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Original Article

Leaf morphoanatomy supporting evolutionary relationships in a recent clade of Asteraceae

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ABSTRACT

This study analyzes the leaf morphoanatomy of representatives of the Chapada Diamantina clade (Asteraceae, Eupatorieae) to identify anatomical characters that aid in the taxonomic delimitation of the group. Leaves from 16 species across six genera were examined, focusing on epidermis, mesophyll, vascular system, and trichomes. The results indicate adaptations to the Campo Rupestre environment, including a thick cuticle, high trichome density, stomatal crypts, and multiseriate parenchyma, suggesting a hypodermis of ground meristem origin and sclerenchyma sheath extensions. We scored 27 structural characters and mapped them onto the most recent phylogenetic tree recovered for the clade. The positioning of secretory ducts in the midrib may represent a synapomorphy of the clade. The presence of isobilateral mesophyll and sclerenchyma fibers in *Agrianthus* and *Arrojadocharis* reinforces their phylogenetic proximity. *Semiria* sp. nov. and *Semiria viscosa* share convergent anatomical traits, but key structural differences support their generic separation. Additionally, *Bishopiella elegans* is distinguished by homogeneous mesophyll and absence of mechanical support tissues, whereas *Lasiolaena* and *Stylotrichium* exhibit revolute margins and multistratified hypodermis. These findings highlight the relevance of leaf anatomy in taxonomic and evolutionary studies, supporting previous phylogenetic hypotheses and emphasizing the importance of integrating morphoanatomical and molecular data in the classification of Asteraceae.

Keywords: Asteraceae, Campo Rupestre, evolution, leaf anatomy, taxonomy.

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Introduction

Comparative micro and macromorphology, combined with molecular phylogenetics, provides insights into the evolution and function of characteristics and organisms, including their diversification and relationships (Jeiter & Smets, 2024). However, to achieve a clearer understanding of evolutionary processes, it is essential to precisely describe morphological variation, which begins with establishing a robust taxonomy, whose knowledge can inform and guide developmental studies (Kellogg, 2006).

Plant anatomy has long been recognized as a valuable and promising field in taxonomic research, providing a wide range of structural characters that offer robust and reliable evidence for the classification of angiosperms (Solereder, 1908; Metcalfe & Chalk, 1950; Cruz *et al.*, 2017; Lusa *et al.*, 2018; Bento *et al.*, 2020). Moreover, anatomical data have played a crucial role in supporting evolutionary hypotheses and clarifying phylogenetic relationships within different angiosperm families (Cetzal-IX *et al.*, 2013; Martínez-Sagarra *et al.*, 2017; Mello *et al.*, 2019; Matos & Araújo, 2021).While plants should be analyzed as a whole, the leaf stands out as the most plastic organ, with its adaptations visibly and rapidly reflecting environmental conditions across diverse habitats (Dickison, 2000).

Asteraceae, one of the largest families of Angiosperms, displays several foliar anatomical traits that serve as important taxonomic markers, including anomocytic stomata, secretory structures, and collateral bundles accompanied by fibers (Solereder, 1908; Metcalfe & Chalk, 1950). Among the anatomical features that may aid in the taxonomy of this family, the outline of epidermal cells, types and distribution of stomata (Adedeji & Jewoola, 2008; Bombo *et al.,* 2016; Budel *et al.,* 2018), diversity of clothing trichomes (Wagner *et al.,* 2014), and characteristics of the mesophyll and fibrovascular system (Melo-de-Pinna, 2004; Ruiz *et al.,* 2016) stand out. Secretory ducts (Lersten & Curtis, 1985) and glandular trichomes, which contain different chemical compounds that aid in protection against herbivores (Fernandes *et al.,* 2016; Muravnik *et al.,* 2016), are also considered significant taxonomic markers for different groups within the family (Castro *et al.,* 1997).

Leaf anatomy has supported phylogenetic hypotheses by identifying synapomorphies within tribes and subtribes of Asteraceae (Lusa *et al.,* 2018; Janaćković *et al.,* 2019; Liesenfeld *et al.,* 2019), enhancing our understanding of the adaptive diversity among its species (Ferraro & Scremin-Dias, 2018; Silva *et al.,* 2019; Liesenfeld *et al.,* 2019). These characteristics also play a pivotal role in plants' responses to both biotic and abiotic environmental factors (Castro *et al.,* 2007; Muniz *et al.,* 2018; Ferraro & Scremin-Dias, 2018; Silva *et al.,* 2019). They are particularly relevant in widely diversified groups, such as the tribe Eupatorieae, which is distributed in the neotropical region (King & Robinson, 1987; Robinson *et al.*, 2009).

The tribe Eupatorieae Cass. comprises 186 genera distributed in 20 subtribes and approximately 2500 species, mainly in the neotropical region (King & Robinson, 1987; Robinson *et al.,* 2009; Rivera *et al.,* 2016b). In South America, Eupatorieae is especially diverse in Brazil, where it includes 88 genera and 611 species, exhibiting high rates of endemism (45% and 72%, respectively; Siniscalchi *et al.,* 2021).

In recent years, molecular research on representatives of the Eupatorieae tribe in Brazil has tested the monophyly of its subtribes (Ferreira, 2010; Hattori, 2013; Rivera *et al.*, 2016a, b) and genera (Fernandes, 2013; Oliveira, 2015; Roque *et al.,* 2017; Amorim, 2019). The most comprehensive of these studies was proposed by Rivera *et al.* (2016a), who recovered monophyletic groups and revealed many polyphyletic genera and subtribes, uncovering novel evolutionary relationships within the tribe. Among these clades, a clade emerged comprising six genera: *Agrianthus* Mart. ex DC. (9 spp.), *Arrojadocharis* Mattf. (2 spp.), *Bishopiella* R.M.King & H.Rob. (1 sp.), *Lasiolaena* R.M.King & H.Rob. (6 spp.), *Semiria* D.J.N. Hind (1 sp.), and *Stylotrichium* Mattf. (6 spp.), all restricted to the campos rupestres (rocky fields) of the Espinhaço Range, Brazil. This same clade was recovered by Roque *et al.* (2017), who sampled five different species compared to the previous analysis.

Considering that the research carried out by Rivera *et al.* (2016a) included only a partial sampling (14 out of 25 species), Amorim (2019) included all genera and 96% of the species in her phylogenetic research. In this study, the clade was named the Chapada Diamantina and was recovered as monophyletic (support of 1.00 PP / 72% BS), with a recent origin during the Pliocene (5.1 Mya) and rapid diversification during the Pleistocene (2.5 Mya). According to the author, except for *Bishopiella*, which is monospecific, the other five genera are not monophyletic.

It is believed that an approach using foliar anatomical characteristics could be an effective tool in taxonomic delimitation (Dickison, 2000) or even evince morphological markers of evolutionary processes among groups (Lusa *et al.,* 2018; Scatena *et al.,* 2005). Thus, this study aims to describe and analyze the leaf anatomy of representatives of the Chapada Diamantina Clade (Asteraceae, Eupatorieae) to identify morphoanatomical characters that may assist in the delimitation of the clade and the taxa involved.

Materials and Methods

Sampling

The ingroup included all six genera and 16 species (60% of the total) represented in the clades recovered by Rivera *et al.* (2016a) and Amorim (2019). Regarding the outgroup, two genera and two species were sampled (Fig. 1, Table 1).

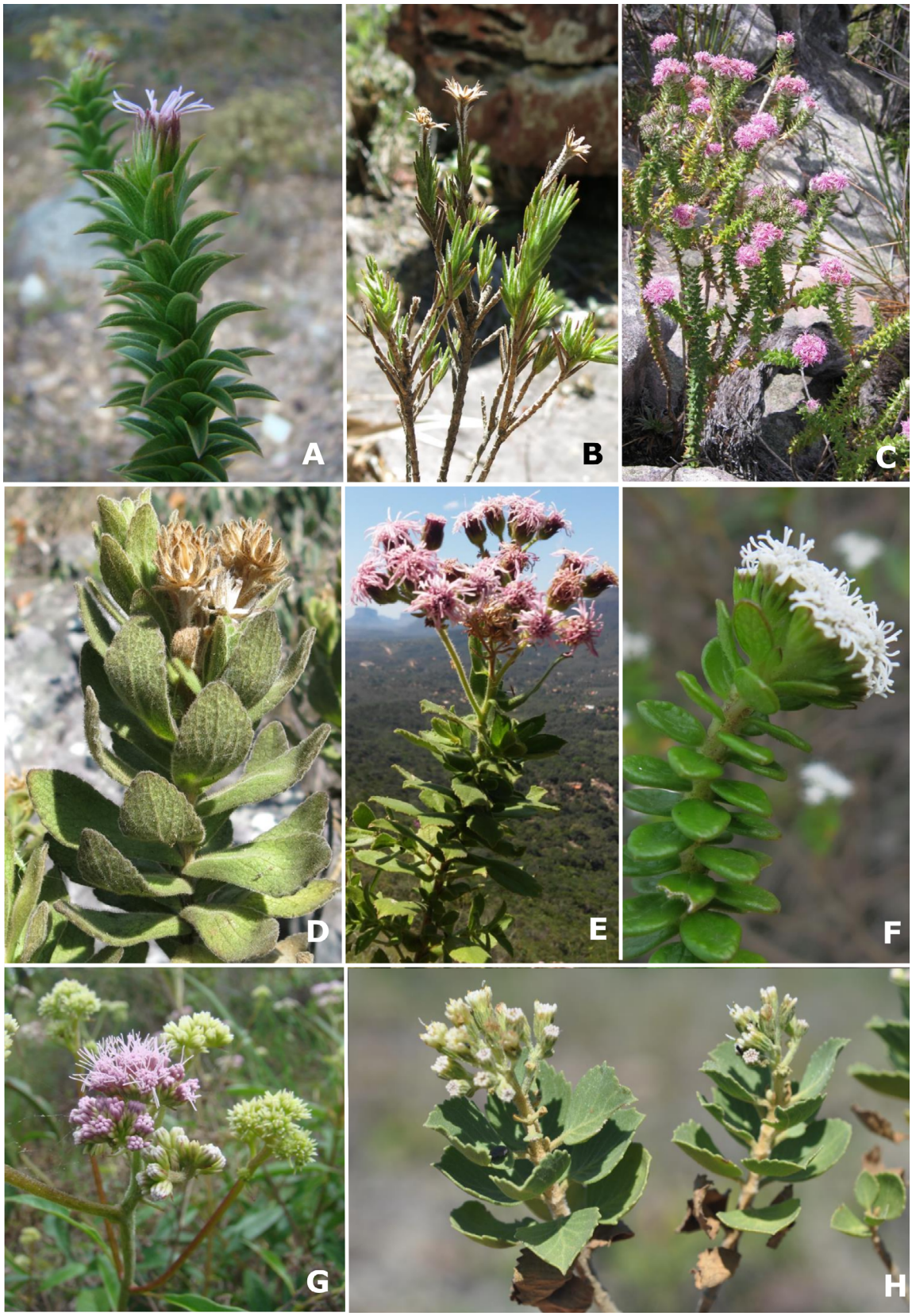

Figure 1. Representative species of the Chapada Diamantina Clade (A-F) and outgroups (G-H). A. *Agrianthus myrtoides* Matt., B. *Arrojadocharis santosii*, C. *Lasiolaena lychnophorioides*, D. *Semiria viscosa*, E. *Semiria* sp. nov., F. *Stylotrichium glomeratum*, G. *Acritopappus confertus*, H. *Lapidia apicifolia*. Photos: A, F, G. N. Roque, B, C, E. V.O. Amorim, D. S. Ferreira, H. L. Barres.

For each species, one individual from three different populations was collected when possible. Three fully expanded leaves were taken from the branch at the third or fourth node from the stem apex, except for *Bishopiella elegans*, which exhibits rosette leaves. The material collected in the field was fixed in 70% FAA - Formaldehyde, Acetic Acid, and preserved in 70% ethyl alcohol after 48 hours (Johansen, 1940). Voucher specimens were herbarium-prepared and deposited at the Alexandre Leal Costa Herbarium (ALCB) of the Universidade Federal da Bahia, with duplicates sent to the HUEFS Herbarium from the Universidade Estadual de Feira de Santana (Table 1).

Table 1. Selected species from the Chapada Diamantina clade (Asteraceae, Eupatorieae) for foliar anatomy analysis. All specimens are deposited in the ALCB Herbarium. *Species that belong to the outgroup.

Species	Populations	Treatment	Elevation	Voucher
Agrianthus giuliettiae D.J.N. Hind	V.O. Amorim *et al.* 480	Herbarium	1612 m	HUEFS 244589
	V.O. Amorim *et al.* 448	Fixed	1558 m	ALCB 135378
	V.O. Amorim *et al.* 362	Herbarium	1068 m	ALCB 036518
Agrianthus luetzelburgii Mattf.	V.O. Amorim *et al.* 375	Herbarium	1127 m	ALCB 036513
	V.O. Amorim *et al.* 468	Fixed	1229 m	HUEFS 244581
	V.O. Amorim *et al.* 357	Herbarium	1459 m	ALCB 140382
Agrianthus microlicioides Mattf.	V.O. Amorim *et al.* 437	Herbarium	1082 m	ALCB 140383
	V.O. Amorim *et al.* 467	Fixed	1433 m	ALCB 138833
Arrojadocharis praxeloides Mattf.	V.O. Amorim *et al.* 494	Fixed	1172 m	ALCB 134774
Arrojadocharis santosii R.M. King & H.Rob.	V.O. Amorim *et al.* 445	Herbarium	1553 m	ALCB 134790
	V.O. Amorim *et al.* 450	Herbarium	1465 m	ALCB 134788
	V.O. Amorim *et al.* 465	Herbarium	1657 m	HUEFS 244579
Bishopiella elegans R.M. King & H.Rob.	V.O. Amorim *et al.* 526	Fixed	1514 m	ALCB 138815
	V.O. Amorim *et al.* 527	Fixed	1465 m	ALCB 138816
Lasiolaena blanchetii (Ach. Bip. *ex* Baker) R.M.King & H.Rob.	V.O. Amorim *et al.* 438	Herbarium	1692 m	ALCB 138825
	V.O. Amorim *et al.* 366	Herbarium	1101 m	ALCB 138827
Lasiolaena duartei R.M. King & H.Rob.	V.O. Amorim *et al.* 381	Herbarium	873 m	ALCB 138808
	V.O. Amorim *et al.* 401	Herbarium	982 m	ALCB 138811
	V.O. Amorim *et al.* 431	Herbarium	1814 m	ALCB 138810
Lasiolaena lychnophorioides Roque, S.C. Ferreira & H.Rob.	V.O. Amorim *et al.* 399	Herbarium	1348 m	ALCB 138196
	Gandara *et al.* 29	Herbarium	1383 m	ALCB 120819
Lasiolaena pereirae R.M.King & H.Rob.	V.O. Amorim *et al.* 419	Herbarium	1215 m	ALCB 141514
Lasiolaena santosii R.M. King & H.Rob.	V.O. Amorim *et al.* 479	Fixed	1689 m	HUEFS 244588
Semiria viscosa D.J.N. Hind	V.O. Amorim *et al.* 447	Fixed	1553 m	ALCB 134776

Table 1. Cont.

Species	Populations	Treatment	Elevation	Voucher
Semiria sp. nov.	V.O. Amorim *et al.* 416	Herbarium	1058 m	ALCB 141511
	V.O. Amorim *et al.* 425	Herbarium	1151 m	ALCB 141516
Stylotrichium corymbosum Mattf.	V.O. Amorim *et al.* 497	Fixed	717 m	ALCB 134799
Stylotrichium rotundifolium Mattf.	V.O. Amorim *et al.* 415	Herbarium	1228 m	ALCB 134770
	V.O. Amorim *et al.* 491	Herbarium	1307 m	HUEFS 244594
	V.O. Amorim *et al.* 516	Herbarium	–	HUEFS 244608
Stylotrichium sucrei King & H.Rob.	V.O. Amorim *et al.* 372	Herbarium	1230 m	ALCB 134778
	V.O. Amorim *et al.* 409	Herbarium	1537 m	ALCB 134780
**Acritopappus confertus* (Gardner) R.M.King & H.Rob.	A.V.F. Guterres 382	Fixed	903 m	ALCB 137861
	A.V.F. Guterres 385	Fixed	937 m	ALCB 137863
**Lapidia apicifolia* Roque & S.C.Ferreira	A.V.F. Guterres 376	Fixed	903 m	ALCB 135976
	M.G. Staudt *et al.* 118	Fixed	1013 m 1013 m	ALCB 75951 ALCB 75951

Sample Processing and Light Microscopy (LM) Analysis

For the anatomical study, FAA-fixed samples were used (Johansen, 1940). Herbarium materials were used when necessary, undergoing rehydration, which involved placing the leaves in 5% sodium hydroxide for two days, then washing them with distilled water five times for 20 minutes each and subsequently dehydrating them in an ascending ethanol series before storing them in 70% ethyl alcohol (Anderson, 1963).

Analyses of the epidermal surface and cross-sections were conducted at the apex, middle, and base of the leaf blade, and in the mid-region of the petiole. For epidermal analysis, samples were chemically dissociated using the Franklin method (modified by Kraus & Arduin, 1997). The isolated epidermises were then stained with 1% alcoholic Safranin (Kraus & Arduin, 1997), mounted on semi-permanent slides with 50% glycerin, and sealed with clear nail polish.

Cross-sections of the petiole and leaf blade (main vein, mesophyll, and margin) were made from dehydrated material embedded in synthetic resin (Historesin, Leica®) following the manufacturer's instructions. The embedded material was sectioned at a thickness of 5 μm using a Carl Zeiss HM325 rotary microtome (Thermo Scientific). Histological sections were stained with 0.05% Toluidine Blue (Sakai, 1973) and mounted on permanent slides with Entellan synthetic resin (Merck®).

Anatomical analyses of the material and photographic documentation under light microscopy (LM) were performed using a Carl Zeiss Axio Scope A1 photomicroscope with an attached digital camera (EOS, Canon) installed at the Plant Anatomy and Wood Identification Laboratory (LAVIM) at the Instituto de Biologia, Universidade Federal da Bahia.

Descriptions of leaf anatomical structures followed those used by Metcalfe & Chalk (1950) and Fahn & Cutler (1992), while trichome terminology was based on Trindade *et al.* (2014), Budel *et al.* (2018), Payne (1978), Silva *et al.* (2019), Liesenfeld *et al.* (2019), and Martínez-Quezada *et al.* (2022). Only trichomes that were fully intact in paradermal and cross-sections were considered for description. In Table 2, 'unassessed character' indicates structures that could not be described due to their location, either within stomatal crypts or beneath the cuticle.

Table 2. Anatomical characteristics present in the adaxial and abaxial epidermis of species from the Chapada Diamantina clade (Asteraceae, Eupatorieae). Legend: * Character not analyzed due to stomatal crypts; ani – anisocytic stomata; ano – anomocytic stomata.

	Epidermis				Stomata				
Species/Characteristics	**Adaxial outline**	**abaxial outline**	**Layers**	**Cuticle thickness**	**Types**	**Adaxial outline**	**Abaxial outline**	**Leaf blade**	**Crypts**
Agrianthus giuliettiae	Straight	Straight	Uniseriate	Thick	Ano	Straight	Straight	Amphistomatic	Absent
A. luetzelburgii	Straight	Straight	Uniseriate	Thick	Ani/ano	Straight	Straight	Amphistomatic	Absent
A. microlicioides	Straight	Straight	Uniseriate	Thick	Ani/ano	Straight	Straight	Amphistomatic	Absent
Arrojadocharis praxeloides	Straight	Straight	Uniseriate	Thick	Ani/ano	Sinuous	Straight	Amphistomatic	Absent
A. santosii	Straight	Straight	Uniseriate	Thick	Ani/ano	Straight	Straight	Amphistomatic/ Hypostomatic	Absent
Bishopiella elegans	Straight	Straight	Uniseriate	Thin	Ani/ano	Straight	Straight	Amphistomatic	Absent
Lasiolaena blanchetii	Straight	*	Uniseriate	Thin	Ano	Absent	*	Hypostomatic	Present
L. duartei	Straight	*	Uniseriate	Thick	Ano	Absent	*	Hypostomatic	Present
L. lychnophorioides	Straight	*	Uniseriate	Thin	Ano	Absent	*	Hypostomatic	Present
L. pereirae	Straight	Straight	Uniseriate	Thick	Ano	*	*	Amphistomatic	Present
L. santosii	Straight	*	Uniseriate	Thick	Ano	Absent	*	Hypostomatic	Present
Semiria viscosa	Straight	Sinuous	Uniseriate	Thick	Ano	Straight	Sinuous	Amphistomatic	Absent
Semiria sp. nov.	Straight	Sinuous	Uniseriate	Thick	Ani/ano	Sinuous	Sinuous	Amphistomatic	Absent
Stylotrichium corymbosum	Straight	*	Uniseriate	Thick	Ano	Absent	*	Hypostomatic	Present
S. rotundifolium	Straight	*	Uniseriate	Thick	Ano	Absent	*	Hypostomatic	Present
S. sucrei	Straight	*	Uniseriate	Thick	Ano	Absent	*	Hypostomatic	Present
Outgroup									
Acritopappus confertus	Straight	Straight	Biseriate on adaxial	Thick	Ano	Absent	Sinuous	Hypostomatic	Absent
Lapidia apicifolia	Straight	Straight	Uniseriate	Thick	Ano	Straight	Straight	Amphistomatic	Absent

Character coding and mapping

A matrix of binary and/or multistate anatomical characters was elaborated based on the analysis of the dermal and vascular systems of leaf blades. Character coding followed Sereno (2007), and hypotheses of primary homologies were proposed following De Pinna (1991).

All characters were mapped onto a majority-rule consensus tree obtained using Mesquite v.4.02 (Maddison & Maddison, 2025), adapted from the phylogenetic analysis of Amorim (2019), which was based on Bayesian inference (BI) of a concatenated dataset including seven molecular markers: three nuclear regions (ITS, *gsh*1, *shmt*) and four plastid regions (*trn*L-*trn*F, *psb*A-*trn*H, *trn*C-*pet*N, *trn*Y-*rpo*B).

Character mapping was performed under parsimony criteria, and figures were generated for characters recovered as synapomorphies, autapomorphies, or homoplastic traits potentially informative for circumscribing genera or clades.

Results

Anatomical Aspects of the Petiole

The petiole is present in *Agrianthus luetzelburgii*, *Agrianthus microlicioides*, *Bishopiella elegans*, *Lasiolaena santosii*, *Stylotrichium corymbosum*, *Stylotrichium rotundifolium*, and the outgroup species *Acritopappus confertus* and *Lapidia apicifolia*. In cross-section, it exhibits a plano-convex (Fig. 2 A-D) and concave-convex (Fig. 2E-H) shapes.

The epidermis is uniseriate with a thick cuticle (Fig. 2), containing stomata along its entire surface, as well as glandular and non-glandular trichomes of the same types present on the leaf blade (Fig. 2A, D). *Lasiolaena santosii* (Fig. 2D) and the genus *Stylotrichium* (Fig. 2E-F) exhibit stomatal crypts. The ground parenchyma consists of circular cells throughout, with few intercellular spaces. The vascular bundles are collateral, with a central bundle and accessory lateral bundles that have secretory channels exclusively associated with the xylem, displaying variations in quantity and lumen size (Fig. 2D-E).

In the external group species, *Acritopappus confertus* (Fig. 2G) and *Lapidia apicifolia* (Fig. 2H), secretory channels are found not only associated with the xylem but also dispersed throughout the ground parenchyma. The support function in the petiole is provided by sclerenchymatic fibers, which are more noticeable in *Agrianthus* species (Fig. 2B) and *Acritopappus confertus*. Procambial fibers (pericyclic) are observed in *Lasiolaena* (Fig. 2D), *Stylotrichium* (Fig. E-F), and *Lapidia apicifolia* (Fig. 2H). In *Lapidia apicifolia* (Fig. 2H), sclereids are present, scattered within the ground parenchyma. In *Bishopiella elegans* (Fig. 2C), fibers are absent, with collenchyma observed in regions adjacent to and surrounding the entire epidermis.

Anatomical Aspects of the Leaf Blade

Epidermis

In a frontal view (Fig. 3, Table 2), the species displayed polygonal epidermal cells with straight outlines on both surfaces in *Agrianthus* (Fig. 3A-B), *Arrojadocharis* (Fig. 3C-D), *Bishopiella elegans* (Fig. 3E-F), as well as in the outgroup species *Acritopappus confertus* (Fig. 3M-N), and *Lapidia apicifolia* (Fig. 3O). On the other hand, *Semiria* sp. nov. (Fig. 3H-I) and *Semiria viscosa* (Fig. 3J-K) showed straight outlines on the adaxial surface and strongly sinuous outlines on the abaxial surface. In *Lasiolaena* (Fig. 3G) and *Stylotrichium* (Fig. 3L), a straight outline was only present on the adaxial surface. Regarding *Lasiolaena* and *Stylotrichium* species, the anticlinal wall outlines on the abaxial surface could not be observed due to stomatal crypts.

In a cross-sectional view (Figs. 4, 6; Table 2), the cuticle was thick in most species, except in *Bishopiella elegans* (Figs. 4G, 6F), *Lasiolaena blanchetii*, and *Lasiolaena lychnophorioides,* which exhibited thin cuticles when visually compared to other species. The epidermis was classified as uniseriate in all species (Figs. 4, 6, Table 2), except for *Acritopappus confertus* (Fig. 4N), which exhibited a biseriate adaxial epidermis.

Stomata

The predominant stomatal type in the studied species was anomocytic (stomata surrounded by four to six subsidiary cells with straight to sinuous outlines), along with the anisocytic type (stomata surrounded by three subsidiary cells), which was less common (Fig. 3, Table 2). In *Arrojadocharis praxeloides* (Figs. 3C-D), the outline of subsidiary cells was slightly sinuous on the adaxial surface compared to other epidermal cells, similar to *Semiria* sp. nov. (Fig. 3H). In *Acritopappus confertus,* the outline was slightly sinuous on the abaxial surface (Fig. 3N). In cross-section, stomata were at the same level as the other epidermal cells in all species, with stomatal ledges observed upon the ostioles (Fig. 4C), formed by cuticular projections.

Agrianthus (Figs. 4A-B), *Arrojadocharis praxeloides* (Fig. 4D), *Bishopiella elegans* (Fig. 4G), *Lasiolaena pereirae* (Fig. 4I), *Semiria* sp. nov. (Fig. 4K), and *Semiria viscosa* (Fig. 4L) were amphistomatic, while *Arrojadocharis santosii* displayed both amphistomatic (Fig. 4E; V.O. Amorim 465 and 450) and hypostomatic (Fig. 4F; V.O. Amorim 445) leaf blade populations. *Lasiolaena* and *Stylotrichium* are hypostomatic (Figs. 4H-J, M), with abaxial stomata located within crypts (Figs. 4J, M). Among the outgroup, *Acritopappus confertus* was hypostomatic (Fig. 4N), and *Lapidia apicifolia* was amphistomatic (Fig. 4O), lacking stomatal crypts (Table 2).

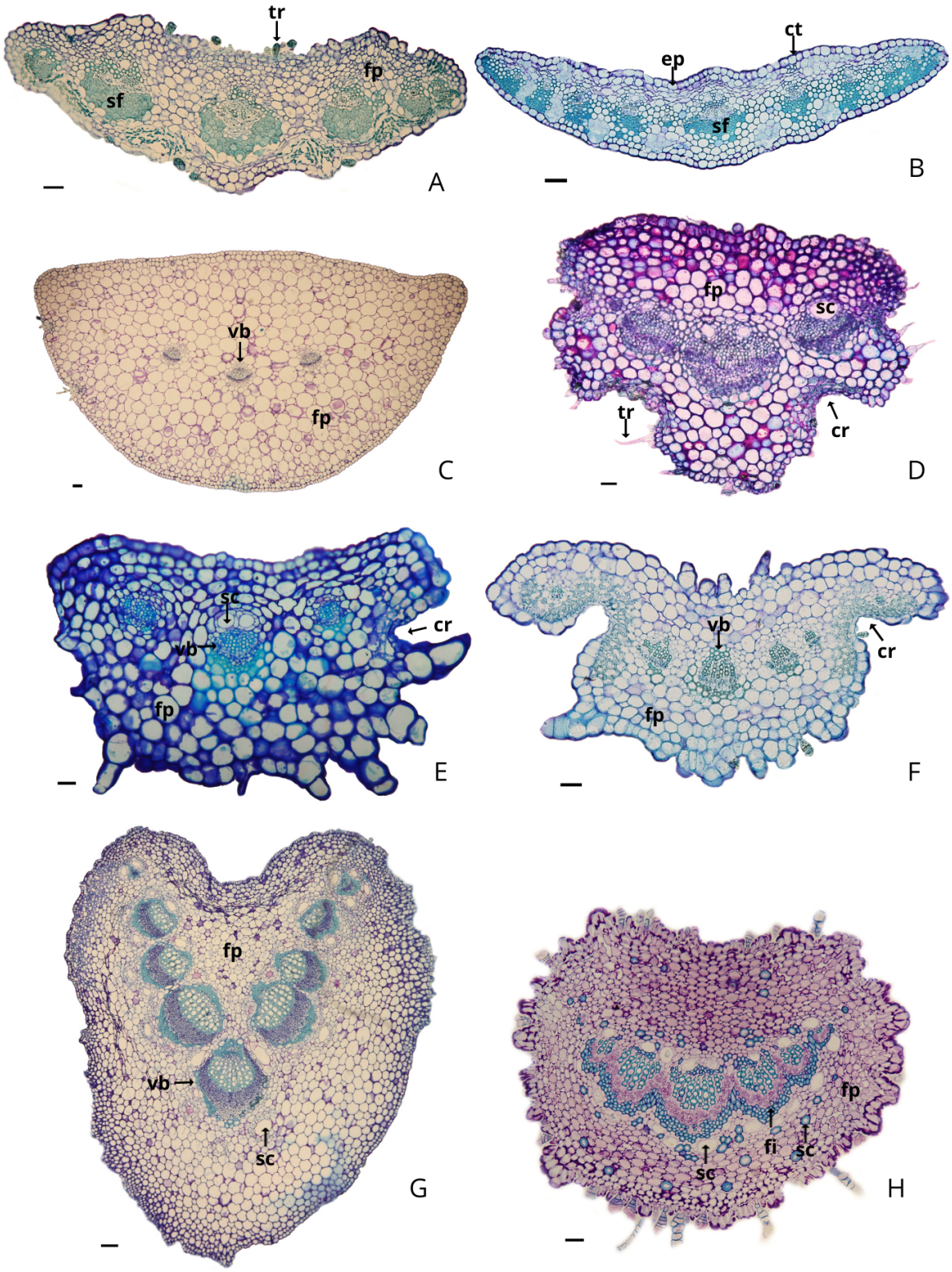

Figure 2. Transverse sections of the petiole with plano-convex (A-D) and concave-convex (E-H) shapes obtained by light microscopy. A. *Agrianthus luetzelburgii*, B. *Agrianthus microlicioides*, C. *Bishopiella elegans*, D. *Lasiolaena santosii*, E. *Stylotrichium corymbosum*, F. *Stylotrichium rotundifolium*, G. *Acritopappus confertus*, H. *Lapidia apicifolia*. Legend: ct – cuticle thickness; ep – uniseriate epidermis; pf – fundamental parenchyma; sc – secretory cavity; sf – sclerenchyma fibers; fi – procambial fibers; tr – trichomes; vb – vascular bundles. Scale bars: 100 μm (A-H).

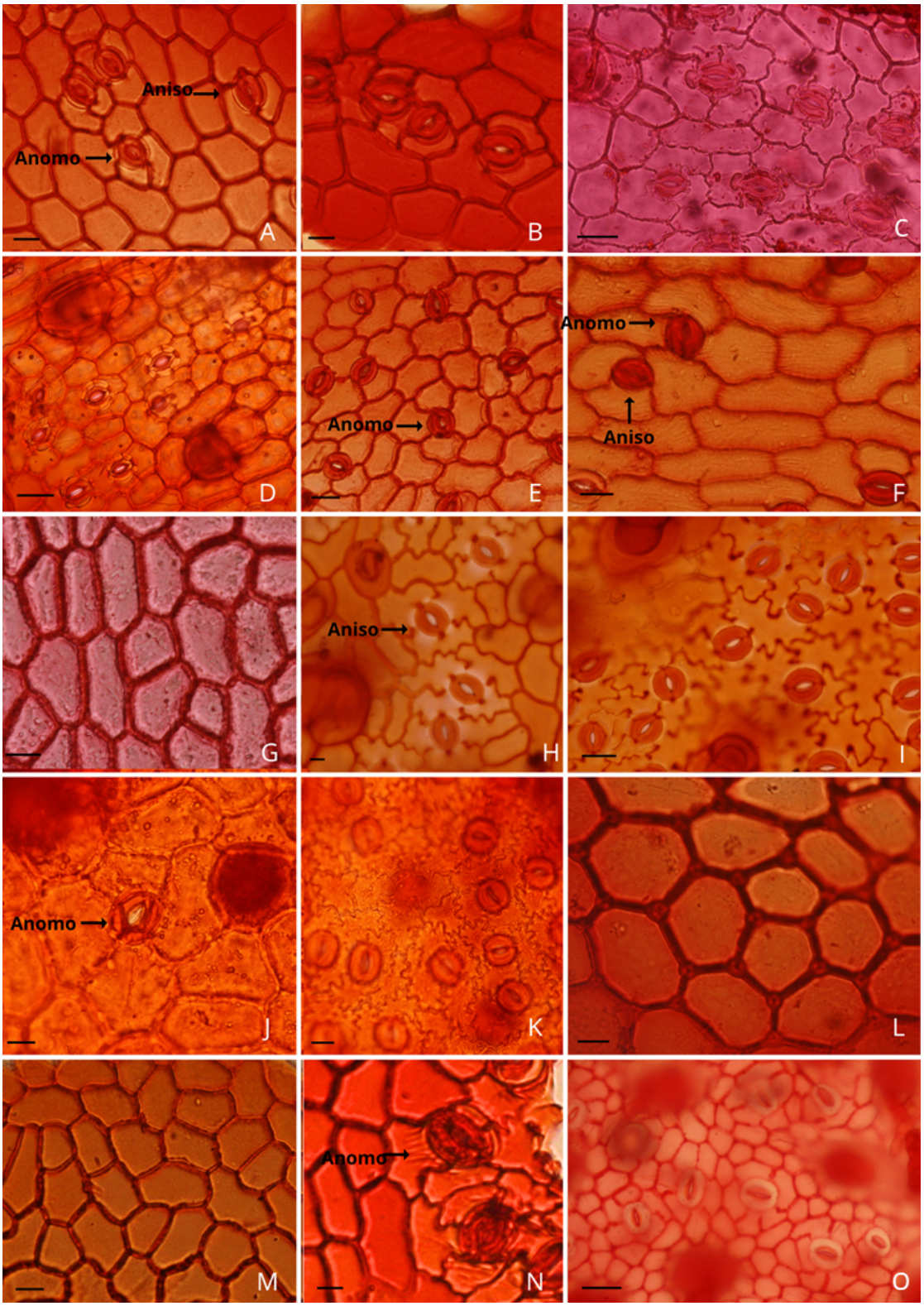

Figure 3. Frontal view of the leaf blade epidermis of Asteraceae species from the Chapada Diamantina Clade, obtained under light microscopy, showing cells with straight to sinuous outlines and anomocytic (A, C, D, E, I, J, K, N, O) and anisocytic (A, F, H) stomata with subsidiary cells with different outlines. *Agrianthus microlicioides*: A. Adaxial surface B. Abaxial surface. *Arrojadocharis praxeloides*: C. Adaxial surface D. Abaxial surface. *Bishopiella elegans*: E. Adaxial surface F. Abaxial surface. *Lasiolaena duartei*: G. Adaxial surface. *Semiria sp. nov.*: H. Adaxial surface. I. Abaxial surface. *Semiria viscosa*: J. Adaxial surface. K. Abaxial surface. *Stylotrichium corymbosum*: L. Adaxial surface. *Acritopappus confertus*: M. Adaxial surface. N. Abaxial surface. *Lapidia apicifolia*: O. Abaxial surface. Legend: Anomo - anomocytic stomata; Aniso - anisocytic stomata. Scale bars: 50 µm (C, D, G-H, I, J-K, O); 100 µm (A-B, E-F, L-M).

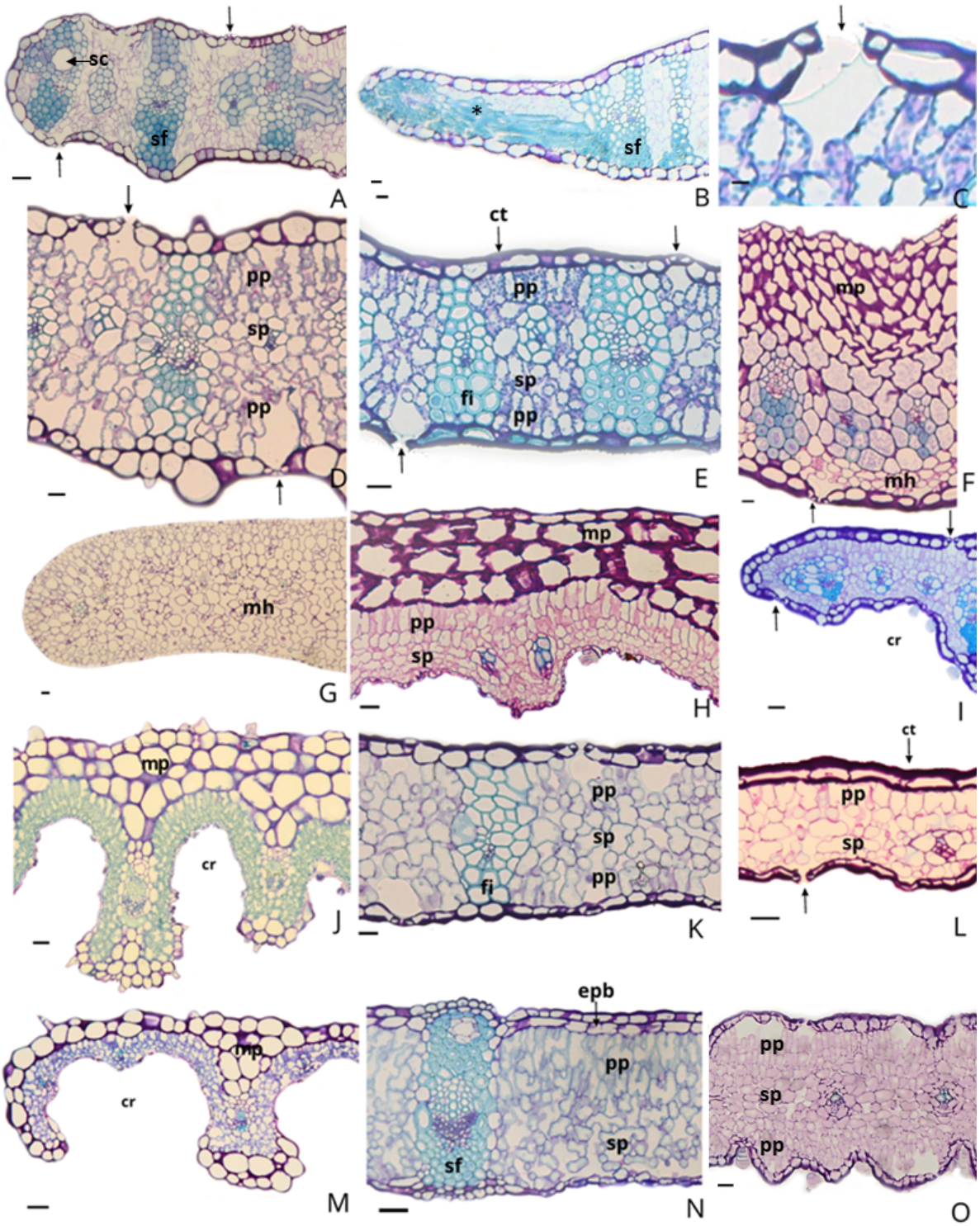

Figure 4. Transverse sections of the mesophyll and leaf margin with straight (A, B, I) and revolute (M) patterns obtained by light microscopy. A. *Agrianthus luetzelburgii*, B. Anatomy of the toothed margin with sclerenchyma fibers in *Agrianthus microlicioides*, C. Detail of the stomatal crest formed by cuticular projection on a stoma in the adaxial surface of *Arrojadocharis santosii*, D. *Arrojadocharis praxeloides*, E. *Arrojadocharis santosii* (isobilateral mesophyll), F. *Arrojadocharis santosii* (homogeneous mesophyll), G. *Bishopiella elegans*, H. *Lasiolaena duartei*, I. *Lasiolaena pereirae*, J. *Lasiolaena santosii*, K. *Semiria sp. nov.*, L. *Semiria viscosa*, M. *Stylotrichium rotundifolium*, N. *Acritopappus confertus*, O. *Lapidia apicifolia*. Legend: * - toothed margin; arrow – stoma; cr – crypts; ct – cuticle thickness; ep – uniseriate epidermis; epb – biseriate epidermis; fi – procambial fibers; vb – vascular bundles; hyp – hypodermis; mh – homogeneous mesophyll; mp - multiseriate parenchyma; sf – sclerenchyma fibers; sp – spongy parenchyma; pp – palisade parenchyma. Scale bars: 100 μm (A-P).

Trichomes

Eleven types of trichomes (Types I-XI) were identified among the studied species, including three glandular types (Types I-III, Fig. 5A-D) and eight non-glandular types (Types IV-XI, Fig. 5E-L). Glandular and non-glandular trichomes varied in occurrence and location on the leaf blades among species (Tables 3-4).

Among the glandular types, Type II (vesicular, biseriate body with 8-10 cells, globoid and unicellular head) is the most common (Figs. 5B-C), while Type III (stipitate, biseriate, multicellular body with 3-5 cells, and two apical cells with dense content forming the head) were found only in *Stylotrichium rotundifolium* (Fig. 5D).

Among the non-glandular types, the types IV (bifid: uniseriate, multicellular body with two terminal cells, bifurcating at the terminal cell with pluricellular arms of 2-5 sharp-tipped cells) and X (uniseriate, multicellular body with 3-5 cells, with a widened basal cell and a pointed apical cell, numerous and often recurved) are restricted to *Lasiolaena santosii* (Fig. 5E, K, respectively). On the other hand, type VI (uniseriate, multicellular body with 3-6 cells, curved with a sharp tip) was exclusively recorded in the genus *Semiria* (Fig. 5G), and type IX (uniseriate, multicellular body with 6-7 cells, straight with rounded apical cell) was described exclusively for *Stylotrichium corymbosum* (Fig. 5J). Finally, type XI (moniliform, uniseriate, multicellular body with 3-6 cells, with a rounded apical cell) was restricted to *Bishopiella elegans* (Fig. 5L).

Characteristics related to the organization of fundamental and vascular tissues in the mesophyll, main veins, and leaf margin are presented in Tables 5, 6, and 7.

Table 3. Description of the types of glandular trichomes observed in the adaxial and abaxial epidermises of species from the Chapada Diamantina clade (Asteraceae, Eupatorieae) (Fig. 5 A-D).

Type	Species	Occurrence	Description	Reference
Type I	*Arrojadocharis* (2 spp.), *Semiria* sp. nov. *Semiria viscosa* and *Stylotrichium sucrei*	Throughout the leaf blade (adaxial and abaxial faces).	Stipitate: biseriate body with 6-8 cells, elongated, ovoid head with 8 apical cells containing dense material in terminal cells.	Trindade *et al.* 2014
Type II	*Agrianthus giuliettiae, A. luetzelburgii, Semiria viscosa, Acritopappus confertus, Lapidia apicifolia*	Throughout the leaf blade (adaxial and abaxial faces).	Vesicular: biseriate body with 8-10 cells, globoid unicellular head.	Budel *et al.*, 2018
	Lasiolaena and *Stylotrichium* spp.	Stomatal crypts on the abaxial face and sparse on the adaxial face.		
Type III	*Stylotrichium rotundifolium*	Stomatal crypts on the abaxial face.	Stipitate: biseriate body, multicellular with 3-5 cells and two apical cells with dense content forming the head.	

Table 4. Description of the types of protective (non-glandular) trichomes observed on the adaxial and abaxial epidermises of the species of the Chapada Diamantina clade (Asteraceae, Eupatorieae) (Fig. 5 E-L).

Type	Species	Occurrence	Description	Reference
Type IV	*Lasiolaena santosii*	Abaxial leaf surface, more external to the crypts.	Bifid: uniseriate body, multicellular with two cells, bifurcated at the terminal cell with pluricellular arms composed of 2-5 cells with acute apex.	Payne 1978, adapted.
Type V	*Arrojadocharis* (2 spp.), *Semiria* sp. nov. and *Semiria viscosa*	On both leaf surfaces, predominantly on the edges.	Uniseriate body, multicellular with 3-10 long cells, straight and with an acute apex.	
Type VI	*Semiria viscosa* and *Semiria* sp. nov.	Adaxial and abaxial faces, predominantly on the veins	Uniseriate body, multicellular with 3-6 cells, curved and with an acute apex.	
Type VII	*Lasiolaena blanchetii, L. duartei* and *L. lychnophorioides*	Abaxial face	Filiform: unicellular body, elongated with an acute apex. Numerous and appear as a tangled mass of trichomes due to length.	Silva *et al.* 2019; Liesenfeld *et al.* 2019

Table 4. Cont.

Type	Species	Occurrence	Description	Reference
Type VIII	*Lasiolaena santosii*, *Stylotrichium corymbosum* and *S. rotundifolium*	Abaxial face	Uniseriate, multicellular body of 5-7 short cells, straight and with an acute apex.	
Type IX	*Stylotrichium corymbosum*	Stomatal crypts on the abaxial face	Uniseriate, multicellular body with 6-7 cells, straight with rounded apical cell.	
Type X	*Lasiolaena santosii*	Adaxial face	Uniseriate: uniseriate body, multicellular with 3-5 cells, with the basal cell expanded and the apical one acute. Numerous and may be seen recurved.	Martínez-Quezada *et al*. 2022
Type XI	*Bishopiella elegans*	On both leaf surfaces, grouped in cavities.	Moniliform: uniseriate, multicellular body with 3-6 cells, with rounded apical cell.	

Table 5. Anatomical characteristics present in the mesophyll of species from the Chapada Diamantina clade (Asteraceae, Eupatorieae).

Species/Characters	Organization	Atypical Palisade	Number of Layers	Multiseriate parenchyma	Chlorophyll parenchyma in vein
Agrianthus giuliettiae	Isobilateral	Present	1 layer	Absent	Absent
A. luetzelburgii	Isobilateral	Present	1 layer	Absent	Absent
A. microlicioides	Isobilateral	Present	1 layer	Absent	Absent
Arrojadocharis praxeloides	Isobilateral	Present	1 layer	Absent	Absent
A. santosii	Homogeneous/ isobilateral	Absent/Present	Absent/1 layer	Absent/Present	Absent
Bishopiella elegans	Homogeneous/	Absent	Absent	Absent	Absent
Lasiolaena blanchetii	Dorsiventral	Present	1-3 layers	Present	Absent
L. duartei	Dorsiventral	Present	1-3 layers	Present	Absent
L. lychnophorioides	Dorsiventral	Present	1-3 layers	Present	Absent
L. pereirae	Dorsiventral	Present	1-3 layers	Present	Absent
L. santosii	Dorsiventral	Present	1 layer	Present	Absent
Semiria viscosa	Dorsiventral	Present	1 layer	Absent	Present
Semiria sp. nov.	Isobilateral	Present	1 layer	Absent	Present
Stylotrichium corymbosum	Dorsiventral	Present	1 layer	Present	Absent
S. rotundifolium	Dorsiventral	Present	1 layer	Present	Absent
S. sucrei	Dorsiventral	Present	1 layer	Present	Absent
Outgroup					
Acritoppapis confertus	Dorsiventral	Present	1-3 layers	Absent	Absent
Lapidia apicifolia	Isobilateral	Absent	2-3 layers	Absent	Absent

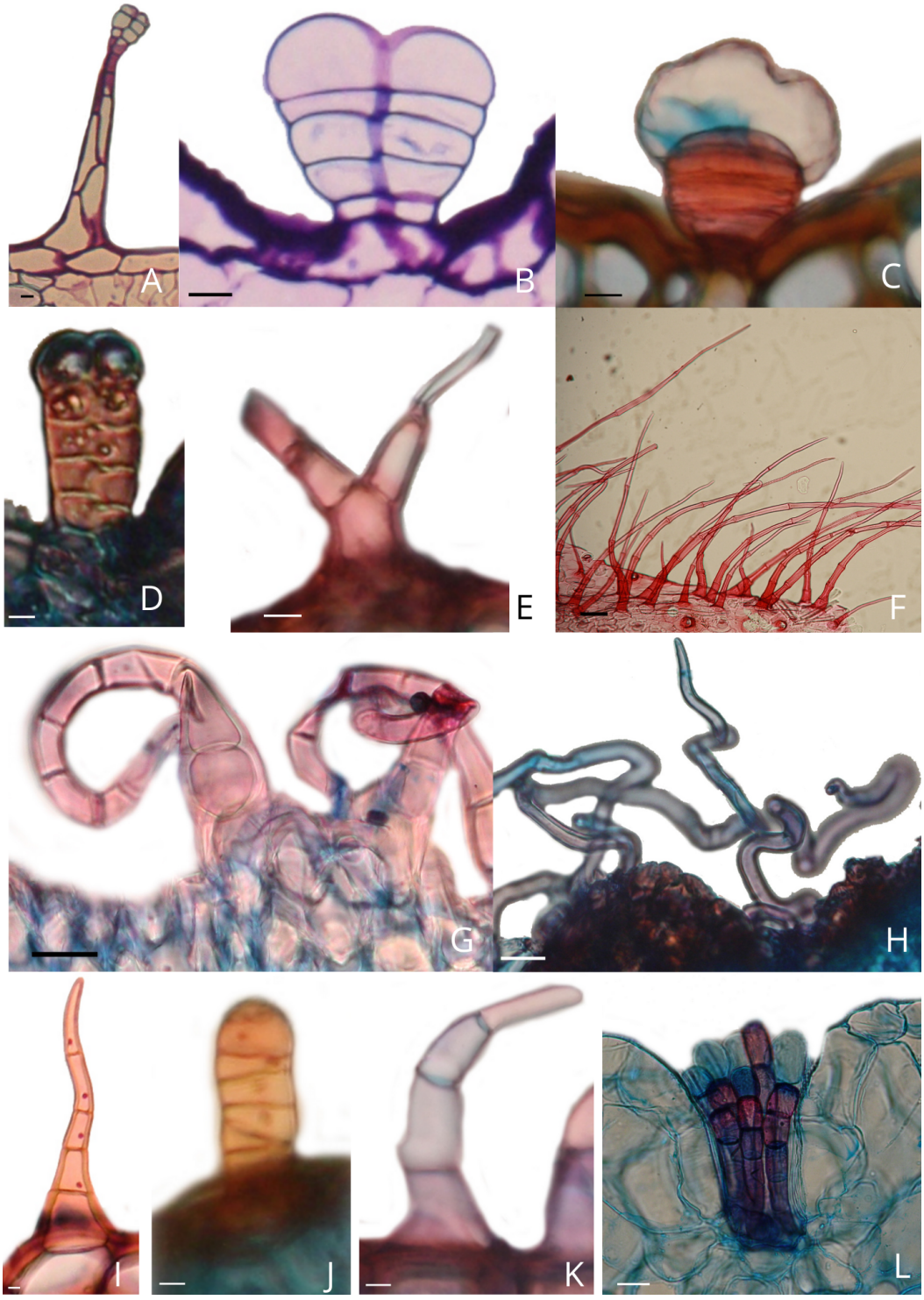

Figure 5. Glandular (A-D) and non-glandular (E-L) trichomes under light microscopy. A. Type I glandular trichome in *Semiria* sp. nov. B. Type II glandular trichome in *Semiria viscosa*. C. Detail of cell division in Type II glandular trichome in *Stylotrichium corymbosum*. D. Type III glandular trichome in *Stylotrichium rotundifolium*. E. Type IV tector trichome in *Lasiolaena santosii*. F. Type V tector trichome in *Arrojadocharis santosii*. G. Type VI tector trichome in *Semiria sp. nov.* H. Type VII tector trichome in *Lasiolaena duartei*. I. Type VIII tector trichome in *Stylotrichium rotundifolium*. J. Type IX tector trichome in *Stylotrichium corymbosum*. K. Type X tector trichome in *Lasiolaena santosii*. L. Type XI tector trichome in *Bishopiella elegans*. Scale bars: 100 µm (A-K) and 50 µm (L).

Table 6. Anatomical characteristics present in the mesophyll and veins of species from the Chapada Diamantina clade (Asteraceae, Eupatorieae).

Species/Characters	Support tissue			
	Collenchyma	Procambial fibers	Sclerenchyma fibers	Sclereids
Agrianthus giuliettiae	Present	Absent	Present	Absent
A. luetzelburgii	Present	Absent	Present	Absent
A. microlicioides	Present	Absent	Present	Absent
Arrojadocharis praxeloides	Present	Absent	Present	Absent
A. santosii	Present	Absent	Present	Absent
Bishopiella elegans	Absent	Absent	Absent	Absent
Lasiolaena blanchetii	Present	Present	Absent	Present
L. duartei	Present	Present	Absent	Present
L. lychnophorioides	Present	Present	Absent	Present
L. pereirae	Present	Present	Absent	Present
L. santosii	Present	Present	Absent	Absent
Semiria viscosa	Present	Present	Absent	Absent
Semiria sp. nov.	Present	Present	Absent	Absent
Stylotrichium corymbosum	Present	Present	Absent	Absent
S. rotundifolium	Present	Present	Absent	Absent
S. sucrei	Present	Present	Absent	Absent
Outgroup				
Acritopappus confertus	Present	Absent	Present	Absent
Lapidia apicifolia	Present	Present	Absent	Absent

Table 7. Anatomical characteristics present in the veins of species from the Chapada Diamantina clade (Asteraceae, Eupatorieae).

Species/Characters	Vascularization			Secretory duct		Prominence of the central vein		Margin
	Type	Number of bundles	Accessory bundles	Location	Quantity	Adaxial	Abaxial	Pattern
Agrianthus giuliettiae	Colateral	1	Absent	Associated with the bundles	1	Flat	Slightly prominent	Straight
A. luetzelburgii	Colateral	1	Absent	Associated with the bundles	1	Flat	Slightly prominent	Straight
A. microlicioides	Colateral	1	Absent	Associated with the bundles	1	Flat	Flat	Straight
Arrojadocharis praxeloides	Colateral	1	Absent	Associated with the bundles	1	Flat	Flat	Straight
A. santosii	Colateral	1	Absent	Associated with the bundles	1	Flat	Slightly prominent	Straight
Bishopiella. elegans	Colateral	1-3	Absent	Associated with the bundles	1-2	Slightly prominent	Slightly prominent	Straight
Lasiolaena blanchetii	Colateral	1-3	Absent	Associated with the bundles	1-2	Flat	Prominent	Revolute
L. duartei	Colateral	1-3	Absent	Associated with the bundles	1-2	Flat	Prominent	Revolute
L. lychnophorioides	Colateral	1-3	Absent	Associated with the bundles	1-2	Flat	Prominent	Revolute
L. pereirae	Colateral	1-3	Absent	Associated with the bundles	1-2	Slightly prominent	Prominent	Straight
L. santosii	Colateral	1-3	Absent	Associated with the bundles	1-2	Flat	Prominent	Revolute

Table 7. Cont.

Species/Characters	Vascularization			Secretory duct		Prominence of the central vein		Margin
	Type	Number of bundles	Accessory bundles	Location	Quantity	Adaxial	Abaxial	Pattern
Semiria viscosa	Colateral	1	Absent	Associated with the bundles	1-2	Flat	Prominent	Straight
Semiria sp. nov.	Colateral	1	Present	Associated with the bundles	1-2	Flat	Prominent	Straight
Stylotrichium corymbosum	Colateral	1-3	Absent	Associated with the bundles	1-2	Flat	Prominent	Revolute
S. rotundifolium	Colateral	1-3	Absent	Associated with the bundles	1-2	Flat	Prominent	Revolute
S. sucrei	Colateral	1-3	Absent	Associated with the bundles	1-2	Flat	Prominent	Revolute
Outgroup								
Acritopappus confertus	Colateral	1-3	Absent	Scattered in the fundamental parenchyma	More than two channels	Flat	Prominent	Straight
Lapidia apicifolia	Colateral	1	Absent	Scattered in the fundamental parenchyma	More than two channels	Slightly prominent	Slightly prominent	Straight

Parenchyma

Multiseriate, composed of 1-5 layers of cells, was observed only in species of *Lasiolaena* and *Stylotrichium* (Figs. 4H-J, M). *Lasiolaena blanchetii* and *L. duartei* (Fig. 4H) showed the greatest variation regarding the number of layers in the vein region, with up to five layers exhibiting more heavily thickened cells compared to other species. *Arrojadocharis* did not show layers of cells (Figs. 4D, E); however, a population of *A. santosii* (Fig. 4F, V.O. Amorim 445) presented a five-layers cells adjacent to the adaxial epidermis.

In the mesophyll, the chlorenchyma ranged from homogeneous in *Bishopiella elegans* (Fig. 4G) and *Arrojadocharis santosii* (Fig. 4F, V.O. Amorim 445, 450), to dorsiventral and isobilateral. The latter two types displayed atypical palisade parenchyma with lobed cells irregularly distributed along the leaf area (Figs. 4A-F, H-N), except for *Lapidia apicifolia* (Fig. 4O), which had the typical palisade parenchyma. The mesophyll is isobilateral in *Agrianthus* (Fig. 4A), *Arrojadocharis santosii* (Figs. 4D-E), *Semiria* sp. nov. (Fig. 4K), and *Lapidia apicifolia* (Fig. 4O), formed by one layer of palisade parenchyma and 2-4 layers of spongy parenchyma. In *Lasiolaena* species (Figs. 4H-J), *Semiria viscosa* (Fig. 4L), *Stylotrichium* (Fig. 4M), and *Acritopappus confertus* (Fig. 4N), the mesophyll is dorsiventral with 1-3 layers of palisade parenchyma and 3-5 layers of spongy parenchyma.

Collenchyma

In the region adjacent to the epidermis, angular collenchyma was observed, varying from 1 to 2 layers on the abaxial face in species of *Agrianthus* (Fig. 6B) and *Arrojadocharis* (Fig. 6D-E). However, in *Arrojadocharis santosii* (V.O. Amorim 445), five to seven layers of this tissue were observed along the entire adaxial epidermis (Fig. 4F). In *Semiria* sp. nov. (Fig. 6G), *S. viscosa* (Fig. 6K), *Acritopappus confertus* (Fig. 6N), and species of *Lasiolaena* (Fig. 6H-J) and *Stylotrichium* (Fig. 6L-M), the collenchyma was presented only in the vein regions, exhibiting 3–5 layers of cells toward the upper side and 1–5 layers toward the lower side in species of *Lasiolaena* (Fig. 6H-J), and 1–2 layers adjacent to the upper side and 1–3 adjacent to the lower side in species of *Stylotrichium* (Fig. 6L-M). *Bishopiella elegans* (Fig. 6F) is the only species in which collenchyma was not present.

Sclerenchyma

In *Agrianthus* (Fig. 4A-B), *Arrojadocharis* (Fig. 4D-F), and *Acritopappus confertus* (Fig. 4N), the sclerenchymatic tissue was present as fiber bundles that form sheath extensions toward the vascular bundles in the mesophyll, connecting the epidermis and vascular tissues. In *Agrianthus*, *Arrojadocharis* (Fig. 4A-B, D-E), and *Acritopappus confertus* (Fig. 4N), the sclerenchyma fibers, in addition to forming sheath extensions, also surrounded the vascular bundles. *Lasiolaena* (Fig. 4H-J), *Semiria* sp. nov. (Fig. 4K), *Stylotrichium* (Fig. 4M), and *Lapidia apicifolia* (Fig. 4O) showed likely pericyclic fibers surrounding or within the vascular bundles. *Bishopiella elegans* was the only species showing mesophyll and main vein without support tissue (Fig. 4G, 6F).

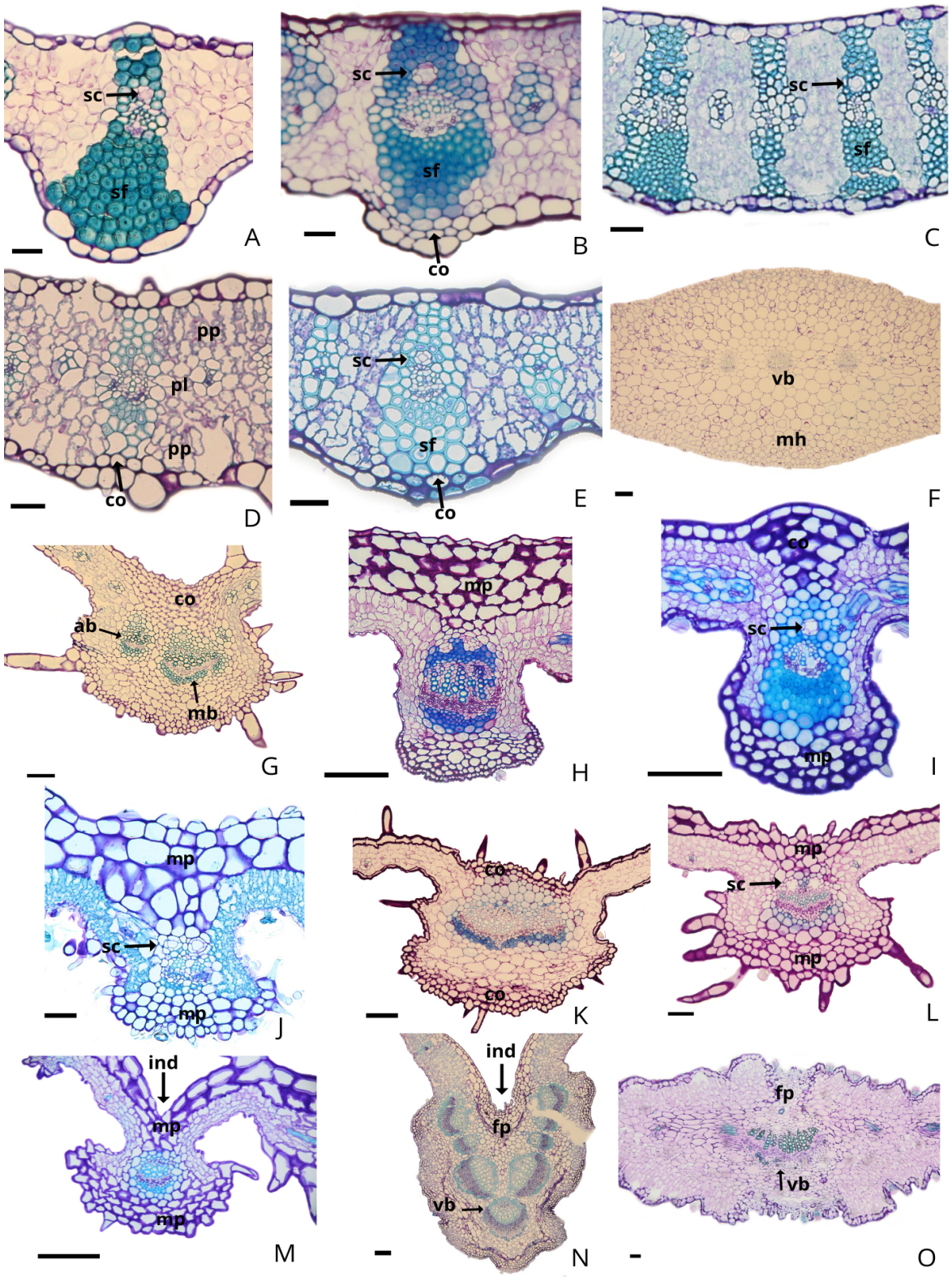

Figure 6. Transverse sections of the main vein obtained by light microscopy, highlighting different prominences and the tissues that fill them. A. *Agrianthus giuliettiae*, B. *Agrianthus luetzelburgii*, C. *Agrianthus microlicioides*, D. *Arrojadocharis praxeloides*, E. *Arrojadocharis santosii*, F. *Bishopiella elegans*, G. *Semiria sp. nov.*, H. *Lasiolaena duartei*, I. *Lasiolaena pereirae*, J. *Lasiolaena santosii*, K. *Semiria viscosa*, L. *Stylotrichium rotundifolium*, M. *Stylotrichium sucrei*, N. *Acritopappus confertus*, O. *Lapidia apicifolia*. Legend: ab – accessory bundle; co – collenchyma; fp – fundamental parenchyma; ind – indentation on adaxial surface; mb – main bundle; mh – homogeneous mesophyll; mp - multiseriate parenchyma; sc – secretory channel; sf – sclerenchyma fibers; vb – vascular bundle. Scale bars: 100 µm (A-O).

Vascularization

Vascular bundles were collateral in all species (Fig. 6A-O). The vascularization of the main vein in *Agrianthus* (Fig. 6A-B), *Arrojadocharis* (Fig. 6C-E), *Semiria viscosa* (Fig. 6K), and *Lapidia apicifolia* (Fig. 6O) consisted of a single central bundle, while in *Bishopiella* (Fig. 6F), *Lasiolaena* (Fig. 6H-J), *Semiria* sp. nov. (Fig. 6G), and *Stylotrichium* (Fig. 6L-M), in addition to the main bundle, one to three vascular bundles in the main vein region were present.

The presence of secretory channels associated with the vascular bundles of the main vein was observed in all the species within the clade, always near the xylem. Secretory channels were found in different quantities scattered in *Acritopappus confertus* (Fig. 6N) and *Lapidia apicifolia* (Fig. 6O). *Agrianthus* and *Arrojadocharis* had only one secretory channel associated with the bundle, and 1–2 channels in other genera (Fig. 6A-E).

Morphoanatomy Aspects of the Leaf Blade

The genera *Agrianthus* and *Arrojadocharis* had a main vein varying from flat on the adaxial side to slightly prominent on the abaxial side (Fig. 6A-E). In *Bishopiella elegans* (Fig. 6F) and *Lapidia apicifolia* (Fig. 6O), the main vein was slightly prominent on both sides. In genera *Lasiolaena*, *Semiria*, and *Stylotrichium*, the main vein was prominent on the abaxial side and flat to nearly flat on the adaxial side (Fig. 6G, H, J-L), except in *Lasiolaena pereirae* (Fig. 6I), which had a main vein prominent on the abaxial side and slightly prominent on the adaxial side, while *Stylotrichium sucrei* and *Acritopappus confertus* presented an indentation on the adaxial side (Fig. 6M-N).

Margin

The margin exhibited a straight pattern in *Agrianthus*, *Arrojadocharis*, *Bishopiella elegans*, *Semiria viscosa*, *Semiria* sp. nov., *Lasiolaena pereirae*, *Acritopappus confertus,* and *Lapidia apicifolia* (Fig. 4A-B, G, I). On the other hand, a revolute pattern (Fig. 4M) was observed in the remaining species of *Lasiolaena* and in the genus *Stylotrichium*. In *Agrianthus microlicioides*, sclerenchyma fibers are observed in its toothed margin (Fig. 4B).

Character state reconstructions

The reconstruction of twenty-seven anatomical characters of the leaf blade revealed distinct patterns of distribution within the Chapada Diamantina clade (Table 8). Among these, eighteen characters were phylogenetically informative, while nine showed no consistent phylogenetic signal or displayed only intraspecific variation.

Among the informative characters, five were recovered as character states shared by species within key internal lineages and may represent synapomorphies supporting the *Lasiolaena* core, the *Stylotrichium* core, their sister-group relationship, and the broader Chapada Diamantina clade: the presence of filiform non-glandular trichomes (C10), the presence of stomatal crypts (C18), the presence of sclereids (C23), the location of secretory ducts scattered in the fundamental parenchyma (C25), and the quantity of secretory ducts exceeding two (C26). Within the *Lasiolaena* core (*L. lychnophorioides*, *L. blanchetii*, and *L. duartei*), type VII filiform trichomes (character 10) was recovered as a shared derived feature (Fig. 7A). The sister group relationship between *Stylotrichium* and *Lasiolaena* is supported by stomatal crypts (Character 18; Fig. 7B), while sclereids (Character 23) occurs in all *Lasiolaena* species except *L. santosii* (Fig. 7C), reinforcing the anatomical cohesion within the genus. Ducts associated with the bundles (character 25; Fig. 7D) and secretory duct quantity (character 26) are shared among the entire Chapada Diamantina lineage, distinguishing it from the outgroups (Fig. 7E).

In contrast, nine characters were recovered as homoplastic when mapped onto the molecular phylogeny, suggesting independent origins in different lineages: the presence of glandular trichomes Type I (stipitate, biseriate, 6–8 cells; C4), glandular trichomes Type II (vesicular; C5), non-glandular trichomes Type V (uniseriate, multicellular, 3–10 cells; C8), non-glandular trichomes Type VIII (uniseriate, multicellular, 5–7 cells; C11), sinuous subsidiary cell contour on the adaxial surface (C15), amphistomatic or amphi-hypostomatic stomatal distribution (C17), dorsiventral mesophyll (C19), the presence of procambial fibers (C21), and midrib prominence (C27).

For instance, dorsiventral mesophyll (C19) is shared by *Agrianthus*, *Lasiolaena santosii*, *L. pereirae*, and *Arrojadocharis praxeloides*, but was recovered as homoplastic, providing no synapomorphic support for closer relationships among these taxa. Similarly, the presence of procambial fibers (C21) occurs in *Lasiolaena* taxa belonging to distinct subclades, suggesting repeated emergence of similar anatomical traits within the lineage.

Autapomorphies were restricted to terminal taxa and reflect species-specific anatomical modifications that are useful for delimitation at the species level. Examples include the presence of glandular trichomes Type III (stipitate, biseriate; C6) in *Stylotrichium rotundifolium*, non-glandular trichomes Type IX (straight with rounded apex; C12) in *S. corymbosum*, non-glandular trichomes Type X (uniseriate, multicellular, 3–5 cells; C13) in *Lasiolaena santosii*, and non-glandular trichomes Type XI (moniliform; C14) in *Bishopiella elegans*. Although phylogenetically uninformative, these features contribute to the recognition of diagnostic anatomical attributes within each lineage.

Uniseriate epidermis, absence of Type III glandular trichomes, and absence of sclerenchymatic fibers (characters 1, 6, and 22, respectively), likely represent plesiomorphic conditions retained from ancestral states. These conditions are widespread and likely represent ancestral anatomical configurations conserved within the Chapada Diamantina clade.

Table 8. List of qualitative and quantitative characters used in character reconstruction.

Character number	Character name	Character coding
C1	Epidermis: layers	(0) Uniseriate; (1) Biseriate.
C2	Epidermis: contour (straight on both surfaces)	(0) Absent; (1) Present.
C3	Epidermis: contour (sinuous on abaxial surface)	(0) Absent; (1) Present.
C4	Glandular trichome: Type I (Stipitate, biseriate, 6-8 cells)	(0) Absent; (1) Present.
C5	Glandular trichome: Type II (Vesicular)	(0) Absent; (1) Present.
C6	Glandular trichome: Type III (Stipitate, biseriate)	(0) Absent; (1) Present.
C7	Non-glandular trichome: Type IV (Bifid)	(0) Absent; (1) Present.
C8	Non-glandular trichome: Type V (Uniseriate, multicellular 3-10 long cells)	(0) Absent; (1) Present.
C9	Non-glandular trichome: Type VI (Curved)	(0) Absent; (1) Present.
C10	Non-glandular trichome: Type VII (Filiform)	(0) Absent; (1) Present.
C11	Non-glandular trichome: Type VIII (Uniseriate, multicellular 5-7 cells)	(0) Absent; (1) Present.
C12	Non-glandular trichome: Type IX (Straight with rounded apex)	(0) Absent; (1) Present.
C13	Non-glandular trichome: Type X (Uniseriate, multicellular 3-5 cells)	(0) Absent; (1) Present.
C14	Non-glandular trichome: Type XI (Moniliform)	(0) Absent; (1) Present.
C15	Stomata: subsidiary cell contour (adaxial surface)	(0) Straight; (1) Sinuous; (2) Absent.
C16	Stomata: subsidiary cell contour (abaxial surface)	(0) Straight; (1) Sinuous; (2) Absent.
C17	Stomata: distribution	(0) Amphistomatic; (1) Hypostomatic; (2) Amphi-hypostomatic.
C18	Stomata: distribution (stomatal crypts)	(0) Absent; (1) Present.
C19	Mesophyll: organization	(0) Homogeneous; (1) Isobilateral; (2) Dorsiventral.
C20	Palisade Parenchyma: atypical	(0) Absent; (1) Present.
C21	Procambial fiber	(0) Absent; (1) Present.
C22	Sclerenchymatous fiber	(0) Absent; (1) Present.
C23	Sclereids	(0) Absent; (1) Present.
C24	Accessory bundles (in the midrib)	(0) Absent; (1) Present.
C25	Secretory duct: location	(0) Associated with the bundles; (1) Scattered in the fundamental parenchyma.
C26	Secretory duct: quantity	(0) 1; (1) 1-2; (2) >2
C27	Midrib prominence	(0) Not prominent; (1) Slightly prominent; (2) Prominent.

Finally, eleven characters were considered non-informative due to intraspecific variability or lack of consistent distribution across the phylogeny: number of epidermal layers (C1), sinuous epidermal contour on the abaxial surface (C3), non-glandular trichomes Type IV (bifid; C7), non-glandular trichomes Type V (uniseriate, multicellular, 3–10 cells; C8), non-glandular trichomes Type VII (filiform; C10), non-glandular trichomes Type VIII (uniseriate, multicellular, 5–7 cells; C11), sinuous subsidiary cell contour on the abaxial surface (C16), stomatal distribution (C17), atypical palisade parenchyma (C20), the presence of sclerenchymatous fibers (C22), and accessory bundles in the midrib (C24).

Some characters, such as epidermis layering (C1), non-glandular trichomes Type V (C8), and non-glandular trichomes Type VII (C10), appear in more than one evolutionary category depending on the clade or taxon analyzed, reflecting their potential roles as plesiomorphic, autapomorphic, or homoplastic under different phylogenetic contexts. Additionally, non-glandular trichomes Type VI (curved; C9), although restricted to *Semiria* species, do not support any specific hypothesis of interspecific divergence, illustrating that not all unique traits necessarily contribute to phylogenetic resolution within the group.

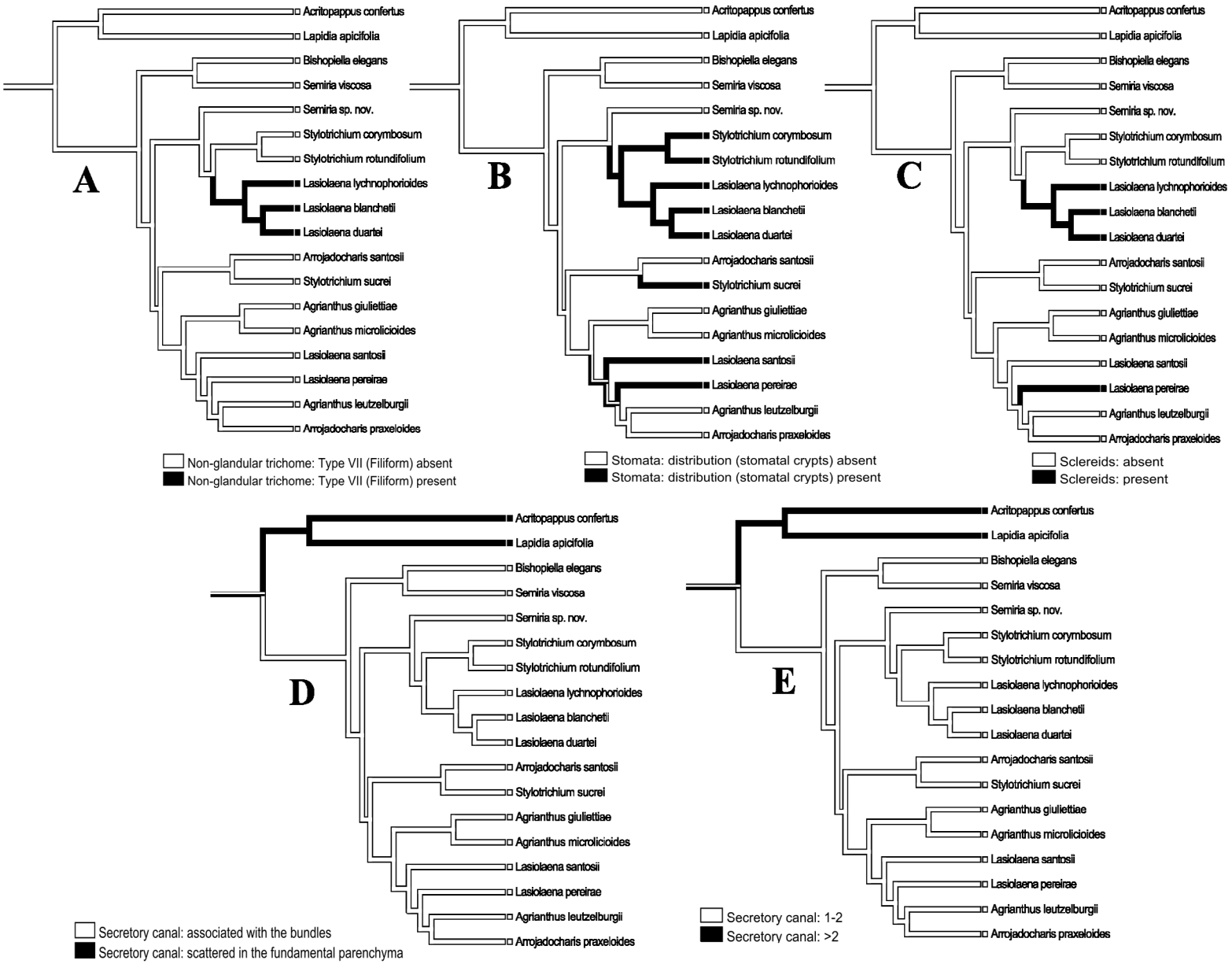

Figure 7. Mapping of anatomical characters recovered as synapomorphies supporting the circumscription of clades and taxa in this study. A. Character 10-Non-glandular trichome: Type VII (filiform). B. Character 18-Stomata: distribution (stomatal crypts). C. Character 23- Sclereids. D. Character 25-Secretory canal: location. E. Character 26-Secretory canal: quantity.

Discussion

Species in the Chapada Diamantina clade exhibit macro and micromorphological traits with adaptive value to the rocky fields (campo rupestre) vegetation. This ecosystem is predominant in high-altitude areas (above 900 m) on sandy, shallow soils with a high proportion of quartzite-sandstone rocky substrates. It is common in the Espinhaço Range, Brazil, where 90% of it is located (Alves & Kolbek, 2010).

The species in this clade exhibit coriaceous, imbricated leaves with reduced leaf blades, thick cuticles, high trichome density, crypted stomata, atypical palisade parenchyma, hypodermis, and sclerenchyma sheath extensions, which aid in water retention within tissues and optimize photosynthetic processes (Fahn & Cutler, 1992; Guerra & Scremin-Dias, 2017; Ariano *et al.,* 2022). However, some of these characteristics, such as a thick cuticle, stomatal crypt, hypodermis, and support tissue, are absent in *Bishopiella elegans*, the only species in the clade that occurs on flooded soil during the rainy season (November-March) in the Chapada Diamantina. In other angiosperm groups, morphoanatomical adaptations in aquatic and amphibious species have been observed as evolutionary responses to environmental pressures (Bedoya & Madrinán, 2014; Leme & Scremin-Dias, 2014).

From the analysis of the foliar morphoanatomy of representatives of the Chapada Diamantina clade, characteristics that may assist in delimiting the clade and the taxa recovered by Rivera *et al.* (2016a), Roque *et al.* (2017), and Amorim (2019) were identified. The location of secretory channels in the main vein may be a synapomorphy for the Chapada Diamantina clade, since all the analyzed species showed 1-2 channels associated with vascular bundles, in contrast to outgroup species, which exhibited more than two channels dispersed in the ground parenchyma, as inferred from the phylogenetic reconstruction (Fig. 7 D-E). The differing positions of secretory channels in the leaves of *Aldama* (Asteraceae) helped Oliveira (2015) and Silva *et al.* (2014) in species distinction.

The straight outline of epidermal cells on the adaxial side was recorded for all clades and outgroup species. On the abaxial side, the outline of the cells with crypted stomata, the presence of crypted stomata (Fig. 7 B), hypostomatic leaf blades, multistratified hypodermis, and revolute margins are shared traits among the genera *Lasiolaena* (except *L. pereirae*) and *Stylotrichium*, supporting their grouping as probable sister groups (Amorim, 2019). In addition to its taxonomic significance, the multistratified hypodermis may represent an important environmental adaptation, as observed in Eriocaulaceae species found in campo rupestre habitats. Here, a hypodermis composed of sclerenchyma provides the plant with resistance to wind, intense light, and water stress (Mascarenhas *et al.,* 2020). Although ontogenetic studies were not conducted, the morphological differences between parenchymatic cells and epidermal cells suggest that the former originate from the ground meristem.

Only *Semiria viscosa* and *Semiria* sp. nov. showed cells with sinuous outline on the abaxial epidermal surface (*vs.* straight). Similarly, in *Aldama*, the variability in epidermal cell outlines allowed Bombo *et al.* (2016) to use this trait for species differentiation, underscoring its taxonomic relevance within the genus. The restriction of sinuous epidermal cell outlines to two non sister species of *Semiria* suggests that this trait represents a homoplastic or species-level diagnostic character rather than a synapomorphy.

Species of *Agrianthus*, along with *Arrojadocharis praxeloides*, *Bishopiella elegans*, *Semiria* sp. nov., and *S. viscosa* exhibited amphistomatic leaves. Furthermore, amphistomatic leaves were also observed in *Lasiolaena pereirae*, a species placed within the *Agrianthus* clade sensu Amorim (2019). Variations in stomatal placement are common in Asteraceae species (Melo-de-Pinna, 2004; Budel *et al.,* 2018) and may be adaptive strategies, contributing to reducing water loss and increasing carbon dioxide diffusion (Lusa *et al.,* 2014). However, since all the studied species are restricted to campos rupestres, hypostomatic leaves with crypted stomata are believed to reflect the common evolutionary history between *Lasiolaena* and *Stylotrichium* (Fig. 7 B).

Among the 11 types of trichomes described (three glandular and eight non-glandular), six species exhibited exclusive trichomes (autapomorphies): *Bishopiella elegans* (type XI), *Lasiolaena santosii* (types IV, X), *Semiria* (2 spp.; type VI), *Stylotrichium corymbosum* (type IX), and *S. rotundifolium* (type III). These trichomes therefore represent reliable taxonomic markers at the species level. The diversity of trichomes was reported by Liesenfeld *et al.* (2019) as an important trait for Asteraceae tribe taxonomy, in addition to studies on genera (Narayana, 1979) and subtribes (Wagner *et al.,* 2014). Glandular trichomes are the most diverse secretory structures for the family, especially for the Eupatorieae tribe (Castro *et al.,* 1997; Martínez-Quezada *et al.,* 2022). These structures also play crucial ecological roles, producing volatile oils and phenolic compounds that may act as defense agents against herbivores and pathogens (Fernandes *et al.*, 2016).

Nevertheless, the greatest diversity of trichomes was observed among the non-glandular trichome types (types IV–XI). In addition to the five types mentioned above (IV, VI, IX, X, and XI), type VII is restricted to three species of *Lasiolaena*, excluding *L. santosii* and *L. pereirae*, and represents a derived character according to our phylogenetic reconstruction (Fig. 7A). Similarly, the presence of non-glandular trichomes was important in identifying species of Lychnophorinae (Asteraceae), suggesting a probable adaptation against water stress (Wagner *et al.,* 2014). Trichomes can also contribute to plant protection against excessive transpiration during the dry season in the Cerrado and campos rupestres by secreting substances and protecting the plant from pathogens and herbivores (Trindade *et al.,* 2014).

Mesophyll organization varied among the analyzed taxa, with isobilateral leaves occurring in species of *Agrianthus*, *Arrojadocharis praxeloides*, *Semiria* sp. nov., and the outgroup *Lapidia apicifolia*, whereas dorsiventral mesophyll was recorded in *Semiria viscosa*, *Lasiolaena*, *Stylotrichium*, and the outgroup *Acritopappus confertus*. *Bishopiella elegans* was distinguished by a homogeneous mesophyll (Table 5). The occurrence of similar mesophyll types in both ingroup and outgroup taxa suggests that this character is evolutionarily labile and likely homoplastic, limiting its value as a synapomorphy within the clade. Rather than reflecting shared ancestry, mesophyll organization appears to be more strongly influenced by ecological or functional constraints.

The species *Arrojadocharis santosii* showed population variation regarding stomatal location (amphi and hypostomatic) and mesophyll, being isobilateral and homogeneous, as well as the presence or absence of hypodermis. Anatomical traits variation reinforces its possible hybrid origin, as discussed by Amorim (2019), and has been observed in other angiosperm groups, such as in Poaceae (Paštová, 2017).

In all the clade species, except *Bishopiella elegans*, mesophyll composition included atypical palisade parenchyma with non-uniform lobed cells (Fahn, 1978), resembling the plicate parenchyma recently reported in *Mikania glomerata* (Milan *et al.,* 2006). According to Fahn & Cutler (1992), atypical parenchyma can increase the area of photosynthetic tissue, benefiting species with reduced leaf areas, considered as a xeromorphic adaptation. The absence of atypical palisade parenchyma in *Bishopiella elegans* distinguishes this species from the remaining members of the clade, highlighting its anatomical and phylogenetic distinctiveness (Table 5).

Support tissues, derived from both the ground meristem and procambium, also proved useful for taxonomic delimitation within the group, particularly at the generic level. Procambial fibers occur in species of *Lasiolaena*, *Semiria*, and *Stylotrichium*, whereas sclerenchymatic fibers were recorded in *Arrojadocharis* and *Agrianthus*. Sclereids were dispersed in species of *Lasiolaena*, contributing to the anatomical circumscription of the genus. The absence of all support tissues in *Bishopiella elegans* is unique within the clade and represents a clear autapomorphy, supporting its monospecific circumscription.

In contrast, the presence of procambial or sclerenchymatic fibers and dispersed sclereids in other taxa is associated with sheath extensions that may enhance mechanical support and hydraulic continuity between vascular tissues and the mesophyll, particularly under xeric conditions typical of campos rupestres environments (Fahn & Cutler, 1992; Lusa *et al.,* 2014). However, because similar combinations of support tissues occur across distantly related ingroup taxa and in outgroup species, these characters exhibit limited phylogenetic signal and are better interpreted as convergent, ecologically driven adaptations rather than synapomorphies.

The analysis of leaf anatomy in the Chapada Diamantina clade (Asteraceae) proved to be a valuable tool for taxonomic and evolutionary studies. The anatomical characters analyzed contributed to reinforcing or clarifying phylogenetic hypotheses previously proposed with molecular data, offering robust morphological support for distinguishing genera and understanding their relationships. These findings emphasize the relevance of anatomical traits in identifying synapomorphies and resolving taxonomic uncertainties within the group. Moreover, the integration of anatomical and molecular evidence emerges as a powerful approach for advancing the delimitation and classification of genera in the Asteraceae family, particularly within the Eupatorieae tribe.

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Authors' Contributions

Bárbara Oliveira performed the anatomical analysis in the anatomy laboratory and carried out the anatomical descriptions. Kelly Leite assisted with anatomical sectioning, preparation of results, and discussion, and also contributed to conceiving the idea. Vivian Oliveira Amorim collected the species and contributed to the discussion of the results, as well as to conceiving the idea. Nádia Roque conceived the idea and contributed to the discussion.

Conflict of Interest

The authors declare that there is no conflict of interest.

Data Availability

The primary datasets generated and analyzed, specifically the character matrix and the phylogenetic tree files, are openly available in editable format in the Figshare repository under the identifier [doi: 10.6084/m9.figshare.30489671.v2]. All other data supporting the findings are included within the article.

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