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Comparative leaf anatomy of *Baccharis* (Asteraceae) from high-altitude grasslands in Brazil: taxonomic and ecological implications

Thales Ornellas, Gustavo Heiden, Bruna Nunes de Luna, and Claudia Franca Barros

Abstract: *Baccharis* L. is an important genus in the high-altitude environments of South America. In the State of Rio de Janeiro, southeastern Brazil, the greatest richness is found at montane and high-montane ecological refuges, known as high-altitude grasslands. The high altitude provides several environmental factors that turn the high-altitude grasslands into singular tropical habitats. In this article we describe the leaf anatomy of six shrubby species of *Baccharis*, pointing out ecological aspects of the structures, and testing the use of statistical analyses to compare the species. Using light and scanning electron microscopy, we compared the anatomical features of the leaves and performed a cluster and principal component analyses. The results show novel features in the leaf anatomy for the genus, such as an undulated cuticle, three secretory ducts in the midrib, secretory ducts associated with the xylem, and biseriate glandular trichomes with an irregular organization of the cells. Our statistical analyses indicate that the anatomical features of the leaves, especially trichomes, are useful for distinguishing the different species, whereas others, such as isobilateral mesophyll, amphistomatic leaves, and an epidermis with thick cell walls correlate with their high-elevation habitat.

Key words: campos de altitude, secretory ducts, trichomes, morpho-anatomy.

Résumé : *Baccharis* L. est un genre important des environnements de haute altitude en Amérique du Sud. Dans l'état de Rio de Janeiro, dans le sud-est du Brésil, la richesse la plus élevée se trouve dans les refuges écologiques montagnards et de hautes montagnes, connus sous l'appellation de prairies de haute altitude. La haute altitude fournit plusieurs facteurs environnementaux qui transforment les prairies de haute altitude en habitats tropicaux singuliers. Les auteurs proposent ici de décrire l'anatomie foliaire de six espèces arbustives de *Baccharis* en signalant les aspects écologiques des structures, et de tester l'utilisation d'analyses statistiques pour comparer les espèces. À l'aide de la microscopie optique et de la microscopie électronique à balayage, ils ont comparé les caractéristiques anatomiques des feuilles et réalisé une analyse de groupement et une analyse en composantes principales. Les résultats présentent des nouveautés en ce qui concerne l'anatomie de la feuille chez ce genre, comme une cuticule ondulée, trois canaux sécrétoires dans la côte, des canaux sécrétoires associés au xylème et des trichomes glandulaires bisériés, dont les cellules ont une organisation irrégulière. L'analyse statistique indique que des caractères anatomiques des feuilles, particulièrement les trichomes, sont utiles pour distinguer les espèces alors que d'autres comme le mésophylle isobilatéral, les feuilles amphistomatiques et l'épiderme à parois cellulaires épaisses sont corrélés avec leur habitat en haute altitude. [Traduit par la Rédaction]

Mots-clés : campos de altitude, canaux sécrétoires, trichomes, morpho-anatomie.

Introduction

Baccharis L. (Asteraceae: Astereae) is a Neotropical plant genus comprising 354 species (Müller 2013) that occupy a wide range of environments, from sea level to high altitudes, and has its main diversity centers in the mountain regions of the northwest and southeast of South America. The dioecy and the tufted indument of the leaves and stems are synapomorphies of the genus (Müller 2006). In Brazil, there are 179 species with a widespread distribution in the different phytogeographical domains, and about 115 endemic species (Heiden 2019). In Rio de Janeiro State, the highest richness of *Baccharis* is found in montane and high-montane ecological refuges, such as the high-altitude grasslands and inselbergs, comprising 55 species, and seven endemics, of which six are restricted to the high altitude regions of the State (Heiden 2009).

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As reported by Safford (1999), the high-altitude grasslands are plant formations belonging to the Atlantic Rain Forest biome, restricted to the summits of the mountains in Brazilian south and southeast, above 1600 m a.s.l. The high altitude provides several environmental factors, such as the low atmospheric pressure that influences the increase in light irradiance and decrease in temperature, which turn the high-altitude grasslands into singular tropical habitats. Safford (1999) characterizes this ecosystem as a grassy archipelago surrounded by the Ombrophilous Forest, forming a mosaic of shrubby and graminoid formations with a low frequency of arboreal species, with their distribution controlled by the topography, drainage, and soil type. The high-altitude grasslands show a high degree of floristic richness and endemism, therefore considered reliquaries of biodiversity with phytogeographic connections with the "Cerrado" and Andean ecosystems. Asteraceae is the most speciose botanical family in high-altitude grasslands, with *Baccharis* being its most significant genus in these habitats (Safford 1999).

Baccharis species are commonly used in traditional medicine, known by the vernacular names "carqueja" (species with winged stem and reduced leaves) and "vassoura" (leafy species) (e.g., Budel and Duarte 2007, 2008*a*, 2008*b*, 2009, 2010; Molares et al. 2009; Rodriguez et al. 2010; Jasinski et al. 2014; Bobek et al. 2015, 2016; Budel et al. 2018). The pharmacological and phytochemical studies have contributed to improving the taxonomy of the genus with the identification of morpho-anatomical characters that can be used to determine species often commercialized as herbal medicines.

While floristic inventories are available (Nesom 1990; Giuliano 2001; Müller 2006; Heiden 2009; Giuliano and Freire 2011; Heiden et al. 2012), there was no infrageneric classification of the Brazilian species, with the exception of Heiden (2009), which is restricted to the 55 species of Rio de Janeiro State and recognized four subgenera and 15 sections. However, Heiden and Pirani (2016*a*, 2016*b*) recently presented novel features for the infrageneric classification, based on a broad phylogenetic study encompassing seven subgenera and 45 sections, which still needs to be tested from a morpho-anatomical point of view to identify new potential synapomorphies of the main clades. A comparative approach of the leaf anatomy of some *Baccharis* species has been explored recently (Bobek et al. 2016; Budel et al. 2018), identifying some characters that express the diversity of the leaf anatomy of the genus, and can be useful to differentiate the species, for example, the trichome types, occurrence, and types of the stomata, the mesophyll organization, among other characters.

In addition to its contribution to taxonomy and systematics, the knowledge of plant anatomy can be useful for identifying local adaptations, when the environmental context is considered (e.g., Starr 1912; Hayden 1919; Metcalfe and Chalk 1979; Morretes 1980; Mott and Michaelson 1991; Somavilla and Graciano-Ribeiro 2011; Aguiar-Dias et al. 2012; Jakovljević et al. 2014; Sanso et al. 2014; Perrone et al. 2015; Gavilanes et al. 2016).

Thus, the major objectives of this study are to describe the leaf anatomy of six shrubby species of *Baccharis* occurring in the high-altitude grasslands of Rio de Janeiro State, to test the usefulness of leaf anatomical characters for the segregation of *Baccharis* species, and to identify those that correlate with their high-altitude habitat.

Materials and methods

Samples of six shrubby species occurring in the high-altitude grasslands of Rio de Janeiro were collected between May and August 2018. The infrageneric classifications are based on Heiden (2009) and Heiden and Pirani (2016*b*). Samples of *Baccharis itatiaiae* Wawra (subgenus *Baccharis* section *Cylindricae*), *B. phylicifolia* DC. (subgen. *Tarchonanthoides* sect. *Canescentes*), and *B. stylosa* Gardner (subgen. *Molina* sect. *Molinae*) were collected at the plateau of the Parque Nacional de Itatiaia located in Serra da Mantiqueira (22°14′33.19″N; 22°27′54.14″S; 44°34′3.52″E; 44°46′10.77″W). This area is classified as having a subtropical highland climate (Cwb), in the Köppen climate classification, with annual precipitation of about 2400 mm, concentrated in the summer, and an annual average temperature around 11.4 °C (FBDS 2000). *Baccharis calvescens* DC. (subgen. *Baccharis* sect. *Racemosae*), *B. parvidentata* Malag. (subgen. *Baccharis* sect. *Cylindricae*), and *B. platypoda* DC. (subgen. *Baccharis* sect. *Agglomeratae*) were collected at the Parque Nacional da Serra dos Órgãos in the Serra do Mar chains of mountains (22°52′N; 22°54′S; 42°09′E; 45°06′W). This area is classified as having a temperate oceanic climate (Cfb) with annual precipitation varying from 1500 to 3600 mm, well distributed along the year, and an annual average temperature around 18.4 °C (ICMBio 2008). Voucher specimens (Supplementary data, Table S1[1]) were deposited in the Herbarium of the Botanical Garden of Rio de Janeiro (RB).

Fully developed leaves were fixed in a solution of formalin–acetic acid–70% ethanol (Johansen 1940) and stored, after 48 h, in 70% ethanol (Berlyn and Miksche 1976).

Samples of the midrib and leaf blade were dehydrated and embedded in glycol methacrylate (Leica Historesin®), following the instructions of the manufacturer. Transverse sections were obtained using a rotary microtome (Leica RM2245), and the sections were stained with Toluidine Blue O (O'Brien et al. 1964).

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[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjb-2019-0035.

To analyse the frontal view of epidermal features, the leaf epidermis was dissociated in a solution of hydrogen peroxide and acetic acid (1:1 *v/v*), washed in distilled water, and stained with 1% safranin (Franklin 1945). The classification of stomata followed the terminology of Metcalfe and Chalk (1950), and the trichomes were described according to the terminology of Freire et al. (2007): conical, aseptate flagellate, filiform flagellate, 1-armed, 2–4-armed, bulbiferous flagellate, and glandular biseriate; and according to Budel et al. (2018): type I and type II flagelliform.

Histochemical tests were performed on freehand sections of fixed leaf samples. Lipophilic compounds were detected using Sudan III (Sass 1951); lignified tissues using hydrochloric phloroglucinol (Foster 1949); and phenolic compounds using ferric chloride (Johansen 1940).

The images were obtained using a digital camera (Olympus DP73) coupled to an optical microscope (Olympus BX50).

Additional leaf samples were fixed in a solution of 2.5% glutaraldehyde and 4.0% formaldehyde buffered with 0.05 mol·L^{-1} sodium cacodylate (pH 7.2). The samples were rinsed three times in the same buffer, and then dehydrated in an increasing series of ethanol and acetone, then completely dehydrated using liquid CO_2 in a critical point dryer (Leica EM CPD030), mounted on aluminium stubs, and sputter-coated with gold (Emitech K550X) for epidermal analysis under a scanning electron microscope (Zeiss EVO 40).

Cluster analysis and principal component analysis (PCA) were performed using the software Statistica 7.0 (StatSoft Inc 2004) using leaf anatomical characters to identify, through the factor analysis, the most significant features in the distinction of species. Leaf anatomical data of an additional eight species of *Baccharis* from Bobek et al. (2016) and Budel et al. (2018) were included in the analyses, totalling 14 species.

Results

Epidermis and cuticle
The epidermis of all the analysed species had thick, straight to slightly undulated, anticlinal walls with conspicuous pits. *Baccharis stylosa* has a smooth cuticle on both leaf sides (Figs. 1I, 1L), showing striations only radiating from the trichome bases. In *B. platypoda*, the cuticle was undulated (Figs. 1H, 1K). In *B. calvescens*, *B. itatiaiae*, *B. parvidentata*, and *B. phylicifolia*, the cuticle was smooth on the adaxial side (Figs. 1A–1C, 1G), with striations around the trichomes or stomata in *B. calvescens* (Fig. 1A) and *B. itatiaiae*, and striated on the abaxial side of several species (Figs. 1D–1F and 1J).

In the transverse sections (Figs. 2A–2R), all of the species have a single-layered epidermis covered with a thin cuticle, showing thick outer periclinal walls (Fig. 3P). *Baccharis calvescens*, *B. parvidentata*, and *B. phylicifolia* have epidermal cells with greater volume on the adaxial side

in relation to the abaxial side (Figs. 2C, 2I, and 2L), whereas the volume of the epidermal cells was relatively uniform in the other species (Figs. 2F, 2O, and 2R).

Stomata
Baccharis calvescens, *B. parvidentata*, and *B. phylicifolia* are hypostomatic (Figs. 2B, 2H, and 2K) and *B. itatiaiae*, *B. platypoda*, and *B. stylosa* are amphistomatic (Figs. 2E, 2N, and 2P), with the latter showing stomata on the adaxial side only along the veins. The stomata are at the same level as the other epidermal cells in *B. platypoda* (Fig. 2N) and *B. stylosa* (Fig. 2Q), at the same level or slightly elevated in *B. calvescens* (Fig. 2B), *B. itatiaiae* (Fig. 2E), and *B. parvidentata* (Fig. 2H), and protruded in *B. phylicifolia* (Fig. 2K). *Baccharis itatiaiae* and *B. parvidentata* present preferentially tetracytic and staurocytic stomata, also occurring, with lower frequency, anomocytic stomata. *Baccharis platypoda* presents tetracytic and staurocytic stomata, rarely ciclocytic and anomocytic. In *B. stylosa*, anisocytic and anomocytic stomata occur. In *B. phylicifolia*, the high stomata density leads to an anomocytic type with indistinct subsidiary cells. This characteristic is also found in *B. calvescens*, which also rarely presents actinocytic stomata.

Indumentum
We found different types of trichomes in the studied species, and these were often located in depressions in the epidermis and usually occurred in tufts, except in *B. parvidentata* and *B. phylicifolia*, which have isolated trichomes. In *B. itatiaiae*, the tufts are composed of biseriate glandular trichomes and type I flagelliform glandular trichomes on both sides of the leaf (Figs. 3B and 3E). *Baccharis stylosa* has trichomes clustered in tufts on both sides of the leaf blade, and these are biseriate on the adaxial side (Fig. 3I), and type II flagelliform on the abaxial one (Fig. 3L). Similarly, tufts of biseriate and capitate uniseriate trichomes are found on the adaxial side of *B. calvescens* (Figs. 1A and 3A), whereas on the abaxial side there are branched uniseriate glandular trichomes (Figs. 1D and 3D). In *B. platypoda*, tufts of trichomes are also present on both sides, with biseriate and capitate uniseriate glandular trichomes on the adaxial side (Fig. 3H), and biseriate glandular trichomes on the abaxial side (Fig. 3K). *Baccharis parvidentata* presents bulbiferous flagellate glandular trichomes, subtype B, singled or rarely tufted, in deep depressions along the veins on the adaxial side, and scattered across the leaf blade on the abaxial side (Figs. 1F, 3C, and 3F). In *B. phylicifolia*, the adaxial indument is composed of conical trichomes with a multicellular pedestal, followed by a globular cell with dense cytoplasm, ending with a series of elongated filiform cells with thick lateral walls (Figs. 1G and 3G). Three types of trichomes densely cover the abaxial side of the epidermis: (*i*) biseriate glandular trichomes with an irregular organization, showing two series of cells without alignment (Figs. 1J and 3J detail); (*ii*) uniseriate conical

Fig. 1. Scanning electron micrographs of leaf epidermis from *Baccharis* species. (A, B, C, G, H, and I) Adaxial side. (D, E, F, J, K, and L) Abaxial side. (A and D) *Baccharis calvescens*. (B and E) *Baccharis itatiaiae*. (C and F) *Baccharis parvidentata*. (G and J) *Baccharis phylicifolia*. (H and K) *Baccharis platypoda*. (I and L) *Baccharis stylosa*. bi, biseriate trichome; br, branched trichome; bu, bulbiferous flagellate trichome; co, conical trichome; fl, flagelliform trichome; ib, irregular biseriate trichome; tt, trichomes in a tuft. Scale bars = 20 μm.
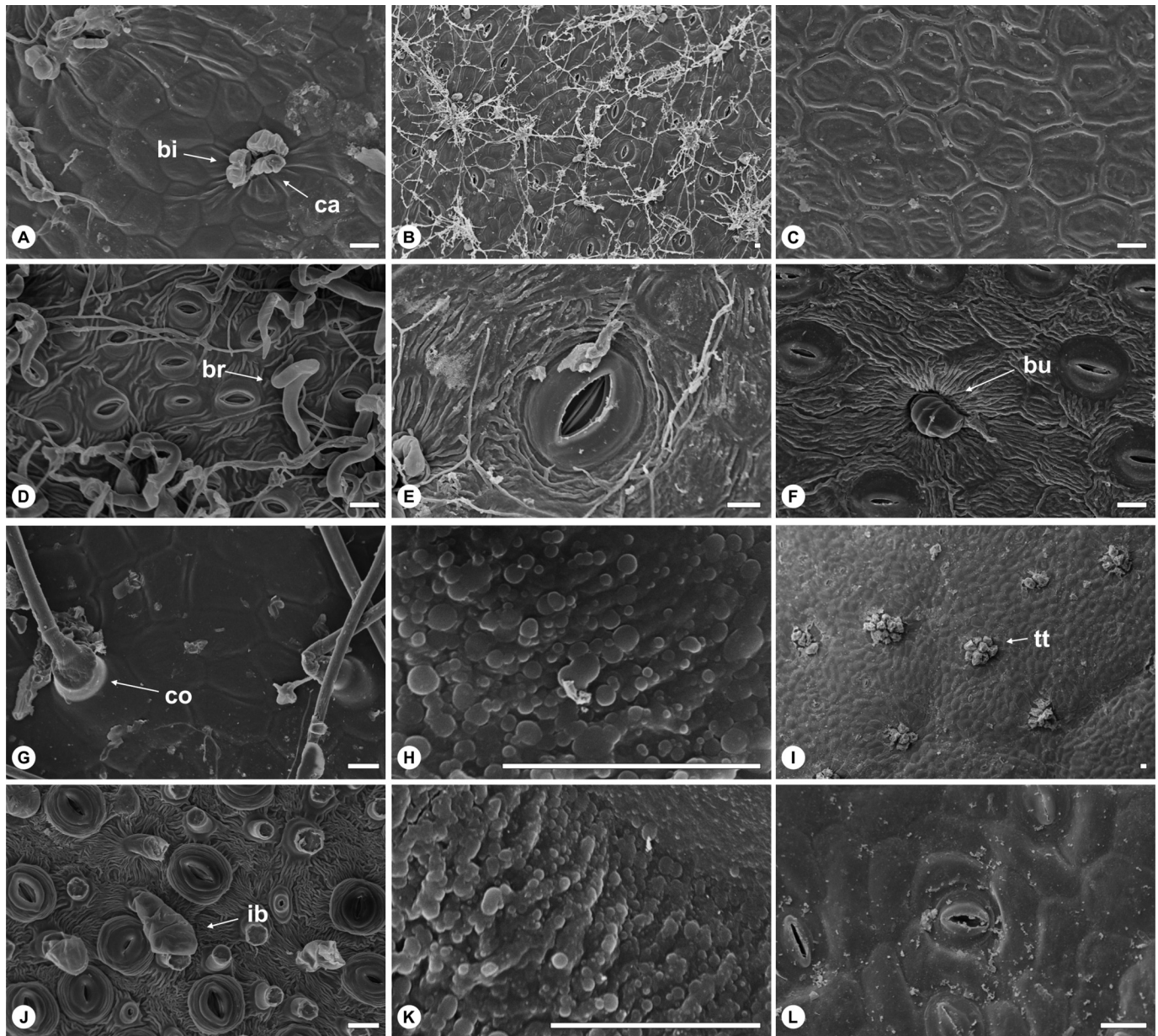

trichomes with a multicellular pedestal, a large cylindrical cell, ending with a series of elongated filiform cells with thick lateral walls; and (*iii*) uniseriate aseptate trichomes with a multicellular pedestal, a small cylindrical cell, and a terminal aseptate filiform cell (Fig. 3J).

Midrib and vascular system

The shape of midrib and the arrangement of vascular bundles and secretory ducts vary among the species we studied. *Baccharis itatiaiae* has a flat–convex shape (Fig. 2D), and in *B. platypoda* it is biconvex (Fig. 2M). The other species have a concave–convex shape (Figs. 2A, 2G,

2J, and 2P). Some layers of angular collenchyma are found in all the species, mainly on the abaxial side. All of the six species present collateral vascular bundles surrounded by sclerenchyma fibres, mainly below the phloem. *Baccharis calvescens*, *B. itatiaiae*, *B. parvidentata*, and *B. phylicifolia* have a single vascular bundle associated with an abaxial schizogenous secretory duct (often inconspicuous in *B. phylicifolia*) composed of a single-layered epithelium with a variable number of cells (Figs. 2A, 2D, 2G, and 2J). In *B. stylosa*, the single vascular bundle has three associated secretory ducts (Fig. 2P), and

Fig. 2. Midrib and leaf blade in transverse sections of *Baccharis* species. (A–C) *Baccharis calvescens*. (D–F) *Baccharis itatiaiae*. (G–I) *Baccharis parvidentata*. (J–L) *Baccharis phylicifolia*. (M–O) *Baccharis platypoda*. (P–R) *Baccharis stylosa*. bu, bulbiferous flagellate trichome; co, conical trichome; ib, irregular biseriate trichome; pa, palisade parenchyma; sd, secretory duct; sp, spongy parenchyma; st, stomata; tt, trichomes in tuft; vb, vascular bundle. Scale bars = 100 μm. [Colour online.]
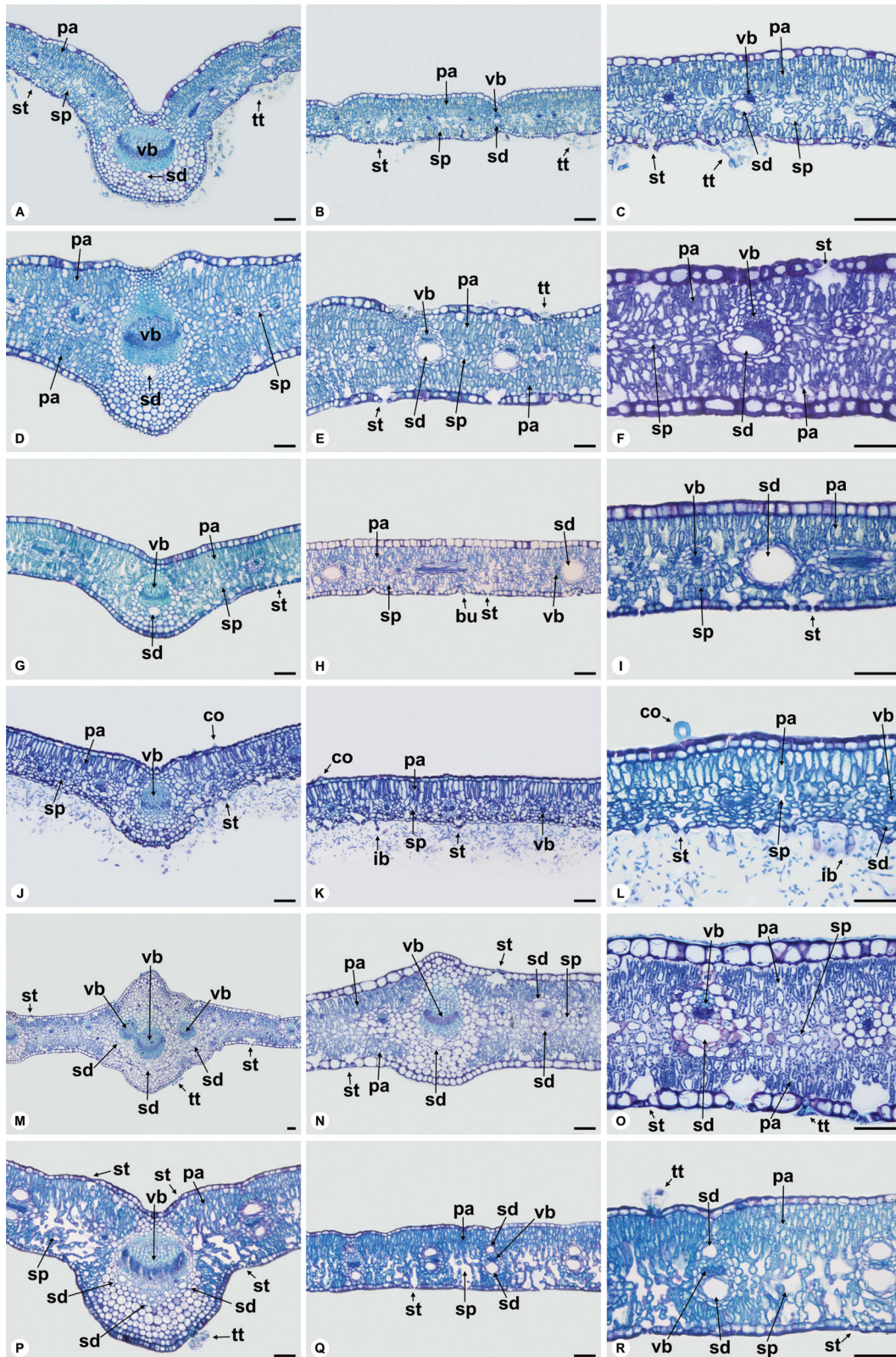
Fig. 3. Frontal view of leaf epidermis (A–L) and histochemical tests (M–R) of *Baccharis* species. (A–C and G–I) Adaxial side. (D–F and J–L) Abaxial side. (M–R) Transverse section. (M, O, P, and R) Section stained with Sudan III. (N) Section stained with ferric chloride. (Q) Section stained with hydrochloric phloroglucinol. (A, D, and M) *Baccharis calvescens*. (B, E, and N) *Baccharis itatiaiae*. (C, F, and O) *Baccharis parvidentata*. (G, J, and P) *Baccharis phylicifolia*. (H, K, and Q) *Baccharis platypoda*. (I, L, and R) *Baccharis stylosa*. as, aseptate trichome; bi, biseriate trichome; br, branched trichome; bu, bulbiferous flagellate trichome; ca, capitate trichome; co, conical trichome; cu, cuticle; et, epidermal thickening; fl, flagelliform trichome; ib, irregular biseriate trichome; lp, lignified parenchyma; ob, oil bodies; pa, palisade parenchyma; sd, secretory duct; sp, spongy parenchyma; tt, trichomes in a tuft; vb, vascular bundle. Scale bars = 100 μm. [Colour online.]
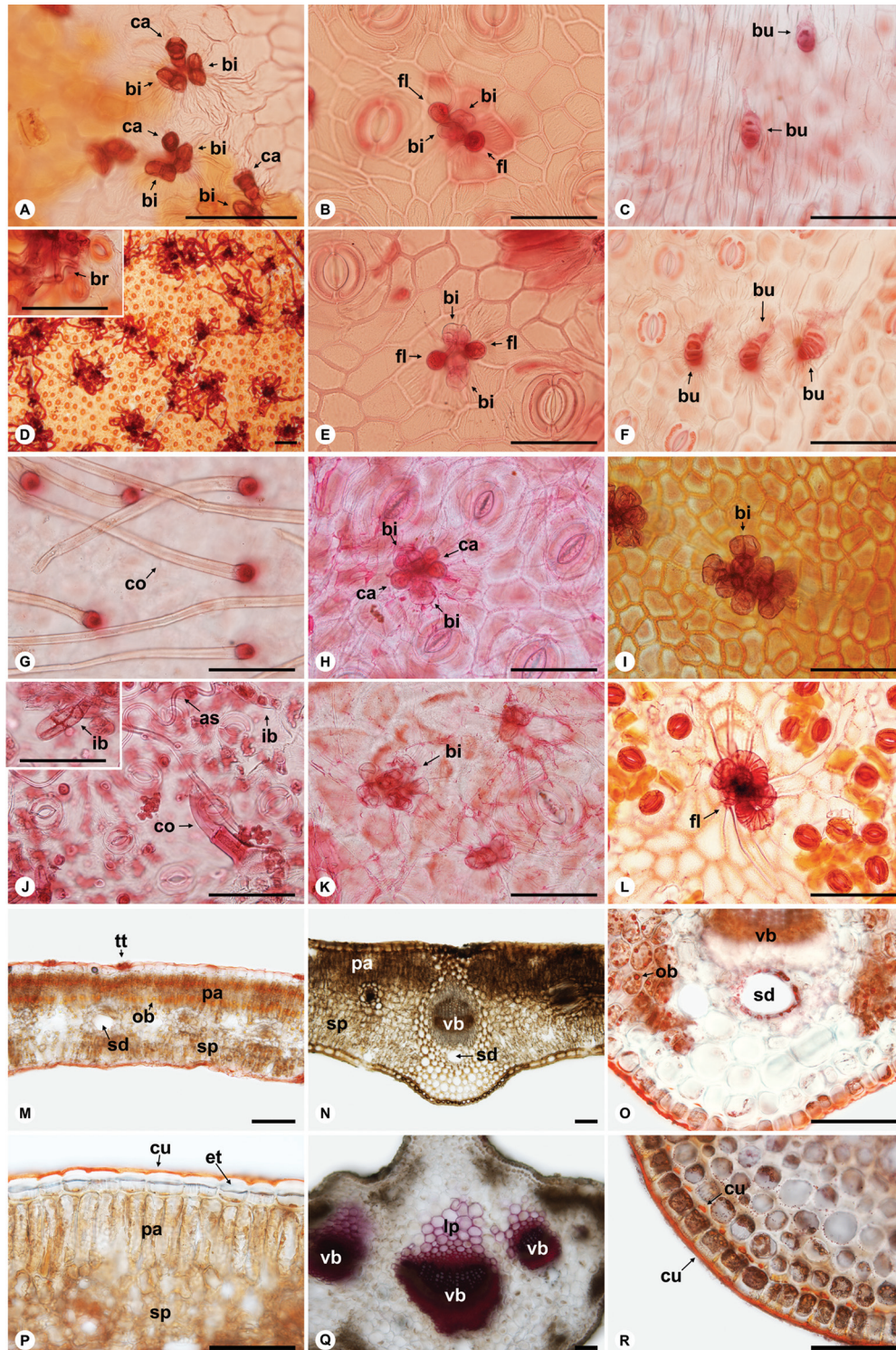
in *B. platypoda* is observed three vascular bundles, owing to the pinnate venation of the leaves, each one associated to one secretory duct (Fig. 2M).

Leaf blade and mesophyll

The mesophyll of *B. itatiaiae* and *B. platypoda* is isobilateral with two to three layers of palisade parenchyma on each side and a compact arrangement of spongy parenchyma (Figs. 2F and 2O). *Baccharis calvescens*, *B. parvidentata*, *B. phylicifolia*, and *B. stylosa* have a dorsiventral organization, presenting one to three layers of palisade parenchyma (Figs. 2C, 2I, 2L, and 2R). In *B. platypoda* there are ridges on the larger lateral vascular bundles (Fig. 2N), whereas in *B. calvescens* there are depressions (Fig. 2B). In all of the analysed species, the collateral vascular bundles are arranged along the mesophyll, surrounded by a parenchymatous sheath and usually associated with an abaxial secretory duct, except in *B. stylosa*, which has a second secretory duct associated with the xylem on the adaxial side (Figs. 2Q and 2R). This adaxial duct also occurs in *B. platypoda*, but less frequently (Fig. 2N). In *B. parvidentata*, the secretory ducts are large and often are arranged laterally or isolated (Figs. 2H and 2I).

Histochemical tests

The hydrochloric phloroglucinol test revealed slightly lignified parenchymatic cells above the sclerenchyma fibres in *B. platypoda* (Fig. 3Q). The test for lipids showed the presence of oil bodies in *B. calvescens*, *B. parvidentata*, and *B. platypoda*, mostly in the palisade parenchyma (Figs. 3M and 3O) and the epithelium of the secretory ducts (Fig. 3O). The presence of an internal cuticle in the epidermis of the midrib in *B. stylosa*. (Fig. 3R) was also observed. The presence of phenolic compounds was detected in the epidermis of the midrib of *B. itatiaiae* (Fig. 3N) and *B. stylosa*, and in the palisade parenchyma of all the species (Fig. 3N).

Statistical analyses

The cluster analysis and PCA were performed from a presence/absence matrix of 38 characteristics of the leaf anatomy (see the Supplementary data, Table S2[1]). The PCA results show that the three first factors explain 59.16% of the total variance. The factor analysis (Table 1) indicates that the features that most influenced the distinction of the species were cuticle ornamentation, stomata type, stomata position, stomata occurrence, mesophyll organization, number of secretory ducts in the midrib and leaf blade, number of vascular bundles in the midrib, and trichome type. The first factor (26.57%) was weighted for stomata at the same level as the epidermal cell, protruded stomata, tetracytic stomata, staurocyric stomata, hypostomatic leaves, isobilateral mesophyll, and dorsiventral mesophyll. The second factor (19.70%) was weighted for anisocytic stomata, the single secretory duct in the leaf blade, the double secretory duct in the leaf blade, the single secretory duct in the

midrib, and three secretory ducts in the midrib. The third factor (12.89%) was weighted for undulated cuticle, capitate trichomes, single vascular bundle in the midrib, and multiple vascular bundles in the midrib. The 3D graphic of the PCA (Fig. 4) shows the distinction between two groups and the isolation of *B. platypoda* and *B. stylosa*.

The cluster analysis (Fig. 5) segregates the studied species into two major groups, distinguished by the mesophyll organization. In each respective group, *B. stylosa* and *B. platypoda* segregate first due to the presence of a second secretory duct in the leaf blade and the conformation of the vascular bundles and secretory ducts in the midrib, followed by the segregation of a group characterized by hypostomatic leaves in the cluster of dorsiventral mesophyll and a group of amphistomatic and amphi-hypostomatic leaves in the cluster of isobilateral mesophyll. The proposed anatomical characters did not distinguish *B. illinita* and *B. reticularioides*.

Discussion

Taxonomic evaluation

In general, the cuticle in *Baccharis* species is described as smooth or striated, on both or one of the leaf sides, or even presenting striations only around stomata and trichomes (Müller 2006; Jasinski et al. 2014; Bobek et al. 2015, 2016; Budel et al. 2018). In the studied species, only *B. platypoda* presented a different pattern, showing an undulated cuticle on both sides of the leaf, which is reported herein for the first time for *Baccharis*.

Regarding the anticlinal walls, Budel et al. (2018) suggest that sinuous walls can be a common character in species of the subgenus *Molina*. However, *B. stylosa* belongs to this subgenus (Heiden 2009) and has straight anticlinal walls on both sides of the epidermis.

Amphistomatic leaves are frequent in *Baccharis*, being reported in *B. brevifolia* (Bobek et al. 2016), *B. caprariifolia* (Bobek et al. 2015), *B. cognata* (Budel et al. 2013), *B. dracunculifolia* (Budel et al. 2004), *B. glaziovii* (Jasinski et al. 2014), *B. obovata* (Molares et al. 2009), *B. pauciflosculosa* (Bobek et al. 2016; Budel et al. 2018), *B. sphenophylla* (Budel et al. 2018), *B. spicata* (Oliveira et al. 2011), *B. trilobata* (Bobek et al. 2016), and *B. junciformis* (as *B. usterii*) (Budel and Duarte 2010). Hypostomatic leaves also occur in the genus, as reported in *B. anomala* (Budel and Duarte 2008b), *B. erioclada* (Bobek et al. 2015), *B. microdonta* (Bobek et al. 2016), *B. ochracea* (Barreto et al. 2015), *B. punctulata* (Budel et al. 2018), *B. singularis* (Souza et al. 2011), and *B. uncinella* (Budel and Duarte 2008a). In the current study, *B. itatiaiae*, *B. platypoda*, and *B. stylosa* were found to have amphistomatic leaves, whereas *B. calvescens*, *B. parvidentata*, and *B. phylicifolia* have hypostomatic leaves.

Analysing 38 medicinal species of *Baccharis*, Freire et al. (2007) reported seven types of trichomes occurring isolated or in tufts composed of similar or distinct trichomes. Biseriate glandular trichomes and uniseriate flagelliform trichomes are among the most com-

Table 1. Factor loadings and percentage of variance for the three principal components obtained from the 38 characters analysed.

	Factor 1	Factor 2	Factor 3
Adaxial epidermal cells > abaxial epidermal cells	0.591347	−0.483444	0.065340
Adaxial epidermal cells = abaxial epidermal cells	−0.591347	0.483444	−0.065340
Striated cuticle	0.542772	−0.522609	0.165202
Smooth cuticle	0.464356	0.216000	−0.320855
Undulated cuticle	−0.365225	0.407033	**0.810206**
Cuticle with striations around stomata and trichomes	−0.271418	0.345645	−0.601147
Stomata at the same level of the epidermal cells	**−0.743205**	0.069009	−0.301896
Protruded stomata	**0.743205**	−0.069009	0.301896
Tetracytic stomata	**−0.820462**	−0.338856	0.066411
Staurocytic stomata	**−0.820462**	−0.338856	0.066411
Anisocytic stomata	0.087128	**0.774042**	−0.443198
Actinocytic stomata	−0.201368	−0.576355	−0.099945
Anomocytic stomata	0.577823	0.532138	0.342219
Cyclocytic stomata	−0.482046	0.123530	0.192958
Amphistomatic leaves	−0.591347	0.483444	−0.065340
Hypostomatic leaves	**0.913022**	−0.079147	0.211251
Amphi–hypostomatic leaves	−0.284621	−0.501960	−0.152961
Isobilateral mesophyll	**−0.907635**	−0.341415	0.039042
Dorsiventral mesophyll	**0.907635**	0.341415	−0.039042
Tufted trichomes	−0.509642	0.268681	−0.154666
Isolated trichomes	0.509642	−0.268681	0.154666
Biseriate trichomes	−0.169211	0.260716	−0.043486
Type I flagelliform trichomes	−0.528905	−0.570730	−0.254002
Type II flagelliform trichomes	0.228928	0.605091	−0.371078
Capitate trichomes	0.046010	0.284899	**0.760316**
Branched trichomes	0.427740	−0.019931	0.222859
Bulbiferous flagelliform trichomes, subtype b	0.169211	−0.260716	0.043486
Conical trichomes	0.582077	−0.073834	0.187336
Aseptate trichomes	0.582077	−0.073834	0.187336
Single secretory duct in the leaf blade	0.204674	**−0.869247**	−0.270111
Double secretory duct in the leaf blade	−0.204674	**0.869247**	0.270111
Single secretory duct in the midrib	−0.087128	**−0.774042**	0.443198
Three secretory ducts in the midrib	0.087128	**0.774042**	−0.443198
Single vascular bundle in the midrib	0.365225	−0.407033	**−0.810206**
Multiple vascular bundles in the midrib	−0.365225	0.407033	**0.810206**
Biconvex midrib	−0.462733	−0.270372	0.462946
Flat–convex midrib	−0.162389	0.053264	−0.218718
Concave–convex midrib	0.570656	0.198192	−0.222949
Percentage of total variance (%)	26.57227	19.70277	12.89229

Note: Bold font indicates the values for loadings greater than |0.7|.

mon types found in the genus. Flagelliform trichomes have been considered as nonglandular, but Budel et al. (2018) suggest that these trichomes exhibit secretory activity, owing the presence of lipophilic compounds in their cells, classifying them as flagelliform glandular trichomes. In the leaves of the studied species, the trichome type was the most diagnostic feature, showing different morphologies and configurations among species. It is worth mentioning that in *B. calvescens* the indumentum is composed of tufts of branched trichomes, which was also seen in *B. coridifolia* (Budel and Duarte 2007), *B. dracunculifolia* (Budel et al. 2004), *B. erioclada* (Bobek et al. 2015), and *B. uncinella* (Budel and Duarte 2008a), all of which belong to section *Racemosae* (Heiden 2009), except *B. coridifolia*, which belongs to section *Coridifoliae* (Giuliano 2011). In *B. phylicifolia* there are four

distinct types of trichomes in its leaves. The conical trichomes present on the adaxial side are similar to those illustrated by Hellwig (1992) for the adaxial side of *B. helichrysoides*, which also belongs to section *Canescentes* (Heiden and Pirani 2016b), but in *B. phylicifolia* the filiform portion is composed of a series of cells instead of a single one. Hellwig (1992) also depicts trichomes on the abaxial side of *B. grisebachii* leaves, similar to the aseptate trichomes in *B. phylicifolia*. These trichomes and the abaxial conical trichomes have similar cylindrical cells, only differing in size. The biseriate trichomes with an irregular organization of the cells are reported for the first time herein.

The occurrence of single secretory ducts associated with the phloem in *Baccharis* species is well-documented in the literature for the genus, and it is present in the species analysed in this study, except in *B. platypoda* and

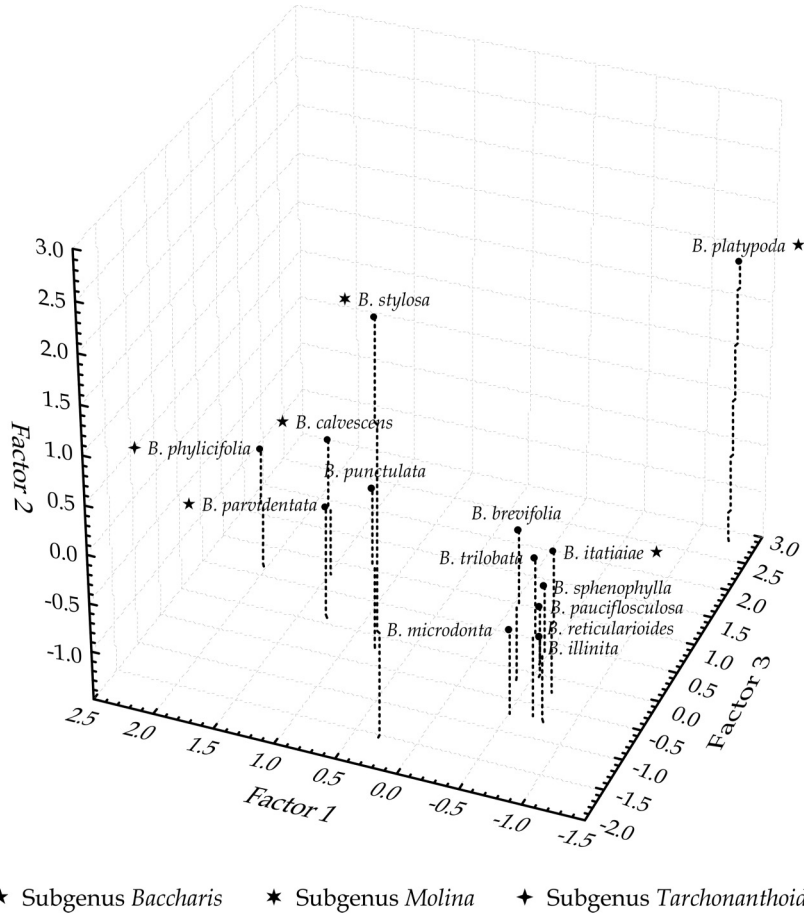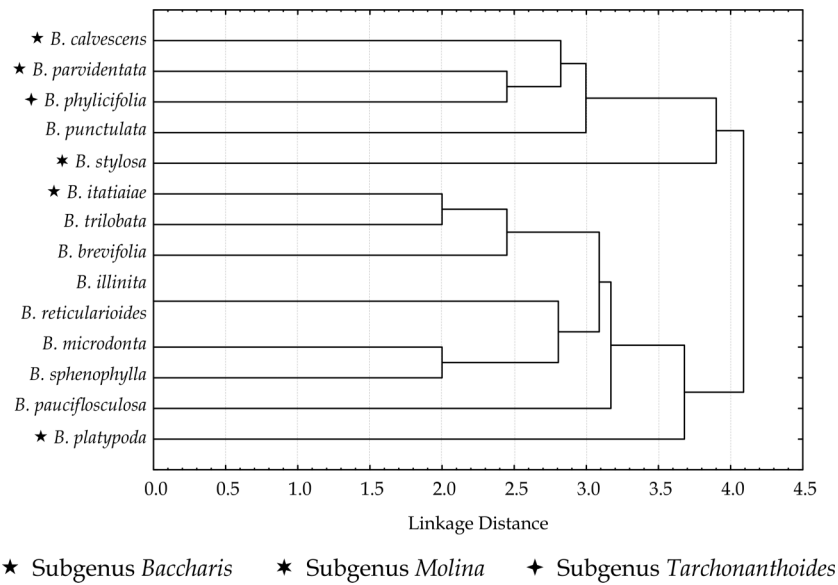**Fig. 4.** Scatterplot based on principal component analysis.

★ Subgenus *Baccharis*   ✳ Subgenus *Molina*   ✦ Subgenus *Tarchonanthoides*

Fig. 5. Dendrogram based on cluster analysis.

★ Subgenus *Baccharis*   ✳ Subgenus *Molina*   ✦ Subgenus *Tarchonanthoides*

B. stylosa, where there is a second duct associated with the xylem. This feature is commonly found in the Asteraceae family in species of the tribes Eupatoriae and Helianthae, and rarely in the tribe Astereae (Castro et al. 1997). Smiljanic (2005), studying the leaf anatomy of As-

teraceae species, *B. platypoda* and *B. stylosa* among them, reported for the first time the presence of a second duct associated with the xylem in *Baccharis*, but only recording it for *B. stylosa*. This study provides the first reported occurrence of the second duct for *B. platypoda*. The pres-

ence of three secretory ducts associated with the vascular bundle on the midrib of *B. stylosa* is especially noteworthy, as it is a novel finding for the genus.

The results of the cluster analysis and PCA concurs with the formation of two major groups distinguished by the mesophyll organization and stomata occurrence, as well as the segregation of *B. platypoda* and *B. stylosa*, both of which characterized by the presence of a second secretory duct in the leaf blade. Considering the six species studied here, their grouping does not reflect the current infrageneric classification (Heiden 2009; Heiden and Pirani 2016*b*), as demonstrated by the separation of the species belonging to the subgenus *Baccharis*.

Ecological interpretation

All of the studied species present a thickening in the periclinal walls of the ordinary epidermal cells; a feature that is also found in other plants from high altitudes (Körner et al. 1989; Gavilanes et al. 2016). Such thickening can be an adaptation to the environmental conditions present at high altitudes, serving as a filter to reflect or absorb the high UV-radiation (Robberecht et al. 1980), thus compensating for the thin cuticle layer observed in all of the studied species.

The sinuosity and thickness of the anticlinal walls can also reflect ecological influences (Wilkinson 1979). Straight and thick walls tend to decrease the excessive loss of water (Medri and Lleras 1980; Fahn and Cutler 1992); an advantageous feature in a mountaintop environment where the shallow soils make water retention difficult. These features are common in *Baccharis* (Oliveira et al. 2011; Souza et al. 2011; Barreto et al. 2015; Bobek et al. 2015, 2016; Budel et al. 2018) and were found in all of the studied species. *Baccharis stylosa* also shows an additional anatomical strategy to minimize the water evaporation, presenting an internal cuticle on the inner side of the epidermis (Pesacreta and Hasenstein 1999).

Amphistomatic leaves are common in *Baccharis*. Mott et al. (1982) suggested that the presence of stomata on both sides of the leaf has an adaptative significance, being a derived character and ecologically correlated with the increased leaf conductance of carbon dioxide in plants growing in sunny environments with seasonal or perennial variations of water availability on the soil. Such environmental characteristics, in addition to the greater diffusion of gases due to the low atmospheric pressure, are present in regions of high altitude, where there is great diversity of *Baccharis* species. The occurrence of stomata and the mesophyll organization seem to be correlated in the studied species, and in the genus in general: amphistomatic leaves tend to be isobilateral, whereas hypostomatic leaves present dorsiventral mesophyll. Even *B. stylosa*, which has dorsiventral mesophyll and amphistomatic leaves, shows a tendency to have hypostomatic leaves with few scattered stomata along the veins on the adaxial side.

The presence of oil bodies in the mesophyll of *Baccharis* was first reported by Budel et al. (2018), and it was observed here in the mesophyll of *B. calvescens*, *B. parvidentata* (including in the epithelium of secretory ducts), and *B. platypoda*. The absence of oil bodies in the other studied species is not conclusive, owing to the use of ethanolic fixative in specimens preparations, which can dissolve lipids. Oil bodies are widely found in leaves of Asteraceae and other families (Lersten et al. 2006), despite their uncertain physiological function, some studies suggest that they can be a site of energy storage, a chemical source for defence responses to pathogens and an adaptation to low temperatures (Pihakaski et al. 1987; Lersten et al. 2006; Shimada et al. 2014).

Regarding the groups established in the statistical analyses (supported by the mesophyll organization and the stomata occurrence), it is not possible to assign an ecological influence in the anatomical patterns, because species sharing the same habitat show different features, and species with the same features occur in different habitats. Considering that the mesophyll organization and distribution of stomata are also features associated with physiological traits, we suggest that two functional patterns are present in *Baccharis* from the same environment.

Conclusions and future perspectives

This study increases the knowledge about the anatomy of the genus *Baccharis*, presenting first reports on the leaf anatomical characters, such as undulated cuticle, irregular biseriate glandular trichomes, adaxial secretory ducts in the leaf blade, and three secretory ducts on the midrib. The leaf structure of the studied species shows two functional patterns formed by the mesophyll organization and the distribution of stomata, and some characters, such as thick anticlinal walls and oil bodies in the mesophyll, can be narrowly linked to the environmental conditions. Features of the leaf anatomy showed potential for segregating the species, but considering the morpho-anatomical diversity of *Baccharis*, further studies integrated with phylogenetic analysis are required to address the evolution of the anatomical features and their ecological significance.

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