Braz. J. Bot
DOI 10.1007/s40415-013-0022-5

Leaf anatomy with emphasis on separation of two species of *Varronia* P.Br. (Cordiaceae) of the Brazilian semi-arid region

Elisabeth Emília Augusta Dantas Tölke · José Iranildo Miranda De Melo · Sandra Maria Carmello-Guerreiro · Ana Paula Stechhahn Lacchia

Received: 23 July 2013 / Accepted: 12 September 2013

Abstract This work points out some variations in leaf anatomy useful in the separation of two species of the genus *Varronia* P.Br., *V. globosa* Jacq. and *V. leucocephala* (Moric.) J. S. Mill., and some anatomical adaptations of the semiarid climate. These species differ in stomata distribution, types of glandular trichomes, non-glandular trichomes density, accumulation of substances in *V. leucocephala*, crystal types, colenchyma type in midrib and petiole, and the vascular bundles in petiole. As unifying characters, both have uniseriate epidermis, glandular and non-glandular trichomes, dorsiventral leaves, crystals, collateral vascular bundles in leaf blades, and petiole with three vascular traces. The morphological study of trichomes has been extensively explored since it is one of the main characteristics differing the species from the genus, and being recognized several types of glandular trichomes, particular to each species. Some anatomical typical features of plants occurring in xeric environments were also identified: stomatal distribution, abundant trichomes with micropapillae on its surfaces, and lipid accumulation.

Keywords Boraginaceae s.l. · Caatinga · Cordia · Cystolith · Crystal sand · Trichomes · *Varronia*

E. E. A. D. Tölke (✉)
Postgraduate Program in Plant Biology, Institute of Biology, State University of Campinas, Campinas, SP CEP 13083-970, Brazil
e-mail: elisabeth.tolke@gmail.com

J. I. M. De Melo · A. P. S. Lacchia
Department of Biology, Center for Biological and Health Sciences, State University of Paraíba, Campina Grande, PB CEP 58459-500, Brazil

J. I. M. De Melo
Fellow of Research's Productivity, National Council for Scientific and Technological Development, Brasília CEP 71605-001, Brazil

S. M. Carmello-Guerreiro
Plant Biology Department, Institute of Biology, State University of Campinas, Campinas, SP CEP 13083-970, Brazil

Introduction

Cordiaceae R.Br. ex Dumort. had been placed in Boraginaceae sensu lato as one of their subfamilies (=Cordioideae A. Gray) for a long time. Traditionally, Boraginaceae comprises four subfamilies: Ehretioideae, Cordioideae, Heliotropioideae, and Boraginoideae (Diane et al. 2002; Miller and Gottschling 2007). However, recent phylogenetic studies supported by molecular data (Chase et al. 1993; Olmstead et al. 1993; Böhle and Hilger 1997; Ferguson 1999; Gottschling et al. 2001) focusing mainly on the subclass Asteridae demonstrated that Boraginaceae is a paraphyletic group. Thus, the four subfamilies of Boraginaceae s.l. had became at the level of family and along with Hydrophyllaceae and Lennoaceae constitute the order Boraginales.

Cordiaceae is a monophyletic group supported by molecular data and apomorphies such as the presence of undivided endocarp, four stigma lobes and plicate cotyledons (Gottschling et al. 2001, 2004, 2005; Miller and Gottschling 2007; Gasparino and Barros 2009). This family has around 350 species (Miller 2001) and about 65 of them occurring in Brazil (Gasparino and Barros 2009). Currently, the family includes the genera *Coldenia* L., *Cordia* L. and *Varronia* P.Br. (Barroso et al. 2007; Miller and Gottschling 2007; Gasparino and Barros 2009).

The largest genus is *Cordia* L. with species distributed in tropical and subtropical regions (Miller and Gottschling

Published online: 29 September 2013

2007). Gottschling et al. (2005) proposed the subdivision of this genus into four clades: *Varronia*, *Sebestena*, *Collococcus*, and *Myxa*. However, the strong morphological diversity of the genus resulted in different opinions regarding its taxonomic division and Miller and Gottschling (2007) proposed the reinstatement of the neotropical genus *Varronia,* that now includes the species *Cordia globosa* (Jacq.) Kunth and *C. leucocephala* Moric. (=*Varronia globosa* Jacq. and *V. leucocephala* (Moric.) J. S. Mill. respectively). Previous studies (e.g., Nowicke and Miller 1990; Gottschling et al. 2001, 2005) have suggested that the section *Varronia* should be considered as a distinct genus, but only the work of Miller and Gottschling (2007) generated molecular data correlated with the morphological and palynological data and allowed the separation of *Varronia* as one of the genus in the family Cordiaceae.

Varronia P.Br. comprises about 100 species distributed in the New World tropics and warm temperate regions from Arizona to Argentina (Miller and Gottschling 2007). The main morphological difference between the genera *Cordia* and *Varronia* consists of the habit and inflorescences types: trees with paniculate or cymose inflorescences in *Cordia* and shrubs with condensed inflorescences, capitate or spicate, small and compact in *Varronia* (Miller and Gottschling 2007).

Varronia leucocephala is a characteristic shrub of the Caatinga vegetation, and it is restrict to the Northeastern Brazil, Caatinga and restinga forest (Melo and Sales 2005). The species is often found in sandy soils (Melo and Sales 2005; Melo and Andrade 2007; Melo and Lyra-Lemos 2008). *V. globosa* is widely dispersed in the Caatinga vegetation (Melo and Sales 2005; Melo and Andrade 2007; Freitas et al. 2008) and resembles morphologically to *V. leucocephala*, which is distinguished by having nectary thickened at the ovary base and the corolla size, which is larger in *V. leucocephala* (Melo and Sales 2005; Melo and Andrade 2007). However, the species are quite similar in vegetative stage, requiring alternatives to zdifferentiate them, being frequentely encountered as sympatrics.

Anatomical studies for species from this family are still incipient (Metcalfe and Chalk 1950; Dasti et al. 2003; Ventrella and Marinho 2008; Souza 2008; Ló and Duarte 2001), evidencing the need to expand knowledge about anatomy, searching for characters that contribute to the identification and separation of species of this family. The anatomical characters of vegetative organs are used as important additional data to external morphology (Metcalfe and Chalk 1983), especially when it has only vegetative specimens for analysis. This work has aimed at performing the anatomical and histochemical study of the leaf blade and petiole of *V. globosa* Jacq. and *V. leucocephala* (Moric.) J. S. Mill. (Cordiaceae), in order to search distinctive characters of this species, especially regarding the trichomes morphology.

Materials and methods

Plant material

The study was conducted at middle, top, and base region segments of fully expanded leaf and petiole of three individuals of *V. globosa* Jacq. and *V. leucocephala* (Moric.) J. S. Mill. The species were collected in Junco do Seridó and Patos municipalities, both located in Caatinga vegetation of Paraíba state, Northeastern Brazil. The material was fixed in FAA (formalin, acetic acid, 50 % alcohol) for 24 h following the Johansen (1940) protocol and NBF (neutral-buffered formalin) in 0.1 M pH 7.0 sodium phosphate buffer for 48 h as Lillie (1965) advised, and preserved in 70 % ethyl alcohol using the Jensen (1962) technique. The testimony material was placed in the herbarium ACAM (Herbário Manuel de Arruda Câmara), State University of Paraíba (UEPB), Campus I. *V. leucocephala*: BRAZIL.PARAÍBA: Patos, 17-IV-2009, Tölke et al. *52* (ACAM); *V. globosa*: BRAZIL. PARAÍBA: Junco do Seridó, 17-IV-2009, Tölke et al. *53* (ACAM).

Light microscopy

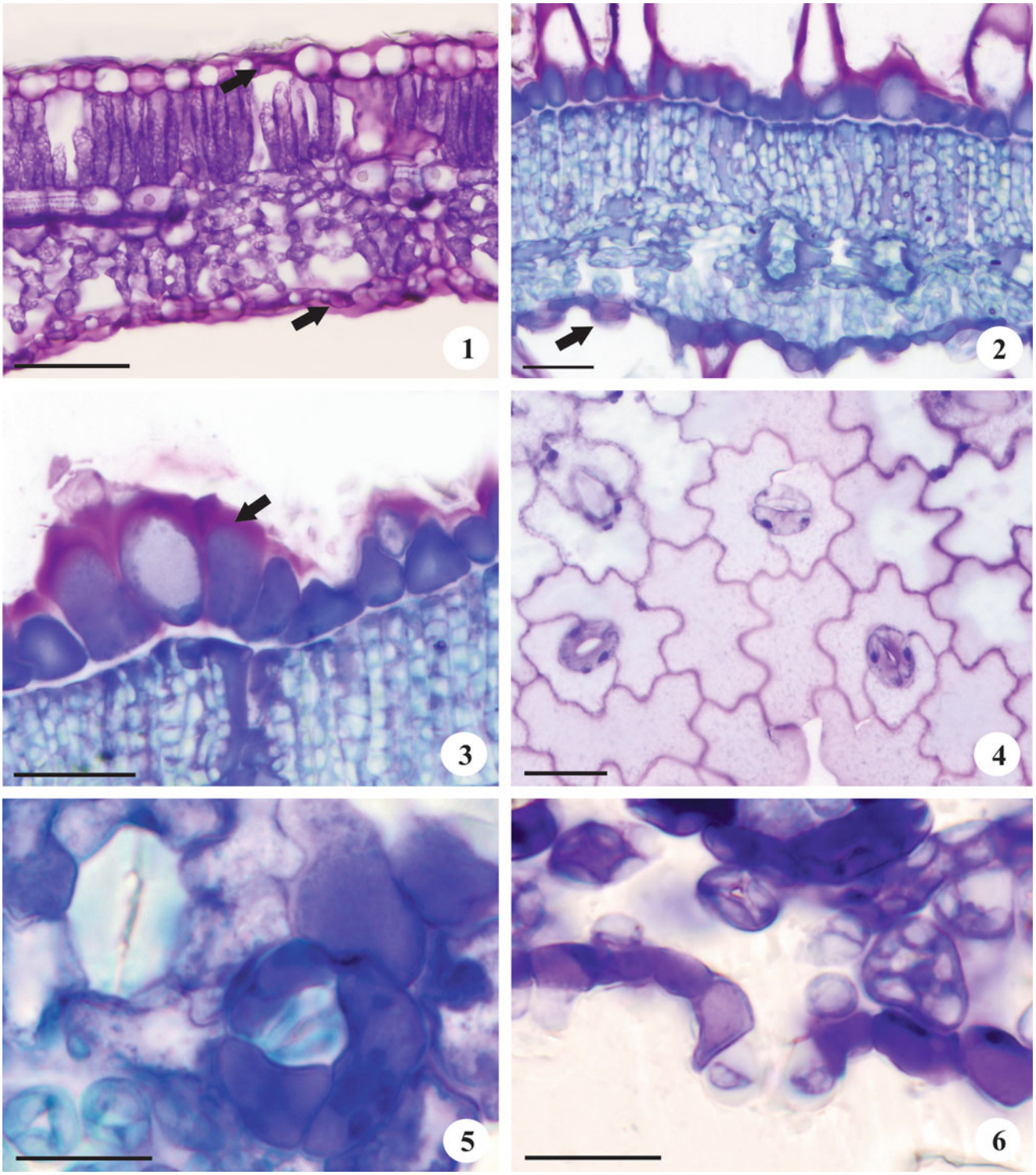
The leaf blades and petioles were dehydrated in ethanol series and embedded in hidroxyethyl methacrylate resin (Historesin® Leica) according to technique of Gerrits and Smid (1983) following the manufacture's recommendations. Cross and longitudinal sections (5–7 μm tick) were obtained with a rotary microtome and stained with 0.05 % Toluidine Blue in sodium acetate buffer (O'Brien et al. 1964). All blades were temporarily prepared in slides with water and the results were recorded by photomicrographs under optical microscope (Olympus® BX51) with attached camera (Olympus® DP71).

Scanning electron microscopy (SEM)

For the micromorphological analysis, the material was fixed in FAA, dehydrated in ethanol series, dried by critical point method, mounted on stubs and metalized with gold. The observations and images were obtained using scanning electron microscope (SEM) Jeol® JSM 5800 LV at 10 kV with a digital camera attached.

Histochemistry

For the histochemical analysis, the leaf blades and petioles were fixed in FAA (for hydrophilic substances) (Johansen



Figs. 1–6 Leaf blade in transverse and paradermical sections of *Varronia globosa* Jacq. (**1**, **4**) and *V. leucocephala* (Moric.) J. S. Mill. (**2**, **3**, **5**, **6**). **1** General appearance of *V. globosa* leaf blade showing uniestratified epidermis and amphistomatic distribution in cross-section (*arrow* = stomata). **2** General appearance of *V. leucocephala* blade showing uniestratified epidermis and heterogeneous aspect of the epidermal cells (*arrow* = stoma). **3** Cuticle (*arrow*). **4** Paradermical section in *V. globosa* highlighting the dyacitic stomata on adaxial surface. **5** Paradermical section in *V. leucocephala* showing anysocitic stomata on adaxial surface. **6** Abaxial surface of *V. leucocephala* highlighting stoma above the level of other epidermal cells. *Bar* = 50 μm (**1**); 20 μm (**2**–**4**); 10 μm (**4**–**6**)

1940) or NBF (for lipophilic substances) (Clark 1973), then the material was dehydrated in butyl series and embedded in Paraplast. Then, the sections were submitted to the following reagents: Sudan Black B (Pearse 1980) for detection of total lipids; Lugol (Berlyn and Miksche 1976) for starch; Ferric Chloride (Johansen 1940) for phenolic compounds; Wagner's Reagent (Furr and Mahlberg 1981) for alkaloids; Schiff's Reagent (PAS) (McManus 1948) for total polysaccharides; Tanic Acid/Ferric Chloride (Pizzolato and Lillie 1973) for mucilage and Red Ruthenium (Johansen 1940) for pectins.

Results

Epidermis

The leaf blades of *V. globosa* and *V. leucocephala* have unistratified epidermis (Figs. 1, 2). In *V. leucocephala,* it is observed that the epidermis possesses larger and elongated cells in adaxial surface compared to the lower epidermis, besides having a thick cuticle surrounding the adaxial surface (Fig. 3). In paradermic sections of *V. globosa,* the common epidermal cells have sinuous anticlinal walls in both surfaces of the leaf blade (Fig. 4). The leaf is amphistomatic with the guard cells at same level of other epidermal cells (Fig. 1) and the stomata are diacytic (Fig. 4), uniformly distributed. In *V. leucocephala,* the leaf is hypostomatic (Fig. 2) with anisocytic stomata (Fig. 5) above the level of other epidermal cells (Fig. 6). The epidermal cells of *V. leucocephala* store large amounts of lipids (Fig. 34; Table 1).

Two basic types of trichomes can be distinguished on leaf blades of both species: non-glandular and glandular trichomes. On the adaxial surface of *V. globosa,* the non-glandular trichomes are more abundant than on the abaxial surface; on the other hand, the opposite occurs with the glandular trichomes (Figs. 7, 8), midrib shelters both types (Fig. 9). In *V. leucocephala*, the glandular trichomes are more abundant on the adaxial surface (Fig. 10) while the non-glandular trichomes are more abundant on the abaxial surface (Fig. 11), the central rib also has both types of trichomes. The trichomes density on the leaf surface of *V. leucocephala* is more pronounced than in *V. globosa* (Figs. 10, 11, 12).

The non-glandular trichomes of *V. globosa* are unicellular, simple, conical and the base is formed by protruding special cells responsible for lifting the trichomes above the epidermis level (Figs. 13, 14). The trichomes are long, uniform in length, and have papillae on the external wall. In *V. leucocephala,* at least two types of non-glandular trichomes are distinguished, a very similar to that described for *V. globosa*, simple, unicellular, long, conical with the base high above the epidermis and covered by papillae; however, the adjacent cells of the epidermis are not salient as those found in *V. globosa* (Figs. 15, 16), this being most frequently found in midribs and on the abaxial surface. A second type, also simple, unicellular and conical, presents a bulbous base and papillae on its external surface (Fig. 17), slightly shorter than the previous one, more common on the adaxial surface, and a cystolith at its base is observed in the histological section (Fig. 18).

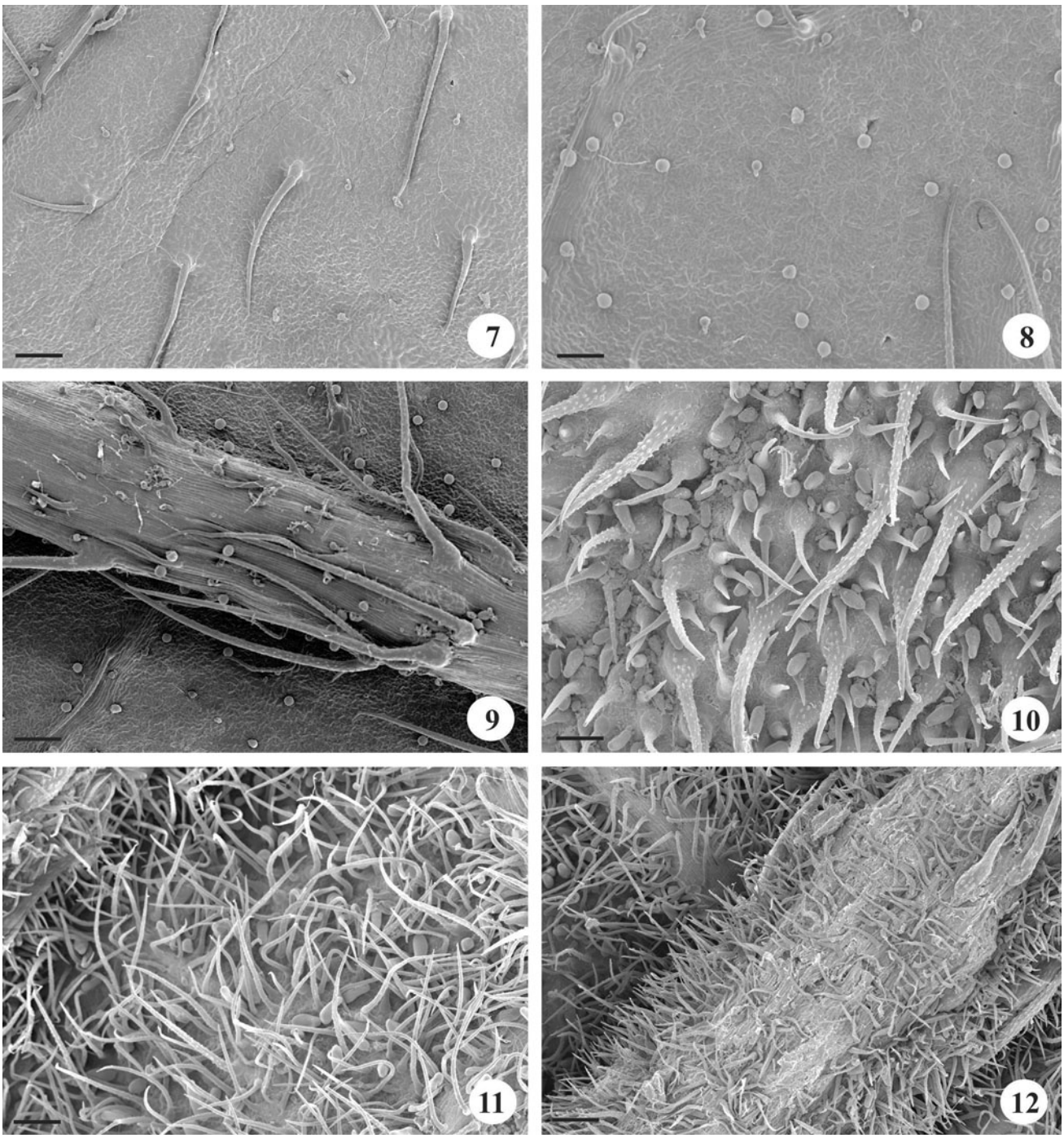
Table 1 Histochemical tests in the leaf blade and petiole of *Varronia globosa* Jacq. and *V. leucocephala* (Moric.) J. S. Mill

Test	Substance	*V. globosa*	*V. leucocephala*
Sudan black B	Lipids	(−)	(++) ep, pp, idv, idp
Ferric chloride	Phenolic compounds	(−)	(−)
Wagner's reagent	Alkaloids	(−)	(−)
Schiff's reagent	Polysaccharides	(++) cw	(++) cw
Ruthenium red	Pectins	(++) cw	(++) cw
Tanic acid/ ferricchloride	Mucilage	(−)	(−)
Lugol	Starch	(−)	(++) me

++ strongly positive, − negative, *cw* cell wall, *ep* epidermis, *pp* paraveinal parenchyma, *idv* idioblasts of vascular system, *idp* idioblasts of petiole, *me* mesophyll

The glandular trichomes can be subdivided into subsessile (*V. globosa*) or pedunculate (*V. globosa* and *V. leucocephala*). Only one type of subsessile trichome was verified, which shows a very short unicellular stalk, followed by a globular secretory multicellular head (Figs. 19, 20), and it is distributed on both surfaces of *V. globosa*. Two types of pedunculate secretory trichomes are found in these species, one of them was observed only in *V. globosa* and the other only in *V. leucocephala*. The pedunculate secretory trichome of *V. globosa* distributed on both surfaces of the leaf blade has globular secretory multicellular head (2–4 cells) and the unicellular peduncle raised by a multicellular base formed by epidermis cells (Figs. 21, 22). The pedunculate secretory trichome of *V. leucocephala* is also distributed on the two leaf surfaces, but it has the peduncle formed by a cell, followed by a club-shaped and elongated secretory unicellular head (Figs. 23, 24).

The subsessile trichomes of *V. globosa* reacted positively to Sudan Black B, showing the presence of lipids (Fig. 31). At the long stalk trichome of the same species, the reactions in the secretory head were positive for lipids (Fig. 32), and mucilage evidenced by Tanic Acid and Ferric Chloride was observed in the subcuticular space of secretory head (Table 2), but in the glandular trichome of *V. leucocephala,* the reactions were positive only for lipids in the peduncle region (Fig. 34). Reactions were negative

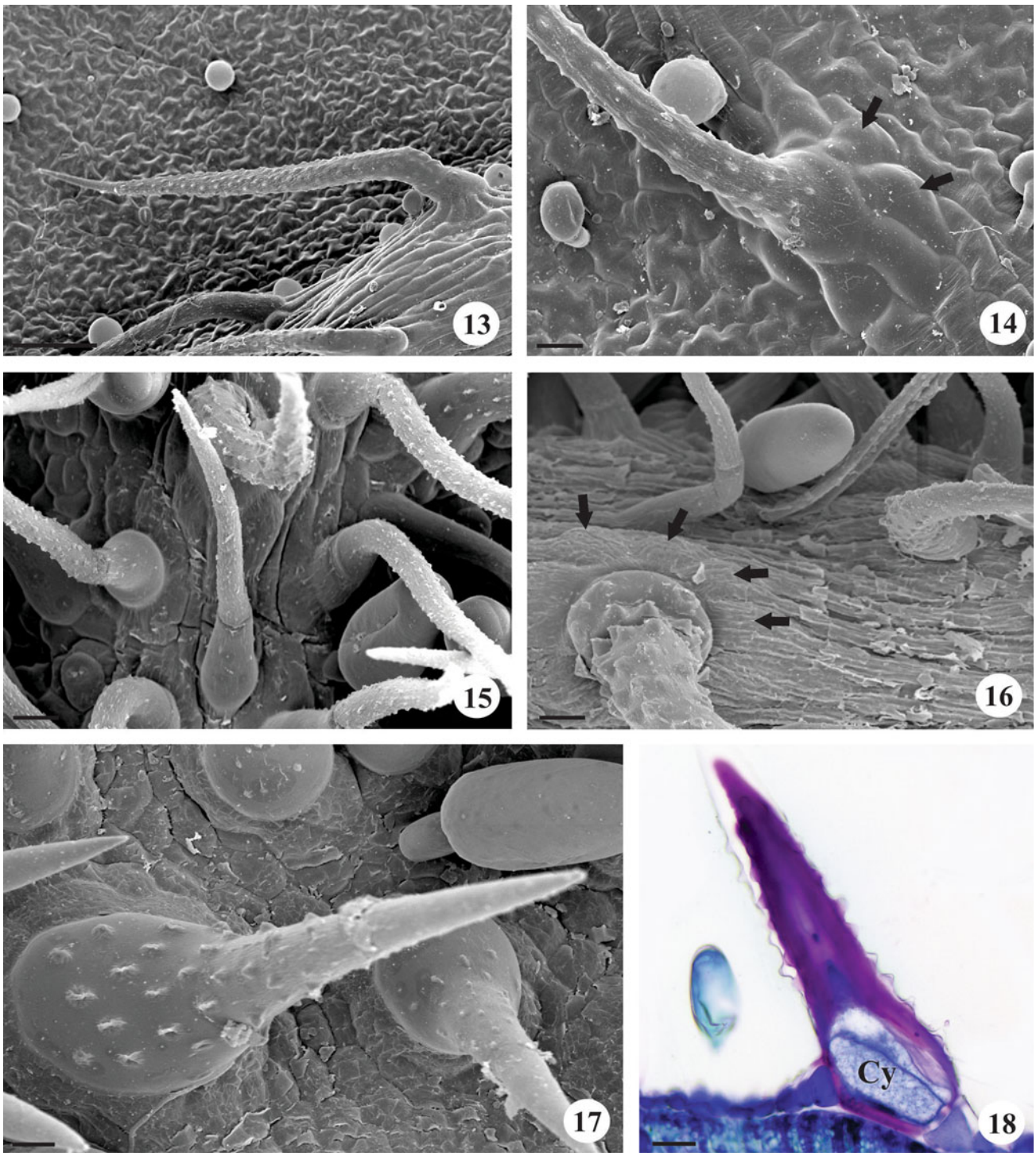


Figs. 7–12 Density of trichomes in *Varronia globosa* Jacq. (**7–9**) and *V. leucocephala* (Moric.) J. S. Mill. (**10–12**).**7**, **10** Adaxial view. **8**, **11** Abaxial view. **9**, **12** The central rib. *Bar* = 100 µm (**7–9**; **12**); 80 µm (**10**, **11**)

to the secretory head and peduncle in all other tests, the results are summarized in Table 2.

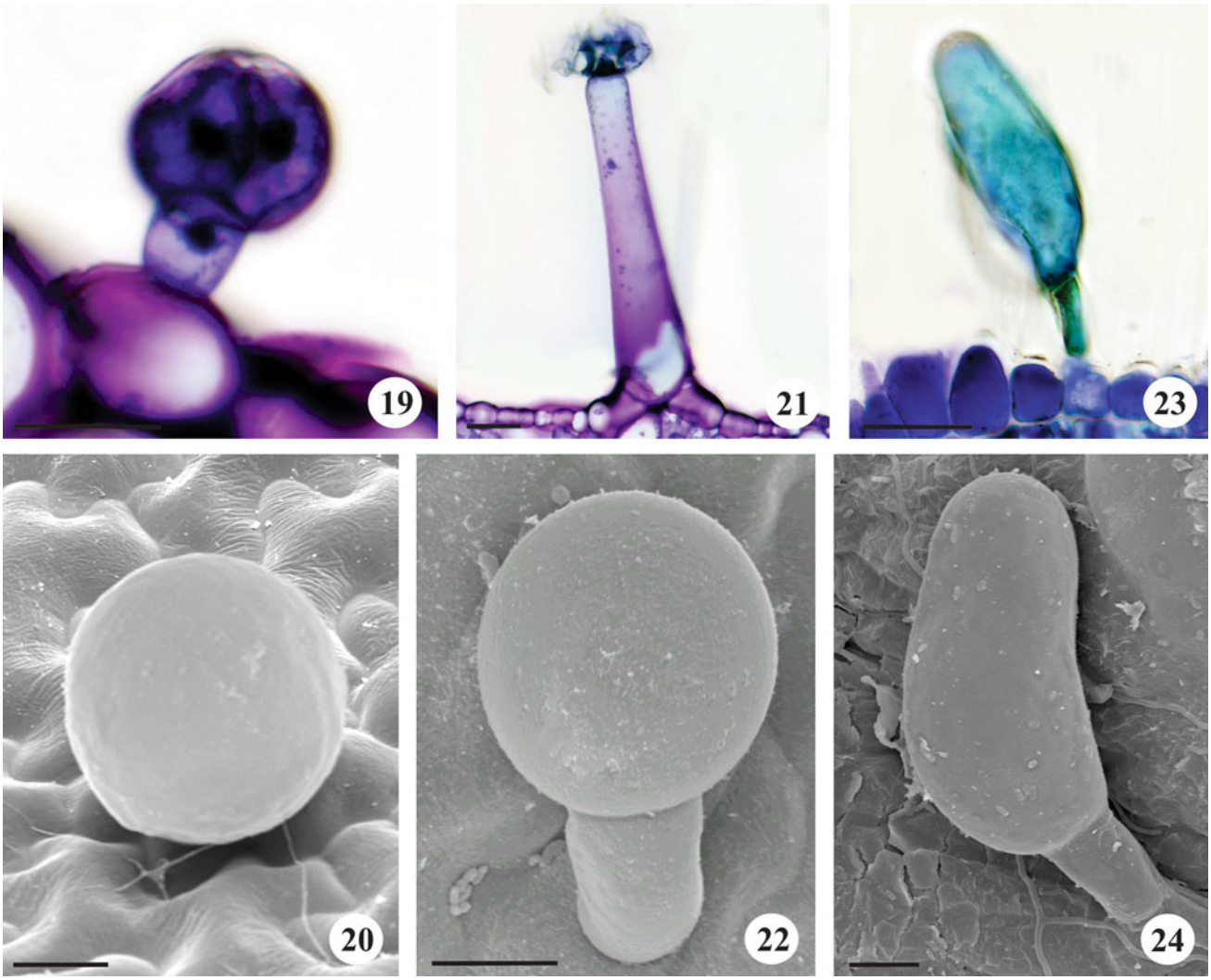
Mesophyll

The leaf blades of both studied species are dorsiventral (Figs. 1, 2). The palisade parenchyma is composed of one layer in both species and the spongy has 6–8 layers of cells in *V. globosa* and 3–5 layers in *V. leucocephala*. In *V. globosa*, there is also a row of idioblasts containing druses, located between the cells of palisade parenchyma and the spongy parenchyma, close to vascular bundles (Fig. 25). Druses were not found in *V. leucocephala* mesophyll, but there are cells containing crystalline sand inside it at



Figs. 13–18 Non-glandular trichomes in *Varronia globosa* Jacq. (**13**, **14**) and *V. leucocephala* (Moric.) J. S. Mill. (**15–18**). **13** General appearance of the non-glandular trichome in *V. globosa*. **14** Base of non-glandular trichome in *V. globosa* highlighting salients cells (*arrows*). **15** General view of trichomes type 1 in *V. leucocephala*. **16** Base of non-glandular trichome type 1 in *V. leucocephala* highlighting salients cells (*arrows*). **17** General view of trichomes type 2 in *V. leucocephala* highlighting bulbous base. **18** Histological section of non-glandular trichome type 2 in *V. leucocephala* showing the cystolith in the trichome base (*Cys*). *Bar* = 100 μm (**13**); 20 μm (**14–16**; **18**); 10 μm (**17**)

various points in the mesophyll (Fig. 26). Both species have a paraveinal parenchyma (Figs. 1, 2, 25, 26). There is starch storage in *V. leucocephala* parenchyma (Fig. 33; Table 1) and the paraveinal parenchyma showed positive reaction for lipids, as observed in epidermal cells (Fig. 34) (in the same Table).



Figs. 19–24 Glandular trichomes in *Varronia globosa* Jacq. (19–22) and *V. leucocephala* (Moric.) J. S. Mill. (**23–24**). **19–20** Subsessile glandular trichome in *V. globosa* with unicellular short stalk and multicellular secretory head. **21**, **22** Stalked glandular trichome in *V. globosa* with unicellular long peduncle and multicellular secretory head. **23**, **24** Stalked glandular trichome in *V. leucocephala* with unicellular stalk and unicellular secretory club-shaped head. *Bar* = 10 μm (**19**, **20**, **22**, **24**); 20 μm (**21**, **23**)

Vascular system

Collateral bundles of small and medium thick are arranged along the mesophyll of both species. The midrib of *V. globosa* is plane convex and the vascular system performs in an arc shape (Fig. 27), *V. leucocephala* has midrib biconvex (Fig. 28). The vascular system of *V. leucocephala*, arc-shaped with two very close dorsal lines, is completely surrounded by idioblasts containing lipophilic substances (Fig. 35; Table 1). In both species, vascular bundles are collateral. In *V. globosa*, there are one to three layers of tangencial collenchyma below the epidermis on both faces and in *V. leucocephala* there are three to five layers of annular collenchyma (Figs. 27, 28).

Petiole

Varronia globosa has a D-shaped slightly sulcate petiole with obtuse margins while in *V. leucocephala,* petiole is D-shaped obovate, not sulcate with obstuse margins. There were non-glandular and glandular trichomes similar to those described for leaf epidermis. The epidermis of both species is uniseriate and the outer cortex is formed by collenchyma cells (Figs. 29, 30). In *V. leucocephala,* there are five to seven layers of annular collenchyma and in *V. globosa,* there are two to three layers of angular collenchyma. The organization pattern of the vascular bundles is constituted for three free lines, the highest one is in arc-shaped (Figs. 29, 30) formed by collateral bundles; however, the traces of the dorsal region are composed of

Table 2 Histochemical tests in glandular trichomes of *Varronia globosa* Jacq. and *V. leucocephala* (Moric.) J. S. Mill

Test	Substance	Trichome A[a]	Trichome B[a]	Trichome C[a]
Sudan black B	Lipids	(++) sh, pd	(++) sh	(++) pd
Ferric chloride	Phenolic compounds	(−)	(−)	(−)
Wagner's reagent	Alkaloids	(−)	(−)	(−)
Schiff's reagent	Polysaccharides	(++) cw	(++) cw	(++) cw
Ruthenium red	Pectins	(++) cw	(++) cw	(++) cw
Tanic acid/ferric chloride	Mucilage	(++) cw	(++) ss, cw	(++) cw
Lugol	Starch	(−)	(−)	(−)

++ strongly positive, − negative, *sh* secretory head, *pd* peduncle, *cw* cell wall, *ss* subcuticular space

[a] Trichome type A, subsessile trichome of *V. globosa*; Trichome type B, pedunculate trichome of *V. globosa*; Trichome type C, pedunculate trichome of *V. leucocephala*

amphicribal bundles in *V. leucocephala* (Fig. 30). The parenchyma of *V. leucocephala* has idioblasts containing crystalline sand, just it as observed in mesophyll and idioblasts containing lipophilic substances in the ventral parenchyma and around vascular bundles (Table 1). The main anatomical features distinguishing between *V. globosa* and *V. leucocephala* are summarized in Table 3.

Discussion

The results of *V. globosa* and *V. leucocephala*, especially the presence of crystals and non-glandular and glandular trichomes, agree with the remarks made by Metcalfe and Chalk (1950) for the genus *Cordia* sensu lato; many of these species currently included in the genus *Varronia*. According to Dasti et al. (2003), the epidermal features have little taxonomic significance for the family Boraginaceae sensu lato, such as straight or sinuous walls and stomatal types; individuals of the same species collected from different sites show a wide diversity of stomatal types, evidencing that this feature may vary depending on the habitat where the plant lives.

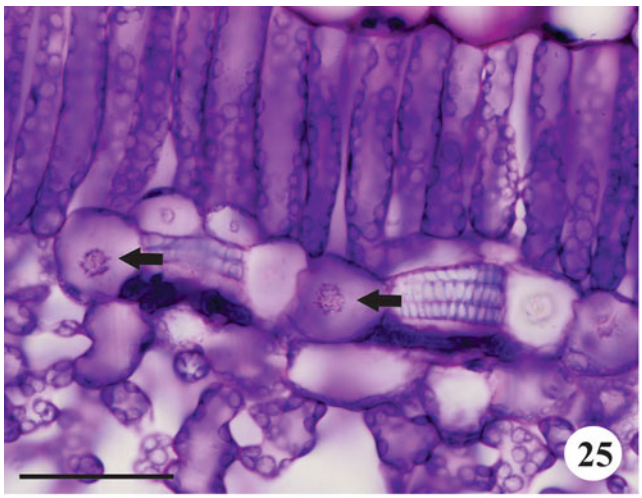
The morphology of trichomes can vary greatly according to the species, and more than 300 descriptions are shown in the literature to characterize the different morphological types of trichomes, often used in plant classifications (Theobald et al. 1979; Wagner 1991). Particularly for Cordiaceae, different types of trichomes offer an alternative for identification of the species when it gets only vegetative material for analysis, because some species are quite similar, making it difficult to be identified in field.

Figs. 25–30 Mesophyll, midrib and petiole cross sections of *Varronia globosa* Jacq. (**25**, **27**, **29**) and *V. leucocephala* (Moric.) J. S. Mill. (**26**, **28**, **30**). **25** Druses (*arrows*) lined in the middle portion of *V. globosa* mesophyll. **26** Idioblast containing crystalline sand (*arrow*) in *V. leucocephala*. **27** Midrib of *V. globosa*. **28** Midrib of *V. leucocephala* with idioblasts (*Id*) containing lipid substance inside. **29** *V. globosa* petiole showing three arc-shaped vascular traces. **30** *V. leucocephala* petiole showing three arc-shaped vascular traces, the unique vascular trace situated in dorsal region, highlighting secretory idioblasts (*Id*); detail to ampicribal bundle (*Xy* = xylem; *Ph* = phloem). *Bar* = 20 μm (**26**); 25 μm (**25**); 100 μm (**27**–**29**); 200 μm (**30**) ▶

In the family Boraginaceae s.l., non-glandular trichomes associated with cystoliths into the base are quite common (Metcalfe and Chalk 1950; Diane et al. 2003; Ventrella and Marinho 2008), these trichomes are usually single-celled with basal cells that stand out in comparison to other epidermal cells (Metcalfe and Chalk 1950; Diane et al. 2003; Fariña et al. 2003), as observed on the species of this work. The presence of cystoliths into the base of non-glandular trichomes was not evident in *V. globosa*. It was possible to detect the presence of cystoliths in the shorter non-glandular trichome of *V. leucocephala*. These cystoliths were also observed by Ventrella and Marinho (2008) in the species *V. verbenacea* (DC.) Borhidi in its short trichomes.

The functions of non-glandular trichomes depend on their morphology, the organ where they are located and their position (Werker 2000). Those occurring in leaves have the function of maintaining the atmosphere saturated with water vapor around the leaf, reducing transpiration in xeric environments, and these appendages are also capable to regulate the temperature, reflecting the solar radiation that reaches the leaf, which reduces the water loss (Larcher 2001; Valkama et al. 2003). The trichomes substantially increase the leaf ability to reflect all wave lengths of solar radiation while restrict the absorption of these rays, which results in decreased heat load (Rotondi et al. 2003). The micropapillae present in these trichomes emphasize the reflection of solar rays and further can reduce the temperature (Selvi and Bigazzi 2001). The same authors reported the presence of these micropapillae in many species of Boraginaceae s.l., a feature repeated in *V. globosa* and *V. leucocephala*.

The morphology and distribution of glandular trichomes were quite similar to those described in other studies addressing representatives of the family Boraginaceae (Metcalfe and Chalk 1950; Ventrella and Marinho 2008) with stalk unisseriate and typical secretory head. The two types found in *V. globosa* were very similar to those described to *V. verbenacea* (Ventrella and Marinho 2008), with short or elongated peduncle and globular secretory head, the reniform type described in this same work was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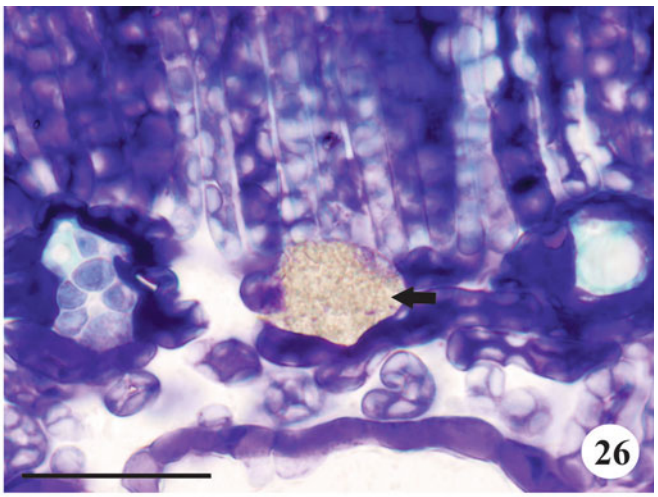
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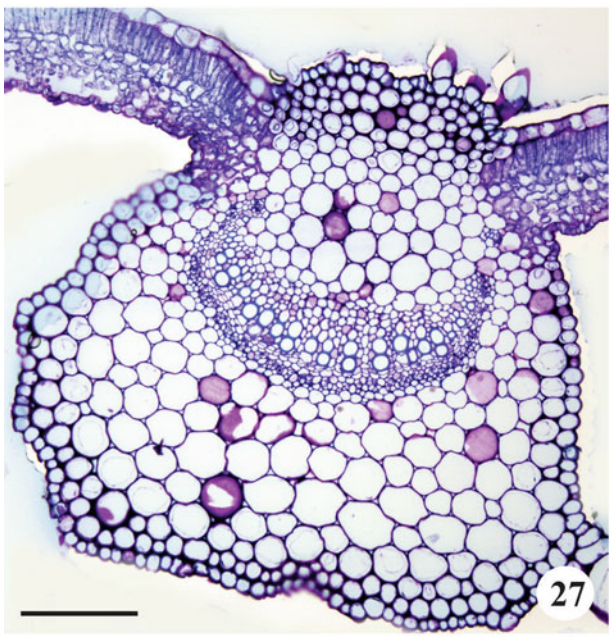
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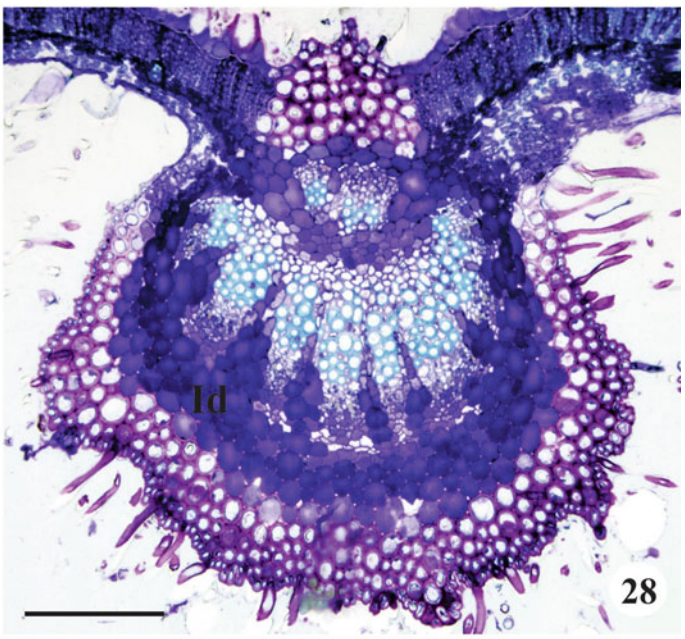
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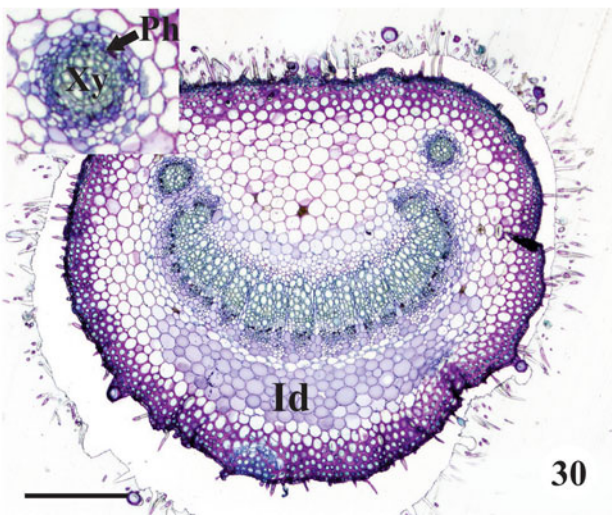
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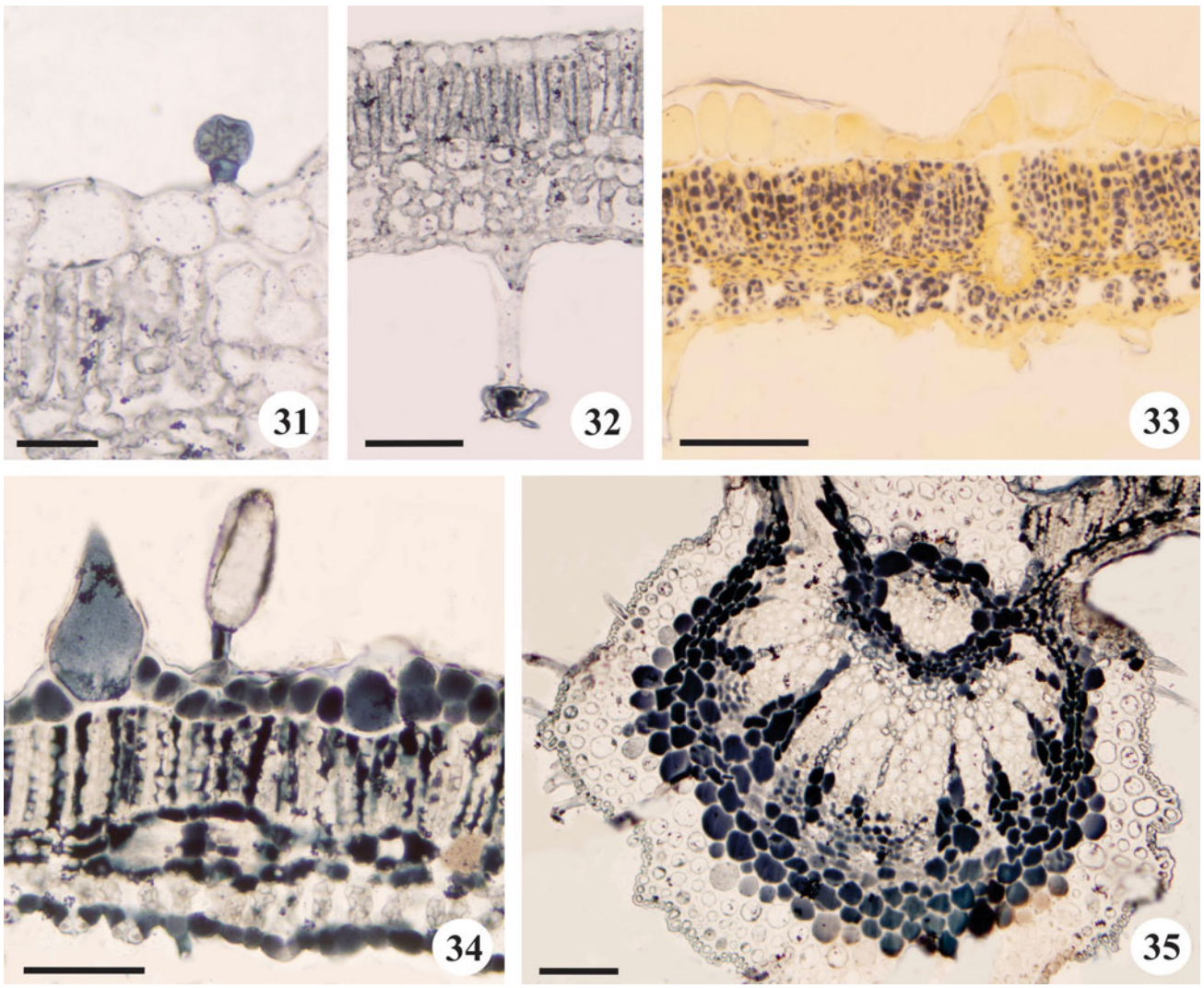
not found. The glandular trichomes of *V. leucocephala* with elongated secretory club-shaped head is being first described for the genus *Varronia*. The lipids accumulated in the peduncle cells of some trichomes here described are pointed out as responsible for blocking the influx of the secreted material and it is quite common trichomes secreting essential oils (Fahn 1979; Werker 2000; Ventrella and Marinho 2008). According to Werker (2000), lipophilic substances are related to chemical protection against herbivores and pathogens.

In the epidermis, besides the types of differentiated trichomes between the two species, the stomata distribution is an important characteristic once the leaf is amphistomatic in *V. globosa*, while in *V. leucocephala* is hypostomatic. The stomata location in the same level as the other epidermal cells in *V. globosa* is related to the improvement of gas exchange by increasing the photosynthetic efficiency (Ciccarelli et al. 2010). Amphistomatic leaves generally have higher amount of stomata on the abaxial side preventing photoinhibition, since the adaxial surface is more exposed to the radiation (Smith et al. 1997), besides having increased photosynthetic capacity (Fahn 1990). Selvi and Bigazzi (2001) studied several species of Boraginaceae s.l. and attributed the occurrence of amphistomatic leaf as adaptive feature to the arid environments or high altitudes, what can also be applied to this situation once the species were collected in the semiarid environment.

Hypostomatic leaves are referred in Lakusic et al. (2010) as a characteristic common to species living in xeric environments. This may be an alternative to reduce the water



Figs. 31–35 Histochemical tests on *Varronia globosa* Jacq. (**31**, **32**) and *V. leucocephala* (Moric.) J. S. Mill. Positive reactions to: **31** Lipids on subsessile glandular trichome. **32** Secretory head on pedunculate glandular trichome. **33** Starch. **34** Lipids on epidermis, parenchyma cells, and peduncle of glandular trichome. **35** Lipids of idioblasts on midrib. *Bar* = 20 μm (**31**); 40 μm (**33**, **34**); 50 μm (**32**, **35**)

Table 3 Main anatomical features distinguishing *Varronia globosa* Jacq. from *V. leucocephala* (Moric.) J. S. Mill

Characteristic	*V. globosa*	*V. leucocephala*
Stomata classification	Diacytic	Anisocytic
Stomata distribution	Amphistomatic	Hypostomatic
Stomata position	In the same level of other epidermal cells	Above the level of other epidermal cells
Abundance of non-glandular trichomes	Adaxial surface	Abaxial surface
Abundance of glandular trichomes	Abaxial surface	Adaxial surface
Types of glandular trichomes	Subsessil and pedunculate with globular head	Pedunculate with club-shaped hair
Cystolith on the basis of non-glandular trichomes	Absent	Present
Crystal types	Druses	Crystalline sand
Collenchyma type on midrib and petiole	Tangencial and angular	Annular
Petiole shape	D-shaped slightly sulcate	D-shaped obovate, not sulcate
Petiole vascular system	Collateral	Collateral and amphicribal
Accumulation of lipids	Absent	Present
Accumulation of starch	Absent	Present

loss, since stomata are better protected from solar radiation on the abaxial surface. Selvi and Bigazzi (2001) stated that the absence of stomata in the adaxial epidermis can also be an ancestral trait retained by some genera that were once common in woods or forests. To sustain this hypothesis it is required a study comparing the current biogeography of these species with the habitats present in the earlier geological periods in the same area. The stomata location above the epidermic level is also very common in *Cordia* species (Metcalfe and Chalk 1950), many of them currently in *Varronia*, it is suggested that this factor may influence the increased efficiency of gas exchange in *V. leucocephala*, since the trichome density is quite pronounced.

Particularly in *Cordia* s.l. the presence of cystoliths is a determinant character for species identification (Metcalfe and Chalk 1950), mainly consisting of calcium oxalate (Metcalfe and Chalk 1950; Binzet and Açkin 2009) and it is one more feature that separates the two species of this work. In *V. globosa*, druses can be observed in the mesophyll while in *V. leucocephala* it idioblasts containing crystal sand are observed. These crystals may be related to the plant ionic balance (Molano-Flores 2001; Volk et al. 2002; Paiva and Machado 2005) and defense against herbivory (Molano-Flores 2001; Korth et al. 2006), and play a substantial role in the resistance to water stress (Rotondi et al. 2003).

The lipid accumulation in epidermic cells and in some mesophyll cells, as well as in idioblasts located near the midrib and near the vascular bundles of the petiole are suggested as adaptive characteristics to xeric environment where *V. leucocephala* is inserted, for promoting water retention and avoiding excessive loss to the environment (Silva et al. 2011). The starch present in the parenchyma of the same species may act to control the osmotic pressure under water deficit, accumulating solutes from the conversion of starch into simple carbohydrates (Rotondi et al. 2003). In *V. globosa* lipids and starch were absent.

Our observations suggests that the combination of morphological and anatomical leaf characteristics may assist in distinguishing these species, such as stomata distribution, glandular trichomes types, non-glandular trichomes density, the accumulation of substances in *V. leucocephala*, crystal types, petiole shape, colenchyma type in midrib and petiole and the amphicribal vascular system in *V. leucocephala* petiole, once this results allowed the identification of several unique characteristics to each one. The anatomical descriptions of species are performed for the first time, which contributes to the expansion of knowledge about leaf anatomy for the Cordiaceae family.

The non-glandular trichomes are very similar between the two species, not being a good distinguishing feature; however, the glandular trichomes of both species and *V. verbenacea* (Ventrella and Marinho 2008) are morphologically different and represent a good feature differentiating species groups of this genus, as well as the density of non-glandular trichomes. Several unifying characteristics among species may be cited, such as uniseriate epidermis, dorsiventral mesophyll, bundles collateral type, presence of non-glandular and glandular trichomes, and petiole containing three free vascular traces.

The anatomical observations confirm the generalized adaptive properties to environmental conditions, high temperatures and intense solar radiation, such as amphistomatic leaf in *V. globosa*, retention of lipids and starch in the *V. leucocephala* parenchyma and high density of non-glandular trichomes. This species uses different strategies in adapting to the climate despite sharing the same environment. From the analysis of other representatives of the genus, it can be established more accurately, several anatomical features that can be used in delimitation of

their species, as well as checking that they are directly related to the natural habitat.

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