

Foliar anatomy and micromorphology of southern South American Alstroemeriaceae: Alstroemerieae, and its systematic implications in *Alstroemeria*

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Alstroemerieae is an exclusively Central and South American tribe belonging to Alstroemeriaceae, which comprises two large genera, *Alstroemeria* and *Bomarea*. *Alstroemeria* has two areas of distribution, mediterranean Chile and central southeastern Brazil. Most *Bomarea* species grow in forests and hedges in moist areas, however, some species are adapted to dry Andean valleys and high altitudes. Previous leaf anatomical data were obtained from a limited group of species. To assess the value of the anatomical characters for the systematics and their importance as adaptations to different environments, we compared representative species from different geographical areas and habitats. Data regarding leaf anatomy and micromorphology were obtained from light microscopy and scanning electron microscopy and were combined with macromorphology for 27 Alstroemerieae species. In accordance with earlier studies, our results show variation in relation to several leaf morpho-anatomical characters. Based on these we define seven types. We furthermore analyzed the morpho-anatomical characters in a phylogenetic context. Morpho-anatomical characters are highly homoplastic within the family. Leaf anatomy may support monophyly of Baker's informal grouping of *Alstroemeria* Brazilian species with rigid leaves, however, a more thorough study of Brazilian *Alstroemeria* species are needed to confirm this.

An update of the Angiosperm Phylogeny Group classification (APG III 2009) considers Alstroemeriaceae Dumort. to include two tribes, Alstroemerieae Bernh. and Luzuriageae Benth. & Hook. The first one is an exclusively Central and South American tribe, which comprises two large genera, *Alstroemeria* L., including *Schickendantzia* Pax and *Taltalia* Ehr. Bayer, (ca 70 species) and *Bomarea* Mirb. (ca 120 species), including *Leontochir* Phil. Alstroemerieae species are long lived, perennial herbs, with the exception of *A. graminea* Phil., which was reported as annual by Bayer (1998). Alstroemerieae species have terminal umbel-like inflorescences, rarely unifloral, showy flowers, and thickened roots modified to store nutrients and water (Sanso and Xifreda 2001). Additionally, nearly all members of the tribe have resupinate leaves: the leaves are twisted near the base or along the lamina, placing the morphological adaxial surface as the functional lower surface. According to Napp-Zinn (1974), the inverse dorsiventrality in leaves of *Alstroemeria pelegrina* L. raised the attention of Treviranus as early as 1835. Some of the earlier descriptions of leaf anatomy of Alstroemerieae species (formerly Alstroemeriaceae)

are those of Haberlandt (1882) and Dufour (1886) on *Alstroemeria psittacina* Lehm.; Colozza (1898, 1901) and Schulze (1893) studied species of *Bomarea* and *Alstroemeria*; Scharf (1892), Goebel (1913) *Bomarea*; Kugler (1928) and Arber (1925) *A. aurea* Graham (= *A. aurantiaca* D. Don), and finally Sanso and Xifreda (1995) *B. edulis* (Tuss.) Herb. The most recent studies were made by Sanso and Xifreda (1998), by Lyshede (2002), mainly on Chilean *Alstroemeria* species and by Hofreiter and Lyshede (2006), mostly on Peruvian species of *Bomarea*.

The genus *Alstroemeria* has two main areas of distribution: Chile and central southeastern Brazil. Whereas Lyshede (l. c.) focused on twelve species from Chile, the present study includes *Alstroemeria* species from both geographical areas. The sister genus *Bomarea* is mainly distributed in the Andean mountains between Colombia and Bolivia with most species found in forests and hedges in moist areas (Hofreiter and Tillich 2002). However, some species of *Bomarea* subgenus *Wichuraea* are adapted to dry Andean valleys and high altitudes (Hofreiter and Tillich 2003). The present study includes representative species from these

geographical areas to examine the value of leaf anatomical and morphological characters for the systematics and the adaptations to different environments.

One of the first attempts to make an infrageneric *Alstroemeria* classification was published by Baker (1888), who distinguished two major groups: the Chilean and the Brazilian species. Further, in each of these two groups, the species were divided according to the leaf texture and morphology. This author (l. c.) circumscribed as an informal group twelve Brazilian *Alstroemeria* species with rigid leaves. On the other hand, Assis in her thesis (2001) divided the Brazilian species into 4 informal groups, three of these (groups 1–3) including species with more or less rigid leaves, but differentiated by presence or absence of fibres associated with the vascular bundles.

Phylogenetic studies based on DNA sequence data (Aagesen and Sanso 2003, Chacón et al. 2012) have confirmed the monophyly of *Alstroemeria* and *Bomarea* but failed to provide detailed resolution at subgeneric level. For this reason, an additional goal for us was to explore whether the morpho-anatomical foliar characteristics include qualitative traits that may contribute to resolve phylogenetic relationships within the genera.

Material and methods

Taxon sampling

Fully grown leaves were collected in the field. Those of Chilean origin were obtained from plants cultivated at the Copenhagen Botanical Garden (Denmark). When it was not possible to obtain living material, samples were taken from herbarium specimens.

In the anatomical analysis 23 *Alstroemeria* taxa, including *A. graminea* and *A. pygmaea* (previously included in the monotypic genera *Taltalia* and *Schickendantzia*) and 11 *Bomarea* species (including *B. ovallei*) were used. The studied taxa and their provenance are shown in the Table 1. For macromorphological considerations, observations made by Bayer (1987), Sanso and Xifreda (1995), Sanso (1996), Assis (2001), and Aagesen and Sanso (2003) were also taken into account.

Scanning electron microscopy

SEM of leaves were carried out at the SEM Service of La Plata Museum, Argentina. Lamina samples were mounted

Table 1. Taxa and origin of plant material used in this study. * = only SEM data; + = species analyzed for the first time.

Taxon	Origin	Voucher
<i>Alstroemeria andina</i> Phil. subsp. <i>andina</i> *+	Chile	Castillo w/n (SGO 100086)
<i>Alstroemeria andina</i> Phil. subsp. <i>venustula</i> (Phil.) M. Muñoz+	Argentina	Kiesling 9062 (SI), Fortunato and Kiesling 5630 (BAB)
<i>Alstroemeria angustifolia</i> Herb. subsp. <i>angustifolia</i>	Chile	Cult. Copenhagen Botanical Garden P1995-5003 (SI)
<i>Alstroemeria apertiflora</i> Baker+	Paraguay	Hassler 5811, 10099 (G)
<i>Alstroemeria aurea</i> Graham	Argentina	Xifreda and Sanso 2015, 2030 (SI)
<i>Alstroemeria exserens</i> Meyen	Chile	Cult. Copenhagen Botanical Garden P1995-5009 (SI)
<i>Alstroemeria graminea</i> Phil.*+	Chile	von Bohlen 1275 (SGO 131118)
<i>Alstroemeria hookeri</i> Lodd. subsp. <i>hookeri</i>	Chile	Cult. Copenhagen Botanical Garden P1995-5010 (SI)
<i>Alstroemeria isabellana</i> Herb.+	Argentina	Sanso et al. 5 (SI)
<i>Alstroemeria ligtu</i> L. subsp. <i>ligtu</i>	Chile	Cult. Copenhagen Botanical Garden P1995-5026 (SI)
<i>Alstroemeria magnifica</i> Herb. subsp. <i>magnifica</i>	Chile	Cult. Copenhagen Botanical Garden P1995-5013 (SI)
<i>Alstroemeria pallida</i> Graham	Chile	Cult. Copenhagen Botanical Garden P1995-5034 (SI)
<i>Alstroemeria patagonica</i> Phil.+	Argentina	Xifreda and Sanso 2035 (SI)
<i>Alstroemeria pelegriana</i> L.	Chile	Garaventa 3262, 4196 (SI)
<i>Alstroemeria presliana</i> Herb. subsp. <i>presliana</i>	Argentina	Xifreda and Sanso 2012 (SI)
<i>Alstroemeria pseudospathulata</i> Ehr. Bayer+	Argentina	Xifreda and Sanso 2004 (SI)
<i>Alstroemeria psittacina</i> Lehm.	Argentina	Cult. FCEN, University of Buenos Aires (SI)
<i>Alstroemeria pulchra</i> Sims subsp. <i>pulchra</i>	Chile	Cult. Copenhagen Botanical Garden P1995-5046 (SI)
<i>Alstroemeria pygmaea</i> Herb.+	Argentina	Sanso and Pereyra 8 (SI)
<i>Alstroemeria revoluta</i> Ruiz & Pav.+	Chile	Cult. Copenhagen Botanical Garden P1995-5049 (SI)
<i>Alstroemeria sellowiana</i> Seub.+	Brazil	Kummron 3357 (ESA)
<i>Alstroemeria versicolor</i> Ruiz & Pav.	Chile	Cult. Copenhagen Botanical Garden P1995-5052 (SI)
<i>Alstroemeria werdermanii</i> Ehr. Bayer+	Chile	Cult. Copenhagen Botanical Garden P1995-5054 (SI)
<i>Bomarea andimarcana</i> (Herb.) Baker	Peru	Sanso and Pereyra 26, Sanso and Pereyra 27 (SI)
<i>Bomarea boliviensis</i> Baker+	Argentina	Cult. Hunziker 22 (SI)
<i>Bomarea dulcis</i> (Hook.) Beauverd	Peru	Sanso and Pereyra 21 (SI)
<i>Bomarea edulis</i> (Tuss.) Herb.+	Argentina	Xifreda and Sanso 867, Sanso and Pereyra 9 (SI)
<i>Bomarea glaucescens</i> (H.B.V.) Baker*+	Ecuador	Lehmann 499 (K)
<i>Bomarea macrocephala</i> Pax+	Argentina	Sanso and Pereyra 11 (SI)
<i>Bomarea ovallei</i> (Phil.) Ravenna	Chile	Macaya Berti (w/n) (SI), Ricardo w/n (SGO 73065)
<i>Bomarea ovata</i> (Cav.) Mirb.+	Peru	Sanso and Pereyra 22 (SI)
<i>Bomarea salsilla</i> (L.) Herb.*+	Chile	Silva w/n (SGO 104724)
<i>Bomarea setacea</i> (Ruiz & Pav.) Herb.*	Colombia	Hunziker 11407 (SI)
<i>Bomarea stans</i> Kraenz.*+	Argentina	Kiesling et al. 3767 (SI)

onto SEM stubs using double-sided adhesive tape, coated with gold using a Jeol JFC-1100 ion sputter coater and subsequently examined in a Jeol JSM-T100 SEM.

Light microscopy

Material for LM were fixed in 3% glutaraldehyde in 0.25 M phosphate buffer (pH 6.8) for 24 h, dehydrated in a graded ethanol series and acetone for 2 h in each step and embedded in Spurr's resin. Transverse sections (TS) of leaf laminae of 1 μm or 2 μm thickness were stained with Toluidine blue (O'Brien and McCully 1981), as metachromatic stain and Ruthenium red (Luft 1971). Epidermal samples were stripped from the leaf, stained with Toluidine blue and mounted in glycerol jelly.

Phylogeny

Selected qualitative morpho-anatomical characters analysed in the present study (Appendix 1) were added to the DNA sequence data set published by Chacón et al. (2012). Species for which voucher material was lacking were coded according to information found in the literature, mainly Assis (2001, 2009). We extracted sequences of *matK*, *matR*, *ndhF*, and *rbcL* from GenBank (<www.ncbi.nlm.nih.gov>) (Supplementary material Appendix A1). The matrix was assembled with the program GB-to-TNT (Goloboff and Catalano 2012) calling Mafft for the alignment step (Katoh and Toh 2008). The combined matrix was analyzed in TNT ver. 1.1 (Goloboff et al. 2003), searching for the most parsimonious trees and branch supports with gaps coded as missing data.

Results

Micromorphology

Epidermal features observed with SEM showed variation in relation to the pubescence, cell shape, sinuosity of anticlinal cell walls, orientation pattern and type of wax particles. In particular, regarding this last point, new data were obtained for *Bomarea* species.

Alstroemerieae leaves are glabrous or with indumenta, sometimes trichomes on the ribs (Fig. 1A). Midvein protruding adaxially, but in some species, ribs protruding on both surfaces (Fig. 1B). In paradermal view, adaxial cells longitudinally elongated (Fig. 1C) or intercostal ones asymmetrical, like a jigsaw puzzle (Fig. 1D), and with more wax than those on the abaxial surface, with or without sinuous anticlinal walls (Fig. 1E). Stomata are anomocytic in parallel rows (Fig. 1F). Abaxial cells are more or less rectangular with straight or undulating anticlinal walls.

Distinctive epicuticular waxes form parallel rows of longitudinally aligned platelets, in regular (Fig. 1G) or occasionally random orientation (Fig. 1H–I), in characteristic patterns around the stomata, sometimes, radiating around them (Fig. 2A). Wax particles of different shapes and sizes, from flakes to filaments and granules. In some species the two surfaces have different types of wax (*A. pygmaea*, *A. patagonica*); adaxial epidermis with a granular, warty layer

(Fig. 2B); in other ones, warty coating composed of crust-like particles (*B. stans*, Fig. 2C), dense wax deposit, consisting of rods or threads randomly orientated and superimposing (*B. salsilla*, Fig. 2D), irregular scales arranged in upright angles with random orientation (*B. setacea*, Fig. 2E) or, concave, plate-like wax with cuticle areas free of wax (*B. dulcis*, Fig. 2F).

Stomatal guard cells are with wax particles (Fig. 2A) but in *A. magnifica* subsp. *magnifica*, without them. Stomatal rim raised, aperture long and narrow (Fig. 2G). Stomata slightly sunken and surrounded by curved ridges of cuticle on the subsidiary cells (Fig. 2C) but in species as those of the Brazilian group, the curved ridges are not outstanding (Fig. 2G–H).

Anatomy

Resupination

Leaf anatomy is inverted, in nearly all Alstroemerieae species, with stomata and indument, when present on only one side, at the morphological adaxial surface (indicated by the position of vascular tissues in TS). In species having stomata on both surfaces, the pattern is recognizable by the protuberance of main nerves or the hooded apex, both characters for the adaxial surface.

Lamina thickness

Lamina thickness between midrib and margin in cross-section ranges from 100 (*A. psittacina*, 4 mesophyll cell layers) to 620 μm (*A. andina* subsp. *venustula*, 9–10 layers). The thickest lamina was observed in *A. andina* subsp. *venustula*, *A. patagonica*, *A. pseudospathulata* and *A. werdermannii*.

Epidermis

Single cell layer. In *Alstroemeria* adaxial and abaxial epidermal cells are of equal height, except in *A. pelegriana* (Fig. 4G) and *A. pulchra*. In most *Bomarea* species, the abaxial epidermis is more developed. Adaxial cells are generally taller above the midrib (Fig. 5H), and in extreme cases, distinctly large above vascular bundles partly hiding intercostal areas (Fig. 4G); cell walls of variable thickness, with irregular or smooth borders. Abaxial outer cell walls are thickened; in *Bomarea* subg. *Wichuraea*, very thick and convex, whereas adaxial outer cell walls are relatively thin (Fig. 3H).

Anomocytic stomata. In *Bomarea* generally restricted to the adaxial side; in *Alstroemeria*, on both surfaces (amphistomatic leaves, *A. andina* subsp. *venustula*, *A. patagonica*), but fewer stomata on the abaxial surface or only on the adaxial. More or less sunken stomata, except in *A. psittacina* (Fig. 4H), with an outer cuticular rim and thick-walled guard cells (Fig. 3A) or minute/absent outer and inner rim with very thick-walled stomatal apparatus cells (Fig. 3B). In *Bomarea* each pair of guard cells is surrounded by subsidiary cells, very thick cuticular ridges, as hood-like projections on the guard cells, in the boundary with the ostiole; cuticular layer continued on the inner tangential walls, in contact with a very wide substomatic chamber.

Mesophyll distribution

Dorsiventral laminae in all investigated species of *Bomarea* and most *Alstroemeria* taxa, but in some Chilean–Argentinian

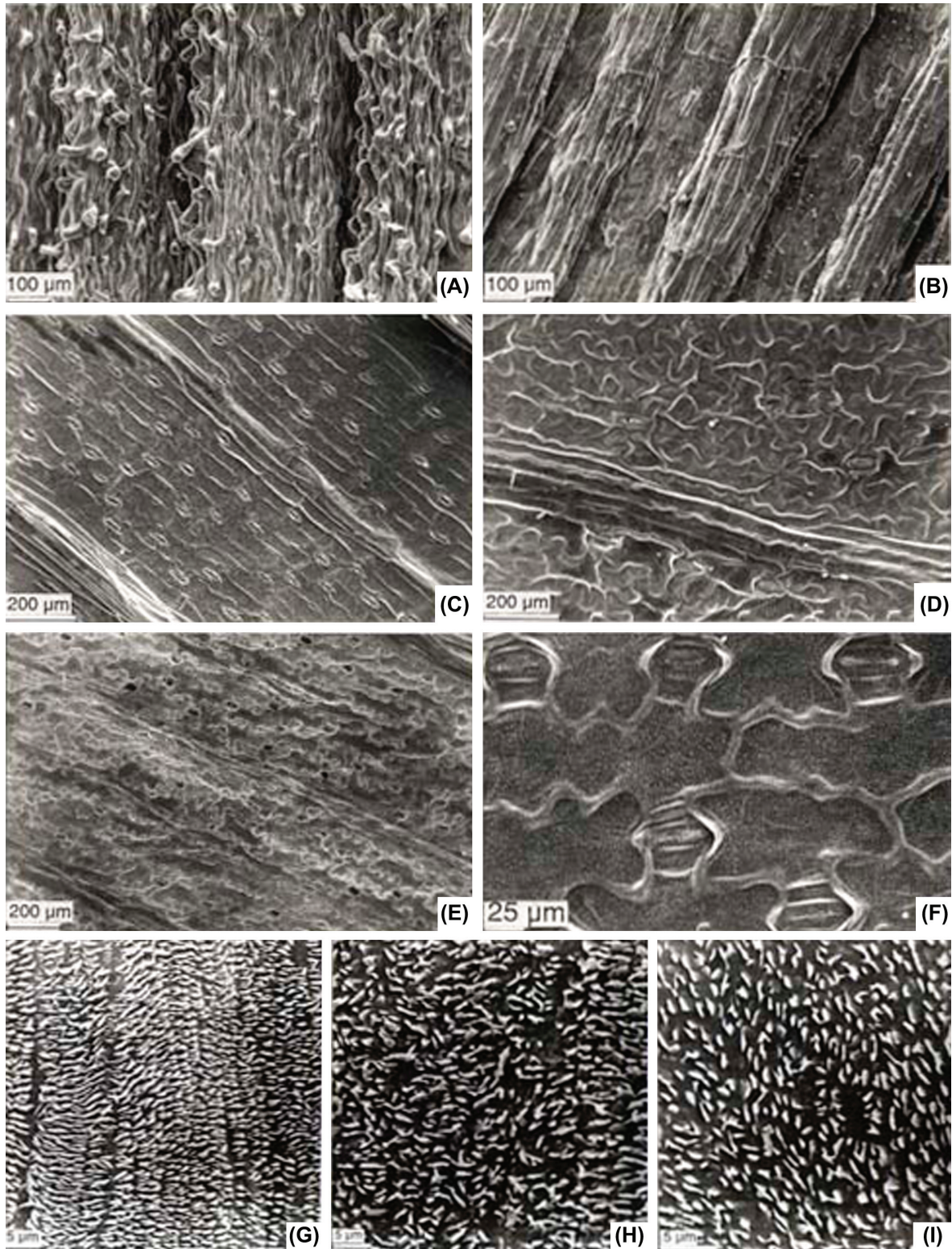


Figure 1. Micromorphological characters in Alstroemerieae. Surface cells and waxes. Electron micrographs (SEM). (A)–(B) *B. macrocephala*; (A) adaxial surface, trichomes abundantly distributed mostly on leaf ribs; (B) abaxial surface, glabrous and with veins protruding also on this leaf surface. (C) *A. isabellana*, abaxial cells arranged in paralleled rows. (D)–(E) cell walls with undulating outline, (D) *A. psittacina*, (E) *A. aurea*. (F) *B. stans*, anomocytic stomata arranged in paralleled rows. (G) *A. revoluta*, short wax striations with regular orientation. (H)–(I) wax platelets with random orientation. (H) *A. ligtu* subsp. *ligtu*, (I) *A. magnifica* subsp. *magnifica*.

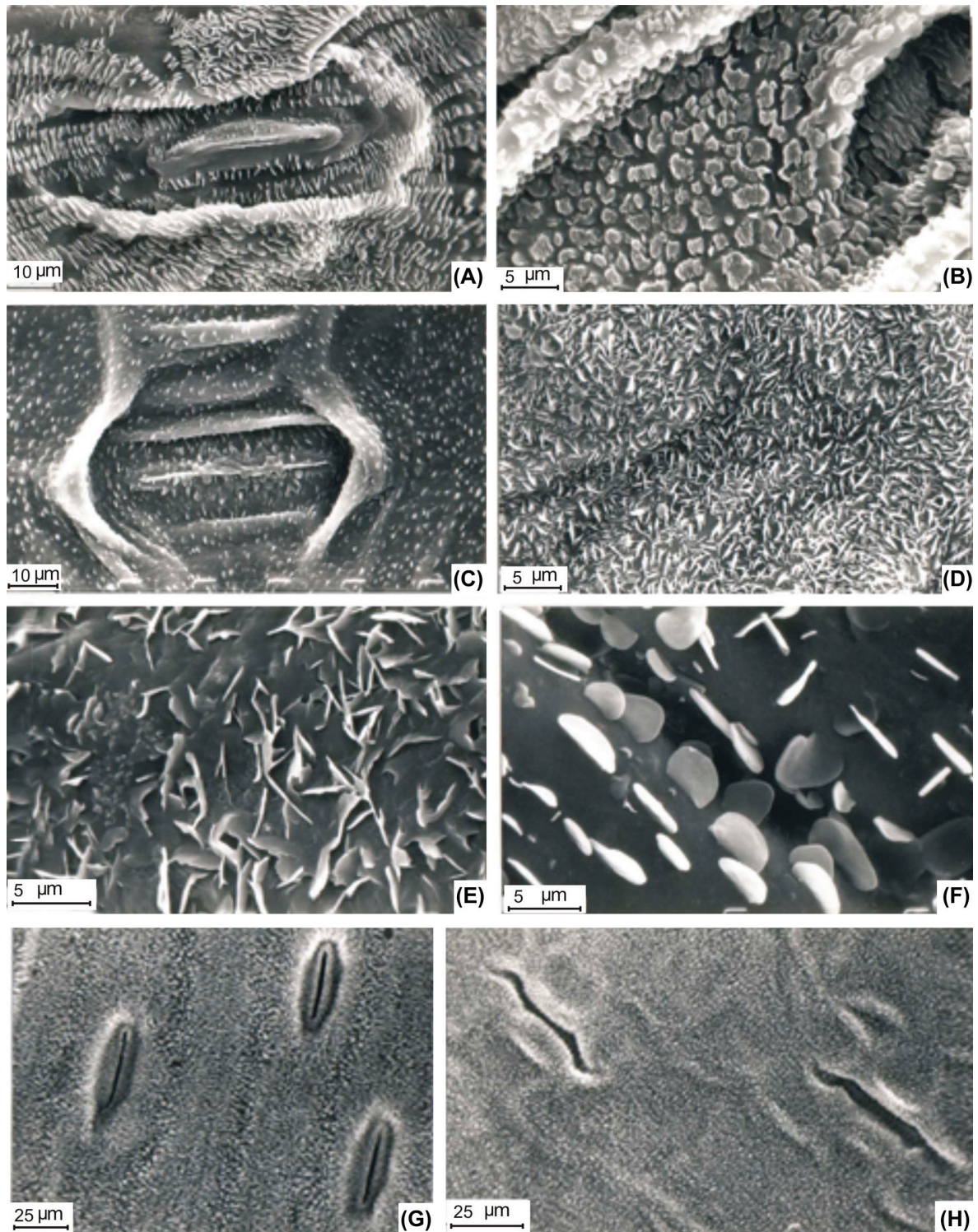


Figure 2. Micromorphological characters in Alstroemerieae. Wax arrangement pattern. Electron micrographs (SEM). (A) *B. macrocephala*, wax particles in characteristic pattern, radiating around the stoma. (B) *A. pygmaea*, adaxial surface with granular warty layer. (C) *B. stans*, sunken stoma with crustose wax particles. (D) *B. salsilla*, dense wax deposit consisting in rods or threads randomly orientated. (E) *B. setacea*, irregular scales arranged in upright angles with random orientation. (F) *B. dulcis*, concave plate-like particles. (G) *A. apertiflora*, stomata almost superficial without curved ridges. (H) *A. isabellana*, sunken stomata without curved ridges.

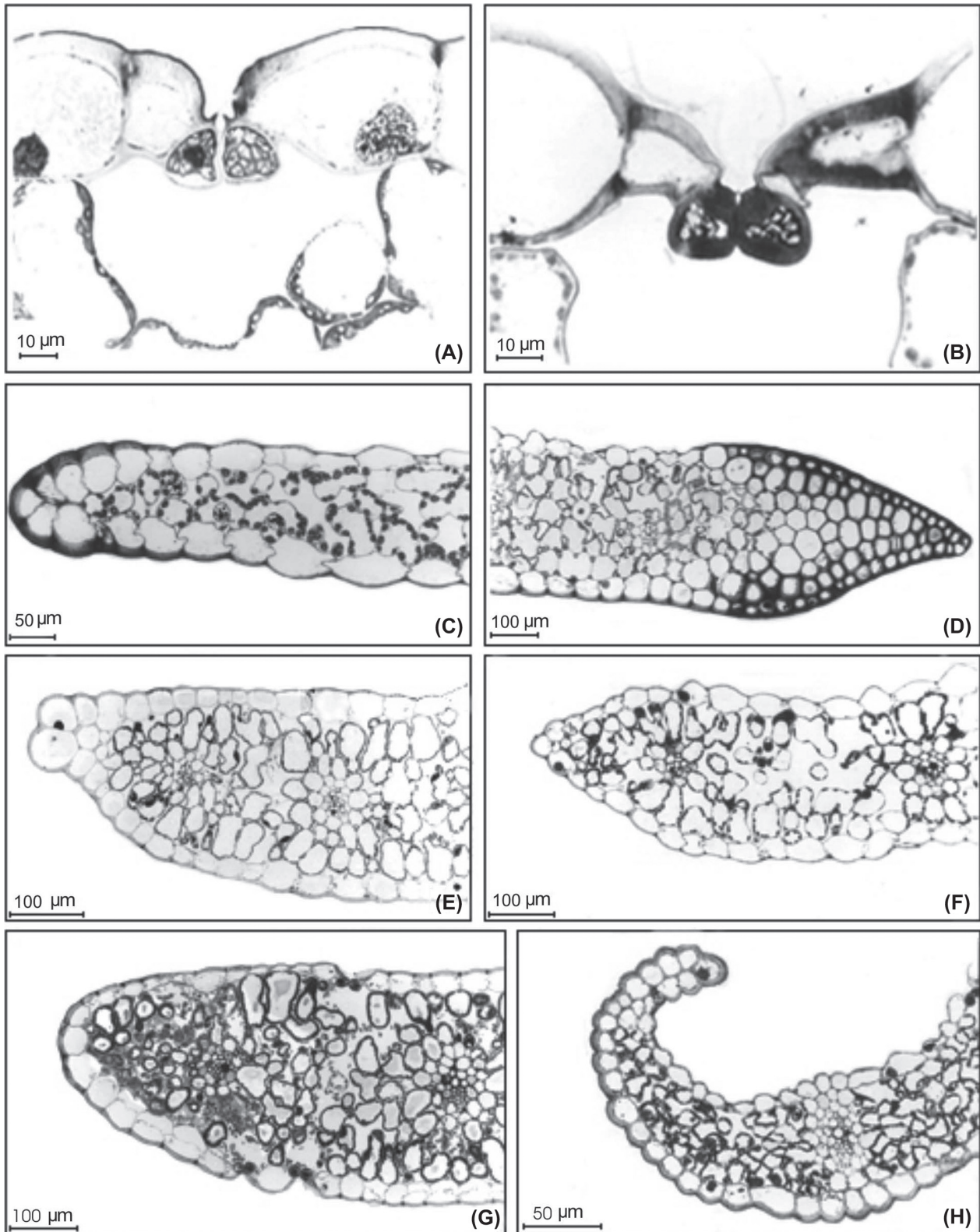


Figure 3. Stomata and leaf margin in Alstroemerieae. TS at light microscopy. (A) *A. isabellana*. stoma with outer cuticular rim and guard cells with thick walls. (B) *A. andina* subsp. *venustula*, stoma with minute outer rim cuticular ridges and very thickened walled stomatal apparatus cells. (C) *A. psittacina*, rounded margin with around 9 to 10 cells of the apex presenting outer cell walls thickened. (D) *A. isabellana*, fusiform margin, edges thickened with one to two layer of around 20 smaller and schlerenchymatous cells occurring on both epidermis sides. (E) *A. angustifolia* subsp. *angustifolia*, leaf margin blunty shaped by tiers of two or three large cells. (F) *A. hookeri* subsp. *hookeri*, blunty shaped apex. (G) *A. versicolor*, rounded margin. (H) *B. macrocephala*, involute leaf margin finishing in two and finally one cell.

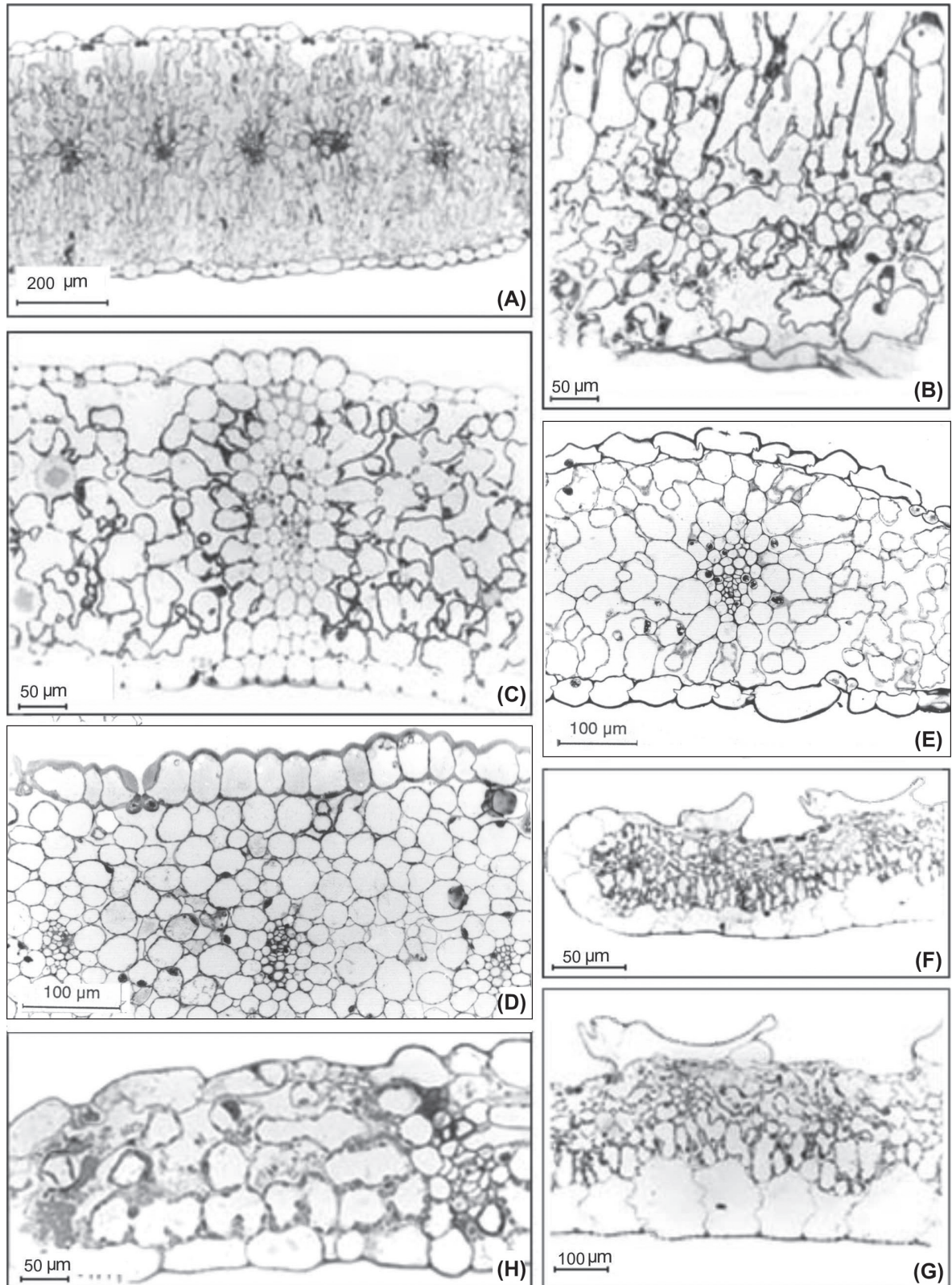


Figure 4. Morpho-anatomical types in Alstroemerieae. TS at light microscopy. (A) *A. andina* subsp. *venustula*, slightly dorsiventral and wide lamina. (B) *A. pseudospathulata*, mesophyll with conspicuous lobed cells. (C) *A. pygmaea*, chlorenchyma with cells fairly irregular in shape. (D) *A. patagonica*, rather compact chlorenchyma tissue, composed of small, isodiametric cells and a vascular bundle. (E) *A. isabellana*, mesophyll poorly differentiate with cells irregularly shaped and large intercellular spaces. (F)–(G) *A. pelegrina*, dorsiventral lamina with one layered palisade mesophyll contiguous the abaxial surface. (G) adaxial surface with extremely large epidermal cells on the vascular bundle. (H) *A. psittacina*, thin mesophyll with one palisade layer occurring beneath the abaxial surface, some of these cells bone-like shaped.

ones, such as *A. patagonica*, and some Brazilian species with rigid leaves, such as *A. isabellana*, the mesophyll is only slightly differentiated; in *A. patagonica* tightly packed, circular to oval, thin-walled cells and abundant chloroplasts with mostly small, triangular intercellular spaces were observed (Fig. 4D); in *A. isabellana* mesophyll not compact, irregularly shaped cells with rather conspicuous intercellular spaces (Fig. 4C). Palisade cells rectangular, as in *A. hookeri* subsp. *hookeri* (Fig. 5C), or multi-lobed, as in *A. psittacina* (Fig. 4H). In *A. pseudospathulata*, arrangement of the mesophyll basically bifacial, tending to an intermediate condition, with the addition of discontinuous palisade on the abaxial side (Fig. 4B). Cells of spongy parenchyma difficult to distinguish from the palisade ones, irregularly shaped and vertically oriented, nevertheless, on the phloem side, cells less lobed and more closely packed.

Vascular tissue

Closed vascular bundles, oval in shape, collateral, organized centrally in the mesophyll as a single row, and usually surrounded by a distinct parenchymatous sheath. In *A. andina* subsp. *venustula*, sheath with elongated cells radially arranged (Fig. 4A); in *Bomarea* (Fig. 5G) and *Alstroemeria* Brazilian taxa (Fig. 4C), associated with sclerenchyma.

Leaf margin

Leaves dorsiventrally flattened, with rounded (Fig. 3C) to acute margins (Fig. 3D). In *A. psittacina*, rounded margin, 9–10 cells with thickened outer walls; in *A. isabellana*, fusiform margin, with edges thickened and stramineous, 1–2 layers of about 20 cells, one smaller and sclerenchymatous (Fig. 3D); in *A. angustifolia* subsp. *angustifolia* (Fig. 3E) and *A. hookeri* subsp. *hookeri* (Fig. 3F), margins blunt and shaped by tiers of 2–3 large cells; in *A. ligtu*, *A. pelegrina*, *A. exserens* and *A. versicolor* (Fig. 3G), rounded margins; in *B. edulis*, cells with thickened outer and lateral walls and in *B. macrocephala*, involute margins (Fig. 3H), cuticle thicker in the margin, with the epidermal cells often distinctly domed.

Indument

Alstroemeria leaves generally glabrous but sometimes with papillae covering the whole surface (adaxial surface of *A. andina* subsp. *venustula*), only the margins or the apex (some Brazilian *Alstroemeria* species with rigid leaves, *A. apertiflora*, *A. isabellana*, *A. sellowiana*). *Bomarea* leaves with or without hairs (*B. ovallei*, *B. salsilla*), finely pubescent (*B. boliviensis*, *B. edulis*, *B. macrocephala*) to densely pilose (*B. dulcis*, *B. ovata*), trichomes hyaline, unbranched, uniseriate, uni- to multi-cellular, 1–4 to 10-celled.

Morpho-anatomical types

Seven morpho-anatomical types based on leaf characters were defined in accordance with the studied material. Major histological features of these types are described below.

1) *Alstroemeria andina* group. *A. andina* subsp. *venustula* (Fig. 4A), *A. pseudospathulata* (Fig. 4B), *A. werdermannii*

Very thick leaves, maximum intercostal width from 400 (*A. pseudospathulata*) to 620 μm (*A. andina* subsp. *venustula*)

or 800 μm in vascular bundles (*A. pseudospathulata*). Epidermal cells elongated along the leaf short axis or isodiametric (30–40 μm). Cuticle thick to very thick (*A. pseudospathulata*, 10 μm) on both surfaces. Stomata sunken to deeply sunken (*A. andina* subsp. *venustula*), almost exclusively or more densely distributed on the xylem side and smaller or absent on the other surface. Papillae on adaxial surface in *A. andina* subsp. *venustula*. Laminas slightly dorsiventral, with conspicuously lobed cells in the mesophyll (8–9 layers).

2) *A. isabellana* group. *A. isabellana* (Fig. 4C), *A. apertiflora*, *A. sellowiana*

Leaves coriaceous. Lamina thickness from 300 to 420 μm , or ca 520 μm in the main vascular bundle (*A. isabellana*); thinner lamina in *A. apertiflora*, 160–180 μm . Abaxial epidermal cells isodiametric (30–40 μm); adaxial, less regular in size and larger on the midrib. Cuticle thin. Stomata on both surfaces, but more numerous on the adaxial one. Mesophyll only slightly differentiated into palisade (one layer on abaxial side) and spongy parenchyma, with cells irregularly shaped and large intercellular spaces. Sclerenchyma surrounding the vascular bundles, as girders from the vascular bundle sheaths towards both epidermises, except in some minor ones.

3) *A. patagonica* group. *A. patagonica* (Fig. 4D), *A. pygmaea* (Fig. 4E)

Laminas equifacial, without differentiation between palisade and spongy mesophyll. Epidermal cells of both sides equal in size; sunken stomata, less frequent on abaxial side. Cuticle thick in *A. patagonica*, less so in *A. pygmaea*. Chlorenchyma tissue rather compact and composed of small, isodiametric cells (*A. patagonica*, 9–13 layers) or of fairly irregular cells, with intercellular spaces (*A. pygmaea*, 5–6 layers). Mechanical tissues absent.

4) *A. pelegrina* group. *A. pelegrina* (Fig. 4F–G), *A. pulchra*

Laminas dorsiventral, one-layered palisade mesophyll contiguous on the abaxial surface, with thin spongy mesophyll (4–5 layers). Abaxial epidermis with large cells, uniform in size (60–70 μm high), without stomata. Adaxial epidermis with flat cells in the intercostals areas; above veins characteristically thin walled, free of chloroplasts; bulliform cells (Fig. 4G), in *A. pelegrina*, 10–15 times larger than the rest of the epidermal cells (50–60 μm height). Stomata slightly sunken.

5) *A. psittacina* group. *A. psittacina* (Fig. 4H), *A. angustifolia* subsp. *angustifolia*, *A. aurea* (Fig. 5A), *A. exserens* (Fig. 5B), *A. hookeri* subsp. *hookeri* (Fig. 5C), *A. ligtu* subsp. *ligtu*, *A. magnifica* subsp. *magnifica*, *A. pallida*, *A. presliana* subsp. *presliana*, *A. revoluta*, *A. versicolor* (Fig. 5D)

Leaves membranaceous, widest in *A. angustifolia* subsp. *angustifolia* and *A. versicolor*; thinnest in *A. psittacina* (100–150 μm). Mesophyll of 3–7 layers, one cell layer regular and lined up contiguously to one or both epidermises. In *A. psittacina*, a palisade layer towards abaxial epidermis with bone shape-like cells and high density of chloroplasts (Fig. 4H). Spongy mesophyll with rounded and irregular cells and intercellular spaces, quite dense (*A. revolute*) or with

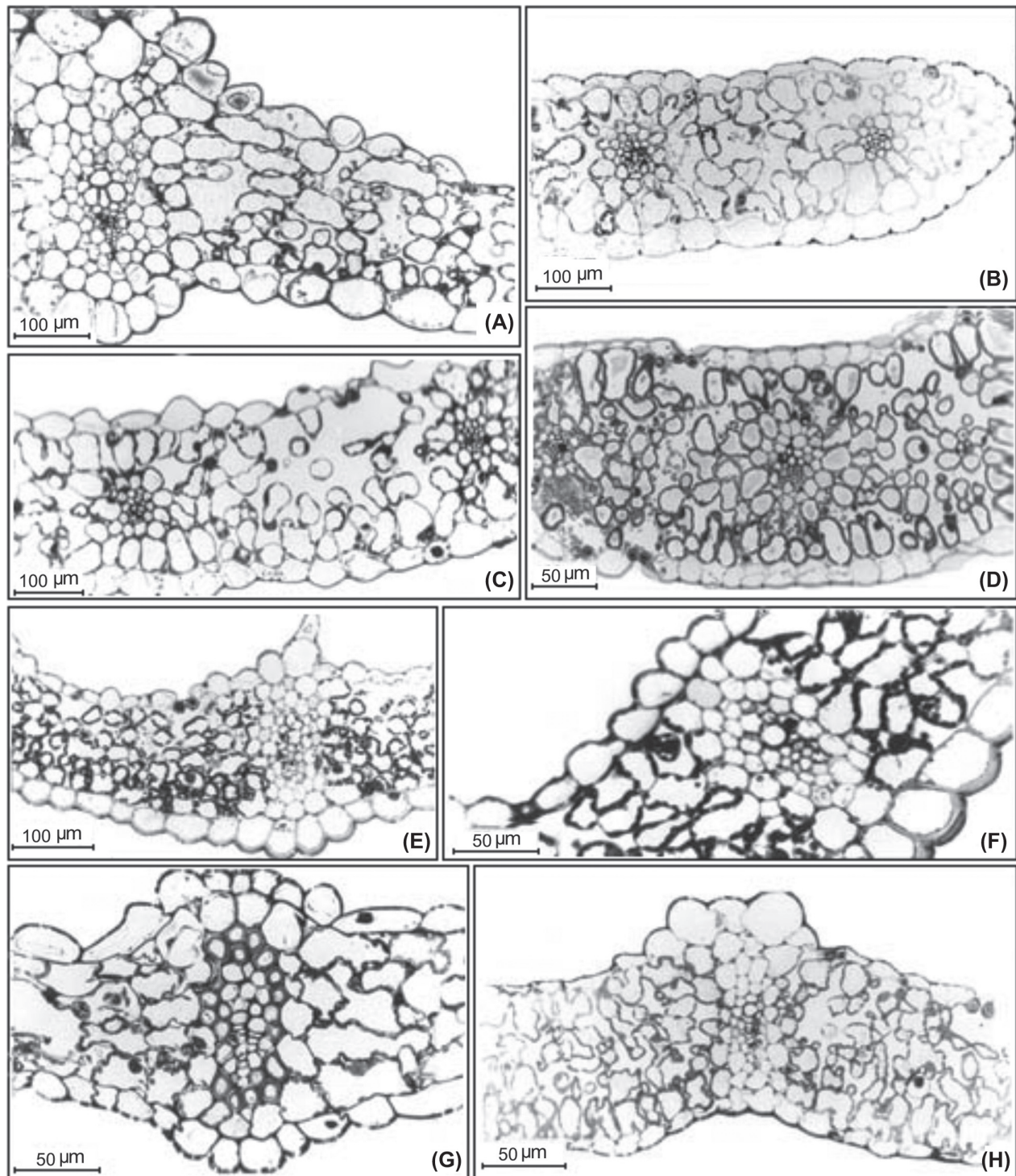


Figure 5. Morpho-anatomical types in Alstroemerieae. TS at light microscopy. (A) *A. aurea*, on the left, midrib with larger epidermal cells above it. (B) *A. exserens*, mesophyll slightly dorsiventral and conspicuous intercellular spaces. (C) *A. hookeri* subsp. *hookeri*, mesophyll with important intercellular spaces. (D) *A. versicolor*, some mesophyll cells regular and lined up contiguous to each epidermis side and remaining ones, isodiametric and irregularly distributed. (E)–(F) *B. macrocephala*, dorsiventral lamina, the adaxial surface with sunken stomata and a trichome. (F) detail of a vascular bundle; abaxial surface with thick cuticle. (G) *B. edulis*, principal vascular bundle surrounded by a schlerenchymatous sheath. (H) *B. boliviensis*, slightly dorsiventral lamina showing the principal vascular bundle with larger epidermal cells above it.

more intercellular spaces (*A. aurea*, *A. hookeri* subsp. *hookeri* (Fig. 5A, C). Sunken stomata on both/adaxial side or superficial stomata on one side (*A. psittacina*).

6) *Bomarea macrocephala* group. *B. macrocephala* (Fig. 5E–F), *B. andimarcana*, *B. dulcis*

Involute and dorsiventral laminas. Epidermal cells elongated along the short axis of the leaf, with undulating anticlinal walls, larger on the veins. Adaxial surface pubescent, with sunken stomata; abaxial, glabrous or pubescent; trichomes more abundant on the veins (Fig. 5E), very thick cuticle, paradermal cell walls thickened (up to 14 µm) (Fig. 5F). Mesophyll of 5–10 layers, maximum width 300 µm (*B. andimarcana*); cells arrangement more compact on the phloem side, forming a palisade layer. Sclerenchymatous sheaths composed of one or several cell layers.

7) *B. edulis* group. *B. edulis* (Fig. 5G), *B. boliviensis* (Fig. 5H), *B. ovallei*

Laminas equifacial or slightly dorsiventral, from 210 (*B. edulis*) to 320 µm (*B. ovallei*) or 600 µm in the major vascular bundle (*B. boliviensis*). Epidermal cells with thin cuticle, and slightly sunken stomata on adaxial side. Spongy mesophyll with rounded or irregular cells (4–5 layers) and intercellular spaces; palisade mesophyll contiguous to the abaxial epidermis, of bone-like or lobed cells (Fig. 5H). Vascular bundles surrounded by parenchymatous sheaths, the larger bundle, by sclerenchymatous cells.

Phylogeny and taxonomical considerations

The qualitative morpho-anatomical character matrix is found in Supplementary material Appendix A2. Note, only species for which DNA sequence data was available could be included in the phylogenetic analysis, hence *Alstroemeria pulchra* and *Bomarea andimarcana* were excluded. The final matrix included 119 taxa and 13 morpho-anatomical characters. The combined data set yielded more than 10 000 optimal trees (length = 1021, CI = 0.71). Figure 6 shows a selected optimal tree with branch support and branches that are collapsed in the strict consensus tree. With the present species sampling available for morpho-anatomical analyses the fraction of missing data is high, and the morpho-anatomical characters add limited structure at subgeneric level. None of the morpho-anatomical types formed monophyletic groups, and most characters may have evolved several times within the family. The Brazilian species with rigid leaf (species group I and III, Assis 2001) do not form a monophyletic group (Fig. 6). However, only species from group III were examined in the present study, and these were found to be monophyletic with the exception of *A. longistyla* (for which voucher material was not available). Presence of sclerenchyma in the vascular bundle sheaths (character 12) and sclerenchyma in bundle sheaths extended as girders from the vascular bundles towards both leaf surfaces (character 13) define the Brazilian species with rigid leaves. Both are well defined qualitative characters, which may show to support monophyly of the Brazilian species with rigid leaves (species group I + III) if coding of these characters are completed among the Brazilian *Alstroemeria* species.

Discussion

In this comparative study on representative species of the Alstroemeriaceae, Alstroemerieae showed variation in relation to several morpho-anatomical characters of the leaf, confirming previous results obtained mainly by Lyshede (2002), but also by Xifreda and Sanso (1998).

Papillae were found in few species of *Alstroemeria*; very inconspicuous ones at the apex in *A. apertiflora*, *A. isabellana* and *A. sellowiana*, and more visible ones in the trans-Andean *A. andina* subsp. *venustula*. According to Assis (2001) other Brazilian species with papillae are: *A. foliosa*, *A. speciosa* and *A. viridiflora*. On the contrary, trichomes are widespread in *Bomarea*.

The epicuticular wax arrangement and the orientation of parallel platelets found in most of the studied species have been described as ‘Convallaria-type’ (Barthlott and Frolich 1983). Flat crystalloids with entire margin and regular shape, ‘Entire platelets type’ (Barthlott et al. 1998) were exclusively found in some *Bomarea* species, e.g. *B. dulcis*. Epicuticular wax may appear with an upright orientation or in different angles from 0° to 90°. Orientation and distribution of the particles modify the capacity to scatter light. Also, contrary to traditional thoughts, the cuticle's thickness and overall wax load do not control water evaporation. Instead, thick cuticles probably play a much more general, structural, and protective role (Kerstiens 1996).

Leaf anatomy and epicuticular wax of the *B. setacea* complex were studied by Hofreiter (2006). Some differences found between his and our results, such as the lack of epicuticular wax in both leaf surfaces (Hofreiter l. c., Hofreiter and Lyshede 2006) or the presence of irregular scales arranged in upright angles (this paper), may be the consequence of his more narrow species.

Amphistomatous leaves are frequently associated with plants growing under dry conditions, but the primary relationship is probably with increased leaf thickness, for reasons concerned with optimized gas exchange under water stress (Parkhurst 1978). Correlated with this, the thickest laminas among the studied species have developed in parallel in *A. andina* subsp. *venustula*, which inhabits stony or sandy slopes of the Andean mountains, *A. patagonica* and *A. pseudospathulata* from the Patagonian meseta, and *A. werdermannii* from the Atacama desert, all environments with pronounced water shortage.

Bulliform cells are larger than the typical epidermal cells, thin-walled, and with a large vacuole, placed only on the veins. They were observed in ‘*A. pelegriana*-type’ species and previously by Lyshede (l. c.) but without using the present denomination. Similarly, Rudall (1994) described a small groups of enlarged, bulliform cells in distinct regions of the leaf epidermis, or over larger vascular bundles in Iridaceae. As pointed out by Fahn (1990) they may have a functional significance in relation to the involution of leaves, a characteristic of xerophytes, as result of their loss or uptake of water by hydrostatic movements.

The palisade mesophyll commonly comprises round cells, but sometimes presents deeply lobed cells appearing branched (*A. andina* subsp. *venustula*), or with intermediate forms (*A. psittacina*). Rudall et al. (1986) recorded them in



Figure 6. A selected optimal tree. The numbers above the branches are jackknife values from 10000 replicates, those below the branches are Bremer supports. Grey branches are collapsed in the strict consensus tree. The numbers in the column showing infrageneric divisions refer to formal and informal divisions discussed in the text. The numbers in the column showing morpho-anatomical types refer to the grouping discussed in the text. Habitats are recorded for species in Alstroemeriaceae, following the biomes of Olson et al. (2001).

Sisyrinchium (Iridaceae) and considered that the adaptive advantages of this mesophyll type were unknown. However, Fahn (1990), supposed that shape and arrangement of these specialized mesophyll cells increase the internal surface area of the leaf and could result in a more efficient photosynthesis.

Neither *A. pygmaea*, *A. graminea* nor *B. ovallei* present unique foliar character states. Thus, to all existing arguments for not retaining *Schickendantzia*, *Taltalia* nor *Leontochir* as separate genera (Sanso and Xifreda 1999, 2001, Sanso 2002, Agesen and Sanso 2003), we can add the morpho-anatomical foliar group of characters.

Our data would support the group including species of *Bomarea* subgenus *Wichuraea* ('*Bomarea macrocephala*-type'). However, phylogenetic analyses based on DNA sequence data (Fig. 6 and Alzate et al. 2008, Chacón et al. 2012) do not recover clades that represent the recognized subgenera of *Bomarea* (Baker 1888, Hofreiter and Tillich 2002). *Bomarea* subgenus *Wichuraea* species, have lanceolate to linear leaves that are sometimes small and closely appressed to the stem, ascending, marcescent at anthesis, with involute margins, which determine an adaxial funnel (kennel), and have a high degree of uniformity in relation with the leaf anatomy. All *Wichuraea* grow in full sun, only some of the climbing species may occur in light shadow inside the fog forests (Hofreiter and Tillich 2003) in high mountains. Perhaps as a consequence of habitat, this group presents leaf features that might be regarded as xeromorphic according to Fahn and Cutler (1992), e.g. with a reduced leaf area, strongly involute margins, and on the adaxial epidermis with outer cell walls that are conspicuously convex (domed) in transversal sections. The occurrence of sclerenchyma could also be interpreted as an adaptation to xeromorphy. However, this character needs to be interpreted with care in a broad survey of *Bomarea* species.

Assis (2001) assembled Brazilian species with rigid leaves and sclerenchyma in vascular bundles in two groups, the '*A. gardneri* group' and the '*A. longistyla* group' species, group I and III, respectively. With the present character coding leaf morpho-anatomical data do not support the monophyly of the Brazilian species group with rigid leaves (here the '*Alstroemeria isabellana*-type'). However, only species from group III were examined in the present study. According to Assis (l. c) both groups have an extension of the sclerenchymatous sheaths, but the second group possess fewer fibres than the first. Coding presence of sclerenchyma in the vascular bundle sheath and extension of the sclerenchymatous sheaths (character 12 and 13) in all species of groups I and III proposed by Assis (2001) would render these groups monophyletic (results not shown). The species are distributed in marshy regions of the neotropical savannas (group III) and in the Cerrado (group I). According to the dated phylogeny in Chacón et al. (2012) the species with rigid leaves diverged from other *Alstroemeria* species less than 10 Ma years ago, towards the end of a phase of global cooling, in agreement with the estimated age of other Cerrado lineages (Simon et al. 2009). The distinctive anatomy in *A. isabellana* and other species from its group, showing hygromorphic characters (large intercellular spaces, spongy tissue, thin cuticle) and the conspicuous sclerenchyma

distribution giving mechanical support to the leaf would be an adaptation to marshy regions, never fully drying, and wet 'campos' with tall grasses. These species form a monophyletic group (with exception of *A. longistyla* that was not examined in the present study). Among the other species groups mentioned by Assis (2001), group IV correspond to the '*A. psittacina*' type in the present study, the leaf type that appears to be plesiomorphic within *Alstroemeria* (Fig. 6). Voucher material of species from group II was not available in the present study.

Conclusions

We analyzed morpho-anatomical characters from a wide range of Alstroemeriaceae species from different geographic regions and different habitats. Our assessment allows to infer the value of the foliar anatomy and morphology as taxonomical characters in Alstroemeriaceae tribe Alstroemerieae and their systematic implications on subgeneric delimitation. Further studies to group the taxa with thin leaves in different taxonomical sections remain to be done, for example, the scanning in other species for bulliform cells such as those observed in *A. pelegriana*. A broader foliar anatomical survey, especially on more Brazilian species of *Alstroemeria* and species of *Bomarea*, may allow recognizing further phylogenetic relationships within the group.

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Appendix 1. Qualitative morpho-anatomical character used in the phylogenetic analysis.

1. Leaves resupinate: *no* (0); *yes* (1). In nearly all species belonging to Alstroemeriaceae the leaves are resupinate making the adaxial surface as the functional lower surface.
2. Resupination of leaves: *partial, twisting only some leaves* (0); *complete* (1).
3. Surface of the functional abaxial side of the leaf: *glabrous* (0); *with papillae* (1); *with hyaline hairs* (2). The character has been coded as additive in the analyses as we consider the hyaline hairs to be prolonged papillae, hence state 1 and 2 are homologous and should be coded as interrelated states.
4. Papillae at the apex of the leaves: *absent* (0); *present* (1). In the Brazilian *Alstroemeria* species with stiff rigid leaves, inconspicuous papillae are found at the leaf apex. The character has also been coded as present in species having papillae covering the whole surface.
5. Cuticle thickness: *thin* (0); *intermediate condition* (1); *thick* (2). The character is coded additive.
6. Stomata present on both the adaxial and abaxial surface of the leaves: *absent from abaxial surface* (0); *present on both surfaces* (1).
7. Cells of both epidermises conspicuously different in size: *no* (0); *yes* (1).
8. Bifacial lamina: *no* (0); *yes* (1).
9. Presence of lobed cells in the mesophyll: *no* (0); *yes* (1).
10. Lobed cells: *some and not outstanding lobed* (0); *conspicuously lobed* (1).
11. Conspicuously distinct vascular bundle sheath: *no* (0); *yes* (1).
12. Sclerenchyma in vascular bundle sheath: *no* (0); *yes* (1).
13. Sclerenchyma in bundle sheaths extended as girders from the vascular bundles towards both leaf surfaces: *no* (0); *yes* (1).

Supplementary material (Appendix njb-00470 at www.nordicbotany.org/readers/appendix). Appendix A1–A2.