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Original Article

What about the striated phyllaries in *Calea* (Asteraceae: Neurolaeneae)? Anatomical analyses and the implications for the genus taxonomy

Vinícius R. Bueno[1,2,*], Marcelly A. Martins[3], Hugo H. Araújo[4], Jaqueline D. Pereira[5]

[1] Fundação de Parques e Zoobotânica da Prefeitura de Belo Horizonte, Herbário BHZB, Belo Horizonte, MG, Brazil.
[2] Universidade Federal de Uberlândia, Departamento de Botânica, Uberlândia, MG, Brazil.
[3] Universidade Federal de Viçosa, Instituto de Ciências Biológicas e da Saúde, Rio Paranaíba, MG, Brazil.
[4] Universidade Federal de Viçosa, Departamento de Biologia Vegetal, Viçosa, MG, Brazil.
[5] Universidade Federal de Viçosa, Instituto de Ciências Biológicas e da Saúde, Rio Paranaíba, MG, Brazil.
*Corresponding author: vrbueno@outlook.com, vnrbueno@gmail.com

ABSTRACT

Calea L. (Asteraceae Bercht. & J.Presl) has 166 species that can be characterized by striated phyllaries, modified leaves that surround the flowers forming an involucre to protect these structures, yellow florets, and pappus scales. Despite being one of the diagnostic characters for the genus, this is the first in-depth paper about the striated phyllaries of *Calea*. We analyzed 166 different *Calea* species to verify if there are differences in the width of these striations and their taxonomic value. Additionally, we examined 16 *Calea* species for anatomical characterization and investigated the nature of the phyllary striations. Each stria is characterized by a pair of secretory ducts that run side by side with a vascular bundle surrounded by fibers. The lumen of the secretory ducts was characterized according to its width: narrow striae (46.5–80 µm) and wide striae (100–265.4 µm). The characterization of the type of striae has great informative taxonomic potential for *Calea*. We present images with anatomical sections of the phyllaries of the species analyzed. We also provide the classification of all *Calea* species analyzed macroscopically regarding the type of striae on the inner phyllaries.

Keywords: Anatomy, Capitula, Compositae, Heliantheae Alliance, Microcharacter

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Introduction

Neurolaeneae Rydb. is one of the 50 tribes of Asteraceae Bercht. & J.Presl (Susanna *et al.*, 2020), and it is included in the Heliantheae Alliance (Susanna *et al.*, 2020). Members of this tribe are commonly shrubby, having opposite leaves, cymose capitulescences, heterogamous capitula, pistillate ray florets, and pappus with scales, rarely absent, bristles or coroniform (Bueno *et al.*, 2021; Bueno, 2023). Neurolaeneae comprises 187 species (Bueno, 2023; Pruski, 2023) of neotropical and pantropical distribution (in the latter, only *Enydra* Lour.) (Bueno *et al.,* 2021; Bueno, 2023). All the species are classified in six genera: *Calea* L., *Enydra*, *Greenmaniella* W.M.Sharp, *Heptanthus* Griseb., *Neurolaena* R.Br., and *Staurochlamys* Baker (Bueno, 2023).

Calea is the most species-rich genus, with 166 species (Bueno, 2023; Pruski, 2023; Bueno *et al.,* 2025; Melquíades *et al.*, 2025; Silva *et al.*, 2025), occurring from Mexico to Argentina, as well as Jamaica and Trinidad and Tobago (Bueno, 2023). *Calea* species are morphologically diagnosed by their shrubby habit, opposite leaves, dichasial cymose or umbelliform capitulescences or solitary capitula, heterogamous capitula, striated phyllaries, yellow to brown anthers, and cypselae with pappus scales (Bueno *et al.*, 2021; Bueno, 2023). Pruski (2023) proposed the segregation of the genus into seven genera based on morphological characters. However, Bueno (2023) provided the first phylogeny for *Calea* and showed its monophyly; these analyses took into account molecular, morphological, and geographic characters.

Phyllaries are modified leaves grouped, enclosing, or closely subtending an inflorescence in Asteraceae (Roque *et al.*, 2009). The phyllaries differ from the leaves in terms of the position associated with the inflorescence, as well as in color, shape, and texture (Roque *et al.*, 2009). In the capitulum, the phyllaries act functionally as sepals, protecting the inflorescence during development, and can be attractive for insects (Roque *et al.*, 2009).

The application of microcharacters in the taxonomy of Asteraceae has been performed since Cassini (1821) until today (Robinson, 2009; Martínez-Quezada *et al.*, 2023). These studies used microscopy techniques to study taxonomically informative characters at an anatomical level, such as trichomes and ducts (Martínez-Quezada *et al.*, 2023), and morphologically, such as receptacles, corollas, anthers, styles, and pappi (Robinson, 2009). Examples of microcharacter applications have even been reported throughout the history of *Calea*: anatomical studies of the cypsela of *Calea* species provided subsidies to justify the transfer of some species of *Calea* to *Alloispermum* Willd. (Robinson, 2009).

Several studies on *Calea* (Pruski, 1984; Pruski & Urbatsch, 1983; 1988; Roque & Carvalho, 2011; Reis-Silva & Nakajima, 2020; 2021; Bueno & Heiden, 2021; 2022a;b; Bueno, 2023; Bueno *et al.*, 2024a) have cited the striated phyllaries as diagnostic characters for the genus taxonomy. Other studies (Pruski & Urbatsch, 1988; Pruski, 1997; 2011; Bueno & Heiden, 2022a) have used phyllaries as a source of important taxonomic information for species comparison, but only their external morphology, such as shape, dimensions, indument, and number of series. Although Robinson (1975) superficially suggested that the striae are resinous ducts, he does not indicate which species he analyzed or which methodology he used, and he does not discuss it. Therefore, to date, no paper has investigated what the *Calea* phyllary striations are and whether these structures are taxonomically informative.

Furthermore, all the *Calea* literature that cites the striated phyllaries refers to the striae without mentioning differences in these striations (Robinson, 1975; Pruski, 1984; Pruski & Urbatsch, 1983; 1988; Roque & Carvalho, 2011; Reis-Silva & Nakajima, 2020; 2021; Bueno & Heiden, 2021; 2022a;b; Bueno, 2023; Bueno *et al.*, 2024a).

In *Calea*, there are species with two types of phyllaries or only one (*Calea* in Flora e Funga do Brasil, 2025). The foliaceous phyllaries are the outermost series in several species, while the innermost ones are always scarious. In this way, through anatomical analyses on the inner phyllaries of *Calea* species, we intend to investigate the nature of the striae in phyllaries. Additionally, we morphologically examined whether there are differences in the width of these striations and their taxonomic value.

Materials and Methods

Macromorphological analyses were performed on inner phyllaries of at least one specimen from each of the 166 species of *Calea*, using stereoscopic microscopes (Leica M205C, Leica M60) and Leica Application Suite X 3.7.5. The list of the examined materials is provided in Supplementary material. These specimens are from 53 herbaria: ALCB, BHCB, BHZB, BLA, BM, BRIT, CEN, CESJ, DIAM, ECT, EFC, ESAL, FLOR, FUEL, FURB, G, HAS, HBR, HDJF, HEPH, HRB, HUCS, HUEFS, HUFSJ, HUFU, HURB, IBGE, ICN, K, MBM, MO, NYBG, P, PACA, PAMG, PEL, R, RB, RFA, SJRP, SMDB, SP, SPF, SPSF, TEPB, UB, UEC, UFG, UPCB, US, VIC, and VIES (Thiers, 2025). We analyzed all available *Calea* specimens in these herbaria. Some species are only known from the type specimen, whereas others have a large number of specimens available, all of which were analyzed. The analyses were performed on the inner phyllaries, which are always scarious and are present in all *Calea* species (Bueno, 2023).

The striae of the 166 species of *Calea* were previously classified based on macroscopic and qualitative analyses of the herbarium specimens using the Leica Application Suite X 3.7.5. Based on qualitative macroscopic analyses of the striae width in herbarium specimens, two distinct groups of striae were identified: narrow and wide. This classification, which divided the *Calea* species into two groups, is easily observed using a stereoscopic microscope.

To confirm this macroscopic classification, we performed anatomical analyses on a subset of the material from 16 species (Table 1), representing six subgenera (60 % of *Calea*) (Bueno, 2023). For anatomical analyses and diaphanization, we used 16 species that had at least three specimens available, except for *Calea triantha* (Vell.) Pruski, for which only two specimens were found (Table 1).

Diaphanization was performed according to a modified protocol from Handro (1964). The samples were placed in sodium hydroxide (2 %) for two hours, washed in distilled water (four successive times), clarified in sodium hydroxide solution (11 %), washed again in distilled water (four times sequentially), stained with safranin, and finally washed with 50 % ethanol to remove excess safranin (Kraus & Arduin, 1997). The material was then mounted on a slide with glycerin (Kraus & Arduin, 1997) and analyzed under a light microscope (Olympus CX 41).

For anatomical analyses, the herborized samples were rehydrated (Smith & Smith 1942), dehydrated in an ethyl series (10–70 %), and stored in 70 % ethanol. The samples were subsequently dehydrated in an ethyl series (85 %–95 %) and embedded in Historesin (Leica Biosystems, Heidelberg, Germany). Cross-sections and longitudinal sections (5 µm thick) were obtained with a rotary microtome (RM 2155, Leica Microsystems, Heidelberg, Germany). The samples were stained with toluidine blue (O'Brien *et al.*, 1964). The slides were mounted with Permount synthetic resin (Fisher Scientific, New Jersey, USA) and analyzed under an Olympus CX 41 microscope. Analyses and photographic documentation were carried out with a Nikon photomicroscope (Eclipse E200) equipped with a digital camera.

The width of the lumen of the secretory ducts was measured in cross-sections images using the ImageJ software (Schneider *et al.*, 2012). Each measurement was made in triplicate, with three replicates in each sample and three replicates per species whenever possible. The measurements taken are available in the supplementary material. To test the measurements and determine whether the two morphological groups of stretch marks were significantly different, we performed a t-test to compare the measurements taken for the two groups. The statistical analyses were performed using R (version 4.5.1; R Development Core Team, 2025; http://www.r-project.org/).

Results

Anatomically, in species classified as narrowly striate based on macroscopic analysis, the lumen of the secretory ducts ranged from 46.5 to 80 µm in width, whereas in widely striate species, it ranged from 100 to 265.4 µm (Supplementary Material). Statistical analysis of the data objectively reinforced the distinction between narrow and wide striae, confirming what was already observed macroscopically under a stereoscopic microscope (Figure 1A–B). Welch's t-test revealed a highly significant difference between the two groups ($p < 0.00000000002$), with averages of approximately 64 µm for the narrow striae and 155 µm for the wide striae. Furthermore, the confidence intervals did not overlap, reinforcing the robustness of the separation between the categories.

We propose here the characterization of all *Calea* species regarding the type of striae on the inner phyllaries based on macroscopic classification (Table 2). It was seen that there is no variation within each species. As can be seen in Fig. 1A–B, in addition to the width being visibly different in the stereoscopic images, the narrow striae (Fig. 1B) are clearly less prominent, less delimited and less conspicuous than the wide striae (Fig. 1A), this is uniform for all 166 species classified here.

Table 1. *Calea* species anatomically analyzed and its accession number.

Species	Materials analyzed
Calea acaulis Baker	SP 16823, NYBG 3365341, US 3403650
Calea candolleana (Gardner) Baker	SP 341416, HUFU 75931, HUFU 75833
Calea clausseniana Baker	HUFU 81150, ICN 203005, HALP 1347
Calea clematidea Baker	ICN 203021, ICN 167350, ICN 155275
Calea cymosa Less.	ECT 4947, HUFU 75775, US 2454190
Calea graminifolia Sch.Bip. ex Krasch.	ICN 203030, HUFU 81147, HUFU 12237
Calea hymenolepis Baker	ICN 203035, ICN 203036, HUFU 66990
Calea lantanoides Gardner	CEN 76898, NYBG 309467, US 3433771
Calea lemmatioides Sch.Bip. ex Krasch.	ICN 203039, HUFU 24911, US 3396210
Calea lutea Pruski & Urbatsch	HUFU 64399, NYBG 2482363, NYBG 3075534
Calea mediterranea (Vell.) Pruski	ECT 2392, HUFU 1230, HUFU 71938
Calea pinnatifida (R.Br.) Less.	ECT 3539, ICN 60765, ICN 164002
Calea quadrifolia Pruski & Urbatsch	CEN 54924, HEPH 3221, IBGE 30452, UB 24893
Calea sickii (G.M.Barroso) Urbatsch, Zlotsky & Pruski	ICN 203000, ICN 203068, ICN 203069, ICN 203070
Calea teucriifolia (Gardner) Baker	HUFU 26096, HUFU 63140, ICN 203072
Calea triantha (Vell.) Pruski	FUEL 37148, ICN 203052

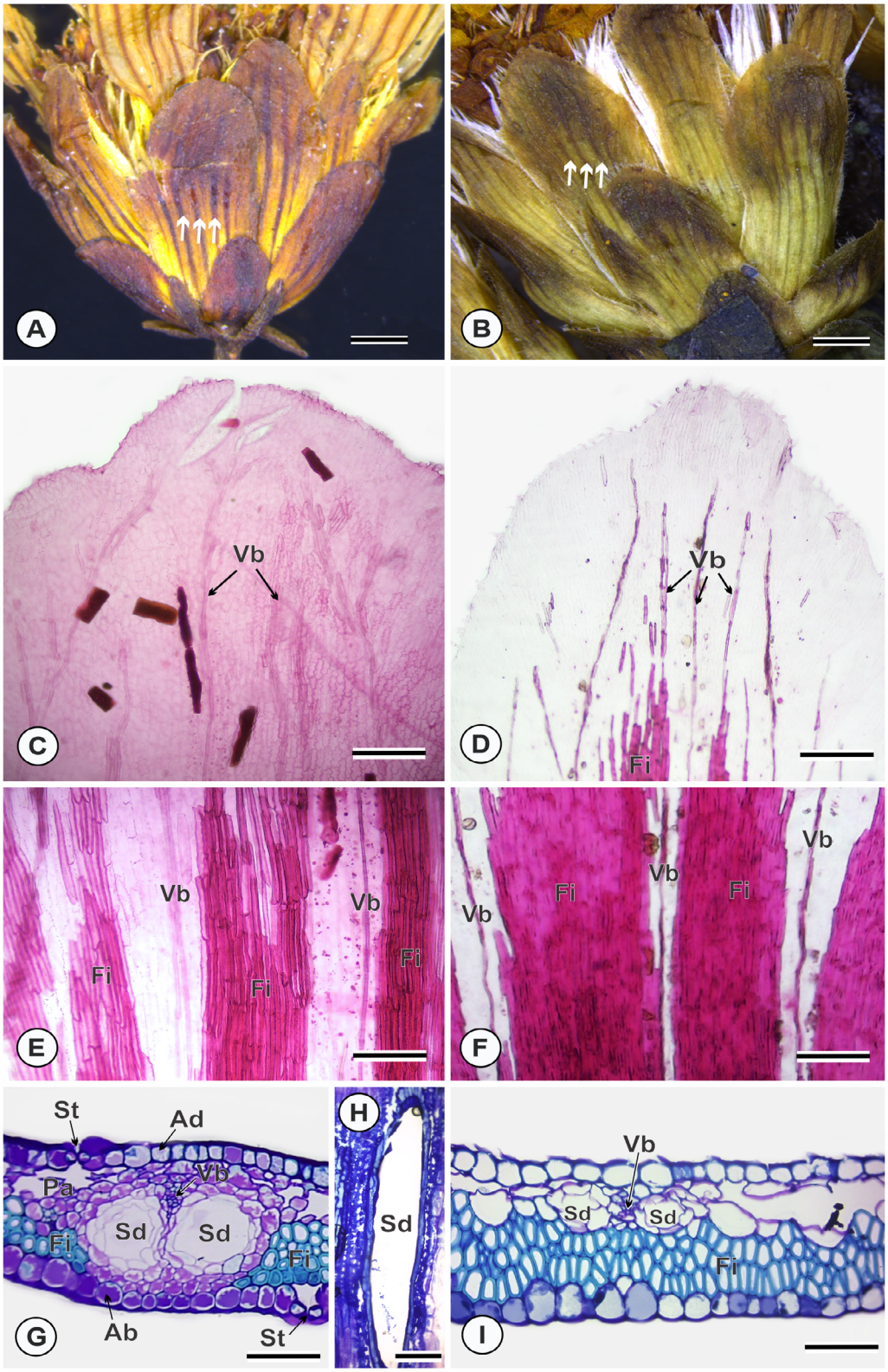

Figure 1. External morphology and anatomy of inner phyllaries of *Calea graminifolia* (A, C, E, G, and H) and *C. pinnatifida* (B, D, F, and I). Two types of striae in the inner phyllaries of *Calea*: Widely striate - 100–180.7 µm (A) and Narrowly striate - 46.5–86 µm (B). Frontal view of inner phyllaries in light microscopy – diafanization (C-F). General viewer of inner phyllaries (C and D). Detail of vascularization and fibers of inner phyllaries (E and F). Anatomy of inner phyllaries in cross (G and I) and longitudinal (H) section – light microscopy. Ad: adaxial surface epidermis; Ab: abaxial surface epidermis; Fi: Fibers; Vb: Vascular bundles; St: Stomata; Sd: Secretory duct; White arrows: striae inner phyllaries. Scales: A and B = 1 mm; C and D = 500 µm; E and F = 200 µm; G and I = 45 µm; H = 150 µm.

Table 2. *Calea* species regarding the inner phyllaries striae types.

Type of striae	Species
Narrow	*C. aggregata* V.R.Bueno & M.L.Campos; *C. angosturana* Hieron.; *C. arachnoidea* G.A.Reis-Silva & J.N.Nakaj.; *C. berteriana* DC.; *C. brittoniana* Pruski; *C. bucaramangensis* Pruski; *C. chapadensis* Malme; *C. colombiana* Gand.; *C. coriacea* DC.; *C. coridifolia* Pruski; *C. crassifolia* Standl. & Steyerm.; *C. crenata* Chodat; *C. crocinervosa* Wussow; *C. divaricata* Benth.; *C. fruticosa* (Gardner) Urbatsch, Zlotsky & Pruski; *C. funkiana* V.R.Bueno & G.Heiden; *C. gargantae* Cuatrec.; *C. grandiflora* V.R.Bueno & G.Heiden; *C. granitica* Pruski; *C. grazielae* J.U.Santos; *C. harlingii* H.Rob.; *C. hassleriana* Chodat; *C. heteropappa* Pruski & Urbatsch; *C. huigrensis* S.F.Blake; *C. ilienii* Malme; *C. intermedia* Pruski & Urbatsch; *C. irwinii* G.M.Barroso; *C. jamaicensis* L.; *C. myrtifolia* (DC.) Baker; *C. neblinensis* (Maguire & Wurdack) Pruski; *C. nelsonii* B.L.Rob. & Greenm.; *C. nitida* Less.; *C. oxylepis* Baker; *C. parvifolia* (DC.) Baker; *C. perijaensis* Cuatrec.; *C. perimbricata* Cuatrec.; *C. peruviana* Benth. ex S.F.Blake; *C. phelpsiae* Lasser & Maguire; *C. phyllolepis* Pruski & Urbatsch; *C. pinnatifida* (R.Br.) Less.; *C. prunifolia* Kunth; *C. pruskiana* V.R.Bueno & G.Heiden; *C. punctata* Maguire & Wurdack; *C. quadrifolia* Pruski; *C. reticulata* Gardner; *C. rhombifolia* S.F.Blake; *C. riopardensis* V.R.Bueno, Prata-Silveira & Bentes; *C. roqueana* V.R.Bueno, Prata-Silveira & Bentes; *C. rotundifolia* (Less.) Baker; *C. santanderensis* Pruski; *C. saxatilis* Cuatrec.; *C. septuplinervia* Hieron.; *C. serrata* Less.; *C. sessiliflora* Less.; *C. sessilifolia* V.R.Bueno & G.Heiden; *C. sickii* (G.M.Barroso) Urbatsch, Zlotsky & Pruski; *C. solidaginea* Kunth; *C. wedelioides* (Baker) S.F.Blake; *C. yariguiensis* B.V.Rodríguez & S.Díaz; *C. yuruparina* Cuatrec.
Wide	*C. abbreviata* Pruski & Urbatsch; *C. abelioides* S.F.Blake; *C. acaulis* Baker; *C. aldamoides* G.H.L.Silva, Bringel & A.M.Teles; *C. angusta* S.F.Blake; *C. anomala* Hassl.; *C. asclepiifolia* Hassl.; *C. bahiensis* (Mattf.) H.Rob.; *C. bakeriana* Chodat; *C. breviflora* V.R.Bueno & M.S.Silva; *C. cabrerae* Pruski; *C. caleoides* (Benth.) H.Rob.; *C. camani* Maguire & K.D.Phelps; *C. candolleana* (Gardner) Baker; *C. chodatii* Hassl.; *C. clausseniana* Baker; *C. clematidea* Baker; *C. coronopifolia* Sch.Bip. ex Baker; *C. cuneifolia* DC.; *C. cymosa* Less.; *C. dalyi* Pruski & Urbatsch; *C. diamantinensis* G.A.Reis-Silva & J.N.Nakaj.; *C. diffusa* Pruski; *C. divergens* Sch.Bip. ex Baker; *C. elongata* (Gardner) Baker; *C. esposi* Maguire & K.D.Phelps; *C. ferruginea* Sch.Bip. ex Baker; *C. fluviatilis* S.F.Blake; *C. formosa* Chodat; *C. gardneriana* Baker; *C. gentianoides* DC.; *C. graminifolia* Sch.Bip. ex Krasch.; *C. grandibracteata* Melq., A.K.Koch, A.M.Teles & V.R.Bueno; *C. harleyi* H.Rob.; *C. hatschbachii* Pruski & D.J.N. Hind; *C. huanchacana* Pruski; *C. hymenolepis* Baker; *C. hypericifolia* (Gardner) Baker; *C. kirkbridei* H.Rob.; *C. kunhardtii* Maguire; *C. lucidivenia* Gleason & S.F.Blake; *C. lutea* Pruski & Urbatsch; *C. martiana* Baker; *C. mediterranea* (Vell.) Pruski; *C. melissifolia* Baker; *C. microphylla* (Gardner) Baker; *C. multiplinervia* Less.; *C. nana* Maguire; *C. nematophylla* Pruski; *C. nervosa* G.M.Barroso; *C. oliveri* B.L.Rob. & Greenm.; *C. orbiculata* Maguire & Aristeg.; *C. ottohuberi* Pruski; *C. papposa* Malme; *C. paraguayensis* (Kuntze) Deble; *C. pilosa* Baker; *C. pinheiroi* H.Rob.; *C. pohliana* Sch.Bip. ex Baker; *C. politii* Maguire; *C. polycephala* (Baker) H.Rob.; *C. purpurea* G.M.Barroso; *C. ramosissima* Baker; *C. repanda* V.R.Bueno, Gostel & G.Heiden; *C. robinsoniana* Pruski; *C. rupicola* Chodat; *C. semirii* Pruski & D.J.N.Hind; *C. senecioides* Baker; *C. sipapoana* Maguire; *C. teucriifolia* (Gardner) Baker; *C. tocantina* Pruski; *C. tricephala* Maguire; *C. tridactilyta* Sch.Bip. ex Krasch.; *C. uniflora* Less.; *C. venosa* Pruski; *C. verticillata* (Klatt) Pruski; *C. villosa* Sch.Bip. ex Baker; *C. woodii* Pruski & Urbatsch

As observed in the diaphanized samples (Fig. 1C–F), the striae are the unstained regions between the fibers. In cross-section, the striae are characterized by a pair of secretory ducts that run side by side with a vascular bundle surrounded by fibers (Fig. 1G–H). In some cases, the analyzed inner phyllaries region may present a continuous layer of fibers on the abaxial side, below the secretory ducts (Fig. 1I). The secretory ducts are arranged longitudinally in the phyllaries of all the species evaluated (Figs. 1–2).

In the diaphanized samples, we observed the presence of fibers, vascular bundles, and stomata in all species evaluated (Fig. 1C–F; Fig. 2A–F). In some species, the inner phyllaries' surface is glabrous when viewed from the median frontal region, such as in *C. graminifolia*, *C. pinnatifida* (Fig. 1C–F), *C. candolleana*, *C. hymenolepis*, *C. lemmatioides,* and *C. sickii* (Fig. 2A–B and 2E–F). However, in other species, such as *C. mediterranea* (Fig. 2C) and *C. acaulis* (Fig. 2D), the inner phyllaries' surface presents both tector and glandular trichomes. Furthermore, it was possible to observe variations in the organization of vascular bundles (venation patterns) and distribution of fibers in the species evaluated in the diaphanized material. Some species have fibers that extend to the apex of the phyllaries, such as *C. lemmatioides* and *C. sickii* (Fig. 2E-F); others have less elongated fibers restricted to the base of the phyllaries, such as *C. graminifolia, C. pinnatifida* (Fig. 1C-F), *C. candolleana, C. hymenolepis, C. mediterranea*, and *C. acaulis* (Fig. 2A-D).

In anatomical cross-sections, the inner phyllaries of all the species evaluated have a unstratified epidermis with stomata that can occur on both the adaxial and abaxial surfaces (Fig. 1G, 1I; Fig. 3). The mesophyll of all the species evaluated was composed of parenchymatic cells and fibers (Fig. 1G–I; Fig. 3). The secretory ducts (with longitudinally elongated internal space delimited by a secretory epithelium; Fig. 1H) are associated with the vascular bundles (Fig. 1G–I; Fig. 3A–N). In some species, such as *Calea acaulis*, *C. candolleana*, *C. cymosa*, and *C. mediterranea,* there are tector and glandular trichomes on the surface of the epidermis (Fig. 3G–J).

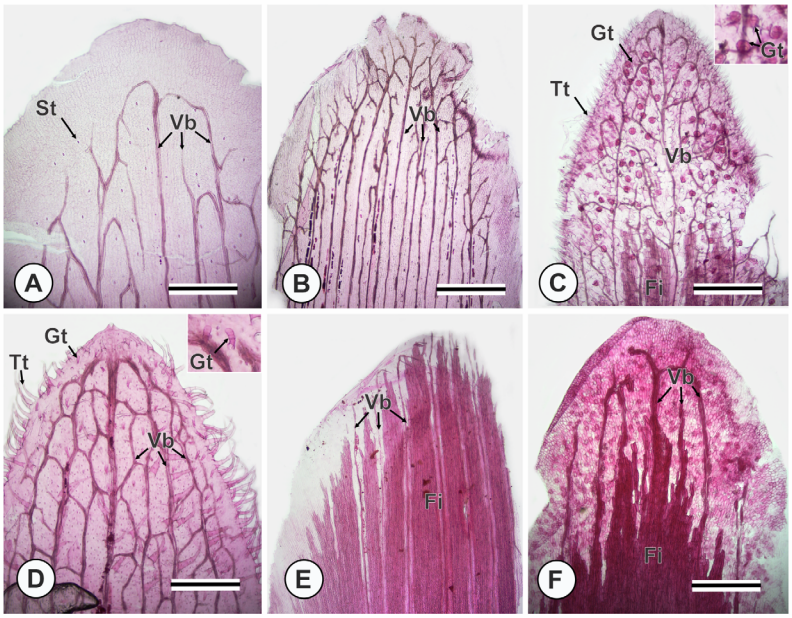

Figure 2. Frontal view of inner phyllaries of *Calea* in light microscopy - diafanization (A-F). Widely striate (A, B, C, and D) and narrowly striate (E and F). *Calea candolleana* (A); *C. hymenolepis* (B); *C. mediterranea* (C); *C. acaulis* (D); *C. lemmatioides* (E), and *C. sickii* (F). St: Stomata; Vb: Vascular bundles; Fi: Fibers; Tt: Tector trichome; Gt: Glandular trichome. Scales: A-F = 600 µm.

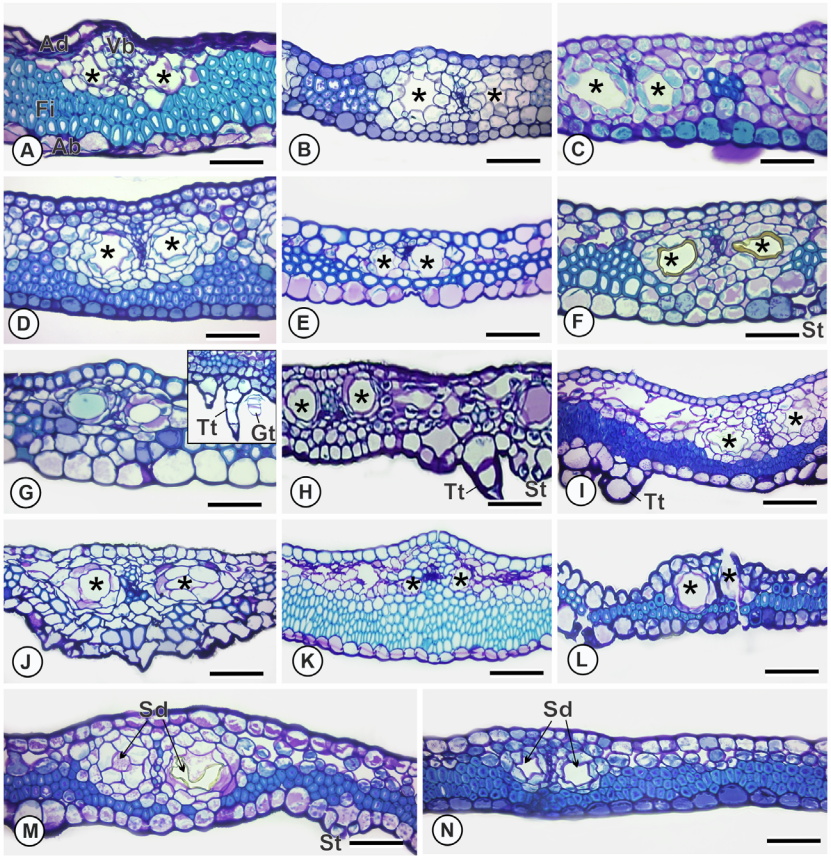

Figure 3. Anatomy of inner phyllaries of *Calea* in cross section - light microscopy (A-N). Widely striate (B, C, D, F, G, H, I, J, and M) and narrowly striate (A, E, K, L, and N). *C. quadrifolia* (A); *C. lutea* (B); *C. hymenolepis* (C); *C. teucriifolia* (D); *C. lemmatioides* (E); *C. clematidea* (F); *C. mediterranea* (G); *C. cymose* (H); *C. candolleana* (I); *C. acaulis* (J); *C. sickii* (K); *C. lantanoides* (L); *C. clausseniana* (M) and *C. triantha* (N). Ad: adaxial surface epidermis; Ab: abaxial surface epidermis; Fi: Fibers; Vb: Vascular bundles; St: Stomata; Sd: Secretory duct; Gt: Glandular trichome; Tt: tector trichome; asterisk: secretory duct. Scales: A-N = 45 µm.

Discussion

Our data demonstrated that the striae in *Calea* phyllaries are secretory ducts arranged longitudinally, associated with vascular bundles, and may be surrounded by fibers, as demonstrated in the anatomical analyses. Among all the *Calea* literature concerning the striae in the phyllaries, only Robinson (1975) makes a superficial mention of structures that could anatomically correspond to striae in the inner phyllaries. We confirm that Robinson's hypothesis (1975) is correct: the *Calea* inner phyllaries' striae are longitudinal ducts associated with the vascular bundle. As pointed out by Robinson (1975), this is one of the few characteristics that are shared by all species of *Calea*. Although this author originally used the term 'resinous duct', no chemical analyses of the secretion were performed, so we prefer to refer to them as 'secretory ducts'.

The secretory ducts we describe in *Calea* phyllaries exhibit anatomical similarities to those in other Asteraceae species (Fahn, 1979; Martínez-Quezada *et al.,* 2023). In Asteraceae, it is reported that the predominant internal secretory structures are ducts, also described as secretory canals and resinous ducts, and they are widely distributed in vegetative organs and floral units (Robinson, 1981; Metcalfe & Chalk, 1983; Mauseth, 1988; Martínez-Quezada *et al.,* 2023). Secretory ducts, as we verified, are characterized by the presence of a secretory epithelium that delimits a lumen, an elongated internal space in which secretion accumulates and is generally associated with vascular bundles (Fahn, 1979; Prado & Demarco, 2018). The composition of the duct secretion can vary, but it is predominantly resinous in some families in which it occurs, including Asteraceae, and has taxonomic value (Metcalfe & Chalk, 1983; Prado & Demarco, 2018; Martínez-Quezada *et al.,* 2023). The presence of ducts is common in Asteraceae, especially in Asteroideae Lindl., Mutisioideae Lind. and Carduoideae Cass. ex Sweet (Robinson, 1981; Metcalfe & Chalk, 1983; Martínez-Quezada *et al.,* 2023).

Even though the striations have never been studied in depth in *Calea*, there are records of ducts in the inner phyllaries of other groups. Anderberg (2009) reported a unique duct in the inner phyllaries of *Iphionopsis* Anderb. (Inuleae Cass.); Funk *et al. (*2009) reported the presence of a single duct in inner phyllaries of *Feddea* Urb. (Feddeae Urb.); Robinson (1981) (as Coreopsidineae Lindl.) and Crawford *et al.* (2009) noted that there are few or many ducts in the inner phyllaries of Coreopsideae Lindl.; Pruski *et al.* (2015) also found ducts in the inner phyllaries in *Electranthera* Mesfin, D.J.Crawford & Pruski (Coreopsideae); and Lizarazu & Freire (2019) documented these ducts in the phyllaries for *Heterosperma* Cav. (Coreopsideae). Except for *Iphionopsis* (Inuleae), all the other groups mentioned are included in the Heliantheae Alliance (Susanna *et al.*, 2020). Interestingly, although the presence of secretory ducts in these species has been noted, their anatomical structure remains unexplored. Anatomical data on these structures in *Calea* are notably scarce in the literature. In this context, our study is pioneering, as it provides a detailed description of the anatomy of secretory ducts in the phyllaries of *Calea*, thus serving as a foundation for future anatomical and taxonomic studies.

As for *Electranthera* and *Narvalina* (Pruski *et al.*, 2015) and *Heterosperma* (Lizarazu & Freire, 2019), we propose that all *Calea* species have ducts. This is supported by our anatomical analyses, which found ducts within the striae that are macromorphologically visible in all *Calea* species. Robinson (1975) or any of the other authors who mention *Calea*'s striae (Pruski, 1984; Pruski & Urbatsch, 1983; 1988; Roque & Carvalho, 2011; Reis-Silva & Nakajima, 2020; 2021; Bueno & Heiden 2021; 2022a; 2022b; Bueno 2023; Bueno *et al.*, 2024a) do not discuss the informative taxonomic potential of these striae within *Calea*. All genera of Neurolaeneae have striate phyllaries (Bueno, 2023). We demonstrated that herbarium specimens can be used in anatomical studies of phyllaries and that this approach can be extended to other genera within Neurolaeneae.

No *Calea* species presents both types of striae, which shows that this is a stable character for each species. Because the striae types are easily distinguishable macroscopically, their characterization has several taxonomic applications. This information can help to differentiate infrageneric groups and species, as seen in recently published subgroups: *Calea* subgen. *Teucriifoliae* V.R. Bueno, Gostel & G.Heiden (Bueno *et al.,* 2024a) and *C.* ser. *Candolleanae* V.R. Bueno, Gostel & G. Heiden (Bueno *et al.*, 2024b) are both characterized by widely striate phyllaries, whereas *Calea* subg. *Meyeria* can be characterized by narrowly striate phyllaries (Bueno *et al.* 2025).

Bueno & Heiden (2022b) compared *C. arachnoidea* G.A. Reis-Silva & J.N.Nakaj., *C. sessilifolia* V.R.Bueno & G.Heiden, *C. heteropappa* Pruski, and *C. semirii* Pruski & D.J.N.Hind due to the similar pappus. According to Table 2, our results could contribute to the key, since the presence of widely striate phyllaries only exists in *C. semirii*, while the other three species have narrowly striate phyllaries.

Furthermore, Bueno (2023) discussed the morphological relationships of the taxonomy of the Nana clade of *Calea* that includes five species: *Calea coridifolia* Pruski, *Calea linearifolia* Maguire & Wurdack, *Calea nana* Maguire, *Calea saxatilis* Cuatrec., and *Calea spiralis* V.R.Bueno, Gostel & G.Heiden. With our results, we can see that *Calea nana* presents widely striate phyllaries, while the remaining species have narrow striate phyllaries. The type of striate phyllaries is also informative for *Calea* sect. *Haplocalea* (Less.) Pruski, which has eight species (Pruski, 1998) that present similar morphology regarding their capitulescence. However, three have widely striate phyllaries (*Calea acaulis*, *C. cymosa*, and *C. mediterranea*) and five have narrowly striate phyllaries (*C. crenata* Chodat, *C. hassleriana* Chodat, *C. reticulata* Gardner, and *C. rhombifolia* S.F.Blake).

In taxonomic studies of *Calea* with geographic scope, the differentiation of the types of striate phyllaries could also be very informative. Silva & Teles (2018) listed 30 species for Goiás state, Brazil, and produced a key for these species. Our results contribute directly to the differentiation of these species, since seven of these species have narrowly striate phyllaries and the other 23 have widely striate phyllaries. Pruski (1997) listed the *Calea* species for the Flora of the Venezuelan Guayana; 26 species have already been published, of which 14 are widely striate phyllary species and 12 are narrowly striate. Therefore, this character could be quite useful in the taxonomy of these species.

Our results contribute novel insights that fill gaps in the understanding of the anatomy of *Calea* phyllaries. These insights may enhance the characterization of the genus and support comparative studies across related taxa. Additionally, although our study was directed towards the analysis of inner phyllaries striae, anatomical analyses also revealed that only some species of *Calea* species present tector and glandular trichomes on the surface of their inner phyllaries. Thus, we suggest that in addition to striae, other anatomical characters of inner phyllaries, such as the shape of epidermal cells, venation patterns, and distribution of fibers, may also be potentially useful in taxonomic studies of *Calea*. However, this study provides a foundation for future research, and additional anatomical studies are needed to better understand the chemical nature of secretory duct secretion and the usefulness of other anatomical structures for taxonomic purposes.

This study is the first to identify anatomically what these striations are and their significant potential for generating taxonomic information within the genus. Anatomical analyses solved the great mystery surrounding the striated inner phyllaries of *Calea* by elucidating that they are secretory ducts associated with vascular bundles. We propose that these striae can be classified morphologically into two types: narrowly and widely striate. This characteristic is present in all *Calea* species analyzed, and our analyses demonstrate that it is highly informative for the genus, with the potential to characterize groups and to be applied in taxonomic keys. Further studies could elucidate whether the inner phyllaries of other genera of Neurolaeneae (Bueno *et al.,* 2021; Bueno, 2023) show structural differences when compared with *Calea* and among themselves. Furthermore, other anatomical characters of inner phyllaries, such as vascularization and distribution of fibers, may be potentially useful in these investigations.

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Supplementary Materials

The following online material is available for this article:

Supplementary Material 1. List of striation phyllaries measurements of the *Calea* species.

Supplementary Material 2. List of the specimens examined for morphological characterization: Taxon, locality, collection date, collector, and number (Herbarium code and accession number).

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Authors' Contributions
The authors' contribution are: Conceptualization (VRB, JDP); Data curation (VRB, MAM, JDP); Formal analysis (VRB, MAM, HHA, JDP); Funding acquisition (VRB, JDP); Investigation (VRB, MAM, HHA, JDP); Methodology (VRB, MAM, HHA, JDP); Project administration (VRB, JDP); Software (MAM, HHA); Resources (VRB, JDP); Supervision (VRB, JDP); Validation (VRB, HHA, JDP); Visualization (VRB, MAM, HHA, JDP); Writing – original draft (VRB, MAM, HHA, JDP); Writing – review & editing (VRB, HHA, JDP).

Conflict of Interest
The authors declare no conflict of interest.

Data Availability
No new data were created or analyzed in this study.

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