

# Leaf anatomy of six species of Heliotropiaceae Schrad. from the Brazilian semi-arid region

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Submetido em 12/08/2014  
Aceito para publicação em 19/06/2015

## Resumo

**Anatomia foliar de seis espécies de Heliotropiaceae Schrad. da região semiárida do Brasil.** A família Heliotropiaceae possui mais de 450 espécies distribuídas principalmente nas regiões tropicais e subtropicais. No Brasil está representada pelos gêneros *Euploca* Nutt., *Heliotropium* L., *Myriopus* Small e *Tournefortia* L. O objetivo do presente trabalho é descrever a anatomia foliar de seis espécies de Heliotropiaceae da região semiárida do Brasil: *E. polyphylla* (Lehm.) J.I.M. Melo & Semir, *E. procumbens* (Mill.) Diane & Hilger, *H. angiospermum* Murray, *H. curassavicum* L., *M. rubicundus* (Salzm. ex DC.) Luebert e *M. salzmannii* (DC.) Diane & Hilger. Além de contribuir para o conhecimento da anatomia de representantes da família na Caatinga (Savana brasileira), este trabalho busca elencar e destacar características anatômicas adaptativas à região, bem como buscar caracteres com potencial valor diagnóstico. Foram realizados procedimentos usuais em Microscopia Óptica (MO) e Microscopia Eletrônica de Varredura (MEV). Também foram realizados testes adicionais para detectar a presença de amido e de lipídios totais. A variação morfológica e a distribuição dos tricomas foram as características mais relevantes no diagnóstico das espécies. A distribuição dos estômatos em *M. salzmannii* e *H. angiospermum* é distinta das descrições apresentadas em outros trabalhos, o que demonstra plasticidade anatômica nestas espécies. A anatomia foliar de *M. rubicundus* é descrita pela primeira vez neste trabalho.

**Palavras-chave:** *Euploca*; *Heliotropium*; *Myriopus*; *Tournefortia*; Tricomas

## Abstract

The family Heliotropiaceae has more than 450 species, mainly distributed in the tropics and subtropics. In Brazil, it is represented by the genera *Euploca* Nutt., *Heliotropium* L., *Myriopus* Small and *Tournefortia* L. The aim of this study was to describe the leaf anatomy of six species of Heliotropiaceae recorded in the semi-arid region of Brazil: *E. polyphylla* (Lehm.) J.I.M. Melo & Semir, *E. procumbens* (Mill.) Diane & Hilger, *H. angiospermum* Murray, *H. curassavicum* L., *M. rubicundus* (Salzm. ex DC.) Luebert and *M. salzmannii* (DC.) Diane & Hilger. Besides contributing to the knowledge of the anatomy of the representatives of the family in

the Caatinga (Brazilian savannah), we also sought to determine and highlight the anatomical features adaptive to the region and to identify features with potential diagnostic value. Leaf structures were analyzed by light microscopy and scanning electron microscopy. Additional tests were also carried out to detect starch and total lipids. The morphological variation and the distribution of trichomes were the most relevant features for species diagnosis. The distribution of stomata in *M. salzmannii* and *H. angiospermum* differed from that described in other papers, which demonstrates the anatomical plasticity of these species. This is the first report describing the leaf blade of *M. rubicundus*.

**Key words:** *Euploca*; *Heliotropium*; *Myriopus*; *Tournefortia*; Trichomes

## Introduction

The family Heliotropiaceae is mainly distributed in the tropics and subtropics and comprises five genera and approximately 450 species (DIANE et al., 2002; 2003; GOTTSCHLING et al., 2004; LUEBERT et al., 2011). In Brazil, it is represented by the four genera *Euploca* Nutt., *Heliotropium* L., *Tournefortia* L. and *Myriopus* Small (MELO et al., 2009).

*Tournefortia* comprises approximately 150 species distributed in the Neotropics. Its representatives are allocated into two sections: *Tournefortia* sect. *Tournefortia* I.M. Johnst., with straight embryo and slightly lobed fruits; and *T.* sect. *Cyphocyema* I.M. Johnst., with curved embryo and a four-lobed fruit (CAVALHEIRO et al., 2011). In Brazil, both sections are represented by approximately 25 species (MELO et al., 2009). The inclusion of all species of *T.* sect. *Cyphocyema* in the genus *Myriopus* has been suggested by phylogenetic studies combined with morphological data, showing *T.* sect. *Tournefortia* as a polyphyletic group (DIANE et al., 2002). The species chosen for this study are in the genus *Myriopus*.

*Euploca* has approximately 50 species distributed in tropical, subtropical and temperate regions with centers of diversity in Africa, Australia and tropical America, especially in South America and Mexico. Its representatives are predominantly herbaceous, subshrubs and rarely shrubby (MELO; SEMIR, 2010). In Brazil, it is represented by 17 species (MELO et al., 2009).

The genus *Heliotropium* includes approximately 300 species, distributed in tropical and subtropical regions around the world (DIANE et al., 2004; IBÁÑEZ et al., 2011). It includes herbaceous, subshrubs, and more rarely, shrub representatives, where they are

mostly characterized by scorpioid inflorescences, free stamens, and schizocarp fruit with two locules and two seeds (MELO; SEMIR, 2008). In Brazil, the genus is found along the coast (Restinga and Atlantic Forest formations) and on rocky outcrops and is widespread in the Cerrado as well as Caatinga (Brazilian savannah) and Pampas (Brazilian grassland) (MELO; SEMIR, 2008). *Heliotropium* comprises nine species (MELO et al., 2009).

Anatomical features have been used in taxonomic and phylogenetic analysis of Heliotropiaceae, but few representatives of the Brazilian flora, particularly from the Caatinga, have been included (DIANE et al., 2003; FARIÑA et al., 2003; MUHAIDAT et al., 2011). In the Caatinga, studies on Heliotropiaceae are limited to taxonomic aspects (MELO; SALES, 2005; MELO; ANDRADE, 2007; VIEIRA et al., 2013).

This study aimed to describe the leaf anatomy of six species of Heliotropiaceae found in the Brazilian semi-arid region, Caatinga biome: *Euploca polyphylla*, *E. procumbens*, *Heliotropium angiospermum*, *H. curassavicum*, *Myriopus rubicundus* and *M. salzmannii*. Besides contributing to the anatomical knowledge of the representatives of Heliotropiaceae in the Caatinga, we sought to determine and highlight the anatomical features adaptive to the semi-arid region, looking for characters with potential diagnostic value.

## Material and Methods

Samples were collected in Caatinga areas in the states of Paraíba and Rio Grande do Norte, Northeastern Brazil: *E. polyphylla*. BRAZIL. **Rio Grande do Norte**. Porto do Mangue, 13-V-2007 (fl, fr), *Melo et al. 610*

(MOSS). *E. procumbens*. BRAZIL. **Rio Grande do Norte**. Tenente Laurentino da Cruz, 6°6'45.3"S, 36°42'10.7"W, 09-IV-2004 (fl, fr), *Melo & Xavier 449* (MOSS, PEUFR). *H. angiospermum*. BRAZIL. **Rio Grande do Norte**. Florânia, 6°7'32.8"S, 36°46'36.7"W, 09-IV-2004 (fl, fr), *Melo & Xavier 446* (MOSS, PEUFR). *H. curassavicum*. BRAZIL. **Paraíba**. Soledade, City's water reservoir, 17-IV-2009, *Tölke 51* (ACAM). *M. rubicundus*. BRAZIL. **Rio Grande do Norte**. Serra Negra do Norte, Seridó Ecological Station, 20-IV-2007 (fl, fr), *Melo et al. 594* (MOSS). *M. salzmannii*. BRAZIL. **Rio Grande do Norte**. Patu, Serra do Lima, 30-I-2008, *Melo et al. 661* (MOSS).

Three fully expanded leaves from different individuals (five individuals of each species) were fixed in FAA (formaldehyde, acetic acid, 50% ethanol, 1:1:18 v/v) for 24 h (JOHANSEN, 1940) and preserved in 70% ethanol. The samples were dehydrated in an ethanol series and embedded in HistoResin® Leica (GERRITS; SMID, 1983). Transverse and longitudinal sections of 5-7 µm thickness were obtained with a rotary microtome and stained with 0.05% toluidine blue in acetate buffer (pH 4.7) (O'BRIEN et al., 1964). All slides were temporarily mounted in water. The images were captured with a digital camera (Olympus DP71) coupled to a light microscope (Olympus BX51). Histochemical assays for starch (BERLYN; MIKSCHE, 1976) and total lipids (PEARSE, 1980) were performed using Lugol and Sudan black B, respectively.

For micro-morphological analysis, the samples were dehydrated in an ethanol series, dried by the CO<sub>2</sub> critical point method, mounted and metalized with gold. The observations and images were obtained by scanning electron microscopy (SEM) using a Jeol JSM 5800 LV at 10 kV with a coupled digital camera. The morphological description of the trichomes was according to Diane et al. (2003) and Metcalfe and Chalk (1979).

## Results

The following descriptions are based on the results of light microscopy and SEM.

### *Euploca polyphylla* and *E. procumbens*

The leaf blades of *E. polyphylla* and *E. procumbens* have a uniseriate epidermis on both surfaces (Figure 1A-B). In paradermic sections, both have curved anticlinal walls (Figure 1C-D). The epidermal cells are slightly rounded and have irregular size in transversal sections (Figure 1A-B). The leaf blades are amphistomatic (Figure 1A-D). The stomata of *E. polyphylla* are anomocytic (Figure 1C), while those of *E. procumbens* can be anomocytic and tetracytic (Figure 1D). *E. polyphylla* has dorsiventral leaves; the palisade parenchyma is single-layered and the spongy parenchyma has 5-7 layers (Figure 1A). *E. procumbens* has isobilateral leaves with a layer of palisade parenchyma facing each of the sides and 3-4 layers of spongy parenchyma between the palisade parenchyma (Figure 1B). The parenchyma cells of both species accumulate starch in their interior, evidenced by the positive reaction with Lugol (Figure 1E-F). The midribs are plano-convex, and the midrib region follows the pattern of the mesophyll; however, the palisade parenchyma is only on the upper surface (Figure 1G-H). *E. polyphylla* has a starch sheath in the midrib and lateral bundles (C4 photosynthesis) (Figure 1A, E and G), while in *E. procumbens* there are no chloroplasts in the sheath (C3 photosynthesis) (Figure 1B, F and G).

Unicellular non-glandular, simple and tapered trichomes were found in the two species (Figure 2A-F) on both surfaces. The abaxial surface of *E. polyphylla* has a homogeneous distribution of trichomes (Figure 2A), while the adaxial surface has trichomes only in the central region (Figure 2B). The trichomes of *E. polyphylla* are relatively short (90-250 µm), appressed to the leaf surface and have protuberances on the surface, and the adjacent epidermal cells form a high base (Figure 2C-D). In *E. procumbens*, the trichomes are long (400-500 µm), curved on the surface, but not appressed or with micropapillae on the surface (Figure 2E-F). The adjacent epidermal cells also form a high base around the trichomes (Figure 2F). The density of the trichomes is more pronounced in *E. procumbens* with homogeneous distribution on the adaxial and abaxial surfaces (Figure 2E).

FIGURE 1: General aspects of the leaf blade of *Euploca polyphylla* (A, C, E and G) and *E. procumbens* (B, D, F and H). A and B: leaf blade in cross section. C and D: stomata and cell wall of the upper surface in paradermic sections. E and F: positive reaction to Lugol, demonstrating the presence of starch. G and H: midrib in cross section. Abbreviations: St, stoma; SS, starch sheath; Xy, xylem; Ph, phloem. Scales: 25  $\mu\text{m}$  (C-D), 50  $\mu\text{m}$  (A-B, E-H).

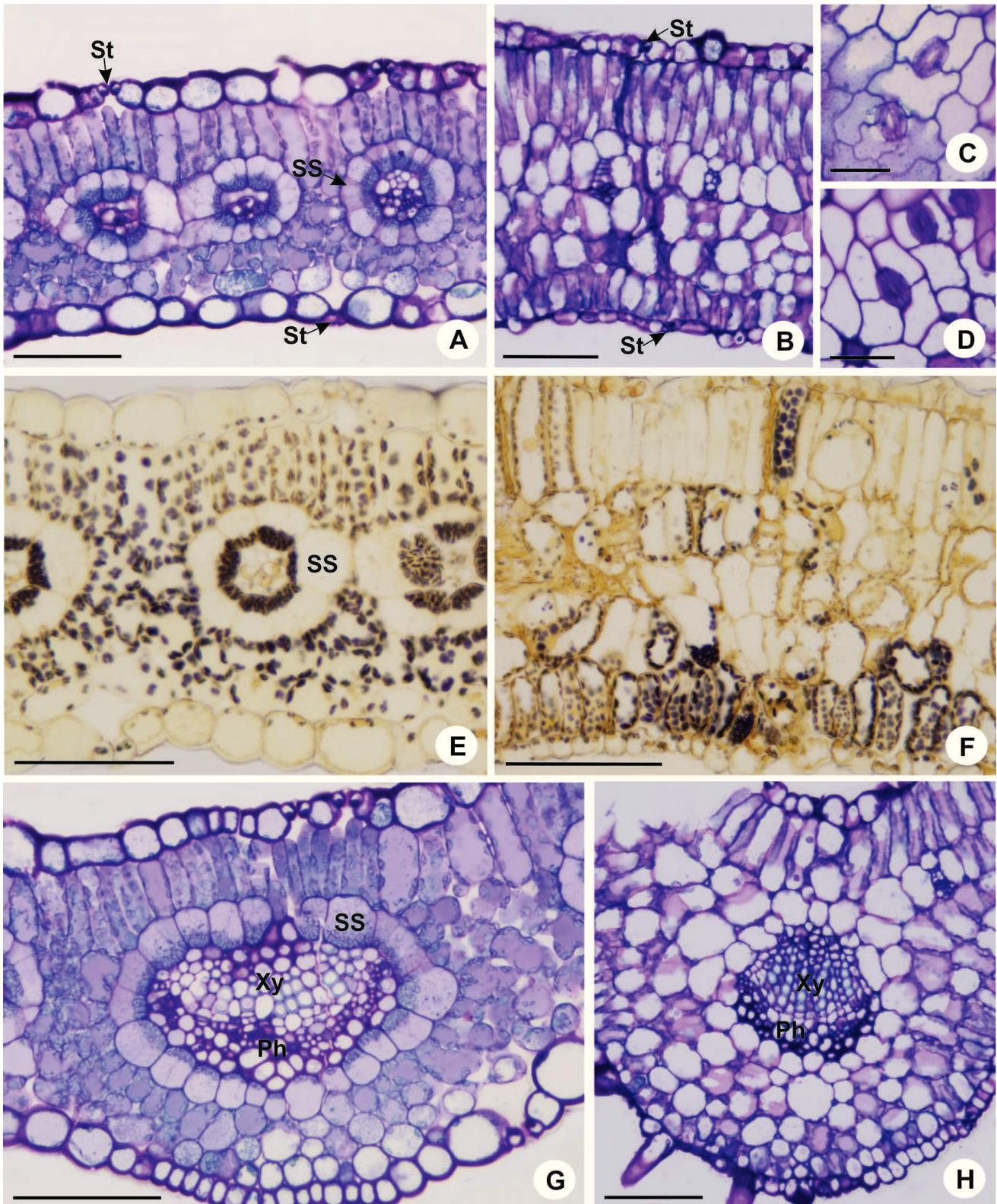
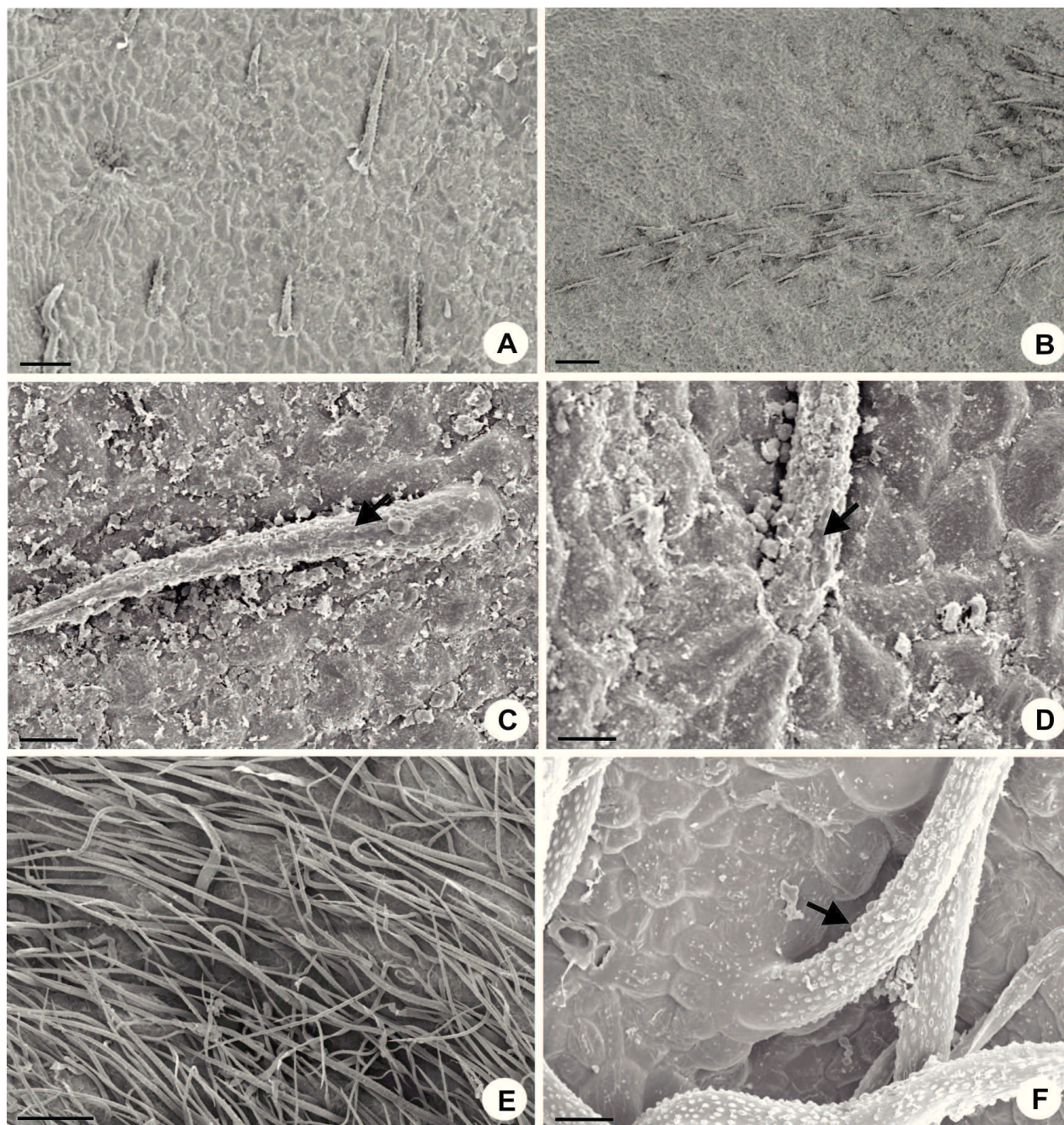


FIGURE 2: Non-glandular trichomes of *E. polyphylla* (A-D) and *E. procumbens* (E-F). A: distribution of the trichomes on the abaxial surface. B: adaxial surface with trichomes concentrated in the central region of the leaf blade. C and D: trichome appressed with protuberances (arrow) on adaxial surface. E: distribution of the trichomes on the abaxial surface. F: trichomes not appressed on adaxial surface with micropapillae (arrow). Scales: 20  $\mu\text{m}$  (C-D, F), 100  $\mu\text{m}$  (A), 200  $\mu\text{m}$  (B and E).



### *Heliotropium angiospermum* and *H. curassavicum*

*H. angiospermum* and *H. curassavicum* have a uniseriate epidermis with sinuous anticlinal walls on

both surfaces (Figure 3A-C). The epidermal cells are slightly rounded and have irregular size in transversal sections, and they are more elongated vertically in *H. curassavicum* (Figure 3 A-B). *H. angiospermum* has a hypostomatic blade (Figure 3A) and *H. curassavicum* an

amphistomatic blade (Figure 3B), both with anomocytic stomata (Figure 3C). *H. angiospermum* has dorsiventral leaves, the palisade parenchyma is single-layered and the spongy parenchyma has 3-6 layers (Figure 3A). *H. curassavicum* has isobilateral leaves with the palisade parenchyma showing 3-4 layers and parenchyma cells (3-5 layers) in the central region of the blade, not characterized as spongy parenchyma (Figure 3B). Lugol tests were negative for both species. Lithocysts can be seen (Figure 3A-B and D). Collateral vascular bundles

are present in the two species (Figures 3E-F and 4B); the midrib of *H. angiospermum* is convex-concave and the cortical region is composed exclusively of parenchyma cells (Figure 3E). The lateral bundles have a sheath of parenchyma cells that stains purple with toluidine blue, (Figure 3F); histochemical tests with Sudan black B indicated the production of lipids (Figure 4A). The midrib of *H. curassavicum* is plano-convex and the midrib region follows the pattern of the mesophyll (Figure 3B).

FIGURE 3: General aspects of the leaf blade of *H. angiospermum* (A, C, E and F) and *H. curassavicum* (B and D). A and B: leaf blade cross section. C: sinuous walls and anomocytic stomata on the adaxial surface. D: lithocyst in epidermis. E: midrib with collateral vascular bundle and cortical region composed of parenchyma cells. F: lateral bundle with sheath of parenchyma cells. Abbreviations: St, stoma; Li, lithocyst; Xy, xylem; Ph, phloem; SS, sheath. Scales: 20  $\mu\text{m}$  (D and F), 50  $\mu\text{m}$  (A-B), 100  $\mu\text{m}$  (E), 200  $\mu\text{m}$  (C).

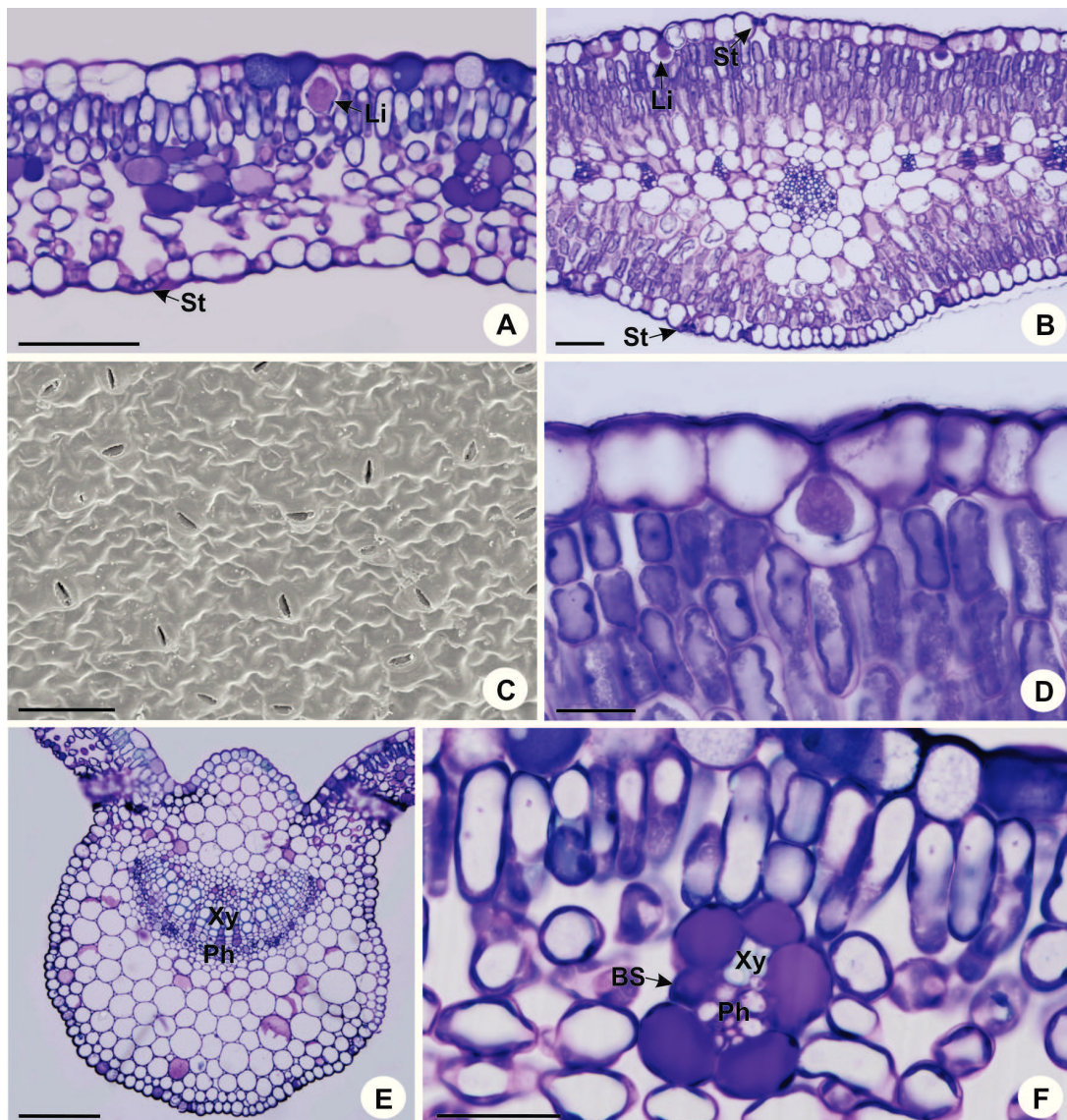
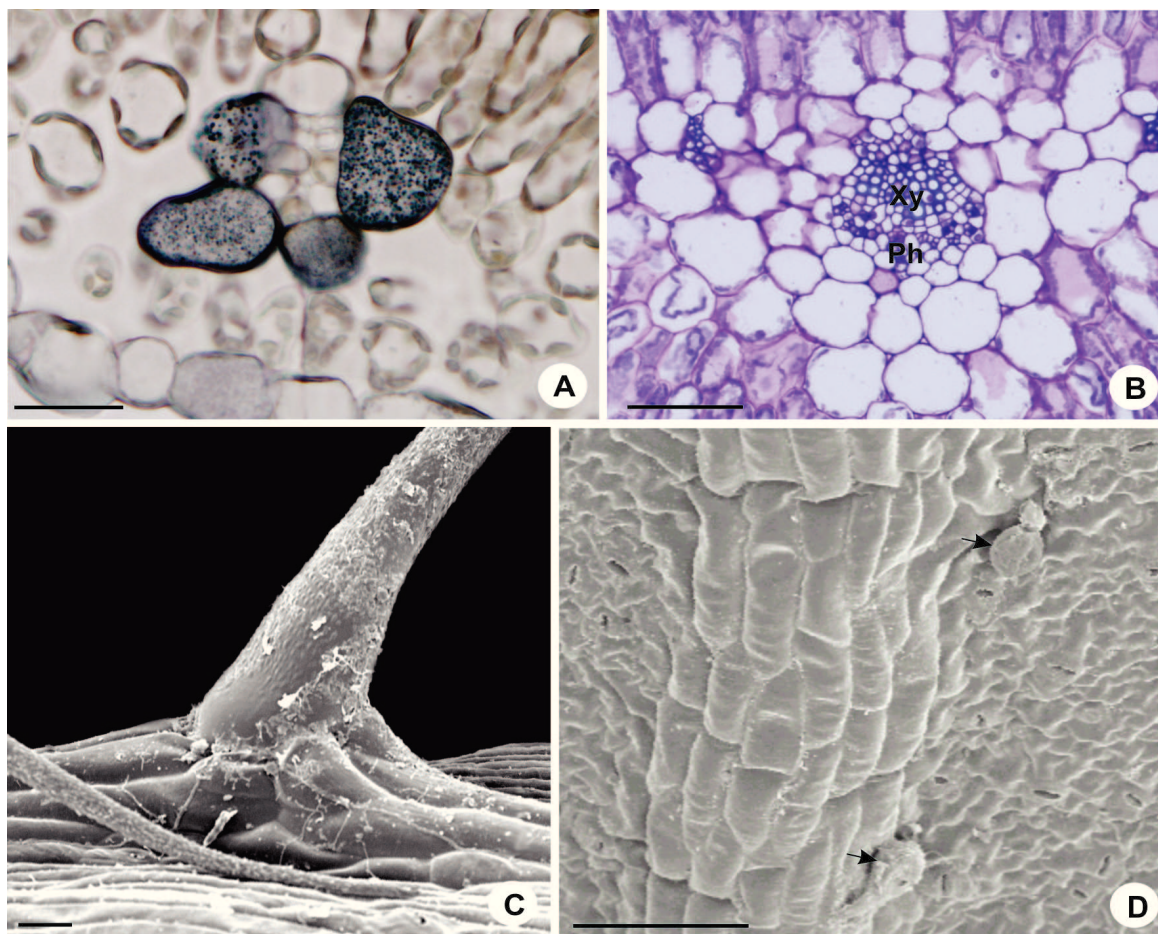


FIGURE 4: General aspects of the leaf anatomy of *H. angiospermum* (A, C, D) and *H. curassavicum* L. (B). A: positive staining with Sudan black B in the sheath cells. B: midrib showing side organization. C: non-glandular trichome detail around the midrib with micropapillae on the abaxial surface. D: glandular trichomes on the abaxial surface consisting of peduncle and secretory portion (arrows). Abbreviations: Xy, xylem; Ph, phloem. Scales: 20  $\mu\text{m}$  (A), 30  $\mu\text{m}$  (C), 50  $\mu\text{m}$  (B), 100  $\mu\text{m}$  (D).



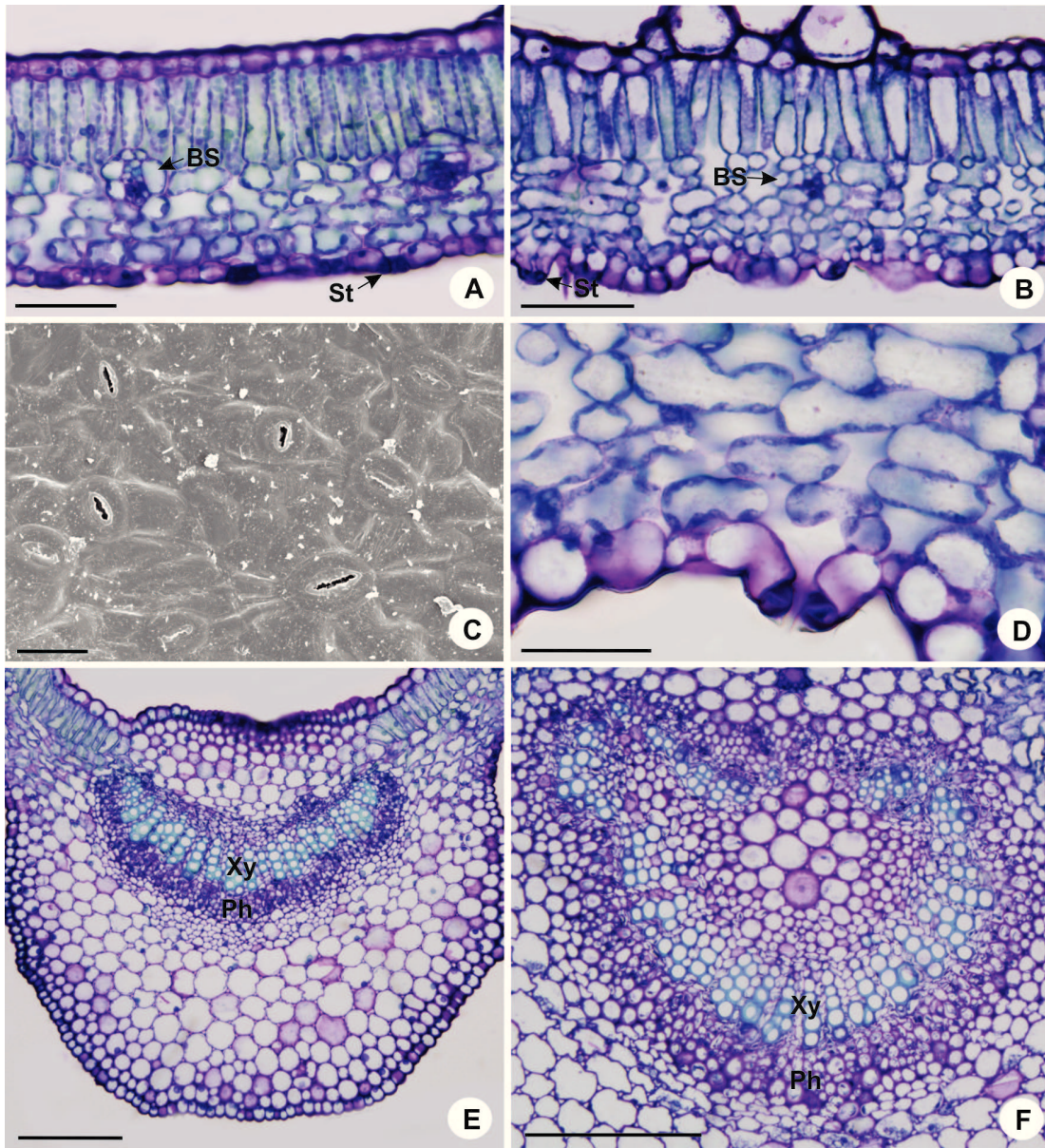
The leaf surface of *H. curassavicum* is glabrous on both surfaces, while *H. angiospermum* has non-glandular, simple, tapered trichomes, located only on the main vein on both sides. The trichomes are long (540-600  $\mu\text{m}$ ), not appressed and with small micropapillae on the surface, and the adjacent epidermal cells form a high base around the trichome (Figure 4C). In the same species, we also observed glandular trichomes consisting of peduncle and secretory portion (Figure 4D).

### *Myriopus rubicundus* and *M. salzmannii*

*Myriopus rubicundus* and *M. salzmannii* have a uniseriate epidermis with curved walls and anomocytic stomata on both surfaces (Figure 5A-C), and they have hypostomatic leaves (Figure 5A-B). The epidermis of

*M. salzmannii* is sinuous in paradermic sections (Figure 5 B). The epidermal cells are slightly rounded and are irregular in size in transversal sections (Figure 5 A-B), and the stomata are elevated in relation to neighboring cells (Figure 5D). Both have dorsiventral mesophyll containing one layer of palisade parenchyma; the spongy parenchyma in *M. rubicundus* contains 4-5 layers, and in *M. salzmannii*, there are 6-7 layers (Figure 5A-B). Collateral vascular bundles are found in both species (Figure 5E-F) and lateral bundles exhibit a parenchyma sheath (Figure 5A-B). The midrib of *M. rubicundus* is convex-concave (Figure 5E), and the cortical region displays angular collenchyma (Figure 6A). In *M. salzmannii*, the vascular bundle of the midrib forms a semi-arc, open at the top end (Figure 5F), and the cortical region has annular collenchyma (Figure 6B).

FIGURE 5: General aspects of the leaf anatomy of *M. rubicundus* (A, C and E) and *M. salzmannii* (B, D and F). A and B: leaf blade cross section. C: curved cell wall and anomocytic stomata on the abaxial surface. D: high stoma above the level of the epidermis. E and F: midrib in cross section. Abbreviations: BS, vascular sheath; St, stoma; Xy, xylem; Ph, phloem. Scales: 20  $\mu\text{m}$  (C-D), 40  $\mu\text{m}$  (A), 50  $\mu\text{m}$  (B), 100  $\mu\text{m}$  (E-F).

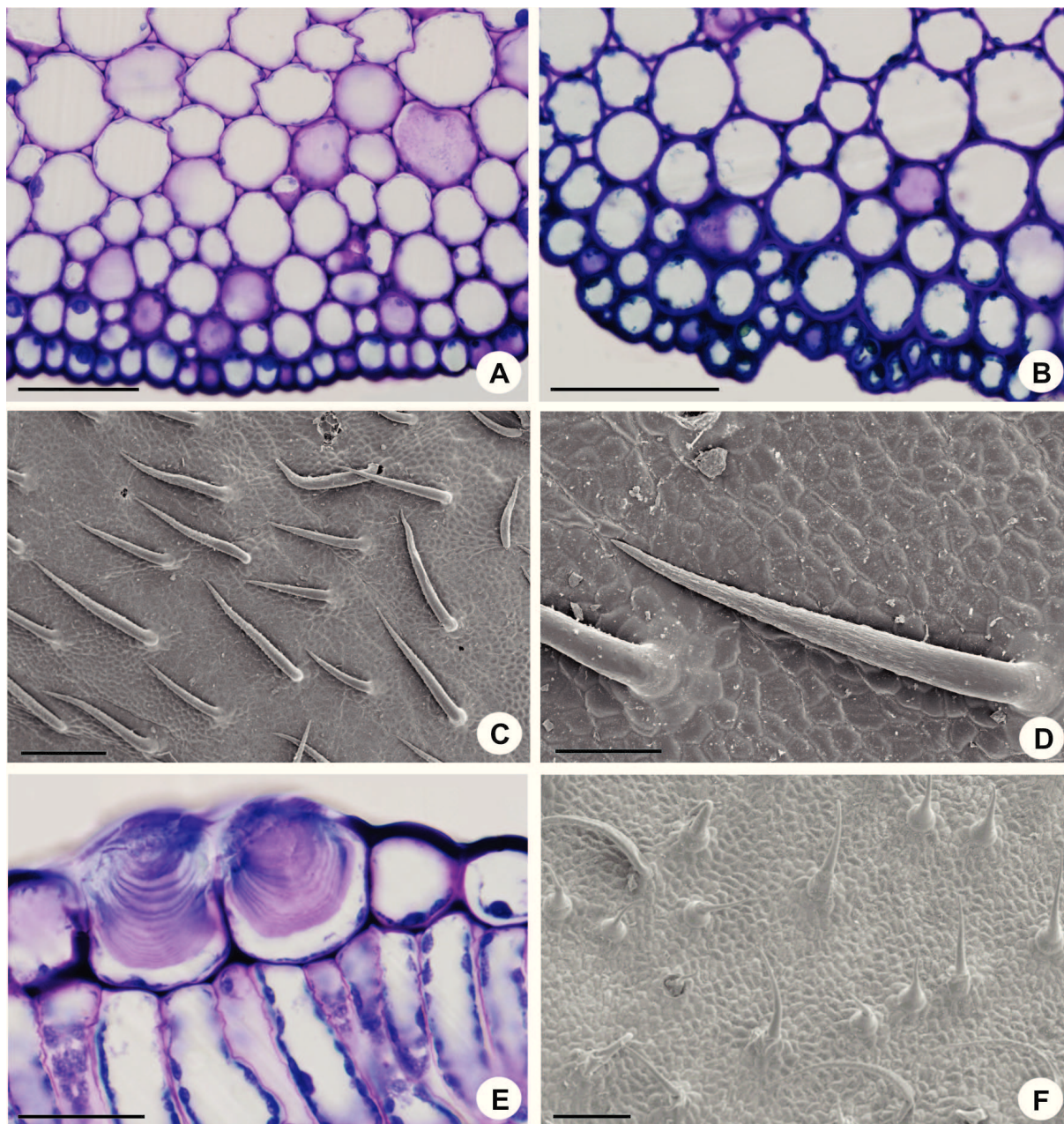


Unicellular, non-glandular, simple and tapered trichomes are present in the two species on both surfaces (Figures 6 C-F and 7 A-C). The trichomes of *M. rubicundus* are quite variable in size (280-550  $\mu\text{m}$ ) and appressed (Figure 6C-D), and they have a rough surface (Figure 6D), in addition to possessing cystoliths at their base (Figure 6E). In *M. salzmannii*, the density of the trichomes is more pronounced on the abaxial surface (Figures 6F and 7A), and two types of

trichomes are distinguished. The first one is distributed on both surfaces (Figures 6F and 7A), has a bulbous base, is erect, measures 100-500  $\mu\text{m}$  and has a smooth surface (Figure 7B). The second type, found in a smaller number, is quite similar to the trichomes described in *E. procumbens*. It is long (400-500  $\mu\text{m}$ ), with the surface covered by micropapillae, curved in relation to the leaf surface and distributed on the abaxial surface, primarily on the main vein (Figure 7C).



FIGURE 6: General aspects of the leaf anatomy of *M. rubicundus* (A, C-E) and *M. salzmännii* (B and F). A and B: cross section of the midrib, displaying the cortical region. C: trichomes on the adaxial surface. F: upright, non-glandular trichome of bulbous base on the adaxial surface. D: non-glandular trichome appressed to the adaxial surface. E: cells that form the basis of the non-glandular trichome displaying cystoliths in their interior. F: trichomes of bulbous base on the adaxial surface. Scales: 20  $\mu\text{m}$  (E), 40  $\mu\text{m}$  (A-B), 100  $\mu\text{m}$  (D), 200  $\mu\text{m}$  (F), 300  $\mu\text{m}$  (C).



Among the species studied, we observed four types of trichomes, all simple and tapered: type 1 – appressed, with micropapillae on the surface, without cystoliths in the cells of the trichome base; type 2 – curved, with micropapillae on the surface and the adjacent epidermal

cells forming a high base; type 3 – appressed, with micropapillae on the surface and with cystoliths in the epidermal cells; type 4 – upright, bulbous and smooth surface. In Table 1, we summarize the main features found in the species studied.

FIGURE 7: General aspects of the leaf anatomy of *M. salzmannii*. A: abaxial surface with non-glandular trichomes. B: upright, non-glandular trichome of bulbous base on the adaxial surface. C: non-glandular trichome, simple and tapered on the midrib. Scales: 30  $\mu\text{m}$  (B), 100  $\mu\text{m}$  (C), 200  $\mu\text{m}$  (A).

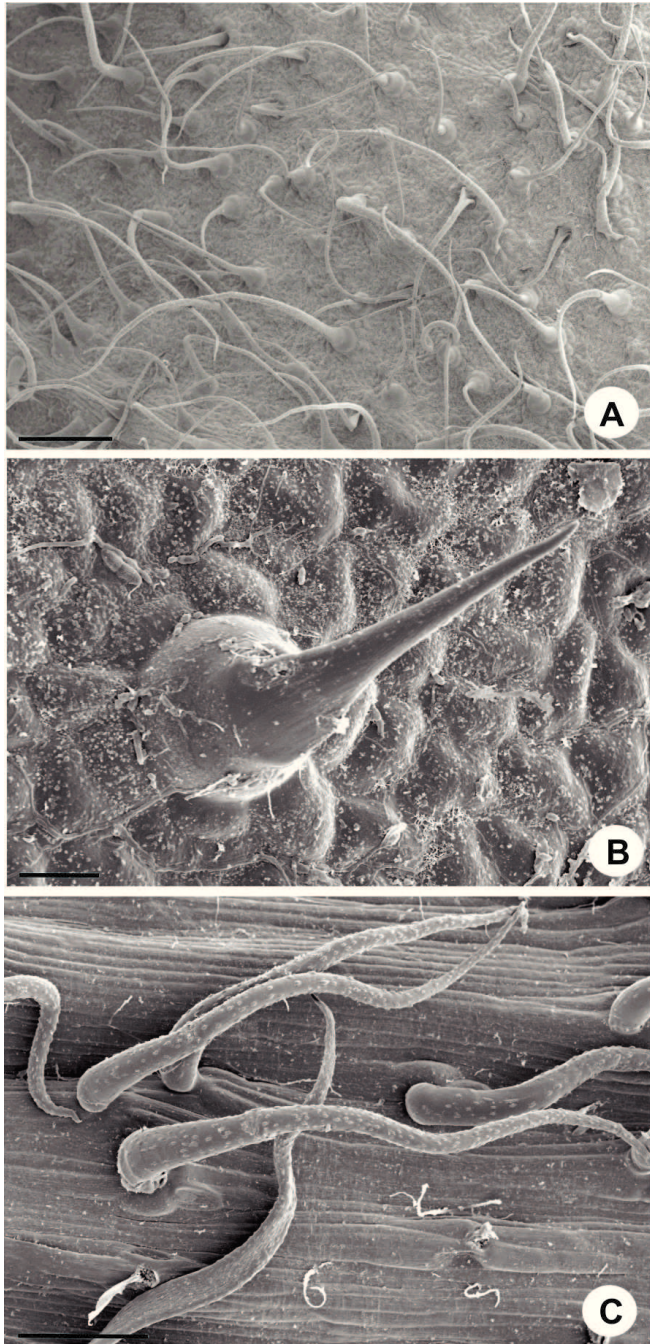


TABLE 1: Summary of the anatomical features of the leaf blade of the species studied.

Features/Species	E1	E2	H1	H2	M1	M2
- Epidermis	Uni	Uni	Uni	Uni	Uni	Uni
- Anticlinal wall	Cur	Cur	Sin	Sin	Cur	Cur
- Stomatal distribution	Amp	Amp	Hyp	Amp	Hyp	Hyp
-Type(s) of stoma(ta)	Ano	Ano/ Tet	Ano	Ano	Ano	Ano
- Mesophyll	Dor	Iso	Dor	Iso	Dor	Dor
- Reserve substance	Sta	Sta	Abs	Abs	Abs	Abs
- Midrib	PC	PC/ Sta	CC	PC	CC	SA
- Lateral vein	PS	LS	LS	PS	PS	PS
- Lithocysts	Abs	Abs	Pre	Pre	Abs	Abs
- Glandular trichomes	Abs	Abs	Pre	Abs	Abs	Abs
- Types of non-glandular trichomes	Type 1	Type 2	Type 2	Abs	Type 3	Types 2/4
- Distribution of non-glandular trichomes	Ad	Ad/ Ab	Ad/ Ab	Ab	Ad/ Ab	Ad/ Ab

\* Abbreviations: E1 – *E. polyphylla*; E2 – *E. procumbens*; H1 – *H. angiospermum*; H2 – *H. curassavicum*; M1 – *M. rubicundus*; M2 – *M. salzmannii*; Uni – uniseriate; Cur – curved; Sin – sinuous; Amp – amphistomatic; Hyp – hypostomatic; Ano – anomocytic; Tet – tetracytic; Dor – dorsiventral; Iso – isobilateral; Sta – starch; Abs – absent; PC – plano-convex; CC – convex-concave; SA – semi-arc; PS – parenchyma sheath; LS – lipid sheath; Pre – present; Ad – adaxial surface; Ab – abaxial surface.

## Discussion

This study showed that there was variation in the leaf anatomy of the species examined. However, certain features were present in all of them and are typical of the Heliotropiaceae. The presence of crystals (lithocysts) and non-glandular trichomes are important structures for species of the family (METCALFE; CHALK, 1983).

Non-glandular trichomes are often used in the separation of Heliotropiaceae species, extensively described in the work of Diane et al. (2003). The morphological variation of trichomes is broadly discussed as a useful feature for identifying species in several families of Angiosperms (WERKER, 2000), including Heliotropiaceae (METCALFE; CHALK, 1983; DASTI et al., 2003).

Variations in length, curvature and presence/absence of cystoliths occur in the trichomes of different species of the same genus, or between species belonging to different genera (METCALFE; CHALK, 1983). For

Heliotropiaceae, Diane et al. (2003) described seven types of non-glandular trichomes. In this study, we found three types of non-glandular trichomes fitting the descriptions given by Diane et al. (2003): unicellular, unbranched trichomes with rough surface and adjacent epidermal cells forming a high base (type 1); multicellular complex – adjacent epidermal cells containing cystoliths (type 3); and unicellular, unbranched trichomes with bulbous base (type 4). Type 2 (curved, with micropapillae on the surface and adjacent epidermal cells forming a high base) was not described in the work of Diane et al. (2003). In their work, the trichomes with adjacent epidermal cells forming a high base are always appressed, whereas the type 2 is curved in relation to the leaf surface.

Crystals also have taxonomic value in the family (METCALFE; CHALK, 1983; DIANE et al., 2003). According to Diane et al. (2003), crystals are often found in the species of the genera *Tournefortia* (including *Myriopus*) and *Heliotropium*. In this study, crystals were reported in the species of *Heliotropium* (in the lithocysts) and also in the cells of the non-glandular trichome base of *M. rubicundus*. Cystoliths in the basal part of the trichomes are common in representatives of the family, and sometimes in the epidermal cells adjacent to the trichomes (METCALFE; CHALK, 1983). The lithocysts observed in the two species of the genus *Heliotropium* are considered by Diane et al. (2003) as reduced unicellular trichomes. However, to confirm such a hypothesis, an in-depth study is required aimed at reconstructing the evolutionary history of trichome morphology in the family.

The mesophyll showed great diversity between the species examined, ranging from isobilateral to dorsiventral in a single genus. Therefore, it should not be used as a diagnostic character between species of different genera. The same is true for characteristics such as size and shape of epidermal cells and type of stomata.

Overall, the leaf anatomy of *M. salzmannii* described here corresponds to the observations made by Diane et al. (2003) for the same species. However, differing from the data described in the above cited work, the leaf of *M. salzmannii* in the Caatinga environments was hypostomatic, while the specimen examined by

Diane et al. (2003) was amphistomatic. The same occurs in *H. angiospermum*, described by Diane et al. (2003) as amphistomatic; in our study, leaves were found to be hypostomatic. According to Kundu and Tigerstedt (1998), hypostomatic leaves are typical of xeric environments, which allow great water savings by reducing the rate of transpiration. Another different aspect was shown in relation to the types of trichomes of *M. salzmannii*: Diane et al. (2003) described only type 2 trichomes, and in the present investigation, we also found non-glandular trichomes of bulbous base.

In relation to *Euploca*, we also found differences in relation to what is described in the literature. In this study, we found only one type of non-glandular trichome in *E. procumbens*, i.e., curved over the surface, not appressed and with micropapillae. Diane et al. (2003) described two types of trichomes for this species: (1) trichomes with micropapillae on the surface and (2) multicellular complex of lithocysts and slender trichome with the base at the level of the epidermis. This feature may be related to the general environment of the species (Caatinga).

The leaf anatomy of *H. curassavicum* was in accordance with that described by Diane et al. (2003). However, the authors stated that the species had druses in the mesophyll, structures not found in the present study. The number and size of the crystals vary in different periods of the year and also according to the availability of limestone material in the soil (METCALFE; CHALK, 1983), which explains their absence in this work.

Among the species belonging to the genera studied, we found characters with potential diagnostic value. The main feature that differentiates *E. procumbens* from *E. polyphylla* is the Kranz anatomy present in the latter, which was already observed in the work of Muhaidat et al. (2011). In addition, the type and distribution of the trichomes are also important. The main difference between *H. curassavicum* and *H. angiospermum* is the absence of trichomes in the former, while the latter displays non-glandular and glandular trichomes. The species *M. salzmannii* and *E. procumbens* differ anatomically primarily by the types of trichomes they have.

The results obtained can assist in identifying the leaf features of Heliotropiaceae species found in the Caatinga. The anatomical description of *M. rubicundus* is presented for the first time. Several features were different in relation to the studies already carried out for the same species, for example, the distribution of the stomata in *M. salzmannii* and *H. angiospermum*, which may be related to environmental factors, since the leaf blade is the structure that changes the most in response to environmental changes (ELIAS et al., 2003).

Because of the great importance of the morphology of trichomes in Heliotropiaceae, we suggest a study encompassing a greater number of species of this family, also including the genus *Ixorhea* Fenzl, in which species are monospecific and endemic to Argentina. The overall aim would be to determine the types of trichomes and their use in phylogenetic approaches regarding this family.

## Acknowledgments

We thank the Electron Microscopy Laboratory of the State University of Campinas (UNICAMP), Plant Anatomy Laboratory of the State University of Campinas and Botanical Laboratory of the State University of Paraíba (UEPB) for the use of the equipment. J.I.M. Melo thanks the National Council for Scientific and Technological Development (CNPq) for the Research Productivity Scholarship (PQ-2 Process number 302751/2012-2). Dr. A. Leyva helped with English editing of the manuscript.

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