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Palynotaxonomy of *Calea* sect. *Meyeria* (Asteraceae: Neurolaeneae)

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ABSTRACT

Calea comprises 157 species that occur in the Neotropics. The genus is organized into eight subgenera and 18 sections. *Calea* sect. *Meyeria* consists of eight species occurring exclusively in south-central Brazil. In this study, pollen grains from these eight species were sampled from herborized flower buds in pre-anthesis. Pollen samples were acetolyzed, measured, and photographed under a light microscope. Unacetolyzed pollen grains were examined under a scanning electron microscope. The results were used to characterize pollen morphology and construct a palynotaxonomic key for *Calea* sect. *Meyeria*. The analyzed pollen grains were monads, isopolar, usually medium-sized, oblate spheroidal, tricolporate, with lalongate endoaperture, the sexine echinate and thicker than the nexine, and six spines in the apocolpium region. The eight species have very similar pollen grains, but some characters are informative for this section, namely pollen size, polar area, sexine ornamentation, and endoaperture characteristics. Despite the similarity of the pollen grains, palynological analysis was able to contribute to the delimitation of *Calea* species, providing new information for species distinction within *Calea* sect. *Meyeria*.

KEYWORDS
Calea myrtifolia group; *Calea triantha* group; compositae; palynology

1. Introduction

Asteraceae comprises ca. 24,000–30,000 species and 1600–1700 genera with a cosmopolitan distribution (Mandel et al. 2019). The family is monophyletic (Susanna et al. 2020), as supported by phylogenetic analysis of molecular data (Mandel et al. 2019) and morphological characters, such as capitulate inflorescence, synantherous anthers, inferior ovary, and cypselate fruits, commonly with pappus (modified calyx) (Roque and Bautista 2008; Funk et al. 2009; Roque et al. 2017). Members of the family are classified into 16 subfamilies and 50 tribes (Susanna et al. 2020). One of these tribes, Neurolaeneae, was re-established based on molecular studies (Panero et al. 1999; Panero and Funk 2002; Panero 2007). The tribe contains six genera and ca. 185 species, with the vast majority occurring in the tropical regions of the Neotropics (Bueno et al. 2021; Bueno 2023). Bueno et al. (in press) proposed that Neurolaeneae should be divided into six genera.

Calea L. comprises 157 species generally characterized by opposite leaves, 3–8 seriate involucre, yellow corolla, and pappus scales (Bueno 2023). In Brazil, the genus is represented by 91 species, 59 of which are endemic (Bueno et al. 2022; Reis-Silva and Nakajima 2021). *Calea* is currently subdivided into eight subgenera and 18 sections; *Calea* section *Meyeria* Benth. & Hook.f. consists of eight species occurring exclusively in south-central Brazil (Bueno et al. 2021, 2022; Bueno 2023). Members of *Calea* sect. *Meyeria* are characterized by shrubby habitat, ovate leaves, involucre with more than three foliaceous phyllaries, radiate capitula, and cypsela larger than the pappus (Bueno 2023). This group of eight species is also referred to in the literature as the *Calea myrtifolia* complex (Pruski and Urbatsch 1988; Pruski 2005; Bueno et al. 2021, 2022). Bueno et al. (in press) supported the existence of two morphological groups in this section, the *C. myrtifolia* group and the *C. triantha* group.

Palynological analysis can be used in research on pollination, pollen dispersion, paleoecology, melissopalynology and forensics. Furthermore, it has fundamental importance in the study of ecological and evolutionary characteristics and phylogenetic relationships of different groups of plants (Cancelli et al. 2005; Cui et al. 2019; Mezzonato-Pires et al. 2019; Gonçalves-Esteves et al. 2022; Quamar et al. 2022; Cartaxo-Pinto et al. 2022a, 2022b, 2022c, 2023). The study of pollen grains is a useful tool for elucidating the taxonomy of several botanical families, and circumscribing genera and subgeneric categories (Gonçalves-Esteves et al. 2022).

Numerous palynological studies have been conducted on Asteraceae (Gonçalves-Esteves 1976, 1977a, 1977b; Gonçalves-Esteves and Esteves 1986, 1988a, 1989a, 1989b; Mendonça

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et al. 2002; Cancelli et al. 2005, 2010; Magenta et al. 2010; Moreira et al. 2019; Reshmi and Rajalakshmi 2019; Ulukuş and Tugay 2020; Marques et al. 2021; Souza-Souza et al. 2021, 2022; Tellería et al. 2023). However, there are no specific papers describing the palynology of the tribe Neurolaeneae, only studies focused on Asteraceae that evaluated the pollen of some Neurolaeneae species: *Calea* species (e.g. Roubik and Moreno 1991; Melhem et al. 2003; Cancelli et al. 2010; Stanski et al. 2014; Souza-Souza et al. 2022) and *Enydra* Lour. species (Perveen 1999; Cancelli et al. 2010). Among the cited authors, only Stanski et al. (2014) analyzed two species of *Calea* sect *Meyeria*.

This is the first study focused exclusively on describing the pollen of Neurolaeneae species with a taxonomic scope. The objective was to analyze pollen grains of the eight species of *Calea* sect. *Meyeria* to identify characters that contribute to the palynology of the section and genus and generate data to elucidate the taxonomy of this group.

2. Material and methods

2.1. Pollen material

We analyzed pollen grains from eight species of *Calea* sect. *Meyeria*, in the *Calea myrtifolia* group (*C. marginata* S.F. Blake, *C. myrtifolia* (DC.) Baker, *C. parvifolia* (DC.) Baker and *C. phyllolepis* Baker), and the *Calea triantha* group (*C. funkiana* V.R. Bueno & G. Heiden, *C. pruskiana* V.R. Bueno & G. Heiden, *C. subintegerrima* (Malme) V.R. Bueno & G. Heiden and *C. triantha* (Vell.) Pruski). Pollen grains were collected from fertile anthers of flowers in anthesis or flower buds in pre-anthesis. The botanical material was obtained from specimens deposited in the following Brazilian herbaria: ICN, SP, SPSF, and UPCB (acronyms according to Thiers 2023). The specimens are listed in the Appendix.

2.2. Light microscopy

For observation by light microscopy, pollen samples were prepared according to the acetolysis method of Erdtman (1952), with the modifications proposed by Melhem et al. (2003). Acetolyzed pollen grains were measured up to 7 days after preparation (Salgado-Labouriau 1973). Microscope slides of pollen grains were deposited in the pollen collection of the Álvaro Xavier Moreira Laboratory of Palynology, Department of Botany, National Museum, Federal University of Rio de Janeiro, Brazil.

2.3. Scanning electron microscopy

Unacetolyzed pollen grains were mounted on stubs with carbon tape (Cartaxo-Pinto et al. 2022a) and examined using an FEI Quanta 450 field-emission scanning electron microscope at the Nanotechnology Characterisation Center (CENANO), National Institute of Technology, Brazil.

2.4. Measurement of pollen grains

A total of 25 measurements were randomly taken of polar and equatorial diameters. Additionally, 10 measurements were randomly taken of the equatorial diameter in polar view, apocolpium side, aperture length and width, exine thickness, and echinus dimensions. The results were subjected to statistical analysis to obtain the arithmetic mean (*x\bar*), standard deviation of the sample (*s*), standard deviation of the average mean ($s_{x\backslash bar}$), coefficient of variation, and 95% confidence interval (CI). Table 1 presents the arithmetic mean (*x\bar*), standard deviation of the average mean ($s_{x\backslash bar}$), and 95% CI values. The arithmetic mean of equatorial diameter in polar view, apocolpium side, aperture length and width, exine thickness, and echinus dimensions are presented in Tables 2 and 3. For each specimen, a minimum of three permanent slides of acetolyzed pollen grains were analyzed to ensure sample standardization (Salgado-Labouriau et al. 1965).

2.5. Terminology

The terminology adopted was that of Punt et al. (2007), which takes into account pollen size, shape, aperture number, and sexine ornamentation pattern. Descriptions of polar area and aperture size follow the classification of Faegri and Iversen (1966) for calculation of the polar area index. Photomicrographs of pollen grains were captured using a digital camera coupled to a Zeiss Axiostar Plus microscope.

3. Results

The following species were analyzed: *Calea funkiana* (Plate 1, figures 1–3), *C. marginata* (Plate 1, figures 4–6), *C. myrtifolia* (Plate 1, figures 7–9), *C. parvifolia* (Plate 1, figures 10–12), *C. phyllolepis* (Plate 2, figures 1–3), *C. pruskiana* (Plate 2, figures 4–6), *C. subintegerrima* (Plate 2, figures 7–9), and *C. triantha* (Plate 2, figures 10–12).

Table 1. Measurements (in μm) of *Calea* sect. *Meyeria,* serie Myrtifolieae (Asteraceae) species in equatorial view ($n = 25$).

	Polar diameter (P)			Equatorial diameter (E)				
Species	Range	$x̄ \pm s_{x̄}$	95% CI	Range	$x̄ \pm s_{x̄}$	95% CI	P/E	Shape
C. funkiana	35.0–50.0	40.2 ± 0.9	38.3–42.1	35.0–50.0	40.7 ± 0.6	39.3–42.0	0.98	Oblate spheroidal
C. marginata	45.0–52.5	47.5 ± 0.4	46.5–48.4	45.0–52.5	50.0 ± 0.4	49.0–50.9	0.95	Oblate spheroidal
C. myrtifolia	40.0–47.5	43.2 ± 0.5	42.2–44.2	40.0–47.5	44.0 ± 0.5	40.0–50.0	0.98	Oblate spheroidal
C. parvifolia	32.5–47.5	43.8 ± 0.6	42.5–45.0	42.5–47.5	45.4 ± 0.3	44.7–46.0	0.96	Oblate spheroidal
C. phyllolepis	37.5–45.0	42.0 ± 0.4	41.1–42.9	40.0–45.0	42.9 ± 0.2	42.4–43.4	0.98	Oblate spheroidal
C. pruskiana	37.5–45.0	40.6 ± 0.5	39.6–41.6	37.5–45.0	41.1 ± 0.5	40.0–42.2	0.99	Oblate spheroidal
C. subintegerrima	40.0–55.0	45.6 ± 0.9	43.6–47.6	40.0–55.0	47.4 ± 0.8	45.7–49.1	0.96	Oblate spheroidal
C. triantha	45.0–57.5	53.4 ± 0.6	52.1–54.6	47.5–57.5	52.9 ± 0.4	51.9–53.8	1.00	Oblate spheroidal

x̄: arithmetic mean; s: standard deviation; sx̄: standard deviation of the mean; CI: confidence interval; PD/PE ratio: shape.

Table 2. Measurements (in µm) of *Calea* sect. *Meyeria*, serie Myrtifolieae (Asteraceae) species in polar view ($n = 10$).

Species	EDPV		AS		Polar area	
	Range	x	Range	x		
C. funkiana	50.0–60.0	55.5	10.0–17.5	12.5	0.22	Very small
C. marginata	52.5–62.5	57.0	7.5–10.5	10.5	0.18	Very small
C. myrtifolia	50.0–60.0	55.5	10.0–15.0	11.7	0.21	Very small
C. parvifolia	47.5–55.5	52.0	12.5–15.0	13.5	0.26	Small
C. phyllolepis	37.5–45.0	42.4	5.0–6.6	6.6	0.16	Very small
C. pruskiana	40.0–45.0	42.5	7.5–12.5	9.5	0.22	Very small
C. subintegerrima	42.5–52.5	47.5	15.0–20.0	17.0	0.36	Small
C. triantha	47.5–57.5	52.0	15.0–17.5	15.8	0.30	Small

EDPV: equatorial diameter in polar view; SA: Side Apocolpium; x\bar: arithmetic mean; PAI: polar area index.

Table 3. Measurements (in µm) of apertures, exine layers and echinae dimensions of *Calea* sect. *Meyeria*, serie Myrtifolieae (Asteraceae) species in polar view ($n = 10$).

Species	Colpus		Endoaperture		Exine layers				Echinae		
	Length	Width	Length	Width	Exine	Sexine	Nexine	Cavea	Length	Width	DBE
C. funkiana	16.9	4.0	2.7	11.7	7.2	5.9	0.7	0.6	5.4	4.2	11.0
C. marginata	18.3	4.6	3.7	14.3	8.7	6.7	1.0	1.0	6.2	3.8	14.2
C. myrtifolia	33.2	3.3	2.9	13.5	13.9	10.9	2.2	0.8	8.8	3.2	10.7
C. parvifolia	19.8	7.1	4.8	11.3	8.4	6.9	0.6	1.0	6.2	2.5	14.0
C. phyllolepis	24.1	1.0	5.4	12.4	8.0	6.6	0.7	0.7	5.8	4.7	9.4
C. pruskiana	15.4	4.9	5.1	12.0	8.6	7.2	1.0	0.8	5.2	3.9	9.9
C. subintegerrima	25.7	3.2	6.9	13.6	10.1	7.1	2.0	1.0	5.2	3.6	10.5
C. triantha	22.1	2.0	3.6	11.7	7.2	4.6	1.4	1.0	3.6	3.9	8.9

Exine: sexine + nexine + cavea; DBE: distance between echinae.

3.1. Dispersion units, polarity, size, amb, shape, and polar area

Pollen grains from the eight analyzed species are monads, isopolar, oblate-spheroidal, medium-sized (25.0–50.0 µm) in most species and large only in *C. triantha* (50.0–100.0 µm), with subcircular or subtriangular amb. Furthermore, the polar area is very small in most species, being small only in *C. parvifolia*, *C. subintegerrima* and *C. triantha* (Tables 1 and 2).

3.2. Aperture

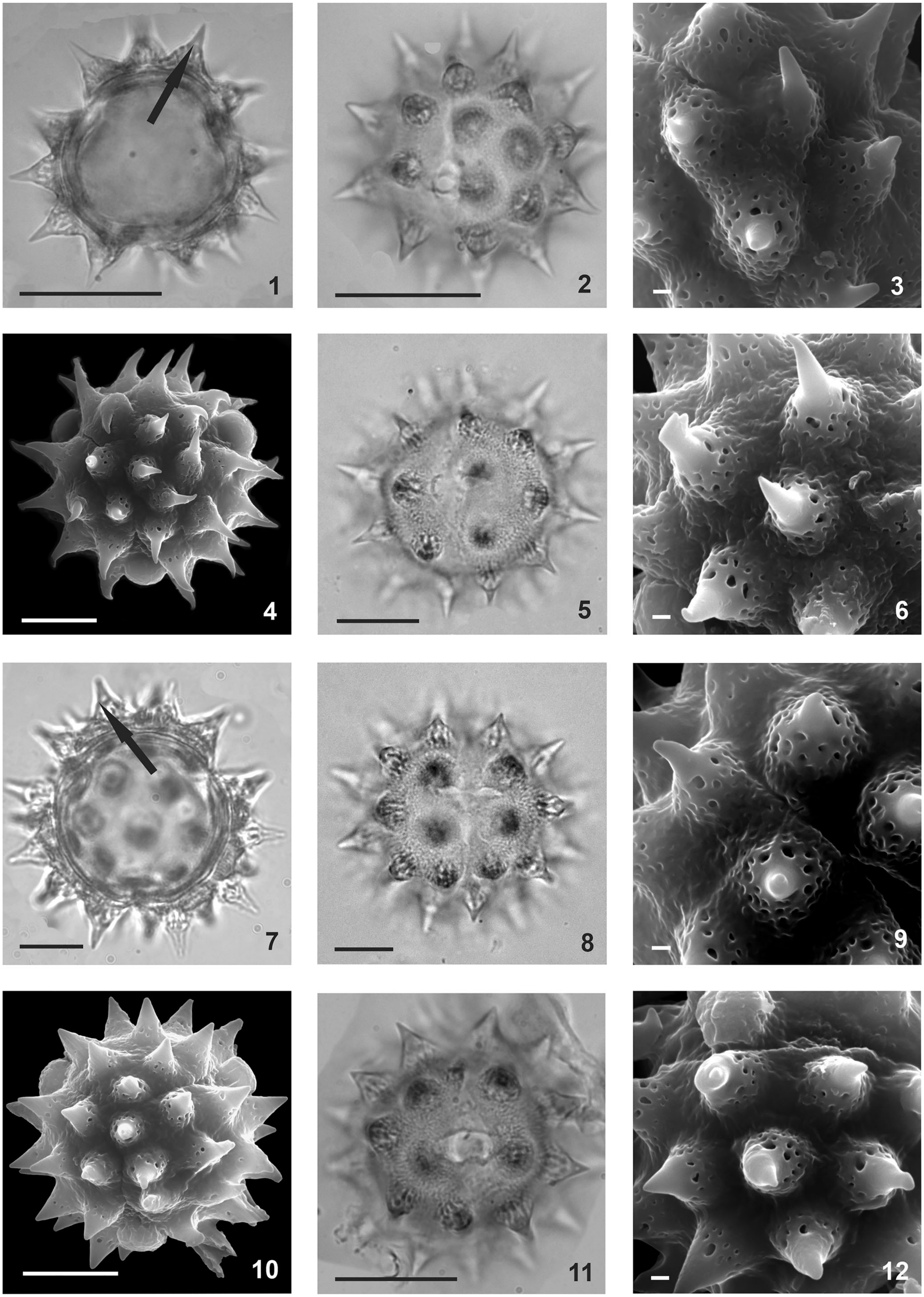
Pollen grains are tricolporate (Plate 1, figures 1, 4, 7, 10 and Plate 2, figures 1, 4, 7, 10). Membrane ornamentation is present in all species. Colpi are very long in most species and long in *C. parvifolia*, *C. subintegerrima* and *C. triantha*. Colpi with larger dimensions (ca. 33.2 µm) were found in *C. myrtifolia* and colpi with smaller dimensions (ca. 15.4 µm) were found in *C. pruskiana*. The largest colpi (ca. 7.1 µm) were recorded in *C. parvifolia* and the narrowest (ca. 1.0 µm) in *C. phyllolepis*. The endoaperture is lalongate (Plate 1, figures 2, 5, 8, 11 and Plate 2, figures 2, 5, 8, 11), tapered in most species (Plate 1, figures 8, 12 and Plate 2, figures 2, 5, 8), and very tapered in *C. triantha* (Plate 2, figure 11) (Table 3). Constrictions were observed in the median region of the endoaperture only in *C. marginata* (Plate 1, figure 5), *C. myrtifolia* (Plate 1, figure 8), *C. parvifolia* (Plate 1, figure 11), and *C. pruskiana* (Plate 2, figure 5).

3.3. Exine ornamentation and structure

The sexine is echinate, with echinae distributed in an organized way. In equatorial view, echinae are arranged in rows of three on each side of the aperture (Plate 1, figures 8, 11, and Plate 2, figure 5). In polar view, six echinae are seen in the apocolpium region (Plate 1, figures 4, 7, 10 and Plate 2, figures 1, 7). Echinae have a tapered apex, an enlarged basis (Plate 1, figures 3, 6, 9, 12 and Plate 2, figures 3, 6, 9, 12), and perforations in the median region (Plate 1, figures 1, 7 and Plate 2, figure 4) and at the basis (Plate 1, figures 3, 6, 9, 12 and Plate 2, figures 3, 6, 9, 12), with high and apparent columellae (Plate 1, figures 1, 7 and Plate 2, figures 4, 7, 11). Granules are present between the sexine and echinae (Plate 1, figures 6, 9, 12 and Plate 2, figures 3, 6, 9). Echinae are short (3.6 µm) in *C. triantha*; long (5.2–6.2 µm) in *C. funkiana*, *C. marginata*, *C. parvifolia*, *C. phyllolepis*, *C. pruskiana*, and *C. subintegerrima*; very long (ca. 8.8 µm) only in *C. myrtifolia*; narrow (2.5–3.2 µm) in *C. myrtifolia* and *C. parvifolia*; and wide (3.6–4.7 µm) in *C. funkiana*, *C. marginata*, *C. phyllolepis*, *C. pruskiana*, *C. subintegerrima*, and *C. triantha*. The echinae are close to each other (8.9–9.9 µm) in *C. phyllolepis*, *C. pruskiana*, and *C. triantha*; far apart (10.5–11.0 µm) in *C. funkiana*, *C. myrtifolia*, and *C. subintegerrima*; and very far apart (14.0–14.2 µm) in *C. marginata* and *C. parvifolia*. The sexine is always thicker than the nexine. The thickest sexine (6.9–10.9 µm) was observed in *C. myrtifolia*, *C. parvifolia*, *C. pruskiana*, and *C. subintegerrima*, followed by (5.9–6.7 µm) *C. funkiana*, *C. marginata*, and *C. phyllolepis*. The narrowest sexine was observed in *C. triantha* (ca. 4.6 µm). All species have conspicuous cavea, ranging in thickness from 0.6 to 1.0 µm (Table 3).

3.4. Artificial pollen key for Calea section Meyeria species

1. Large pollen grains, larger than 50 µm .... *C. triantha*
   1. Medium pollen grains, smaller than 50 µm
   2. Small polar area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3. Colpus ca. 19.8 μm, echinae ca. 6.2 μm long ……………… *C. parvifolia*
3. Colpus ca. 25.7 μm, echinae ca. 5.2 μm long ……………… *C. subintegerrima*
2. Very small polar area
4. Endoarpeture without a median constriction
5. Colpus ca. 16.9 μm long, endoaperture 2.7 × 11.7 μm ……………… *C. funkiana*
5. Colpus ca. 24.1 μm long, endoaperture 5.4 × 12.4 μm ……………… *C. phyllolepis*
4. Endoarpeture with a median constriction
6. 95% CI polar diameter = 39.6–41.6 μm, colpus ca. 15.4 μm long ……………… *C. pruskiana*
6. 95% CI polar diameter ≥ 42.2 μm, colpus ≥ 18.3 μm long
7. 95% CI polar diameter = 46.5–48.4 μm, colpus ca. 19 μm long, echinae ca. 6.2 μm long ……………… *C. marginata*
7. 95% CI do polar diameter = 42.2–45.0 μm, colpus ca. 33.2 μm, echinae ca. 8.8 μm long ……………… *C. myrtifolia*

4. Discussion

Analysis of palynological measurements and characters of *Calea* sect. *Meyeria* revealed that the eight species have very similar pollen grains. However, some characters allow the differentiation of the species, namely pollen size, polar area, and sexine ornamentation, in addition to endoaperture characteristics.

Bueno et al. (in press) performed a taxonomic study of the genus and investigated the sporophyte morphology of *C.* section *Meyeria*. They observed that the species (the same as those analyzed here) are similar to each other. The authors supported the existence of two morphological groups in this section, the *C. myrtifolia* group and the *C. triantha* group. Based on our results and according to the pollen key organized here, only one trait strictly agrees with such a grouping, namely echinus length smaller than 5.4 μm in species of the *C. triantha* group (vs. greater than 5.8 μm in species of the *C. myrtifolia* group). Another informative characteristic is endoaperture constriction. Species of the *C. triantha* group, except *C. pruskiana*, do not show constriction, whereas the *C. myrtifolia* group shows constriction, except *C. phyllolepis*. Species of the *C. triantha* group were treated as *C. triantha* until recently (Bueno et al. 2021, 2022).

Analysis of pollen morphology and size provides new evidence to justify the distinction among the three most recently proposed species (*C. funkiana*, *C. pruskiana*, and *C. subintegerrima*) and *C. triantha*. *Calea funkiana* differs from *C. triantha* in having colpus length less than 17 μm (vs. 22.1 μm), echinus length of 5.6–6.2 μm (vs. ca. 3.6 μm), and polar diameter 95% CI of 38.2–42.1 μm (vs. 52.1–54.6 μm). *Calea pruskiana* differs from *C. triantha* in having colpus length less than 16 μm (vs. ca. 22.1 μm), endoaperture constriction present (vs. absent), sexine thickness of 6.9–10.9 μm (vs. ca. 4.6 μm), and polar diameter 95% CI of 39.6–41.6 μm (vs. 52.1–54.6 μm). *Calea subintegerrima* differs from *C. triantha* in having echinus length of 5.6–6.2 μm (vs. ca. 3.6 μm), sexine thickness of 6.9–10.9 μm (vs. ca. 4.6 μm), and polar diameter 95% CI of 43.6–47.6 μm (vs. 52.1–54.6 μm).

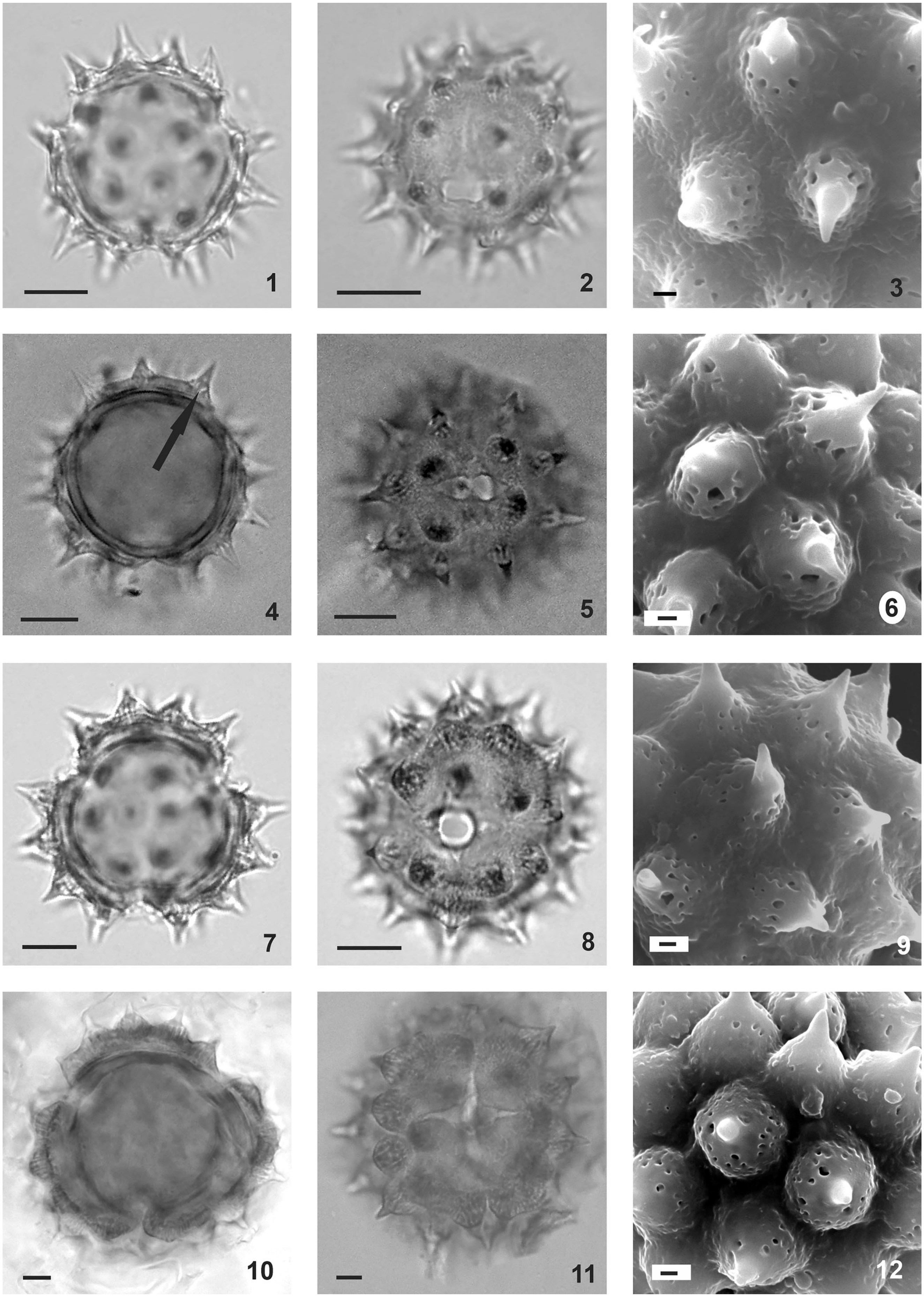
In the *C. myrtifolia* group, two pairs of species are very similar to each other. *Calea marginata* and *C. parvifolia* are commonly misidentified (Bueno et al. in press); palynologically, the species can be distinguished by analyzing colpus width (ca. 3.6 vs. ca. 7.1 μm) and equatorial diameter (95% CI of 49.0–50.9 vs. 44.7–46 μm). The other pair is *C. myrtifolia* and *C. phyllolepis*. *Calea myrtifolia* differs from *C. phyllolepis* in colpus dimensions (33.2 × 3.3 vs. 24.1 × 1 μm), endoaperture constriction present (vs. absent), echinae length ca. 8.8 μm (vs. 5.6–6.2 μm), exine thickness ca. 13.9 μm (vs. ca. 8 μm), sexine thickness ca. 2.1 μm (vs. ca. 0.8 μm), and nexine thickness ca. 2.2 μm (vs. ca. 0.7 μm).

Roubik and Moreno (1991) described the pollen grains of *Calea prunifolia* H.B.K. as isopolar, oblate-spheroidal, tricolporate, with echinate sexine, conical echinae, inconspicuous colpi, and lalongate endoaperture. Compared with species of *Calea* sect. *Meyeria*, *C. prunifolia* pollen differs only in having inconspicuous colpi (vs. conspicuous). Melhem et al. (2003) described the pollen grains of *C. pinnatifida* Less. as medium-sized, spherical, and tricolporate, with elongate endoaperture and pointed echinae. Cancelli et al. (2010) analyzed three *Calea* species (*C. clematidea* Baker, *C. kristiniae* Pruski, and *C. pinnatifida*). Cancelli et al. (2010) considered that the species of *Calea* present 12 echinae in equatorial view, a different result from that found here for the species of *Calea* sect. *Meyeria* (six echinae).

Stanski et al. (2014) analyzed *C. pinnatifida* and confirmed the characters previously mentioned in other articles (Melhem et al. 2003; Cancelli et al. 2010). Stanski et al. (2014) investigated two species that were studied here, *C. parvifolia* and *C. triantha* (= *Calea hispida* (DC.) Baker, Reis-Silva and Nakajima 2020). Stanski et al. (2014) described *C. parvifolia* as having pollen small to medium in size, prolate-spheroidal, tricolporate endoaperture with central constriction; cavea well delimited, echinae longer than wide at the base, dome-shaped, with straight apex; and sexine thicker than the nexine. In our study, similar characteristics were observed, except for pollen shape, relationship between echinus length and width, and cavea not always well delimited.

Recently, Souza-Souza et al. (2021) analyzed the pollen grains of *C. serrata*, a species whose sporophyte is very

Plate 1. Pollen grains of *Calea* (Asteraceae) species analyzed using light microscopy (LM) and scanning electron microscopy (SEM). *C. funkiana* – 1. polar view, optical section, arrow indicating perforation; 2. equatorial view, aperture; 3. surface detail. *C. marginata* – 4. polar view, general aspect; 5. equatorial view, aperture; 6. surface detail. *C. myrtifolia* – 7. polar view, optical section, arrow indicating perforation; 8. equatorial view, aperture; 9. surface detail. *C. parvifolia* – 10. polar view, general aspect; 11. equatorial view: aperture; 12. surface detail. Scale bars: 1, 2, 4, 5, 7, 8, 10, 11 = 5 μm; 3, 6, 9, 12 = 1 μm.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similar to that of *C. pinnatifida*. Here, we found that the species differ in endoaperture type (almost circular) and sculptural characteristics in the region between echinae (scabrate-perforate). Although *C. serrata* was not analyzed in the present study, it can be said that its pollen characters are similar to those of other *Calea* species.

5. Conclusion

Calea species have similar pollen characters. This is the first study to focus on *Calea*, particularly on species of *C.* sect. *Meyeria*. The results of this palynotaxonomic approach were very positive, as different characters that allow species distinction were identified, such as pollen size, polar area, sexine ornamentation, endoaperture type, and distance between echinae, even considering the phylogenetic proximity among the studied species. The results indicate that, even though species of the section are very similar, they can be differentiated through palynological analyses, which points to palynotaxonomy as a promising tool to enhance knowledge within the tribe Neurolaeneae in general and within *Calea* specifically. Here, we developed a pollen key to the section. Further studies with other genera of Neurolaeneae and other infragenera of *Calea* may allow a better understanding of the palynology of the genus, and this information could be combined in the phylogenetic reconstruction based on molecular data obtained by Bueno (2023) to gain greater insight into the evolution of pollen traits within the tribe and the genus.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Plate 2. Pollen grains of *Calea* (Asteraceae) species analyzed using light microscopy (LM) and scanning electron microscopy (SEM). *C. phyllolepis* – 1. polar view, optical section; 2. equatorial view, aperture; 3. surface detail. *C. pruskiana* – 4. polar view, optical section, arrow indicating perforation; 5. equatorial view, aperture; 6. surface detail. *C. subintegerrima* – 7. polar view, optical section; 8. equatorial view, aperture; 9. surface detail. *C. triantha* – 10. polar view, optical section; 11. equatorial view, aperture; 12. surface detail. Scale bars: 1, 2, 4, 5, 7, 8, 10, 11 = 5 µm; 3, 6, 9, 12 = 1 µm.

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Appendix

Examined material

C. funkiana V.R. Bueno & G. Heiden – Brazil, Minas Gerais, Santana do Riacho, A.P. Duarte, 8801, 31 January 1965 (ICN).

C. marginata S. F. Blak – Brazil, Paraná, R. Gonçalves, 58, 12 December 2002 (UPCB).

C. myrtifolia (DC.) Baker – Brazil, Minas Gerais, Diamantina, V.R. Bueno, J.F. Castro; S.M. Gonçalves, 108, 10 January 2020 (ICN).

C. parvifolia (DC.) Baker – Brazil, Paraná, Balsa Nova, A. Christ, M. Buchowski, C. Ribeiro, 646, 02 April 2019 (ICN).

C. phyllolepis Baker – Brazil, Rio Grande do Sul, Osório, V.R. Bueno, P.R. Bueno, 113, 01 February 2020 (ICN).

C. pruskiana V.R. Bueno & G. Heiden – Brazil, São Paulo, Campos do Jordão, M.J. Robim, J.Pm Carvalho, 530, 04 February 1988 (SPSF).

C. subintegerrima (Malme) V.R. Bueno & G. Heiden – Brazil, Santa Catarina, Rio do Alemães, O.S. Ribas et al., 2255, 14 January 1998 (MBM).

C. triantha (Vell.) Pruski – Brazil, Santa Catarina, Rio Negrinho, C. Ribeiro, M. Buchowski, A. Christ, 313. 23 December 2018 (SP).