Rodriguésia 75: e01232023. 2024
http://rodriguesia.jbrj.gov.br
DOI: http://dx.doi.org/10.1590/2175-7860202475014

Plant anatomy: history and future directions

Morphoanatomy and histochemistry of *Baccharis palustris*: insights into a highly endangered endemic species from Southeastern America

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Abstract

Morphoanatomy and histochemistry of *Baccharis palustris*: insights into a highly endangered endemic species from Southeastern America. The phenotypic plasticity of the *Baccharis* genus makes species identification difficult, even at the flowering stage. In this context, morphoanatomical studies are a powerful tool for botanical authentication, mainly emphasizing the recognition of diagnostic characteristics that may be useful for distinguishing similar species. Given the limited knowledge available about the endemic species *B. palustris*, this work aimed to characterize the morphoanatomy and histochemistry of its vegetative aerial parts to identify characters with diagnostic value and to elucidate the sites of synthesis and accumulation of metabolites of medicinal importance. *B. palustris* leaf showed pinnate, camptodrome-brochidodrome venation patterns. Blade with dorsiventral mesophyll, aerenchymatous spongy parenchyma, collateral vascular bundles, and different types of stomata and trichomes, including glandular trichomes with a multi-layered base evidenced and described for the first time in the genus. The petiole was winged, with three collateral vascular bundles. The stem showed a penta-lobulated contour with unusual growth, starch, and crystals in the pith. The presence of secretory ducts and glandular trichomes, which synthesized lipids, terpenes/polyacetylenes, and phenolic compounds, was observed. The morphological/histochemical characteristics described in this work contribute to the knowledge of the species, highlighting the importance of its preservation as a valuable resource.

Key words: *Aphyllae*, Asteraceae, micromorphology, scanning electron microscope, venation pattern.

Resumen

Morfoanatomía e histoquímica de *Baccharis palustris*: ampliando el conocimiento de una especie endémica altamente amenazada del sureste de América. La plasticidad fenotípica del género *Baccharis* dificulta la identificación de las especies, incluso en la etapa de floración. En este contexto, la descripción morfoanatómica es una herramienta poderosa para la autenticación botánica, haciendo hincapié en las características de valor diagnóstico que pueden ser útiles para distinguir especies similares. Dado el escaso conocimiento disponible sobre la especie endémica *B. palustristis*, el objetivo de este trabajo fue caracterizar la morfoanatomía y la histoquímica de las partes aéreas vegetativas de *B. palustris* y establecer los sitios de síntesis y acumulación de metabolitos de importancia medicinal. La hoja de *B. palustris* mostró venación pinnada, camptódroma,

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broquidódromo. Lámina con mesofilo dorsiventral con parénquima esponjoso aerenquimático, haces vasculares colaterales y distintos tipos de estomas y tricomas, entre los que se destacan tricomas glandulares de base pluriceriada nunca antes descriptos para el género. El pecíolo se presentó alado, con tres haces vasculares colaterales. El tallo mostró contorno pentalobulado con crecimiento poco usual, almidón y cristales en la médula. Se observó la presencia de conductos secretores y tricomas glandulares, los cuales pueden sintetizar lípidos, terpenos/poliacetilenos y compuestos fenólicos. Las características morfológicas/histoquímicas descritas en este trabajo contribuyen al conocimiento de la especie, destacando la importancia de su preservación como un recurso valioso.

Palabras clave: *Aphyllae*, Asteraceae, micromorfología, microscopía electrónica de barrido, patrón de venación.

Introduction

Baccharis L. is considered one of the most emblematic and diverse genera belonging to Asteraceae with approximately 442 accepted species (Barroso 1976; Heiden 2021; Fernandes *et al*. 2021). All members of this genus are considered aromatics with commercial, ethnomedicinal, and pharmacological importance (Heiden 2021; Manfron *et al.* 2021a; Minteguiaga *et al.* 2021).

The morphoanatomical records of the genus show a high degree of phenotypic plasticity leading to difficulties in the identification of the species even at the flowering stage (Simões-Pires *et al.* 2005; Schneider 2009; Martinez *et al.* 2018). Thus, research activities involving morpho-anatomical studies are required to better understand the species diversity (Bobek *et al.* 2016; Budel *et al*. 2018a; Manfron *et al*. 2021a). The most important morpho-anatomical useful characters in *Baccharis* species identification can be assessed at the vegetative stage and include the shape, size, and margins of the leaves, contour of anticlinal epidermal cell walls, type and density of stomata, type of indumentum, organization of mesophyll and vascular tissues, type of trichomes, secretory ducts and crystals morphotypes (Rodriguez *et al.* 2010; Martinez *et al.* 2018; Manfron *et al.* 2021a, b; Raeski *et al.* 2023a, b).

Baccharis palustris Heering is an endemic dioecious species from Southeastern America (Heering 1904), described as occurring in Southeastern Brazil (Minas Gerais, Rio Grande do Sul, and Santa Catarina states) and Uruguay (Canelones and Florida Departments), which is found in marshy or humid habitats. Traditionally, this species has been classified by its macromorphology within the infrageneric sect. *Caulopterae* DC. (Heiden *et al.* 2009; Schneider & Boldrini 2011), but recently, a revision made on DNA sequencing and phylogenetic analyses allocated it into the sect. *Aphyllae* Baker (Heiden *et al.* 2019; Heiden 2021). Due to its limited distribution and the loss of its natural habitat caused by the expansion of urbanization, the National System of Protected Areas from Uruguay (SNAP) following the International Union for Conservation of Nature (IUCN) criteria, considered *B. palustris* as a rare and highly endangered species (Heiden *et al.* 2009; Marchesi *et al.* 2023).

Among the 26 species within sect. *Aphyllae*, only 10 have documented comprehensive anatomical studies. These species are *B. articulate* (Lam.) Pers. (Cortadi *et al.* 1999; Rodriguez *et al.* 2010; Martinez *et al.* 2018), *B. penningtonii* Heering (Martinez *et al.* 2018), *B. sagittalis* DC. (Petenatti *et al.* 2007; Martinez *et al.* 2018), *B. burchellii* Baker, *B. organensis* Baker (Zuccolotto *et al.* 2019), *B. glaziovii* Baker (Jasinski *et al.* 2014), *B. megapotamica* Spreng. (Budel *et al.* 2012), *B. milleflora* (Less.) DC. (Pereira *et al.* 2014), *B. pentaptera* DC. (Budel *et al.* 2015), and *B. regnellii* Sch.Bip. *ex* Baker (Lima *et al.* 2023). Particularly for *B. palustris*, the available literature is restricted to a single micromorphological investigation, specifically focused on the cypselae surface patterns (Schneider & Boldrini 2011).

A morpho-anatomical description is considered the first and preferred method of botanical authentication, emphasizing diagnostic features that may be useful in distinguishing similar species. In fact, individual structural elements are relatively common within the same organs of related plant taxa. However, how these elements are organized in a species constitutes its characteristic fingerprint (Upton *et al.* 2011). Many *Baccharis* species have analogous structures making their macro-morphological identification somehow difficult (Freire *et al.* 2007; Budel *et al.* 2018a; Manfron *et al.* 2021b).

On the other hand, *Baccharis palustris* essential oil can be distinguished from those oils obtained from other representatives of the genus by the presence of C_9/C_{10}-polyacetylenes as main components (*ca*, 90%), which are responsible for the species characteristic aroma (Minteguiaga *et al*. 2022, 2023). Polyacetylenes are a group of low-molecular-weight bioactive compounds, occurring mostly in Asteraceae, Apiaceae, and Araliaceae families exhibiting great structural diversity (Christensen 2010; Minteguiaga *et al*. 2022). This group of compounds has a relevant physiological function in nature, exerting ecological roles as phytoalexins, anti-feedants, antifungals, antibacterials, allelopathic agents, or acting against abiotic stresses such as metal salts, detergents, and UV radiation (Christensen & Brandt 2006; Minto & Blacklock 2008; Christensen 2010; Konovalov 2014). Naturally occurring polyacetylenes have multiple pharmacological effects including anesthetics, anti-inflammatories, and antimicrobials (Christensen & Brandt 2006, Christensen 2010; Negri 2015; Xie & Wang 2022) and are of value primarily as cytotoxic-anticancer agents (Christensen & Brandt 2006; Christensen 2010, 2020).

The frequent use of the same common names for different *Baccharis* species leads to indiscriminate applications for similar therapeutic purposes (Freire *et al*. 2007; Manfron *et al*. 2021a). This work aimed to characterize the morpho-anatomy and histochemistry of the vegetative aerial parts of *B. palustris* to identify distinctive diagnostic characters as well as to elucidate the sites of synthesis and accumulation of metabolites with medicinal significance.

Material and Methods

Plant material

Vegetative aerial parts of *B. palustris* (leaves and stems) were collected in July 2021 in a marshy environment at "Paso Carrasco" (Canelones Department, Uruguay) (Fig. 1a-b). The taxonomic identification was conducted by A. H. González at the National Museum of Natural History (MNHN, Montevideo), and a voucher specimen was deposited at MNHN herbarium (MVM 23488 González).

Morpho-anatomical and histochemical analysis

Fully expanded leaves in good phytosanitary conditions and stem fragments from the second and fourth internode of three *B. palustris* specimens, were fixed in formalin-acetic acid 50%-ethanol, 5:5:90 v/v/v (FAA) during 24 to 48 h, or dried at room temperature for anatomical and histochemical characterization respectively.

Five fixed leaves, per plant specimen, were diaphanized according to Dizeo de Strittmatter (Argüeso 1986), cleared with commercial bleach (NaOCl) in water 1:1 and stained with cresyl violet 1% w/v and mounted in glycerol 50%. Leaf architecture and stomata types were described according to Dilcher (1974) and Ellis *et al.* (2019).

Pieces of 2 cm^2 of FAA fixed leaves containing the midvein and 2 cm longitude stem sections from the three individuals, were sectioned (25–35 μm) by microtome MICROM HM 315 (Mercado & Ponessa 2021), cleared with NaOCl (50% v/v), stained with astra blue and safranin O and mounted

Figure 1 – a-b. *Baccharis palustris* – a. collection site at Paso Carrasco, Canelones Department, Uruguay; b. general aspect of the plant. Scale bars: a = 15 cm; b = 5 cm.

in glycerol 50% (Zarlavsky 2014).

For histochemical characterization, dry leaves and stems were rehydrated for 10 min in distilled water, sectioned as previously described, and analyzed by light and fluorescence microscopy. To visualize phenolic compounds, samples were treated with $FeCl_3$ (10% w/v) in MeOH. Flavonoids and hydroxycinnamic acids were detected by their differential fluorescence (yellow-orange or green-bluish fluorescence, respectively) under UV light after treating the samples with Neu's reagent (2-aminoethyl-diphenylborinate), also known as NP or NPR (Natural Products Reagent), 1% in absolute methanol (Neu 1956; Merck 1980; Wagner & Bladt 1996). A saturated solution of Sudan IV in EtOH was used for the detection of lipids (Zarlavsky 2014). Essential oil terpenoids and polyacetylenes were evidenced with Nadi reagent (David & Carde 1964; Minteguiaga *et al.* 2023). Samples without staining were used as a control for comparative purposes.

Microscopy

For light microscopy, sections were viewed under a Zeiss Axiolab (Carl Zeiss Ltd, Oberkochen, Germany) microscope coupled to a Zeiss Axiocam ERc 5s digital camera. Photomicrographs were taken and scales were calculated using the AxioVision software version 4.8.2 (Carl Zeiss Ltd, Herts, UK). Fluorescence microscopy images were obtained in a Nikon Optiphot microscope (Nikon Corp., Tokyo, Japan) equipped with a UV light filter UV-1A using a 365 nm excitation filter and a 400 nm barrier filter.

For scanning electron microscopy (SEM), leaf and stem segments were fixed in glutaraldehyde phosphate, dehydrated properly in EtOH series, coated with gold (FineCoat Ion Sputter JEOL JFC-1100), and analyzed with a Carl ZEISS SUPRA-55 VP (Carl Zeiss Ltd, Oberkochen, Germany) at the Centro Integral de Microscopía Electrónica (CIME-CONICET-UNT, Tucumán, Argentina).

Results and Discussion

Leaf architecture

The leaves were simple, alternated, and short petiolated. Petiole of approximately 0.3 cm longitude, slightly winged. The leaf blade was subcoriaceous, symmetrical, narrowly elliptical-lanceolate to oblong-obovate, 2–4 cm longitude by 1–2.5 cm wide, hispid on both sides, with entire slightly revolute margin, obtuse apex occasionally shortly mucronate and symmetric decurrent base. The observed characters correspond to those previously described by Heering (1904) and Schneider (2009). Heering (1904), recognizes that obtuse ovate minutely mucronate leaves correspond to female specimens.

The primary vein framework was pinnate, camptodrome, and brochidodrome. The primary vein showed right course and moderate size (1.6%). Two secondary veins ran parallel to the primary vein while other decurrent secondary diverged forming moderate to wide acute angles. Secondary veins with moderate to wide acute angle of divergence (Fig. 2a). Admedial and external tertiary veins showed ramified or irregular poorly developed reticulated patterns, the external ones forming loops. Intercostal tertiary veins that ran perpendicular between two major secondaries parallel and decurrent secondaries were scarce. Quaternary veins showed ramified or reticulated patterns forming irregular areoles with absent, simple, or branched freely ending veinlets. Finally, the marginal ultimate venation was looped forming an intramarginal vein (Fig. 2b).

Leaf anatomy

The front view shows the cuticle smooth, both epidermis presented rectangular to isodiametric cells with straight to curved anticlinal walls, and the cells of the abaxial surface were smaller than the ones of the adaxial surface (Fig. 3a-b). In *Baccharis* species anticlinal epidermal cell walls can be straight to wavy generally with striated cuticles (Bobek *et al.* 2016; Minteguiaga *et al.* 2018) although smooth cuticles can also occur as in the leaves of *B. microdonta* DC. (Bobek *et al.* 2016; Manfron *et al.* 2021a).

In *Baccharis palustris* mainly anomocytic stomata occurred in the abaxial epidermis, although occasionally cyclocytic, anisocytic, hemi-amphibrachyparacitic, and twin stomata were observed (Fig. 3b-f), with a density of 135.43 ± 34.47 stomata per mm^2. The guard cells exhibited an average length of 39.33 ± 3.48 µm of longitude by 32.92 ± 3.16 µm of wide. Interestingly in *Baccharis*, both amphistomatic and hyposthomatic leaves can occur (Bobek *et al.* 2016; Budel *et al.* 2018a; Manfron *et al.* 2021a). Anomocytic stomata have been described as characteristic of Asteraceae (Metcalfe & Chalk 1972). Anomocytic, anisocytic (Freire *et al.* 2007; Petenatti *et al.* 2007; Cortadi *et al.* 1999; Freire *et al.* 2007; Rodriguez *et al.* 2013; Bobek *et al.* 2016; Minteguiaga *et al.* 2018; Budel *et al.* 2018a) and cyclocytic (Freire *et al.*

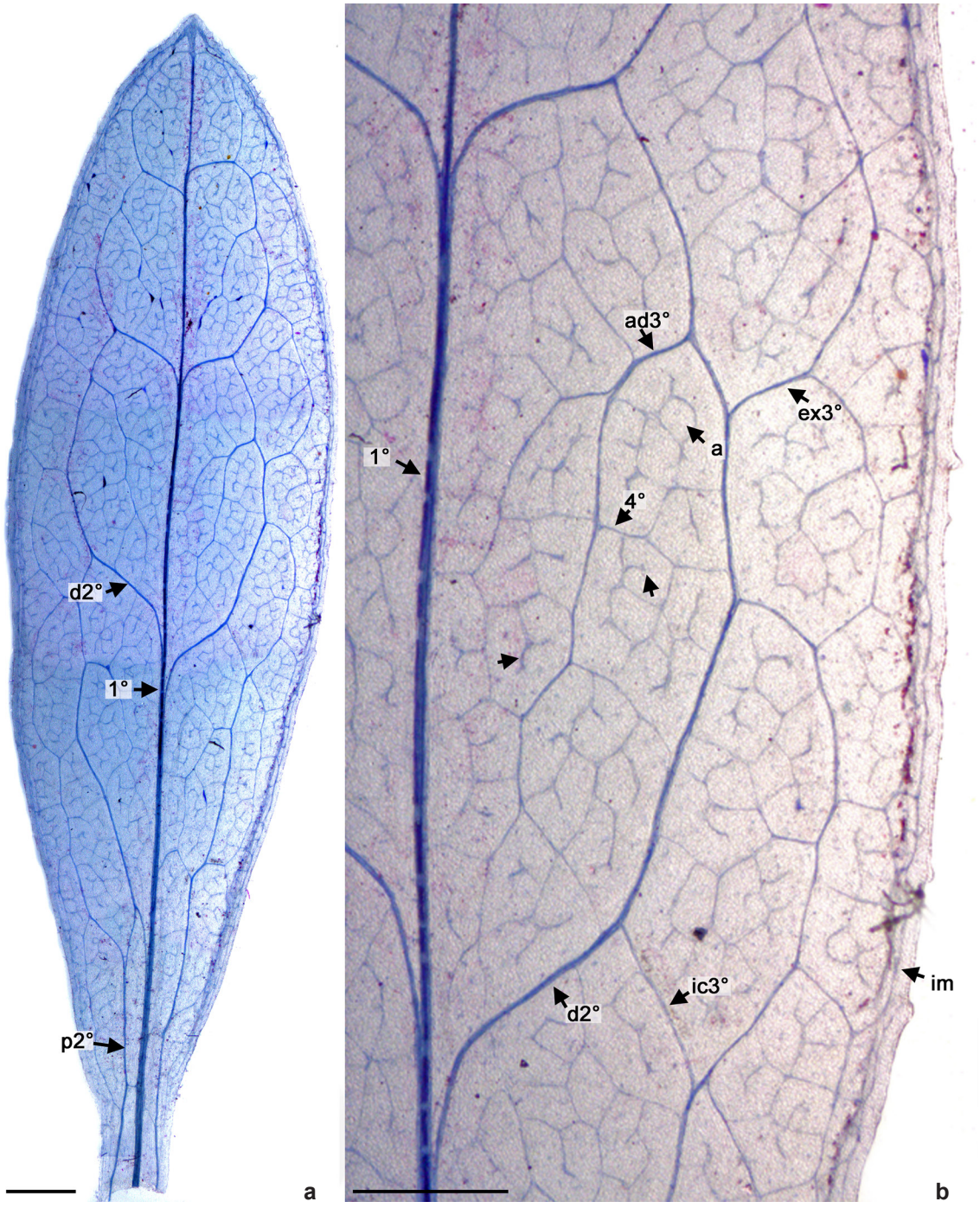

Figure 2 – a-b. *Baccharis palustris*. Leaf architecture – a. simple leaf with pinnate venation; b. leaf architecture detail. (1° = primary vein; 2° = secondary vein; a = areole; ad3° = admedial tertiary vein; arrow = simple or branched freely ending veinlets; d2° = decurrent secondary vein; ex3° = exterior tertiary vein; ic3° = intercostal tertiary vein; im = intramarginal ultimate venation; p2° = parallel secondary vein). Scale bars: a = 2 mm; b = 1 mm.

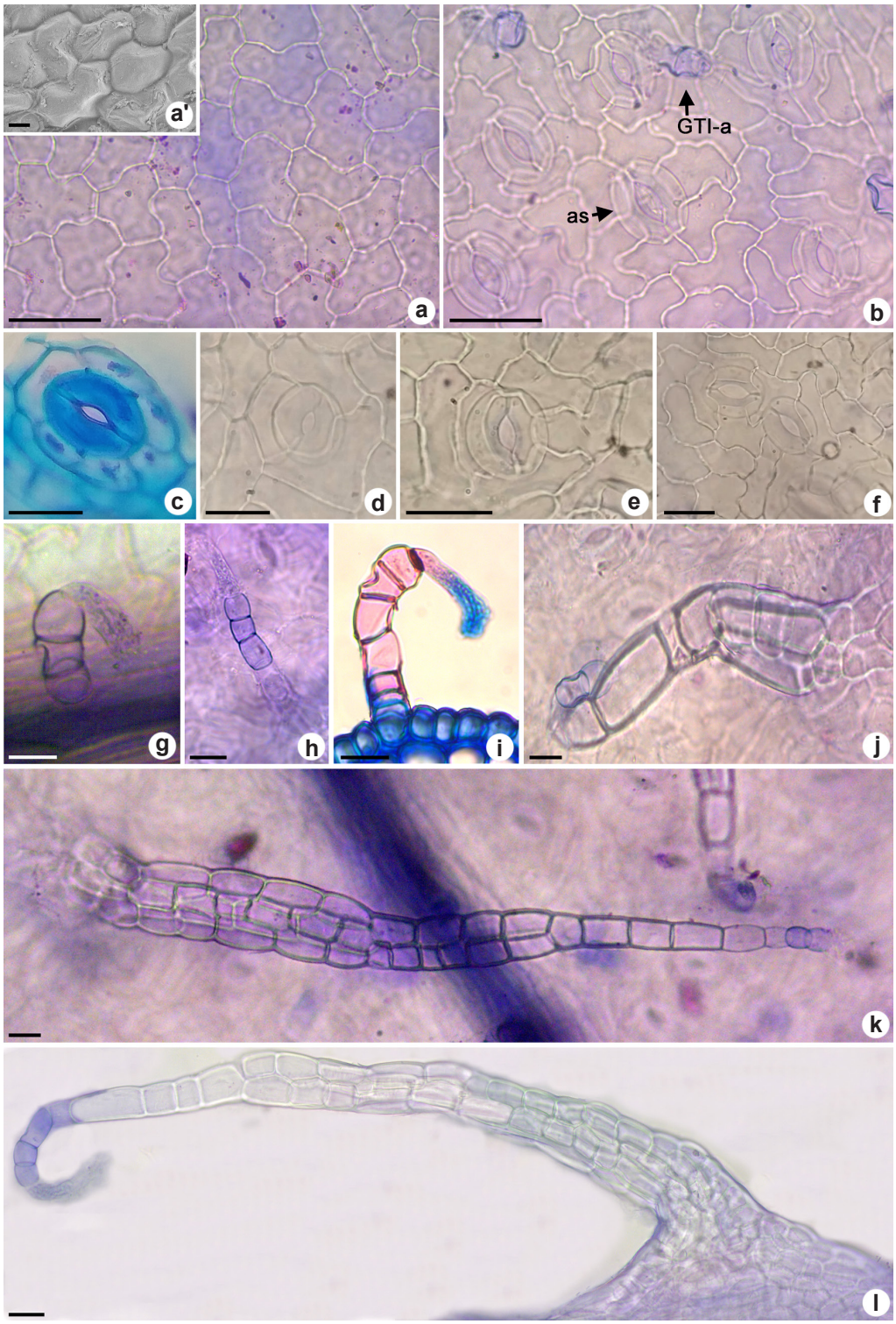

Figure 3 – a-l. *Baccharis palustris* – Leaf superficial view – a. adaxial epidermis; a'. the smooth cuticle in SEM; b. abaxial epidermis; c. cyclocytic stomata; d. anisocytic stomata; e. hemi-amphibrachyparacitic stomata; f. twin stomata; g-h. types of GTIa trichomes; i. types of GTIb trichomes; j. types of GTIIa trichomes; k. types of GTIIb1 trichomes; l. types of GTIIb2 trichomes. (GTIa = glandular trichome type; as = anomocytic stomata). Scale bars: a-b = 50 µm; detail in a = 10 µm; c-f = 30 µm; g-l = 20 µm.

2007; Petenatti *et al.* 2007; Rodriguez *et al.* 2013; Bobek *et al.* 2016; Budel *et al.* 2018a) stomata were reported for most *Baccharis* species, however staurocytic (Freire *et al.* 2007; Bobek *et al.* 2016; Budel *et al.* 2018a), tetracytic (Freire *et al.* 2007; Bobek *et al.* 2016; Budel *et al.* 2018a), hexacytic (Bobek *et al.* 2016) and actinocytic types (Freire *et al.* 2007; Budel *et al.* 2018a) have also been reported (Manfron *et al.* 2021a). Some authors demonstrated that species of *Baccharis* with similar foliar indument, can be differentiated based on the form of their epidermal anticlinal cell walls and by their stomata types, densities, and lengths (Freire *et al.* 2007; Martinez et *al.* 2018). Therefore, the results here presented might contribute to the differentiation between *B. palustris* and other *Baccharis* species from sect. *Aphyllae*.

Baccharis palustris showed isolated multicellular glandular trichomes, which were differentiated into two mayor groups with types and sub-types:

— GTI showing a basal cell, a uniseriate body (with voluminous cells), and a unicellular hyaline club-shaped to rounded head (generally collapsed exhibiting a flagelliform or whip-like appearance). This group was divided into two types according to their body cell number: GTIa, with the body formed by 2 to 4 cells (Fig. 3g-h), and GTIb, with the body formed by 6 to 8 cells (Fig. 3i). They measured around 55.58 ± 10.50 µm of longitude and were located on both leaf surfaces being more abundant and homogeneously distributed on the abaxial epidermis in a density of 4236.64 ± 2501.27 trichomes/mm^2.

— GTII presents a multiseriate base and a variable number of cells, a uniseriate body and a unicellular club-shaped head (also usually collapsed exhibiting a flagelliform or whip-like appearance), longitude ranging around 402.34 ± 123.89 µm. Among this group two types were defined according to their body cell number; GTIIa, short, multiseriated, and ending with 1 or 2 cells, with a club-shaped head (Fig. 3j), located on both leaf surfaces; and GTIIb, with 6 to 8 cells composing the body. In turn, this last type showed two sub-types characterized by the trichome form and distribution: GTIIb1, straight, located on the leaf blade and veins (Fig. 3k) and GTIIb2, curved-antrorse, located exclusively on the leaf margins (Fig. 3l).

Trichomes have been traditionally considered to be one of the most important anatomical characteristics for the diagnosis in *Baccharis* species (Freire *et al.* 2007; Budel *et al.* 2018a; Martinez *et al.* 2018; Ornellas *et al.* 2019). They usually appear isolated or inserted in epidermal depressions forming clusters or tufts (Manfron *et al.* 2021a). All the trichomes described in this work for *B. palustris* were isolated multicellular glandular, corresponding to flagelliform trichomes variations commonly found in numerous *Baccharis* species (Manfron *et al.* 2021a). Interestingly, the trichomes belonging to GTII, have not been described for any other representative of the genus, pointing out their value as diagnostic characters for the identification of *B. palustris* when compared to other related species.

Freire *et al.* (2007), Jasinski *et al.* (2014), Ornellas *et al.* (2019), and Budel *et al.* (2018a,b) demonstrated that the apical and body cells of some flagelliform trichomes presented amber contents, suggesting that this type of trichomes have mixed functional properties (mechanical protection and metabolites secretion), thus being an intermediate trichome type between glandular and non-glandular ones. In *B. palustris* the apical cells of both groups of trichomes (GTI and GTII) broke easily, releasing their contents to the leaf surface.

In cross section, *B. palustris* lamina was slightly flat in outline, with a revolute margin and a prominent midrib towards the hypophyll, in correspondence with a slight depression towards the epiphyll (Fig. 4a). A thin cuticle covered the one-layered epidermis, consisting of cells with thick outer walls, with the adaxial epidermal cells being larger than the abaxial ones. The mesophyll was dorsiventral, hypostomatic, with stomata slightly raised in relation to the epidermal cells, it showed 2 to 3 layers of palisade parenchyma and 3 to 6 of aerenchymatous spongy parenchyma (Fig. 4b). The leaf margin presented one layer of subepidermal laminar collenchyma (Fig. 4a insert). At the midrib toward both epidermal surfaces 1–3 layers of subepidermal laminar to angular collenchyma were observed, the vascular system consisted of a collateral bundle with sclerenchyma caps towards the phloem and xylem, accompanied by one or two schizogen secretory ducts located towards the abaxial surface adjacent to the phloem and surrounded by a complete or incomplete parenchyma sheath (Fig. 4b). The secretory ducts showed a one layered epithelium of 5 to 6 cells with dense cytoplasm and amber contents (Fig. 4c), typical coloration of this species essential oil (Minteguiaga *et al*. 2022). The lower-order veins presented similar characteristics, sometimes

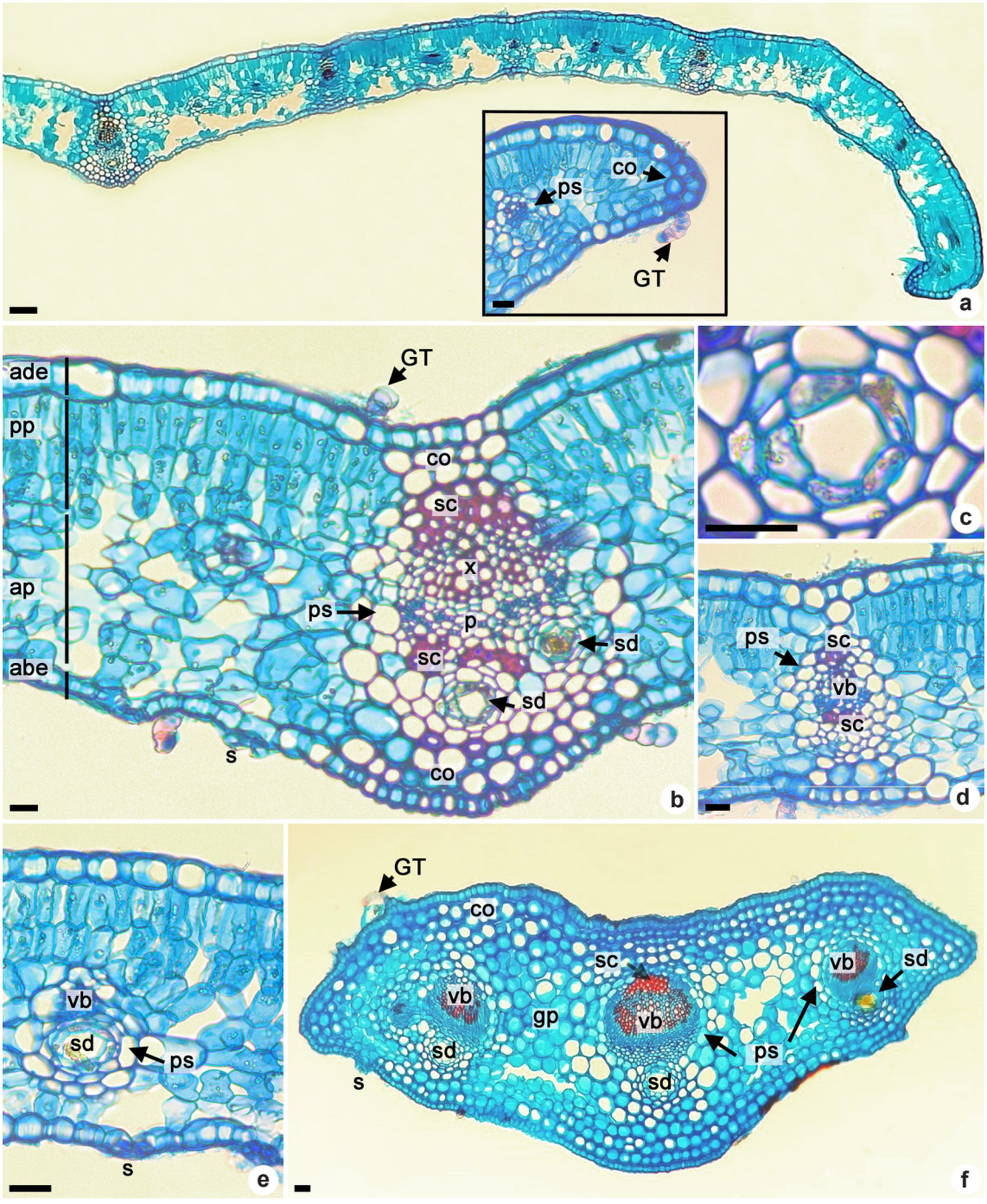

Figure 4 – a-f. *Baccharis palustris*. Leaf anatomy in cross section – a. the lamina with slightly flat in outline, insert detail the of leaf margin; b. mesohyll and midrib; c. secretory duct; d-e. lower order secondary and tertiary vein respectively; f. petiole. (abe = abaxial epidermis; ade = adaxial epidermis; ap = aerenchymatous parenchyma; co = collenchyma; gp = ground parenchyma; GT = glandular trichome; p = phloem; pp = palisade parenchyma; ps = parenchymatous sheath; s = stomata; sc = sclerenchyma; sd = secretory duct; vb = vascular bundle; x = xylem. Scale bars: a = 100 µm; inset in a, b-f = 20 µm.

without exhibiting the secretory ducts and the sclerenchyma caps (Fig. 4d-e).

The shape of the midrib in a transverse section has diagnostic value in *Baccharis* (Bobek *et al.* 2016; Budel *et al.* 2018a). Most of the species possess isobilateral mesophyll in the leaf (Manfron *et al.* 2021a). However, a dorsiventral arrangement was observed in *B. anomala* DC. (Budel & Duarte 2008), *B. singularis* (Vell.) G.M. Barroso (Souza *et al.* 2011), *B. ochracea* Spreng. and *B. punctulata* DC. (Budel *et al.* 2018a). Moreover, the aerenchyma exhibited in *B. palustris* spongy mesophyll could be interpreted as an adaptation to the wetland environment where this species grows, providing a pathway of low resistance for oxygen diffusion (Björn *et al.* 2022).

Secretory ducts have been extensively described in *Baccharis* (Bobek *et al*. 2016; Almeida *et al.* 2021; Manfron 2021a). Usually, a single secretory duct occurs in the midrib, however in some species such as *B. paucifloscula* DC. and *B. organensis* two and three secretory ducts were reported, respectively (Budel *et al.* 2018a; Zuccolotto *et al.* 2019), suggesting that this feature may be usefully for species identification.

Petiole anatomy

In cross-section *B. palustris* petiole showed a subeliptical shortly winged outline with pointed ends. As in the leaf, the midrib was slightly prominent towards the hypophyll, in correspondence with a slight depression towards the epiphyll. The epidermis was one layered with a thin and smooth cuticle; laminar to angular subepidermal collenchyma (1 to 2 layers) was evident towards both surfaces, continued by 6 to 8 layers of ground rounded parenchyma cells. Three to five collateral vascular bundles were arranged in line, the largest one was located in a central position showing collenchyma or sclerenchyma caps toward the xylem and phloem (Fig. 4f). The trichomes were similar to the ones described for the lamina, being more abundant the GTIb, GTIIa and GTIIb types.

Similar petiole characteristics were reported for *B. spicata* (Lam.) Baill. (Oliveira *et al*. 2011), *B. glaziovii* (Jasinski *et al.* 2014) and *B. microdonta* (Bobek *et al.* 2016).

Stem anatomy

Baccharis palustris stems showed striated hispidus surfaces when young. In paradermal view, the stem epidermis presented rectangular cells with straight walls, cyclocytic stomata slightly elevated in relation to the epidermal cells, and multicellular glandular trichomes of types GTIa, GTIIa, and GTIIb. In cross-section, the stem exhibited a subpentagonal outline with 5 lobes and concave valleys (Fig. 5a). In each lobe a collateral vascular bundle with a sclerenchyma cap toward the phloem and surrounded by a aerenchymatous sheath with an endodermis was observed; being these bundles externally accompanied by a secretory duct toward the phloem. The stem showed unusual growth, due to the simultaneous presence of the lobes bundles and a continuous cambium with secondary phloem and xylem. The stem's epidermis was one layered with thin cuticles. A continuous subepidermal ring of laminar and lacunar collenchyma (1 to 3 layers, thicker at the lobes) was observed, followed by cortical parenchyma (1 to 5 layers), which internally developed a continuous 1 to 2 layered angular collenchyma. The endodermis was the last cortical layer before the vascular cylinder. Schizogenic secretory ducts were evident in the inner cortex, while sclerenchyma strands with or without lignification are visible next to the phloem (Fig. 5b-c). Finally, the pith of *B. palustris* stem was aerenchymatous and lignified in the outer layers, it contained simple amyloplasts, and prismatic calcium oxalate crystals of various shapes (Fig. 5d).

A distinctive characteristic feature found in many *Baccharis* species is the presence of minor collateral vascular bundles, which are surrounded by an endodermis and arranged either on the wings of the cladodes or externally to the vascular cylinder within the cortical parenchyma (Bobek *et al.* 2016; Budel *et al.* 2018a; Minteguiaga *et al.* 2018).

Petenatti *et al.* (2007) mentioned that in *Baccharis* stems the subepidermal collenchyma can be discontinuous and alternating with chlorenchyma, as in *B. sagittalis* and *B. triangularis*; while in other species as observed in *B. palustris* the subepidermal collenchyma forms a continuous stratum. Meanwhile, the secretory ducts were always located in the inner portion of the cortex next to the parenchyma sheathes or endodermal layers in all *Baccharis* species. (Manfron *et al*. 2021a).

Different crystal morphotypes have been reported for the leaves and stems of several species of *Baccharis*, showing characteristics shapes, and composition of crystals within the same species. Thus, the crystal macro patterns aid in species

identification and provide data for the *Baccharis* taxonomy (Manfron 2021a; Bobek *et al.* 2016; Raeski *et al.* 2023a, b).

Histochemistry

Several members of the *Baccharis* genus, are high producers of essential oils (Manfron *et al.* 2021b; Fernandes *et al.* 2021), and *B. palustris* was not an exception, being differentiated by the presence of C_9/C_{10}-polyacetylenes as main components (Minteguiaga *et al.* 2022, 2023). These volatile oils are usually found in glandular trichomes and secretory ducts (Budel *et al.* 2012; Jasinski *et al.* 2014; Minteguiaga *et al.* 2018).

In this work, due to the delicate nature of GTII trichomes, the histochemical analyses were performed exclusively on leaf and stem secretory ducts and GTI trichomes. The glandular trichomes and secretory ducts of the control sections (Fig. 6a-d) exhibited evident amber/brown contents, which was reported for this species of essential oil (Minteguiaga *et al.* 2022). Within both GTIa

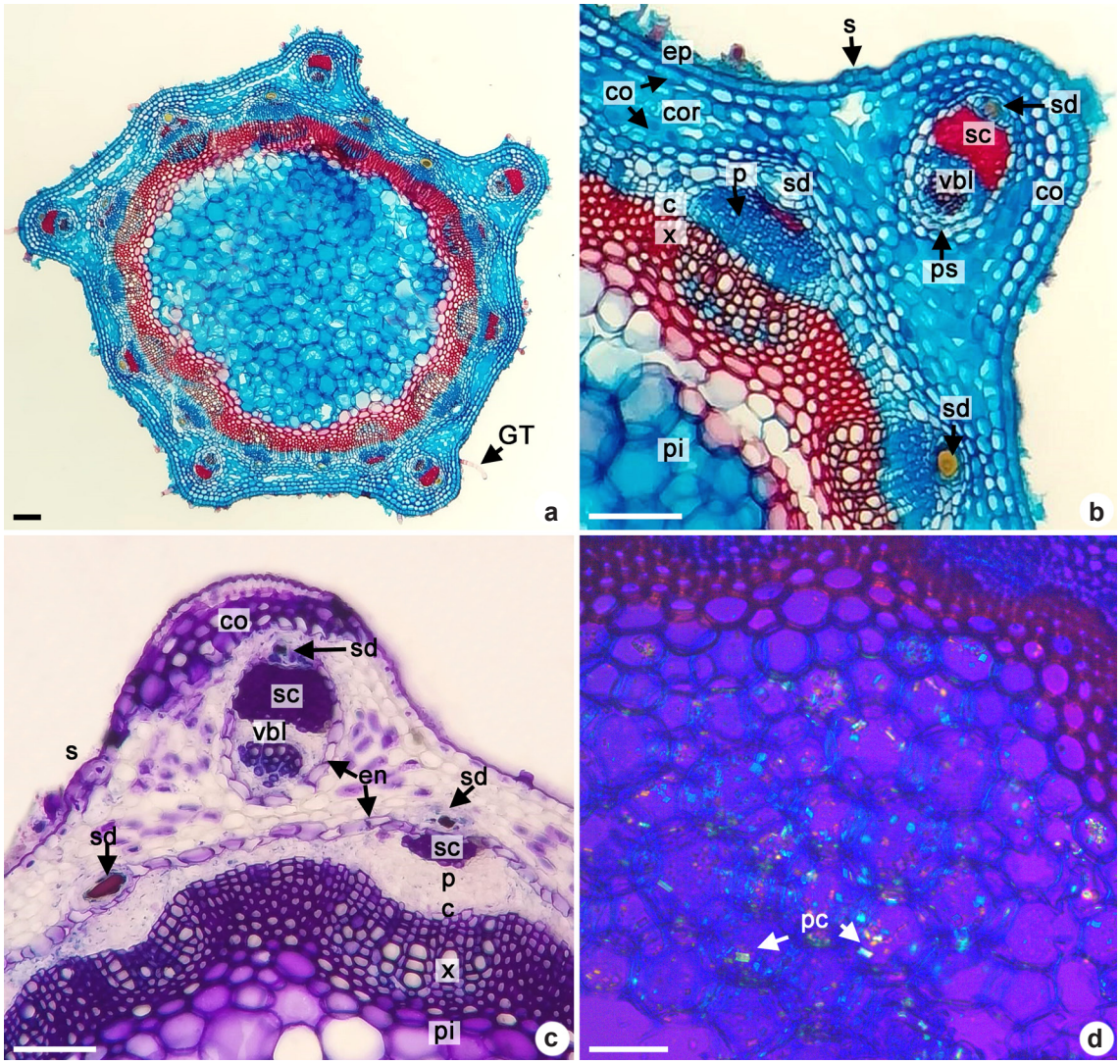

Figure 5 – a-d. *Baccharis palustris*. Stem anatomy in cross section – a. subpentagonal stem with 5 lobes; b-c. detail of lobe; d. pith with calcium oxalate crystals under polarized light microscope. (c = cambium; co = collenchyma; cor = cortex; en = endodermis; ep = epidermis; GT = glandular trichome; p = phloem; pb = aerenchymatous bundle; pc = prismatic crystal; pi = pith; s = stomata; sc = sclerenchyma; sd = secretory duct; vbl = vascular bundle of the lobe; x = xylem). Scale bars: a-d = 50 µm.

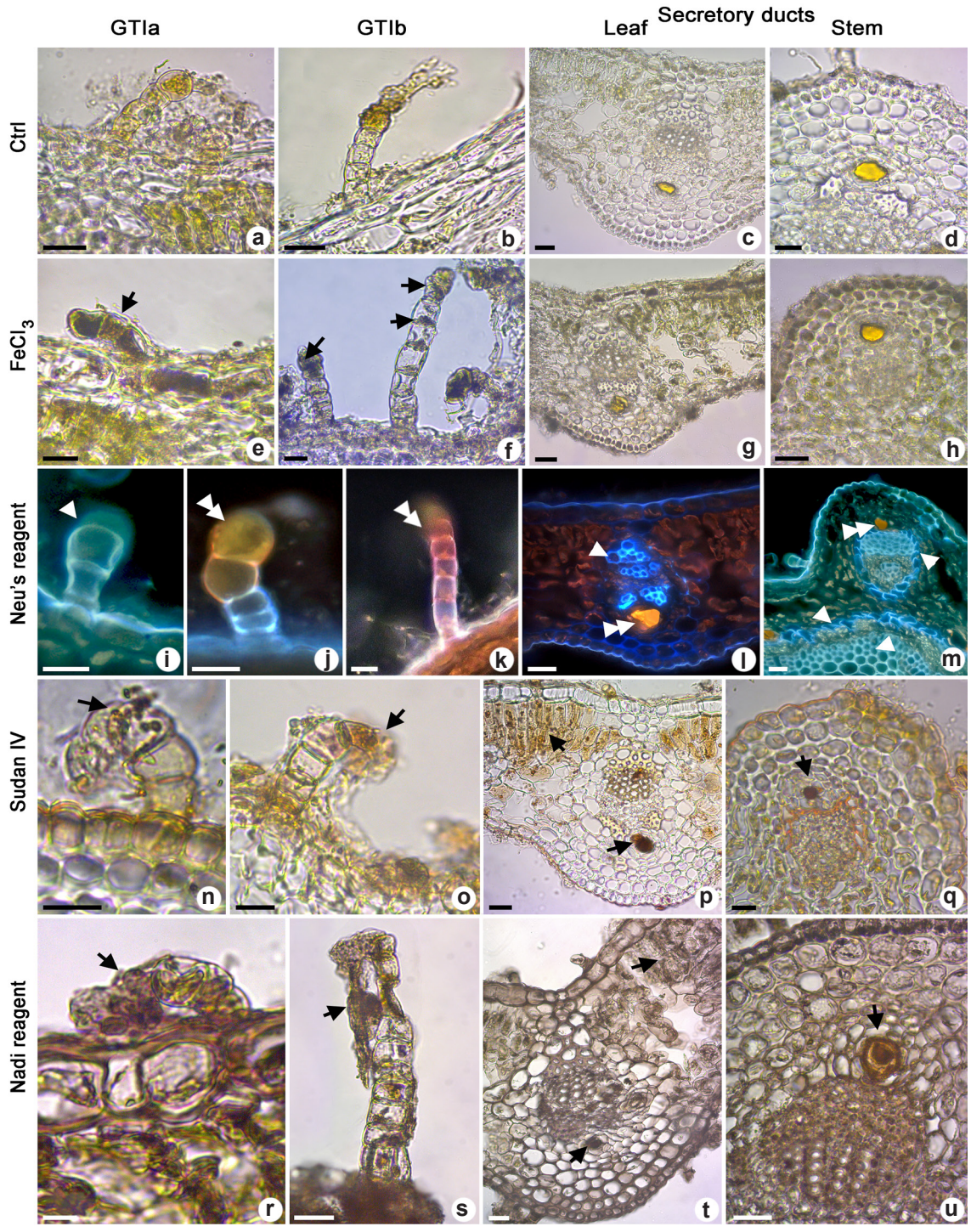

Figure 6 – a-u. *Baccharis palustris*. Histochemistry – a, e, i, n, r. glandular trichome type GTIa; b, f, j-k, o, s. glandular trichome type GTIb; c, g, l, p, t. leaf secretory ducts; d, h, m, q, u. stem secretory ducts; a-d. control (abbreviated as Ctrl in the image); e-h. $FeCl_3$ to identify phenolic compounds (arrow); i-m. Neu's reagent to detect hydroxycinnamic acid derivatives by their green-bluish fluorescence (arrowhead) and flavonoids by their yellow-orange fluorescence (double arrowhead). Red fluorescence is due to the chlorophylls. n-r. Sudan IV for the detection of lipids; s-v. Nadi reagent for the identification of terpenes and polyacetylenes (arrow). Scale bars: a-u = 20 µm.

and GTIb, as well as in the secretory ducts, the presence of lipids, terpenes/polyacetylenes, and phenolic compounds were identified (see Tab. 1; Fig. 6e-u). Additionally, terpene was observed in the palisade mesophyll of the leaf (Fig. 6p-t). Comparable findings were previously reported for other *Baccharis* species (Freire *et al.* 2007; Budel *et al.* 2012; Jasinski *et al.* 2014; Ornellas *et al.* 2019; Budel *et al.* 2018a, b; Minteguiaga *et al.* 2018; Almeida *et al.* 2021).

The occurrence of flavonoids and other phenolic compounds was reported in about 15% of *Baccharis* species. These compounds exert antioxidant activity and constitute an interesting source of metabolites with hepatoprotective, antimicrobial, anti-inflammatory, antitumoral, and antiviral, among other activities (Bastos & Arruda 2021; Grecco *et al.* 2021).

We provide here, for the first time, the description of *Baccharis palustris* leaf architecture, as well as the leaf and stem anatomy. Many macroscopic and microscopic characteristics of *B. palustris* aerial vegetative organs correspond with common features described within the broader *Baccharis* genus and for other members of the section *Aphyllae*. However, *B. palustris* presented a set of distinctive characteristics possessing diagnostic value: simple leaves with entire revolute margins, foliar epidermal cells with straight to curved anticlinal walls, different types of stomata and glandular trichomes (including multiseriate GTIIs, as novel within the genus), dorsiventral mesophyll featuring aerenchymatous spongy tissues, five-lobed stems, secretory ducts and prismatic calcium oxalate crystals. Furthermore, this study revealed the sites of synthesis and accumulation of lipids, terpenes/polyacetylenes, and phenols within the glandular trichomes and secretory ducts, providing valuable insights into their ecological role. Also contributing to the knowledge of this species highlighting the importance of its preservation as a valuable resource for pharmaceutical research.

Table 1 – *Baccharis palustris* histochemistry.

Compound	Reagent / specific staining	Positive reaction	GT Ia and Ib		Ducts	
			Body cells	Head cell	Secretory cells	Lumen content
Phenols	$FeCl_3$	Green-grey	+	+	-	-
Flavonoids	Neu's reagent	Yellow orange fluorescence	+	-	+	+
Hydroxycinnamic acids	Neu's reagent	Green-blue fluorescence	+	-	-	-
Lipids	Sudan IV	Red	+	+	+	++
Terpenes/ polyacetylenes	Nadi reagent	Blue-purple	+	+	+	+

References: (+) positive, (-) negative, identified by comparison to control.

Acknowledgements

For funding to projects B-0001-1 and B-0002-1, Fundación Miguel Lillo.

Data availability statement

In accordance with Open Science communication practices, the authors inform that all data used in this manuscript are available upon request to the corresponding author.

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Area Editor: Dra. Mariana Alves
Received on July 31, 2023. Accepted on November 21, 2023.