

## *Alcantarea* (Bromeliaceae) leaf anatomical characterization and its systematic implications

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*Alcantarea* (Bromeliaceae) has 26 species that are endemic to eastern Brazil, occurring mainly on gneiss–granitic rock outcrops ('inselbergs'). *Alcantarea* has great ornamental potential and several species are cultivated in gardens. Limited data is available in the literature regarding the leaf anatomical features of the genus, though it has been shown that it may provide valuable information for characterizing of Bromeliaceae taxa. In the present work, we employed leaf anatomy to better characterize the genus and understand its radiation into harsh environments, such as inselbergs. We also searched for characteristics potentially useful in phylogenetic analyses and in delimiting *Alcantarea* and *Vriesea*. The anatomical features of the leaves, observed for various *Alcantarea* species, are in accordance with the general pattern shown by other Bromeliaceae members. However, some features are notable for their importance for sustaining life on rock outcrops, such as: small epidermal thick-walled cells, uneven sinuous epidermal walls, hypodermis often differentiated into lignified layers with thick-walled cells, aquiferous hypodermis bearing collapsible cells, and the presence of well developed epicuticular stratum. *Alcantarea* leaves tend to show different shapes in the spongy parenchyma, and have chlorenchymatous palisade parenchyma arranged in more well-defined arches, when compared to *Vriesea* species from the same habitat.

The monocot family Bromeliaceae is almost exclusive of the New World but exhibits a great morphological and ecophysiological versatility (Smith and Downs 1974, Benzing 2000a). Including 58 genera and 3172 species (Luther 2008), Bromeliaceae can be seen in a wide variety of habitats across tropical and subtropical Americas, ranging from coastal sandy plains – like the Brazilian 'restingas' – to humid tropical forests to deserts and high altitudes vegetation in the Andes (Smith 1934, Pittendrigh 1948, Rundel and Dillon 1998). Currently, Bromeliaceae is considered to be the first lineage to emerge within the Poales order, and is close to Typhaceae and Rapateaceae (Soltis et al. 2005, Chase et al. 2006). Several bromeliad species are key elements in stressful environments (e.g. restingas, swamps, high altitude fields, rock outcrops inside the Atlantic rainforest domain), usually providing more favorable conditions for the establishment (germination and growth) of other species, thus being known as 'nurse plants' (Scarano 2002).

The Atlantic rainforest of eastern Brazil has been considered a center of diversity for Bromeliaceae. A pattern of decreasing numbers of genera and species when approaching the drier inland territory with its associated vegetation has been observed (Smith 1934, Reitz 1983, Versieux and Wendt 2007). Most bromeliads, however, present adapta-

tions to save water, such as the rosettes formed by the spirally distributed leaves. In this case, the overlapping bases form a cavity that can be filled up with rain water (Pittendrigh 1948) forming a reservoir. A second key characteristic is the presence of peltate foliar trichomes, also referred to as lepidote, stellate, or multi-cellular hairs considered to be the most distinguishing feature of the family (Tomlinson 1969). Their functions include e.g. retarding transpiration, reducing heat load, and photo-injury to absorbing water and nutrients (Benzing 1976, 2000b). Concomitant to the evolution of absorptive trichomes, there was a progressive structural and functional reduction of the root system, formation of parenchyma and support structures, all important for the transition to epiphytic and rupicolous life forms (Braga 1977). Due to these adaptations and others, like endurance to drought and impoverished substrates (such as xeromorphic leaf structure, 'crassulacean acid metabolism' (CAM) photosynthesis, and heterophylly), Bromeliaceae are able to occupy exceptional habitats and become the most exclusive and dominant plant in a particular environment (Reinert and Meirelles 1993, Por-embski et al. 1998, Benzing 2000a).

Along eastern Brazil, a type of habitat that can be considered stressful for plant communities are the inselbergs

(from German *insel* = island, *berg* = mountain), which are very old dome shaped gneiss–granitic mountains, presenting a scattered distribution and popularly known in Brazil as ‘sugar loaf’. Most Brazilian *inselbergs* are situated within the Atlantic rainforest domain (Safford and Martinelli 2000), where water supply through precipitation can not be considered a limiting factor to plant growth. Nevertheless, over the *inselberg* bare rock surface, water is quickly lost through run-off, as soil cover is restricted. Also, rock temperatures is affected by solar radiation and can reach nearly 61°C, contributing to accelerated evapotranspiration, characterizing these habitats as ‘xeric islands’ (Carauta and Oliveira 1984, Szarzynsky 2000). In this habitat, plant communities are usually restricted to small mats of vegetation, where Bromeliaceae can be the most important plant family with regards to species number (Porembski et al. 1998, Safford and Martinelli 2000). From the biodiversity conservation point of view, *inselbergs* are important because in several localities they are the last refuge for vegetation, as they lack agricultural interest (Porembski et al. 1998), and can act as barriers to urban growth.

*Alcantarea* (E. Morren ex Mez) Harms, Bromeliaceae, subfamily Tillandsioideae, with 26 rupicolous species is a genus endemic to eastern Brazil, occurring mostly on *inselbergs* within the Atlantic rainforest of Bahia, Minas Gerais, Espírito Santo, Rio de Janeiro, and São Paulo States. A few species occur in open grassland outcrops in ‘campo rupestre’ vegetation in Bahia and Minas Gerais as well (Versieux and Wanderley 2007a). Usually *Alcantarea* species present large habits, sometimes reaching 5 m high while blooming and forming broad rosettes that can hold up to 40 liters of water (Martinelli 1997, Versieux and Wanderley 2007b, 2007c).

Currently, there is a debate about the generic limits in Bromeliaceae and many new combinations have been proposed. It has long been considered that assigning species to a genus may be extremely difficult, particularly in the Bromelioideae and Tillandsioideae subfamilies (Smith and Downs 1979). In particular, the generic status of *Alcantarea* has been discussed. *Alcantarea* used to be considered a subgenus of the much larger genus *Vriesea* (Mez 1894, Smith and Downs 1977). The key characteristics that are believed to separate *Alcantarea* from *Vriesea* are the presence of long-ligulate, ephemeral and spiralecent petals, semi-inferior ovaries, together with seeds bearing apical and basal appendages (Harms 1930, Grant 1995). Because *Alcantarea* taxa are specialized to grow only on rock outcrops, while *Vriesea* species grow either in rocky outcrops or as epiphytes inside mesic environments, it is expected that foliar anatomy may reflect this habitat selection, and thus, provide some additional morphological characters that could support the systematic boundaries of these taxa.

This work is a contribution to the knowledge of the anatomy of Bromeliaceae leaves, particularly of *Alcantarea*, for which the only reference available is the work of Reinert and Meirelles (1993). We aim to describe the leaf anatomical characteristics that vary among species within *Alcantarea*, comment on the implications for systematics, compare our results with other previously published work or new data presented here for *Vriesea* and add data to the discussion about their systematic status. In addition, we will

cover the adaptive significance of several features presented by this genus while growing in the harsh environments where they occur: *inselberg* and/or ‘campo rupestre’.

## Material and methods

Leaf samples from nine species of *Alcantarea* and two species of *Vriesea* were investigated, and the material was obtained during fieldwork in various Brazilian states. Samples were taken from the middle of the leaf blade, fixed in the field in a solution of FAA 70% (formalin–acetic acid–ethanol; Ruzin 1999) and then transferred and stored in 70% ethanol. The epidermis from the middle of the leaf blade was cut and subjected to a progressively dehydrating alcoholic series ranging from 50% up to 100% EtOH, then dried to a critical point, mounted on stubs, stained with platinum and observed by scanning electron microscopy (SEM) in a digital scanning microscope DSM940 at the electronic microscopy laboratory, Biosciences Inst., Univ. of São Paulo. For epicuticular wax observation, the samples were directly dried in an oven at 40°C for three days, mounted and then spur-coated with platinum. Chloroform tests were performed to confirm the presence of epicuticular wax. Transverse sections were taken using a razor blade, bleached by the use of sodium hypochlorite, stained in Safrablau (Bukatsch 1972), dehydrated through an ethanol series and mounted in glycerin. The epidermis was dissociated through immersion in the Franklin’s solution (Franklin 1945 modified by Kraus and Arduin 1997), a mixture (1:1) of 30% of hydrogen peroxide and glacial acetic acid, for 24 h and then mounted according to the same procedure described for transverse sections. Sections were observed under light microscopy (LM) and digital pictures were taken.

A herbarium specimen for each studied sample was prepared and deposited in the herbarium Inst. de Botânica, São Paulo, Brazil (SP). Voucher materials are indicated below, followed by its provenance and collector number: *Alcantarea burle-marxii* (Leme) J. R. Grant, Pedra Azul, Minas Gerais, Versieux 420; *Alcantarea duarteana* (L. B. Sm.) J. R. Grant, Diamantina, Minas Gerais, Versieux 255; *Alcantarea extensa* (L. B. Sm.) J. R. Grant, Cachoeiro do Itapemirim, Espírito Santo, Versieux 380; *Alcantarea farneyi* (Martinelli & A. F. Costa) J. R. Grant, Santa Maria Madalena, Rio de Janeiro, Versieux 247; *Alcantarea geniculata* (Wawra) J. R. Grant, Petrópolis, Rio de Janeiro, Versieux 285; *Alcantarea glaziouna* (Leme) Leme, Rio de Janeiro, Rio de Janeiro, Versieux 343; *Alcantarea nahoumii* (Leme) J. R. Grant, Santa Teresinha, Bahia, Versieux 418; *Alcantarea nigripetala* Leme & L. Kollmann, Pancas, Espírito Santo, Louzada 22; *Alcantarea patriae* Versieux & Wand., Jerônimo Monteiro, Espírito Santo, Versieux 365; *Alcantarea roberto-kautskyi* Leme, Baixo Guandú, Espírito Santo, Versieux 398 and Santa Maria do Salto, Minas Gerais, Versieux 430; *Alcantarea trepida* Versieux & Wand., Baixo Guandú, Espírito Santo, Versieux 394, 395; *Alcantarea turgida* Versieux & Wand., Conceição do Mato Dentro, Minas Gerais, Versieux 260; *Vriesea minarum* L. B. Sm., Sabará, Minas Gerais, Versieux 140; *Vriesea stricta* L. B. Sm., Santana do Riacho, Minas Gerais, Versieux 301.

## Results

### Overall leaf morphology

*Alcantarea* species present simple entire leaves, as do all the other members of the Tillandsioideae subfamily. Leaves are rosulate, clearly divided into a leaf sheath and a leaf blade; have a coriaceous texture and some prominent veins. The leaf sheath is densely lepidote on both faces and is generally thicker than on the leaf blade. The leaf sheath is variable in shape, but is often ovate. The leaf blade is usually ligulate, sometimes linear–triangular, and canaliculate at least close to the base. The apex may be acute or obtuse, but is most frequently acuminate. Rosette size and form are quite variable within the genus.

### Epidermis and mesophyll

In all examined species, epidermal cells on both surfaces are organized in a single layer (Fig. 1a–k, 2a–d, 2h–o). The anticlinal and the internal periclinal cell walls are thick. Cell shapes vary in cross-section: appearing u-shaped in *A. extensa*, *A. trepida*, *A. vinicolor*, *A. geniculata*, *A. naboumii*, *A. patriae* and in *A. burle-marxii*, to irregularly polygonal in *A. farneyi*. The epicuticular wax together with the cuticle forms a thick layer over the outer periclinal cell epidermis wall as can be observed in most species (Fig. 2a, 2h, 2i, 2k, 2m) and also on the SEM pictures (Fig. 4, 5). One nearly spherical silica body can be observed inside each cell (Fig. 2b, 3g, 3k, 3l, 3m, 4a, 4n, 5n). In front view (Fig. 3a, 3c, 3e, 3h–3n) the anticlinal walls of the epidermal cells are sinuous, with different cell shapes varying from more oblong (Fig. 3a) to more parallel sided (Fig. 3h, 3k). This variation can be noticed among different species or between the adaxial and abaxial face of the same leaf. The abaxial epidermis is also uniseriate, plane to slightly undulate in *A. duarteana* and *A. farneyi* (Fig. 1c, 1k, 2e–f). The periclinal walls are thick, the outer covered by the epicuticular stratum. The cells has an extremely reduced lumen due to the anticlinal wall thickening. In LM and SEM it is possible to see that the abaxial epidermis is costate with very narrow costal and intercostal zones (Fig. 3b, 3f, 4h, 4m). Stomata complexes are tetracitic and restricted to the intercostals zones. The subsidiary cells are parallel to the guard cell and have distinct shapes in cross sections (Fig. 2s–y). Other ordinary epidermal cells that surround the stomata may be arranged in a kind of ring pattern and show slightly thicker and straighter walls than their neighbors (Fig. 3g, 3h, 3l).

A multi-cellular aquiferous hypodermis is present in all studied species but its extension may vary in different taxa. In eight species, however, the hypodermis is a lignified tissue with 2–5 layers of thick-walled cells. Fibrous clusters, not associated with the vascular bundle, have a scattered distribution in all species, forming islands in the hypodermis or in the mesophyll. Eventually, sheath bundle extensions join with the mesophyll fibers (island fibers), as can be seen in some species (Fig. 1b, 1d, 1f, 1i–j). Under the lignified layer of the hypodermis, there are 3–12 layers of aquiferous hypodermis that usually present rounded cells that constitute the water storing tissue or aquiferous parenchyma. The cells of these layers are collapsible, and

the thin anticlinal walls can be seen as sinuous (Fig. 1i) during the desiccation, or shifting to turgid nearly globose cells with stretched walls when well watered (Fig. 1a). The first hypodermic layers with thick-walled cells seem to be absent in *A. farneyi* and in *A. duarteana* (Fig. 1c, 1k), where the entire hypodermis is aquiferous. The water storing tissue is variable in width in *Alcantarea* species and in *A. duarteana* it reaches its largest number of layers (Fig. 1k). Internally within the hypodermis, there is a chlorenchymatous palisade parenchyma that can be observed in transverse sections forming arches that can be contiguous (*A. patriae*, Fig. 1h) or interrupted by vascular bundles, or even by expansions of pericyclic fibers (Fig. 1a–b, 1e). Below the palisade parenchyma, there is a spongy parenchyma, with stellate cells positioned in aeration channels (or air-lacunae in the sense of Tomlinson 1969). Analyzing Fig. 1m, it is possible to notice the presence of spongy parenchyma in the entire air channel. This spongy parenchyma in the air channels alternate with the vascular bundle and has different contours among the distinct species (Fig. 1a–k). In cross section, the air channels can be elliptic (Fig. 1a–b), or elliptic with a weak constriction close to the middle (Fig. 1e–f, 1h, 1j), oblageniform (Fig. 1c) i.e. resembling an upside-down bottle, obovate (Fig. 1i), or obtriangular (Fig. 1k). This spongy parenchyma is disposed all along the mesophyll (Fig. 1m), is flanked by the vascular bundle and is connected to the sub-stomatic chambers on the abaxial surface (Fig. 2y). Between the spongy parenchyma and below each vascular bundle there is a short area filled with the abaxial hypodermis. We observed idioblasts in the spongy parenchyma of *A. duarteana* (Fig. 1l).

All the species present collateral vascular bundles in a single series, where the thicker ones alternate with the thinner ones. The larger vascular bundles are completely surrounded by lignified pericyclic fibers and more externally by the endodermis, the mesophyll inner layer. The pericyclic fibers may be of different sizes, sometimes with extended projections toward the adaxial and abaxial aquiferous parenchyma (Fig. 1a–b, 1e) or they can be reduced and surround the vascular tissues (Fig. 1h, 1k). These fiber extensions usually do not overpass the arched clorenchyma. A layer of thick-walled cells that separate the xylem from the phloem can also be observed in most species. The thin commissural vascular bundle can be seen in the longitudinal section (Fig. 1m).

The epidermis in front view presents rectangular to quadrangular cells with sinuous and thick anticlinal walls and one nearly spherical silica body (Fig. 3). These cells may be laterally compressed along the coastal zones and slightly different from the neighboring cells located in the intercostal zones of the same leaf (Fig. 3b, 3g, 3k, 3l). Trichomes are observed on both surfaces of the leaf blade in all the analyzed species, but their densities vary from sparsely to densely lepidote (Fig. 4d, 4f). The trichomes emerge from a lower level than the other epidermal cells (Fig. 2f, 2j, 2l, 2n, 2p) and the stalk is uniseriate and composed of few cells. The trichome wing cells can be radially symmetric (Fig. 3j, 3m, 4l) or slightly asymmetric (Fig. 3d, 4d).

Figure 6 shows transversal sections for the leaf blade of two species of *Vriesea* that we analyzed for comparison. It is possible to see that in cross section the epidermis is similar

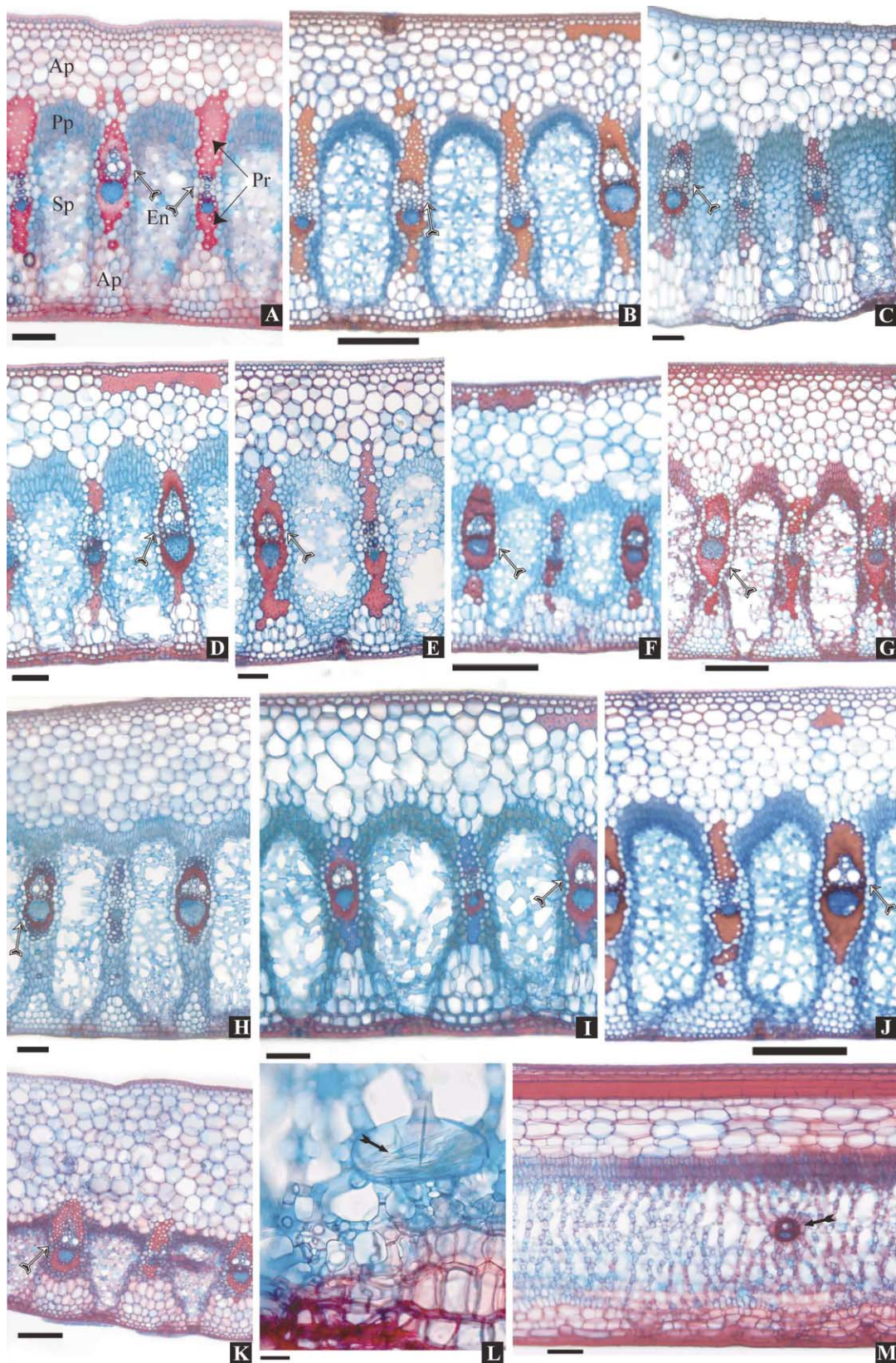


Figure 1. (A)–(K) transversal sections of the leaf middle portion (5× scale = 250 μm, 10 × = 100 μm, black and white arrows indicate the endodermis), (L)–(M) longitudinal sections of the leaf middle portion, (A) *Alcantarea burle-marxii* (10×), Ap = aquiferous parenchyma, Pp = palisade parenchyma, Sp = spongy parenchyma, En = endodermis, Pr = pericyclic fibers, (B) *A. extensa* (5×), (C) *A. farneyi* (10×), (D) *A. geniculata* (10×), (E) *A. naboumii* (10×), (F) *A. nigripetala* (5×), (G) *A. vinicolor* (5×), (H) *A. patriae* (10×), (I) *A. roberto-kautskyi* (10×), (J) *A. trepida* (5×), (K)–(L) *A. duarteana*, (K) transversal section (5×), (L) longitudinal section with a raphide (arrow) (20× scale = 50 μm), (M) longitudinal section of *A. burle-marxii* (10×) with a commissural vascular bundle (arrow).

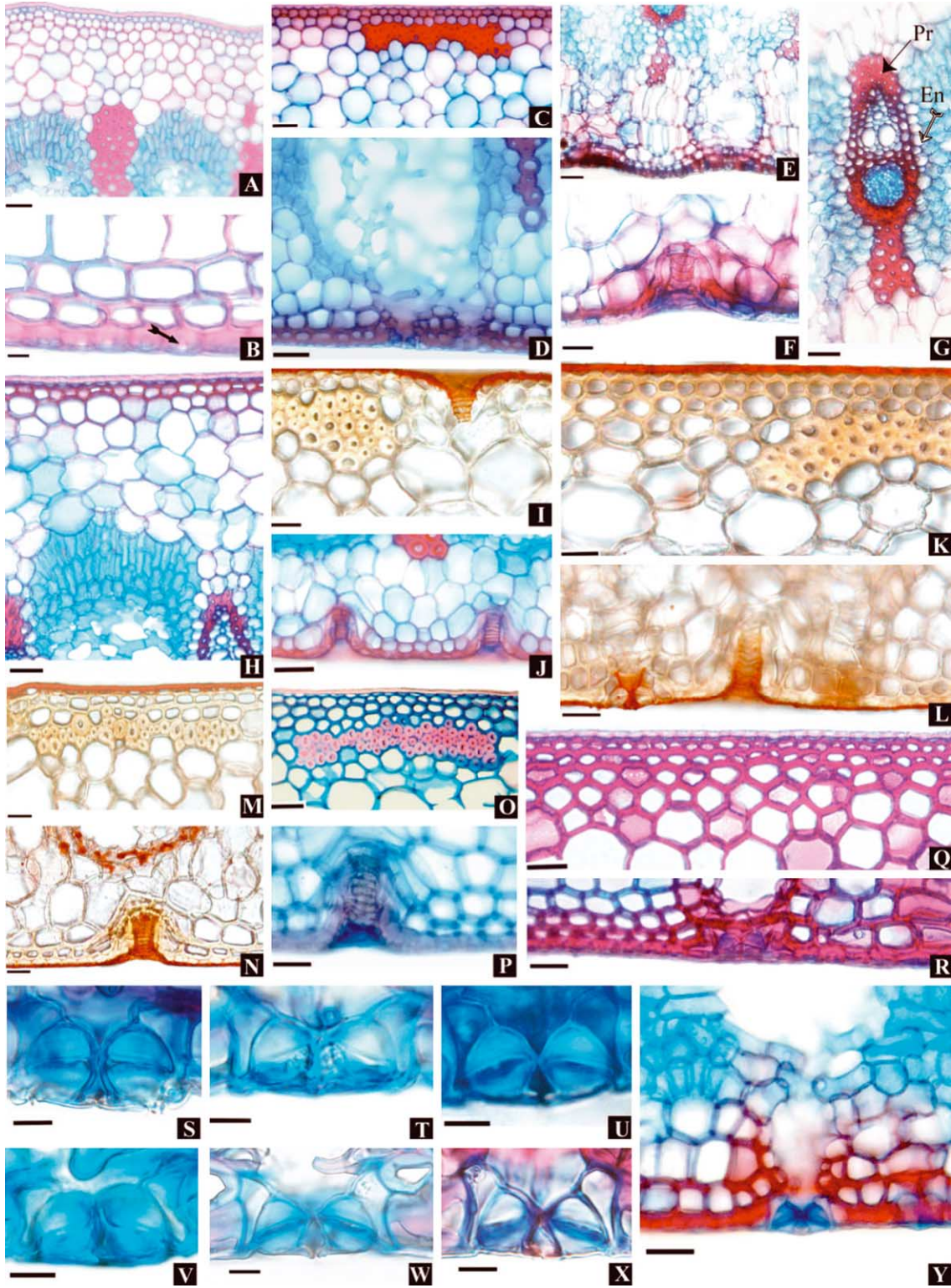


Figure 2. Details of adaxial and abaxial epidermis (scales:  $20\times = 50\ \mu\text{m}$ ,  $40\times = 25\ \mu\text{m}$ ,  $100\times = 10\ \mu\text{m}$ ). (A)–(B) *A. burle-marxii*, (A) adaxial surface ( $20\times$ ), (B) abaxial surface ( $100\times$ ) with cells containing silica bodies (arrow), (C)–(D) *A. extensa*, (C) adaxial surface ( $20\times$ ), (D) abaxial surface ( $20\times$ ), (E)–(G) *A. farneyi*, (E)–(F) abaxial surface ( $40\times$ ), (G) vascular bundle ( $20\times$ ) showing pericyclic fibers (Pr) and endodermis (En), (H) *A. geniculata* adaxial surface ( $20\times$ ), (I)–(J) *A. nigripetala*, (I) adaxial surface ( $40\times$ ), (J) abaxial surface ( $20\times$ ), (K)–(L) *A. patriae*, (K) adaxial surface ( $40\times$ ), (L) abaxial surface ( $40\times$ ), (M)–(N) *A. roberto-kautskyi*, (M) adaxial surface ( $40\times$ ), (N) abaxial surface ( $40\times$ ), (O)–(P) *A. trepida*, (O) adaxial surface ( $20\times$ ), (P) abaxial surface ( $40\times$ ), (Q)–(R) *A. vinicolor*, (Q) adaxial surface ( $20\times$ ), (R) abaxial surface ( $40\times$ ), (S)–(Y) abaxial leaf surface showing stomata and subsidiary cells in transversal sections ( $100\times$ ), (S) *A. farneyi*, (T) *A. geniculata*, (U) *A. heloisae*, (V) *A. nigripetala*, (W) *A. roberto-kautskyi*, (X) *A. vinicolor*, (Y) *A. patriae*, leaf abaxial surface ( $40\times$  scale =  $25\ \mu\text{m}$ ) showing the sub-stomatic chamber.

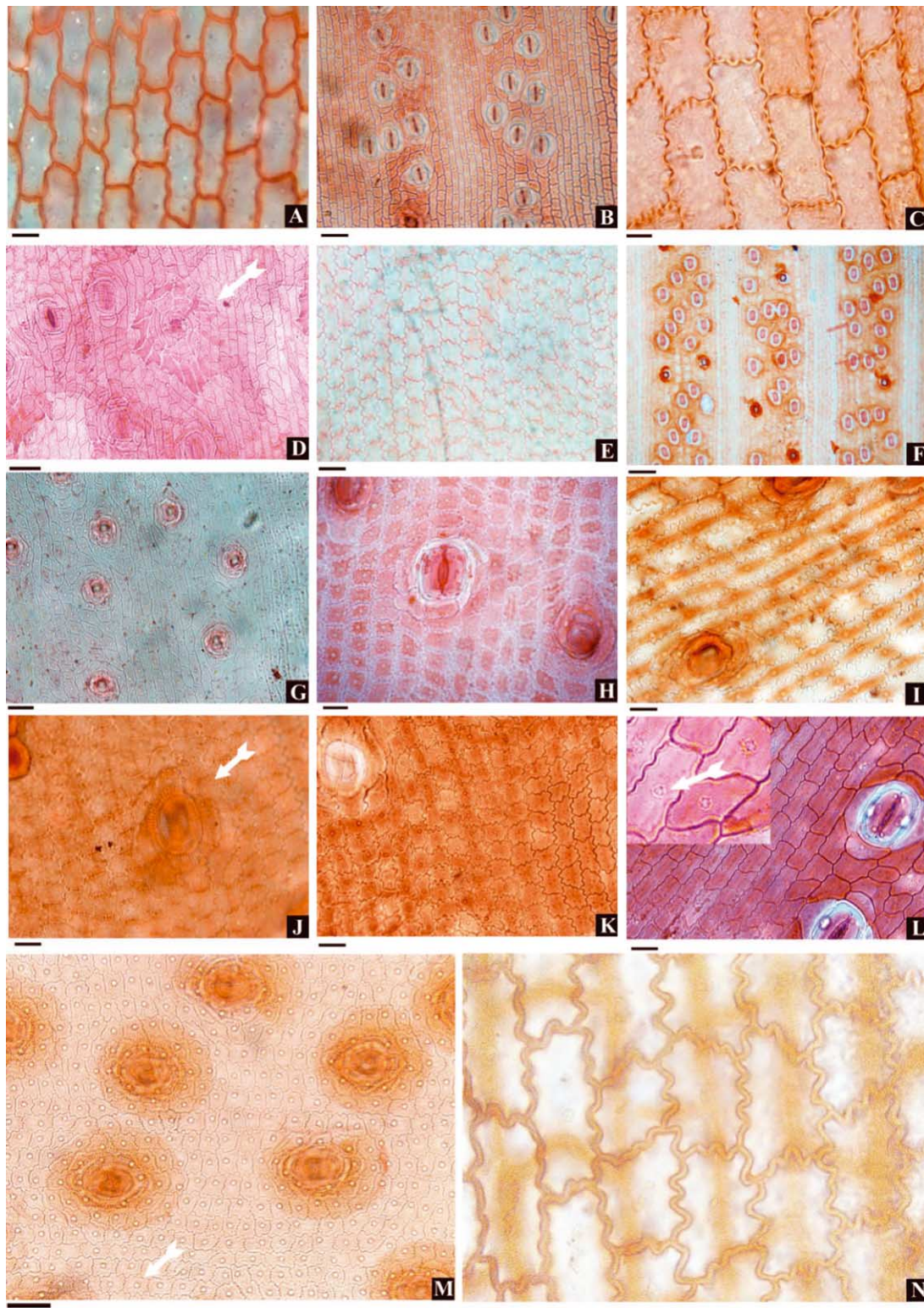


Figure 3. (A)–(N) adaxial and abaxial epidermis in frontal views (10× scale = 100 μm, 20× = 50 μm, 40× = 25 μm, 100× = 10 μm), (A)–(B) *A. burle-marxii*, (A) adaxial surface (100×), (B) abaxial surface (20×), (C)–(D) *A. duarteana*, (C) adaxial surface (100×), (D) abaxial surface with a slightly asymmetric trichome (arrow), (E)–(F) *A. extensa*, (E) adaxial surface (40×), (F) abaxial surface showing stomata and vestiges of trichomes arranged in rows (10×), (G)–(H) *A. geniculata*, (G) adaxial surface (20×), (H) Abaxial surface showing stomata and trichome (40×), (I)–(J) *Alcantarea patriae*, (I) adaxial surface showing epidermal anticlinal and periclinal sinuous walls and trichomes (40×), (J) abaxial surface depicting radially symmetric trichomes with cell disposed in 4 + 8 + 64 concentric rings (arrow), (K)–(L) *Alcantarea vinicolor* (40×), (K) adaxial surface, (L) abaxial surface, showing enlarged detail of the silica body (arrow), (M) *A. burle-marxii* abaxial surface with trichomes and silica bodies (arrow) (20×), (N) *A. roberto-kautskyi* adaxial surface showing sinuous walls (100×).

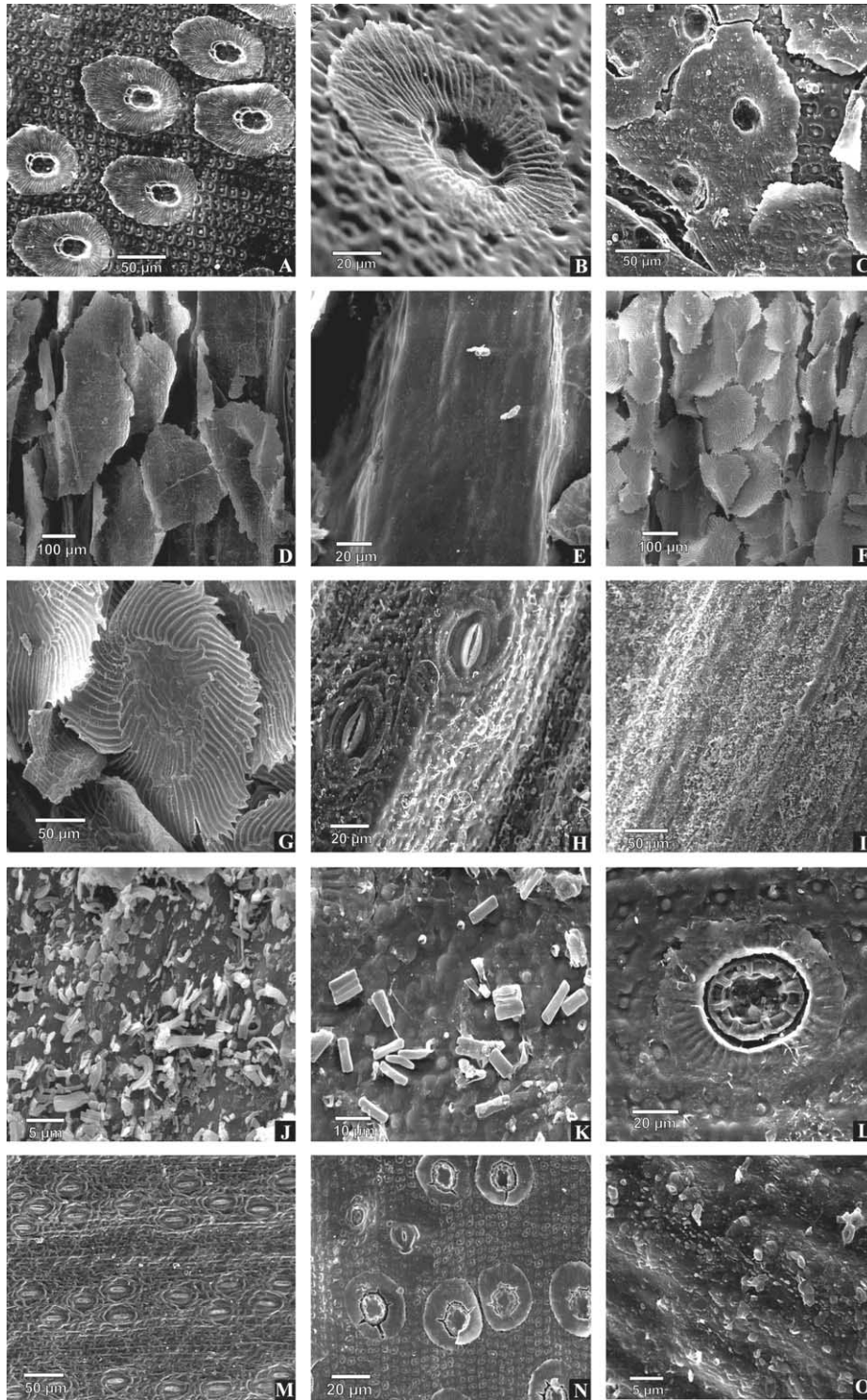


Figure 4. Leaf sheath and leaf blade epidermis scanning electron microscopy (SEM) images. (A)–(C) *A. burle-marxii* leaf sheath, (A) abaxial view showing trichomes and silica bodies, (B) detail of trichome of abaxial face, (C) trichomes on the adaxial face, (D)–(G) *A. duarteana*, middle portion of the leaf blade, (D)–(E) abaxial surface, (F)–(G) adaxial surface, (D) trichomes covering the whole surface of the leaf, (E) detail of the epidermis without wax under trichomes, (F) trichomes slightly asymmetric and densely disposed over the abaxial surface, (G) detail of trichome showing the 4+8+16+32+64 concentric cell rings, (H)–(J) *A. patriciae*, (H) abaxial leaf sheath showing epicuticular wax and two stomata, (I) adaxial leaf blade showing epicuticular wax, (J) detail view of individual epicuticular wax crystalloids, (K)–(M) *A. trepida* abaxial view of the leaf sheath epicuticular wax crystalloids, (L) radially symmetric trichome on the adaxial leaf sheath with the 4+8+32 concentric ring cells pattern, (M) middle abaxial portion of the leaf blade showing stomata complexes arranged in rows, (N)–(O) *A. vinicolor*, (N) adaxial leaf sheath trichomes, (O) middle adaxial leaf surface showing epicuticular wax.

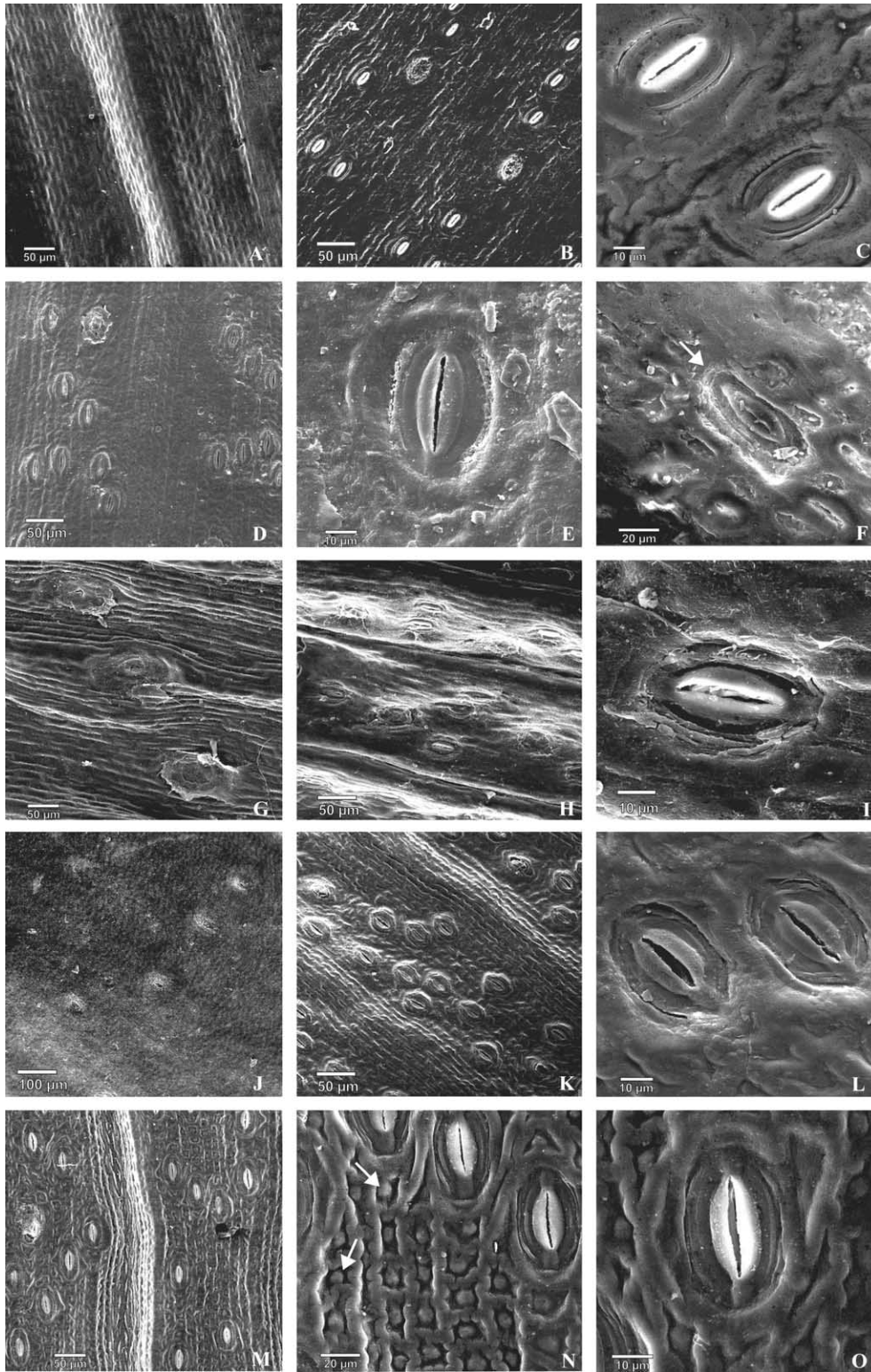


Figure 5. Leaf blade and rachis epidermis scanning electron microscopy (SEM) for samples treated with dehydrating alcoholic series. (A)–(C) *A. burle-marxii*, (A) adaxial view, (B) abaxial view showing stomata and trichomes vestiges arranged in rows, (C) detail of stomata and the different position of the ordinary cells that surround stomata, (D)–(E) abaxial view of *A. extensa*, (D) stomata and vestiges of trichomes, (E) stomata partially covered by epicuticular wax, (F) detail of stomata present on the inflorescence rachis of *A. glazouana* (arrow), (G)–(I) *A. farneyi*, (G) adaxial view showing remnants of trichomes, (H) stomata in rows, (I) detail of the stomata surrounded by thick cuticle, (J)–(L) *A. trepida*, (J) glabrescent adaxial surface, (K) abaxial view showing stomata in rows, (L) detail of stomata in the abaxial view, (M)–(O) *A. vinicolor*, (M) abaxial surface showing stomata, (N) detail of silica bodies and sinuous epidermal walls (arrows), (O) detail of stomata.



to that observed in *Alcantarea* species and the air channels are filled with stellate cells which present forms broader rather than longer, almost orbicular (Fig. 6a, 6c). In *Vriesea minarum* and *V. stricta* the adaxial and abaxial hipodermis is aquiferous, as in *Alcantarea*. But the abaxial hipodermis is apparently interrupted by fewer stomata. The extension of the vascular system occupies nearly 1/4 the width of the leaf blade. The larger vascular bundles are completely surrounded by the lignified pericyclic fibers and by the endodermis (Fig. 6a–b). The pericyclic fibers do not show extended projections toward the adaxial and abaxial aquiferous parenchyma. Trichomes are sparsely distributed on both faces of the leaf blade in both species (Fig. 6a, 6c). The trichomes emerge from a lower level than the other epidermal cells and the stalk is uniseriate and composed of fewer cells (Fig. 6b).

## Discussion

Overall leaf morphology (size, shape, apex format) and rosette shape can aid in the identification of *Alcantarea* species. The latter is a variable character that can range from grass-like or tubular in few species that have limited capacity to hold water (*A. benzing*, *A. duarteana*, *A. farneyi*, *A. hatschbachii*) to broad, infundibuliform, or crateriform rosettes holding up to 40 liters of water in *A. imperialis*, *A. roberto-kautskyi*, *A. patriae* (Martinelli 1997, Versieux and Wanderley 2007a, 2007b, 2007c).

The leaf anatomical features observed for *Alcantarea* species are in accordance with the general pattern known for other Bromeliaceae (Robinson 1969, Tomlinson 1969, Braga 1977, Reinert and Meirelles 1993, Arruda and Costa 2003, Palací et al. 2004, Scatena and Segecin 2005).

The presence of trichomes have been reported as one of the most characteristic features of Bromeliaceae (Tomlinson

1969). We observed foliar trichomes on both leaf surfaces of all studied species. Usually, adaxial trichomes can be noticed only by vestiges on the glabrescent leaves whereas in *A. duarteana* and *A. farneyi*, trichomes are densely arranged over both surfaces and are easily seen even on adult leaves. In these particular species, no crystalloids of epicuticular wax have been observed under the trichome shield and apparently this could be a pattern for densely lepidote Tillandsioideae leaves, as it was also observed by Scatena and Segecin (2005) in the genus *Tillandsia*.

Specialized epidermal trichomes have functions that range from retarding transpiration, reducing both heat load and photoinjury, water and nutritive ion absorption, avoiding herbivory and pathogens, attraction of pollinators and seed dispersers, and secretion of possible digestive enzymes used to process prey (in few species of *Brocchinia*) (Benzing 1976, 2000b). Though all analyzed species here are from open and well-illuminated habitats, where trichomes could be playing most of the possible roles mentioned above, the presence of more densely arranged trichomes over the leaf of *A. duarteana* and *A. farneyi* may have evolved as a compensatory function. Both *A. duarteana* and *A. farneyi* have very reduced phytotelma compared to the large-tank *Alcantarea*, and both live at high altitude (near 2000 m.a.s.l.) where mist and dew formation is common. These sources of water could be better absorbed by a denser indumentum.

In all species the trichomes are randomly arranged on the leaf sheath and they are organized in rows (i.e. concentrated on the intercostal zone) along the leaf blade as previously observed in *Vriesea splitgerberi* (Mez) L. B. Sm. & Pittendrigh (Braga 1977), and in few species of *Aechmea* (Sousa et al. 2005). Reinert and Meirelles (1993) studied juvenile leaves of *Alcantarea glazionana* (Lem.) Leme, then identified them as *Vriesea geniculata* (Wawra) Wawra, and reported that trichome density changed over the lifetime of

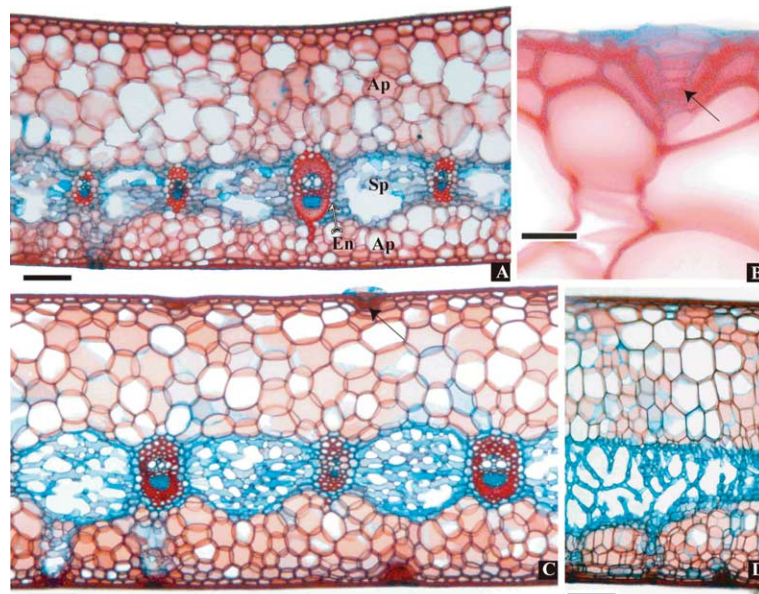


Figure 6. (A)–(B) transversal sections of the leaf middle portion of *Vriesea minarum*, (A) Ap = aquiferous parenchyma, Sp = spongy parenchyma, En = endodermis (10× scale = 100 µm), (B) detail of the trichome stalk cells (arrow) (40× scale = 25 µm), (C)–(D) *Vriesea stricta* (10× scale = 100 µm), (C) transversal sections of the leaf middle portion, showing trichomes (arrow), (D) longitudinal section showing the air channel filled with spongy parenchyma.

the plant, and that they occurred in the coastal and intercostal zones of the leaves when they were young but were restricted to the intercostal bands in adult leaves. Our observations confirm the occurrence of glabrescent leaves (i.e. that lose their trichomes during the development) in *Alcantarea* as usually mentioned in taxonomic descriptions (Versieux and Wanderley 2007a, 2007b), but we also report here, that for a few species, the trichomes extend along the entire leaf. Reinert and Meirelles (1993) observed that juvenile leaves of *A. glaziouana* were narrow, strongly canaliculate, succulent, and did not form a tank, while adult *A. glaziouana* present nearly flat leaves, broad, and covered by epicuticular wax, which form a well developed tank. Heterophyllous bromeliads, with juvenile forms densely covered by trichomes were also observed by Adams and Martin (1986), who noticed that juveniles of *Tillandsia deppeana* were more tolerant to a prolonged period of drought than adults were, and furthermore, that it would show higher levels of photosynthesis following a brief wetting after a period of desiccation. These adaptations could favor and protect the young plant until it is completely developed and established.

A central silica body is characteristic for the epidermal cells of most Bromeliaceae, as well as for some other monocot families (Robinson 1969, Tomlinson 1969). In *Alcantarea* the silica body can be noticed in the paradermal sections as well as on SEM images. Silica bodies are commonly found within the Poales, and their morphology and presence may define taxonomic groups in plant families such as Cyperaceae and Poaceae (Mehra and Sharma 1965, Zhang and Clark 2000). This characteristic was used already by Gilmartin and Brown (1987) in a morphological phylogenetic analysis and supported the proximity of Bromeliaceae to other monocot families such as Commelinaceae, Rapateaceae and Strelitziaceae. Recently, the presence of silicon dioxide bodies in leaves, together with epicuticular waxes of the *Strelitzia* type, are considered to be a synapomorphy of the larger Commelinid clade (Soltis et al. 2005). An anti-herbivory role and also a growth promotor activity have been attributed to silica in other plant families within the Poales (MacNaughton et al. 1985). The function of silica bodies was once conjectured as being photoprotective because it possibly contributed to the reflection of sunlight, preventing overheating and water loss (Baumert 1907 in Krauss 1949).

The following xeromorphic traits were observed in *Alcantarea* leaves: small epidermal thick-walled cells which exhibit considerable resistance to collapse (Oertli et al. 1990 in Fahn and Cutler 1992); uneven sinuous epidermal walls that strengthen the leaves and help to prevent the separation of epidermis from the underlying hypodermis, as mentioned by Benzing (2000b). The hypodermis often differentiated into 2–5 lignified layers with thick-walled cells, an adaptation to avoid water loss and to protect the inner water storage tissue layer. In addition, we observed the aquiferous hypodermis bearing collapsible cells that possess flexible walls that can tolerate cell volume change from a well-watered to a dry situation; presence of a well-developed epicuticular stratum with a thick cuticle often covered by *Strelitzia* type of epicuticular wax, frequently protecting the stomata complexes.

In all analyzed species, stomata are restricted to the abaxial face (hypostomatic leaf), and this is a general trend, with few exceptions for the entire family and confirmed by other authors for designated Bromeliaceae genera (Braga 1977, Reinert and Meirelles 1993, Arruda and Costa 2003). In most species, stomata are positioned at the same level as common cells of the epidermis. In *A. farneyi* and *A. duarteana* the stomata are at the same level as the adjacent cells, but the epidermis itself is slightly sinuous. Hypostomatic leaves and stomata arranged on the same level as neighboring cells are not considered to be typical xeromorphic adaptations. Several plant families from arid zones, particularly succulents, have stomata deeply sunken in crypts (e.g. Agavaceae, Fahn and Cutler 1992, Cactaceae, Calvente et al. 2008). In all species, stomata are situated above a multi-layered hypodermis that delimits a sub-stomatal chamber. According to Fahn and Cutler (1992), sub-stomatal chambers like the ones seen in *Alcantarea*, establish a long and shallow diffusion gradient between the chlorenchyma and the environment, with a high diffusion resistance. Fahn and Cutler (1992) argued that stomata on both sides of the leaf tended to be more common on the leaves of xeric plants. Nevertheless, in all *Alcantarea* species analyzed here, and in most bromeliad species, stomata are restricted to the abaxial face. Scatena and Segecin (2005) argued that the presence of hypostomatic leaves (a character assumed to be typical of mesic taxa) may be more related to the phylogenetic ancestry of the bromeliads than to an ecological or environmental selection response. In other words, these authors considered that this character would be maintained in Bromeliaceae as a plesiomorphic condition. Other authors, like Nobel (1999) discuss that hypostomatic leaves may constitute a strategy to minimize water loss by convection currents or breezes, that could remove water vapor from the leaf surface, and also that stomata surrounded by a thick cuticle have their resistance to water loss slightly enhanced.

Though in cross section the epidermal cell shapes appear rather invariable, the paradermal sections (face view), show that the epidermal cell wall morphology vary. Actually, the significance of epidermal cell morphology for systematics has been assessed for other families and a great number of potentially informative characteristics have been defined (Calvente et al. 2008). In *Tillandsia*, Scatena and Segecin (2005) report that the shape of the epidermal cellular wall may vary from nearly straight to curved or undulate. In the analyzed species of *Alcantarea*, the epidermal walls are always sinuous. Krauss (1949) noticed that ordinary epidermal cells that surround the stomata may be differently distributed, being arranged in a ring form, showing a slightly thicker and straighter wall that could help protect the stomata from longitudinal stress. This pattern of modification was also observed here and we speculate that the remaining sinuous interdigitated thin-walled cells could be elastic and have the ability to better tolerate turgor shifts.

Epicuticular wax has been reported to be present in several Bromeliaceae species and its importance in prevention of water loss is broadly cited (Palací et al. 2004). Other protective functions of wax may include minimizing mechanical damage to cells and inhibiting fungal and insect attacks (Eglinton and Hamilton 1967). The pattern observed for epicuticular wax deposition in *Alcantarea* is

known as the *Strelitzia* type (Barthlott and Frölich 1983) and is characterized by the presence of rod-like structures.

The wax composition of one species may differ between parts of the same plant and may vary with season, site, and plant age (Eglinton and Hamilton 1967). Barber (1955), presented data on the glaucousness of *Eucalyptus* species (Myrtaceae) and observed changes in wax deposition according to frost activities. He discovered that more glaucous populations occurred in the more frosty localities. As epicuticular wax may vary according to the environment, it is important to carefully consider this variation prior to using such characteristics for taxonomic decisions and species circumscriptions. Palací et al. (2004) suggests that wax can affect Bromeliaceae leaves by increasing their water impoundment ability and maintain its capability of gas exchange through water repellency. Traditionally, waxes have been cited for their importance in reducing sunlight interception by leaves in exposed habitats.

All the species present leaves with collateral vascular bundles in a single series, enveloped by the pericycle and the endodermis as already mentioned for Bromeliaceae by Pita (1997), Arruda and Costa (2003), and Scatena and Segecin (2005) following the nomenclature adopted by Van Fleet (1961), Esau (1965), and Menezes et al. (2006). These authors consider the inner mesophyll layer of the leaf as the endodermis (parenchymatous sheath). Commissural vascular bundles can be seen in longitudinal sections and this feature has also been observed in other species by different authors (Braga 1977, Sousa et al. 2005).

Leaf anatomy provides potential characteristics to be explored in detailed morphological phylogenetic studies in the future. However, for very closely related species or species complexes anatomical features are very homogeneous within the genus *Alcantarea*. This seems to be particularly true for species of the so-called *Alcantarea extensa* complex defined by Versieux and Wendt (2006). *Alcantarea extensa* and a closely related species, *A. trepida*, can barely be differentiated based on their leaf anatomy.

*Alcantarea* has always been considered a close relative of *Vriesea*. In fact, for most of its taxonomic existence it was treated as a subgenus of *Vriesea*. Harms (1929) elevated *Alcantarea* to the generic rank, albeit his decision was not followed by subsequent authors (Smith 1934, 1955, Smith and Downs 1977). A recent molecular phylogeny that sampled two *Alcantarea* species demonstrated that *Alcantarea* forms a clade within the larger genus *Vriesea* and suggested that its recognition as an independent genus turns *Vriesea* into a paraphyletic taxon (Barfuss et al. 2005). Nevertheless, the same work (Barfuss et al. 2005) indicated that if we consider *Vriesea* a polyphyletic taxon, it is possible to recognize at least three monophyletic genera (that corresponds to tribe Vrieseae W. Till & Barfuss), i.e. *Alcantarea*, that is sister to a monophyletic group of eastern Brazilian *Vriesea* and that this clade is sister to the remaining species of *Vriesea* from central America and the Andean region. It would be desirable to have leaf anatomy data that could provide additional information regarding the relationship of these two genera.

Comparing our results on leaf anatomy of *Alcantarea* to the works of Braga (1977) and Arruda and Costa (2003) that included *Vriesea* specimens, and to the new data presented here for *V. minarum* and *V. stricta* (both

rupicolous species that grow in the same habitat as some *Alcantarea* species), it is possible to see an apparent distinction between the two genera concerning the shape of the air channels. These air channels are filled with spongy parenchyma, composed by stellate cells. The channels seem to be narrower and more elongated in *Alcantarea*, while *Vriesea* shows leaves with broader air channels, almost sub-orbicular in outline and filled with a more diffuse spongy parenchyma. Additionally, the presence of a chlorenchymatous palisade parenchyma constitute another difference. In *Alcantarea*, all analyzed species present palisade parenchyma that usually have more than one cell layer and are arranged as well-defined arches, whereas in *V. minarum* and *V. stricta* it is not evident, but in other species it has a planar arrangement (e.g. *V. bituminosa* and *V. fosteriana* in Arruda and Costa 2003). The aquiferous hypodermis in *Vriesea* is usually thicker, with more cell layers. Externally, the leaves of *Alcantarea* usually present greater dimensions and more prominent nerves. The abaxial hypodermis in *Vriesea* is interrupted by fewer stomata. Stomata density may be an additional character to be examined in a broader sampling of *Vriesea* as it is a potential character to segregate these genera. Furthermore, the presence of fibers in the mesophyll is more characteristic of *Alcantarea*. The extension of the vascular system provides another distinction between these genera, while in *Vriesea* species it usually occupies nearly 1/4 of the leaf blade width and in *Alcantarea* it occupies about 1/3.

Measuring such external morphological features across the genera with a quantitative evaluation of the anatomical features (above), combined with an increased sampling of *Vriesea* would lead to a less qualitatively based and more natural classification. It is still important to stress that *Vriesea* is one of the largest genera in Bromeliaceae with more than 200 species (Smith and Downs 1977). The data available here for comparison is just a small fraction of the great variability that would be expected for such large and broadly distributed genus. Leaf anatomy alone may not answer all questions related to species and genera delimitation because there is a considerable variation in some traits. For example, epidermis cell shape can vary within the same individual and between the adaxial and abaxial surfaces, and so may even the foliar trichome morphology, which limits their use at the species level. Variation in the anatomical feature of Bromeliaceae leaves were reported by Robinson (1969) in *Connelia*, *Cottendorfia* and *Navia* where many species possess an intermediate type of leaf anatomy from what would be expected from each genus. Regarding trichome morphology, the number of cells in each trichome may also vary, as mentioned by Palací et al. (2004).

In conclusion, these results show that despite a reduction in the number of anatomical variable characters among closely related species, the anatomy of *Alcantarea* presents differences in size and number of layers of the lignified and aquiferous hypodermis. In addition, interspecific differences can be seen in air channel shape, density and form of leaf blade trichomes as well as in the amount epicuticular wax. Hence, foliar anatomy can be considered an additional tool for taxonomic and evolutionary studies in *Alcantarea* and allied taxa.

*Acknowledgements* – This paper is part of the PhD thesis of the first author, Univ. of São Paulo. We acknowledge the financial support received from the Brazilian agencies FAPESP and CNPq. We thank Rachel Costa and Robert Smythe for linguistic advice.

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