



# A study of the morphoanatomical characters of the leaves of *Chamaecrista* (L.) Moench sect. *Apoucouita* (Leguminosae-Caesalpinioideae)

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## ABSTRACT

Little attention has been paid to species of *Chamaecrista* sect. *Apoucouita* (Leguminosae-Caesalpinioideae), especially regarding anatomical studies. When only vegetative material is available, the identification of such species may be difficult. Additionally, vegetative material of some species of *C.* sect. *Apoucouita* may be even harder to identify because they can resemble species of *Inga* Mill. (Leguminosae-Mimosoideae). The present study focused on recognizing morphoanatomical characters of leaves that are taxonomically useful for the species of *C.* sect. *Apoucouita* by employing standard anatomical techniques. The arrangement of the vascular system in the petiole/rachis, dorsiventral mesophyll, mucilage idioblasts in the epidermis of leaflets and hypostomatic leaves were some of the characters shared by all species studied. Length of the petiole, position and type of extrafloral nectaries, leaflet venation, presence and type of papillae on the epidermis of the leaflet blades and sclereids in the mesophyll were some of the characters useful in the distinction of taxa. The vascular arrangement of the petiole/rachis is a promising character in the distinction of species of *C.* sect. *Apoucouita* and *Inga*. Based on morphoanatomical data, the taxonomic revision of some species and varieties ascribed to *C.* sect. *Apoucouita* is suggested.

**Keywords:** Amazon Rainforest, applied botany, Atlantic Forest, extrafloral nectaries, papillae, secretory idioblasts, secretory structures, taxonomy

## Introduction

*Chamaecrista* is one of the most diverse genera of Leguminosae-Caesalpinioideae, including about 330 species (Lewis 2005). Species of *Chamaecrista* can be readily recognized by the presence of paripinnate leaves (Fig. 1A-C) or less commonly bifoliolate, yellow flowers (Fig. 1D-F), androecium (erratically) actinomorphic, anthers pubescent along the sutures, a pair of bracteoles at the flower pedicel,

elastically dehiscent pod with coiling valves (Fig. 1G) and either the presence of extrafloral nectaries (Fig. 1A-C) on the leaves or sticky glandular hairs usually all over the plant body (Irwin & Barneby 1982). Species of *Chamaecrista* are classified into six sections: *C.* sect. *Apoucouita*, *Absus*, *Caliciopsis*, *Chamaecrista*, *Grimaldia* and *Xerocalyx*.

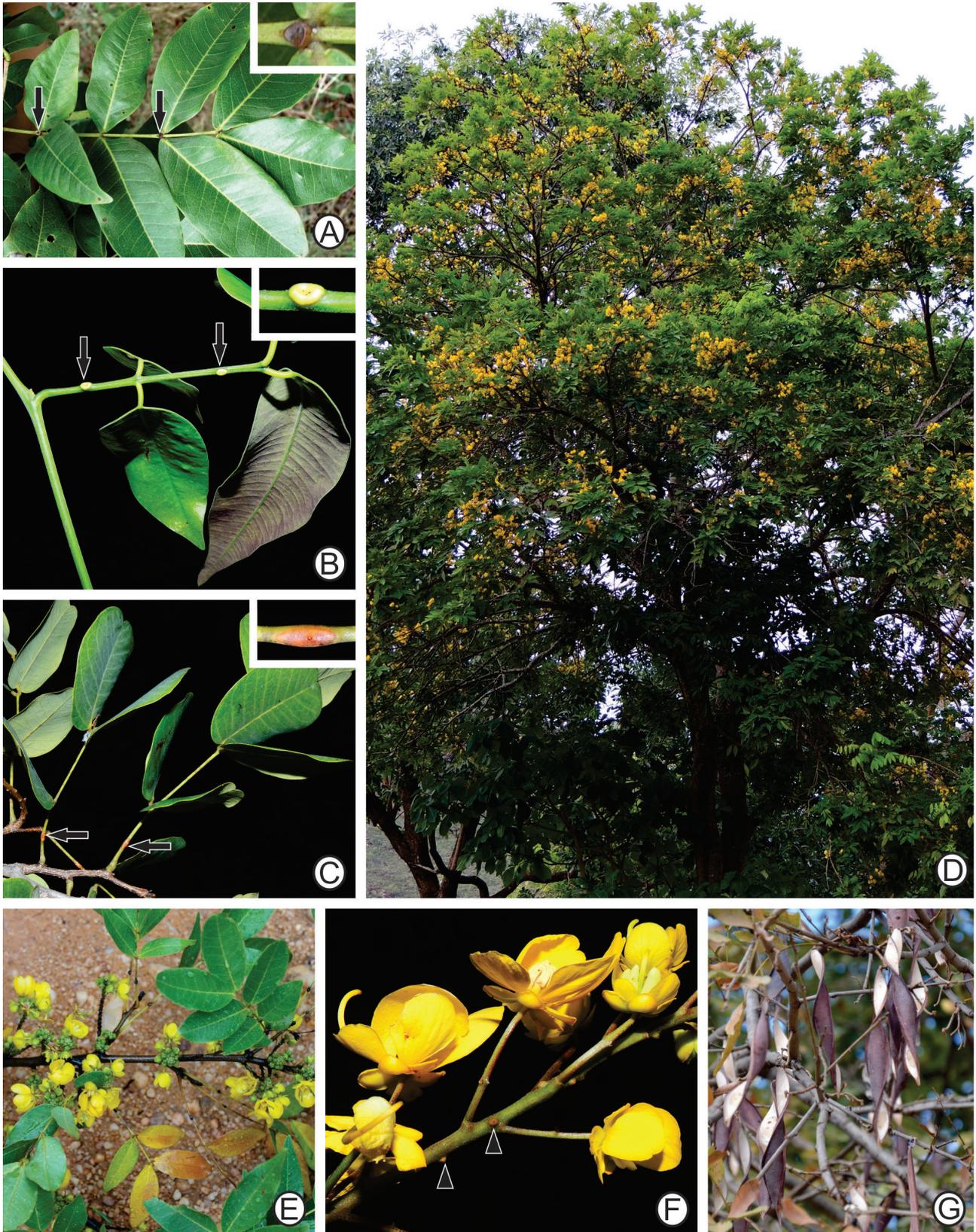
*Chamaecrista* sect. *Apoucouita*, composed of 21 species (26 taxa), contains most of the arborescent species of the genus. Besides including arborescent species (Fig. 1D), *C.*

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**Figure 1.** Species of *Chamaecrista* sect. *Apoucouita*: Extrafloral nectaries (EFN, arrows) on the petiole/rachis of (A) *C. eitenorum* var. *regana*, (B) *C. hymenaeifolia* and *C. polystachya*. Note the detail of EFNs in A-C. (D, E) *C. ensiformis* var. *ensiformis*. Note the ramiflorous inflorescences in (E); (F) Raceme of *C. polystachya* charged with glands (arrowheads); (G) Pendant elastically dehiscent pods of *C. ensiformis* var. *ensiformis*.

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sect. *Apoucouita* may be characterized as having cauliflorous or ramiflorous inflorescences (Fig. 1E) with minute, truncate or depressive inflorescence glands (Fig. 1F) and pendant elastically-dehiscent pods (Fig. 1G). Such arborescent species are usually found in neotropical rain forests, and are most diverse in the Amazon and Brazilian Atlantic forests (Irwin & Rogers 1967; Irwin & Barneby 1977; Conceição *et al.* 2009).

*Chamaecrista* has received much attention in the last 10 years with studies on their anatomy (Francino *et al.* 2006; Coutinho *et al.* 2012; 2013; 2015; Meira *et al.* 2014), molecular biology (Conceição *et al.* 2008; 2009; Torres *et al.* 2011; Rando *et al.* 2016), taxonomy (Camargo & Miotto 2004; Dantas & Silva 2013; Rando *et al.* 2013), ecology, geography (Rando & Pirani 2011), and ecological and economic importance (Morris 2012). Little attention, however, has been paid to the species of *C. sect. Apoucouita*, especially regarding their anatomy.

Additionally, non-reproductive individuals of *C. sect. Apoucouita* can be quite difficult to identify since vegetative material may resemble species of the genus *Inga*, especially because of the winged petiole in some species and the always-present leaf extrafloral nectaries. Morphoanatomical characters have proven to be useful in taxonomic and phylogenetic research of not only Leguminosae (Solereder 1908; Metcalfe & Chalk 1950; 1979; Lackey 1978; Luckow 2002; Arambarri *et al.* 2006) but also *Chamaecrista* (Coutinho *et al.* 2013; 2015; Francino *et al.* 2015).

Moreover, the delimitation of the varieties within species of *C. sect. Apoucouita* seems to be rather complex. For instance, varieties of *C. adiantifolia* were once defined based on pubescence and flower size (Irwin & Rogers 1967), then later on length of pulvinule and shape of the leaflet apex (Irwin & Barneby 1977). These authors themselves stated that 'the varietal key might be emended as follows, but requires testing against the full range of material available to the taximetric study' (Irwin & Barneby 1977). Morphoanatomy has proven useful in supporting molecular studies within *Chamaecrista*, which elevated all varieties to the species level (Conceição *et al.* 2008; Coutinho *et al.* 2013).

Owing to the importance of morphoanatomical characters as an additional tool in taxonomic and phylogenetic studies, we addressed the following questions: Are there recognizable leaf morphoanatomical characters that support the taxonomy of the species of *C. sect. Apoucouita*? If so, could such characters be used to build an identification key? Can morphoanatomy support the varieties ascribed to *C. sect. Apoucouita*?

## Materials and methods

We sampled 23 (18 spp.) of the 26 taxa (21 spp.) which belong to *C. sect. Apoucouita* (Irwin & Barneby 1982; 1985; Barneby 1999) (S1 in Supplementary Material). Taxonomic authorities for all taxa used in the paper are given in the Supplementary Material. Both field-collected and herbarium

material were used. Voucher specimens were deposited at the herbarium of the Universidade Federal de Viçosa (VIC), Universidade de São Paulo (SPF), and Universidade Estadual de Feira de Santana (HUEFS) (acronyms according to Thiers 2016, continuously updated).

Fully expanded leaves were collected in the field and fixed in FAA (formaldehyde, acetic acid and 50% ethanol; 1:1:18 by volume) for 48 h and stored in 70% ethanol (Johansen 1940). Material sampled from herbarium specimens was rehydrated by microwaving in distilled water for 7 min and left to rest overnight. Rehydrated samples were then treated with 2% potassium hydroxide for 1-2 h and dehydrated in an ethanol series and stored in 70% ethanol (Smith & Smith 1942). For identification of the venation pattern and stomata types, whole and/or fragments of mature leaflets stored in 70% ethanol of both field-collected and herbarium material were cleared with 10% sodium hydroxide and 20% hypochlorite solutions, stained with diluted fuchsin and mounted in glycerinated gelatin (Johansen 1940).

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) and the petiole/rachis (base of the petiole right after the pulvinus and rachis right after the insertion of the first pair of leaflets) were taken. The fragments from both the herbarium and field-collected specimens stored in 70% ethanol were embedded in methacrylate (Historesin Leica, Leica Microsystems Nussloch GmbH, Heidelberg, Germany) as recommended by the manufacturer. Cross and longitudinal 4µm-thick sections were made using an automatic rotary microtome (Leica RM2155, Deerfield, IL, USA), placed on glass slides, stained with toluidine blue at pH 4.4 (O'Brien & McCully 1981), dried at room temperature and mounted in resin (Permout, Fisher Scientific, NJ, USA).

Both observations and image captures were conducted using an Olympus Optical AX70TRF (Tokyo, Japan) equipped with a U-Photo system and digital camera (AxioCam HRc – Carl Zeiss – Gottingen, Germany). Classification of the venation and stomata types followed Ellis *et al.* (2009) and Wilkinson (1979), respectively. Types of vascular arrangements of the petiole/rachis followed Howard (1979) and Francino *et al.* (2015). The types of leaf extrafloral nectaries and their position, as well as data on the morphology of leaves/leaflets (petiole and blade), were taken from the literature (Irwin & Rogers 1967; Rizzini 1976; Irwin & Barneby 1977; 1979a; b; 1982; 1985; Barneby 1999; Coutinho & Meira 2015).

## Results

### *Petiole/Rachis*

A petiole shorter than 15 mm was observed in fourteen taxa (Tab. 1), longer than 15 mm and shorter than 50 mm in 17 (Tab. 1) and over 50 mm in *C. amorimii* only. Petiole/



**Table 1.** Morphoanatomical characters observed in species of the *Chamaecrista* sect. *Apoucouita*. 0 = absence; 1 = presence; 0/1 absence or presence. **adia adia** - *Chamaecrista adiantifolia* var. *adiantifolia*; **adia pter** - *C. adiantifolia* var. *pteridophylla*; **amab** - *C. amabilis*; **amor** - *C. amorimii*; **apou** - *C. apoucouita*; **aspl** - *C. asplenifolia*; **aspi** - *C. aspidiifolia*; **bahi** - *C. bahiae*; **comp** - *C. campitilis*; **duar** - *C. duartei*; **eite eite** - *C. eitenorum* var. *eitenorum*; **eite rega** - *C. eitenorum* var. *regana*; **ensi ensi** - *C. ensiformis* var. *ensiformis*; **ensi mara** - *C. ensiformis* var. *maranomonica*; **ensi plur** - *C. ensiformis* var. *plurifoliolata*; **hyme** - *C. hymenaeifolia*; **negr albu** - *C. negrensis* var. *albuquerquei*; **negr negr** - *C. negrensis* var. *negrensis*; **onus** - *C. onusta*; **poly** - *C. polystachya*; **scl** - *C. scleroxylon*; **subp** - *C. subpeltata*; **xing** - *C. xinguensis*.

Morphoanatomical character	adia adia	adia pter	amab	amor	apou	aspl	aspi	bahi	comp	duar	eite eite	eite rega	ensi ensi	ensi mara	ensi plur	hyme	negr albu	negr	onus	poly	scl	subp	xing
ENF on the petiole proper	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1
ENF between/immediately below the basal pair of leaflets	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	0
ENF between/immediately below other pairs of leaflets	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1
ENF below other pairs of leaflets	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
ENF impressed	0	0	1	1	1	0	0	1	0	1	0	0	0	0	0	1	1	1	0	1	0	0	0
ENF sessile flat/truncate/convex	0	0	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0
ENF sessile concave	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1	0	1
ENF sessile urceolate	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	1
ENF cupuliform short-stalked	1	1	0	1	1	1	0	1	0	0	1	1	1	1	1	0	1	1	0	0	1	0	1
ENF cupuliform stalked	1	1	0	0	1	1	1	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	1
ENF cupuliform long-stalked	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
ENF cupuliform short-stalked	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
ENF pakelliform stalked or long-stalked	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0
Petiole with pulvinus in millimeters	3-6	3-6	15-25	50-100	15-45	7-13	4-8	15-45	15-30	15-45	14-25	14-25	12-25	12-25	12-25	7-20	8-35	8-35	22-50	20-40	10-20	3-10	15-40
Petiole/rachis canalicate or grooved	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1
Petiole/rachis more or less marginate	1	1	0	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	1	0	0
Petiole/rachis vascular arrangement Type IV, V or XIII	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Petiole/rachis vascular arrangement Type XII or XIV	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Non-secretory trichomes on the petiole	1	1	0	0	1	1	1	0/1	1	0/1	0	0	0/1	0/1	0/1	1	0	0	0	0	0/1	1	1
Non-secretory trichomes on the adaxial/abaxial epidermis	0/1	0/1	0	0	0/1	0/1	1	0	0/1	0	0	0	0/1	1	0/1	0/1	0	0	0	0/1	0/1	1	1
Brochidodromous venation	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1
Brochidodromous-craspedodromous venation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Eucamptodromous-brochidodromous venation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Perimarginal veins	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Number of pairs of leaflets	15-35	15-35	2	5	2-4	10-19	9-13	2	(3-4)-(5)	2-3	2	3-4	(2)3-5	(2)3-5	5-8	2(3)	3-6	2-3	2-3	2-4	2-5	7-9	6-10
Length of leaflets in millimeters	10-20	10-20	40-120	90-200	40-120	9-18	5-25	60-120	15-65	70-160	30-100	30-100	20-100	20-100	20-100	30-75	55-100	80-200	40-200	50-60	35-100	5-25	20-90
Several-dome papillae on the abaxial epidermis	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
One-dome papillae on the adaxial epidermis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
One-dome papillae on the abaxial epidermis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
Palisade parenchyma on the adaxial side at the midvein	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sclereids in the mesophyll	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stipules over 1mm, commonly over 2mm, tardily deciduous	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0

rachis in cross sections varied shape: rounded or slightly angulate, more or less marginate and canaliculate or grooved (Figs. 2, 3). Only four taxa had exclusively rounded or slightly angulate petiole/rachis (i.g. *C. bahiae*, *C. duartei*, *C. negrensis* var. *albuquerquei* and *C. negrensis* var. *negrensis*) (Fig. 2 A-B, D-E). A canaliculate or grooved petiole/rachis was observed in 19 taxa (Tab. 1), including in *C. hymenaeifolia* (Fig. 2C), *C. apoucouita* (Fig. 3B), *C. subpeltata* (Fig. 3C) and *C. adiantifolia* var. *pteridophylla* (Fig. 3E). A more or less marginate petiole/rachis was observed in eight taxa (Tab. 1), as in *C. apoucouita* (Fig. 3A), *C. adiantifolia* var. *pteridophylla* (Fig. 3D) and *C. ensiformis* var. *ensiformis* (Fig. 3F). In *C. apoucouita* (Fig. 3A) and *C. scleroxylon*, the petiole/rachis was canaliculate or grooved but the most distal segment of the rachis was always marginate (winged). Non-secretory trichomes on the petiole/rachis were found in 15 taxa (Tab. 1), of which six may or may not possess them (*C. bahiae*, *C. duartei*, all varieties of *C. ensiformis* and *C. scleroxylon*).

The petiole/rachis of all species studied had the vascular system forming a siphonostele (Figs. 2, 3) made up by a central pith of parenchymatic cells surrounded by layers (three or more) of phloem and xylem, respectively, and then by layers of fibers (Fig. 2A). Sometimes the vascular system did not form concentric layers around the central pith and interruptions were observed as if the siphonostele were incomplete (Figs. 2D, 3E-F). The siphonostele, along with the surrounding layers of fibers, usually displayed a circular or oval shape (Figs. 2A, C-E, 3A-B, D). Occasionally the vascular system appeared more like three interconnected semi-circles (Fig. 2B). The arrangement of the vascular system varied in this manner among and within species.

One or two pairs of accessory bundles on the adaxial side of the petiole were common (Figs. 2B-C, 3A-F), but in rare occasions there could be up to six pairs (Fig. 2A). Such arrangements were consistent with types IV, V and XIII. Type IV was characterized by the presence of a siphonostele and two accessory bundles (Figs. 2B-C, 3B-D). Type V, displayed five or more vascular bundles, consisting of the vascularization of the main petiole and two more accessory bundles, as seen in *C. adiantifolia* var. *pteridophylla* (Fig. 3E) and *C. ensiformis* var. *ensiformis*. (Fig. 3F). Type XIII had a siphonostele and four-six accessory bundles, as seen in *C. apoucouita* (Fig. 3A).

When the petiole/rachis was winged, the accessory bundles were usually located in the wings (Fig. 3). Apart from the previously mentioned types, *C. duartei* and *C. polystachya* were the only two species that also displayed type XII, with the accessory bundle concentrated at the center of the adaxial side of the petiole (Fig. 2D). As in the previously described types, this arrangement of the vascular system tended to form concentric layers around the central pith, resembling a siphonostele. *C. duartei* and *C. polystachya* were also the only species to exhibit a new type of vascular arrangement of the petiole, type XIV (Fig. 2E-F). In this arrangement, the accessory bundles are concentrated at

the center of the adaxial side of the petiole; however, the bundles form another cylinder around a less conspicuous central pith (Fig. 2E-F).

Sclereids were encountered in the cortex of the petiole/rachis of all studied species (Figs. 2A, 3A). Larger leaves had a greater number of sclereids in the cortex of the petiole. Druses were found inside the sclereids and a single-layered sheath of druses was also present encircling the outermost layer of fiber.

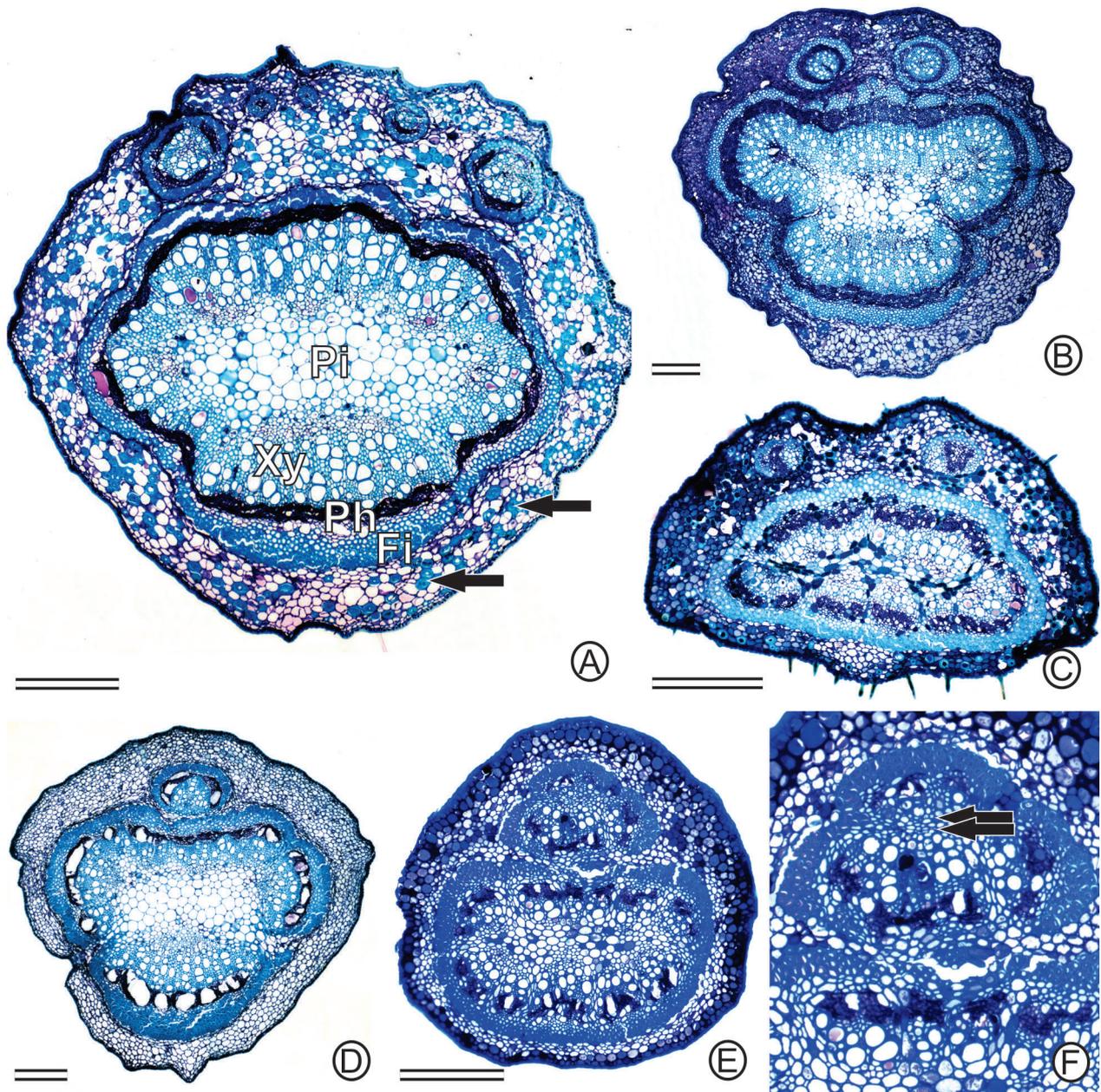
### *Leaflet blade*

The smallest leaflets were found in *C. adiantifolia*, *C. aspidiifolia*, *C. aspleniifolia* and *C. subpeltata*, while the largest were found in *C. amorimii*, *C. duartei*, *C. negrensis* var. *negrensis* and *C. onusta* (Tab. 1). The greatest number of pairs of leaflets was found in *C. adiantifolia*, *C. aspidiifolia* and *C. aspleniifolia*, while the other species varied from having 2 to 10 pairs (Tab. 1). All species had entire pinnate leaves with brochidodromous venation (Fig. 4A-B), except for *C. negrensis*, which had eucamptodromous leaves that became brochidodromous distally (resembling the leaf venation of Lauraceae) (Fig. 4C), and *C. hymenaeifolia* that had craspedodromous-brochidodromous leaves with marginal secondary-type perimarginal veins (Fig. 4D). Marginal ultimate venation was commonly looped (Fig. 4E) for all species, but incomplete marginal ultimate venation (Fig. 4E) was also observed in the same leaflets. Enlarged tracheids (tracheoids) at vein endings were common among all species (Fig. 4F).

Non-secretory trichomes (Fig. 4G-I) were observed on the leaflets in 14 taxa (Tab. 1), of which 10 may or may not have trichomes on leaflets, and when present they were found mainly on the midrib (Fig. 4G) and margins (Fig. 4H-I). Such structures may easily fall off as the leaves grow older. Trichomes were present on all the material collected for only four taxa: *C. aspidiifolia*, *C. ensiformis* var. *maranonica*, *C. subpeltata* and *C. xinguensis*.

All species displayed paracytic laterocyclic stomata (Fig. 4J-K) and hypostomatic leaflets; however, we rarely observed few stomata scattered along the midrib on the adaxial side. As a whole, the anticlinal cell walls of epidermal cells on the adaxial side of leaflets were straight or lightly sinuous (Fig. 4L-M) and sinuous on the abaxial side (Fig. 4J-K). However, few exceptions could be observed in the collections of the two varieties of *C. regana* and *C. xinguensis* (Fig. 4N) and *C. onusta*, as sinuosity on the adaxial side could also be observed. Exceptions were also present in a few collections of *C. amabilis*, *C. amorimii*, *C. bahiae*, *C. compitalis*, *C. ensiformis*, *C. hymenaeifolia*, *C. negrensis*, *C. onusta* and *C. polystachya*, as straight anticlinal cell walls of epidermal cells on the abaxial were observed. Leaflet epidermis was single-layered on both the adaxial and the abaxial sides for all species (Fig. 5).



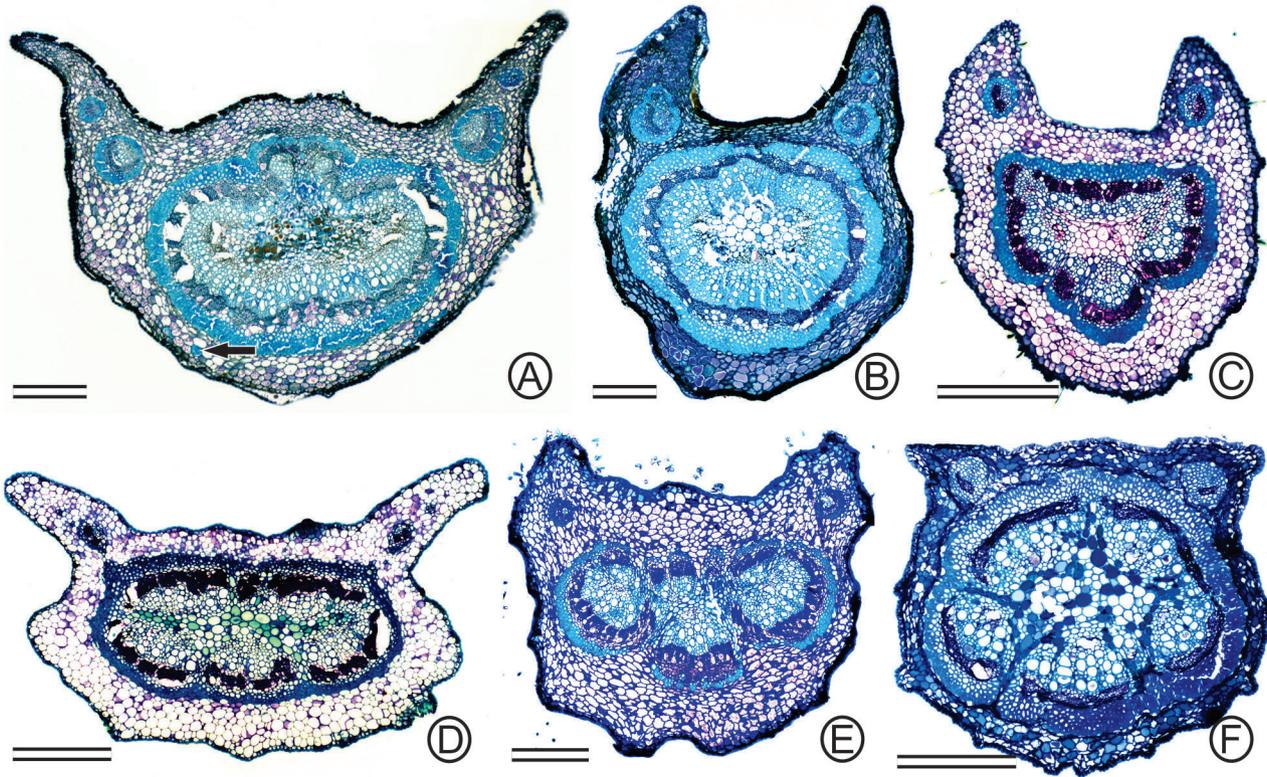


**Figure 2.** Arrangement of the vascular system forming a siphonostele in cross sections of the petiole/rachis in species of *Chamaecrista* sect. *Apoucouita*. (A) *C. negrensis* var. *negrensis*. Type XIII of vascular arrangement of the petiole/rachis. Note the presence of sclereids in the cortex (arrows), fibers (Fi) around the main vascular system composed of phloem (Ph) and xylem (Xy) and a central pith (Pi). (B) *C. bahiae*, type IV. (C) *C. hymenaeifolia*, type IV, note that the petiole/rachis is grooved. (D) *C. duartei*, type XII with accessory bundles concentrated at the center of the adaxial side of the petiole/rachis. (E, F) *C. polystachya*. Type XIV with accessory bundles concentrated at the center of the adaxial side of the petiole, but the bundles form another cylinder around a less conspicuous central pith (double arrows). Bars = 300µm.

Papillae (Fig. 6) were observed in only five taxa (Tab. 1). Two different types of papillae were found: several domes per cell (Figs. 5B-C, 6A-C) and one dome per cell (Figs. 5D-E, 6D-E). One-dome papillae were found in *C. asplenifolia* (Fig. 5A, F), *C. polystachya* (Fig. 6D) and *C. hymenaeifolia* (Fig. 6E), the first species having such papillae on both adaxial

and abaxial sides (Fig. 5A, F), while the other two species had one-dome papillae only on the abaxial side (Fig. 5D). Several-dome papillae were found only in the two varieties of *C. adiantifolia* and were always found on the abaxial side (Fig. 5B, C).

Secretory idioblasts (Fig. 5A, B) were observed on the



**Figure 3.** Arrangement of the vascular system in cross sections of the petiole/rachis of species of *Chamaecrista* sect. *Apoucouita*. (A, B) *C. apoucouita*. Arrow indicates sclereids. (C) *C. subpeltata*. (D, E) *C. adiantifolia* var. *pteridophylla*. (F) *C. ensiformis* var. *ensiformis*. Note the different types of vascular arrangement of the petiole/rachis. Type XIII in (A), type IV in (B-D) and type V in (E-F). (A, D and F) display petiole/rachis more or less marginate while (B-C and E) canaliculate or grooved. Bars = 300µm.

adaxial and abaxial epidermis but were less frequent on the abaxial epidermis, especially when this epidermis had papillae (Fig. 5B). Sometimes the secretory idioblasts displayed a thinner periclinal cell wall, dividing the cell into two, and giving to the epidermis the appearance of a double layer (Fig. 5A, B).

Dorsiventral mesophyll (Fig. 5A-B, E-G) was found in all species. Adaxial palisade parenchyma was composed of 1–3 layers of cells while abaxial spongy parenchyma was composed of 3–12 layers. The number of layers of both palisade and spongy parenchyma varied within species. Sclereids scattered among the mesophyll were exclusive to *C. amorimii* (Fig. 5E).

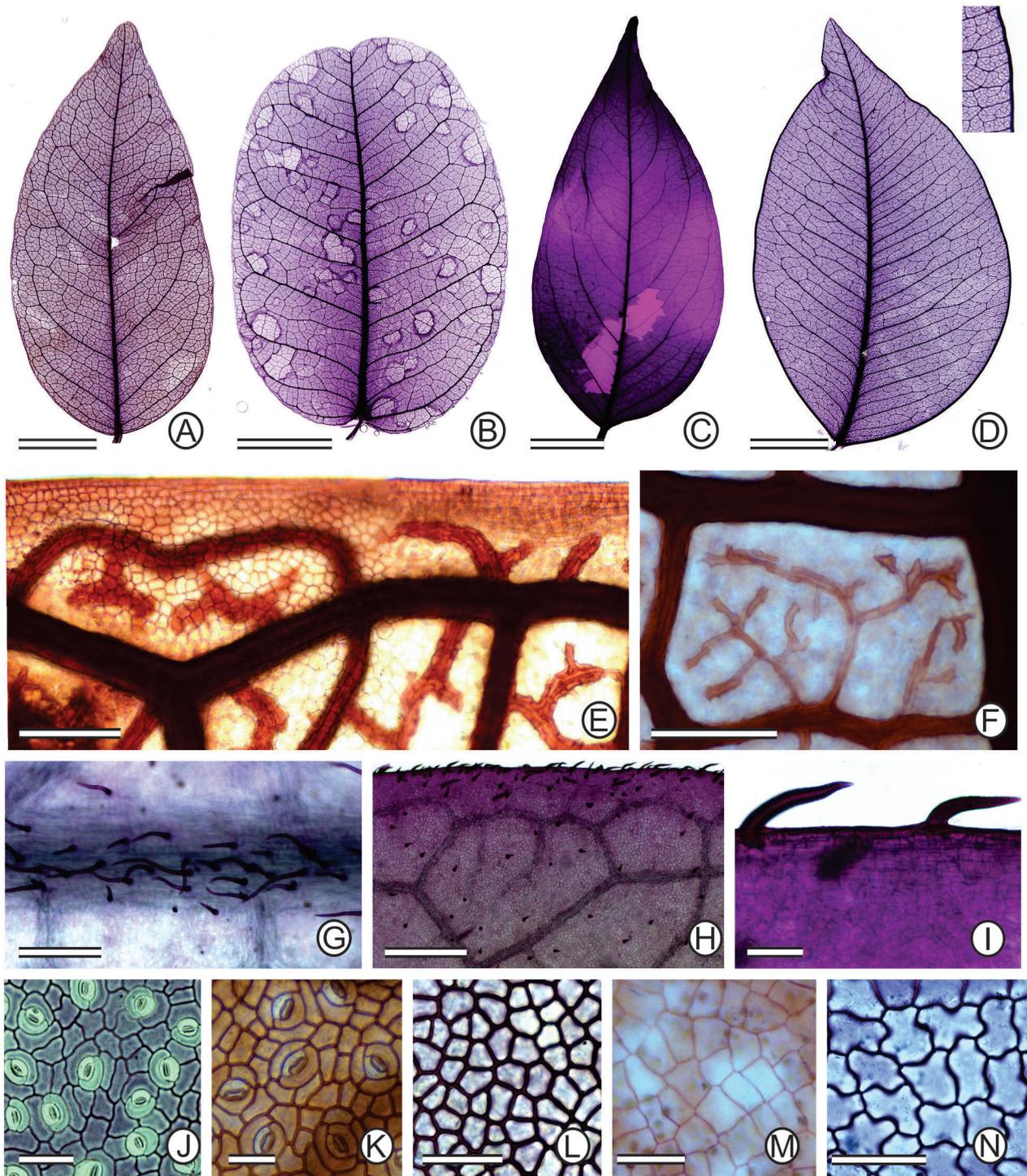
Midribs had the vascular system arranged as collateral vascular bundles (Fig. 5A, G-H). Additionally, we observed that some of the samples of every species had collateral vascular bundles with 3–5 accessory bundles on the adaxial side (Fig. 5H). Similar to the vascular system in the petiole, layers of fibers surrounded the vascular system of the midrib. In the area of the midrib, secretory idioblasts in the epidermis were rarely observed (Fig. 5G, H). Layers of collenchyma below the epidermis were followed by layers of chlorenchyma in all species except the two varieties of *C. adiantifolia* and *C. aspleniifolia*. However, this character was

not fixed as sometimes collenchyma was not found and just a few layers of chlorenchyma or parenchyma were present instead. The chlorenchyma on both the adaxial and abaxial side of the midrib could not be classified into palisade or spongy parenchyma since only compact isodiametric cells were found. Only the two varieties of *C. adiantifolia* and *C. aspleniifolia* had palisade parenchyma on the adaxial side of the midrib area (Tab. 1).

### Extrafloral Nectaries

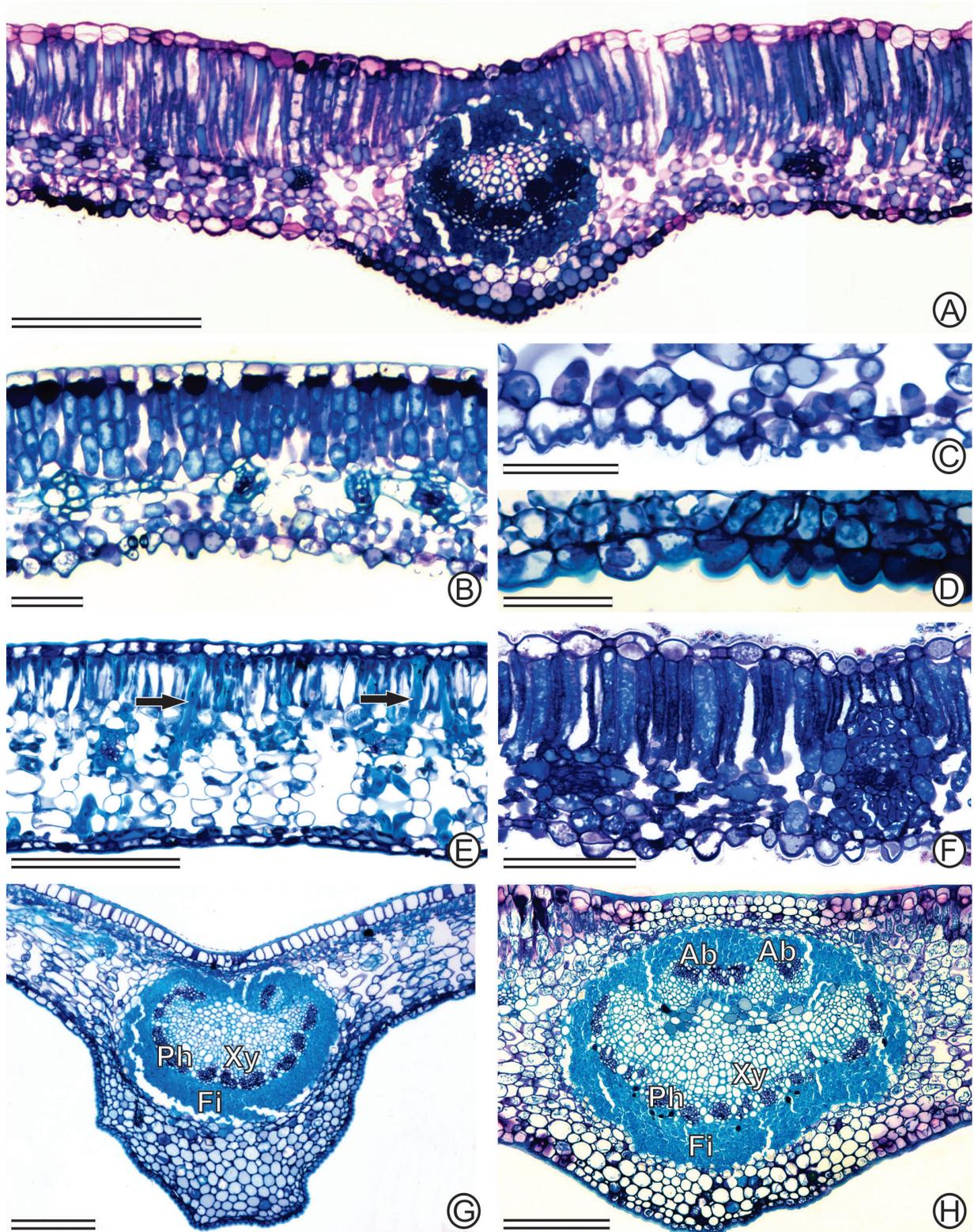
Six taxa exhibited the most basal extrafloral nectary (EFN) exclusively on the petiole proper, such as *C. hymenaeifolia* (Fig. 1B) and *C. polystachya* (Fig. 1C) (Tab. 1). Seventeen taxa had the most basal EFN between/immediately below the basal pair of leaflets (Tab. 1), as in *C. eitenorum* var. *regana* (Fig. 1A). Other EFNs found on the leaves of all species were located between/immediately below other pairs of leaflets, except in *C. hymenaeifolia* and *C. polystachya* where they were located only below the other pairs of leaflets.

Impressed EFNs were observed in nine taxa (Tab. 1), including *C. amabilis* (Fig. 7A), *C. hymenaeifolia* (Fig. 7B) and *C. polystachya* (Fig. 7C); sessile flat/truncate/convex in 13

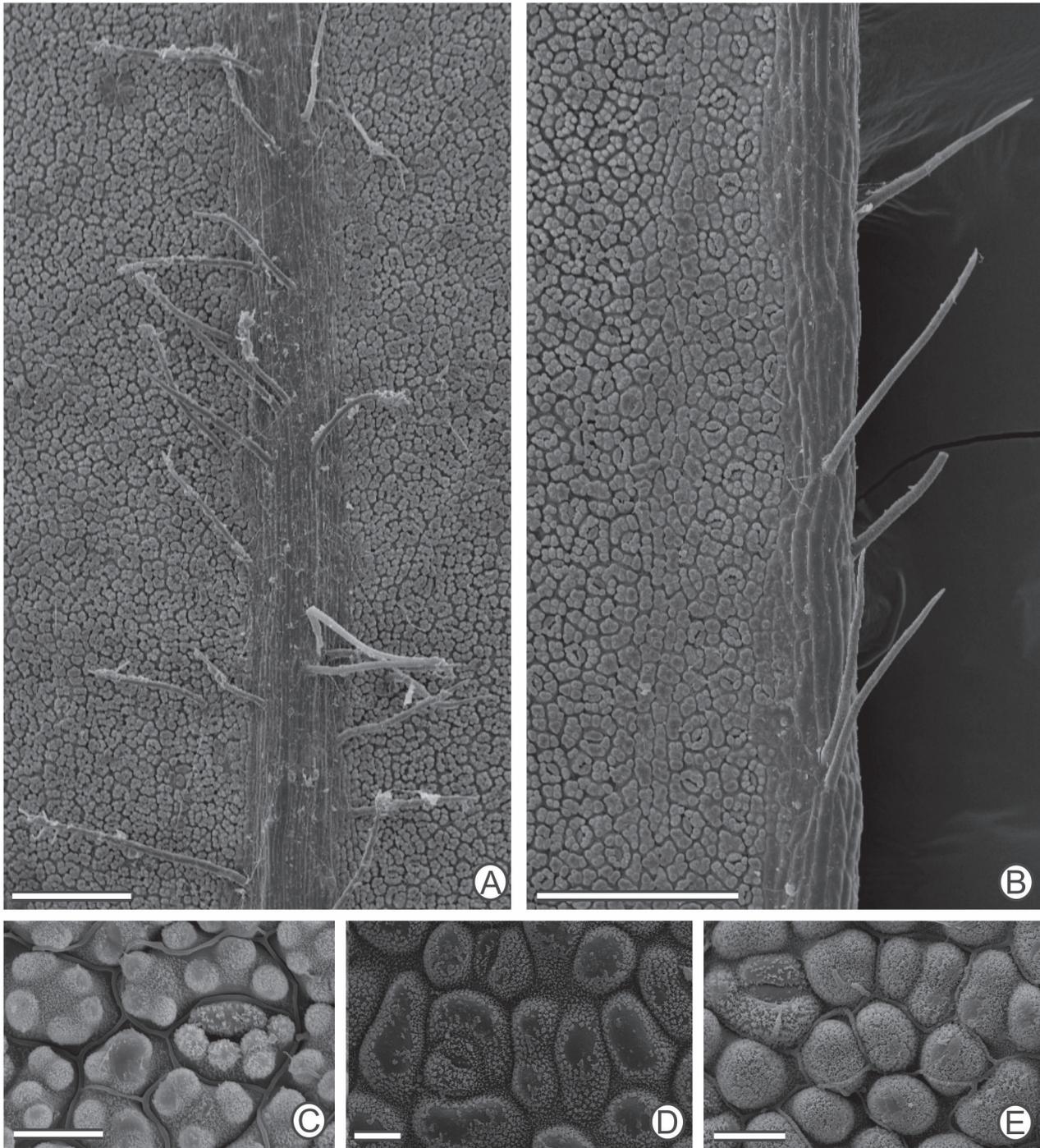


**Figure 4.** Leaflet clearings of species of *Chamaecrista* sect. *Apoucouita*. (A) *C. ensiformis* var. *ensiformis* and (B) *C. polystachya* with brochidodromous venation. (C) *C. negrensis* var. *negrensis* with eucamptodromous becoming brochidodromous distally. (D) *C. hymenaeifolia* with craspedodromous-brochidodromous with detail of the perimarginal veins of marginal secondary type. (E) *C. polystachya* showing marginal ultimate venation looped and incomplete. (F) *C. eitenorum* var. *eitenorum* with enlarged tracheids (tracheoids) at the vein endings. (G) Non-secretory trichomes on the midvein in *C. ensiformis* var. *maranonica* and mostly on the leaflet margin in *C. subpeltata* (H, I). (J) *C. apoucouita* and (K) *C. amorimii* with paracytic laterocyclic stomata on the abaxial side of the epidermis. Note the sinuous outline of anticlinal walls of the epidermal cells on the abaxial side in (J) and straight in (K). (L) *C. amorimii* and (M) and *C. eitenorum* var. *eitenorum* with straight outline of the anticlinal walls of the epidermal cells on the adaxial side and (N) *C. xinguensis* with sinuous. Bars = A–D, 20 mm; E–G, 200  $\mu$ m; H, 500  $\mu$ m, I–N, 50  $\mu$ m.

A study of the morphoanatomical characters of the leaves of  
*Chamaecrista* (L.) Moench sect. *Apoucouita* (Leguminosae-Caesalpinioideae)



**Figure 5.** Cross sections of leaflets of species of *Chamaecrista* sect. *Apoucouita*. (A) *C. aspleniifolia* with one-dome papillae on both sides of the epidermis and collateral arrangement of the vascular bundles. (B–C) *C. adiantifolia* var. *pteridophylla* with several-dome papillae on the adaxial side of the leaflet. Note mucilage idioblasts in the epidermis and a thin periclinal cell wall dividing some of the cells into two (B). (D) *C. hymenaeifolia* with one-dome papillae on the abaxial side. (E) *C. amorimii* with sclereids (arrows) in the mesophyll. (F) *C. aspleniifolia*, note epidermis with one-dome papillae on both sides. (G) *C. onusta* with midvein displaying collateral vascular bundles and (H) *C. amabilis* with collateral vascular bundles with accessory bundles. Bars = A, E–H, 200  $\mu$ m; B–D, 50  $\mu$ m.



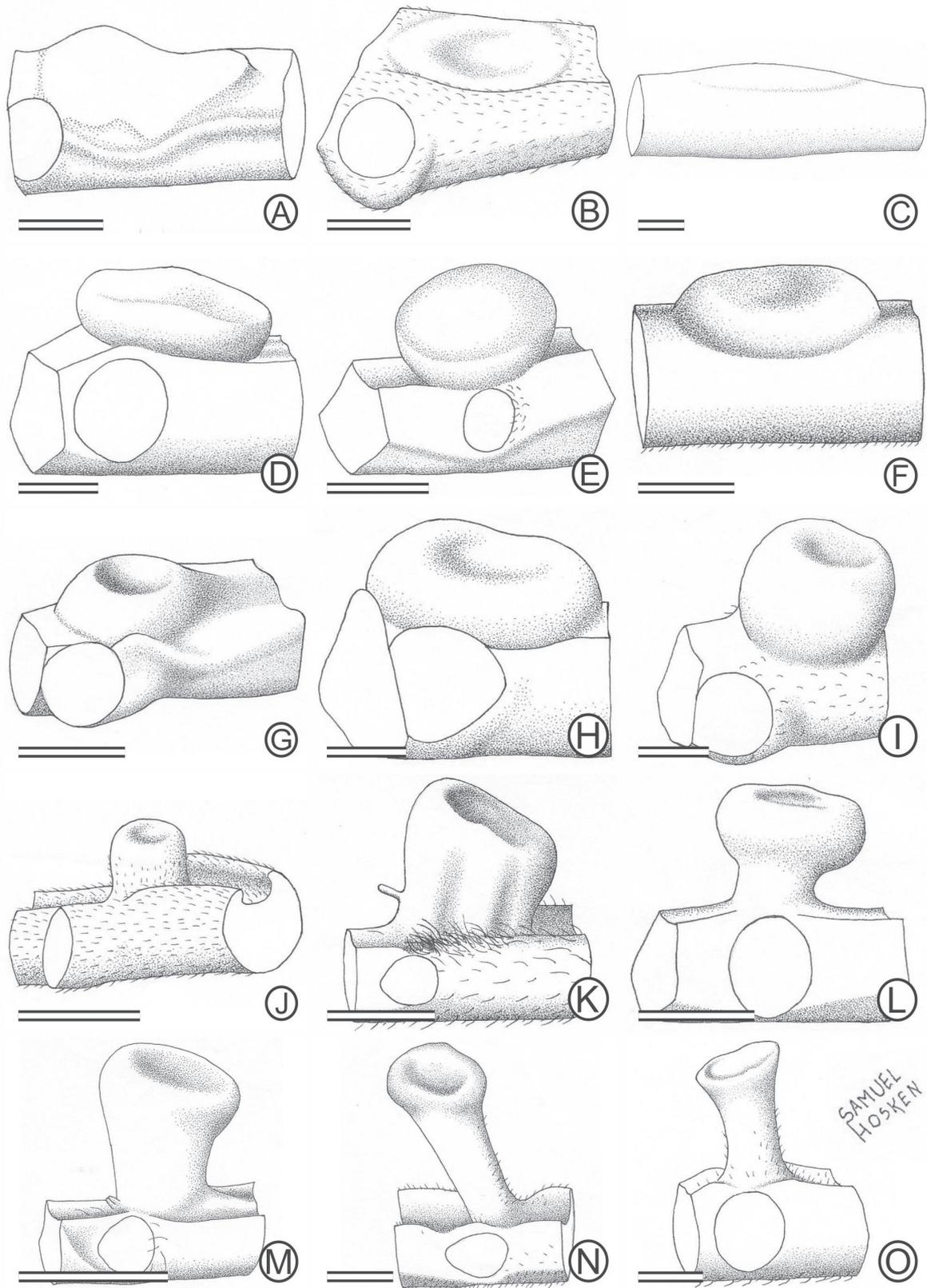
**Figure 6.** Scanning electron microscopy of the abaxial side of leaflets of species of *Chamaecrista* sect. *Apoucouita*. (A–C) *C. adiantifolia* var. *pteridophylla*. Note the non-secretory trichomes on the midvein (A) and (B) margins of the leaflets and the several domes per cell papillae (C). (D) *C. polystachya* and (E) *C. hymenaeifolia* displaying one dome per cell papillae. Bars = A–B, 200  $\mu$ m; C–E, 20  $\mu$ m.

taxa (Tab. 1), such as in *C. bahiae* (Fig. 7D) and *C. eitenorum* var. *regana* (Fig. 7E); sessile and concave in 19 taxa (Tab. 1) (Fig. 7F–K); urceolate in eight (Tab. 1); cupuliform and short-stalked in fifteen taxa (Tab. 1), such as in *C. ensiformis* var. *ensiformis* (Fig. 7L); cupuliform and stalked

in 10 taxa (Tab. 1), such as in *C. aspleniifolia* (Fig. 7M); cupuliform and long-stalked in six taxa (Tab. 1), such as in *C. subpeltata* (Fig. 7N); patelliform and short-stalked in three taxa (Tab. 1); and patelliform and stalked and/or long-stalked in four (Tab. 1), such as in *C. compitalis* (Fig. 7O).



A study of the morphoanatomical characters of the leaves of  
*Chamaecrista* (L.) Moench sect. *Apoucouita* (Leguminosae-Caesalpinioideae)



**Figure 7.** Extrafloral nectaries (EFN) on leaves of species of *Chamaecrista* sect. *Apoucouita*. (A) *C. amabilis*, (B) *C. hymenaeifolia* and (C) *C. polystachya*, EFN impressed. (D) *C. bahiae* and (E) *C. eitenorum* var. *regana*, sessile convex. (F) *C. hymenaeifolia*, (G) *C. amabilis*, (H) *C. onusta*, (I) *C. ensiformis* var. *ensiformis*, (J) *C. xinguensis* and (K) *C. aspleniifolia*, sessile concave. (L) *C. ensiformis* var. *ensiformis*, cupuliform short-stalked. (M) *C. aspleniifolia*, cupuliform stalked. (N) *C. subpeltata*, cupuliform long-stalked. (O) *C. compitalis*, patelliform stalked. Bars = 1mm.



Identification key for *Chamaecrista* sect. *Apoucouita*

An identification key for *Chamaecrista* sect. *Apoucouita* based on morphoanatomical characters of leaves:

1. Leaflets numerous 7-35 pairs; leaflets 5-35 mm long; petiole generally <15 mm long (except in *C. ensiformis* var. *plurifoliolata* 12-25 mm long) ..... 2
  2. Extrafloral nectary on the petiole proper and between/immediately below other pairs of leaflets; extrafloral nectary types variable; epidermis papillose at least on one side of leaflet blades; palisade parenchyma on the adaxial side at the midvein ..... 3
    3. Papillae with one dome per cell on the adaxial and abaxial epidermis; leaflets oblong-elliptic ..... *C. aspleniifolia*
    - 3'. Papillae with several domes per cell on the abaxial epidermis only; leaflets oblong ..... 4
      4. Apex of leaflets obtuse to shallowly emarginate ..... *C. adiantifolia* var. *adiantifolia*
      - 4'. Apex of leaflets abruptly emarginate ..... *C. adiantifolia* var. *pteridophylla*
  - 2'. Extrafloral nectary between/immediately below the basal pair of leaflets as well as other pairs of leaflets; extrafloral nectaries exclusively cupuliform; epidermis papillose on leaflet blades absent; epidermis papillose at least on one side of the leaflet blades absent; palisade parenchyma on the adaxial side at the midvein absent ..... 5
    5. Petiole with pulvinus 12-25 mm; leaflets ovate to narrowly-elliptic, base cuneate to obtuse, apex acute or acuminate ..... *C. ensiformis* var. *plurifoliolata*
    - 5'. Petiole with pulvinus 3-10 mm; leaflets oblong, base oblique, apex obtuse or retuse ..... 6
      6. Distributed in Bahia, Brazil ..... *C. aspidifolia*
      - 6'. Distributed in Maranhão, Brazil ..... *C. subpeltata*
- 1'. Leaflets less numerous 2-6 pairs (except in *C. ensiformis* var. *plurifoliolata* and *C. xinguensis* up to 10); leaflets 20-200 mm long; petiole >12 mm long ..... 7
  7. First extrafloral nectary on the petiole proper ..... 8
    8. Extrafloral nectaries between/immediately below other pairs of leaflets; 6-10 pairs of leaflets, ovate to narrowly ovate, base obtuse, apex acute or acuminate; papillae on the abaxial epidermis of the leaflet blades absent ..... *C. xinguensis*
    - 8'. Extrafloral nectaries below other pairs of leaflets; 2-4 pairs of leaflets; leaflets asymmetrically ovate-elliptic or subsymmetrically obovate-suborbicular; papillae with one dome per cell on the abaxial epidermis of the leaflet blades present ..... 9
      9. Petiole with pulvinus 7-20 mm; extrafloral nectaries impressed only; leaflets asymmetrically ovate-elliptic short-acuminate and midrib curved; brochidodromous-craspedodromous venation with perimarginal veins of secondary type ..... *C. hymenaeifolia*
      - 9'. Petiole with pulvinus 20-40 mm; extrafloral nectaries impressed, sessile flat/truncate/convex or sessile concave; leaflets subsymmetrically obovate-suborbicular, broadly obtuse or emarginate, and midrib straight; brochidodromous venation; perimarginal veins of secondary type absent ..... *C. polystachya*
  - 7'. Extrafloral nectary between/immediately below basal and other pairs of leaflets ..... 10
    10. Petiole with pulvinus >50 mm long; sclereids in the mesophyll; leaflets obovate or oblong-obovate, base cuneate or narrowly cuneate, apex acuminate ..... *C. amorimii*
    - 10'. Petiole with pulvinus <50 mm long; sclereids in the mesophyll absent ..... 11
      11. Petiole/rachis canaliculate, grooved or more or less marginate absent ..... 12
        12. Brochidodromous venation; leaflets exactly 2-3 pairs ..... 13
          13. Leaflets usually 2 pairs of leaflets ..... *C. bahiae*
          - 13'. Leaflets usually 3 pairs of leaflets ..... *C. duartei*
        - 12'. Eucamptodromous-brochidodromous venation; leaflets 2-6 pairs ..... 14
          14. Leaflets 2-3 pairs, usually 2 pairs and broadly ovate with apex short-acuminate ..... *C. negrensis* var. *negrensis*
          - 14'. Leaflets 3-6 pairs, usually 4-6 pairs and narrowly ovate with apex caudately long-acuminate ..... *C. negrensis* var. *albuquerquei*
      - 11'. Petiole/rachis canaliculate, grooved or more or less marginate present ..... 15
        15. Stipules subulate-setiform over 1 mm, commonly over 2 mm; extrafloral nectary cupuliform or patelliform only, both stalked or long-stalked ..... *C. compitalis*
        - 15'. Stipules obsolete (not over 1mm), not observed or early caduceus; extrafloral nectary types variable .. ..... 16



- 16.** Sessile to subsessile leaflets; blade of leaflets cordate or slightly so at base usually on either side present ..... 17
- 17.** Petiole/rachis canaliculate or grooved but the most distal segment of the rachis always marginate (winged) ..... *C. apoucouita* or *C. scleroxylon*
- 17'.** Petiole/rachis canaliculate or grooved but the most distal segment of the rachis never marginate (winged) ..... 18
- 18.** Leaflets exactly 2 pairs ..... *C. eitenorum* var. *regana*
- 18'.** Leaflets 3-4 pairs ..... *C. eitenorum* var. *albuquerquei*
- 16'.** Sessile to subsessile leaflets; blade of leaflets cordate at base usually on either side absent ..... 19
- 19.** Leaves ample; petiole usually over 25 mm; most distal pair of leaflets over 120 mm ... *C. onusta*
- 19'.** Leaves smaller; petiole up to 25 mm; most distal pair of leaflets up to 120 mm ..... 20
- 20.** Leaflets exactly 2 pairs ..... *C. amabilis*
- 20'.** Leaflets 3-8 pairs, rarely 2 ..... *C. ensiformis*

taxa (Tab. 1), such as in *C. bahiae* (Fig. 7D) and *C. eitenorum* var. *regana* (Fig. 7E); sessile and concave in 19 taxa (Tab. 1) (Fig. 7F-K); urceolate in eight (Tab. 1); cupuliform and short-stalked in fifteen taxa (Tab. 1), such as in *C. ensiformis* var. *ensiformis* (Fig. 7L); cupuliform and stalked in 10 taxa (Tab. 1), such as in *C. aspleniifolia* (Fig. 7M); cupuliform and long-stalked in six taxa (Tab. 1), such as in *C. subpeltata* (Fig. 7N); patelliform and short-stalked in three taxa (Tab. 1); and patelliform and stalked and/or long-stalked in four (Tab. 1), such as in *C. compitalis* (Fig. 7O).

## Discussion

The position and type of the extrafloral nectary (EFN) provided an important set of data that were very useful in the identification of taxa. EFN Position was especially useful in the recognition of *C. adiantifolia*, *C. aspleniifolia*, *C. aspidiifolia*, *C. hymenaeifolia*, *C. onusta*, *C. polystachya*, *C. subpeltata* and *C. xinguensis* since EFNs on the petiole proper could always be observed. EFN type was also quite important when there was little variation in shape, such as in *C. hymenaeifolia*, *C. onusta* and *C. polystachya*. For the remaining species, EFN type was generally quite variable, thereby limiting taxonomic usefulness. EFN position has previously been used successfully in the taxonomy of species of *C. sect. Absus* subsect. *Baseophyllum* (Conceição *et al.* 2008) as well as in Leguminosae (Lersten & Brubaker 1987; Pascal *et al.* 2000; Marazzi *et al.* 2006; Melo *et al.* 2010) and other plant families (Keeler & Kaul 1979; Oliveira & Leitão-Filho 1987; So 2004; Weber & Keeler 2013).

The presence of non-secretory trichomes is quite tricky to use as a character in the identification of taxa belonging to *C. sect. Apoucouita*. Non-secretory trichomes may or may not be present in many of the species, especially on leaflet blades, and they may even fall off, an issue that limits their taxonomic usefulness.

Having the vascular arrangement of the petiole similar to a siphonostele was common for all species of *Chamaecrista* sect. *Apoucouita*. This arrangement pattern, as well as the presence of accessory bundles (with accessory bundles

in the wings, when such wings are present), have already been reported in the literature for *Chamaecrista* belonging to different sections (Metcalf & Chalk 1950; Coutinho *et al.* 2013; Francino *et al.* 2015). Although type XII and XIV of vascular arrangement of the petiole were exclusively observed in *C. duartei* and *C. polystachya*, this character has proven difficult to use in the identification of both species since other types of vascular arrangements in the petiole were also found.

Although the amount of sclereids in the cortex of the petiole varied, such character was not taxonomically promising but could be associated with the size of leaves instead. The presence of a greater number of sclereids in the petiole of larger leaves indicates that such cells may be involved in the mechanical support of leaves (Evert 2006). The arrangement of the vascular tissue in the petiole/rachis, as well as the presence of sclereids scattered among the cortex, may be useful in distinguishing vegetative branches of species of *Chamaecrista* sect. *Apoucouita* that resemble species of *Inga* when specimens are not flowering. For *Inga punctata* and *Inga verna* subsp. *affinis*, the transverse sections of the petiole/rachis also exhibits a continuous or interrupted ring of bundles, accompanied by accessory bundles in the wings (Metcalf & Chalk 1950; Arambarri *et al.* 2006). However, the main difference between the species of *C. sect. Apoucouita* and species of *Inga* is the presence of one/two large, accessory, centric, medullary bundle with central phloem and the lack of sclereids in *Inga* (Metcalf & Chalk 1950; Arambarri *et al.* 2006). Although more species of *Inga* should be checked to confirm if such an arrangement pattern of the vascular system is common for all species of the genus, the arrangement of the vascular tissue in the petiole/rachis is a promising character in the separation of *C. sect. Apoucouita* and *Inga*. Such differences can be easily observed in the field upon staining cross sections of the petiole/rachis with phloroglucinol, a dye that stains lignified cell walls such as fibers and xylem cells (Johansen 1940).

Brochidodromous venation was found in all species except *C. hymenaeifolia*. Brochidodromous venation agrees with the pinnate venation described for the *C. sect.*



*Apoucouita* (Irwin & Rogers 1967; Irwin & Barneby 1977; 1982). Craspedodromous-brochidodromous venation with perimarginal veins of secondary-type was unique to *C. hymenaeifolia* while eucamptodromous-brochidodromous venation was unique to *C. negrensis*. Druses associated with the vascular bundles and enlarged tracheids at the vein endings were also observed in all species of *C. sect. Apoucouita* and has already been reported for other species of *Chamaecrista* belonging to other sections (Coutinho *et al.* 2013; Francino *et al.* 2015;) in a way that such characters seem to be common for the genus.

All studied species had hypostomatic leaves, a character that is not widely spread in the genus, as other species from other sections (*sect. Absus*, *Chamaecrista*, *Grimaldia* and *Xerocalyx*) usually display amphistomatic leaves, except *C. sect. Absus* subsect. *Baseophyllum* with species having epistomatic leaves (Coutinho *et al.* 2013; Francino *et al.* 2015). In *Chamaecrista*, hypostomatic leaves are common in species found in rain forests (*C. sect. Apoucouita*) while amphistomatic/epistomatic leaves are usually found in species from open sunny areas (*sect. Absus*, *Chamaecrista*, *Grimaldia* and *Xerocalyx*) which demonstrates that *Chamaecrista* display the typical ecological pattern of stomatal position described in the literature (Fahn & Cutler 1992; Dickson 2000).

Paracytic laterocyclic stomata were present in all species of *C. sect. Apoucouita* and agrees with previous reports in Caesalpinioideae (Solereider 1908; Metcalfe & Chalk 1950; Cowan 1981; Saheed & Illoh 2010) and *Chamaecrista* (Francino *et al.* 2015).

Even though a few exceptions were observed, a straight or lightly sinuous outline of the anticlinal cell wall of the epidermal cells on the adaxial side and sinuous outline on the abaxial side was common for all species of *C. sect. Apoucouita*. This character is not very common in *Chamaecrista* since species tend to have a straight contour on both sides (Francino *et al.* 2015). The few exceptions observed regarding the outline of the epidermal cells may have been a result of the exposure of leaves to sun or shade; leaves exposed to sun usually have a straight outline while leaves exposed to shade display a sinuous outline (Pyykkö 1979). Since most of our material came from herbarium specimens, we do not know if they were exposed to sun or shade. Epidermal papillae were one of the most distinctive characters for species of *C. sect. Apoucouita* since only five species displayed them. Papillae on the adaxial and/or abaxial epidermal cells is not a common trait for *Chamaecrista* as they are usually restricted to the midvein (Francino *et al.* 2015).

The secretory epidermal cells observed are most probably mucilage idioblasts since the reddish-purple staining of the cells indicates the presence of pectins (O'Brien & McCully 1981). The secretory idioblasts found in the mesophyll of species of *C. sect. Absus* subsect. *Baseophyllum* were revealed by histochemical analyses to be mucilage idioblasts

(Coutinho *et al.* 2013); these idioblasts also displayed a reddish purple color when stained with toluidine blue (Coutinho *et al.* 2013), similar to our results. Although *Chamaecrista ensiformis* var. *ensiformis* had already been anatomically studied Carvalho (1983-1985), the author failed to describe the presence of secretory idioblasts in the epidermis, even though a histochemical study was carried out. Epidermal mucilage idioblasts were also observed in other species of *C. sect. Absus* as well as *C. sect. Grimaldia* (Francino *et al.* 2015). Coutinho *et al.* (2013) suggests that mucilage idioblasts may represent an adaptation to aridity as *Chamaecrista* is a genus typically found in open sunny areas. However, species included in *C. sect. Apoucouita* are commonly found in the Amazon and Brazilian Atlantic forests, habitats well supplied with water. Nevertheless, some species are found in savannah-like areas, as is the case with *C. aspidiifolia*, *C. polystachya*, *C. compitalis* and *C. eitenorum*, an environment where the presence of mucilage in the epidermis could readily contribute to water retention.

Dorsiventral mesophyll and open arch collateral vascular bundles which may have 3–5 accessory bundles on the adaxial side were common for all species studied and had already been reported for other species of *Chamaecrista* (Metcalf & Chalk 1950; Coutinho *et al.* 2012; Francino *et al.* 2015). Considering only *C. sect. Apoucouita*, the presence of sclereids scattered in the mesophyll is a unique character for *C. amorimii*. Interestingly, *C. amorimii* has the largest leaves among the species of *Chamaecrista*, indicating that the presence of sclereids in the mesophyll is an important evolutionary character for this species, aiding in the mechanical support of its large leaves (Evert 2006).

Morphoanatomical characters of leaves proved to be a useful additional tool in the identification of species of *Chamaecrista sect. Apoucouita*, as such characters could be readily used to build an identification key. A number of other characters were shared by all species: the arrangement of the vascular system in the petiole/rachis and leaflets, dorsiventral mesophyll, mucilage idioblasts in the adaxial and abaxial epidermis, outline of the anticlinal walls of the epidermal cells and position and type of stomata. Even though such characters may be sparsely found in species of other sections of *Chamaecrista* (Coutinho *et al.* 2012; Francino *et al.* 2015), since they are present in all species of *C. sect. Apoucouita*, they may still be taxonomically promising by contributing to the consolidation of this group of species as a monophyletic section as demonstrated by Conceição *et al.* (2009).

Apart from the difference in the leaf apex, no other morphoanatomical characters that support the distinction of *C. adiantifolia* var. *adiantifolia* and *C. adiantifolia* var. *pteridophylla* were observed. Even with the difference in the leaflet apex, there are collections that may make us wonder which of the two varieties a specimen under consideration represents. This may be explained on the basis of the leaflet



apexes. *C. adiantifolia* var. *adiantifolia* have leaflet apexes varying from obtuse to shallowly emarginated which may overlap with the emarginate or abruptly emarginate leaflet apexes of *C. adiantifolia* var. *pteridophylla*. Two collections that demonstrate this issue are Maguire *et al.* 41983, 02.XI.1957 (NY, SP, US) and Silva *et al.* 239, 03.XII.1985 (NY, US). The overlapping of this character in these collections leads us to question whether such taxa should be treated as two distinct varieties.

No remarkable morphoanatomical differences were observed between *C. aspidiifolia* and *C. subpeltata* that would support the distinction of these species. *C. aspidiifolia* usually displays a higher number of leaflets (9-13 pairs) than *C. subpeltata* (7-9 pairs), but these ranges clearly demonstrate that the number of leaflets may overlap. The geographic distributions of these two species seems to be the only justification for their separation at the species level. An effort to collect more specimens between the known distributions (i.e. states of Bahia and Maranhão, Brazil, for *C. aspidiifolia* and *C. subpeltata*, respectively) should be made in order to confirm whether there are populations with characters that are intermediate between the two species.

Although in our identification key does not separate *C. bahiae* and *C. duartei* properly separated, rising racemes have been widely used to distinguish *C. duartei* from *C. bahiae*; contemporary leaves at the end of hornotinous branchlets in the former, and old wood below contemporary leaves in the later (Irwin & Barneby 1985). However, *C. bahiae* may also display racemes as observed in *C. duartei* (field observations) and vice versa, indicating that other characters should be found to distinguish these species.

Differing from Irwin & Barneby (1977), we found that *C. negrensis* var. *albuquerquei* may have specimens with 6 pairs of leaflets instead of only 3-4. Although differences between the two varieties of *C. negrensis* were observed, we believe that they deserve a taxonomic review, especially because their leaflet and apex shape (broadly ovate with apex short-acuminate against narrowly ovate with apex caudately long-acuminate) may overlap. It seems that the number of leaflets would better distinguish these varieties of *C. negrensis*; *C. negrensis* var. *negrensis* with 2-3 pairs of leaflets and *C. negrensis* var. *albuquerquei* with 4-6 pairs.

Based solely on vegetative characters, *C. apoucouita* was not distinguished from *C. scleroxylon*. Irwin & Barneby (1977) mentioned that in *C. apoucouita* the androecium is glabrous while in *C. scleroxylon* villosulous or velutinous. However, a shift of focus away from characters of pubescence and towards other characters has already been demonstrated by the same authors in the separation of two different taxa (i.e. the varieties included in *C. adiantifolia*) (Irwin & Barneby 1977). Another difference pointed out between *C. apoucouita* and *C. scleroxylon* is that the former has a trunk that is deeply sulcate lengthwise while that of *C. scleroxylon* is sulcate. This character may be misleading, however, since the trunk of specimens is rarely represented or described

in voucher material deposited in herbaria.

For *C. eitenorum* var. *regana* and *C. eitenorum* var. *albuquerquei*, the type of extrafloral nectary has been used as an additional character for their distinction (Irwin & Barneby 1977). However, Coutinho & Meira (2015) have already demonstrated that both varieties have the same types of extrafloral nectaries. Therefore, the type of EFNs should no longer be used to distinguish these varieties. Moreover, Irwin & Barneby (1977) mentioned that the length of the leaf stalk is longer in *C. eitenorum* var. *regana* than in *C. eitenorum* var. *albuquerquei*, a character that is not surprising since the number of pairs of leaflets is higher in the later (3-4 pairs). Perhaps it is better that these varieties are described using only the difference in the number of leaflets. However, a greater number of collections of both varieties should be analyzed before amending taxon circumscriptions.

Unfortunately the three varieties included in *C. ensiformis* could not be distinguished base on the vegetative morphoanatomical analysis of the present study. Besides, floral characters are not constant for any one of the varieties (Irwin & Barneby 1977). The characters used in the description of the varieties are sometimes overlapping, such as the number of pair of leaflets and pubescence of leaves ("thinly puberulente" or "± densily pilosulous"). As shown in our results, pubescence is not a reliable character for the identification of such varieties, as it may vary equally for all of them. Only by having a petiole with a pulvinus smaller than 15mm and 5-8 leaflets can *C. ensiformis* var. *plurifoliolata* be set apart. A revision of this species for a better circumscription of the varieties is needed.

## Conclusion

Morphoanatomical characters of leaves provide an important set of data that may be used as diagnostic characters for some species of *Chamaecrista* sect. *Apoucouita*. The identification key presented herein is an important tool for species identification when only vegetative material is available. Our data provide evidence for the need of taxonomic re-evaluation of some taxa, especially varieties, since no anatomical differences were observed and some of the characters used in the circumscription of such taxa may overlap. When only vegetative material is available, species of *C. sect. Apoucouita* may be confused with the genus *Inga*. However, the arrangement of the vascular tissue and the presence of sclereids scattered among the cortex in the petiole/rachis seem to be promising characters that may aid the distinction of species of *C. sect. Apoucouita* and *Inga*. Moreover, a complete phylogenetic analysis of *Chamaecrista*, with the addition of the morphoanatomical characters common to all species of *C. sect. Apoucouita*, would confirm the usefulness of such data for taxonomy by providing data that could aid in demonstrating that this group of species as a monophyletic section.



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