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Pollen morphology and its relation with meiotic irregularities in ten species of *Campuloclinium* (Eupatorieae, Asteraceae)

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Abstract

Pollen grains of ten species of *Campuloclinium* (Eupatorieae, Asteraceae) are described and illustrated using light and scanning electron microscopy. The species included in this study are *C. burchelli*, *C. campuloclinioides*, *C. chlorolepis*, *C. hirsutum*, *C. irwinii*, *C. macrocephalum*, *C. megacephalum*, *C. parvulum*, *C. purpurascens* and *C. riedelli*. Pollen grains of *Campuloclinium* are typically radially symmetric, echinate, tectate, oblate-spheroidal to prolate-spheroidal (P/E ratio: 0.94–1.10). Pollen is mostly triaperturate, but tetraaperturate or biaperturate pollen also occurs in some species. The pollen diameter was highly variable ranging from 10.54 μm to 36.18 μm . The significance of the variation in type and size of pollen is discussed in relation to diverse meiotic abnormalities and possible reproductive apomictic processes within the polyploid species.

Keywords: *exine*, *2-colporate*, *3-colporate*, *4-colporate*, *polyploidy*

The genus *Campuloclinium* (Eupatorieae, Asteraceae) is derived from the taxonomic reorganisation of *Eupatorium* s.l. It comprises 15 species distributed in North and South America (Robinson 2006). Most of the species occur in various Brazilian biomes, 11 of which are endemic (Nakajima et al. 2014). *Campuloclinium macrocephalum* has the widest geographical distribution occurring in Mexico, Honduras, Guatemala, Venezuela, Colombia, Brazil, Paraguay, Uruguay and Argentina (King & Robinson 1987; McConnachie et al. 2011). In contrast, *C. parvulum* is in danger of extinction and has been included in the Red List of Brazilian plants (Nakajima et al. 2013). The importance of palynology as a subsidy to the systematics of major groups of plants is evident in the contributions of The Angiosperm Phylogeny Group II, III and IV (APG II 2003; APG III 2009; APG IV 2016). In the Asteraceae, the use of pollen features is recognised due to its importance for taxonomy hierarchical levels and, more recently, to establish the phylogenetic position of genera, subfamilies and tribes (Funk et al. 2009).

Asteraceae show some of the most complex and diverse pollen grains among the flowering plants (Skvarla & Turner 1966). The pollen is typically isopolar and radially symmetric, with compound apertures (Stix 1960). Pollen ornamentation is psilate, echinate or lophate (Wodehouse 1926, 1929). Three main patterns of exine stratification, antheroid, helianthoid, and senecioid, were established by Skvarla and Larson (1965) and Skvarla and Turner (1966). Based on the exine structure, Stix (1960) described several pollen types for the Asteraceae using light microscopy (LM) and transmission electron microscopy (TEM). Pollen grains of the tribe Eupatorieae were described as the *Eupatorium* type characterised by the presence of a large cavity between two layers of the exine (Blackmore 1984). Subsequently Skvarla et al. (1977) analysed pollen grains of other species of the tribe Eupatorieae and described these grains as the Helianthoid type. This latter type is characterised by a caveate exine with internal foramina. In recent years, studies on pollen morphology in some species of the tribe Eupatorieae

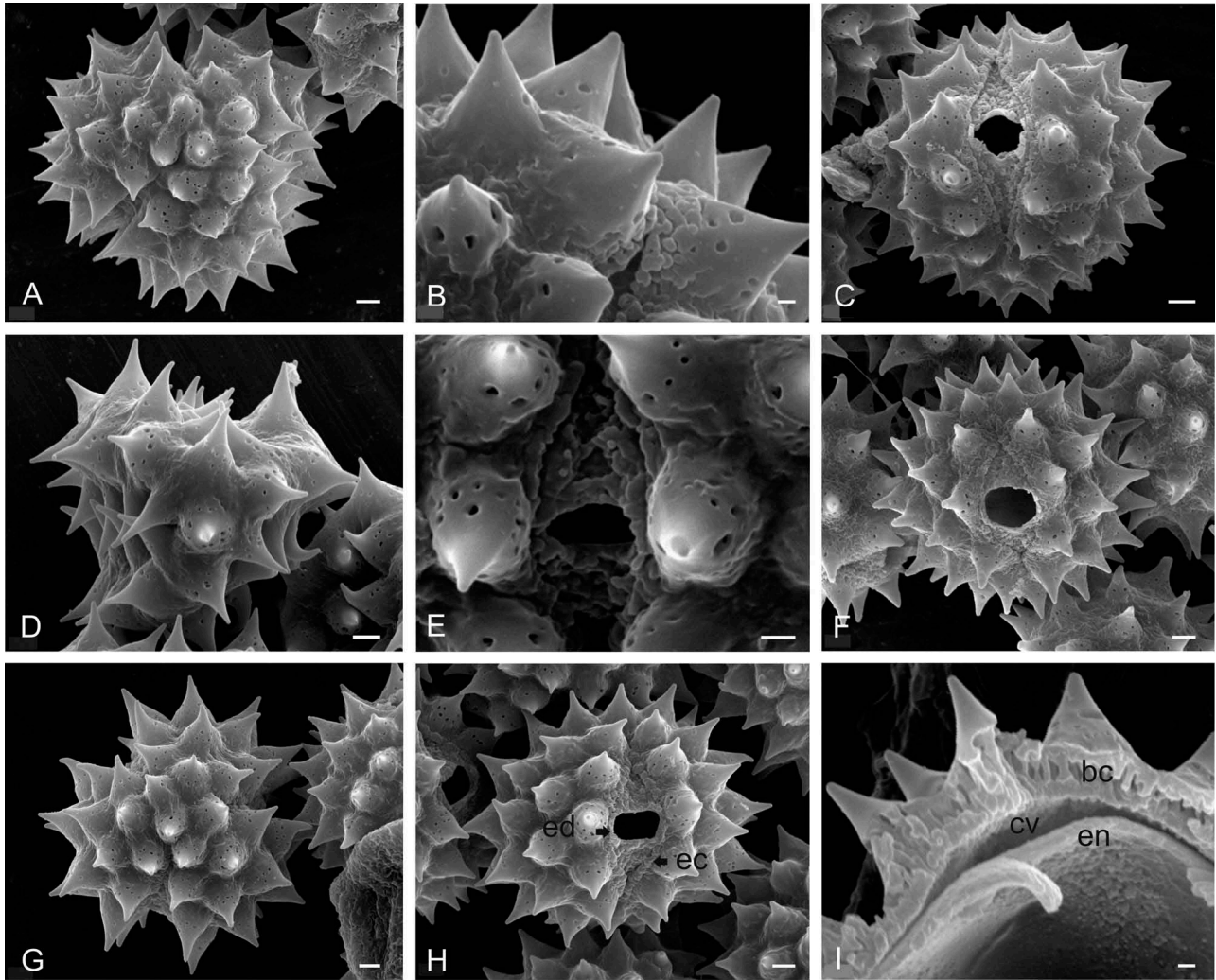


Figure 1. Pollen morphology in *Campuloclinium* (SEM micrographs). **A.** *Campuloclinium chlorolepis*, polar view, apocolpium. **B, C.** *Campuloclinium macrocephalum*, detail of the spines with perforations of different sizes on the basis (**B**), equatorial view, colpus (**C**). **D–F.** *Campuloclinium megacephalum*, polar view, spines covering the folded colpi (**D**), detail of colpore (**E**), equatorial view (**F**). **G–I.** *Campuloclinium purpurascens*, polar view, apocolpium (**G**), equatorial view, colporus (**H**), inner structure (**I**). Abbreviations: bc, basal region of columellae; cv, cavea; ec, ectoaperture; ed, endoaperture; en, endexine. Scale bars – 2 μm (A, C, D, F, G, H), 1 μm (E), 500 nm (B, I).

were performed by Salgado Labouriau (1973), Mendonça and Gonçalves- Esteves (2000), Esteves (2001), Cancelli et al. (2005, 2010) and Cancelli et al. (2007).

The evolution of the pollen grains in the Eupatorieae has been poorly studied and currently, evolutionary trends are known only for five groups of the Asteraceae: Cardueae, Arctotideae, Cichorieae, Vernonieae and Inuleae (Funk et al. 2009). Studies of pollen grains in this big group show that, in general, the Eupatorieae pollen is the most elaborate in the family. The Eupatorieae pollen is spheroidal with acute colpus ends, foramina, uniform distribution of columellae, caveate and ornamentation echinate (Funk et al. 2005). The pollen morphology of several tribes does not appear to have been studied at all in

recent years (Wortley et al. 2012). This lack of interest perhaps reflects the relatively homogenous nature of pollen morphology within the most derived subfamilies of Asteraceae (Wortley et al. 2009). For pollen of *Campuloclinium*, there are only a few morphological and ultrastructural studies and pollen wall architecture was not previously investigated using scanning electron microscopy (SEM).

Pollen features are often claimed as important in elucidating taxonomic problems. In the Asteraceae pollen morphology appears to be useful in taxonomic discussions and has resulted in certain changes at the generic and subgeneric level. In *Campuloclinium*, palynological data are only available for the species *C. macrocephalum* (Ines et al. 1981; Cancelli et al. 2010). The aim of this work is, therefore, to contri-

bute to our knowledge of this genus by performing a comparative study on pollen morphology including most *Campuloclinium* species. Furthermore, the study also investigates possible correlation between pollen morphology and the meiotic irregularities of polyploid cytotypes.

Material and methods

Pollen samples from 33 populations belonging to ten species of *Campuloclinium* were obtained from herbarium specimens of the Instituto de Botánica del Nordeste (CTES), Corrientes, Argentina, the New York Botanical Garden (NY), New York, USA, the University of Uberlândia (HUFU), Uberlândia, Brazil and the Botanical Garden of Rio de Janeiro (RB), Brazil. See the 'Specimens investigated' list for details.

Pollen material was obtained from flowers at anthesis and/or from preanthetic buds. The flower buds were processed using the acetolysis method of Erdtman (1960). Random measurements included 15–30 pollen grains for each sample. For LM, pollen grains were mounted on glass slides using glycerin jelly and were subsequently examined with an oil immersion objective (100×) of a Zeiss Axioplan light microscope. For SEM analyses, the acetolysed pollen grains were first washed in alcohol 96% and absolute alcohol, then coated with gold-palladium and examined with a JEOL 5800 LV scanning electron microscope.

The following characters were considered: exine structure (in cross-section), surface sculpture (ornamentation of the pollen surface), size, shape and apertures (also known as 'pollen classes', Faegri and Iversen, 1989). The characters were partitioned, measurements and calculated: polar axis (*P*), equatorial diameter (*E*), *P/E* ratio, exine thickness and cavus (EC), length of spine (*S*), length of colpus (LC), width of colpus (WC) and distance between spines (DS). Pollen size is measured across the diameter, from base spine to opposite base spine. Small pollen grains include those below 21 µm and large pollen grains those above 28 µm in diameter. Pollen shape is described using the ratio of polar to equatorial diameters (*P/E* ratio; Erdtman 1952). The terminology applied to describe pollen morphology is that suggested by Punt et al. (2007) and Funk et al. (2009).

Results

Shape

Pollen grains of the *Campuloclinium* species analysed here are radially symmetric and isopolar, oblate-spheroidal to prolate-spheroidal (Table I).

Surface sculpture

Mean length of the spines ranges from 2.78 µm to 5.18 µm (Table I). Spine perforations can be homogeneous or heterogeneous, circular, elliptical or amorphous and these continue around the spine base. The spines possess channels or projections formed by the layers of columellae at their base, with the width of the base of the spines being major than the area between spines (Figure 1D). The perforations are wide in the lower half of the spine and decrease in number around of it. In equatorial view, a row with four to five pairs of spines can be seen bordering the aperture. *Campuloclinium megacephalum* is unique by having folded spines that cover the aperture (Figure 1D).

Exine structure

The exine structure in *Campuloclinium* pollen grains is caveate, tectate and columellate, with a mean exine thickness from 2.56 µm to 5.32 µm (including cavea). The tectum is continuous, with numerous microperforations (lesser than 1 µm) and usually echinate microperforated in SEM. Perforations are either elliptical or circular. The infratectum is separated by a caveate region and supported by columellae (Figure 1I).

Apertures

The pollen grains of the analysed species are zonoaperturate and with compound apertures. The aperture type in ectoaperture is a colpus (mean length of colpi) ranged from 10.2 µm to 20.4 µm (Table I) and showed acute apices (Figure 1C, E). The apertural membrane is scabrous (Figure 1E, F) and the endoaperture is distinctly lalongate with (Figure 2B) or without a median constriction (Figure 2F). The grains are typically triaperturate (Figures 2A–F; 3B–C, E, F), less frequently tetraaperturate or biaperturate. In some specimens of *Campuloclinium hirsutum*, *C. irwinii*, *C. parvulum*, *C. riedelli*, *C. macrocephalum*, *C. megacephalum* and *C. purpurascens* 2-, 3- and 4-colporate grains were produced from the same plant (Tables II, III).

Size of pollen grains

The variation in size range is wide within the genus and also within species. The mean polar axis ranges from 16.32 µm to 29.92 µm, and the equatorial axis from 16.00 µm to 29.92 µm (Table I). From a total of ten species analysed, eight presented small and/or large pollen grains. These were: *Campuloclinium chlorolepis*, *C. hirsutum*, *C. irwinii*, *C. parvulum*, *C. macrocephalum*, *C. megacephalum*, *C. purpurascens* and *C. riedelli*. For

Table I. Summary of pollen grain measurements in different *Campuloclinium* species.

Species and populations	P	E	P/E	Shape	Colpus size	Endoaperture	Exine	Spine length	Spine width	Distance between spines
<i>C. burchellii</i> (25 302)	20.40(21.76)24.48	20.40(23.03)24.48	0.94	O-S	13.60-17.00 × 3.40-4.76	2.04(2.98)3.40 × 3.40(4.08)4.76	2.72(2.89)4.08	4.08(4.33)5.44	2.72(3.57)4.08	4.08(5.01)5.44
<i>C. burchellii</i> (12 418)	20.40(22.98)24.48	21.76(23.36)25.84	0.98	O-S	13.60-17.00 × 3.40-6.12	2.04(3.17)4.08 × 3.40(4.82)6.12	2.04(2.69)2.72	3.40(3.85)4.76	2.72(3.58)4.08	3.40(4.44)5.44
<i>C. campuloclinoides</i> (22 823)	17.68(19.17)20.40	17.68(19.44)20.40	0.99	O-S	12.24-13.60 × 4.08-5.44	2.72(3.94)4.76 × 4.08(4.76)5.44	2.04(2.58)2.72	2.72(2.78)3.40	3.40(3.46)4.08	3.40(4.08)4.76
<i>C. campuloclinoides</i> (374)	17.00(18.15)19.04	16.32(17.81)19.04	1.01	P-S	10.20-13.60 × 4.08-5.44	2.72(3.46)4.08 × 4.08(4.89)5.44	2.72	2.72	3.40	3.40(4.01)4.76
<i>C. campuloclinoides</i> (21 871)	16.32(17.81)19.04	17.68(18.70)20.40	0.95	O-S	10.88-13.60 × 4.08-5.44	2.72(3.67)4.08 × 3.40(4.21)5.44	2.72	2.72(2.78)3.40	2.72(3.06)3.40	3.40(3.87)4.76
<i>C. chlorolepis</i> (49)	24.48(25.59)25.84	24.48(26.33)28.56	0.97	O-S	13.60-19.04 × 4.08-5.44	2.72(4.20)5.44 × 4.08(5.25)4.55	2.72(5.12)6.80	2.72(3.53)5.44	2.72(4.14)5.44	4.08(5.91)6.80
<i>C. hirsutum</i> *3 colp. (60)	-	23.80(27.33)29.24	-	-	-	-	2.72(4.12)5.44	2.72(3.46)4.08	2.72(3.67)4.08	4.08(5.00)5.44
<i>C. hirsutum</i> *4 colp. (60)	24.48(26.28)29.24	21.76(25.16)27.20	1.04	P-S	10.88-16.32 × 3.40-6.80	2.72(4.18)5.44 × 3.40(5.61)6.80	4.08(4.87)6.12	2.72(3.40)4.08	2.72(3.85)4.76	4.76(5.21)5.44
<i>C. hirsutum</i> (7227)	21.76(25.59)27.20	21.76(23.67)28.56	1.08	P-S	10.88-16.32 × 3.40-5.44	2.04(3.06)4.08 × 3.40(4.42)5.44	2.04(3.03)4.08	2.72(3.91)4.76	2.72(3.71)5.44	4.76(5.80)6.80
<i>C. hirsutum</i> (69)	20.40(23.80)27.20	21.76(23.32)25.84	1.02	P-S	13.60-16.32 × 3.40-5.44	2.72(3.21)4.08 × 3.40(4.26)5.44	2.72(3.40)4.08	4.08(4.31)5.44	3.40(4.21)4.76	4.76(5.78)6.80
<i>C. irevini</i> *3 colp. (8543)	21.76(24.18)27.20	22.44(23.91)25.84	1.01	P-S	12.24-16.32 × 4.76-6.80	4.08(4.66)6.13 × 4.76(5.53)6.80	4.08(5.32)6.80	2.72(3.51)4.08	3.40(3.74)4.08	4.76(4.76)5.44
<i>C. irevini</i> *4 colp. (8543)	-	21.76(24.02)25.84	-	P-S	-	-	3.40(3.85)4.76	3.40(3.96)4.08	3.40(3.51)4.08	4.76(4.87)5.44
<i>C. irevini</i> (27695)	21.76(24.90)27.20	21.76(24.39)27.20	1.02	P-S	12.24-15.13 × 5.44-6.80	4.08(4.84)5.44 × 5.44(5.78)6.80	3.40(4.76)6.12	3.40(3.74)4.08	3.40(3.74)4.08	4.76(5.18)6.12
<i>C. irevini</i> *3 colp. (28 326)	24.48(26.38)27.20	25.84(26.92)28.56	0.97	O-S	14.96-16.32 × 5.44-6.80	4.08(4.42)4.76 × 5.44(5.71)6.80	3.40(5.03)6.80	3.40(3.80)4.08	3.40(3.80)4.08	4.76(5.30)5.44
<i>C. irevini</i> *4 colp. (28 326)	-	27.20(28.22)29.92	-	-	-	-	2.72(3.23)4.08	2.72(4.08)3.74	2.72(3.74)4.08	4.76(5.27)5.44
<i>C. macrocephalum</i> (541)	21.76(23.39)25.84	23.12(25.19)27.20	0.93	O-S	10.88-16.32 × 4.08-6.80	1.36(2.60)3.40 × 4.08(5.73)6.8	2.72(3.33)4.08	2.72(2.82)4.08	2.72(3.11)4.76	4.76(5.18)6.12
<i>C. macrocephalum</i> (4127)	23.12(25.16)27.20	25.84(25.79)27.20	0.98	O-S	10.88-16.32 × 4.08-5.44	3.40(4.42)5.44 × 4.08(4.76)5.44	2.72(3.15)4.08	4.08(4.42)5.44	2.72(3.33)4.08	4.76(6.05)6.80
<i>C. macrocephalum</i> (31)	23.12(27.84)29.92	23.12(28.20)35.36	0.99	O-S	13.60-17.68 × 4.08-5.44	2.04(3.57)4.08 × 4.08(5.13)5.44	3.40(4.65)6.80	2.72(2.96)3.40	4.08(4.35)5.44	5.44(5.59)6.80
<i>C. macrocephalum</i> (290)	19.04(21.20)24.48	20.40(22.29)24.48	0.95	O-S	12.24-16.32 × 2.72-4.76	1.36(2.68)4.76 × 2.72(3.59)4.76	2.72	2.04(2.86)3.40	2.72(3.06)4.08	2.72(4.37)5.44
<i>C. megacephalum</i> (194)	23.12(25.61)29.92	20.40(27.25)32.64	0.94	O-S	13.60-14.96 × 5.44-2.72	2.72(3.17)4.08 × 2.72(3.96)5.44	2.72(4.39)5.44	4.08(5.18)5.44	5.44(6.26)6.80	5.44(6.54)6.80
<i>C. megacephalum</i> (59 955)	21.76(24.11)25.84	23.12(24.96)32.64	0.97	O-S	10.88-16.32 × 2.72-5.44	2.04(2.72)3.40 × 2.72(4.17)5.44	3.20(4.64)6.40	2.72(4.02)4.08	2.72(4.32)5.44	4.08(5.82)6.08
<i>C. megacephalum</i> (35 372)	-	28.56(41.18)47.60	-	-	-	-	5.44(6.31)6.80	2.72(3.34)4.08	4.08(5.98)6.80	8.16(12.83)16.32
<i>C. parvulum</i> (805)	20.40(23.73)25.84	16.00(24.27)27.20	0.97	O-S	13.60-16.32 × 4.08-6.12	4.08(4.51)5.44 × 4.08(5.12)6.12	3.40(4.19)5.44	3.40(4.30)5.44	4.08(5.10)6.12	4.76(6.09)6.80
<i>C. parvulum</i> (810)	17.68(19.98)21.76	19.04(20.13)24.48	0.99	O-S	10.88-14.96 × 3.40-5.44	2.73(3.89)5.44 × 3.40(4.23)5.44	2.04(3.02)4.08	2.72(2.94)4.08	3.40(3.58)4.08	3.40(4.57)5.44
<i>C. parvulum</i> *3 colp. (843)	24.48(26.81)28.56	23.12(26.81)29.92	1.00	O-S	10.88-16.32 × 4.08-5.44	4.08(4.37)4.76 × 4.08(4.95)5.44	2.72(3.59)4.08	2.72(3.49)4.08	3.4(3.98)4.08	4.08(5.14)5.44
<i>C. parvulum</i> *4 colp. (843)	-	25.84(27.71)28.56	-	-	-	-	2.72(3.82)5.44	2.72(3.65)4.08	3.40(4.16)5.44	4.08(4.93)5.44
<i>C. parvulum</i> (3850)	17.68(20.06)23.12	19.04(21.21)24.48	0.94	O-S	10.20-11.56 × 2.72-5.44	2.72(3.51)5.44 × 2.72(3.96)5.44	2.72(4.08)5.44	2.72(3.21)4.08	3.40(4.08)5.44	4.76(5.48)6.80
<i>C. parvulum</i> (3341)	20.40(22.44)27.20	20.40(21.64)23.12	1.03	P-S	10.88-16.32 × 2.72-6.12	2.72(3.00)4.08 × 2.72(4.08)6.12	2.72(3.68)4.08	2.72(3.40)4.08	4.08(4.25)4.76	5.44(6.12)6.80
<i>C. parvulum</i> (1004)	25.84(26.52)27.20	23.12(25.75)27.20	1.02	P-S	17.00-17.68 × 3.40-4.02	3.40(3.74)4.08 × 3.40(3.74)4.08	4.08(4.33)5.44	2.72(3.82)4.08	3.40(4.33)4.76	4.08(5.18)5.44
<i>C. purpurascens</i> (60 629)	21.76(24.36)27.20	21.76(24.32)28.56	1.00	O-S	13.60-17.68 × 4.08-6.8	4.08(4.96)5.44 × 4.08(4.94)6.80	2.72(3.60)4.08	1.36(2.08)2.72	2.04(2.74)3.40	2.72(3.76)5.44
<i>C. purpurascens</i> (6 559)	21.76(23.89)25.84	21.76(24.54)27.20	0.97	O-S	12.24-16.32 × 2.72-5.44	2.72(3.78)4.76 × 2.72(4.71)5.44	2.72(3.15)4.08	3.40(4.08)5.44	2.72(3.55)4.08	4.08(5.87)6.80
<i>C. purpurascens</i> (36 478)	21.76(22.00)24.48	20.40(21.94)24.48	1.00	O-S	10.88-14.96 × 4.08-5.44	2.04(3.60)5.44 × 4.08(5.10)5.44	2.04(2.60)2.72	3.40(4.03)4.76	2.04(2.90)4.08	3.40(4.60)5.44
<i>C. purpurascens</i> (11 200)	19.72(21.62)23.12	17.68(20.49)23.12	0.95	O-S	10.88-20.40 × 2.72-5.44	12.24(15.95)20.40 × 2.72(4.36)5.44	2.04(2.56)2.72	3.40(3.92)4.08	3.40(4.08)5.44	2.72(4.73)6.12
<i>C. riedeltii</i> (10 574)	23.12(23.80)24.48	21.76(23.80)25.84	1.00	O-S	14.96-16.32 × 4.08-5.44	3.40(3.91)4.08 × 4.08(4.93)5.44	2.72(3.23)4.08	2.72(2.89)3.40	3.40(3.57)4.08	3.40(3.91)4.08
<i>C. riedeltii</i> (4562)	17.68(18.49)19.72	16.32(18.63)20.40	0.99	O-S	10.88-13.60 × 4.08-5.44	2.72(4.28)5.44 × 4.08(4.82)5.44	2.72(2.79)3.40	2.72(2.99)4.76	2.72(3.19)3.40	3.40(4.21)4.76
<i>C. riedeltii</i> *3 colp. (25 217)	21.76(23.25)24.48	20.40(21.08)21.76	1.10	P-S	12.24-14.96 × 4.76-5.44	2.72(3.94)4.76 × 4.76(5.30)5.44	2.72(3.06)3.4	2.04	2.72(3.06)3.40	3.40(4.08)4.76
<i>C. riedeltii</i> *4 colp. (25 217)	20.40(23.12)25.84	21.76(23.29)24.48	-	-	-	-	2.72	2.72	3.40(3.57)4.08	3.40(4.08)4.76
<i>C. riedeltii</i> *3 colp. (10 404)	23.12(25.16)27.20	20.40(20.85)21.76	1.10	P-S	12.24-13.60 × 4.76-5.44	2.72(4.08)4.76 × 4.76(5.21)5.44	2.72	2.72(2.94)3.40	2.72(2.94)3.40	3.40(3.62)4.08
<i>C. riedeltii</i> *4 colp. (10 404)	23.12(27.84)29.92	21.76(23.80)24.48	-	-	-	-	2.72	2.04(2.55)2.72	2.72(3.06)3.40	4.08(4.25)4.76

Note: P, polar axis; E, equatorial diameter; P/E, P/E ratio; O-P, oblate/spheroidal; P-S, prolate/spheroidal; *3-colp. and *4-colp., measurements of pollen grains tricolporates and tetralporates, respectively.

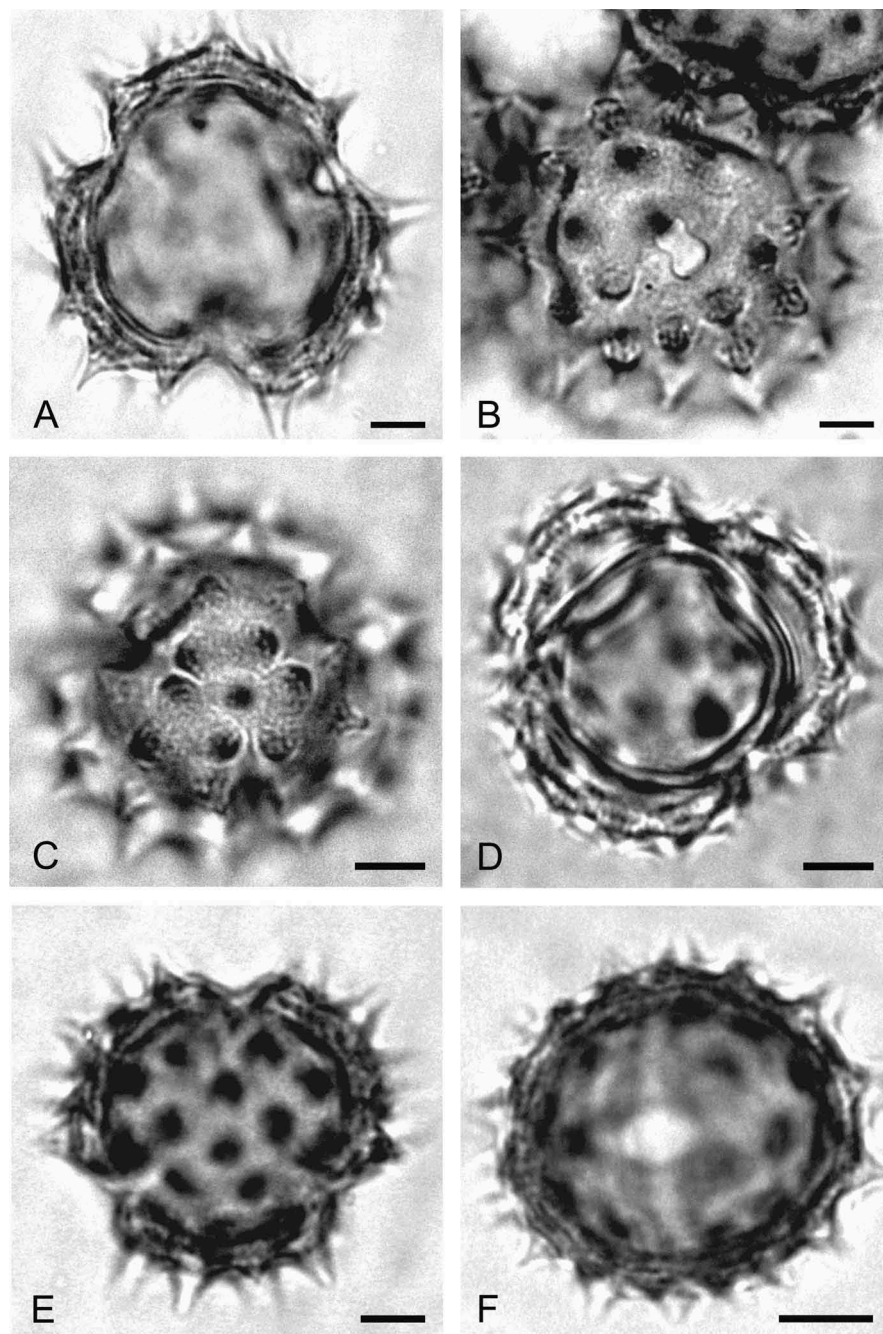


Figure 2. Pollen grains of *Campuloclinium* (LM). **A, B.** *Campuloclinium burchellii* (12418), cross-section (**A**), equatorial view (**B**). **C, D.** *Campuloclinium chlorolepis* (49), polar view (**C**), cross-section, cavea (**D**). **E, F.** *Campuloclinium campuloclinioides* (21871), polar view (**E**), equatorial view (**F**). Scale bars – 5 µm.

example, in *C. irwinii* pollen grains range in diameter from 10.88 µm to 34.00 µm ($n = 28\ 326$), in *C. macrocephalum* from 13.60 µm to 30.94 µm ($n = 3646$) and in *C. parvulum* from 13.05 µm to 31.82 µm ($n = 843$) (Figures 3D, 4B, Table II).

Discussion

Pollen morphology is often relatively constant within a particular plant species (Bravo-Hollis & Sánchez-Mejorada 1978; Pire et al. 2004). Our study shows

that the pollen of *Campuloclinium* display typical characters of the Asteraceae, which is typically tri-zono-colporate with a columellate exine. However, observations using LM and SEM also revealed features such as size, shape, apertures, exine structure and surface sculpture significant to understanding the morphology of Eupatorieae.

All *Campuloclinium* pollen studied here have caveate exine. Further the *Campuloclinium* pollen have a columellate layer similar to other Eupatorieae such as *Fleischmannia incarnata* (Walter) R.M. King et H.Rob. and

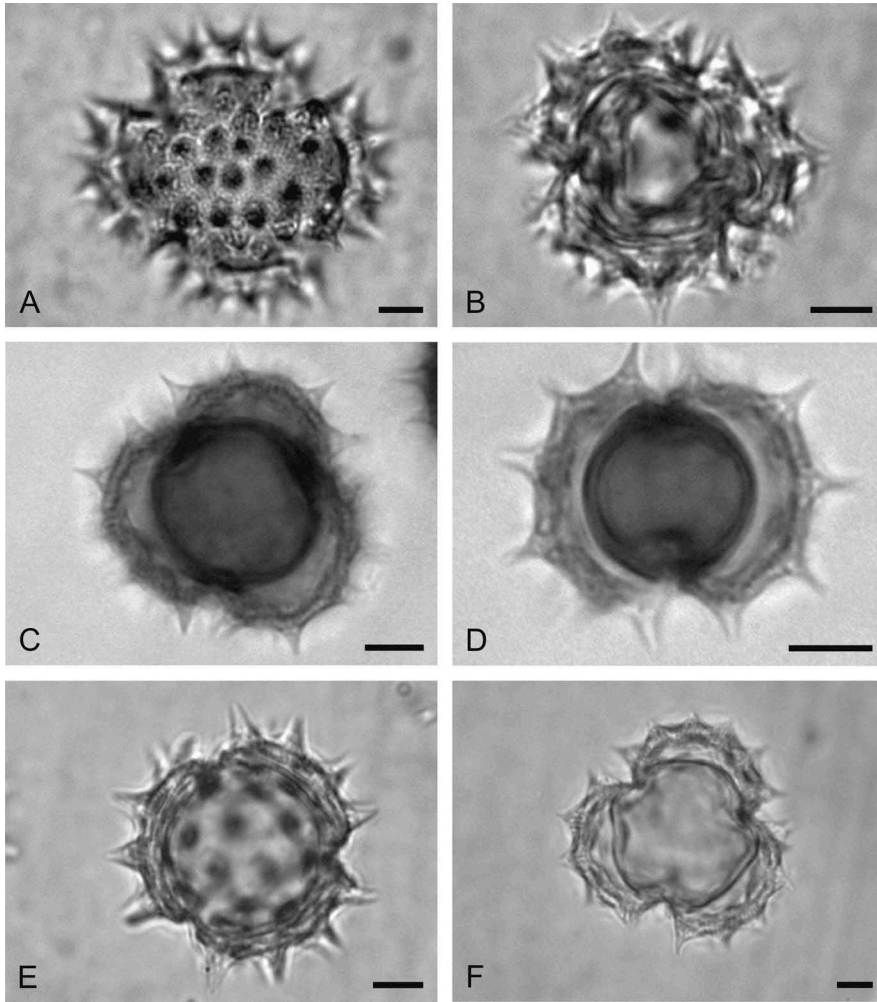


Figure 3. Pollen grains of *Campuloclinium* (LM). **A, B.** *Campuloclinium hirsutum* (60), equatorial view of a tetracolporate pollen grain (**A**), polar view of a small tricolporate pollen grain. **C, D.** *Campuloclinium irwinii* (8543), cross-section of a tricolporate pollen grain (**C**), bicolporate pollen grain (**D**). **E.** *Campuloclinium macrocephalum* (4127), polar view. **F.** *Campuloclinium macrocephalum* (31), cross-section, cavea. Scale bars – 5 µm.

Carphephorus bellidifolius (Michx.) Torr. et A.Gray (Blackmore et al. 2010). The spines are characterised by a basal part with channels and a solid apical part. Similar structures are observed in pollen of *Graphistylis* (Senecioneae; Souza et al. 2016) and may correspond to the helianthoid pollen type of Skvarla et al. (1977), which is common throughout the Asteroideae including the Astereae and Gnaphalieae. However, TEM studies would be required to determine whether the exine of *Campuloclinium* has internal foramina as would be expected of the helianthoid pollen type that is otherwise common in Eupatorieae (Funk et al. 2009). This pollen type is present in some species of tribe Astereae such as *Aster alpinus* L., *A. amellus* L., *A. tripolium* L., *Bellis perennis* L., *Dittrichia graveolens* (L.) Greuter, *Solidago graminifolia* (L.) Salibs., among other species (Punt & Hoen 2009; Wortley et al. 2012).

However, pollen grains of *Campuloclinium* are similar externally to many other Asteraceae subfamilies, such as Athroismeae, Cichorioideae, Barnadesioideae and Gnaphalieae (in genera studied such as

Castroviejoa and *Rhetinocarpha*; Funk et al. 2005; Wortley et al. 2012).

Pollen size in *Campuloclinium burchelli*, *C. campuloclinioides*, *C. chlorolepis*, *C. hirsutum*, *C. irwinii*, *C. macrocephalum*, *C. megacephalum*, *C. parvulum*, *C. purpurascens* and *C. riedelii* is heterogeneous. Specimens collected from different localities show variations in mean *P*, *E*, and minimal and maximal measurements. The Eupatorieae has pollen grains between 16 µm and 27 µm in diameter (Torres 2000). However, King and Robinson (1987) described pollen grains ranging from 21 µm to 25 µm in diameter for *Campuloclinium*. In this study, the regular equatorial diameter varied between 16.32 µm in *C. campuloclinioides* to 47.60 µm in *C. megacephalum*. Similar variations in pollen size were observed in several genera of Asteraceae such as *Eupatorium chinense* var. *simplicifolium* (Makino) Kitam. (Watanabe et al. 1982), *Scorzonera* (Türkmen et al. 2010), *Stevia rebaudiana* (Bertoni) Bertoni (Oliveira et al. 2004), some species of *Achillea* sect. *Achillea* (Akyaçın et al. 2011), *Lessin-*

Table II. Presence and absence of small and large pollen grains and aperture number in *Campuloclinium*.

Species	Populations	Small pollen grains	Large pollen grains	Aperture numbers
		Mean (μm)	Mean (μm)	
<i>C. burchellii</i>	25 302	—	—	3
	12 418	—	—	3
<i>C. campuloclinioides</i>	22 823	—	—	3
	374	—	—	3
	21 871	—	—	3
<i>C. chlorolepis</i>	49	15.14	—	2, 3
<i>C. hirsutum</i>	60	13.15	—	2, 3, 4
	7227	16.77	—	3
<i>C. irwini</i>	69	15.64	34.45	3
	8543	13.15	31.11	2, 3, 4
	27 695	11.39	31.96	2, 3
<i>C. macrocephalum</i>	28 326	10.88	34.00	2, 3, 4
	541	—	—	3, 4
	4127	—	—	2, 3
	31	—	—	3, 4
<i>C. megacephalum</i>	3646	13.60	30.94	3, 4
	194	16.32	—	3
	59 955	—	—	3
<i>C. parvulum</i>	35 372	—	—	3
	805	—	—	3
	810	—	—	3
	843	13.05	31.82	2, 3, 4
	3850	—	—	3
<i>C. purpurascens</i>	3341	—	—	3
	1004	13.71	28.56	3, 4
	60 629	13.60	31.28	3
	6559	14.05	32.25	2, 3
<i>C. riedelii</i>	36 478	—	—	3
	11 200	19.04	36.18	3
	10 574	—	—	3
	4562	—	—	3
	25 217	10.54	—	3, 4
	10 404	13.11	—	3, 4

giantus (Dematteis & Pire 2008; Angulo & Dematteis 2010). Also this was seen in other plant groups such as Cactaceae (*Pachycereus weberi* (J.M. Coult.) Backeb.; Aguilar-García et al. 2012).

Campuloclinium macrocephalum is the first polyploid species in the genus that was studied in detail in mitosis, megagametogenesis and microsporogenesis (Dematteis et al. 2007; Farco et al. 2012). Also it presents mixed populations in their natural state and its polyploid individuals have cytomixis, B-chromosomes, apomixis, meiotic irregularities and low pollen fertility (Farco & Dematteis 2014). In microsporogenesis, numerous micronuclei found in polyploid individuals are a result of abnormal microspores due to unbalanced chromosome segregation (Farco et al. 2012) leading to subsequent production of the heterogeneous pollen grains observed here. Previous studies have shown abnormal meiotic behaviour and its correlation with pollen grains; for example, in *Inula cuspidata* C.B. Clarke (Asteraceae, Inuleae), an accession forms a

large number of malformed pollen grains (Kaur et al. 2010). In other families such as Fabaceae (e.g. *Lathyrus*) and Begoniaceae (e.g. *Begonia*), differences in grain size are linked to irregularities producing unbalanced gametes during meiosis (Dewitte et al. 2009; Chalup et al. 2012). Also, in a study carried out by Noyes and Allison (2005), some mixed populations with unreduced grains in low frequencies were found in two plants of *Erigeron strigosus* Muhl. ex. Willd. (Asteraceae, Astereae) that include polyploid apomicts. The triploid individuals of *C. macrocephalum* has asexual reproduction or apomixis (Farco et al. 2012), and this could affect pollen morphology, such as in *Taraxacum* (Asteraceae, Cichorieae) (Blackmore 1976) and *Hesperomannia* (Asteraceae, Vernoniaeae; Marticorena & Parra 1975). Based on all this antecedents, we suggest that the small pollen grains observed in this study could be the resulted of the meiotic irregularities in polyploid individuals within genus *Campuloclinium*.

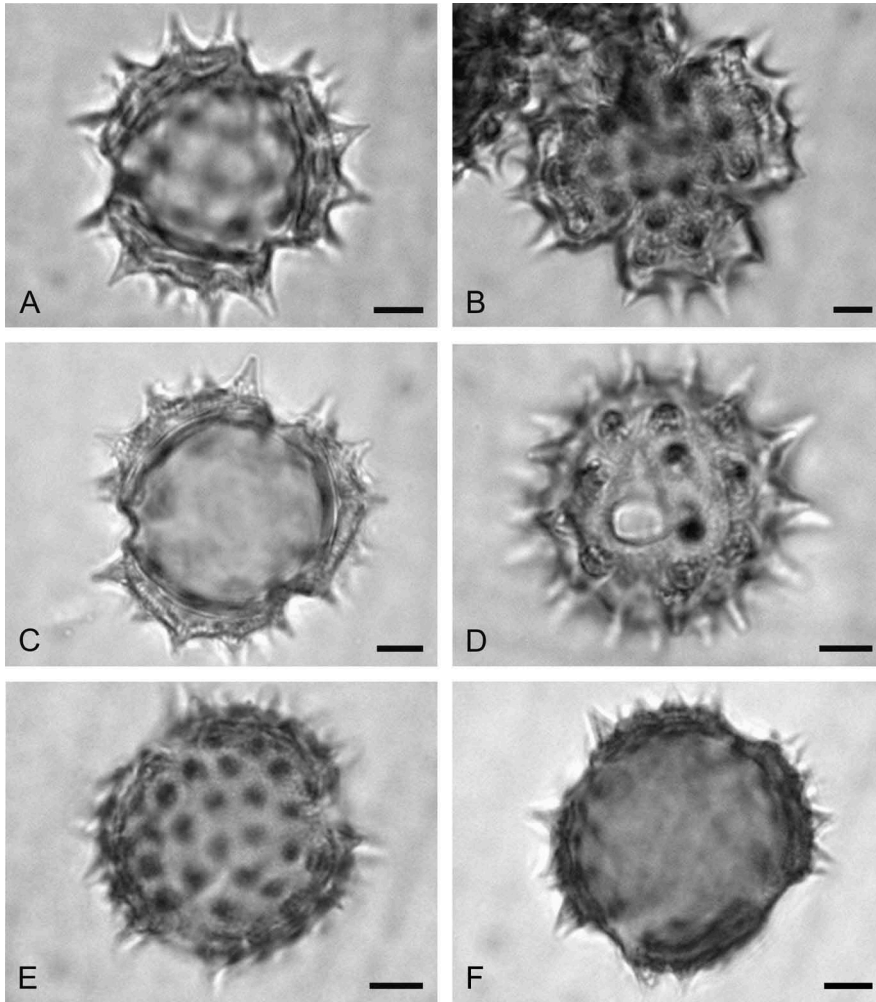


Figure 4. Pollen grains of *Campuloclinium* (LM). **A.** *Campuloclinium megacephalum* (59.955), cross-section, cavea. **B.** *Campuloclinium parvulum* (843), polar view of tetralcolporate pollen grain. **C, D.** *Campuloclinium purpurascens* (6.559), cross-section of tricolporate pollen grain (C), equatorial view, colporo (D). **E, F.** *Campuloclinium riedelii* (10404), polar view of tricolporate pollen grain (E), tetralcolporate pollen grain (F). Scale bars – 5 µm.

King and Robinson (1967) described multiple pollen forms that probably reflect different cytological races in two species of the genus *Stevia* (Asteraceae, Eupatorieae). *Campuloclinium* was reported as 3-colporate in previous studies of the genus (King & Robinson 1987) and we here report for the first time 2-colporate (micro-pollen grains) and 4-colporate pollen grains. Similar aperture numbers have been observed in other genera such as *Eremothamnus*, *Hoplophyllum* (Asteraceae), *Centaurium pulchellum* (Sw.) Druce (Gentianaceae), *Matricaria matricarioides* (Less.) Porter ex Britton (Asteraceae) and *Tarhonanthus* (Asteraceae), which has 3- and 4-colporate pollen grains (Wortley et al. 2007; Via do Pico & Dematteis 2010; Çeter et al. 2013). However, there is no information about 2-colporate pollen grains (small pollen grain) for any of these species. Studies on the aperture numbers in *Nicotiana tabacum* L. (Solanaceae) were attributed to the DNA strands that were exchanged through communication channels within microspores in the tetrad form (Ressayre et al. 2003). However, Robinson and

Skvarla (2014) found different number of apertures in *Oocephala* and *Polydora* (Vernonieae) attributing the position in the tetrads or mother cell.

Skvarla et al. (2003) observed small pollen grains in different species of *Eupatorium*, but attributed their presence to ‘incompatibilities’ in the development of pollen grains. Viral infection might be a possible factor for abnormal pollen grains in *Ageratum houstonianum* Mill. (Asteraceae, Eupatorieae). This would cause the formation of 4-colporate grains, collapsed grains, exine ruptures, irregular colpus development, fused grains, and grains with ‘satellites’ (or small pollen grains). However, these aberrations also could be caused by hybridisation, polyploidy and/or meiotic irregularities (Morzenti 1962; Wells 1971; Chatuverdi et al. 1990; Brochman 1992; Via Do Pico & Dematteis 2010).

Some authors attributed the pollen heteromorphism to variations in pollen size and aperture type (Nair & Kaul 1965; Inceoglu 1973; Till-Bottraud et al. 1995). In species of *Viola* (Violaceae) and *Petunia hybrida* E. Vilm. (Solanaceae), the different pollen forms are not

Table III. Pollen grains percentages of 2-, 3- and 4-colporate in three species of *Campuloclinium* with different apertures.

Species	Population	2-Colporate	3-Colporate	4-Colporate
<i>C. hirsutum</i>	60	8.60	16.12	75.28
<i>C. irwinii</i>	8543	21.84	23.52	54.62
<i>C. irwinii</i>	28 326	9.16	39.69	51.14
<i>C. parvulum</i>	843	31.53	44.14	24.32
<i>C. riedelli</i>	25 217	0	25.50	74.50
<i>C. riedelli</i>	10 404	0	59.16	40.86

due to the production of reduced and unreduced pollen grains (Dajoz et al. 1995). Later studies in *Viola* show that the occurrence of pollen heteromorphism is correlated with sporophytic polyploidy (Nadot et al. 2000).

Some authors suggested that an evolutionary increase in aperture number in angiosperms since the Mesozoic (Walker & Doyle 1975; Van Campo 1976), indicates a possible correlation with pollen fitness. There is even a developmental basis for the asymmetry in the distribution of the pores, more obvious in *Polydora* and less evident in the more specialised *Oocephala*. Thus, until now, the distribution of the pores is influenced by early stages in pollen development, beginning with position in the tetrads or mother cell (Robinson & Skvarla 2014).

Conclusion

In this work, we confirmed the presence of radially symmetric, echinate, tectate, oblate-spheroidal to prolate-spheroidal pollen grains in ten *Campuloclinium* species examined. However, TEM studies would be required to determine whether the exine has internal foramina to be included into the helianthoid pollen type. Our study shows that *Campuloclinium* produces small and large pollen grains and mostly triaperturate, but tetraaperturate or biaperturate pollen also occurs in some species. We demonstrated that all examined *Campuloclinium* species showed considerable variation, both at the interspecific and the intraspecific level. Until now, meiotic irregularities could be related with the development of pollen grains, similar as seen in previous studies of *C. macrocephalum*. However, factors such as hybridisation, meiotic irregularities, apomixis or occurrence of B-chromosomes in the genus could be related to the observed variations here, but cytogenetic studies are needed to confirm this hypothesis.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Specimens investigated

- Campuloclinium burchellii* (Baker) R.M.King et H.Rob. (25 302): Argentina. Corrientes. Depto. Santo Tomé. Loc. Route 40, 18 km northeast from Santo Tomé. 13-IV-1974. A. Krapovickas et al. 25.302. (CTES).
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- Campuloclinium campuloclinioides* (374): Brasil. Minas Gerais. Parque Nacional Grande Sertao Veredas. 15° 13' 01" S, 45° 50' 15" W. 01-V-1999. R. Rodriguez da Silva, T. S. Filgueiras and F. C. A. Oliveira 374. (RB).
- Campuloclinium campuloclinioides* (21 871): Brasil. Minas Gerais. Diamantina, 27 km from Diamantina around Gouveia. Height: 1300 m. 13-I-1969. H.S. Irwin, R. Ries dos Santos, R. Souza and S. F. da Fonseca 21871. (RB).
- Campuloclinium chlorolepis* (Baker) R.M.King et H.Rob. (49): Brasil. Goiás. Goiania. Serra Dourada. Reserva Biológica Prof. José Angelo Rizzo. 16° 03' 59.9" S, 50° 10' 47.4" W. Height: 987 m. 27-I-2012. G. E. Farco, et al. 49. (CTES).
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- Campuloclinium hirsutum* (60 629): Brasil. Rio Grande do Sul. Loc. Rod. RS-110, proximate to Tainhas (mun. Sao Francisco de Paula). 17-IV-1994. G. Hatschbach and E. Barbosa 60.629. (CTES).
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- Campuloclinium irwinii* (27 695): Brasil. Minas Gerais. Sierra del Espinazo. 18 km east of Diamantina. 900 msnm. 16-III-1970. H. S. Irwin, S. F. Fonseca, R. Souza, R. Reis dos Santos, J. Ramos 27695. (NY).
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- Campuloclinium macrocephalum* (31): Argentina. Corrientes. Depto. Berón de Astrada. 8.8 km north of Berón de Astrada over route 12° 28' 19" S, 57° 31' 06" W. Height: 60 m. 06-XII-2010. G. E. Farco 31. (CTES).
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- Campuloclinium megacephalum* (194): Brasil. UB Gama. Parque Recreativo e Reserva Ecológica do Gama. Center-Western, 16° 03' S, 48° 03' W. Height: 850 m. 12-I-2001 B. M. Gomes et al. 194. (CTES).
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