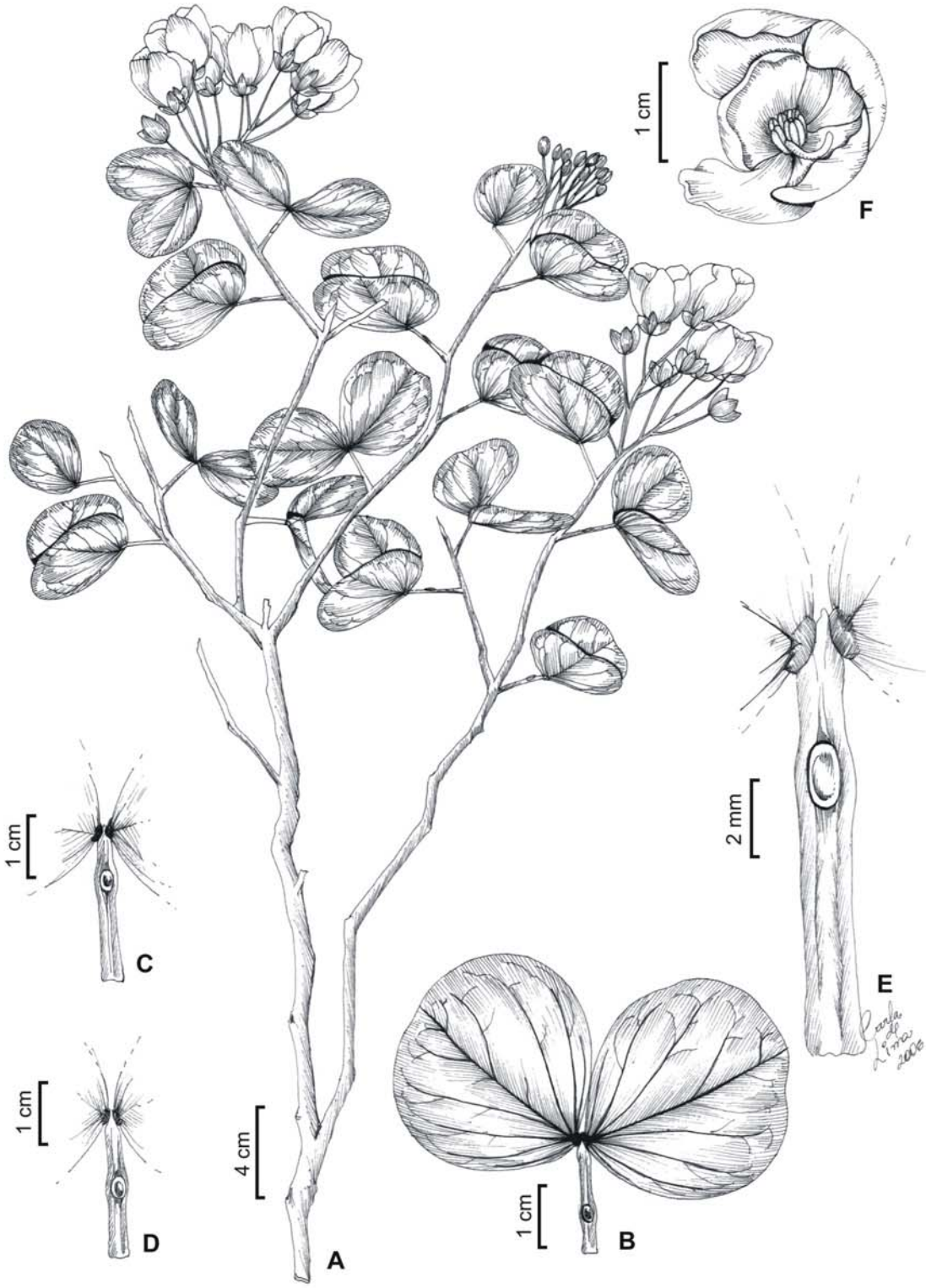


Fig. 7. *C. unijuga*. **A** habit; **B-** leaf; **C-E** details of extrafloral nectaries; **F** flower from A.S. Conceição *et al.* 820.

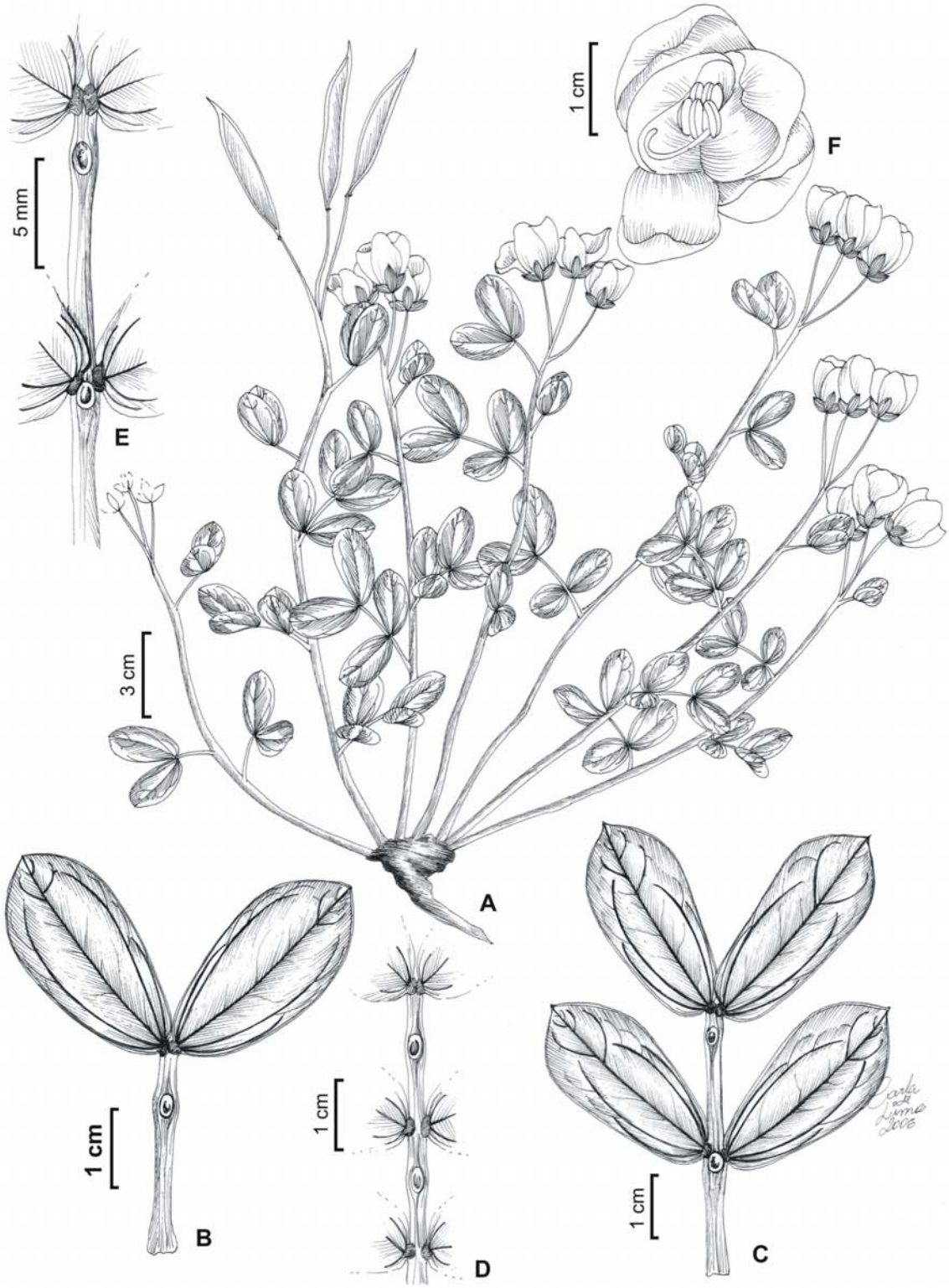


Fig. 8. *C. coriacea*. **A** habit ; **B-C** leaves; **D-E** detail of extrafloral nectaries; **F** flower from A.S. Conceição *et al.* 869.

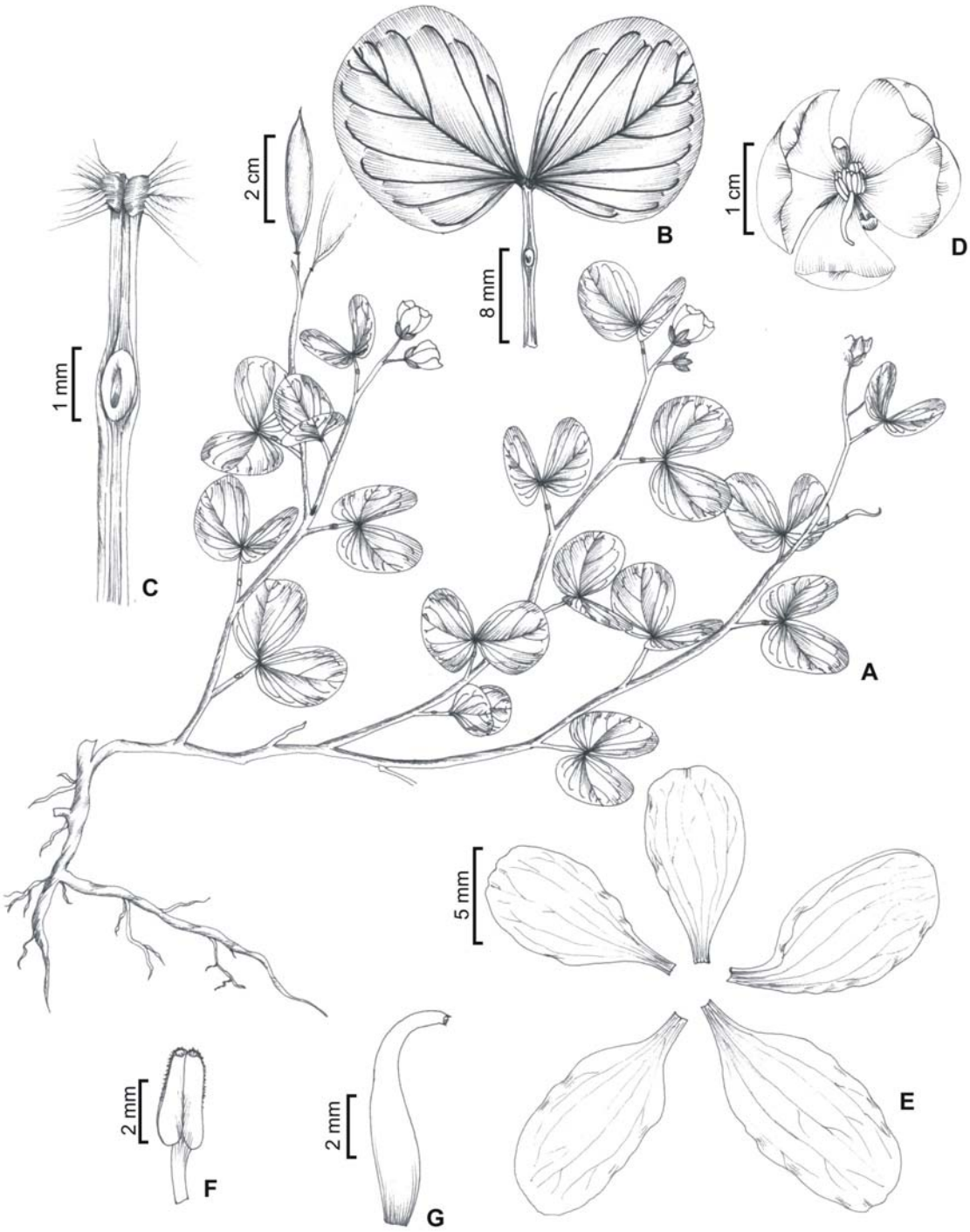
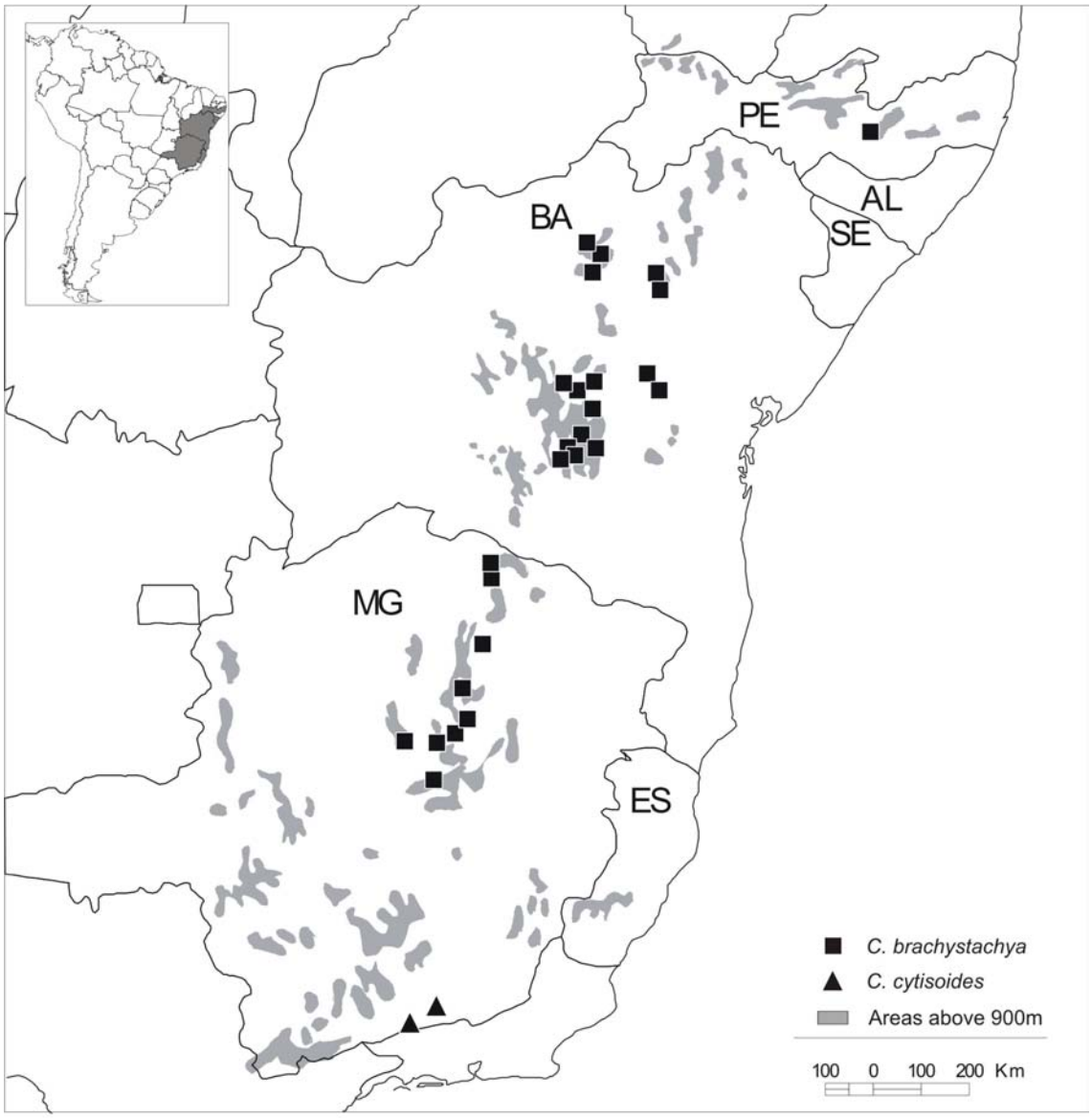
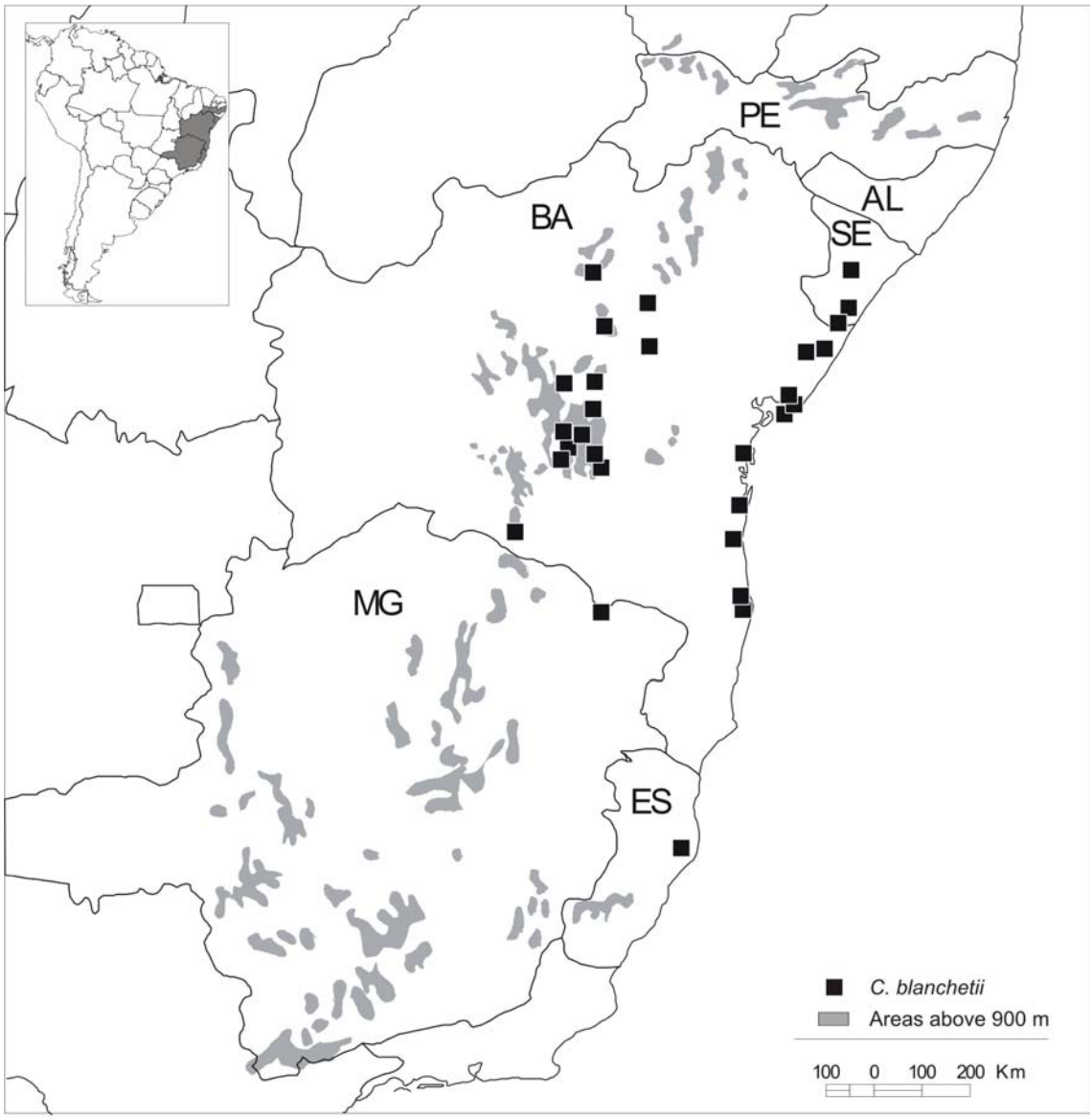


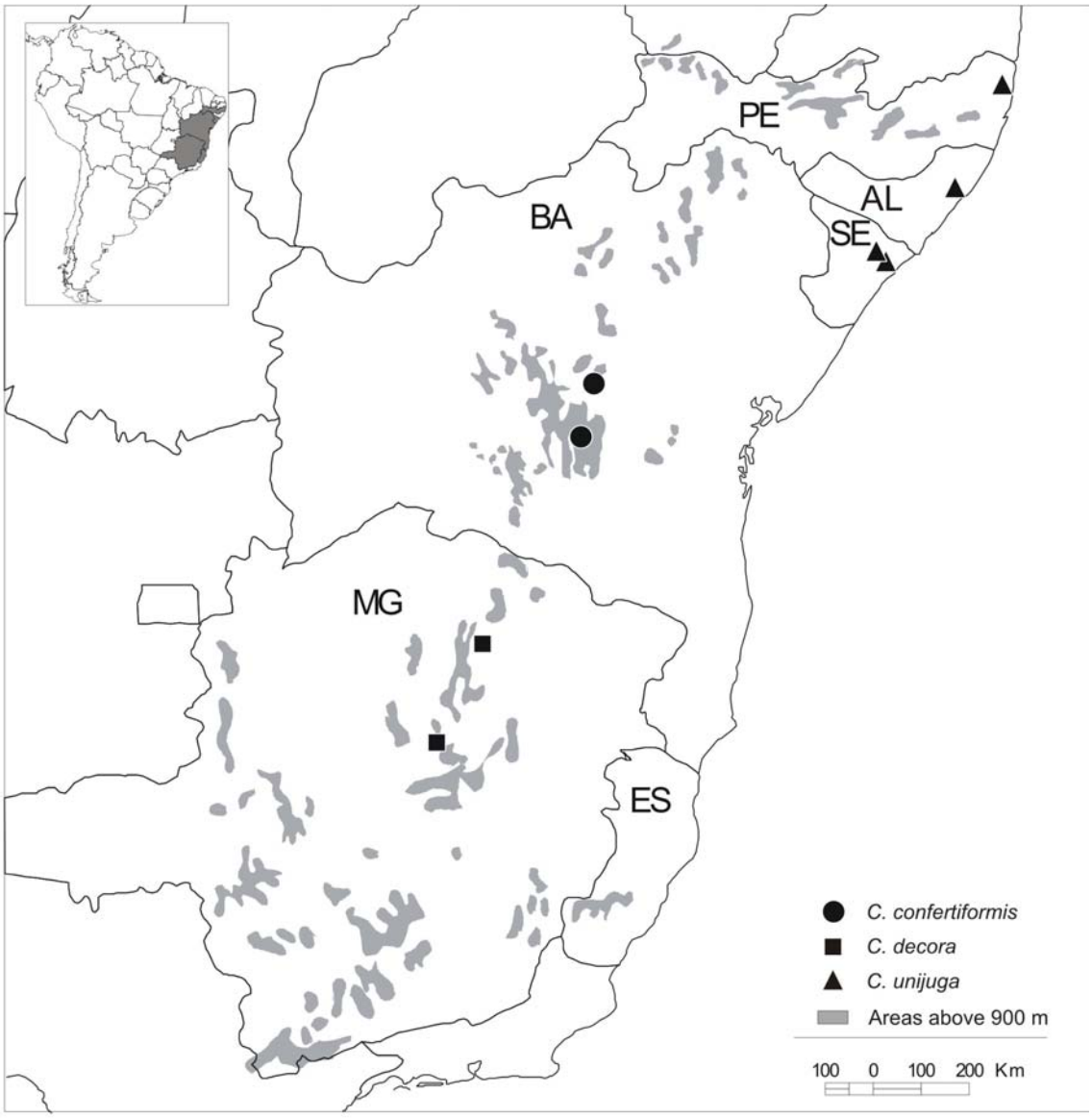
Fig. 9. *C. depauperata*. **A** habitt; **B** leaf; **C** detail of extrafloral nectary; **D** flower; **E** petals; **F** stamen; **G** pistil from A.S. Conceição *et al.* 775.



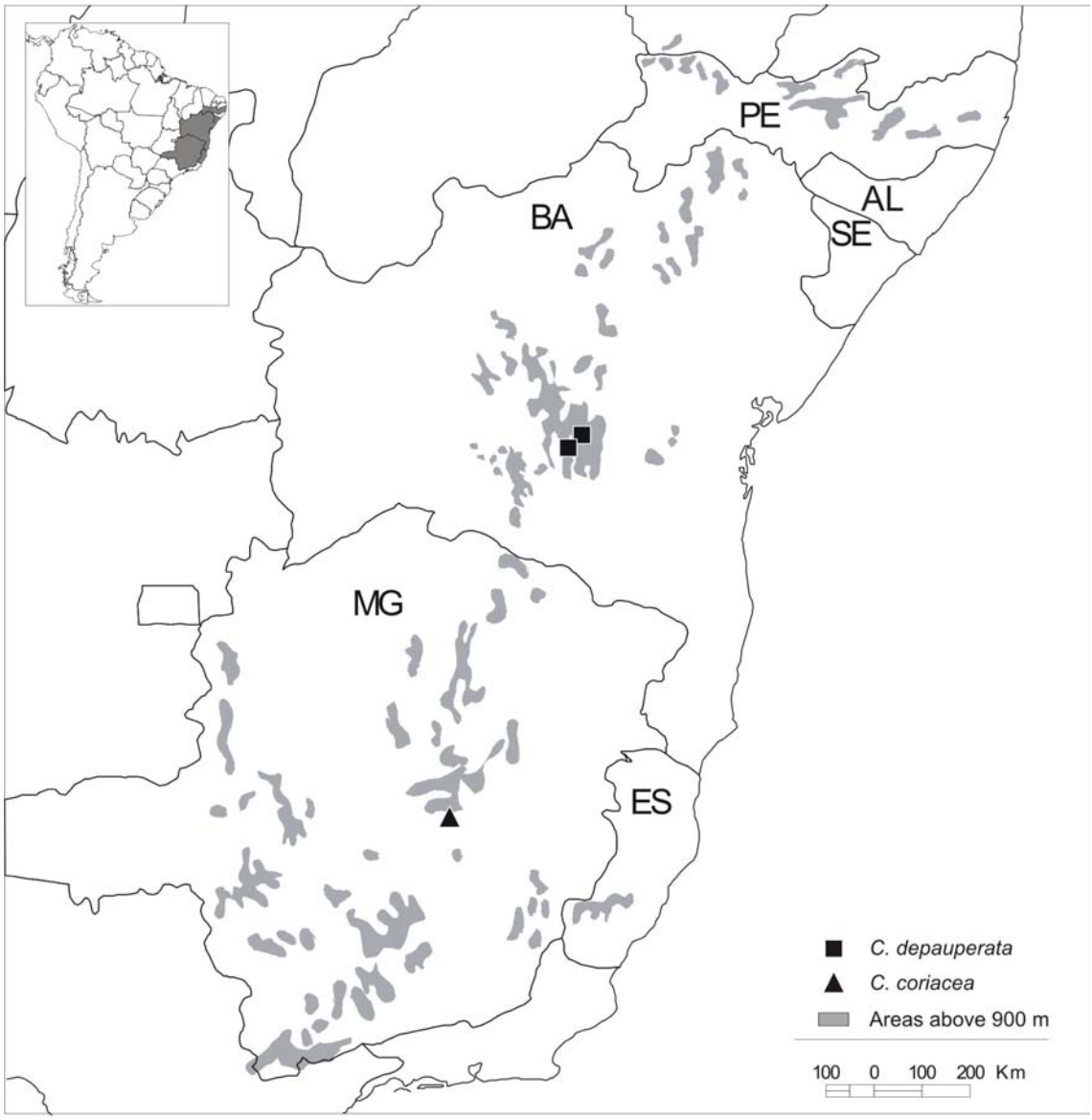
Map. 1. Distributions of *C. brachystachya* and *C. Cytisoides*.



Map. 2. Distribution of *C. blanchetii*.



Map. 3. Distributions of *C. confertiformis, C. decora* and *C. unijuga*.



Map. 4. Distributions of *C. depauperata* and *C. coriacea*.

CONCLUSÕES GERAIS

Como base nos resultados das análises genéticas, morfológicas e filogenéticas chegamos as seguintes conclusões:

1. *Chamaecrista* subseção *Baseophyllum* é um grupo monofilético. No entanto, as análises filogenéticas mostraram que o *Baseophyllym* é um mais relacionado com um clado herbáceo que agrupa as seções *Caliciopsis*, *Chamaecrista* e *Xerocalyx.* Com base nesses resultados propomos elevar a subseção *Baseophyllym* para o nível de seção.

2. *Chamaecrista* seção *Baseophyllum* inclui oito espécies: *C. blanchetii* (Benth.) Conc., L.P.Queiroz & G.P.Lewis, *C. brachystachya* (Benth.) Conc., L.P.Queiroz & G.P.Lewis*, C. confertiformis* (H.S.Irwin & Barneby) Conc., L.P.Queiroz & G.P.Lewis*, C. coriacea* (Benth.) H. S. Irwin *&* Barneby*, C. cytisoides* (Collad.) H.S Irwin & Barneby*, C. decora* (H.S. Irwin & Barneby) Conc., L.P.Queiroz & G.P.Lewis*, C. depauperata* Conc., L.P. Queiroz & G.P. Lewis*, C. unijuga* (Benth.) Conc., L.P.Queiroz & G.P.Lewis. *Chamaecrista cytisoides* var. *micrantha* foi sinonimizada a *C. brachystachya*.

3. Todos os táxons de *Chamaecrista* sect. *Baseophyllum* apresentam baixa variabilidade genética dentro de suas populações. A baixa variabilidade genética deve estar associada ao comportamento do polinizador e aos padrões de distribuição geográfica das espécies desse grupo.

4. Hibridização natural ocorre entre três espécies gênero *Chamaecrista*: *C. blanchetii* x *C. confertiformis* e *C. confertiformis* x *C. depauperata*. Esse processo deve ser favorecido pela ocorrência de simpatria entre esses pares de espécies, compartilhamento dos mesmos polinizadores e sobreposição de período de floração.

5. O gênero *Chamaecrista* é um grupo monofilético. No entanto, as relações de parentesco entre *Chamaecrista* e os demais gêneros da subtribo Cassiinae (*Cassia* e *Senna*) não estão bem definidas.

6. As seções *Apoucouita* e *Xerocalyx* são grupos monofiléticos. A seção *Absus* é parafilética em relação ao um clado herbáceo que inclui representantes das seções *Chamaecrista*, *Caliciopsis* e *Xerocalyx*. A seção *Chamaecrista* é parafilética em relação às seções *Xerocalyx* e *Caliciopsis*.

7. Dentro da seção *Chamaecrista*, as séries *Coriaceae* e *Chamaecrista* parecem ser grupos monofiléticos, enquanto a série *Prostratae* aparece polifilética. Na subseção *Absus*, a série *Absoideae* e *Rigidulae* aparecem parafiléticas.

8. As análises filogenéticas sugerem que os padrões de diversificação em *Chamaecrista* ocorreram inicialmente pela mudança de árvores de florestas úmidas para um grupo mais diverso de arbustos de cerrado.

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