Chapter 9
Morpho-anatomical Characteristics of Species of *Baccharis*

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Abstract *Baccharis* L. is one of the largest and most diversified genera of Asteraceae. Two groups of species, namely, "vassouras" and "carquejas," are generally recognized based on their habit and branching pattern. "Vassouras" plants possess regular stems and leaves and are often broom-like in appearance, whereas "carquejas" have stems that are modified as cladodes and are devoid of leaves or have diminutive, scalelike leaves. Despite these major differences in their growth forms, stems, and leaves, the species within these groups show close resemblances in their morphologies. This chapter provides a comprehensive review of the morphology and anatomy of the genus and discusses the main morpho-anatomical features helpful in the identification of various *Baccharis* species. Further research involving comparative morpho-anatomical studies are required to better understand the species diversity as well as to develop a more accurate classification of *Baccharis* as well as of Asteraceae.

1 Introduction

Morpho-anatomy is the study of morphological and anatomical forms and structures with emphasis on features, which may be useful in distinguishing the species. A morphological examination is considered the first and preferred method of botanical authentication when the required features are available.

Plant anatomy is valuable for the evaluation of dried plant material, especially for fragmented or powdered material. Dried or fragmented plant parts do not result in the loss of most microscopic characteristics, which makes plant anatomy the

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most stable technique for the evaluation of plant characteristics when it comes to authentication.

Morphological and anatomical analyses are inherent procedures in almost all pharmacopoeias and are the main identification tests required for the herbal industry. Even though the individual structural elements are relatively common within the same type of plant parts, how the elements are organized gives a plant species its characteristic fingerprint (Upton et al. 2011). Additionally, morphology and microscopy of botanical samples and microscopic descriptions of impurities are included in most pharmacopoeias demonstrating the importance of these characteristics in the quality control of medicinal plants.

Previous workers have stated that many *Baccharis* species have analogous morphologies making their morphological identification problematic (Freire et al. 2007; Budel et al. 2008). Species within the section *Caulopterae*, such as *B. crispa* Spreng. and *B. articulata* (Lam.) Pers., show a high degree of phenotypic plasticity leading to difficulties in species identification even in flowering stages (Simões-Pires et al. 2005).

In this context, microscopic characteristics can provide helpful information in solving taxonomic problems (Bobek et al. 2016; Budel et al. 2018a).

Another problem is that different species of *Baccharis* are called by the same folk names and used indiscriminately for the same therapeutic indications (Budel and Duarte 2010; Bobek et al. 2015a, 2016; Budel et al. 2018a). Considering traditional names, "carquejas" (plants with winged stems or cladodes) and "vassouras" (plants with broom-like and without winged stems) are the most common, creating confusion in the identification of *Baccharis* species. The confusion due to similar popular names is one of the main causes of misidentification of plant drugs (Upton et al. 2011).

2 Morphology

Baccharis L. is one of the largest genera in the subfamily Asteroideae Lindley within the family Asteraceae (Compositae) and comprises about 440 species (Heiden et al., 2019). All of the species are native to New World with about 90% of them occurring in South America (Nesom 1990). It is also introduced into several parts of the world including Europe, Transcaucasus, and Australia. *Baccharis* is commonly called "Groundsel tree" although the name "Groundsel" is also applied to different species of *Senecio* L. (Asteraceae). Species of *Baccharis* comprise mostly dioecious shrubs and show a wide range of morphological diversity. The most important morphological characters useful in *Baccharis* species identification include the growth form; branching pattern; the shape, size, and margins of leaves; type of indumentum; shape and arrangement of flower heads; and number of nerves on cypselae (fruits). Both staminate and pistillate specimens are important for species identification. Natural hybridization between different species occurs, resulting in the development of intermediate forms showing interspecific variations. For example, hybridization between *B. halimifolia* L. and *B. neglecta* Britton and between *B. halimifolia* and *B. angustifolia* Michx. has been reported. Intermediate forms between *B. thesioides* Kunth and *B. bigelovii* A. Gray have been observed in southern Arizona (eFloras 2008).

The genus is broadly characterized by the tufted indumentums of the leaves and stems, which consists of fused trichomes with a single adjoining basal cell. Dioecy is a common occurrence (Müller 2006).

The genus *Baccharis* has not been taxonomically revised, as a whole. Due to the high number of species, the revisionary works in the past have been carried out at the level of geographical regions. In South America, there are works in Colombia (Cuatrecasas 1969), central Argentina (Ariza-Espinar 1973), and Brazil (Barroso and Bueno 2002). Barroso (1976) and Oliveria et al. (2006) have studied the species for Brazil and reported 125 and 146 species, respectively. Nesom (1990) studied 43 species from the USA, Mexico, and Central America and grouped those under 6 sections.

Several authors have proposed different infrageneric classifications of *Baccharis* mainly based on morphological characters. De Candolle (1836) was the first author to classify *Baccharis* into eight sections based on leaf morphology. Baker (1882–1884) classified the Brazilian species into six series also using leaf characteristics. Cuatrecasas (1967) revised the Colombian species into sections. Giuliano (2001) subdivided 96 Argentinean species of *Baccharis* into 15 sections. Of these, the sect. *Caulopterae* DC. is characterized by the presence of longitudinal wings on the stems. This section was previously named *Alatae* Less. (Ariza-Espinar 1973) and *Trimera* group (Barroso 1976).

Heering (1904) provided the first subgeneric classification of *Baccharis* with five subgenera, namely, *Baccharis*, *Molina*, *Pteronioides*, *Stephananthus*, and *Tarchonanthoides*. In the most recent subgeneric classification of the genus, Müller (2006) accepted four of the five subgenera proposed by Heering (1904) and placed the subgenus *Stephananthus* as *incertae sedis* ("of uncertain placement"). Heiden and Pirani (2012) provided a taxonomic revision of the subgen. *Tarchonanthoides* with four sections, namely, *Canescentes*, *Coridifoliae*, *Curitybensis*, and *Tarchonanthoides*. This subgenus consists of 21 species and is characterized mainly by the corollas of the female florets with 5 papillose teeth and the male florets with a stigma nearly fully divided into lanceolate or ovate branches (Heiden and Pirani 2012). Recently, based on phylogenetic studies, Heiden et al. (2019) classified *Baccharis species into 7 subgenera and 47 sections.*

In some instances, different species of *Baccharis* exhibit similar morphological features leading to confusion in species identification. For example, *B. brevifolia* DC., *B. microdonta* DC., *B. pauciflosculosa* DC., and *B. trilobata* A.S. Oliveira & Marchiori are all commonly called "vassouras" (meaning "broom") in Brazil, due to their similar appearances (Bobek et al. 2016) as well as their use as brooms to clean the house. The name "carqueja" is applied to multiple species, including *B. crispa*, *B. articulata*, and *B. pentaptera* (Less.) DC. (Budel et al. 2005), due to their close resemblances in the morphological features. As a result, different species are used interchangeably (Gianello et al. 2000).

The number of striations (ribs) on the stems can be useful in recognizing species. Bobek et al. (2016) observed 5-6- ribbed stems in B. *brevifolia and B. pauciflosculosa and 4-ribbed stems in B. microdonta* and *B. trilobata*, using anatomical evaluation. The shape of the stem in the cross section is determined by the number of ribs.

Morphological Description

Baccharis are perennial subshrubs, shrubs, or trees, growing 0.1–6 m tall, usually dioecious and rarely monoecious, usually woody at base, rarely rhizomatous [e.g., *B. acaulis* (Wedd. ex R.E.Fr.) Cabrera and *B. davidsonii* Cuatrec.]. In the "vassouras" group of species, including *B. sarothroides* A. Gray, *B. brevifolia* (Fig. 9.1a), *B. dracunculifolia* DC. (Fig. 9.1b), *B. microdonta* (Fig. 9.1c), *B. pauciflosculosa* (Fig. 9.1d), and *B. trilobata*, the plants are often much-branched and broom-like (Fig. 9.1a–d).

Stems in *Baccharis* are erect, ascending, procumbent (*B. caespitosa* (Ruiz & Pav.) Pers., *B. alpina* Kunth, *B. humifusa* Kunth, and *B. pumila* Joch. Müll.) or rarely prostrate (*B. acaulis*), short or well developed, simple (*B. texana* (Torrey & A.Gray) A.Gray) or many-branched, usually striated, rarely terete and smooth, glabrous to hispidulous or villous (*B. plummerae* A.Gray subsp. *plummerae*), often resinous (*B. plummerae* A.Gray subsp. *glabrata*, *B. pteronioides* DC.); young stems are usually green.

In the "carqueja" group of species, the stems are modified into cladodes with 2–5 wings (*B. glaziovii*, *B. junciformis* DC. (syn. *B. usterii* Heering), *B. trimera*, *B. pentaptera* (Fig. 9.2a), and *B. sagittalis* (Less.) DC.), which run along the longitudinal axis of the stem. These stems are either leafless or with sparse leaves that are reduced to scales, such as in *B. glaziovii* (Fig. 9.2b).

Fig. 9.1 Habit of "vassouras" plants. *Baccharis brevifolia* (**a**), *B. dracunculifolia* (**b**), *B. microdonta* (**c**), and *B. pauciflosculosa* (**d**). Scale bar: b = 1 cm; a, c, d = 5 cm. (**a**, **c**, **d** reproduced with permission from Bobek et al. 2016)

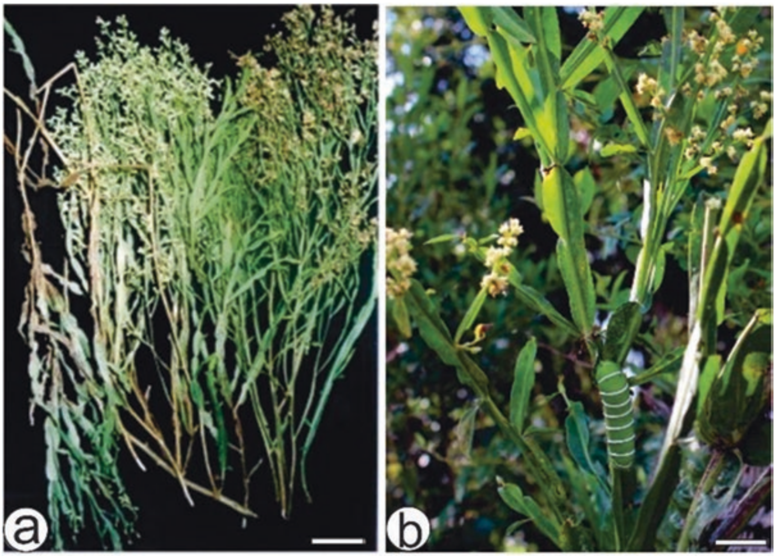

Fig. 9.2 Habit of "carquejas" showing winged stem cladodes. *Baccharis pentaptera* (**a**), *B. glaziovii* (**b**). Scale bar = 1.5 cm. (Reproduced with permission from Budel et al. 2015 (**a**) and Jasinski et al. 2014 (**b**))

Leaves cauline, sometimes deciduous and sparse or absent at flowering (*B. vanessae* R.M.Beauch.), sometimes reduced to scales distally (*B. sarothroides*), simple, alternate, rarely in rosettes, sessile (*B. sergiloides* A. Gray), or petiolate; lamina 1–3-nerved, linear, oblong, lanceolate, ovate, obovate or rhomboid, glabrous or rarely hispidulous or villous, often gland-dotted and resinous, usually acute or obtuse at apex, acute or tapering at base, entire or finely to coarsely serrate along margins (eFloras 2008).

Inflorescences are head-like, sessile or stalked, unisexual, discoid, solitary (*B. acaulis*, *B. alpina*, *B. davidsonii*, *B. pumila*, and *B. tola* Phil.), or in paniculiform, corymbiform, or racemiform (*B. pteronioides*) arrays; involucres cylindrical to campanulate or hemispheric, 3–9 mm in diameter; phyllaries 15–40 arranged in 2–5 series, mid usually green, sometimes red or purple, 1-nerved, ovate to lanceolate, oblique, scarious along margins, often erose, ciliate or keeled, midrib conspicuous or not, obtuse, acute, acuminate or keeled at apex, glabrous or hispid; receptacles flat, tholiform or conical, pitted or smooth, glabrous, tomentose or glandular, and usually epaleate. Staminate florets 10–50; corollas white to pale yellow, tubular; lobes 5, spreading-reflexed, triangular to ovate; pappi 20–40, equal, minutely barbellate or distally with plumose bristles. Pistillate florets 20–150; corollas whitish, narrowly tubular; lobes 5, spreading-reflexed, triangular to ovate; styles glabrate, flattened, and unappendaged (eFloras 2008).

Fruits are cypselae or achenes, obovoid to cylindrical, slightly compressed or 5-angled, 5–10-nerved, glabrous or hispid, light brown; pappi 25–50, whitish, tawny or brownish, minutely barbellate, apically attenuate bristles in 1–3 series, persistent or deciduous, accrescent in fruit (eFloras 2008).

3 Anatomy

Anatomical characteristics are important in the authentication of plants, especially when the plants are dried or fragmented. The drying and the fragmentation of the plants do not result in the loss of the most relevant microscopic characteristics, that is, the anatomical markers. Therefore, these are the reliable characters helpful in the identification and quality control of the botanical samples. Also, microscopy has the advantage of requiring small amounts of material. Microscopic analysis is helpful in the detection of inorganic materials adhered to parts of the plant, such as roots. Microscopy can also detect when different parts of the same plant are present in the sample.

Certain anatomical characteristics helpful in the discrimination of species of *Baccharis* include the epidermal features (contour of anticlinal epidermal cell walls, type of stomata, type of trichomes), the organization of mesophyll and vascular tissues, and the secretory ducts and morphotypes of crystals.

Epidermal Characters

The epidermis is a permanent and complex tissue, including different kinds of cells. Epidermal features can show many different anatomical markers, which may help in the diagnosis of the plant. Several authors have contributed to resolving taxonomic problems in *Baccharis* through the micromorphological analysis of leaves (Muller 2006; Freire et al. 2007; Bobek et al. 2016; Budel et al. 2018b; Almeida et al. 2021).

Epidermal characteristics, such as cuticle, epidermal cells, stomata, and trichomes, have been identified as important tools in the delimitation of some complex genera, such as *Passiflora* (Wosch et al. 2015) and *Piper* (Gogosz et al. 2012), and so is *Baccharis*. They also help in distinguishing medicinal species, since drugs of pharmaceutical use are made up of dried and fragmented parts in which the different macroscopic features of the species are not generally distinguishable (Jackson and Snowdon 1990; Jorge 2000).

Epidermal Cells

The epidermis is the outermost cell layer of a plant protecting against evaporation and attack by microorganisms and herbivores. In a surface view of the epidermis, the anticlinal cell walls may be straight, wavy, or sinuous. Cutin, in the form of the cuticle layer, is deposited on the outer surface of the epidermis to offer a waterproof surface enabling water retention. The cuticle may have a flat surface or display characteristic ornamentation.

In *Baccharis*, the leaves have straight (Fig. 9.3a) to wavy (Fig. 9.3b) anticlinal epidermal cell walls as seen in *B. illinita* DC. (Fig. 9.3a), *B. microdonta*, *B. punctulata* DC. (Fig. 9.3b), *B. sphenophylla* Dusén ex Malme (Budel et al. 2018a), *B. uncinella* DC. (Budel and Duarte 2008a), *B. junciformis* (Budel and Duarte 2010), *B. trilobata*, and *B. brevifolia* (Bobek et al. 2016). However, sinuous anticlinal epidermal walls (Fig. 9.3c) were reported for *B. anomala* DC. (Budel and Duarte 2008b), *B. decussata* (Klatt) Hieron., and *B. pentlandii* DC. (Freire et al. 2007). All of these species belong to *Baccharis* subgen. Molina (Pers.) Heering. This characteristic can be helpful for the identification of the species, as well as for the delimitation of *Baccharis* sections.

In *Baccharis*, the cuticle is typically striated (Fig. 9.3e–i), but it can also be smooth as observed in the leaves of *B. microdonta* (Fig. 9.3d), and winged stems of

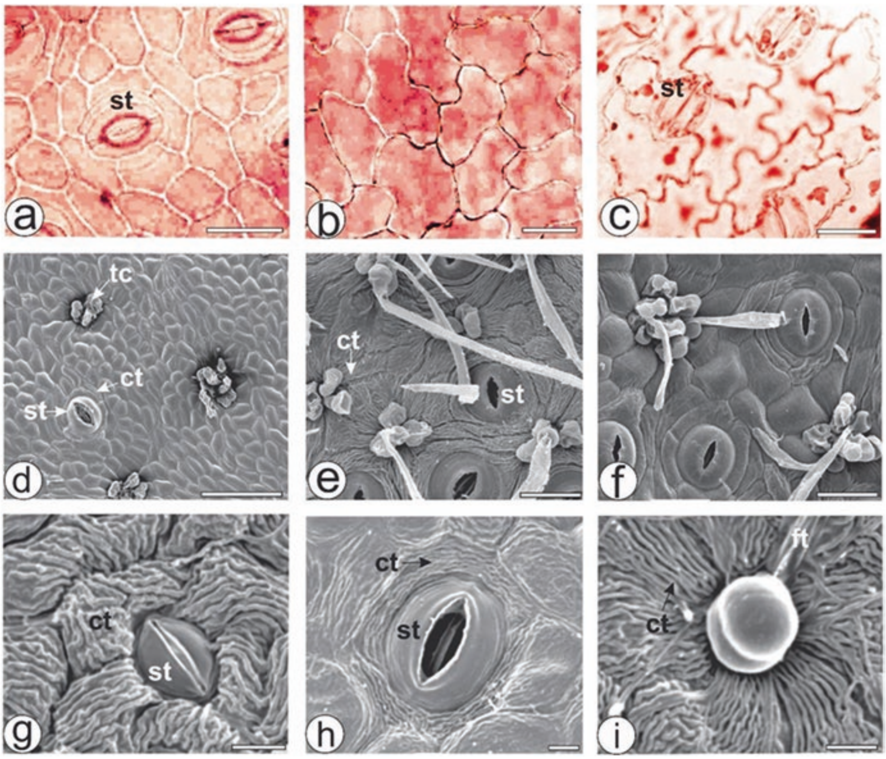

Fig. 9.3 Leaf epidermis in *Baccharis.* [(**a**–**c**) Light microscopy, stained in safranin; (**d**–**i**) scanning electron microscopy]. *B. anomala* (**c**), *B. illinita* (**a**), *B. microdonta* (**d**), *B. pauciflosculosa* (**e**, **i**), *B. punctulata* (**b**, **g**), *B. sphenophylla* (**h**), and *B. trilobata* (**f**). Abaxial side (**c**, **g**, **h**) and adaxial side (**d**, **f**, **i**). Scale bars: b, f = 50 µm, a, c–e, g–i = 100 µm. (**a**, **b**, **d**, **e**, **g**, **h**, **i** reproduced with permission from Budel and Duarte 2008b; from Bobek et al. 2016; and from Budel et al. 2018a; **c**)

B. articulata (Cortadi et al. 1999) and *B. myriocephala* Baker (Sá and Neves 1996). The striate cuticle can occur on both surfaces or either on the abaxial or the adaxial sides (Bobek et al. 2016; Budel et al. 2018a; Almeida et al. 2021). Besides, species of this genus can show different cuticle orientation around the stomata (Fig. 9.3g, h) and the trichomes (Fig. 9.3i). For example, *B. punctulata* presents the cuticle striations arranged in a perpendicular orientation around stomata (Fig. 9.3g); cuticle striations can also occur perpendicular to trichome bases (Fig. 9.3i) or as concentric rings around the stomata (Fig. 9.3h), as reported in *B. pentaptera* (Budel et al. 2015). The cuticle is generally smooth in *B. microdonta* (Fig. 9.3d), but it is striated and radiated around stomata and trichome bases (Budel et al. 2018a).

In *Baccharis*, the leaf epidermis is usually unilayered and covered by a thin cuticle (Budel et al. 2018a). However, a thick cuticle was observed on both sides of *B. coridifolia* DC. (Budel and Duarte 2007), *B. ochracea* Spreng. (Barreto et al. 2015), and *B. spicata* (Lam.) Baill. (Oliveira et al. 2011) and on the adaxial leaf surface of *B. uncinella* (Budel and Duarte 2008a). Thick cuticles are also observed in the stems of some *Baccharis* species, for example, *B. spicata* (Oliveira et al. 2011).

Stomata

According to the arrangement of the surrounding epidermal or subsidiary cells, numerous stomatal types are distinguished. Types of stomata can help narrow the possible identity of unknown plant material. The presence or absence of stomata, size of stomata, stomatal index, and types of stomata are important features for characterizing and differentiating the species. In that sense, Rodriguez et al. (2010) reported that the density of stomata can help differentiate *B. articulata* out of *B. crispa*.

In *Baccharis*, anomocytic (Fig. 9.4a, e) and anisocytic (Fig. 9.4b) stomata types are the most common. However, other types of stomata have also been reported, such as cyclocytic type in *B. articulata*, *B. brevifolia*, *B. illinita*, *B. microdonta* (Fig. 9.4c), and *B. notosergila* Griseb.; actinocytic in *B. brevifolia* (Fig. 9.4d), *B. boliviensis* (Wedd.) Cabrera, *B. conferta* Kunth, and *B. pauciflosculosa*; hexacytic in *B. brevifolia* (Fig. 9.4h); tetracytic (Fig. 9.4f) in *B. reticularioides* Deble & A.S.Oliveira, *B. sphenophylla*, and *B. trilobata*; and staurocytic in *B. conferta*, *B. microdonta* (Fig. 9.4g), and *B. pauciflosculosa* (Freire et al. 2007; Pereira et al. 2014; Bobek et al. 2016; Budel et al. 2018a).

Amphistomatic leaves frequently occur in *Baccharis* (Freire et al. 2007; Molares et al. 2009; Budel et al. 2013; Bobek et al. 2015a, b; Barreto et al. 2015). However, some species, including *B. coridifolia* (Budel and Duarte 2007) and *B. punctulata* (Budel et al. 2018a), possess hypostomatic leaves. This characteristic is helpful in the diagnosis of *Baccharis* species, as demonstrated in the studies of Oliveira et al. (2011), Bobek et al. (2016), and Budel et al. (2018a).

Micro-measurements of stomata revealed that the majority of the species have stomata between 20 and 60 μm long (Freire et al. 2007; Rodriguez et al. 2013; Budel et al. 2018a). However, some species, such as *B. articulata* (60–75 μm) and

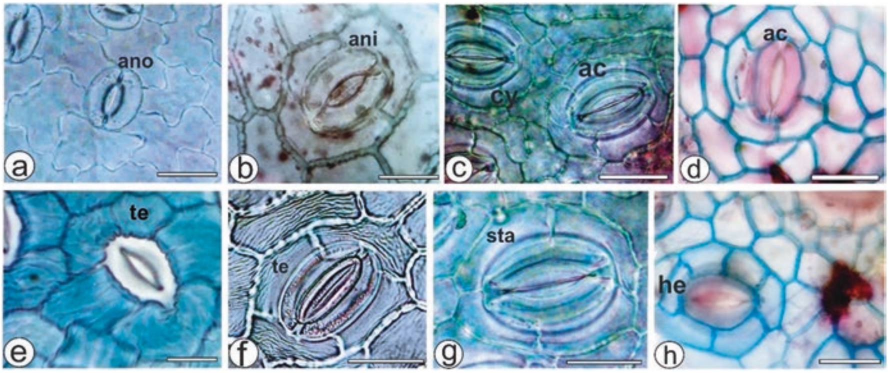

Fig. 9.4 Stomata in *Baccharis* species. *B. brevifolia* (**d**, **h**), *B. curitybensis* Heering ex Malme (**e**), *B. microdonta* (**c**, **g**), *B. junciformis* (**b**), *B. punctulata* (**a**), and *B. reticularioides* (**f**). [ano, anomocytic; ani, anisocytic; ac, actinocytic; cy, cyclocytic; he, hexacytic; te, tetracytic; sta, staurocytic]. Scale bars: a, g–i = 50 μm, b–f = 100 μm. (Reproduced with permission from Budel et al. 2018a (**a**, **c**, **f**, **g**); Oliveira et al. 2011 (**b**, **e**); and Bobek et al. 2016 (**d**, **h**))

B. illinita (75–105 μm), have larger stomata measuring more than 60 μm in length (Freire et al. 2007). The value of the stomatal index (percentage stomata out of the total number of epidermal cells plus stomata) is reported to range from 5 (*B. articulata*) to 16 (*B. punctulata*) (Rodriguez et al. 2013; Budel et al. 2018a).

Trichomes

Trichomes are small hairs or other outgrowths from the epidermis of plants. The two primary types of trichomes are glandular and non-glandular. Glandular trichomes possess the apical cells modified to secrete or store substances. Commonly, the secretions of these trichomes are responsible for biological activities. The recognition of the type and form of trichomes has been helpful in species identification. Non-glandular trichomes, or the covering trichomes, are generally characterized by having an acute apical cell. In *Baccharis*, trichomes are more common in leaves, but they are also sometimes present in stems and flowers.

Trichomes are considered to be the most important anatomical markers for the diagnosis of *Baccharis* species, followed by stomata type and epidermal cell walls (Freire et al. 2007). They usually appear isolated (Figs. 9.3i, and 9.5a, c–g) or in clusters (Figs. 9.3d, e, and 9.5h, i, k, l), arising from epidermal depressions (Fig. 9.5f, g, j). Simple non-glandular trichomes (Fig. 9.5a) formed by around six cells were present only in *B. anomala* (Budel and Duarte 2008b). Considering glandular trichomes, flagelliform (Figs. 9.3e, and 9.5b, c, e, f, g, i) and biseriate (Fig. 9.5f, h–l) types of trichomes have been commonly reported in *Baccharis* species (Budel et al. 2005; Freire et al. 2007; Bobek et al. 2016; Budel et al. 2018a; Almeida et al. 2021).

Biseriate glandular trichomes are formed by two pairs of basal cells and a head with up to four pairs of secretory cells. They appear singly as in *B. uncinella* (Budel

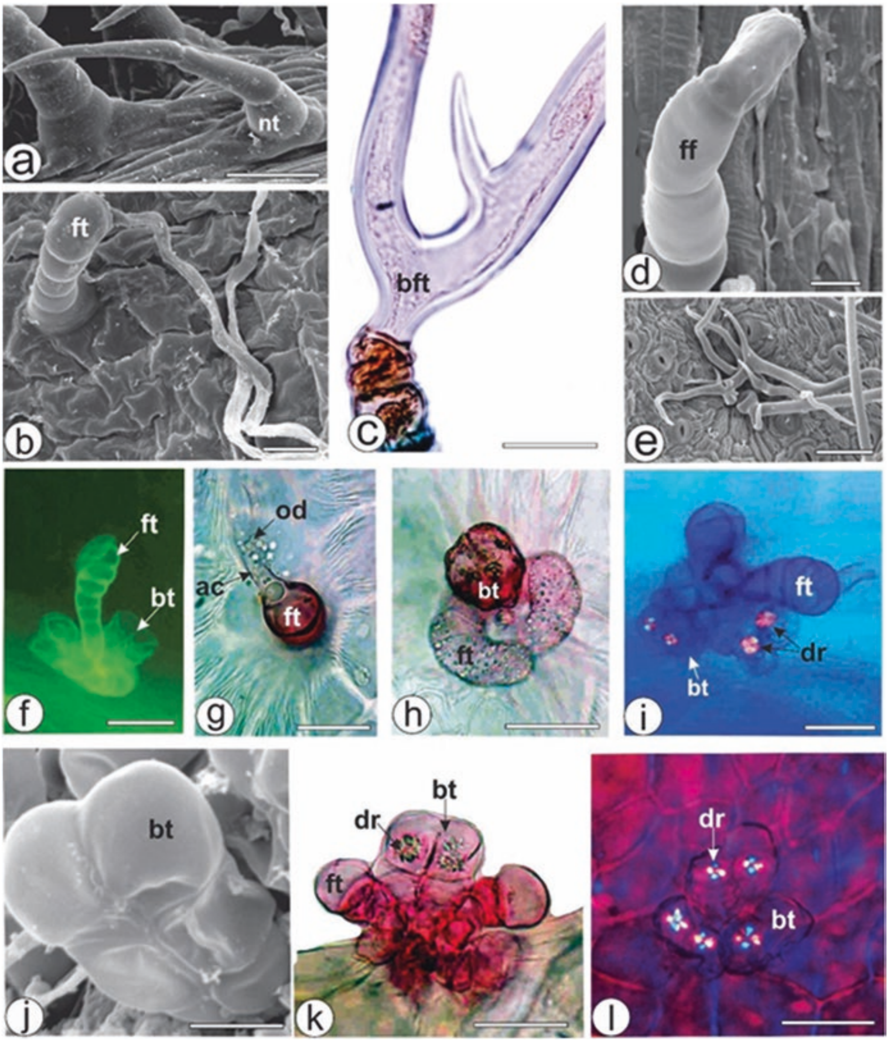

Fig. 9.5 Trichomes in *Baccharis* species [(**a**, **b**, **d**–**f**, **j**) SEM; (**i**, **k**, **l**) polarized light; (**f**) fluorescent light; all others: ordinary light microscopy]. *B. anomala* (**a**, **b**), *B. articulata* (**j**), *B. erioclada* (**e**), *B. microdonta* (**f**, **k**), *B. megapotamica* Spreng. (**d**), *B. pauciflosculosa* (**g**), *B. punctulata* (**h**), *B. sphenophylla* (**i**, **l**), *B. spicata* (**b**), and *B. uncinella* (**c**). ac, apical cell; bft, branched flagelliform trichome; bt, biseriate trichome; dr, druses; ff, filiform flagelliform trichome; ft., simple flagelliform trichome; nt, non-glandular trichome; od, oil droplets. Scale bars: c = 100 μm, a, e, f, g, h = 50 μm, b, i, k, l = 25 μm, d, j = 10 μm. (Reproduced with permission from Budel and Duarte 2008b (**a**, **b**); Bobek et al. 2015a (**e**); and Budel et al. 2018a (**f**, **g**, **k**))

and Duarte 2008a), in clusters of a single trichome type (Fig. 9.5l) as in *B. sphenophylla* (Budel et al. 2018a) or in clusters mixed with flagelliform trichomes (Fig. 9.5h, i, k) as seen in *B. illinita* (Budel et al. 2018a). Some species of *Baccharis* possess a pair of druses within each of the secretory head cells (Fig. 9.5i, k, l), as observed in *B. microdonta*, *B. punctulata*, and *B. sphenophylla* (Budel et al. 2018a).

Flagelliform glandular trichomes have some variations, including simple flagelliform with straight body (Fig. 9.5b, e, f, g, i), as seen in *B. microdonta* (Budel et al. 2018a), *B. pentaptera* (Budel et al. 2015), *B. ochracea* (Budel et al. 2012), *B. singularis* (Souza et al. 2011), *B. spicata* (Oliveira et al. 2011), *B. trilobata* (Bobek et al. 2016), *B. aracatubaensis*, and *B. organensis* (Zuccolotto et al. 2019); branched with straight body (Fig. 9.3c, e), as observed in *B. coridifolia* (Budel and 2007), *B. uncinella* (Fig. 9.5c), and *B. erioclada* (Fig. 9.5e); aseptate simple flagellate in *B. artemisioides* Hook. & Arn. (Freire et al. 2007) and *B. caprariifolia* DC. (Bobek et al. 2015a); filiform flagellate with pointed terminal cell in *B. multiflora* Kunth or pear-like and rounded at the apex (Fig. 9.5d) in *B. megapotamica* (Budel et al. 2012); and flagellate with C-shaped curved body (Fig. 9.5h), as observed in *B. punctulata* (Budel et al. 2018a). The body in these trichomes is secretory, voluminous, and made up of 3–9 cells. The apical cell is whip-like, tubular, and translucent, containing dense oil substances (Fig. 9.5g).

Mesophyll

In *Baccharis*, the organization of leaf mesophyll has correspondence to the chlorenchyma arrangement in the wings of cladodes. The majority of the species of *Baccharis* possesses isobilateral mesophyll (Fig. 9.4a). However, dorsiventral arrangement (Fig. 9.4b) was observed in *B. anomala* (Budel and Duarte 2008b), *B. singularis* (Vell.) (Souza et al. 2011), *B. ochracea* (Fig. 9.6b), and *B. punctulata* (Budel et al. 2018a).

Oil bodies in the leaf mesophyll (Fig. 9.6c) are present in some *Baccharis* species, e.g., *B. illinita*, *B. microdonta*, *B. punctulata*, *B. reticularioides*, and *B. sphenophylla*. They are specifically located in palisade parenchyma cells and some spongy parenchyma cells (Budel et al. 2018a). Minor collateral vascular bundles surrounded by an endodermis traverse the spongy parenchyma (Fig. 9.6d) in all *Baccharis* species studied (Bobek et al. 2016; Budel et al. 2018a).

In the "carquejas," the chlorenchyma in the wing of cladodes consists of palisade parenchyma, comprising approximately three layers of short cells beneath both sides of the epidermis, and spongy parenchyma in the central region (Fig. 9.6e). The isobilateral arrangement of the photosynthetic parenchyma is in correspondence to what is described for the wings of *B. articulata*, *B. myriocephala*, *B. crispa* (Rodriguez et al. 2008), and *B. sagittalis* (Less.) DC. (Petenatti et al. 2007).

At the wing edges, "carquejas" usually have 2–3 layers of angular collenchyma below the epidermis, a collateral vascular bundle with a perivascular fiber cap adjoining the phloem and secretory ducts (Fig. 9.6f), as observed in *B. junciformis*

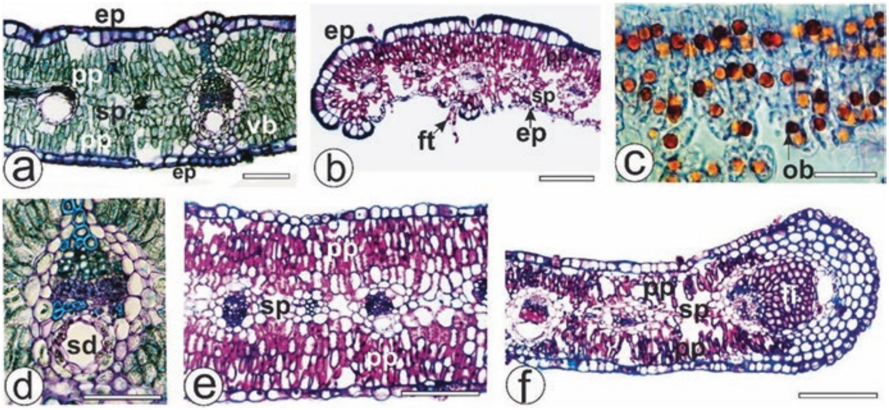

Fig. 9.6 Leaf anatomy of *Baccharis* spp.: leaf blade in cross section [(**c**) treated with Sudan III to stain lipophilic/oil content; (**a**, **d**) stained with toluidine blue; (**e**, **f**) stained with astra blue and basic fuchsin] . *B. ochracea* (**b**), *B. pauciflosculosa* (**a**, **d**), *B. reticularioides* (**c**), *B. microcephala* (**e**), *B. junciformis* (**f**). ep, epidermis; fi, fibers; ob, oil bodies; pp., palisade parenchyma; sd, secretory duct; sp., spongy parenchyma; vb, vascular bundle. Scale bars: a, b, d, f = 100 μm; c, e = 50 μm. (Reproduced with permission from Barreto et al. 2015 (**b**); Budel et al. 2018a (**d**); and Budel and Duarte 2010 (**e**, **f**))

(Budel and Duarte 2010). However, only sclerenchymatous tissue consisting of fibers can be found at the wing edges of some species, such as *B. myriocephala* (Sá and Neves 1996).

Midrib Shape in Cross Section

The shape of the midrib in a transverse section helps in the diagnosis of species as reported for different genera, including *Passiflora* (Wosch et al. 2015), *Mikania* (Almeida et al. 2017), and *Baccharis* (Bobek et al. 2016; Budel et al. 2018a) (Fig. 9.7a–i). Different shapes were reported for *Baccharis* species, such as plano-convex and prominently rounded on the abaxial side (Fig. 9.7a) in *B. anomala*; biconvex in *B. illinita* (Fig. 9.7c), *B. junciformis* (Fig. 9.7i), *B. cognata* (Budel et al. 2013), and *B. reticularioides* (Budel et al. 2018a); biconvex with a rounded projection on the adaxial side in *B. pauciflosculosa* (Budel et al. 2018a); slightly concave-convex in *B. rufescens* (Fig. 9.7f); concave-convex in *B. microdonta* (Fig. 9.7h); almost flat-convex in *B. caprariifolia* (Fig. 9.7b) and *B. megapotamica* (Fig. 9.7c); flat-convex, but truncate on the abaxial side, in *B. ochracea* (Fig. 9.7e); flat on both sides in *B. trilobata* (Bobek et al. 2016); and convex-flat in *B. cultrata* (Fig. 9.7g).

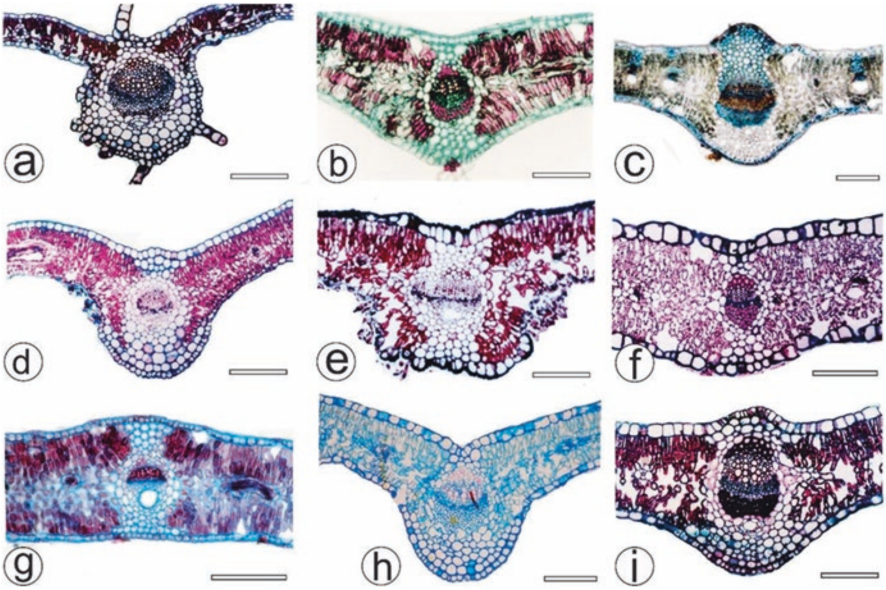

Fig. 9.7 Midrib anatomy in *Baccharis*. *B. anomala* (**a**), *B. caprariifolia* (**b**), *B. illinita* (**c**), *B. megapotamica* (**d**), *B. ochracea* (**e**), *B. rufescens* (**f**), *B. cultrata* (**g**), *B. microdonta* (**h**), and *B. junciformis* (**i**). Scale bars: a = 25 µm; b–h = 100 µm. (Reproduced with permission from Budel and Duarte 2008a (**a**); Bobek et al. 2015a (**b**); Barreto et al. 2015 (**e**); Budel et al. 2018a (**c**); and Bobek et al. 2016 (**g**, **h**))

Vascular System

In the leaves, collateral vascular bundles traverse the mesophyll and are commonly encircled by a parenchymatous sheath (Fig. 9.8a). The midrib vascular system is frequently represented by one collateral and circular bundle that is surrounded by ground parenchyma with lignified perivascular fibers abutting the xylem and phloem, as observed in *B. illinita* (Fig. 9.8b) and *B. microdonta* (Fig. 9.8c).

In the petiole, the vascular system is collateral and commonly similar to the midrib. Otherwise, they can show different organization, such as in *B. singularis* that presents one vascular bundle in open arc (Souza et al. 2011), whereas three or more collateral vascular bundles arranged in an open arc (Fig. 9.8d) are observed in *B. spicata* (Oliveira et al. 2011), *B. glaziovii* (Jasinski et al. 2014), and *B. microdonta* (Bobek et al. 2016).

In the regular stem (Fig. 9.8e) as well as the central axis of the cladodes (Fig. 9.8f), the vascular cylinder presents phloem outward and xylem inward. The xylem tracheary elements are arranged in radial rows and separated by parenchyma cells and fibers (Oliveira et al. 2011; Souza et al. 2011; Jasinski et al. 2014; Budel et al. 2015; Bobek et al. 2015a, b, 2016).

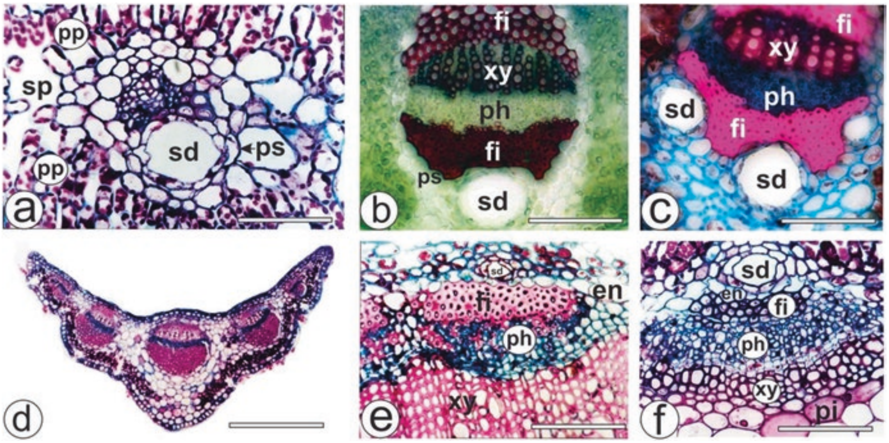

Fig. 9.8 Anatomy of *Baccharis.* (**a**) Leaf blade. (**b**, **c**) Midrib. (**d**) Petiole. (**e**) Stem. (**f**) Caulinar axis. (**a**, **c**–**f**) Stained in astra blue and basic fuchsin; (**b**) treated with phloroglucinol/HCl. *B. pentaptera* (**a**, **f**), *B. illinita* (**b**), *B. reticularioides* (**c**), *B. spicata* (**d**), *B. coridifolia* (**e**). [fi, fibers; ph, phloem; pi, pith; pp., palisade parenchyma; ps, parenchyma sheath; sd, secretory duct; sp., spongy parenchyma; xy, xylem]. Scale bars: a–f = 50 µm. (Reproduced with permission from Budel et al. 2015 (**a**, **f**); Oliveira et al. 2011 (**d**); and Budel and Duarte 2007 (**e**))

Secretory Ducts

Internal secretory structures may have various forms; some are isodiametric, such as the secretory cells and cavities, while others are elongated, such as the secretory ducts. In *Baccharis*, these ducts have uniseriate epithelium of 6–10 cells (Fig. 9.8a, b, c, e, f) containing dense cytoplasm and lipophilic contents. In the mesophyll, the secretory ducts are associated with the minor vascular bundles (Fig. 9.8a), adjacent to the phloem and the parenchyma sheath (Fig. 9.8a, b). In the midrib, usually, a single secretory duct occurs near the phloem (Fig. 9.8b), as observed in *B. coridifolia* (Budel and Duarte 2007), *B. ochracea* (Barreto et al. 2015), and *B. aracatubaensis* Malag. (Zuccolotto et al. 2019). However, *B. pauciflosculosa* (Budel et al. 2018a) has two secretory ducts, and *B. organensis* Baker has three secretory ducts in the midrib (Zuccolotto et al. 2019). Independent of whether regular stems or cladodes are examined, the secretory ducts are localized in the inner portion of the cortex next to the parenchyma sheathes (Fig. 9.8e, f) in all *Baccharis* species (Budel et al. 2003, 2004a, b, 2013; Budel and Duarte 2009; Oliveira et al. 2011; Souza et al. 2013; Pereira et al. 2014; Bobek et al. 2015a, b; Almeida et al. 2021).

Crystals

Several plants accumulate a variety of shapes and sizes of insoluble calcium salts, which, based on their morphology, can be crystalline sand, druses, raphides, styloids, and prismatic crystals. Typically, the morphotypes of crystals as well as their distribution in plant tissues are constant within a specific taxon (Franceschi and Horner 1980; Nakata 2003).

Crystals in plants have several functions, including the elimination of the excess of calcium ions, detoxification of heavy metals and aluminum, cellular ion balance, osmotic regulation, tissue mechanical support, and promotion of mechanical defense (Franceschi and Nakata 2005). Although calcium oxalate is more common in plants, calcium sulfate, magnesium oxalate, and other elements, such as potassium and silicon, can also be found in the crystals (He et al. 2012).

The identification of calcium oxalate crystals is done using a light microscope (LM), polarizing microscope, or scanning electron microscope (SEM). Energy-dispersive X-ray spectroscopy (EDS) coupled with SEM (Brito et al. 2021) is used to identify the elemental chemical composition of the crystals (as shown in Fig. 9.9).

Crystals of calcium oxalate are considered important for authentication purposes because the form, shape, and occurrence of these crystals in plants are species- and tissue-specific; hence, the presence or absence of a particular type of crystal can be used as a taxonomic character (Lersten and Horner 2011; Horner et al. 2012). They are also relevant in the systematic investigations and phylogenetic and ecophysiological characteristics of several plant families (Lersten and Horner 2011).

Different crystals morphotypes have been reported for several species of *Baccharis* and are often found in the stem pith (Budel and Duarte 2008b; Souza et al. 2011; Oliveira et al. 2011; Jasinski et al. 2014; Barreto et al. 2015; Bobek et al. 2015a, b; Budel et al. 2015; Almeida et al. 2021). The most common types are styloids (Fig. 9.10a, d, e, h, i, j, k), bipyramids (Fig. 9.10b, c, e, i), square bipyramids (Fig. 9.10f, l), and elongated square bipyramids (Fig. 9.10j); however, other types such as tile-shaped crystals (Fig. 9.10g) are also rarely observed.

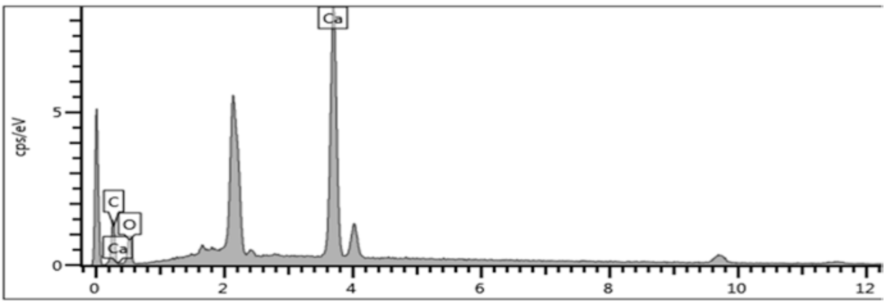

Fig. 9.9 EDS spectrum of a bipyramidal crystal in the stem of *B. pluricaptulata.* The unlabeled peaks in the spectra represent conductive metal used for coating the samples for SEM analysis

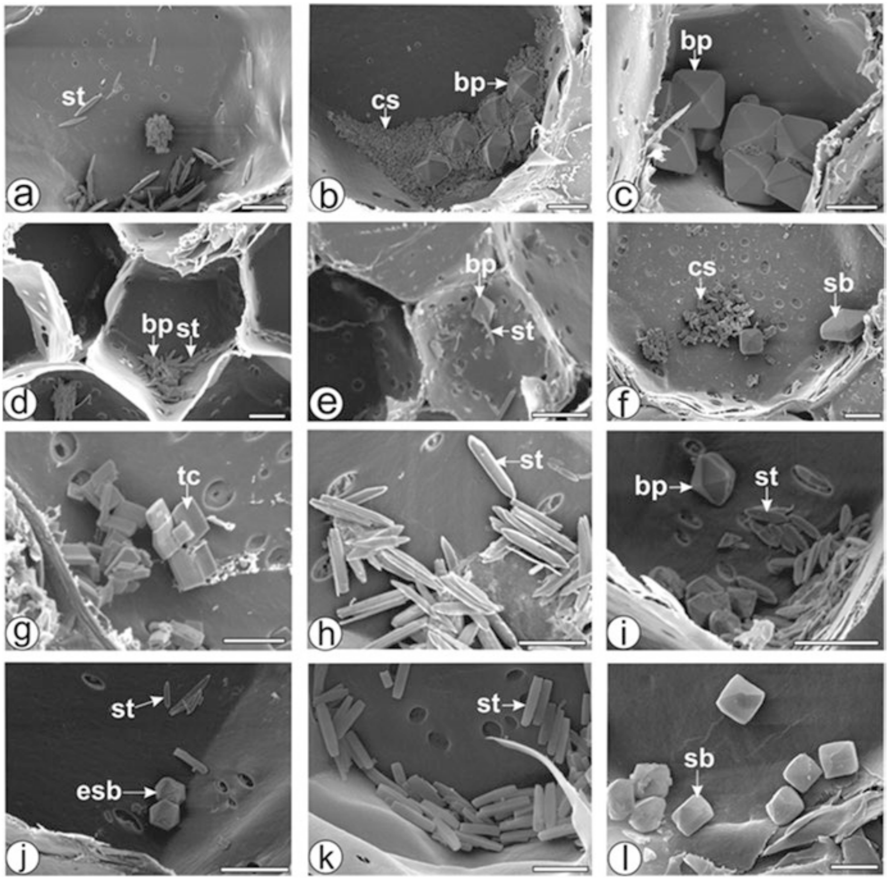

Fig. 9.10 Calcium oxalate crystals in *Baccharis* stem pith (SEM). *B. microdonta* (**a**), *B. pauciflosculosa* (**b**, **c**), *B. punctulata* (**d**, **e**), *B. reticularioides* (**f**, **g**), *B. sphenophylla* (**h**, **i**, **j**), *B. trilobata* (**k**, **l**). [bp, bipyramidal; cs, crystal sand; esb, elongated square bipyramidal; sb, square bipyramidal; st, styloids; tc, tile-shaped crystal]. Scale bar: a–f, h-j = 10 μm; g, k, l = 5 μm

Several species of *Baccharis* show different shapes of crystals within the same species. For example, crystal sand, styloids and square dipyramids in *B. brevifolia*; rare styloids in *B. microdonta*; crystal sand and square dipyramids in *B. pauciflosculosa*; and crystal sand, square dipyramids, elongated square dipyramids, styloids, and tabular crystals in piles that look like a tower in *B. trilobata* (Bobek et al. 2016) are observed. Additionally, a pair of druses is found within the secretory head of biseriate glandular trichomes (Fig. 9.5i, k, l) in some species of *Baccharis* (Budel et al. 2018a).

Morphotypes of calcium oxalate crystals have been described in the caulinar axis of *carquejas*, for instance, crystal sand and square bipyramids in *B. junciformis* (Budel and Duarte 2010); elongated square bipyramids in *B. articulata*; elongated square bipyramids, square bipyramids, cubes, and tetragonal prisms in *B. crispa*

(Cortadi et al. 1999); raphides and hexagonal and tetragonal prisms in *B. triangularis*; raphides and tetragonal prisms in *B. sagittalis* (Petenatti et al. 2007) and square bipyramids in *B. microcephala* (Budel and Duarte 2009); and raphides, styloids, and elongated square bipyramids in *B. glaziovii* (Jasinski et al. 2014).

Other Stem Characteristics

The cortex in *Baccharis*, not only in the stems but also in the caulinar axis, has collenchyma in alternation with chlorenchyma in several species, e.g., *B. anomala*, *B. microcephala*, *B. ochracea*, *B. spicata*, *B. stenocephala*, *B. uncinella*, and *B. junciformis*. This characteristic was also found in *B. sagittalis* and *B. triangularis* (Petenatti et al. 2007). However, a continuous stratum of collenchyma could be observed in some members of the genus, such as *B. caprariifolia* and *B. singularis* (Souza et al. 2011). In addition, one to five layers of angular collenchyma were found in *B. brevifolia*, *B. microdonta*, *B. pauciflosculosa*, and *B. trilobata*, particularly in the ribs (Bobek et al. 2016).

The endodermis is present in stems and roots and occurs as a continuous uniseriate layer separating the central cylinder from the cortical tissues. Ariza-Espinar (1973) reported that Casparian strips were absent in *Baccharis*. On the contrary, endodermis with Casparian strips was observed in the central axis of *B. myriocephala* (Sá and Neves 1996) and *B. crispa* (Cortadi et al. 1999).

Root Characteristics

Anatomical studies involving *Baccharis* roots are uncommon in literature, except for the root of *B. crispa* (syn. *B. trimera* (Less.) DC.), which has been previously studied. In a transverse section, the roots of this species exhibit early secondary growth with persistent unilayered rhizodermis. The cortex has 6–8 layers of assimilation parenchyma containing starch grains, followed by endodermis and pericycle layers. The vascular system is formed by a continuous ring of phloem with fiber caps. The xylem is porous and diffuse with solitary or rarely small groups of vessel elements and numerous fibers (Minteguiaga et al. 2018).

4 Final Considerations

Even though the two groups, carquejas and vassouras, are easily distinguishable by their stem and leaf morphological features, it is often difficult to distinguish the species within the same group due to their close resemblances. To address this problem, anatomical and micromorphological features of the whole plants and their parts can be used. The most important features that can help in the identification of different species of *Baccharis* include the habit of the plant, branching pattern, features of stems, presence or absence of cladodes and wings, leaf characters, and arrangement of the floral heads.

Anatomical and micromorphological features have played important roles in the identification of complex species. Numerous reports stress the usefulness of anatomical characteristics in the identification of *Baccharis* species. Noteworthy features that can help in the quality control and authentication of *Baccharis* species include the epidermis (contour of anticlinal epidermal cell walls and type of stomata and trichomes), mesophyll, vascular tissue, secretory ducts, and the type and macro pattern of calcium oxalate crystals.

Despite the importance of morpho-anatomical studies in solving taxonomic problems, a large portion of the genus remains unexplored. Therefore, future studies focusing on comparative anatomy and micromorphology of *Baccharis* species from different sections will not only help in the quality control and species identification but also aid in the development of a more accurate classification of this large and diversified genus.

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