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Leaf anatomy and micromorphology of six *Posoqueria* Aublet species (Rubiaceae)

Anatomia e micromorfologia foliar de seis espécies de Posoqueria *Aublet (Rubiaceae)*

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Abstract

The present study deals with the leaf anatomy and leaf surface of *Posoqueria acutifolia* Mart., *P. latifolia* Mart., *P. longiflora* Aublet, *P. macropus* Mart., *P. palustris* (Rudge) Roem. and *Posoqueria* sp., collected in fragments of Atlantic rain forest, Rio de Janeiro, Brazil. The epicuticular wax may occur in the form of filaments, granules or crusts. The leaves are covered by a thick cuticular layer that may be smooth or striated. Paracytic stomata, and non-glandular trichomes are limited to the abaxial surface; the latter are numerous in *P. palustris*, and rare in *P. longiflora* and *P. latifolia*. Leaves have a dorsiventral structure, with only one layer of palisade parenchyma and varied amounts of spongy parenchyma. Idioblasts containing crystalliferous sand were observed, and were more abundant in *P. latifolia*. The leaf blade vascular system is formed by collateral bundles with a parenchymatous sheath, associated with fibers. The vascular system of the petiole and the leaf blade forms an arch. Some of the anatomical features observed can be used to distinguish the species studied. Anatomical leaf characters could be used in the recognition of six species of *Posoqueria* studied, such as anticlinal wall of epidermal cells, wax deposition, trichomes and shape of the leaf margin.
Key words: leaf anatomy, scanning electron microscopy, taxonomy, Cinchonoideae.

Resumo

São apresentadas informações sobre a anatomia e a superfície foliar de *Posoqueria acutifolia* Mart., *P. latifolia* Mart., *P. longiflora* Aublet, *P. macropus* Mart., *P. palustris* (Rudge) Roem e *Posoqueria* sp., espécies ocorrentes em fragmentos da Floresta Pluvial Atlântica, Rio de Janeiro, Brasil. A cera epicuticular pode ocorrer na forma de filamentos, grânulos ou crostas. As folhas são recobertas por espessa camada cuticular, que pode ser lisa ou estriada. Na face abaxial a cutícula apresenta-se estriada em *P. latifolia* e *P. palustris*. Os estômatos estão restritos à face abaxial e são do tipo paracítico. Tricomas tectores ocorrem apenas nesta face sendo numerosos em *P. palustris*, raros em *P. longiflora* e *P. latifolia*. As folhas têm estrutura dorsiventral, com apenas uma camada de parênquima paliçádico e variado número no parênquima esponjoso. No mesofilo foram observados idioblastos contendo areia cristalífera, mais abundantes em *P. latifolia*. Na lâmina foliar o sistema vascular é formado por feixes do tipo colateral, envolvidos por bainha parenquimática, e apresentam fibras associadas. No pecíolo e na nervura principal o sistema vascular apresenta-se em arco. Características anatômicas foliares podem ser usadas na distinção das seis espécies de *Posoqueria* estudadas, tais como: a parede anticlinal das células epidérmicas, deposição de cera, tricomas e a forma da margem da folha.
Palavra-chave: anatomia foliar, microscopia eletrônica de varredura, taxonomia, Cinchonoideae.

Introduction

Rubiaceae (Gentianales) is one of the four largest families of angiosperms, with representatives widely distributed worldwide, presenting diverse habits and life forms. The family is monophyletic and includes about 10.700 species, which were recently divided into two subfamilies, the Cinchonoideae and the Rubioideae (Robbrecht & Manen 2006).

Belonging to Cinchonoideae subfamily, *Posoqueria* Aublet is found in the Neotropic region and is composed of approximately 14 species that

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are difficult to be recognized in vegetative state and present great morphological similarity (Macias 1988). To Brazil, six species are described (Macias 1988); most of them are endangered (Leonhardt *et al.* 2008). Some species of this group are used as ornaments, because of their decorative leaves and perfumed flowers. Most species of *Posoqueria* display the characteristic pollen catapult mechanism, which is the trademark feature of the Posoquerieae (Delprete 2009). According to Ariza *et al.* (2007), indolic alkaloids, iridoids, and acetates of hexanile and hexile, among other compounds, have been identified as perfume sources in *Posoqueria latifolia* (Rudge) Roem., popularly known as "açucena-do-mato". For *P. acutifolia* Mart., laboratory tests have identified analgesic and anti-inflammatory activities of the methanolic extracts, which may be related to this species use in popular medicine (Souza *et al.* 2007).

Among the organs that can be useful to taxonomic propositions, the leaf is the most examined one because of its internal architecture, which is a classic source of useful information in the systematics, specially the epidermis and the cuticle (Stuessy 1990; Judd *et al.* 2009).

About micromorphology the studies of Mantovani & Vieira (1993/1997) and Kocsis *et al.* (2004) stand out and are source of important information about the leaf surface of diverse species of this family. Descriptive anatomical studies of the vegetative or reproductive parts also have been carried out with other Brazilian rubioid species (Mantovani *et al.* 1995; Gomes *et al.* 2000; Arruda & Gomes 1996. De Toni & Mariath 2008).

This study describes the leaf anatomy and micromorphology of six Brazilian *Posoqueria* species: *P. acutifolia* Mart., *P. latifolia* (Rudge) Roem. & Schult., *P. longiflora* Aubl., *P. macropus* Mart., *P. palustris* Mart., and *Posoqueria* sp. aiming to identify features that may be useful in recognizing these species.

Materials and Methods

Adult leaves of *Posoqueria latifolia* and *P. longiflora* were collected from plants found at the Parque Nacional da Floresta da Tijuca (22º57'S and 43º18'W), in Rio de Janeiro, (RJ). In this region, the annual avarage temperature varies between 19.3ºC in the winter and 25.5°C in the summer, and the rainfall is about 2000 mm annually (Vieira 1994). *Posoqueria palustris* was collected in Rio das Ostras (RJ, 22º31S'-41°55'W) where the annual average temperature is 22ºC and the annual rainfall between 1500 and 2000 mm (Bidegain & Michael 2003). In this site, the plants are submitted to seasonal flooding in the rainy season, when the water table rises considerably. *Posoqueria acutifolia* was collected at the Parque Estadual da Serra da Tiririca, Niterói, RJ (22º48'S,42º57'W), a fragment of the Atlantic Rain Forest. In this region the annual average temperature is 24ºC, with annual rain fall of 1300mm concentred in the summer. Herborized leaves of *P. macropus* (*Sucre 7678*) and *Posoqueria* sp. (*Martinelli 8804*), obtained from the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro (RB), were rehydrated according to Gomes (2002).

The fresh leaves were fixed in F.A.A. (formalin, acetic acid, ethanol 70%) for 48h and preserved in 70% ethanol (Johansen 1940). For light microscopy, sections of the leaf blade and petiole were dehydrated in graded ethanol-butanol series, embedded in paraffin, sectioned transversely and longitudinally in rotatory microtome at a thickness of 12 mm. The sections were stained with 1% safranin - 1% astra blue in tartaric acid (Luque *et al.* 1996).

The epidermis was studied using sections of the leaf blade dissociated in acetic acid/hydrogen peroxide (Franklin 1945) The epidermis was stained with 1% safranin, alcian blue, or 0.5% fuchsin (modifications in Kraus & Arduin 1997). In order to remove wax and to confirm its presence, sections of herborized leaves were boiled in chloroform three times for one minute, on a hot plate. For the scanning electron microscopy (SEM) analysis, leaf pieces were critical-point dried through a graded series of ethyl alcohols. Leaf fragments were mounted on stubs coated with gold and observed in a JEOL JSM-5310 scanning electronic microscope. Material from herbarium was also mounted on proper stubs coated with gold before being observed in the SEM.

For the histochemical tests, freehand sections of leaf blade were treated with the following reagents: starch grains were localized by Lugol staining (Johansen 1940), lipidic compounds were recognized by Sudan Red 7B in glicerol/ethanol (Kraus & Arduin 1997), pectin substances with 0.02% ruthenium red (Kraus & Arduin 1997); primary walls were identified with zinc iodine chloride (Jensen 1962) and the secondary walls with acid phloroglucin for lignified cells (Foster 1949); phenolic compounds were confirmed with ferric chloride (Johansen 1940). Tests with hydrochloric acid were used to detect calcium oxalate crystals (Jensen 1962).

Results

In most of the studied species, the epidermis, in adaxial surface is composed of ordinary epidermal cells, with sinuous anticlinal walls (Fig. 1 a-f), and thick, notably in *Posoqueria macropus* (Fig. 1 d). Over vascular bundles, such as in the abaxial surface of *Posoqueria* sp. the anticlinal walls can present themselves straight or curved (Fig. 1 f). The abaxial surface is composed by ordinary epidermal cells, stomata and trichomes (Fig. 2 a-f). The epidermal anticlinal cells walls are sinuous in all species (Fig. 2 a-e), with the exception of *Posoqueria* sp in which they are straight. *P. acutifolia* has glabrous leaves (Fig. 2 a), in *P. macropus* numerous, non-glandular, unicellular trichomes, with thick lignified walls were observed (Fig. 2 d). In *P. palustris* there are pluricellular uniseriated trichomes, long, non lignified (Fig. 2 e). *Posoqueria* sp is marked by presence of numerous papillae (Fig. 2 f). Rare short unicellular non-glandular trichomes are observed on the abaxial surface of the leaf of *P. latifolia* and *P. longiflora*, restricted to midvein; in *P. palustris*, numerous pluricellular trichomes are observed.

The stomata belong to the paracytic type and are arranged randomly (Fig 2). The subsidiary cells may be of different sizes; some stomata may be flanked in parallel by more than one subsidiary cell. The six species investigated present some large stomata, around which the epidermal cells are organized radially, and the cuticle layer forms a radiate pattern (Fig. 2 b). In P.macropus and P. palustris the stomata are partly covered by numerous trichomes (Fig. 2 d-e).

The epicuticular wax can occur without defined ornamentation, in the form of filaments, granules and in crusts (Figs. 3 a-h, 4 a-g). The granules can be of varied sizes and are present in *Posoqueria acutifolia* (Fig. 4a), *P. longiflora* (Fig. 3 d, 4d), *P. macropus* (Fig. 4 e), *P. palustris* (Fig. 4 f) and *Posoqueria* sp (Fig. 4 g), including over the stomata (Fig. 4 d, g) and trichomes (Fig. 4 e). The crusts occur in *P. macropus* (Fig. 3 f), *P. palustris* (Fig. 3 g) and are thick in *P. latifolia* (Fig. 3 b); the filaments occur in abaxial surface of *P. latifolia* (Fig. 4 c). The cuticle is smooth on the adaxial surface of all species (Fig. 3 a-h) and on the abaxial surface of *P. longiflora* (Fig. 4 d), and striated on the abaxial surface of the other species (Fig. 4 b, e-g), specially in *P. latifolia* (Fig. 4 c) and in *Posoqueria* sp (Fig. 4 g).

In all species investigated the leaf epidermis is one-layred formed by thick-walled cells and covered by a layer of cuticle and well-developed cuticular-wax strata (Fig 5 a-d). On the abaxial surface, above the stomatal pore, the cuticle layer may form conspicuous ledges (Fig. 5 c). The stomata are located on the same level or slightly below the other epidermal cell and the subsidiary cells can form projections in the region of the substomatal chamber (Fig. 5 c). The trichomes have thick walls (Fig. 5 b), and in *Posoqueria palustris*, the basal region of the septate trichome is pitted (Fig. 5 d).

All the species studied show dorsiventral mesophyll, with one layer of cells in the palisade parenchyma and about 10 to 14 layers in the spongy parenchyma (Fig. 6 a-e). The palisade cells are wider and shorter in *Posoqueria acutifolia* and *P. latifolia* (Fig. 6 a-b) and longer and narrower in the remaining species (Fig. 6 c-e). Idioblasts containing tiny polyhedral crystals of calcium oxalate (crystalliferous sand) are observed on the entire mesophyll in all species studied (Fig. 6 c, f). They are more abundant in *P. latifolia* and *P. palustris.* These cells occur in a greater frequency beneath the palisade parenchyma, or near the epidermis of the abaxial surface, isolated or in groups, especially in *P. latifolia* and *P. palustris* (Fig. 6 e-f). The histochemical tests showed that the crystalliferous cells also accumulate phenolic compounds.

The vascular system of the leaf blade is formed by bundles of the collateral type, of varied calibers (Fig. 6 a, d). The vascular bundles are surrounded by a parenchymatous sheath that may contain chloroplasts, crystalliferous sand, and phenolic compounds. Internally to this sheath lignified fibers occur in variable proportions. In *Posoqueria acutifolia*, *P. latifolia* and *P. macropus* the sclerenchyma occurs in a larger proportion than the vascular tissues (Fig. 6 a, d).

The cross section showed that the outline of leaf margin is truncate-revolute in *Posoqueria longiflora* and slightly revolute in the others species (Fig. 7 a-d). In the epidermis, characteristics similar to those of the rest of the leaf blade can be observed, noticing that the cuticular layer is strongly thickened, forming conspicious cuticular flanges. The common epidermal cells are smaller then in the rest of the leaf blade and in *P. longiflora* have a papillose appearance. Internally, the margin is filled with parenchymatous cells with thickened walls; among them idioblasts with crystals occur. Lignified fibers were observed associated to the vascular system.

In transversal section the outline of petiole can be sulcate, plan-convex or concave-convex (Fig. 8 a-c). On the petiole and along of the midvein, on

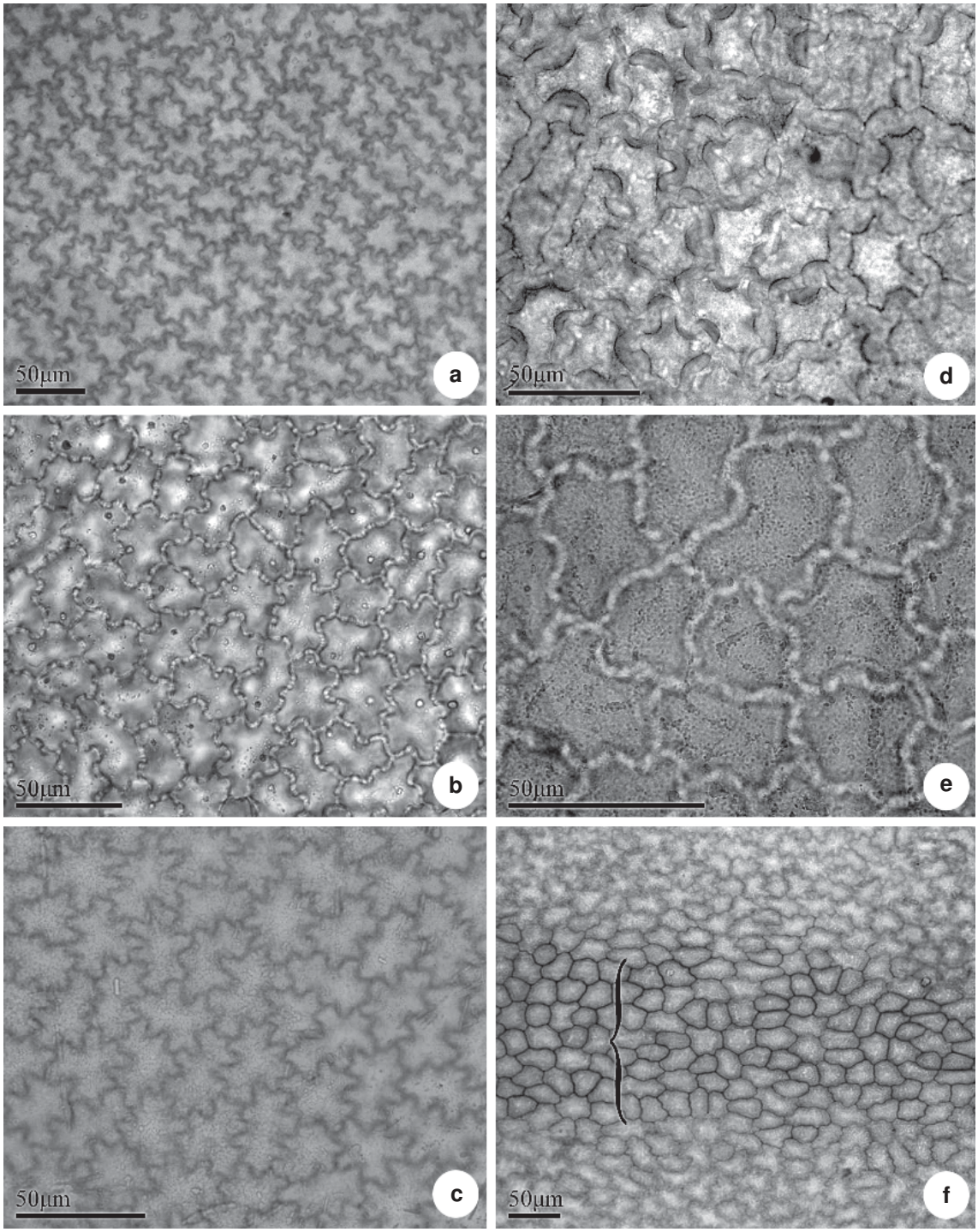

Figure 1 – Leaf epidermis of *Posoqueria* Aublet in frontal view, adaxial surface – a. *P. acutifolia*; b. *P. latifolia;* c. *P. longiflora*; d. *P. macropus*; e. *P. palustris*; f. *P. longiflora*, in the midvein region ({).

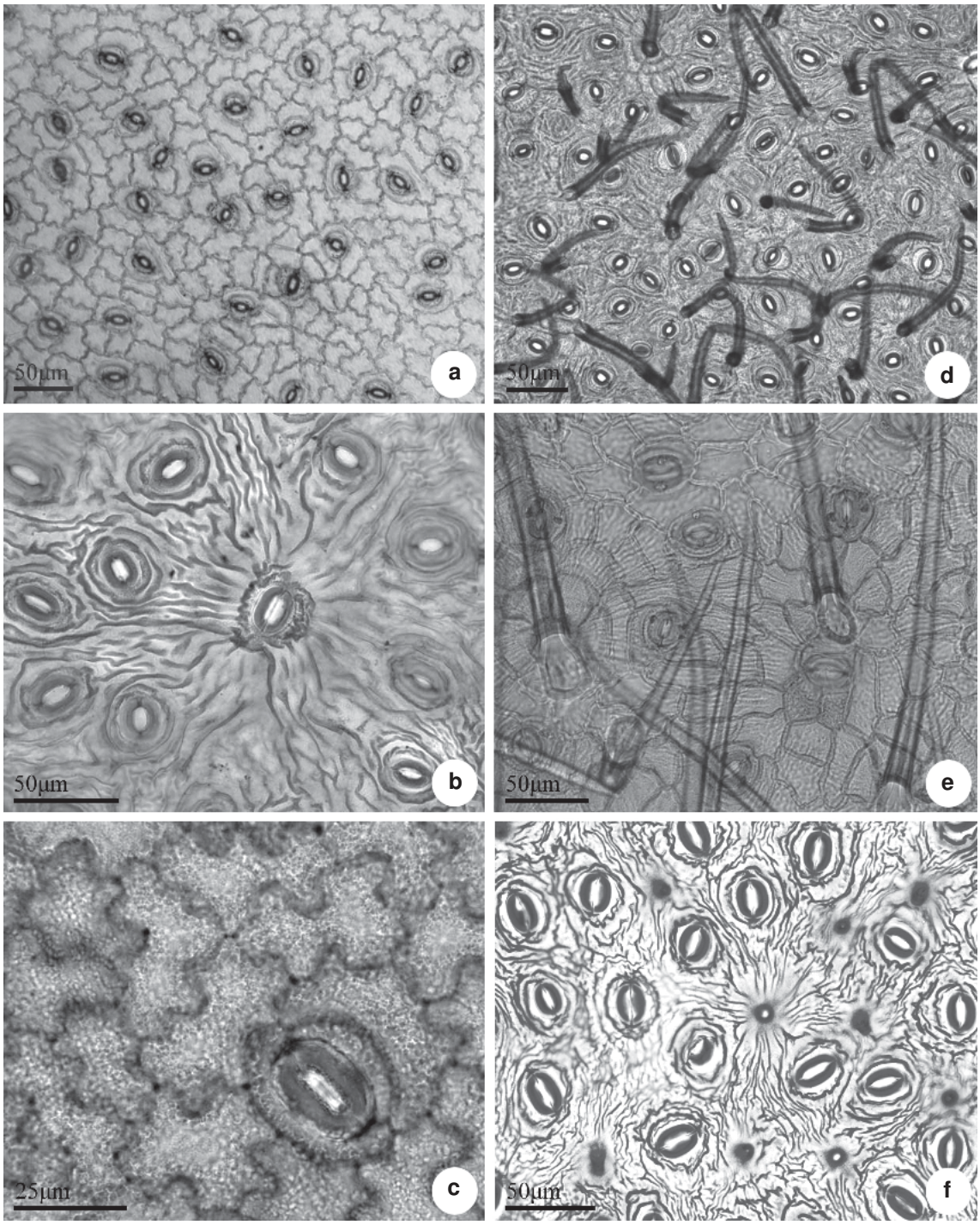

Figure 2 – Leaf epidermis of *Posoqueria* Aublet in frontal view, abaxial surface – a. *P. acutifolia*; b. *P. latifolia;* c. *P.longiflora*; d. *P. macropus*; e. *P. palustris*; f. *Posoqueria* sp. showing the the arrangement of stomata, trichomes (d, e) and papillae (f).

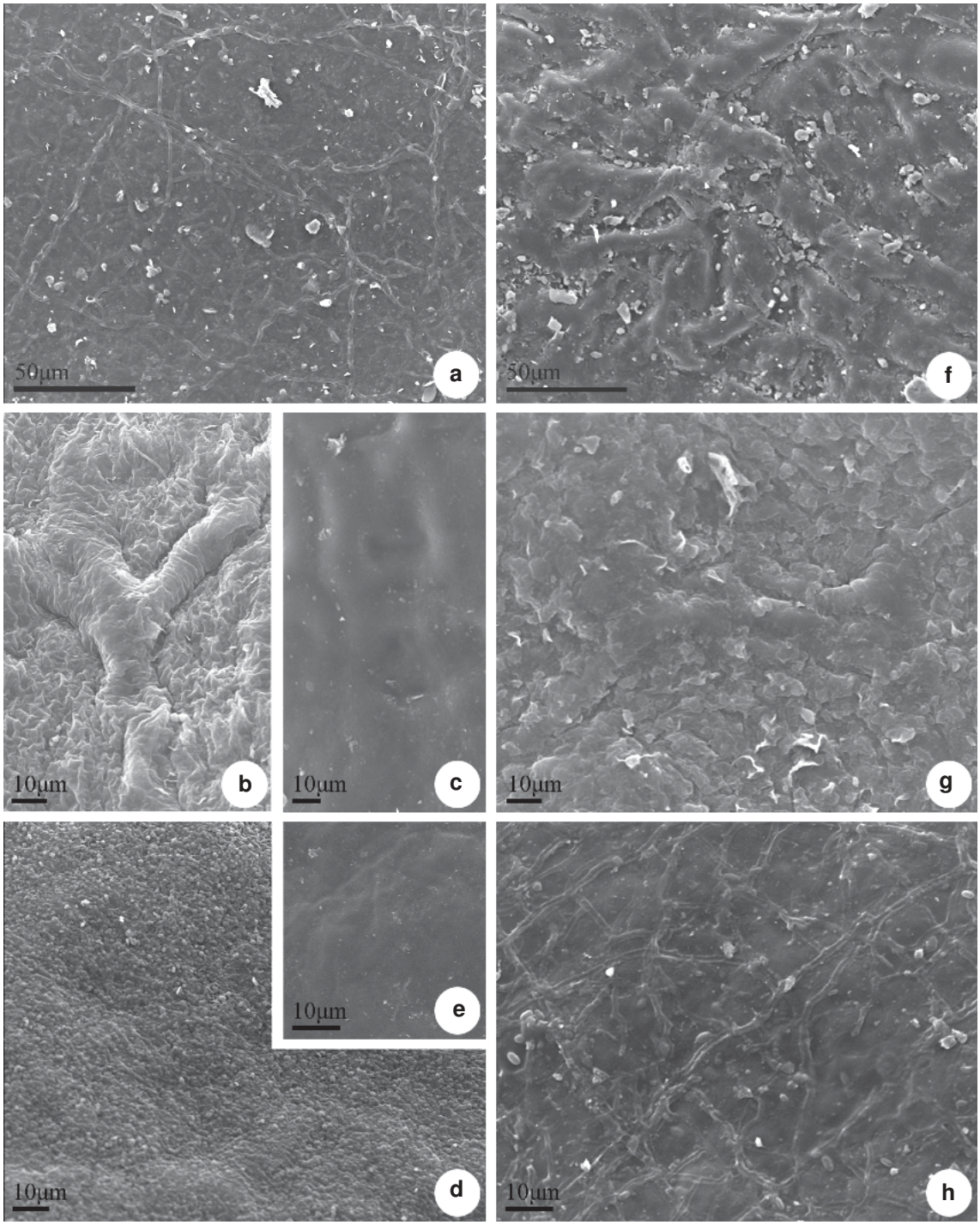

Figure 3 – Scanning electron microscopy of leaf blade of *Posoqueria* Aublet – a. *P. acutifolia*; b-c. *P. latifolia*; d-e. *P. longiflora*; f. *P. macropus*; g. *P. palustris*; h. *Posoqueria* sp. Adaxial surface showing wax in granules (d), in crusts (f) and in thick crusts (b).

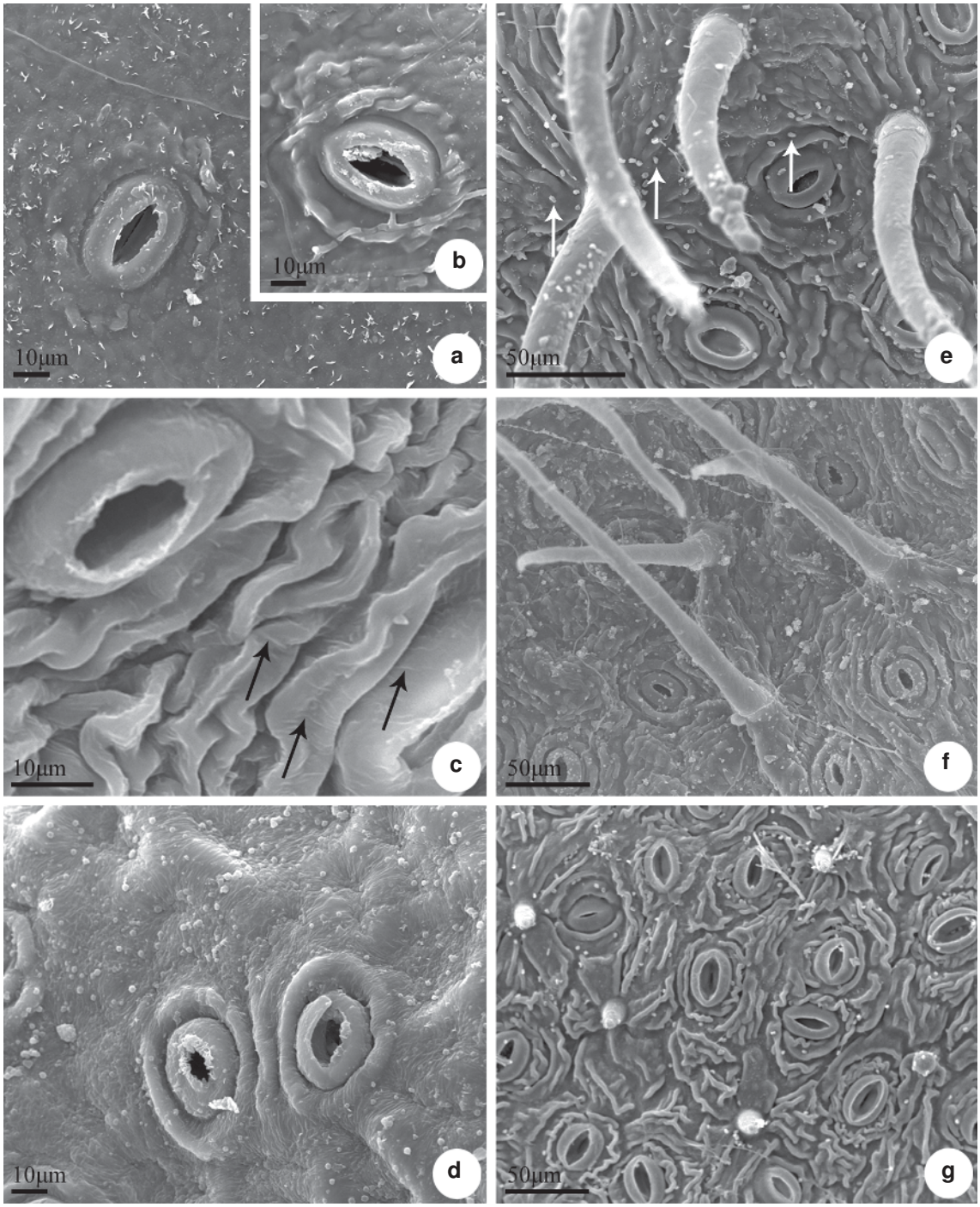

Figure 4 – Scanning electron microscopy of leaf blade of *Posoqueria* Aublet – a. *P. acutifolia;* b. *P. acutifolia* boiled; c. *P. latifolia;* d. *P. longiflora;* e. *P. macropus;* f. *P. palustris;* g. *Posoqueria* sp. Abaxial surface showing stomata (a-g), trichomes in *P. macropus* (e) and *P. palustris* (f) and papillae in *Posoqueria* sp. (g). The surface can be smooth (d) or striated (b-c, e-g). Epicuticular wax (arrow) in filaments (c) and granules (e).

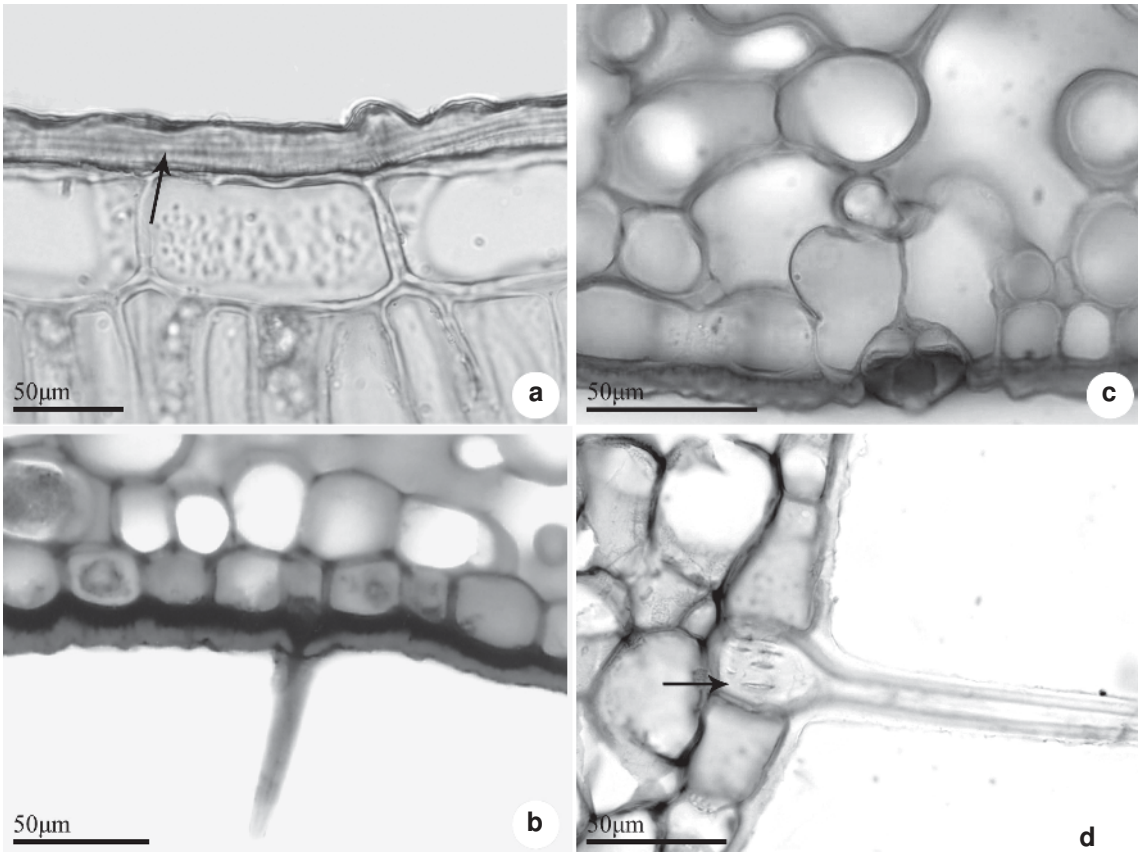

Figure 5 – Cross sections of *Posoqueria* Aublet leaf, details of epidermis – a. *P. latifolia* with a thick cuticular layer (arrow); b. *P. longiflora*, trichome in midvein region; c. *P. longiflora*, stomata with developed rims and subsidiry cells in the substomatal chamber; d. *P. palustris*, lignified trichome with pitted wall (arrow).

both surfaces of the leaf, the epidermal cells are organized in rows and have thicker, nearly straight walls. Stomata can be found in this region of the leaf blade. In the cortical region of the petiole there are two to five layers of angular collenchyma beneath the epidermis. A fundamental parenchyma containing idioblasts with crystals, phenols, or some sclereids are located internally (Fig. 8 d, h). In all species, the vascular system is arranged in an arch (Fig. 8 a-c, e), with the xylem cells arranged in rows and the phloem in the external position, like the collateral type. Additional bundles are observed at the extremities of the vascular system.

In the region of the midvein, beneath the epidermis, the cortical region is occupied by three to five layers of collenchyma that varies between the angular and lamellar types, and underneath there is a palisade parenchyma connecting the two parts of the leaf blade (Fig. 8 f-g). In this portion, the cells of the palisade parenchyma are shorter and wider than the remaining parts of the leaf blade. In the cortical portion below the abaxial surface there is a parenchyma which shows progressively more-voluminous cells with thinner walls towards the vascular system.

In this study the most relevant leaf characteristics to the distinguishing of the species are related in the Table 1.

Discussion

From all the anatomical features examined, the ones that showed a higher potencial of utilization to identify the studied species were: cuticular and wax sculpturing, aspect of the anticlinal walls of epidermal cells and presence and types of trichomes. The variation on the thickness em sinuosity from the anticlinal walls is quoted fot the Rubiaceae by Metcalfe & Chalk (1950) and Gomes *et al*. (2000). Harberlandt (1928) believes that the

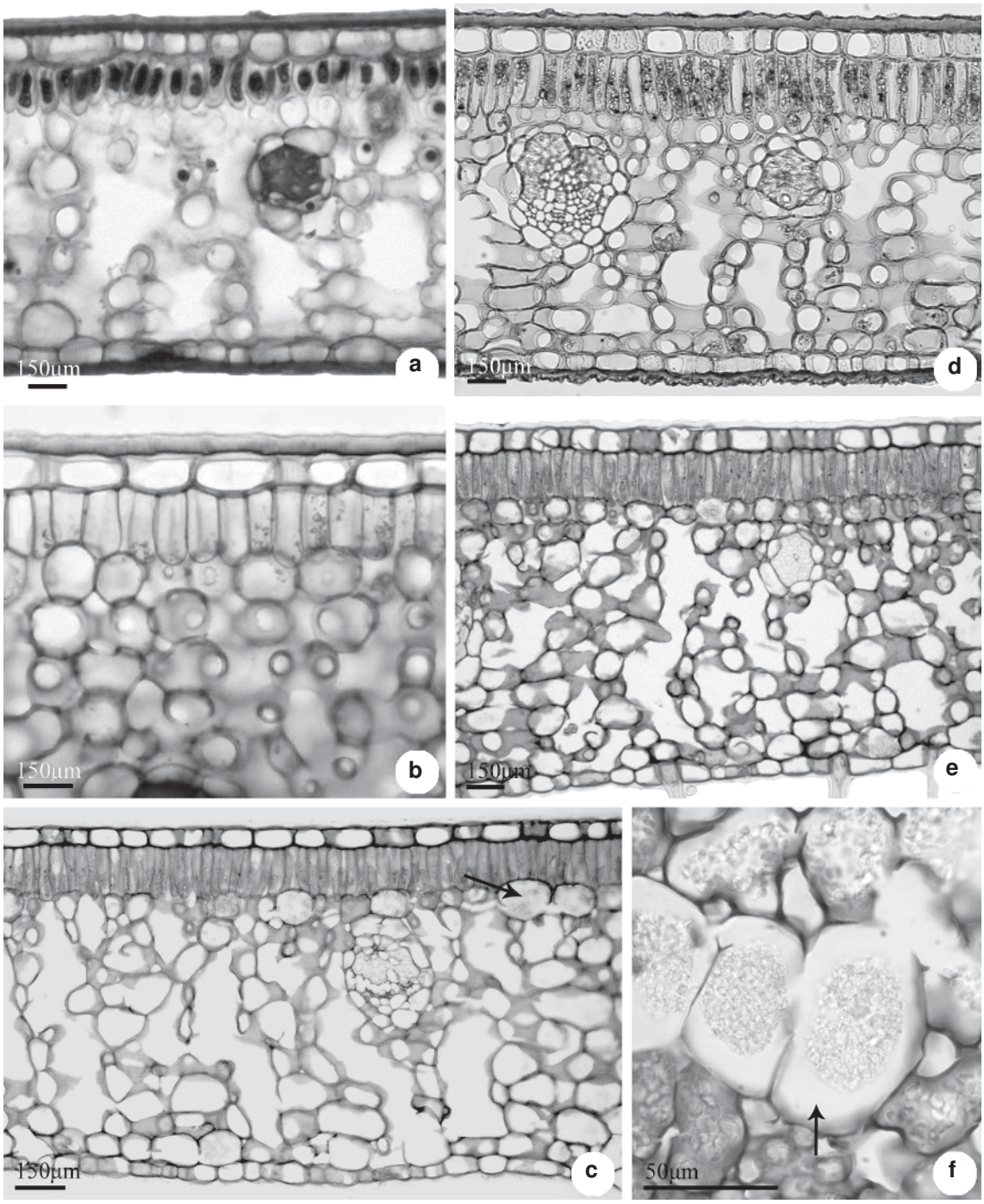

Figure 6 – Cross sections of *Posoqueria* Aublet leaf showing the dorsiventral mesophyll structure – a. *P. acutifolia*; b. *P. latifolia*; c. *P. longiflora*; d. *P. macropus*; e. *P. palustris*; f. *P. palustris* with crystal sand in idioblasts (arrow).

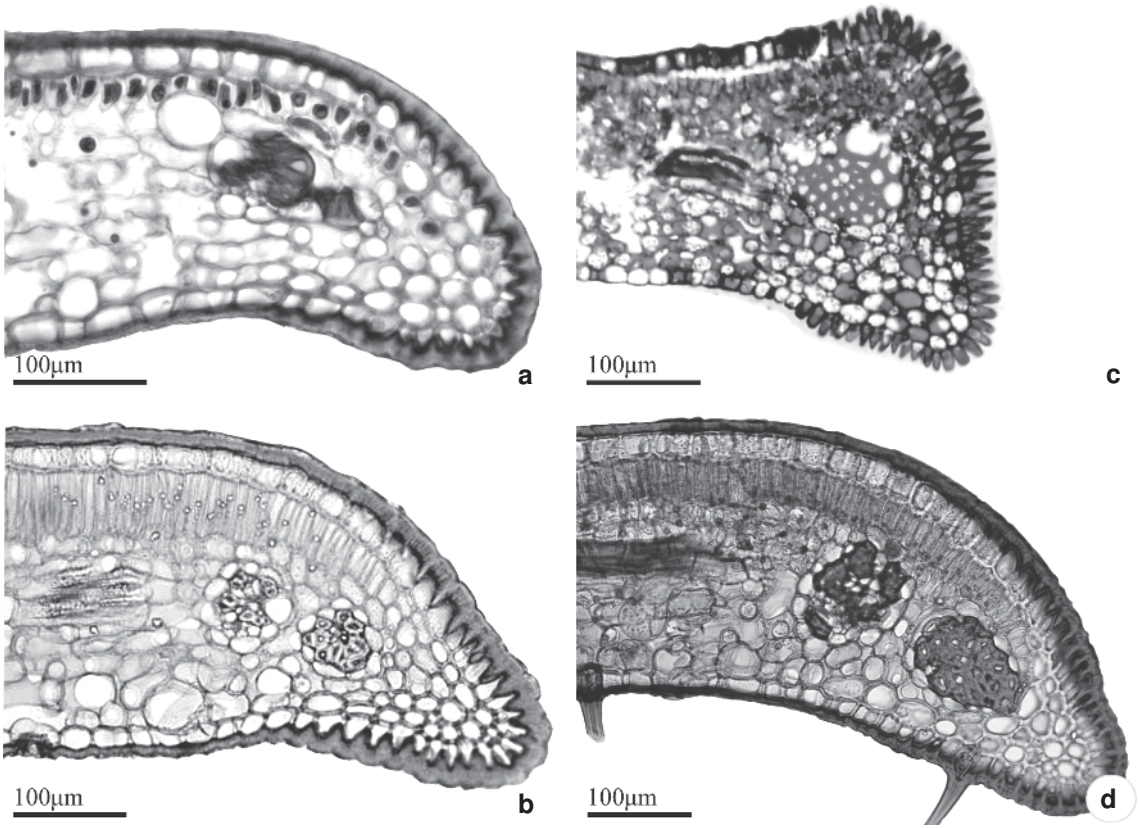

Figure 7 – Cross sections of *Posoqueria* Aublet showing variation outline of the leaf margin – a. *P. acutifolia*; b. *P. latifolia*; c. *P. longiflora*; d. *P. palustris*.

Table 1 – Distinctive characteres to the identification of six *Posoqueria* Aublet (Rubiaceae) species.

Characters/ Species	***Posoqueria acutifolia***	***Posoqueria latifolia***	***Posoqueria longiflora***	***Posoqueria macropus***	***Posoqueria palustris***	***Posoqueria* sp.**
Epidermis, adaxial surface						
Outline of anticlinal wall	sinuous	sinuous	sinuous	sinuous	sinuous	straight
Cuticular surface	smooth	smooth	smooth	smooth	smooth	striated
Epicuticular wax	smooth	crusts	granules	crusts	crusts	smooth
Epidermis, abaxial surface						
Outline of anticlinal wall	sinuous	sinuous	sinuous	sinuous	straight to curves	straight
Cuticular surface	striated	striated	smooth	striated	striated	striated
Epicuticular wax	granules	filaments	granules	granules	granules	granules
Form and distribution of trichomes	absent	unicellular, restrict to midvein region	long, unicellular, restrict to midvein region	long, unicelular, intercoastal region	long, pluricelular, intercoastal region	papillae, intercoastal region
Outline of the leaf margin in transvesal section	revolute	revolute	truncate/ revolute	revolute	revolute	revolute

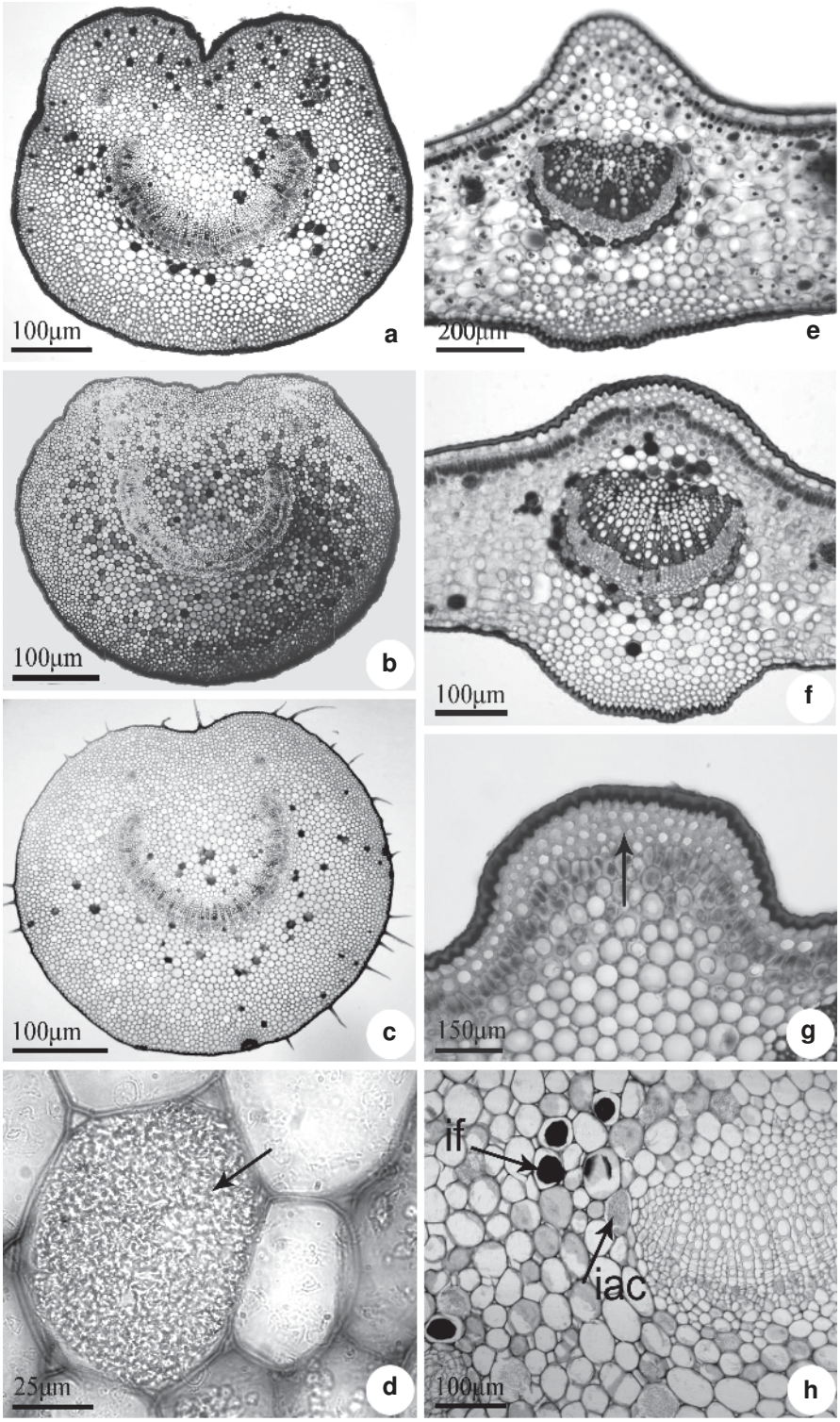

Figure 8 – Petiole and midvein of *Posoqueria* Aublet in cross section – a. *P. latifolia*; b. *P. longiflora*; c. *P. palustris*; d. *P. palustris* showing idioblast with crystal sand in detail (arrow). e. *P. acutifolia;* f. *P. latifolia* g. and h. *P. palustris* with collenchyma and palisade tissue under adaxial epidermis (g, arrow), idioblasts with phenols (if) and crystal sand (iac), on cortical region of petiole (h).

sinuosity from the walls has an important mechanical function, increasing the rigidity of the cell to prevent the parietal collapse in case of lack of water. In *Posoqueria* the walls of epidermic cells that revest the vascular bundles are less sinuous than the ones that revest the mesophyll, what probably is associated to the fact that this region is less susceptible to the environment influences like the loss of water.

A thick cuticular layer is generally observed in plants of dry environment (Fahn & Cuttler 1992). For the plants studied here, which were collected in the interior of the Atlantic Rain Forest, the thick cuticular layer, besides protecting the plants from dehydration and from invasion by fungi and bacteria, also protects them from the heavy rain that is common in the neotropics (Juniper & Jeffree 1983). The different aspects of the cuticular layer of the abaxial surfaces and the ornamentation of epicuticular wax could be used in the characterization of these species, confirming the potencial value of this anatomical characteristics for the taxonomists (Wilkinson 1979).

The presence of hypostomatous leaves appears to be the most common case among the Rubiaceae (Metcalfe & Chalk 1950). In all cases, paracytic stomata predominate, although other patterns such as the anomocytic or anisocytic are reported in the family (Metcalfe & Chalk 1950). Stomata with two or more subsidiary cells arranged parallel to the stomata cells can be classified as parallelocytic, as in the species of *Posoqueria* studied here and also previously described by several members of Rubiaceae (Mantovani *et al.* 1995; Kocsis *et al*. 2004). According to Carpenter (2005), the anomocitic type is an ancestral condition between the basal angiosperms; besides, the paracytic stomatal architecture has been derived independently some families in this group.

Trichomes have great value in taxonomical, ecological, and evolutionary studies (Gomes & Neves 2009). In the Rubiaceae, the leaf trichomes are of the non-glandular type, uni-or multicellular, in variable densities and sizes, from papillae to longer ones that form a dense indumentum (Metcalfe & Chalk 1979; Gomes *et al*. 2000; Kocsis *et al.* 2004). Robbrect & Manen (2006) proposed that whereas the Rubioideae are distinguished by having generally articulated trichomes, those of the Cinchonoideae are distinguished by the cylindrical type (unicellular), as observed in *Posoqueria latifolia* and *P. longiflora*. In *P. palustris*, however, the pattern does not support the proposal of these authors because this species is the only one that shows pluricellular trichomes. In the present study, the type, number of cells (uni or pluricellular) and distributions could be used to recognize the evaluted species as presented in Table 1. This result confirms the importance of these features to taxomic applications as proposed by Metcalfe & Chalk (1979).

The thickness of the leaf blade, however, varies with the amount of light and thin leaves are common in species from the understorey, such as *Psychotria nuda* Wawra, *P. leiocarpa* Mart., *Bathysa gymnocarpa* K. Schum, *B. mendonçaei* K. Schum, *B. cuspidata* (A.St.-Hil.) Hook.f. and *B. australis* (A.St.-Hil.) Benth. & Hook.f. among others (Vieira *et al.* 1992; Gomes *et al*. 2000). Rubiaceae in open areas, such as the coastal dune forests (restingas), has thicker leaves (Arruda & Gomes 1996). The greater leaf thickness may be a result of accentuated development of the palisade or even the spongy parenchyma, under intense irradiation (Dickison 2006). The species of *Posoqueria* studied even though collected in areas where they are relatively protected by the crowns of the canopy plants, have proportionately thicker leaves as a result of the larger number of spongy parenchyma layers. Considering the plastic variation of this character, it has no taxonomic value to the identification of the analized *Posoqueria* species.

Calcium oxalate crystals are an important source of information for defining the subfamilies, tribes, and subtribes of Rubiaceae. Few studies about the development of these idioblasts or ontogenetic and evolutionary relationships of these cells for the subfamilies of Rubiaceae are available in the literature, except the work of Horner & Whitmoyer (1972) with *Psychotria punctata*. Crystals are abundant in the family, and include crystal-sand, raphides, clustered, styloids and other acicular forms. According to Metcalfe & Chalk (1950) the crystal distribution is helpful in genera delimitation. Recently the raphides-type crystals were said to be related to the subfamily Rubioideae, and the absence of this type and the presence of druses or crystalliferous sand, with some exceptions, characterizes the Cinchonoideae (Jansen *et al*. 2003; Andersson & Antonelli 2005; Robbrecht & Manen 2006). In the six species of *Posoqueria* examined, only idioblasts containing crystalliferous sand were observed, corroborating the proposed assignment for the genus. Although this study was not quantitative, it was clear that in

P. latifolia the amount of idioblasts in the leaf blade is comparatively much higher than in the leaf blade of the other two species.

The arrangement of the vascular system in the petiole and the midvein can be useful in the diagnosis of some plant species. In this sense, morphological patterns have been established for taxonomic purposes dealing with several plant families. In the leaves of many Rubiaceae, the vascular tissues may be arranged in a U, O, or V-shape (Metcalfe & Chalk 1950; Kocsis *et al*. 2004). The organization of the vascular system can be used in superior taxonomic levels in Rubiaceae (Martinez-Cabrera *et al*. 2009). In all species analyzed, the vascular system is organized in an arch from the petiole to the principal vein, where it begins to be accompanied by some fibers. For the *Posoqueria* analyzed, a small diagnostic value was attributed to the shape of vascular system in transversal section at species level.

Although the species of *Posoqueria* are considered to be typical shade plants (Macias 1988), some xeromorphic elements were identified in the leaf blade, specially in *P. macropus* e *P. palustris*. These species are eventually affected by the dry and flood periods, a trademark of the flooded region in which they were found, and show, besides the thick cuticular layer, a high amount of fibers associated to the vascular system. The xeromorphic features observed in the studied plants, especially in *P. macropus* and *P. palustris,* can be related to the coastal environment where they are found, marked by a deficiency of nitrogen, phosphorus, calcium and elevated acidity (Henriques *et al*. 1986). The sclerification is considered an important strategy for the survival in poor resourced places, because it gives the plants an augmentation of the longetivity and a higher efficiency in the use of available nutrients (van Arendonk & Poorter 1994).

This study show that anatomical leaf characters could be allied to morphological ones in the recognition of six species of *Posoqueria* evaluated, such as, outline of anticlinal wall of epidermal cells in frontal view, the patterns of the wax and cuticle deposition, the presence and type of trichomes, and the cross-sectional shape of the leaf margin.

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