

Anatomical interpretations of the taxonomy of *Chamaecrista* (L.) Moench sect. *Absus* (Leguminosae–Caesalpinioideae)

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Abstract *Chamaecrista* (L.) Moench (Caesalpinioideae–Leguminosae) with 330 species is organized into six sections: *Apoucouita*, *Absus*, *Grimaldia*, *Chamaecrista*, *Caliciopsis* and *Xerocalyx*. Section *Absus* is the largest section of the *Chamaecrista* and is organized into four subsections, viz., subsect. *Adenophyllum*, subsect. *Baseophyllum*, subsect. *Otophyllum* and subsect. *Absus*. This section is not monophyletic and has a complex taxonomy. This study aims to anatomically characterize 60 taxa of *Chamaecrista*, identifying meaningful morphoanatomical characters that may shed light on the evaluations of the taxonomic and phylogenetic framework of *Chamaecrista*, especially *C. sect. Absus*. Standard light microscopy techniques were used to anatomically characterize the leaves. The anatomical data were subjected to a multivariate analysis (Jacard index). Epidermal papillae on the abaxial side, the

distribution and types of secretory and tector trichomes, variations in the arrangement of the vascular system of the midvein and the type of arrangement of the vascular system of the petiole are useful for separating species or groups of species. The presence and type of secretory structure, the type and position of stomata and the venation pattern are important in establishing the affinities between sections and subsections. The phenetic analysis demonstrates that the anatomical data contribute to the establishment of affinities between sections and subsections, supporting the elevation of the taxonomic status of the clade *Baseophyllum* to section and the insertion of *C. absus* (*C. sect. Grimaldia*) into *C. sect. Absus*, corroborating molecular studies.

Keywords *Chamaecrista* sect. *Absus* · *Chamaecrista* sect. *Caliciopsis* · *Chamaecrista* sect. *Chamaecrista* · *Chamaecrista* sect. *Grimaldia* · *Chamaecrista* sect. *Xerocalyx* · Leaf anatomy · Secretory structures · Taxonomy

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Introduction

Chamaecrista (L.) Moench is a genus that commonly grows in open sunny areas such as rocky fields (Fig. 1a), *cerrados* and *restingas* and primarily contains subshrubs/shrubs (Fig. 1b, c) (Conceição et al. 2001, 2009; Lewis 2005; Rando and Pirani 2011). The genus has ~330 spp from which ~270 spp. are found in the New World (Lewis 2005). *Chamaecrista* is divided into six sections and the species found in the New World are distributed as follows: *Chamaecrista* sect. *Absus* (ca. 180 species), *C. sect. Apoucouita* (ca. 20 species), *C. sect. Caliciopsis* (ca. 2 species), *C. sect. Chamaecrista* (ca. 50 species), *C. sect.*

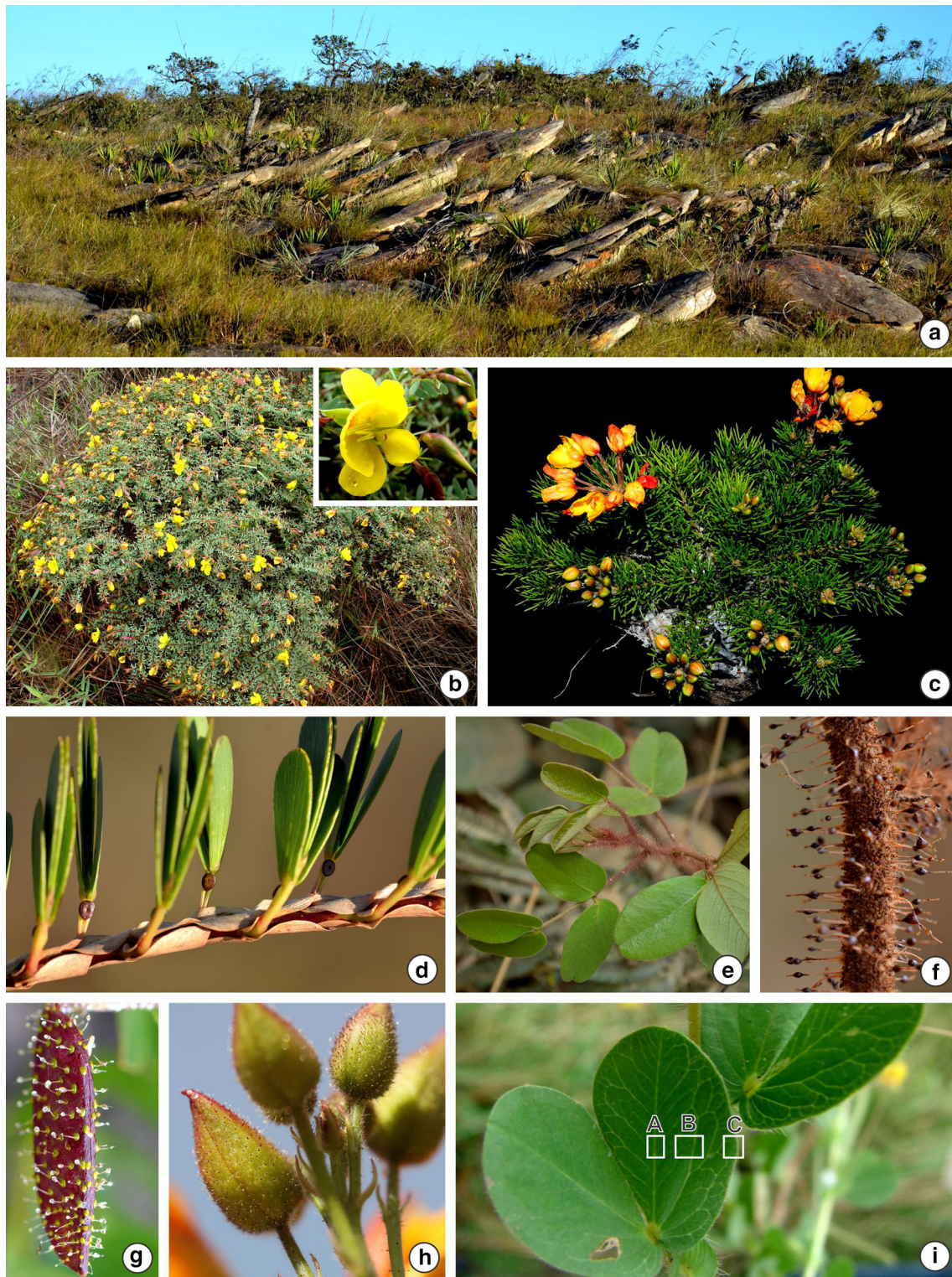


Fig. 1 *Chamaecrista* species occurring in rocky fields in Brazil. **a** General view of a Brazilian rocky field. **b** *C. ramosa*. **c** *C. ericifolia*. **d** Extrafloral nectaries in *C. desvauxii*. **e–g** Sticky glandular hairs: on the leaves of *C. setosa* (**e**), on the stem of *C. setosa* (**f**), on the fruit of

C. semaphora (**g**), and on the inflorescence of *Chamaecrista* sp. (**h**). **i** *C. rotundifolia* var. *rotundifolia* (A, B and C indicate the middle vein, margin and area between the margin and middle vein, respectively)

Grimaldia (01 species) and *C.* sect. *Xerocalyx* (ca. 03 species) (Irwin and Barneby 1982; Conceição et al. 2009). Molecular studies (Conceição et al. 2009) have confirmed the monophyly of the genus *Chamaecrista* but clearly show that most of the sections that were proposed by Irwin and Barneby (1982) do not correspond to natural groups.

Chamaecrista sect. *Absus* is the largest section of *Chamaecrista* and is organized into four subsections: namely, subsect. *Adenophyllum*, subsect. *Baseophyllum*, subsect. *Otophyllum* and subsect. *Absus* (Irwin and Barneby 1978, 1982). According to the molecular phylogeny (Conceição et al. 2009), for sect. *Absus* to be monophyletic, it would be necessary to include the sect. *Grimaldia* (monospecific) and to exclude the subsect. *Baseophyllum*, which must be raised to the sectional level. Additionally, the molecular phylogeny of *Chamaecrista* (Conceição et al. 2009) does not provide enough evidence to support the 31 series proposed for *C.* sect. *Absus* subsect. *Absus* (~170 species) (Irwin and Barneby 1978, 1982) as a limited number of series and species belonging to *C.* sect. *Absus* subsect. *Absus* has been sampled.

Anatomy has proven useful in solving taxonomical problems for several families, especially when leaf characters are used (Solereeder 1908; Metcalfe and Chalk 1950, 1979; Rudall 1993; Diane et al. 2003; Coutinho et al. 2013; Guimarães et al. 2013; Dalvi et al. 2014). In the subfamily Caesalpinioideae, anatomical studies highlight the importance of secretory structures in distinguishing among between close genera as well as in establishing species boundaries (Lersten and Curtis 1993, 1994, 1996; Rudall et al. 1994; Coutinho et al. 2013). In *Chamaecrista* and *Senna*, secretory structures are useful for understanding the evolution of clades at the infrageneric level (Marazzi et al. 2006, 2013; Conceição et al. 2009).

Based on the type of secretory structure, species of the genus *Chamaecrista* may be grouped into two categories: (1) species with EFNs present (Fig. 1d) and sticky glandular hairs absent, represented by the four sections, viz., sect. *Apoucouita*, sect. *Caliciopsis*, sect. *Chamaecrista* and sect. *Xerocalyx* (Conceição et al. 2009), and (2) species lacking EFNs but with such sticky glandular hairs present (Fig. 1e–h), represented by the sections such as sect. *Absus* and sect. *Grimaldia*.

Considering the taxonomical issues in *Chamaecrista* sect. *Absus* and the importance of anatomical studies as additional tools for the taxonomy of *Chamaecrista*, the following questions were raised: (1) do morphoanatomical data support the promotion of *C.* sect. *Absus* subsect. *Baseophyllum* to the sectional status that was proposed by Conceição et al. (2009)? (2) are there morphoanatomical affinities between *C.* sect. *Absus* and the monotypic *C.* sect. *Grimaldia* (*C. absus*)? (3) can anatomy aid in the resolution of the arrangement in *C.* sect. *Absus* subsect. *Absus*? This

study aims to anatomically characterize 60 taxa of *Chamaecrista*, identifying meaningful morphoanatomical characters that may shed light on the evaluations of the taxonomic and phylogenetic framework of *Chamaecrista* (Conceição et al. 2009).

Materials and methods

Species sampling and processing

For this study, 54 species of *Chamaecrista* sect. *Absus* belonging to the *C.* sect. *Absus* subsect. *Absus* (45 spp.), subsect. *Baseophyllum* (8) and subsect. *Otophyllum* (1) were sampled (Online Resource 1). The morphoanatomical characters of species from *C.* sect. *Absus* subsect. *Baseophyllum* were taken from Coutinho et al. (2013). For comparisons, six species from the *C.* sect. *Chamaecrista* (4), sect. *Grimaldia* (1) and sect. *Xerocalyx* (1) were included (Online Resource 1). Taxonomic authorities for all taxa mentioned in the paper (including the figures) are given in the Online Resource 1.

Vegetative and reproductive samples from both field-collected and herbarium materials were used (Online Resource 1). Field-collected material was deposited in the herbarium of the Universidade Federal de Viçosa (VIC) with duplicates sent to the herbarium of the Universidade Estadual de Feira de Santana (HUEFS) and herbarium of the Universidade Estadual da Bahia-Campus Paulo Afonso (HUNEB). These materials were fixed in FAA (formaldehyde, acetic acid and 50 % ethanol; 1:1:18 by volume) and stored in 70 % ethanol (Johansen 1940). The herbarium samples were rehydrated (Smith and Smith 1942) and stored in 70 % ethanol.

During field expeditions, when the secretion of the petiolar extrafloral nectaries was evident, samples of exudates from *C. debilis* and *C. desvauxii* var. *langsдорffii* were randomly blotted on a urine test strip (Alamar Tecnica Científica Ltda., São Paulo, Brazil) for glucose detection.

For structural characterization, fragments from the middle portion of both the leaflet (including the middle vein, margin and area between the margin and the middle vein) (Fig. 1i) and petiole of fully expanded leaves were taken. Both field-collected and herbarium materials were used in an attempt to achieve at least three replicates for each taxon.

The fragments were dehydrated through an ethanol series and embedded in methacrylate resin (Historesin Leica, Leica Microsystems Nussloch GmbH, Heidelberg, Germany) according to the manufacturer's recommendation. Cross and longitudinal sections that were 5 µm thick were made using an automatic rotary microtome (Leica RM2155, Deerfield, IL, USA) with a glass knife. The

sections were stained with toluidine blue at pH 4.4 (O'Brien and McCully 1981) and dried at room temperature. Permanent slides were mounted in synthetic resin (Permout, Fisher Scientific, NJ, USA).

Whole leaflets were cleared with 10 % sodium hydroxide and 20 % sodium hypochlorite and interspersed with distilled water, rinsing at least three times (Johansen 1940, modificado). The cleared leaflets were dehydrated in an ethanol series up to 50 %, stained with 0.1 % basic fuchsin that was diluted in 50 % ethanol, rehydrated and mounted in glycerin jelly (Johansen 1940). Epidermal dissociations were made following the Jeffrey method, and the fragments were stained with a mixture of 2 % astra blue and 1 % safranin and mounted in glycerin jelly (Johansen 1940).

For the scanning electron microscopy (SEM) analysis, the samples that were stored in 70 % ethanol were subjected to critical point drying using CO₂ (CPD 020; Bal-Tec, Balzers, Liechtenstein) and sputter coated with gold (FDU 010; Bal-Tec, Balzers, Liechtenstein). The analyses were performed and documented using SEM (Leo 1430 VP model; Zeiss, Cambridge, England).

Slide analyses and image captures were carried out with a light microscope (Olympus model AX70TRF; Olympus Optical, Tokyo, Japan) that was equipped with a U-Photo System and digital camera (Spot Insightcolour 3.2.0, Diagnostic Instruments Inc., New York, USA). Observations under a stereomicroscope (Olympus SZX7, Japan) were also made.

The following classifications were used: for stomata, Howard (1979); for venation pattern, Hickey (1973); for the arrangement of the petiole vascular system, Wilkinson (1979); and for trichomes, Theobald et al. (1979).

Screening and analysis of taxonomical characters

A total of 76 morphoanatomical characters were selected (Table 1) and binary coded (0 = absent, and 1 = present) and plotted in a matrix of species vs. morphoanatomical characters (Online Resource 2) in Microsoft Excel 2010. A similarity analysis was conducted using software PC-ORD version 5.10 (MjM Software, Gleneden Beach, Oregon, USA). A dendrogram was constructed based on the Jaccard index.

Results

Anatomical characters

The studied taxa of *Chamaecrista* displayed a single-layered epidermis (Fig. 2a–e). In cross sections of leaflets, differences in the height of the epidermal cells from the adaxial and abaxial sides were observed (Table 1; Online

Resource 2). In 16 taxa, the epidermal cells of the adaxial side were higher than those of the abaxial side, as in *C. setosa* (Fig. 2a) and *C. cathartica* var. *paucijuga* (Fig. 2b).

Epidermal papillae (Fig. 2a) were found in only 24 of the studied taxa (Table 1; Online Resource 2), of which 19 were restricted along the middle vein (Table 1; Online Resource 2). In *C. salvatoris* and *C. setosa* (Fig. 2a), papillae were found only on the abaxial side, while in *C. glaucofilix* and *C. hedysaroides*, they were only found on the adaxial side.

The epidermal cell walls were less thick than the cell height (slightly thickened) in 21 taxa (Table 1; Online Resource 2), as in *C. cathartica* var. *paucijuga* (Fig. 2b) and *C. neesiana* (Fig. 2c). In contrast, 31 taxa had outer epidermal cell walls that were thicker than the cell height (or at least as thick as), such as in *C. dalbergifolia* (Fig. 2d) and *C. botryoides* (Fig. 2e).

In the frontal view, the contour of the epidermal cell walls varied from sinuous (Fig. 2f) to straight (Fig. 2g). Most of the species (43 spp.) displayed a straight contour of the epidermal cell walls on both sides of the leaflets (Table 1; Online Resource 2). A sinuous contour of the epidermal cell walls on both sides was observed in 12 species; while in five species, the contour was straight on the adaxial side and sinuous on the abaxial side (Table 1; Online Resource 2). In cross sections, most species of the *Chamaecrista* sect. *Absus* subsect. *Absus* (37 out of 45 spp.) displayed secretory idioblasts on the epidermis (Table 1; Online Resource 2). Such idioblasts, when stained by toluidine blue, displayed a dense purple coloration, as in *C. setosa* (Fig. 2a) and *C. neesiana* (Fig. 2c).

All of the studied species displayed paracytic–laterocyclic stomata (Fig. 2f, g) or modifications of such type. In addition to the paracytic–laterocyclic type of stomata, nine species also had the anisocytic type (Fig. 2g). Six out of the eight species of the *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* had modifications in the paracytic–laterocyclic type, resulting in stomata with 3–6 subsidiary cells. Amphistomatic leaves (Fig. 2b, c, e) were observed in 45 taxa, hypostomatic leaves (Fig. 2a) in five species, amphihypostomatic leaves in three species (Online Resource 2), and epistomatic leaves only in seven species of *C.* sect. *Absus* subsect. *Baseophyllum* (Table 1; Online Resource 2).

The presence of secretory (Fig. 3a–f) and tector trichomes (Fig. 3g–i) was common for *Chamaecrista* and variations, especially regarding the morphology and distribution of such structures. Both types of trichomes could be found together, as in *C. sophoroides* (Fig. 3a). Secretory trichomes were exclusive to *C.* sect. *Absus* subsect. *Absus* (except for *C. conferta*, *C. ochracea* var. *latifolia*, *C. ochracea* var. *purpurascens*, and *C. vauthieri*) and *C. absus*, the only in the species of *C.* sect. *Grimaldia* (Table 1; Online Resource 2). Such typical secretory trichomes

Table 1 Anatomical characters of species of the *Chamaecrista* sect. *Absus* and the related sections that were used in the phenetic analysis (Jaccard index)

| No. | Anatomical characters used |
|-----|---|
| 01 | Epidermal cells of the adaxial side of the leaflets at least two times higher than the cells from the abaxial side |
| 02 | Epidermal cells with similar heights on both the adaxial and abaxial sides of the leaflets |
| 03 | Epidermal papillae on the abaxial side of the leaflets at the midvein only |
| 04 | Epidermal papillae on the abaxial side of the leaflets |
| 05 | Epidermal papillae on the adaxial side of the leaflets |
| 06 | Mucilage idioblasts in the epidermis on both sides of the leaflets |
| 07 | Mucilage idioblasts in the palisade parenchymas of the adaxial side of the leaflets |
| 08 | Mucilage idioblasts in the hypodermis of the abaxial side of the leaflets |
| 09 | Mucilage idioblasts in the spongy parenchyma |
| 10 | Outer epidermal cell walls thick (thickness of the wall at least as high as the cell height) |
| 11 | Outer epidermal cell walls mildly thick (thickness of the wall less than 1/2 of the cell height) |
| 12 | Anticlinal wall of the epidermal cells was sinuous on both sides of the leaflets |
| 13 | Anticlinal wall of the epidermal cells was straight on both sides of the leaflets |
| 14 | Anticlinal wall of the epidermal cells was straight on the adaxial side of the leaflets and sinuous on the abaxial side |
| 15 | Secretory trichomes on both vegetative and reproductive organs |
| 16 | Secretory trichomes on reproductive organs only |
| 17 | Secretory trichomes on both sides of the leaflets |
| 18 | Secretory trichomes restricted to the abaxial side of the leaflets |
| 19 | Secretory trichomes restricted to the leaflet margins |
| 20 | Secretory trichomes scattered throughout the leaflet blade on both sides |
| 21 | Secretory trichomes scattered throughout the leaflet but restricted to the abaxial side |
| 22 | Tector trichomes restricted to the lower leaflet blades |
| 23 | Uniseriate tector trichomes |
| 24 | Short uniseriate tector trichome |
| 25 | Stellate trichomes |
| 26 | Secretory trichomes with a short stalk and a spherical/oblong head |
| 27 | Secretory trichomes with a short stalk and a slightly dilated head |
| 28 | Filiform projection shorter than the basal portion (stalk along with secretory head) of the secretory trichome |
| 29 | Filiform projection longer than the basal portion (stalk along with secretory head) of the secretory trichome |
| 30 | Filiform projection absent |
| 31 | Basal portion (stalk along with secretory head) with elongated cells (similar to tector trichomes) on the secretory head |
| 32 | Dorsiventral mesophyll |
| 33 | Isobilateral mesophyll |
| 34 | Amphistomatic leaflets |
| 35 | Hypostomatic leaflets |
| 36 | Amphihypostomatic leaflets |
| 37 | Epistomatic leaflets |
| 38 | Paracytic–laterocytic stomata |
| 39 | Anisocytic stomata |
| 40 | Modified paracytic–laterocyclic stomata |
| 41 | Prominent midvein on both sides of the leaflets |
| 42 | Prominent midvein on the abaxial side of the leaflets |
| 43 | Mildly prominent midvein |
| 44 | Midvein with open-arch collateral vascular bundles |
| 45 | Midvein with open-arch collateral vascular bundles and two smaller bundles turned to the adaxial side of the leaflets |
| 46 | Midvein with open-arch collateral vascular bundles and three to four smaller bundles turned to the adaxial side of the leaflets |
| 47 | Midvein with fiber caps on both sides of the leaflets |
| 48 | Midvein surrounded by fibers |

Table 1 continued

| No. | Anatomical characters used |
|-----|--|
| 49 | Palisade parenchymas of the adaxial side of the leaflets continuous to the midvein |
| 50 | Lateral veins with sclerenchymatous bundle sheath extension |
| 51 | Petiole vascular arrangement Type I |
| 52 | Petiole vascular arrangement Type II |
| 53 | Petiole vascular arrangement Type III |
| 54 | Petiole vascular arrangement Type IV |
| 55 | Petiole vascular arrangement Type V |
| 56 | Petiole vascular arrangement Type VI |
| 57 | Petiole vascular arrangement Type VII |
| 58 | Petiole vascular arrangement Type VIII |
| 59 | Petiole vascular arrangement Type IX |
| 60 | Petiole vascular arrangement Type X |
| 61 | Petiole vascular arrangement Type XI |
| 62 | Petiole vascular arrangement Type XII |
| 63 | Petiole vascular arrangement Type XIII |
| 64 | Brochidodromous venation |
| 65 | Actinodromous venation |
| 66 | Actinodromous venation at the leaf base and camptodromous–brochidodromous towards the leaflet margin |
| 67 | Conspicuous areoles |
| 68 | Incomplete areoles |
| 69 | Areoles absent |
| 70 | Enlarged tracheoids at the vein endings |
| 71 | Simple tracheoids (not enlarged) at the vein endings |
| 72 | Extrafloral nectaries present |
| 73 | Concave extrafloral nectaries present |
| 74 | Convex extrafloral nectaries present |
| 75 | Sessile extrafloral nectaries |
| 76 | Stalked extrafloral nectaries |

displayed a short stalk, a secretory spherical multiseriate head and a multiseriate apical filiform projection, continuous to the secretory head, as in *C. bracteolata* (Fig. 3c) and *C. campestris* (Fig. 3f). The filiform projection varied in length, being longer than the basal portion (stalk along with secretory head) of the secretory trichome, as in *C. bracteolata* (Fig. 3c), and shorter as in *C. semafora* (Fig. 3d). Such filiform projection was not observed only in *C. hedyaroides*, *C. punctulifera* (Fig. 3e) and *C. strictifolia*. The secretory head also varied in shape, being spherical/oblong (Fig. 3a, c–e) or slightly dilated, as in *C. campestris* (Fig. 3f). In *C. aurivilla* (Fig. 3b) and *C. setosa*, elongated cells (similar to tector trichomes) on the secretory head were observed (Table 1; Online Resource 2).

Most of the taxa (42 spp.) had secretory trichomes on both vegetative and reproductive organs (Table 1; Online Resource 2). Only in *C. cotinifolia*, *C. lundii*, *C. paniculata* and *C. multipennis* were secretory trichomes restricted to reproductive organs. On the leaflets, 25 taxa displayed

secretory trichomes on both surfaces and seven on the abaxial side only, while three were restricted to the margin (Table 1; Online Resource 2).

Three types of tector trichomes (Table 1; Online Resource 2) were observed in all four of the studied sections of *Chamaecrista* (Fig. 3g–i). Long uniseriate tectores (e.g., more than two times the ordinary epidermal cell height) occurred in most species, such as in *C. trichopoda* (Fig. 3g); short uniseriate tectores (e.g., up to two times the ordinary epidermal cell height) occurred in *C. atroglandulosa*, *C. semaphora* (Fig. 3h) and *C. strictifolia*; and stellate trichomes occurred only in *C. aurivilla* (Fig. 3i) and *C. paniculata*. Tector trichomes occurred on both sides of the leaflet blade in 27 species, only on the lower leaflet blade in 19 species and only on the abaxial side in four species (Table 1; Online Resource 2).

Dorsiventral (Fig. 2a, b, e) and isobilateral (Fig. 2c) mesophylls were found in 29 and in 31 taxa, respectively (Table 1; Online Resource 2). Although *C. dalbergifolia*

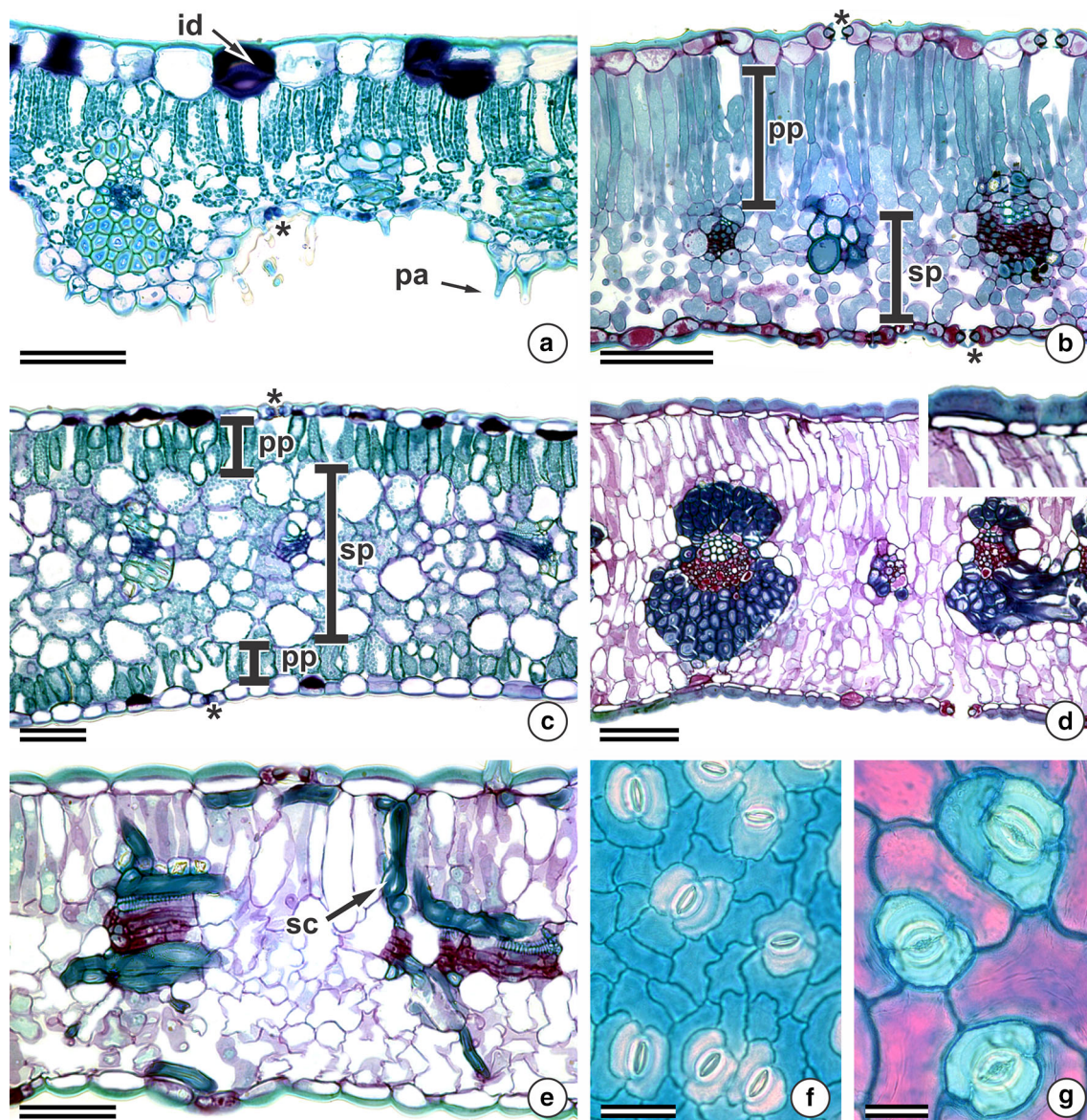


Fig. 2 Leaflet blades of *Chamaecrista* species in cross sections (a–f) and clearings (f–g). **a** Epidermal cells from the adaxial side were higher than the abaxial side, papillae (pa) in the abaxial side, hypostomatic leaflets (asterisk = stomata) and mucilage idioblasts (id) in *C. setosa*. **b** The cell wall was mildly thickened, with amphistomatic leaflets and a dorsiventral mesophyll in *C. cathartica* var. *paucijuga*. **c** Isobilateral mesophyll in *C. neesiana*. **d** Thickened

outer epidermal cell wall (detail) and isobilateral mesophyll with the spongy parenchyma not conspicuous in *C. dalbergifolia*. **e** Sclereids (sc) in the mesophyll of *C. botryoides*. **f** Sinuous contour of the epidermal cells and paracytic–laterocyclic stomata in *C. atroglandulosa*. **g** Straight contour of the epidermal cells and anisocytic stomata in *C. botryoides*. pp palisade parenchyma, sp spongy parenchyma. Scale bars a–f 100 μ m, g–i 50 μ m

(Fig. 2d) and *C. rupestrium* displayed isobilateral mesophyll, the spongy parenchyma was not conspicuous (Fig. 2d). All of the species of the *C. sect. Absus* subsect. *Baseophyllum* displayed a hypodermis on the abaxial side. Sclereids were randomly observed in the mesophyll of *C. botryoides* only (Fig. 2e). Mucilage idioblasts in the epidermis were counted in 39 of the 60 species studied, while mucilage idioblasts in the mesophyll (either in the palisade and/or spongy parenchyma or in the hypodermis) were found only in the species of the

C. sect. Absus subsect. *Baseophyllum* (Table 1; Online Resource 2).

Prominent middle veins in the cross section of the leaflets only on the abaxial side (Fig. 4a) were observed in 28 taxa, while the remaining species, except for *C. urophyllidia*, showed little or no prominence of the middle veins as in *C. debilis* (Fig. 4b). *C. urophyllidia* (Fig. 4c) was the only species that had prominence on both sides of the leaflets. However, the prominence on the adaxial side was mild compared to that on the abaxial side (Fig. 4c).

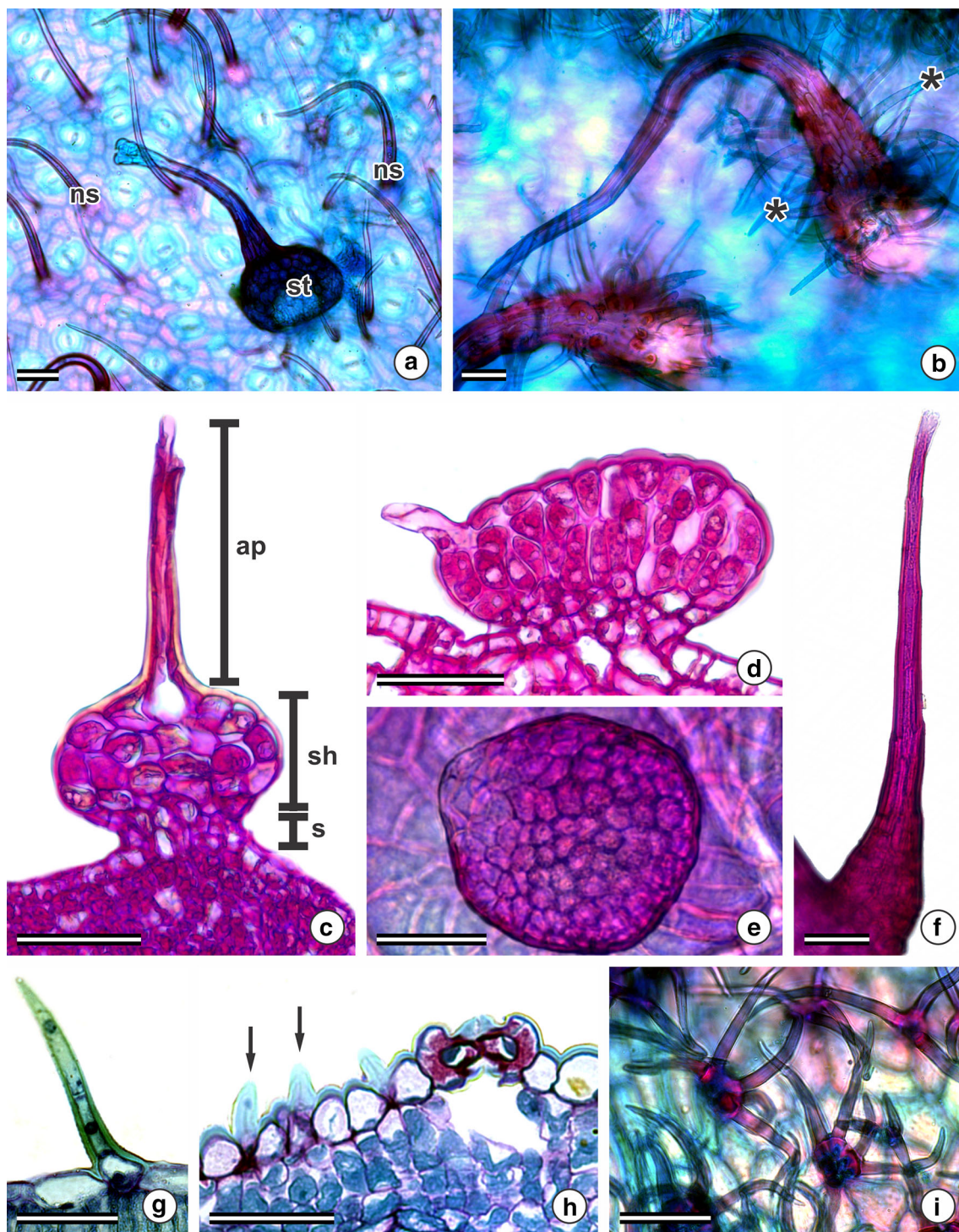


Fig. 3 Secretory and tector trichomes in species of *Chamaecrista*. **a** Secretory (st) and tector (ns) trichomes in *C. sophoroides*. **b** Elongated cells (asterisk) on the secretory head in *C. aurivilla*. **c** Short stalk (s), spherical/oblong secretory head (sh) and apical filiform projection (ap) in the secretory trichomes of *C. bracteolata*. **d** Apical filiform projection was shorter than the secretory head in *C.*

semaphora. **e** Secretory trichome without apical projection in *C. punctulifera*. **f** Slightly dilated secretory head in *C. campestris*. **g** Long uniseriate tector trichome in *C. trichopoda*. **h** Short uniseriate tector trichome in *C. semaphora* (arrow). **i** Stellate trichomes in *C. aurivilla*. Scale bars 50 μ m

At the middle vein, the palisade parenchyma on the adaxial side was continuous in 49 taxa (Table 1; Online Resource 2; Fig. 4a, b). In *C. neesiana*, *C. philippi*, *C.*

setosa, *C. sophoroides* and *C. urophyllidia*, the palisade parenchyma was interrupted by fundamental parenchyma (Fig. 4c); while in *C. aurivilla*, *C. cotinifolia*, *C. desvauxii*,

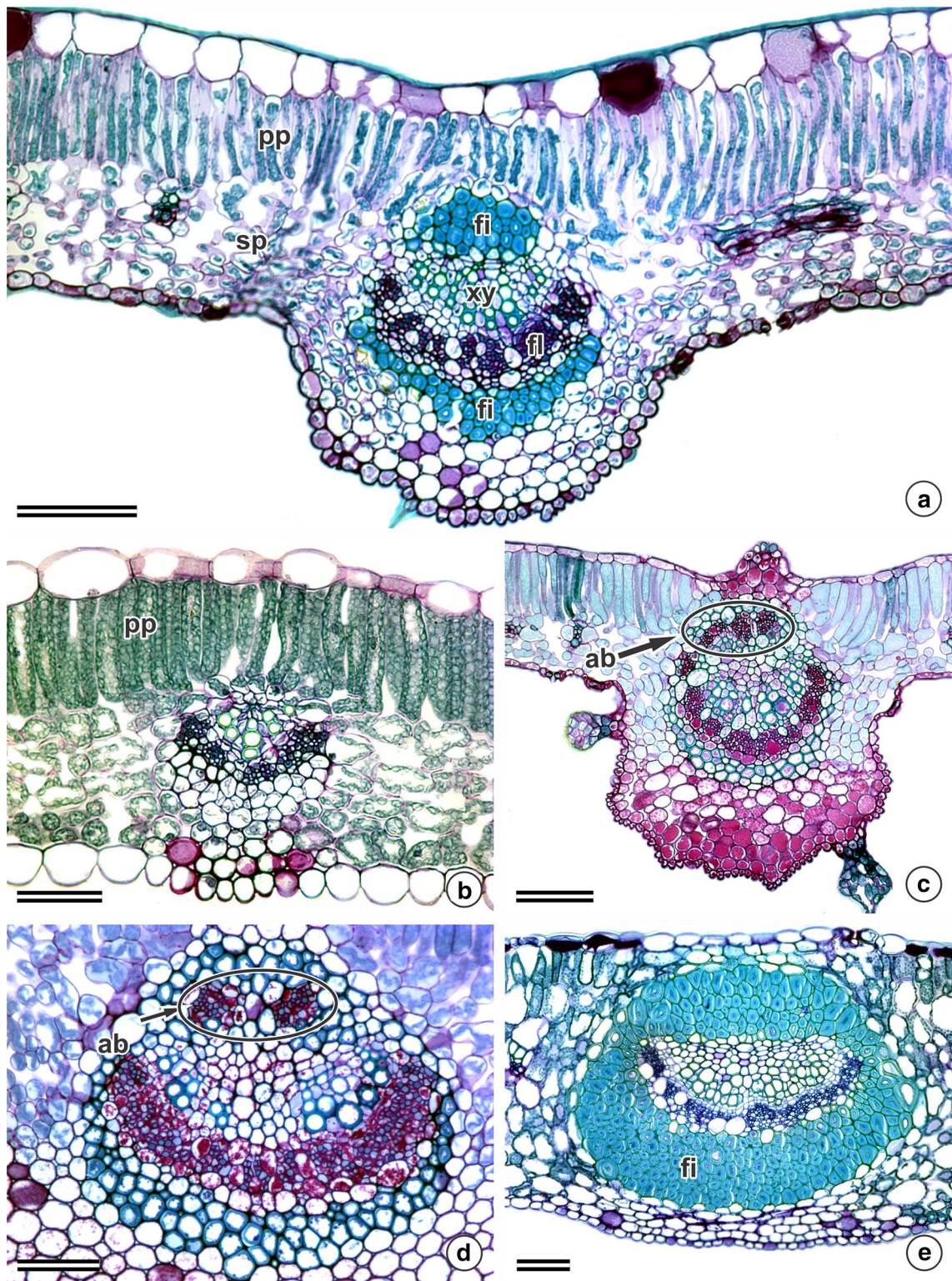


Fig. 4 Cross sections of the leaflet blades of *Chamaecrista* species. **a** Prominent midvein on the abaxial side and open-arch collateral vascular bundles in *C. jacobinae*. **b** Non-prominent midvein in *C. debilis*. **c** Prominence on both sides and accessory bundles (ab) in *C.*

urophyllidia. **d** Two accessory bundles in *C. philippi*. **e** Fibers (fi) surrounding the vascular bundles in *C. neesiana*. fl phloem, pp palisade parenchyma, sp spongy parenchyma, xy xylem. Scale bars 50 μ m

C. geminata, *C. rotundifolia* and *C. speciosa*, it was interrupted by sclerenchyma.

A middle vein composed of open-arch collateral vascular bundles was found in 54 taxa (Table 1; Online Resource 2), such as in *C. jacobinae* (Fig. 4a) and *C. debilis* (Fig. 4b). Accessory bundles were observed in six species (Table 1; Online Resource 2). In *C. orbiculata*, *C. philippi* (Fig. 4d) and *C. speciosa*, two accessory bundles turned to the adaxial side were counted; while in *C. geminata*, *C. setosa* and *C. urophyllidia* (Fig. 4c), there were three to four.

The presence of fibers that were associated with the vascular bundles was evident (Fig. 4a, e), except for *C. absus* and *C. debilis* (Fig. 4b). In most of the studied taxa (50), the fibers formed bundle caps that were associated with both phloem and xylem poles (Fig. 4a). In eight taxa, the fibers completely surrounded the vascular bundles, as in *C. neesiana* (Fig. 4e).

The lateral veins of seven species (*C. aurivilla*, *C. cotinifolia*, *C. desvauxii* var. *langsдорffii*, *C. geminata*, *C. philippi*, *C. speciosa*, and *C. urophyllidia*) presented sclerenchymatous bundle sheath extension (Table 1; Online Resource 2).

Brochidodromous venation was widespread among the studied *Chamaecrista* species, being present in 48 taxa (Table 1; Online Resource 2), such as in *C. semaphora* (Fig. 5a) and *C. geminata* (Fig. 5b). *C. desvauxii* var. *langsдорffii* (Fig. 5c), *C. mucronata*, *C. rotundata* var. *grandistipula* and *C. trichopoda* displayed the actinodromous venation type, while species from *C. sect. Absus* subsect. *Baseophyllum* displayed the actinodromous type at the leaflet base and camptodromous–brochidodromous towards the leaflet margin (Table 1; Online Resource 2).

Closed areoles were observed in 29 taxa (Fig. 5d), while open areoles were observed in 24 taxa (Fig. 5e). Areoles were absent in the four species with the actinodromous venation type as well as in *C. campestris*, *C. debilis*, *C. ericifolia* and *C. strictifolia* (Table 1; Online Resource 2).

Enlarged tracheids (tracheoids) were displayed by 50 taxa (Table 1; Online Resource 2). In some of the taxa, the tracheoids were conspicuous, occupying almost all of the mesophyll height, as in *C. philippi* (Fig. 5f) and *C. vauthieri*. A sheath of crystal-containing idioblasts around the vascular bundles occurred in 29 taxa (Table 1; Online Resource 2).

Apart from the species with sessile leaves (*C. aurivilla*, *C. blanchetti*, *C. geminata*, *C. monticola*, *C. paniculata* and *C. vauthieri*), a uniseriate epidermis in the petiole was found in all of the studied taxa (Fig. 6a–i). Secretory and tector trichomes similar to those on the leaflet blade may occur on the petiole epidermis.

The petiole vascular arrangement was the character that varied the most: 13 types of arrangements were found. In

the Type I, the vascular system is made up of three open-arch collateral vascular bundles (Fig. 6a). This type of arrangement was observed in only three species, *C. cipoana*, *C. hispidula* (Fig. 6a) and *C. multipennis*. Type II, which was observed in 11 taxa (Table 1; Online Resource 2), displayed two accessory bundles that were separated by a groove, turned to the adaxial side of the petiole, in addition to the three open-arch collateral vascular bundles, as in *C. cathartica* var. *paucijuga* (Fig. 6b). Type III, which was found in six taxa (Table 1; Online Resource 2), differed from Type II for having four accessory bundles instead of two, as in *C. belemii*.

Type IV, which was observed in 15 taxa (Table 1; Online Resource 2), was characterized by the presence of several vascular bundles that fuse to form a siphonostele and two accessory bundles turned to the adaxial side of the petiole, as in *C. ochracea* var. *purpurascens* (Fig. 6c). Type V, which was found in seven taxa (Table 1; Online Resource 2), displayed five to ten vascular bundles, constituting the main petiole vascularization, in addition to two accessory bundles, as in *C. sophoroides* (Fig. 6e). In Types IV and V, the accessory bundles were placed in the adaxial projections of the petiole, which is separated by a groove.

Type VI was found in species with a round petiole (*Chamaecrista dentata*, *C. hedysaroides* (Fig. 6d) and *C. cotinifolia*). In these species, the petiole vascular system was composed of seven to eight collateral bundles, varying in size and arranged in a circle, and one or two additional bundles in the pith. Type VII, which was observed in *C. debilis* (Fig. 6f) and *C. rotundifolia*, displayed up to six bundles that were arranged in a circle with a complete ring of fibers surrounding the vascular bundles.

Types VIII and IX displayed collateral vascular bundles concentrated in two different places (Fig. 6g). Type VIII had larger vascular bundles that were turned to the abaxial side of the petiole and a smaller bundle that was turned to the adaxial side; while in Type IX, this arrangement was opposite, with the larger bundle being turned to the adaxial and the smaller to the abaxial side of the petiole. Type VIII was exclusive to *C. conferta*, while Type IX was exclusive to *C. celiae* and *C. glaucofilix* (Fig. 6g).

Types X, XI, XII and XIII were species specific. Type X, which was found in *C. philippi* (Fig. 6h), was composed of eight to nine open-arch vascular bundles with revolute endings and two accessory bundles that were turned to the adaxial side of the petiole. Type XI, which was found in *C. rotundata*, was similar to Type X in the arrangement of the vascular system. However, instead of two accessory bundles, two caps of fibers without an associated vascular system were observed. Type XII, which was exclusive to *C. speciosa* (Fig. 6i), was similar to Type II regarding the arrangement of the main vascular system. However, only one accessory bundle instead of two was turned to the

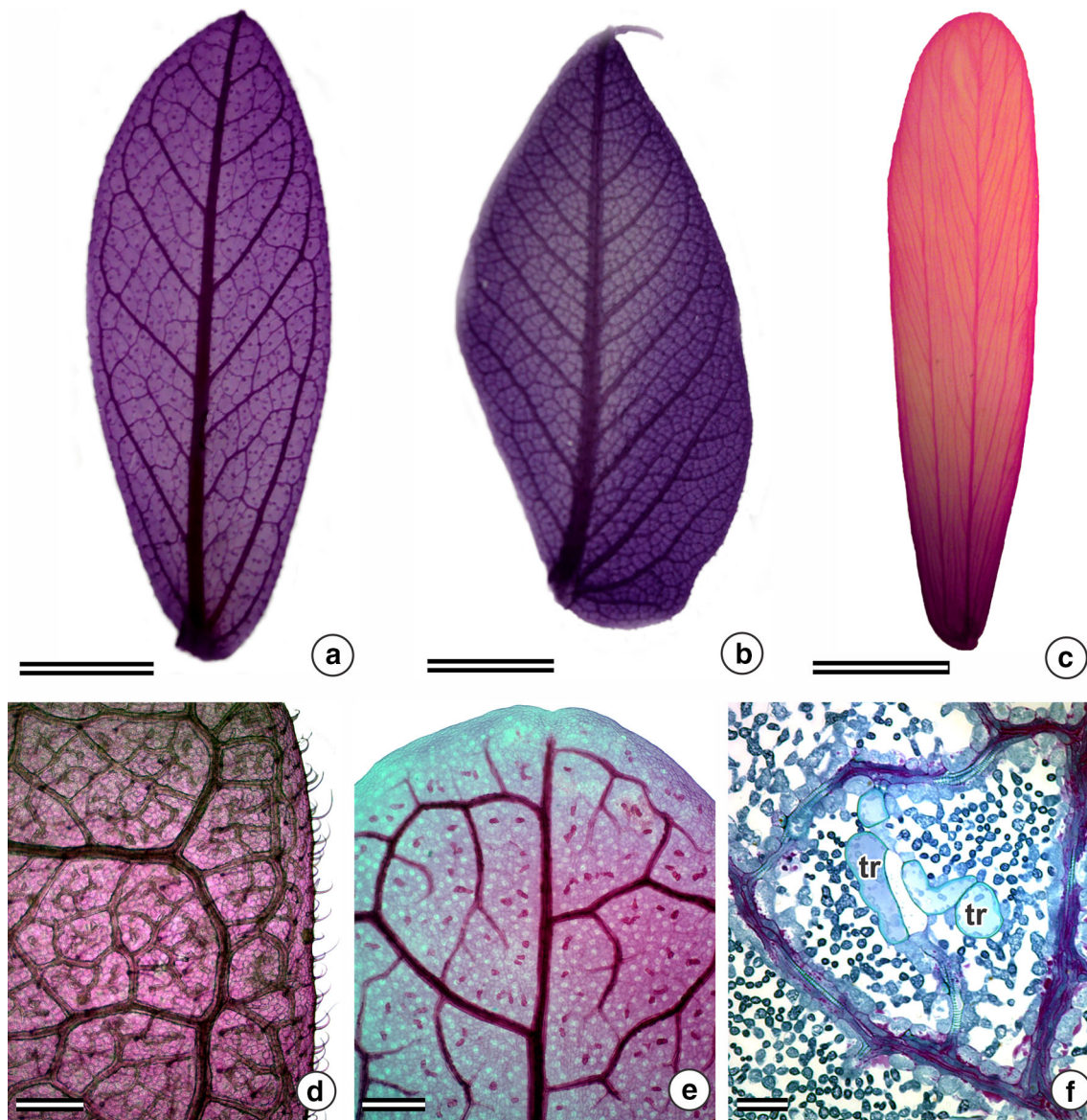


Fig. 5 Venation pattern in cleared leaflets of *Chamaecrista* species. **a, b** Brochidodromous venation in *C. semaphora* and *C. geminata*, respectively. **c** Actinodromous venation in *C. desvauxii* var.

langsdorfii. **d** Closed areoles in *C. jacobinae*. **e** Open areoles in *C. multipennis*. **f** Enlarged tracheids (tr) in *C. philippi*. Scale bars **a–c** 5 mm, **d, e** 400 μ m, **f** 50 μ m

adaxial side of the petiole. Finally, Type XIII, which was specific to *C. decora*, was characterized for presenting the vascular system of the petiole, forming a syphonostele with four to six additional accessory bundles turned to the adaxial side.

Extrafloral nectaries (EFNs) on the petiole (Fig. 7a–c) were found in the species from *Chamaecrista* sect. *Chamaecrista* and sect. *Xerocalyx* and in the species from *C. sect. Absus* subsect. *Baseophyllum* and subsect. *Otophyllum*. The EFNs in *C. sect. Chamaecrista* and sect. *Xerocalyx* were short-stalked with a concave frontal view (Fig. 7a, b). In *C. sect. Absus* subsect. *Baseophyllum*, the

EFNs were sessile with a concave frontal view. *C. debilis* (*C. sect. Absus* subsect. *Otophyllum*) (Fig. 7c) was the only species displaying sessile nectaries with a convex frontal view.

All of the studied EFNs were vascularized by phloem and xylem, had a single-layered epidermis with occasionally two layers, and displayed a nectariferous parenchyma below the epidermis. The vascular system ended near the nectariferous parenchymas. Strips for glucose identification reacted positively to the presence of glucose in the petiolar extrafloral nectaries of *C. debilis* and *C. desvauxii* var. *langsdorfii*.

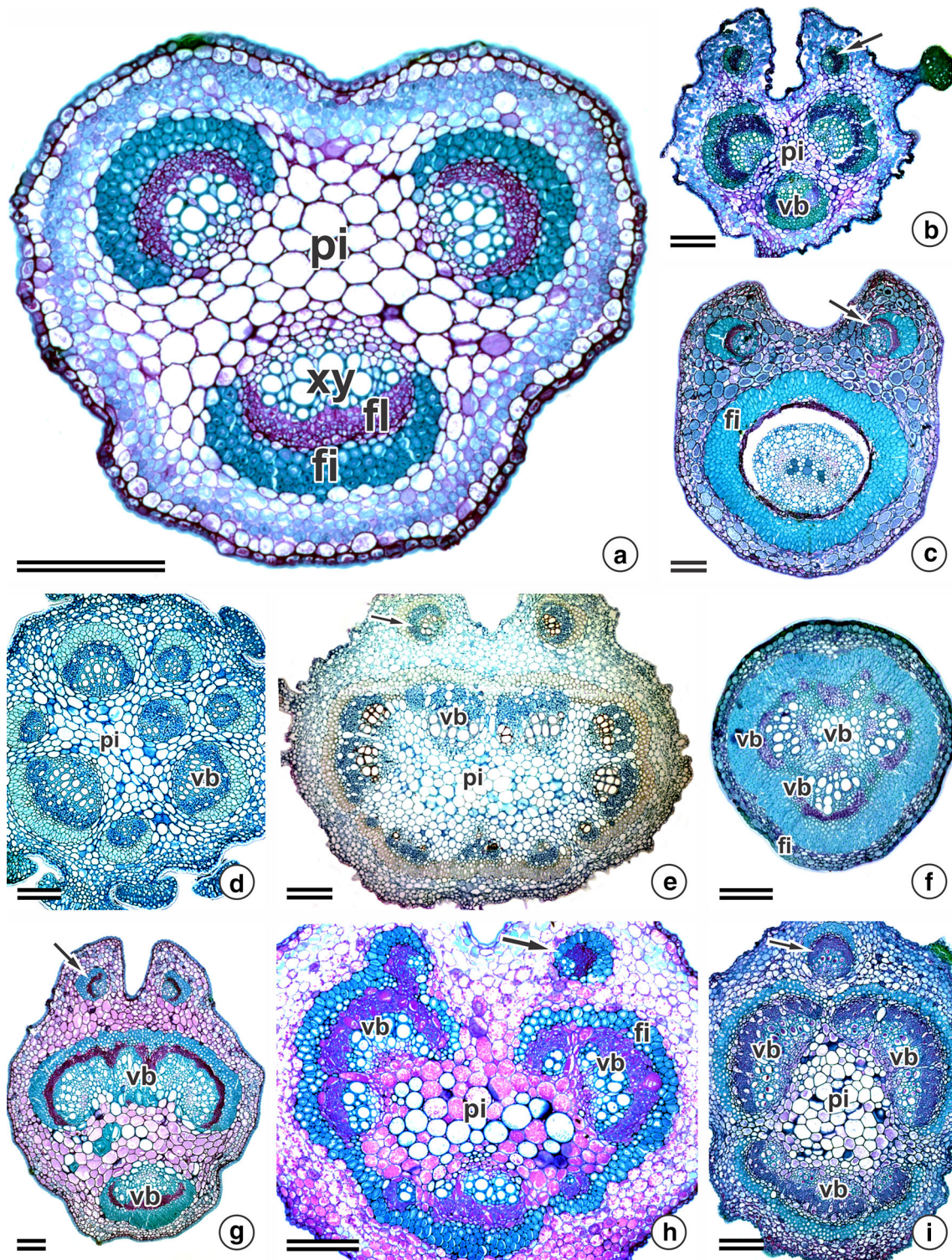


Fig. 6 Cross sections of petiole of *Chamaecrista* species. **a** Type I of vascular arrangement in the petiole in *C. hispidula*. **b** Type II in *C. cathartica* var. *paucijuga*. **c** Type IV in *C. ochnacea* var. *purpurascens*. **d** Type VI in *C. hedyaroides*. **e** Type V in *C. sophoroides*.

f Type VII in *C. debilis*. **g** Type IX in *C. glaucofilix*. **h** Type X in *C. philippi*. **i** Type XII in *C. speciosa*. *fi* fibers, *fl* phloem, *pi* pith, *vb* vascular bundles, *xy* xylem. Scale bars 150 μ m

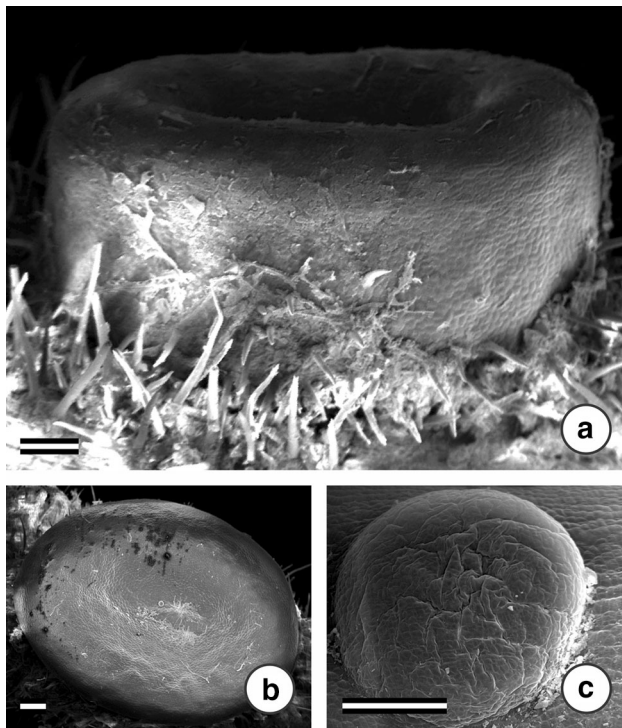


Fig. 7 SEM showing extrafloral nectaries in *Chamaecrista* species. **a, b** Short-stalked concave extrafloral nectaries in *C. rotundata*. **c** Convex sessile extrafloral nectary in *C. debilis*. Scale bars 100 μm

Phenetic analysis

A total of 76 morphoanatomical characters were selected for the multivariate analysis. The dendrogram noted the formation of two main groups, A and B (Fig. 8). Group A was composed of all of the species from *Chamaecrista* sect. *Chamaecrista* and sect. *Xerocalyx* and *C. sect. Absus* subsect. *Baseophyllum* and subsect. *Otophyllum*, while species belonging to *C. sect. Absus* subsect. *Absus* and the monotypic species from *C. sect. Grimaldia* (*C. absus*) were found in group B.

The characters that supported such groupings were the presence of secretory trichomes, enlarged tracheids and brochidodromous venation in group B, while the presence of extrafloral nectaries, epidermal cells on both adaxial and abaxial sides with similar height and little or no prominence of the middle veins were characters of group A.

Group A was composed of two groups (e.g., C and D). Group C was represented by species from *Chamaecrista* sect. *Absus* subsect. *Baseophyllum*, and group D was represented by *C. debilis* (*C. sect. Absus* subsect. *Otophyllum*) and by species from *C. sect. Chamaecrista* and *C. sect. Xerocalyx*. Group C was supported by the exclusive presence of epistomatic leaflets, mucilage idioblasts in the mesophyll (e.g., in the palisade and/or spongy parenchyma) and an actinodromous venation pattern at the leaflet base,

while camptodromous–brochidodromous toward the margin. Group D was supported by the absence of the exclusive characteristics that were found in group C and the presence of non-prominent middle veins. Two groups constituted group D (E and F). Group E (*C. debilis*) differed from groups C and F due to the presence of sessile convex extrafloral nectaries, mucilage idioblasts in the epidermis and a brochidodromous venation pattern. The species that were included in group F differed from those in group C and E based on actinodromous venation.

Within group B, the insertion of *C. absus* (*C. sect. Grimaldia*) was remarkable. Group B was divided into two major groups, G and H. In group G, almost all of the species displayed amphistomatic leaflets in addition to the presence of a collateral bundle in an arch at the midvein. Group G was divided into two groups (I and J), with the species from group I being those that presented an isobilateral mesophyll, while those from group J displayed strongly thickened outer epidermal cell walls, that is, outer epidermal cell walls that were the same height as or higher than the epidermal cells. Such characters (isobilateral mesophyll and strongly thickened cell walls) may eventually be found in species from other groups.

Group H encompassed all of the species that had secretory trichomes with a filiform projection longer than the trichome basal portion and uniseriate tector trichomes. Group H was composed of groups L and K, with K being differentiated from L due to the presence of secretory trichomes on both sides as well as hypostomatic leaflets in all of the species, characters that were absent in K.

Discussion

The groupings that resulted from the phenetic analyses agree with the molecular phylogeny of *Chamaecrista* as described by Conceição et al. (2009). Except for *C. sect. Absus* subsect. *Baseophyllum* (Group C) and *C. debilis* (Group E), most species from *C. sect. Absus* are organized into a main group (Group B). Within Group A, species belonging to *C. sect. Absus* subsect. *Baseophyllum* form a consistent group with species from *C. sect. Chamaecrista* and *C. sect. Xerocalyx* (Group F). This arrangement does not support the classification that was proposed by Irwin and Barneby (1982), as *C. sect. Absus* subsect. *Baseophyllum* should be embedded in *C. sect. Absus*. The affinity of *C. sect. Absus* subsect. *Baseophyllum* with the other groups that were found in group A is based on the presence of petiolar extrafloral nectaries (EFNs), which are found in *C. sect. Chamaecrista* and *C. sect. Xerocalyx* and absent from the other species of *C. sect. Absus* subsect. *Absus*. The distinction of *C. sect. Absus* subsect. *Baseophyllum* (Group C) from the other species that were included in group B

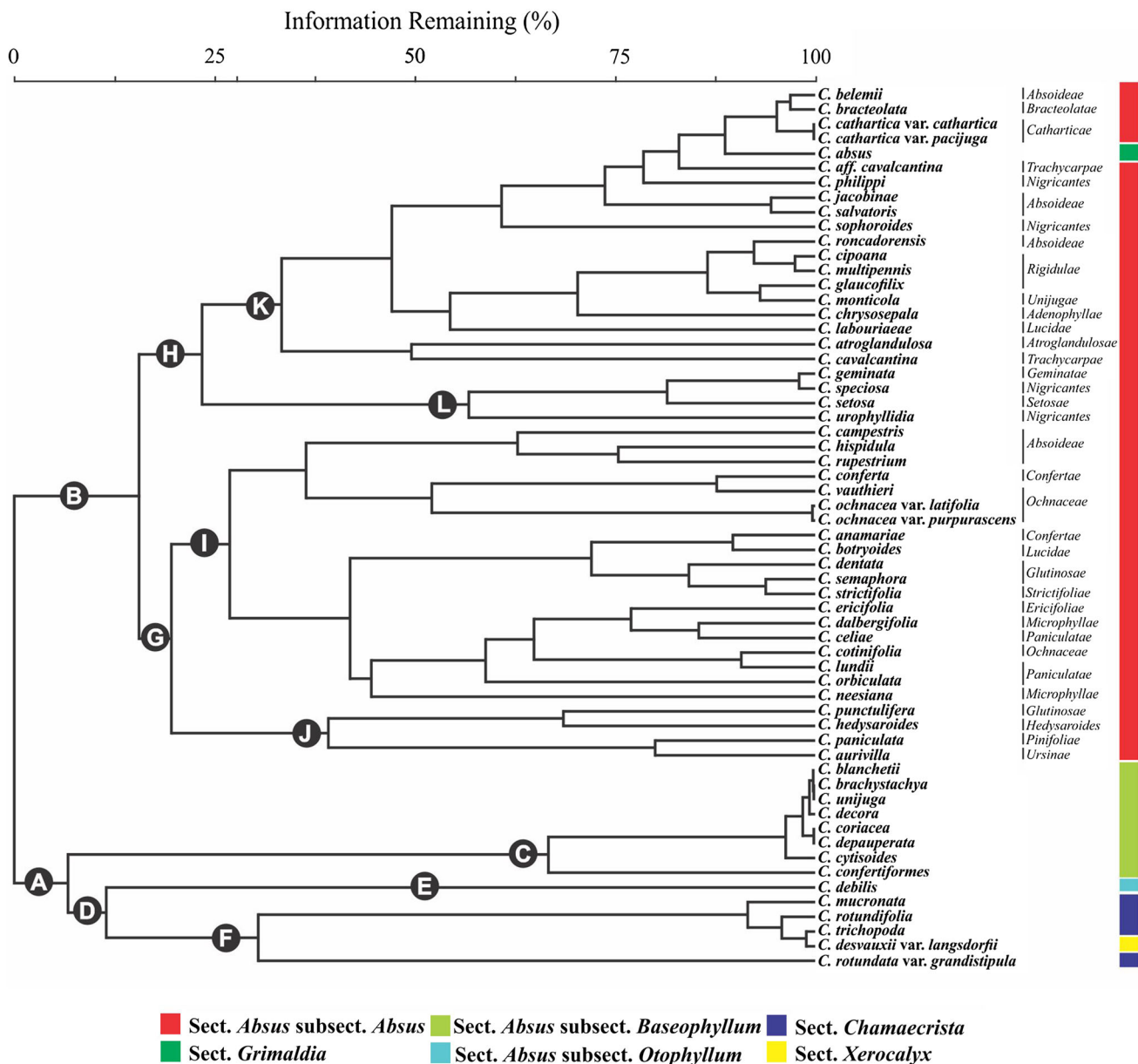


Fig. 8 Dendrogram based on 76 anatomical characters showing the phenetic relationships among 60 taxa of *Chamaecrista*, as given by the Jaccard index

(*C. sect. Absus* subsection. *Absus*) as well from the other species that were found in group A is supported by the presence of epistomatic leaflets, mucilage idioblasts in the mesophyll and an actinodromous venation pattern at the leaflet base, while camptodromous–brochidodromous towards the margin. These results advocate for the exclusion of *C. sect. Absus* subsection. *Baseophyllum* from *C. sect. Absus* and the promotion of this subsection to the sectional status as suggested by molecular and anatomical studies (Conceição et al. 2009; Coutinho et al. 2013).

C. debilis (*C. sect. Absus* subsection. *Otophyllum*) has greater affinity with species from *C. sect. Absus* subsection.

Baseophyllum and *C. sect. Xerocalyx* and *C. sect. Chamaecrista* than with those from *C. sect. Absus*, where it was originally placed (Irwin and Barneby 1982). However, due to the presence of convex EFNs, mucilage idioblasts in the epidermis and a brochidodromous venation pattern, *C. debilis* was placed in a separate branch (Group E). The presence of such anatomical findings indicates the exclusion of *C. debilis* from *C. sect. Absus* in addition to their proximity from *C. sect. Baseophyllum*. Considering that Conceição et al. (2009) did not include *C. debilis* in their molecular study, future molecular phylogenetic studies and/or taxonomic revisions must

include *C. debilis* to allow a conclusive taxonomic placement of these species.

Group B corresponds to all of the species from *C. sect. Absus* subsect. *Absus* along with *C. absus* (the only species of *C. sect. Grimaldia*). These results are in contrast with the classification that was proposed by Irwin and Barneby (1982) and agree with the molecular findings of Conceição et al. (2009), supporting the inclusion of *C. sect. Grimaldia* in *C. sect. Absus* subsect. *Absus* and permitting the establishment of a monophyletic group. The phenetic analysis resulted in the formation of several smaller groups. Although even with sampling 23 series of *C. sect. Absus* subsect. *Absus*, which is larger in number than the number of series (9) sampled by Conceição et al. (2009), the smaller groups within group B do not correspond to the serial organization that was proposed for *C. sect. Absus*. Our results demonstrate the artificiality of the studied series and reinforce the necessity of a wider sampling in molecular and morphological studies of the genus as well as taxonomic revision of *Chamaecrista*.

Among the characteristics with diagnostic taxonomic value at the species level, the petiolar vascular arrangement stood out for having five exclusive types. Variations in the petiolar vascular arrangement have been successfully used in the taxonomy of Leguminosae (Watari 1934; Crow et al. 1997) and other families, such as Melastomataceae (Reis et al. 2005), Celastraceae (Gomes et al. 2005) and Malpighiaceae (Araújo et al. 2010). The vascular system of the petiole forming a continuous ring with accessory bundles on the adaxial side was reported for *Chamaecrista mimosoides* (L.) Greene and *Senna marilandica* (L.) Link (Watari 1934). A prominent midvein on both sides of the leaflets (in *C. urophyllidia*) and convex EFNs (in *C. debilis*) were also significant.

Although among the studied *Chamaecrista* species the trichomes varied regarding their distribution and density, the type/types that were found in a particular species were uniform and may be used as an additional tool in taxonomy. Several authors have used such characteristics in studies of Leguminosae (Lackey 1978; Tripathi and Sahu 1991; Silva 1999; Conceição et al. 2003; Zarre 2003). According to Tripathi and Sahu (1991), trichomes are an additional source of characters that may support taxonomic studies, as their morphology is genetically fixed. Moreover, trichome morphology is quite simple and may be easily observed using ordinary light microscopy techniques, such as leaf clearings. Such variation in morphology may go unnoticed in taxonomic revisions that usually do not make use of microscopic techniques but rely only on dried material.

In *Chamaecrista*, the sticky glandular hairs were anatomically described for *C. dentata* (Meira et al. 2014). Such secretory trichomes were considered as unusual by

the presence of a filiform projection where the secretion is secreted. In this study, we observed the same type of secretory trichome in *C. sect. Absus* subsect. *Absus* and *C. absus* (*C. sect. Grimaldia*). However, variations regarding the diameter of the secretory head as well as the presence and type of the filiform projection that was found continuous to the secretory head demonstrated the taxonomic value of such characters for the genus. The molecular phylogeny of *Chamaecrista* showed that all of the species that were included in the clade constituting *C. sect. Absus* subsect. *Absus* and *C. absus* (*C. sect. Grimaldia*) display sticky glandular hairs (Conceição et al. 2009). However, the increase in the number of species that were sampled for *C. sect. Absus* subsect. *Absus* in this study indicates that not all species from this subsection present sticky glandular hairs.

Secretory trichomes that were similar to *Chamaecrista* were observed on both vegetative (Lersten and Curtis 1994, 1996) and reproductive organs (Rudall et al. 1994) for species belonging to the tribe Caesalpinieae as well as representatives of Papilionoideae (Metcalf and Chalk 1950; Lackey 1978). As secretory trichomes are found in the groups of species that are not related, it is likely that such structures evolved independently and several times in Leguminosae.

Petiolar EFNs were observed only in species belonging to *Chamaecrista* sect. *Chamaecrista*, *C. sect. Xerocalyx* and *C. sect. Absus* subsect. *Baseophyllum* and *C. sect. Absus* subsect. *Otophyllum* in *C. sect. Absus*. Such secretory structures are common in Leguminosae (Solereder 1908; Metcalfe and Chalk 1950; Lewis 2005). In *Chamaecrista*, a protective role against herbivores has been given to both extrafloral nectaries and sticky glandular hairs (Conceição et al. 2009; Coutinho et al. 2013; Meira et al. 2014). Meira et al. (2014) noted that both the viscosity and chemical composition of the sticky glandular hair secretion act as insect deterrents, while Coutinho et al. (2013) suggested that the presence of amino acids in the extrafloral nectary secretion of species from *C. section Baseophyllum* may be more attractive to ants. It is not possible to infer which of these two defensive strategies is more effective as the number of species bearing one of these two defensive strategies is almost the same.

Anatomical characters are taxonomically meaningful and support the promotion of *Chamaecrista* sect. *Absus* subsect. *Baseophyllum* to the sectional status. Anatomical data are useful as diagnostic characters for some taxa in *Chamaecrista*, in addition to demonstrating affinities between *C. sect. Absus* and the monotypic *C. sect. Grimaldia* (*C. absus*). The phenetic analyses using morphoanatomical data clearly note the importance of morphoanatomical studies for this genus.

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