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Importance of anatomical leaf features for characterization of three species of *Mapania* (Mapanioideae, Cyperaceae) from the Amazon Forest, Brazil

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ABSTRACT

Mapania belongs to Mapanioideae, a quite controversial subfamily in Cyperaceae due to the existence of unusual characters in both reproductive and vegetative organs. The genus is represented by seven species in Northern Brazil but taxonomic valuable information related to the leaf organs is still unknown. The present study aimed the anatomical description of the leaf organs (either basal leaves or cataphylls and involucral bracts) of three representative Brazilian species of *Mapania*. Samples of cataphylls, basal leaves and involucral bracts were sectioned and stained for observations under light microscopy. The involucral bracts provide the most elucidative characters (ten) to distinguish the three species The basal leaves provides six distinguishing characters and are useful to *M. macrophylla* and *M. pycnostachya*, as they are absent in *M. sylvatica*. Mesophyll arrangement in the involucral bracts supports the circumscription of *M. macrophylla* and *M. pycnostachya* in *M.* sect. *Pycnocephala* and of *M. sylvatica* in *M.* sect. *Mapania.* Some features as thin-walled epidermal cells, stomata level and aerenchyma were considered to be adaptive to the humid environment in which the species occur. The translucent cells are here considered as aerenchyma precursors and a supportive function is assumed for the bulliform cells on the basal leaves and involucral bracts. No silica bodies were found which confirm it as a diagnostic character of *Mapania* among Hypolytreae genera.

KEYWORDS: aerenchyma, cataphylls, Hypolytreae, involucral bracts, Poales

Importância dos caracteres anatômicos foliares na caracterização de três espécies de *Mapania* (Mapanioideae, Cyperaceae) da Floresta Amazônica, Brasil

RESUMO

Mapania pertence à subfamília Mapanioideae que apresenta caracteres incomuns às demais Cyperaceae, tanto vegetativos como reprodutivos. O gênero é representado por sete espécies no norte do Brasil e apresenta ainda lacunas de informações especialmente relacionadas aos órgãos foliares. O presente estudo objetivou a descrição anatômica dos órgãos foliares (folhas basais, ou catafilos, e brácteas involucrais) de três espécies representativas de *Mapania* no território brasileiro. Amostras de catafilos, folhas basais e brácteas involucrais foram seccionadas e coradas para observações em microscopia de luz. As brácteas involucrais foram as mais elucidativas na separação das espécies, com dez caracteres variáveis, uma vez que ocorrem em todas as espécies durante o estágio reprodutivo. As folhas basais forneceram seis caracteres úteis na distinção de *M. macrophylla* e *M. pycnostachya*, já que não ocorrem em *M. sylvatica.* seguidas das folhas basais. O arranjo do mesofilo das brácteas involucrais suporta a circunscrição de *M. macrophylla* e *M. pycnostachya* em *M.* sect. *Pycnocephala* e de *M. sylvatica* em *M.* sect. *Mapania.* Características adaptativas ao ambiente úmido também foram encontradas, como epiderme com paredes delgadas, níveis dos estômatos e presença de aerênquima. As células translúcidas do mesofilo foram descritas como precursoras de aerênquima e considerou-se que as células buliformes presentes nas folhas e brácteas involucrais apresentam função de suporte desses órgãos. Corpos silicosos não foram encontrados sustentando o seu uso na delimitação de *Mapania* e dos demais gêneros de Hypolytreae.

PALAVRAS-CHAVE: aerênquima, brácteas involucrais, catafilos, Hypolytreae, Poales

INTRODUCTION

Cyperaceae is a cosmopolitan family comprising circa 5,000 species distributed in approximately 100 genera (Goetghebeur 1998; Govaerts *et al.* 2007; Naczi and Ford 2008). The current classification of Cyperaceae supports their division into two subfamilies, Mapanioideae and Cyperoideae, mainly based on molecular data (Simpson *et al.* 2007; Muasya *et al.* 2009). Cyperoideae is predominantly found in grassland environments of tropical and temperate regions, while most representatives of Mapanioideae are associated with tropical forest environments (Goetghebeur 1998).

Mapanioideae currently comprises two tribes: Hypolytreae and Chrysitricheae (Goetghebeur 1998). Among Hypolytreae, *Mapania* Aubl. is particularly well represented in the equatorial regions of South America, Africa, and Asia (Simpson 1989), with 84 species (Govaerts *et al.* 2007). Seven species occur in Northern Brazil, in the states of Amazonas, Amapá, Pará, and Roraima (Alves *et al.* 2014). In Amazonas, *M. macrophylla* and *M. pycnostachya* are both found in flooded areas close to small streams ('igarapés'), while *M. sylvatica* occurs on unflooded lands known as 'terra firme' forests (Ribeiro *et al.* 1999).

Studies on leaf structures (Pfeiffer 1927; Metcalfe 1969; Starr and Ford 2001) have been taxonomically useful at both the genus and species levels within Cyperaceae. Some of these anatomical studies have reported the occurrence of exclusive characters in *Mapania* (or its correlated genus *Hypolytrum*) which are rare amonsgt the other Cyperaceae. These include tetracytic stomata randomly arranged in the leaves, non-conical or absent silica bodies, and spherical, monoporate pollen grains (pseudomonads) (Goetghebeur 1998; Alves *et al.* 2002; Coan *et al.* 2010). Data regarding the anatomy of vegetative organs in *Mapania* are still scarce and limited to two species, *M. pycnocephala* and *M. sylvatica* (Metcalfe 1971; Alves *et al.* 2002), due to the restricted distribution of this genus.

Considering the importance of detailed studies to provide diagnostic features in *Mapania* and its characterization within Hypolytreae (Mapanioideae), the present study aimed to anatomically describe the leaf organs (either basal leaves or cataphylls and involucral bracts) of three representative Brazilian species of this genus.

MATERIALS AND METHODS

The anatomical study of leaf organs of *Mapania* was carried out based on plant material collected during field trips to the Reserva Florestal Ducke (Manaus, AM). The vouchers were deposited at Geraldo Mariz (UFP) and Rioclarense (HRCB) herbaria: *Mapania macrophylla* (Boeck) H.Pfeiff. (HRCB 52754), *M. pycnostachya* (Benth.) T.Koyama (HRCB 52750) and *M. sylvatica* Aubl. (HRCB 52758). Part of the material was fixed in FAA 50 (Johansen 1940) and transferred to 50% alcohol.

Free hand sections (both cross and longitudinal) were made, using razor blades, at the median region of the cataphylls, basal leaves (sheath, pseudopetiole and lamina) and involucral bracts. At least three individuals per species were analyzed, from which three samples of each leaf organs were fixed. The sections were stained with Astra Blue and Basic Fuchsin (Roeser 1962) and mounted in semi-permanent slides with glycerin jelly (Kaiser 1880). The histochemical tests were performed using the following reagents: PAS (Periodic Acid-Schiff Reaction) (Jensen 1962) for carbohydrates; IKI for starch (Johansen 1940); Sudan IV for insoluble lipids; and aqueous ferric chloride for phenolic compounds (Johansen 1940). A polarizing filter was used to detect possible silica bodies.

Permanent slides were also made from fixed material which was dehydrated through a normal butanol (Johansen 1940) or an ethanol series (Sass 1951), according to the consistency of each leaf organ. Dehydrated material was submitted to historesin infiltration (Historesin Embedding Kit, Leica, Wetzlar, Germany) (Feder and O´Brien 1968). The material was sectioned at 8-10µm on a rotary microtome (RM2245, Leica, Wetzlar, Germany) and then the sections were stained with periodic acid-Schiff reaction and 0.05% Toluidine Blue in a 0.1 M sodium phosphate buffer at pH 6.8 (O'Brien *et al.* 1965) and mounted in synthetic resin (Entellan, Merck, Darmstadt, Germany).

Data analysis, interpretation, description and documentation were based on the semi-permanent and permanent slides and the photomicrographs were obtained with a photomicroscope (DMLB, Leica, Wetzlar, Germany) coupled with a digital camera and the projection of micrometric scale.

RESULTS

Among the studied species, only *Mapania sylvatica* does not present basal leaves, which are reduced to cataphylls. In *M. macrophylla* the leaves are differentiated into sheath and blade, while those of *M. pycnostachya* are narrow and possess pseudopetioles (Table 1).

Cataphylls

The cataphylls of *M. sylvatica* show a single-layered epidermis constituted by flat, thin-walled cells (Figure 1A), which are covered by thin cuticle on the adaxial surface and by thick cuticle on the abaxial one (Figure 1A-C). No stomata or bulliform cells were observed. Mesophyll is formed by parenchyma with conspicuous air lacunae (Figure 1A-B). Vascular bundles are collateral and differ in size, with small and large ones alternated on two levels (Figure 1A); the bundles

Table 1. Selected anatomical characters observed in the basal leaves and involucral bracts of *Mapania*. Abbreviations: *Mm*= *Mapania macrophylla*; *Mp*= *Mapania pycnostachya*; *Ms*= *Mapania sylvatica*; AB= absent organ.

	Characters/Taxa	Mm	Mp	Ms
	Basal leaves			
	Basal leaves	well developed	well developed	reduced to cataphylls
	Pseudopetiole	absent	present	AB
Epidermis	**Leaf sheath**			
	Outer periclinal walls on the abaxial surface	convex/ slightly papillose	flat	AB
	Cuticle	thin	thick	AB
Mesophyll	Fiber bundles	scattered	close to the epidermis	AB
	Vascular bundles arrangement	many levels	two levels	AB
Epidermis	**Leaf blade**			
	Bulliform cells on the midvein region	3-4 layered	1-2 layered	AB
	Midvein region with adaxial groove	absent	present	AB
Epidermis	**Involucral bracts**			
	Epidermal cell shape in front view	rectangular	rectangular	irregular
	Stomata	abaxial surface	abaxial surface	both surfaces
	Bulliform cells on the midvein region	3-4 layered	1-2 layered	1-2 layered
	Midvein region with adaxial groove	absent	present	absent
Mesophyll	Aerenchyma	conspicuous	conspicuous	inconspicuous
	Fiber bundles	close to the epidermis	close to the epidermis	absent
	Phenolic idioblasts	absent	absent	present
	Size of vascular bundles	small and large alternated	small and large alternated	same sized
	Vascular bundles arrangement	two levels	two levels	one level
	Vascular bundle sheath extension	present	present	absent

are enclosed by a double sheath: the outer one formed by large, thin-walled cells, and the inner one composed of small, thick-walled cells (Figure 1A). Midvein region has U-shape (Figure 1B) and the margin is formed only by the epidermis (Figure 1C).

Basal leaves - Leaf sheath

Both *M. macrophylla* and *M. pycnostachya* show leaf sheath with single-layered epidermis (Figure 1D-G). *Mapania macrophylla* has small, rectangular, thin-walled epidermal cells (Figure 1D-E). Outer periclinal walls are convex to slightly papillose on the abaxial surface (Figure 1D) or flat to convex on the adaxial one (Figure 1E). Phenolic idioblasts are found in some epidermal cells. *Mapania pycnostachya* is distinguished by its epidermal cells with flat outer periclinal walls on the abaxial surface (Figure 1F-G). A thin cuticle occurs only in *M. macrophylla* (Figure 1D) and no stomata were found in either species.

The mesophyll of the leaf sheath of both species is composed of parenchyma interrupted by collateral vascular bundles interspersed with air lacunae (Figure 1E-G). In *M. macrophylla,* the mesophyll is thicker and presents different-sized bundles on various levels, which are more conspicuous in the central region of the sheath (Figure 1E). In *M. pycnostachya*, small and large vascular bundles are alternated, on two levels (Figure 1F). In both species, vascular bundles

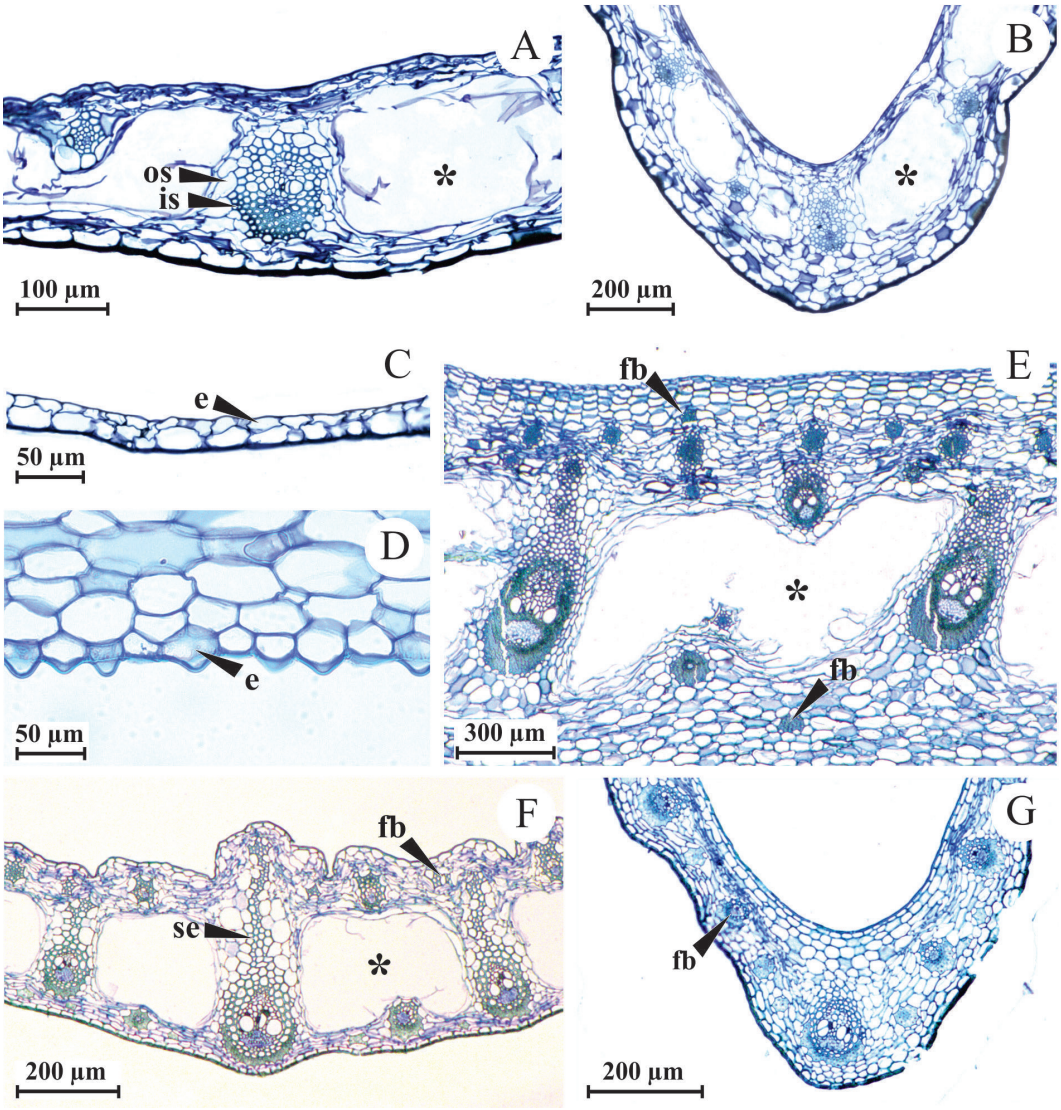

Figure 1. Anatomy of the leaf organs of *Mapania sylvatica* (A-C), *M. macrophylla* (D-E) and *M. pycnostachya* (F-G) (cross sections). A. Cataphyll, median region detail. B. Cataphyll, general view of the U-shaped midvein region. C. Cataphyll, margin detail. D. Basal leaf sheath, detail showing abaxial epidermis with papillose cells. E. Basal leaf sheath, detail of the adaxial surface and mesophyll. F. Basal leaf sheath, median region detail. G. Basal leaf sheath, general view of the U-shaped midvein region. Abbreviations: * (air lacuna), e (epidermis), fb (fiber bundle), is (inner sheath), os (outer sheath), se (sheath extension). Scale bars: A = 100 μm; B, F-G = 200 μm; C-D = 50 μm; E = 300 μm (This figure plate is in color in the electronic version).

are enclosed by a double sheath formed by large, thick-walled cells (Figure 1E-G). Bundle sheath extension may occur in both species (Figure 1E-F). Numerous fiber bundles were observed in *M. macrophylla* (Figure 1E), being scattered and concentrated closer to both epidermal surfaces in *M. pycnostachya* (Figure 1F). The midvein region of both species has U-shape and lacks bulliform cells (Figure 1G).

Basal leaves - Pseudopetiole

The pseudopetiole in *M. pycnostachya* presents a single-layered epidermis formed by rectangular, thin-walled cells with a thick cuticle (Figure 2A). Stomata are paracytic and occur on the abaxial surface (Figure 2B). On the adaxial surface, a hypodermis formed by 1-2 layers of isodiametric cells is observed (Fig. 2A). Chlorenchyma is interrupted by air lacunae formed by the lysis of large translucent cells (Figure 2A) interspersed with small and large vascular bundles arranged on two levels (Figure 2A). Air lacunae are delimited by diaphragms with stellate cells (Figure 2A). Vascular bundles are collateral and enclosed by a double sheath with thick-walled cells (Figure 2A-B). Bundle sheath extensions are composed of small cells with strongly thickened walls (Figure

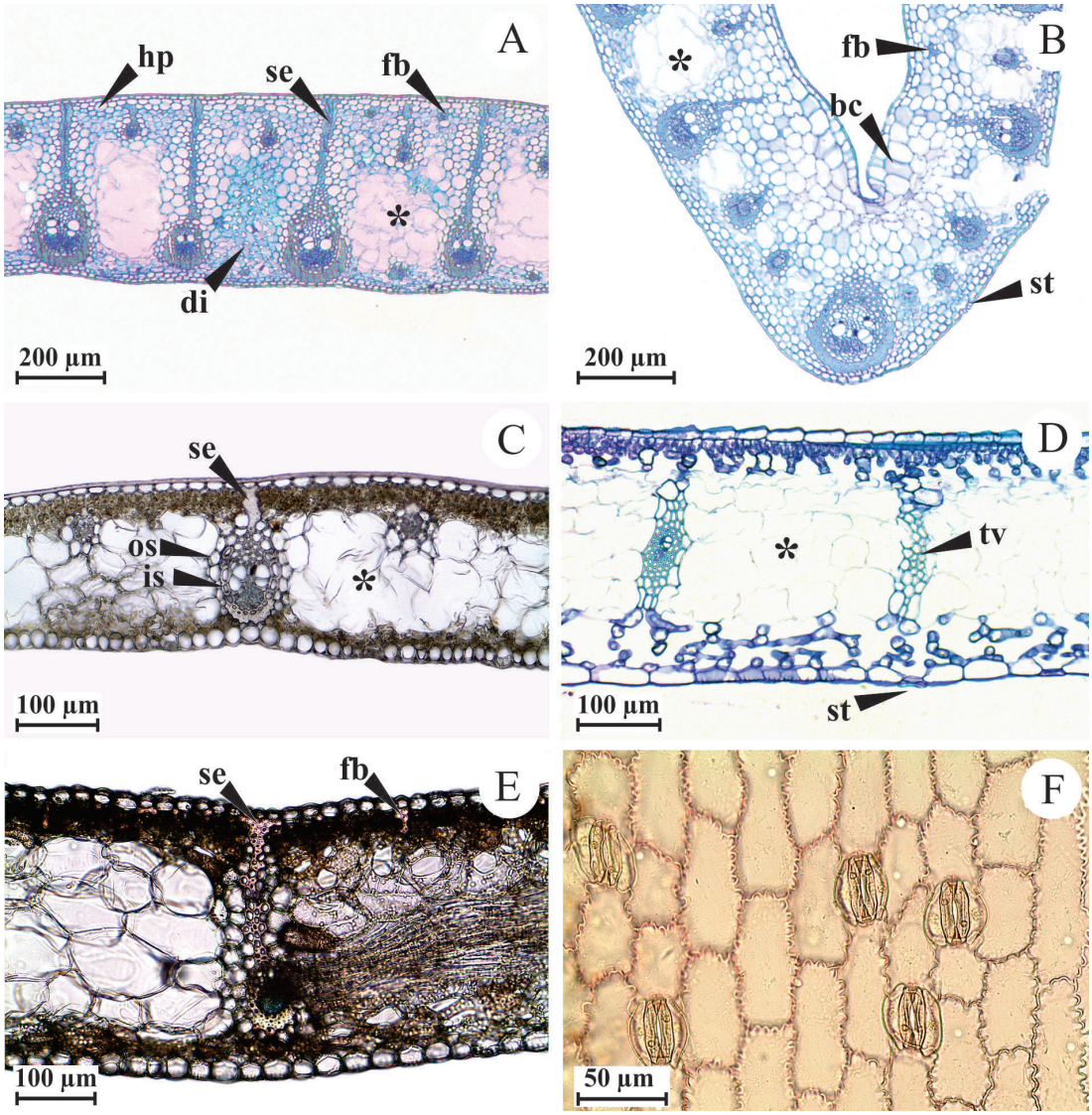

Figure 2. Anatomy of the leaf organs of *Mapania pycnostachya* (A-D, F) and *M. macrophylla* (E). A. Pseudopetiole, median region detail, cross section. B. Pseudopetiole, general view of the V-shaped midvein region, cross section. C. Leaf blade, median region detail, cross section. D. Leaf blade, mesophyll detail, longitudinal section. E. Leaf blade, median region showing transverse vein, cross section. F. Leaf blade, front view of the abaxial surface. Abbreviations: * (air lacuna), bc (bulliform cells), di (diaphragm), fb (fiber bundle), hp (hypodermis), is (inner sheath), os (outer sheath), se (sheath extension), st (stomata), tv (transverse vein). Scale bars: A-B = 200 μm; C-E = 100 μm; F = 50 μm (This figure plate is in color in the electronic version).

2A). Some smaller fiber bundles occur close to the adaxial surface (Figure 2A-B). Midvein region has V-shape with an adaxial median groove; bulliform cells are arranged on 1-2 layers on the adaxial surface (Figure 2B).

Basal leaves - Leaf blades

Mapania macrophylla and *M. pycnostachya* have hypostomatic leaves with single-layered epidermis formed by rectangular, thin-walled cells, which are relatively smaller on the adaxial surface (Figure 2C-E). Cuticle is thin and epidermal cells are rectangular with sinuous walls in front view (Figure 2F). Stomata are paracytic and arranged in rows (Figure 2F), at the same level to the other epidermal cells (Figure 2D).

Mesophyll is heterogeneous, formed by one layer of palisade parenchyma towards the adaxial surface, and multiple layers of spongy parenchyma towards the abaxial surface (Figure 2C-E). Spongy parenchyma is split into two portions by air lacunae and vascular bundles, and it is more conspicuous on the abaxial surface (Figure 2D). The air lacunae (Figure 2C-E) are similar to those described for the pseudopetiole.

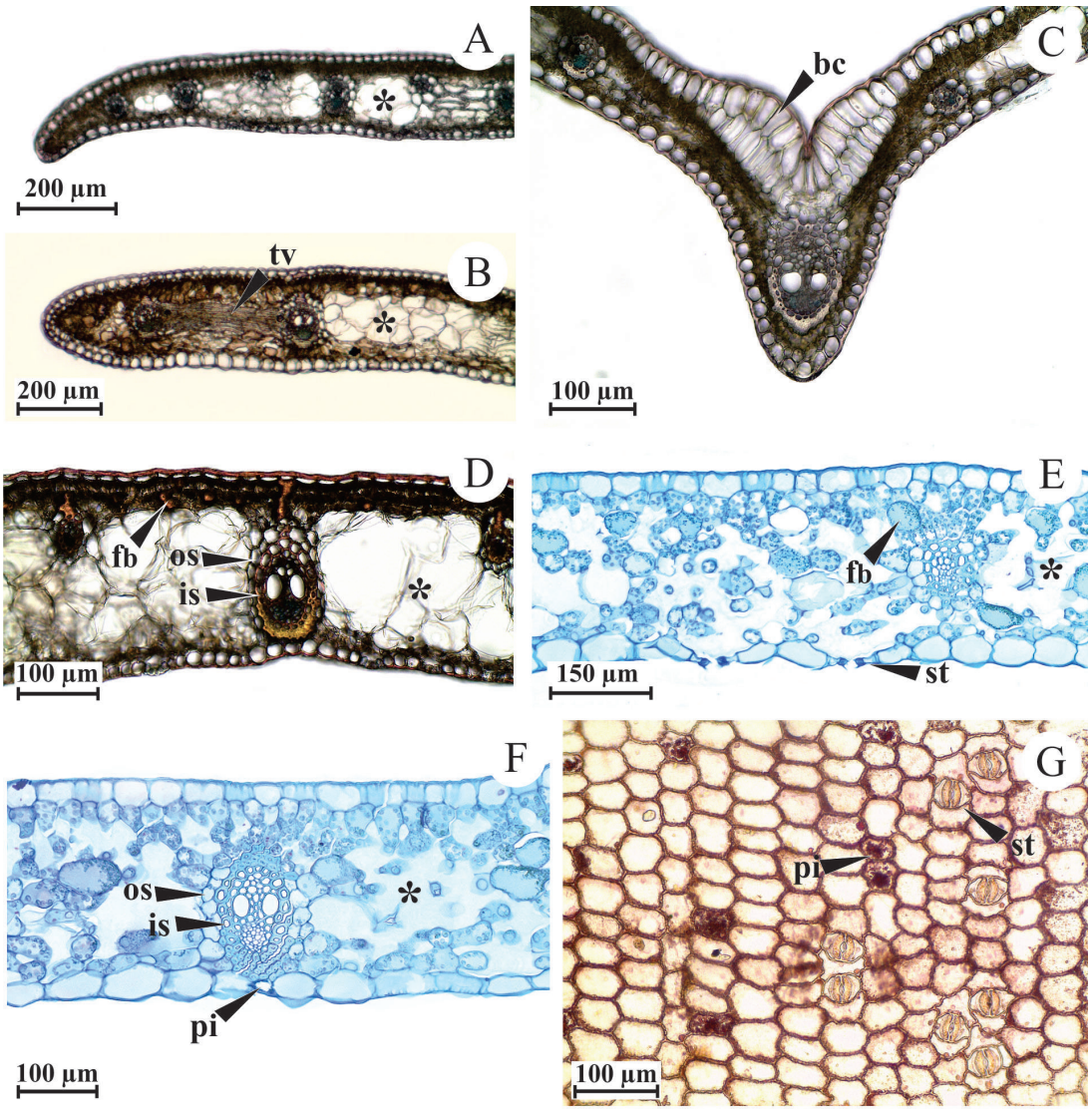

Figure 3. Anatomy of the leaf organs of *Mapania pycnostachya* (A, C-D), *M. macrophylla* (B) and *M. sylvatica* (E-G). A-B. Leaf blades, margins details, cross sections. C. Leaf blade, general view of the midvein region, cross section. D-F. Involucral bracts, general views of the median region showing mesophyll structure, cross sections. G. Involucral bracts, front view of the abaxial surface of the epidermis. Abbreviations: * (air lacuna), bc (bulliform cells), fb (fiber bundle), is (inner sheath), os (outer sheath), pi (phenolic idioblast), st (stomata), tv (transverse vein). Scale bars: A-B = 200 μm; C-D, F-G = 100 μm; E = 150 μm (This figure plate is in color in the electronic version).

Vascular bundles are collateral and differ in size, with small and large ones alternated on two levels (Figure 2C); they are enclosed by a double sheath: the outer one is composed of large, thin-walled cells, and the inner one of small, thick-walled cells (Figure 2C). Larger vascular bundles occur closer to the abaxial surface and the smaller ones, to the adaxial surface, except close to leaf margin (Figure 3A-B). Bundle sheath extension may reach the adaxial surface (Figures 2C, E, 3B).

The midvein region presents several layers of bulliform cells on the adaxial surface in *M. macrophylla* and two layers in *M. pycnostachya* (Figure 3C). Leaf margin is acute/reflex in *M. pycnostachya* (Figure 3A) or slightly obtuse in *M. macrophylla* (Figure 3B), with one layer of subepidermal thin-walled cells. In both species, basal leaves have V-shape, with an adaxial median groove only in *M. pycnostachya* (Figure 3C).

Involucral bracts

The three species studied share involucral bracts with single-layered epidermis formed by thin-walled cells and covered with a thin cuticle (Figure 3D-F). Epidermal cells are small, rectangular in cross section, in *M. macrophylla*

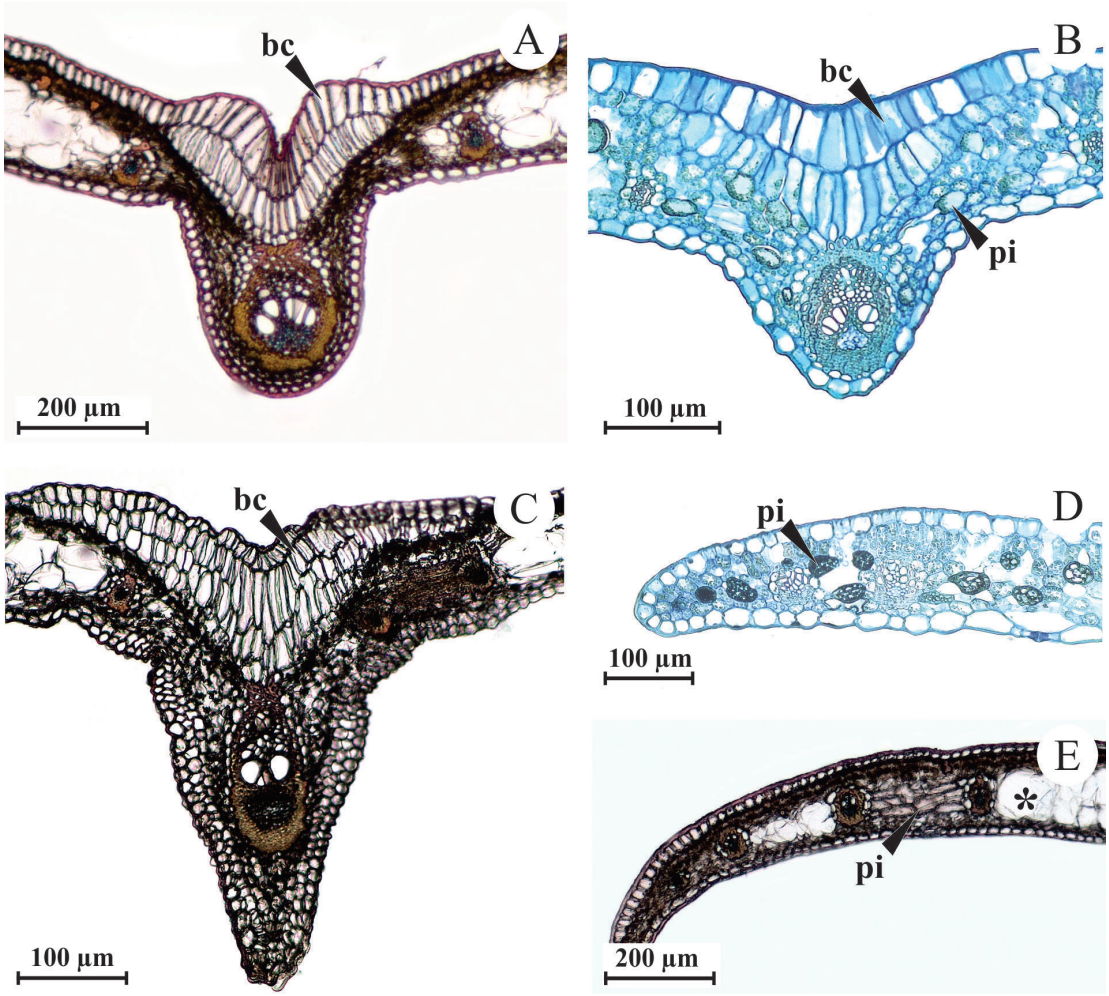

Figure 4. Anatomy of the involucral bracts of *Mapania pycnostachya* (A, E), *M. sylvatica* (B, D) and *M. macrophylla* (C). A-C. Details of the midvein regions showing their shape and distribution of bulliform cells, cross sections. D-E. Margins details, cross sections. Abbreviations: bc (bulliform cells), pi (phenolic idioblast), tv (transverse vein). Scale bars: A, E = 200 μm; B-D = 100 μm (This figure plate is in color in the electronic version).

and *M. pycnostachya*; the outer periclinal walls are flat on the adaxial surface and convex on the abaxial one (Figure 3D). In *M. sylvatica*, epidermal cells are also rectangular on both surfaces, but larger and more conspicuous on the abaxial one (Figure 3E-F). In front view, epidermal cells of all species show sinuous walls; in *M. macrophylla* and *M. pycnostachya* they are rectangular, whereas, in *M. sylvatica*, they are irregular (Figure 3G). Dark epidermal cells with phenolic compounds occur in *M. sylvatica* (Figure 3G).

Involucral bracts are hypostomatic, with paracytic stomata arranged in longitudinal rows (Figure 3G), slightly above the other epidermal cells (Figure 3E). In *M. sylvatica* a few stomata also occur on the adaxial surface. Mesophyll is formed by a layer of palisade parenchyma towards the adaxial surface and various layers of spongy parenchyma towards the abaxial surface (Figure 3D-F). In *M. macrophylla* and *M. pycnostachya*, the mesophyll presents conspicuous air lacunae and small and large vascular bundles alternated (Figure 3D), following the same pattern from their basal leaves. In *M. sylvatica*, mesophyll is interrupted by irregular air spaces formed by translucent cells (Figure 3E-F), and the vascular bundles occur in only one level and a sheath extension is missing (Figure 3D-F).

Conspicuous phenolic idioblasts were found in *M. sylvatica* (Figure 3E-F). Fiber bundles occur close to the adaxial surface in *M. macrophylla* and *M. pycnostachya* (Figure 3D). Vascular bundles are collateral and enclosed by a double sheath: the outer one with thin-walled cells and the inner one with slightly thick-walled cells (Figure 3D-F). The midvein region shows two layers of bulliform cells in *M. pycnostachya* and *M. sylvatica* (Figure 4A-B) and, usually, 3-4 layers in *M.*

macrophylla (Figure 4C). In the latter species, the number of layers vary in the same individual from 3 to 10. In all species studied, involucral bracts have V-shape in cross section, with a median adaxial groove in *M. pycnostachya* (Figure 4A-B). The margins are acute/reflex (Figure 4D-E) and anastomoses were observed between vascular bundles (Figure 4E). A summary of selected anatomical features described for the leaf blade and involucral bract is presented in Table 1.

DISCUSSION

The three species studied here differ morphologically by the absence of basal leaves in *M. sylvatica*, which is reduced to cataphylls, and by the differentiation of a pseudopetiole in *M. pycnostachya* (Simpson 1992). These characters are useful to recognize the three species on the field when only vegetative organs are available. Anatomical differences among them were also found as discussed below.

Anatomical data regarding the involucral bracts were the most elucidative as they allow the comparison among the three species when reproductive organs are present. The mesophyll of *M. macrophylla* and *M. pycnostachya* presents a different pattern when compared to *M. sylvatica*. *Mapania macrophylla* and *M. pycnostachya* share the arrangement of vascular bundles in two levels, the presence of bundle sheath extension, a conspicuous aerenchyma, and fiber bundles close to the epidermis; *M. sylvatica* distinguishes by the arrangement of vascular bundles in one level, absence of bundle sheath extensions, inconspicuous aerenchyma and presence of scattered phenolic idioblasts on the mesophyll. This characterization is firstly reported for the genus and potentially valuable in future analysis in Hypolytreae.

To distinguish the involucral bracts of *M. macrophylla* and *M. pycnostachya*, only a few characters related to the midvein region can be used, as the presence of 2 layers of bulliform cells with adaxial groove in *M. pycnostachya* and usually 3-4 layers of bulliform cells with no adaxial groove in *M. macrophylla.* This character in the latter, however, must be used with care because phenotypic plasticity is often seen.

The cataphylls of *M. sylvatica* present no taxonomic features that separate it from the others. Yet, the basal leaves of *M. macrophylla* and *M. pycnostachya* can complement the data of the bracts. Both have the same mesophyll pattern and diagnostic features found in the involucral bracts. These characters increase our knowledge about *Mapania*, adding significantly data to the anatomical description provided for most genera of Cyperaceae by Metcalfe (1969). It is important to point out that *Mapania sylvatica* belongs to *M.* sect. *Mapania*, while the other two are placed under *M.* sect. *Pycnocephala* (Simpson 1992). Thus, although the basal leaves and involucral bracts of *M. macrophylla* and *M. pycnostachya* are morphologically different, anatomical similarities and the differences found between them and *M. sylvatica* agree with their sections placement.

The leaf organs studied here also show anatomical characters associated to adaptation to humid, tropical forest environments as air lacunae, stomata at the same level or slightly above the other epidermal cells, and epidermal cells with sinuous wall and thin cuticles considered hydromorphic features (Pyykko 1966; Rocha and Martins 2011). However, all these features have already been reported in other Cyperaceae, independently of their phylogenetic position and habitat (Metcalfe 1971; Sharma and Mehra 1972; Alves *et al.* 2002).

The air lacunae observed in the leaf organs of the three species here studied are delimited by diaphragms composed of stellate cells. These air lacunae are formed by large, thin-walled, translucent cells. This character is similar to previous reports on Cyperaceae and Heliconiaceae (Govindarajalu 1968; Simão and Scatena 2004). This resemblance, together with the assumption of tissue continuity from the leaf sheath, pseudopetiole and leaf blade, strengthen our assumption that the translucent cells in the mesophyll of *Mapania* are indeed aerenchyma instead of aquiferous parenchyma, as erroneously indicated by Alves *et al.* (2002) for *M. pycnocephala*, *M. sylvatica* and species of *Hypolytrum*.

Air spaces/lacunae in leaf organs are common in Cyperaceae and other families of monocotyledons (Metcalfe 1969, 1971; Tomlinson and Wilder 1984; Bruhl 1995). Their conspicuousness is often found in plants growing in humid environments as the Amazon forest, where the species studied occur. *Mapania macrophylla* and *M. pycnostachya* present conspicuous aerenchyma and grow in 'igarapés' in 'igapó' forests, whereas *M. sylvatica* lacks this feature and grows in 'terra firme' forests, where seasonal flooding does not occur.

Bulliform cells were observed in all basal leaves and involucral bracts of *Mapania* here studied. This character is considered an adaptation to xeric environments, since they allow leaves to roll under water stress and reduce water loss through transpiration (Moulia 2000; Silva *et al.* 2001; Alvarez *et al.* 2008). However, the species of *Mapania* here studied grow in very humid environments all year round, and this function seems inconsistent. Among the species studied, *Mapania macrophylla* has more layers of bulliform cell and it is more likely that the turgescence of these cells would be important to support the leaf blade. In fact, it is here supposed that the water tension in the intracellular environment could avoid tissue ruptures due to the size of this organ.

Some other features vary among the species studied and should not be related to the environment. The basal leaf sheath in *M. macrophylla* presents more vascular and more fiber bundles, both on various levels. A similar pattern was found in involucral bracts of *Heliconia* (Heliconiaceae, Simão

& Scatena 2004). This feature occurs specially on basal leaves of *M. macrophylla*, in which they are long and robust (up to 1.2 m, based on Simpson 1992) and thus require a more complex supportive tissue. In the pseudopetiole of *M. pycnostachya*, conspicuous fiber bundles and bundle sheath extensions are present, as well as hypodermis, which also seem to contribute for supporting the leaves in this species.

Despite the anatomical similarity between the leaf blades of the species studied here and of those previously reported in Hypolytreae (Metcalfe 1971; Alves *et al.* 2002), the rare occurrence of hypodermis in *Mapania*, as observed in the pseudopetiole of *M. pycnostachya*, should be stressed. Alves *et al.* (2002) used the presence of hypodermis in leaf organs to differentiate *Hypolytrum* from *Mapania* which is clearly not appropriate based on the data here presented.

Martins *et al.* (2012) report a variation between hypodermis and multiple epidermis in various genera of Cyperaceae and present a schematic hypothesis to explain the ontogeny of these tissues up to maturity. In the present study, only mature leaves were analyzed and the tissue pointed out as hypodermis is constituted of cells whose size and shape are quite different from those of the epidermis. So, the ontogeny must be further elucidated to confirm this terminology.

The three species studied also present double-sheathed vascular bundles. Although leaf ontogeny was not the subject of this study, it is possible to suggest that the outer and inner sheaths correspond to the endodermis and pericycle, respectively, as assumed by Martins and Scatena (2011) to other genera of Cyperaceae.

Crystals or silica bodies are common in Cyperaceae and are found in the epidermal and subepidermal cells (Koyama 1966; Dahlgren *et al.* 1985). Alves *et al.* (2002) have confirmed the low occurrence of such structures among *Mapania* and *Hypolytrum* species. Although the dark epidermal cells observed in frontal view of the involucral bracts of *Mapania sylvatica* resemble those silica cells observed in *Trilepis ciliatifolia* by Arruda and Neves (2005), the histochemical tests performed pointed to phenolic compounds instead. Alves *et al.* (2002) cited bridge-shaped silica bodies in *M. pycnocephala* and *M. sylvatica*, although no images were presented. In the present study no silica bodies were found even using polarizing filter, which confirms the lack of silica in the three species of *Mapania* from the Amazon forest.

The present study shows taxonomic characters in basal leaves and involucral bract to distinguish the three species of *Mapania*. Also, the variation, as fiber bundles, vascular bundle sheath extension and conspicuous aerenchyma support the sections where they have been placed. In addition to this, by the observation of diaphragm in this region, it is now clear that the translucent cell in Cyperaceae correspond to aerenchyma and not aquiferous parenchyma. Finally, the lack of hypodermis in both leaf blades and involucral bracts and of silica bodies in epidermal cells are important diagnostic characters to distinguish *Mapania* from other Cyperaceae.

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