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Palynotaxonomy of *Centauropsis* (Compositae, Vernonieae) an endemic genus from Madagascar

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Abstract
Centauropsis is a genus of eight species in the family Compositae, all of which are endemic to Madagascar. There is almost no information about the pollen of this genus, with only one species having its pollen described to date, which hinders systematic studies involving this genus and closely related taxa. In this study, we comprehensively characterise the pollen of *Centauropsis*, with details on morphology and ultrasculpture for six of the eight species of the genus. The pollen of *Centauropsis* is here characterised as 3-colporate, with sublophate ornamentation and nanoreticulate sexine. The species differ from each other mostly in length of the axis, morphology of the colporus endoaperture, and spine shape and size. The correlation between palynological characters and their variation within and between species was explored using principal component analyses (PCA) and cluster analyses (unweighted pair group method with arithmetic mean [UPGMA] and Euclidean distance). Full palynological descriptions, measurements and scanning electron microscopy (SEM) and light microscopy (LM) images are provided for all examined species.

Keywords: *Africa, Asteraceae, paleotropics, palynology, pollen morphology, ultrasculpture*

Centauropsis Bojer ex DC. is a genus belonging to the tribe Vernonieae in the sunflower family, Compositae (or Asteraceae), currently comprising eight species that are all endemic to Madagascar (Robinson 2007; Keeley & Robinson 2009). The genus is characterised by its shrubby habit; one or few capitula, subsessile to long-pedunculate; appendiculate phyllaries; paleaceous receptacles; anthers with broad tails; style without node; glabrous cypselae and pappus formed by numerous bristles (Humbert 1960; Robinson 2007).

The genus was validly published by Candolle (1836), based on specimens collected by W. Bojer. Candolle described two species: *Centauropsis lanuginosa* Bojer ex DC. (= *Oliganthes lanuginosa* (Bojer ex DC.) Humbert) and *Centauropsis fruticosa* Bojer ex DC. Robinson (1999) revised the taxonomy of paleotropical Vernonieae Cass., proposing three new subtribes (Centrapalinae H.Rob., Erlangeinae H.Rob., and Gymantheminae H.Rob.) and several new genera, placing *Centauropsis* within subtribe Gymnantheminae based on the shrubby habit and sweeping hairs often blunt. Nonetheless, molecular phylogenetic studies within Vernonieae have placed *Centauropsis* in a clade with members of the polyphyletic Erlangeinae and Centrapalinae (Keeley et al. 2007, 2021), a clade here referred as 'Erlangeinae/Centrapalinae combined' clade. In those studies, the single species of *Centauropsis* that was sampled is sister to a clade comprising *Hilliardiella* H.Rob. (Centrapalinae), *Crystallopollen* Steetz, *Parapolydora* H.Rob., *Cyanthillium* Blume (Erlangeinae) and *Vernonia subplumosa* O.Hoffm. (unplaced) (Keeley et al. 2007, 2021). According to Keeley and

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Robinson (2009), *Centauropsis* is actually placed in Centrapalinae.

Pollen morphology has been informative for inferring relationships at the family level in Compositae and important characters include the pollen grain shape in polar view (amb), class of endoapertures, thickness of exine layers, and size of spines (Blackmore et al. 2009). Pollen has also been pivotal in the new subtribal classification of paleotropical Vernonieae proposed by Robinson (1999). Historically, pollen characters have been studied in Vernonieae by different authors in the last century and have been of great importance for systematic study of the tribe at the subtribal and generic level (e.g. Wodehouse 1928; Salgado-Labouriau 1973; Jones 1981; Bolick 1991; Robinson 1999).

The pollen of *Centauropsis* has been poorly documented and inconsistently characterised (Kingham 1976; Wortley et al. 2007; Blackmore et al. 2009). A single species of this genus (*Centauropsis fruticosa* Bojer ex DC.) was included in prior palynological studies (Kingham 1976) and its pollen was characterised as medium-sized (35.0 μm), spherical, 3-colporate, subechinolophate/echinate sexine with micropores, appearing to present a reticulation, spines length 5.0 μm, and exine thickness 4.0 μm.

Wortley et al. (2007) suggested that the pollen of *Centauropsis* may be similar to that of the African genera *Cabobanthus*, *Cyanthillium* and *Crystallopollen*, and the South American *Pacourina* sharing the pororate aperture pattern (*sensu* Blackmore et al. 2009; = porate *sensu* Punt et al. 2007) and non-perforated tectum; however, this suggestion was based only on the sole specimen imaged by Kingham (1976) and no other species of *Centauropsis*. In addition, there is also inconsistency in the application of the descriptive terminology in *Centauropsis*, leading to confusion: the pollen grain is said to be 'porate' when the ectoaperture is a pore and not a colpus (i.e. pororate in Wortley et al. [2007] and Blackmore et al. [2009]).

Blackmore et al. (2009), based on the phylogeny of Keeley et al. (2007), suggested that pororate pollen grains is a synapomorphy for the clade Erlangeinae/Centrapalinae combined clade and, therefore, the tricolporate pollen of *Centauropsis* and *Parapolydora* would be a reversion. However, Keeley et al. (2021) have provided a different composition for this clade, in which a tricolporate genus (*Hilliardiella*) and the pororate *Cabobanthus* belong to another clade (Centrapalinae II), thus not recovering a porate clade. Further studies on pollen character evolution, with implications for finding synapomorphies for the clades, are not yet possible due to a lack of data. In this study, the pollen morphology of *Centauropsis* is described in a comprehensive and standardised way for the first time, as a means to support future taxonomic, palynological, and evolutionary studies of Vernonieae.

Material and methods

Taxonomic sampling of the genus comprised six (out of eight) currently recognised species of *Centauropsis*: *C. antanossi* (Scott Elliot) Humbert, *C. cuspidata* Humbert, *C. decaryi* Humbert, *C. fruticosa* Bojer ex DC. var. *fruticosa*, *C. perrieri* Humbert, *C. rhaponticoides* Drake, plus, one variety *C. fruticosa* var. *baronii* Humbert. Unopened mature flower buds were obtained from 13 specimens from K and P herbaria (acronyms following Thiers, [continuously updated]); all specimens examined are listed in the *Specimens Investigated* section. Samples were labelled with an abbreviation of the specific epithet plus the last two numbers of the herbarium voucher or collection number (see Tables I, II, Specimen column).

Pollen grains were treated with acetolysis (Erdtman 1960), as modified by Melhem et al. (2003). Measurements and photomicrographs were performed under a light microscope Leica LMD7 microdissection microscope, and photomicrographs were taken with a video camera Leica DFC 7000 T, supported by LAS software. Permanent slides for light microscopy (LM) were deposited in the pollen reference collection of the Bioimaging Laboratory, Royal Botanic Gardens, Kew, United Kingdom. For scanning electron microscopy (SEM), acetolysed pollen grains were washed and placed on a metal stub with carbon cement and sputter coated with platinum (10 nm) using a quorum-Q150t es Series. Samples were imaged under a Hitachi 8230 scanning electron microscope, with a 5 kV electron beam, at the Bioimaging Laboratory, Royal Botanic Gardens, Kew.

Measurements were taken under LM, using a Leica LMD7 microdissection microscope, on 25 randomly selected pollen grains from each specimen. The polar and equatorial axes were measured in equatorial view, and the equatorial axis in polar view, excluding spines. Ten measurements of the main pollen morphometric parameters were also made: length and width of the colpus, length and width of the endoaperture, the thickness of the nexine and sexine layers (excluding spines, Kingham 1976) and the spine length. Exine measurements were made in the mesocolpium region.

Table I. Centauropsis dimensions (μm) pollen grains in equatorial and polar view using light microscopy.

Specimen	Equatorial axis (EV)			Polar axis (EV)			*P*/*E*	Form	Equatorial axis (PV)			Size
	CI – (*x* ± Sx) CI+	*s*	*V* %	CI – (*x* ± Sx) CI+	*s*	*V* %	*x*		CI – (*x* ± Sx) CI +	*s*	*V* %	CI
ant08	53.7 (54.5 ± 0.4) 55.3	1.9	3.6	49.0 (50.1 ± 0.6) 51.3	2.8	5.7	0.9	OS	54.3 (55.1 ± 0.4) 55.8	1.8	3.2	L
ant92	51.6 (52.3 ± 0.3) 53.0	1.7	3.3	46.6 (47.4 ± 0.4) 48.2	1.8	3.9	0.9	OS	52.5 (53.3 ± 0.4) 54.2	2.0	3.8	M* – L
cus34	44.2 (45.1 ± 0.5) 46.1	2.4	5.2	40.1 (40.8 ± 0.3) 41.1	1.6	4.0	0.9	OS	41.7 (42.5 ± 0.3) 43.2	1.7	4.0	M
cus60	43.0 (44.0 ± 0.5) 45.0	0.5	5.6	41.0 (42.0 ± 0.5) 42.9	2.4	5.7	1.0	OS	42.5 (43.5 ± 0.5) 44.5	2.5	5.7	M
cus63	42.0 (42.8 ± 0.4) 43.5	1.9	4.4	39.1 (39.7 ± 0.3) 40.4	1.6	4.0	0.9	OS	41.7 (42.5 ± 0.3) 43.2	1.7	4.0	M
dec86	45.6 (46.7 ± 0.5) 47.8	2.6	5.6	41.3 (42.3 ± 0.5) 43.2	2.4	5.6	0.9	OS	44.4 (45.2 ± 0.4) 46.0	1.9	4.3	M
dec87	48.2 (49.0 ± 0.4) 48.2	1.9	3.9	41.9 (42.9 ± 0.5) 43.9	2.5	5.8	0.9	OS	46.4 (47.3 ± 0.4) 48.1	2.1	4.5	M
fru36	49.8 (50.9 ± 0.6) 52.1	2.9	5.6	45.3 (46.0 ± 0.3) 46.7	1.6	3.5	0.9	OS	49.0 (50.5 ± 0.7) 52.0	3.6	7.2	M–L
fru54	46.9 (47.6 ± 0.4) 48.4	1.9	3.9	42.3 (43.0 ± 0.3) 43.6	1.6	3.6	0.9	OS	45.4 (46.3 ± 0.4) 47.3	2.2	4.9	M
per62	46.8 (47.3 ± 0.3) 47.8	1.3	2.7	41.1 (41.8 ± 0.3) 42.4	1.6	3.8	0.9	OS	45.1 (45.8 ± 0.4) 46.6	1.8	4.0	M
rha07	55.4 (56.5 ± 0.5) 57.6	2.6	4.7	52.5 (53.3 ± 0.4) 54.1	1.9	3.5	0.9	OS	55.5 (56.5 ± 0.5) 57.6	2.5	4.4	L
rha10	58.6 (59.7 ± 0.6) 60.9	2.8	4.7	55.0 (56.0 ± 0.5) 56.9	2.3	4.1	0.9	OS	58.0 (58.9 ± 0.4) 59.9	2.2	3.8	L
rha11	58.4 (59.2 ± 0.4) 60.1	2.1	3.5	54.5 (55.2 ± 0.3) 55.8	1.6	3.0	0.9	OS	57.6 (58.6 ± 0.5) 59.7	2.5	4.3	L

Note. Specimens were identified by the abbreviation of the epithet and the last two numbers of the herbarium voucher (see Specimen analysed). Equatorial view (EV), polar view (PV), confidence interval (CI) at 95% of probability of the lowest sample values (CI–) and highest sample values (CI+), arithmetic mean (*x*), average standard deviation (Sx), sample standard deviation (*s*), coefficient of variability (*V* %), oblate spheroidal (OS), large (L), medium (M).

The measurements of the polar and equatorial axes were statistically analysed for the arithmetic mean (*x*), average standard deviation (Sx), sample standard deviation (*s*), coefficient of variability (*V*%), and 95% confidence interval (CI) (Zar 2010; Vieira 2011). For exine characters, the arithmetic mean and range were calculated for each pollen grain size class and exine (sexine + nexine). The CI and Sx are present in descriptions in the following order: (EA × PA) × (PV).

A principal component analysis (PCA) was performed to explore whether pollen grain characters corroborate species and specimen grouping. Eleven metric variables (polar axis [PA], equatorial axis [EA], equatorial axis in polar view [PV], colpus length [CL], colpus width [CW], endoaperture length [EL], endoaperture width [EW], nexine thickness [Ne], sexine thickness [Se = infratectum + tectum], exine thickness [Ex = *N* + *S*], spine length [Sp]) and one classes/index (endoaperture class [Ecl]) were analysed for ordination using FITOPAC (Shepherd 1996) and PC-ORD version 7 (McCune & Mefford 2016). To understand how *Centauropsis* specimens relate to each other, based on pollen morphology, a cluster analysis (CA) was performed using the software PC-ORD version 7 (McCune & Mefford 2016). We produced a similarity dendrogram by calculating the unweighted pair group method with arithmetic mean (UPGMA) using the Euclidean distance based on the same PCA metric variables and three classes/indexes.

Pollen terminology follows Punt et al. (2007) and Halbritter et al. (2018). Pollen shape classes and amb types follow Erdtman (1952), colpus length categories follow Mark et al. (2012) and specific sculpturing and apertures type to Vernonieae follow Robinson et al. (2016). The endoaperture classes and sexine/nexine thickness index follow Antonio-Domingues et al. (2022a).

Table II. Dimensions (μm) pollen grains aperture, spine, reticulum and exine layers in equatorial view using light microscopy.

Specimen	Colporus *L* –*W*	Endoaperture *W* (*W*/*L*) *L*	Spine	Exine *S* (*S*/*N*) *N* –*E*
ant08	33.8–8.4	10.3 (1.5) 6.7	3.5	5.9 (2.1) 2.8–8.7
ant92	32.1–9.7	12.5 (1.5) 8.6	3.5	5.3 (2.0) 2.6–7.9
cus34	26.9–5.3	10.9 (2.6) 4.3	3.5	4.3 (2.1) 2.0–6.3
cus60	31.1–6.4	11.1 (1.3) 8.8	2.5	3.6 (1.7) 2.1–5.7
cus63	24.9–4.0	10.0 (3.3) 3.1	4.2	4.3 (2.2) 2.0–6.3
dec86	28.6–5.6	10.7 (3.8) 2.9	5.0	4.3 (1.7) 2.5–6.8
dec87	30.2–7.1	14.8 (2.7) 5.5	5.0	4.3 (1.9) 2.3–6.6
fru36	33.6–8.4	12.7 (1.7) 7.7	4.1	4.4 (2.2) 2.0–6.4
fru54	29.9–6.3	10.3 (2.0) 5.1	4.3	4.5 (2.2) 2.0–6.5
per62	28.4–5.8	8.3 (1.7) 5.1	3.2	4.8 (2.6) 1.8–6.6
rha 07	36.4–9.7	14.1 (1.5) 9.8	5.0	4.7 (1.4) 3.2–8.0
rha 10	40.4–9.7	16.2 (1.6) 10.6	6.5	4.9 (1.5) 3.3–8.1
rha 11	42.0–9.5	13.0 (1.6) 8.2	4.8	4.0 (1.4) 2.7–6.7

Note. Specimens were identified by the abbreviation of the epithet and the last two numbers of the herbarium voucher (see Specimen analysed). Length (*L*), width (*W*), width/length (*W*/*L*), muri (M), lumen (Lum), sexine (*S*), sexine/nexine (*S*/*N*), nexine (*N*), exine (*E*).

Results

General description

Centauropsis *Bojer ex DC.* — Pollen grains are monads, medium to large-sized (43.0 [50.9 ± 4.0] 58.9 × 38.3 [46.5 ± 4.1] 54.6) × (41.5 [50.2 ± 4.4] 58.8) (Table I), radially symmetrical, isopolar (Figures 1A, D, G, J, 2A, D, G, 3A, D, G, J, 4A, D, G), oblate spheroidal to prolate spheroidal (Table I), amb circular or subtriangular, circular to ellipsoidal in equatorial view (Figures 1A, D, G, J, 3A, D, G, J), angulaperturate, polar area large to small (Figures 1B, E, H, K, 3B, E, H). Apertures 3-zonocolporate, colporus narrow, long to short, and non-constricted, (Figures 1A, D, G, J, 2D, G, 3A, D, G, 4A, D, G) apices acute or rounded (Figures 1B, E, H, K, 2G, H, J, 3B, E, H, 4E, H, Table III), margo prominent, membrane granulate, protruding aperture absent; operculum not observed (Figures 1A, D, G, J, 2D, G, 3A, D, G, 4A, D, G); endoaperture lalongate to very lalongate, terminals acute or rounded (Figures 1A, D, G, J, 3A, D, G, Table III). Exine thick, 5.0–10.0 μm, sexine 1.1–3.3 times thicker than nexine (Table II), sculpture sublophate-echinolophate (sublophate) (Figures 2A, D, E, G, H, J, K, 4A, B, D, F, G, H, Table III); spine 1.3–8.3 μm (Table II), base constricted (Figure 2E, F) or non-constricted (Figures 2C, I, K, 4B, C, E, F, H, I, J–L, Table III), apices acute (Figures 2C, E, F, I, 4B, C, J–L, Table III) or obtuse (Figure 4I, Table III); lopha (ridge) nanoreticulate (Figures 2E, F, K, 4F, H, I, Table III) or nanoreticulate-microechinate (Figure 4B, C, J, Table III), sublacuna nanoreticulate; muri perforate, lumina rounded. Tectum tectate perforate (Figures 2C, I, 4C, J–L), infratectum with thick digitate columella supporting the spines in a concordant pattern (Figures 2C, I, 4C, J–L) and thin simple columellae supporting the pluricolumellate lophae (Figure 4C, J–L).

A summary of the measurements is shown in Tables I and II and additional observations and descriptions are provided in Table III.

Centauropsis antanossi *(Scott Elliot) Humbert (Figures 1A–C, 2A–C)*. — Pollen grains are large-sized, rarely medium (52.1 [53.3 ± 0.6] 54.6 × 47.2 [48.8 ± 0.8] 50.3) × (53.0 [54.2 ± 0.6] 55.3), oblate spheroidal (Table I), amb circular to subtriangular (Figure 1A), ellipsoidal in equatorial view (Figures 1A, C, 2A), angulaperturate, polar area medium (Figure 1B). Colporus medium, apices acute (Figure 1B), membrane granulate (Figure 2B); endoaperture lalongate terminals rounded (Figure 1A). Exine 6.8–10.0 μm, sexine 1.6–2.9 times thicker than nexine; spines 3.5 μm (Table II), base non-constricted, apices acute (Figure 2B, C); lophae and sublacunae nanoreticulate.

Centauropsis cuspidata *Humbert (Figures 1D–F, 2D–F)*. — Pollen grains are medium-sized (42.5 [43.9 ± 0.6] 45.3 × 39.2 [40.2 ± 0.4] 42.9) × (41.4 [42.4 ± 0.4] 43.4), oblate spheroidal to prolate spheroidal (Table I), amb circular to subtriangular (Figure 1E), circular to ellipsoidal in equatorial view (Figures 1D, F, 2D), angulaperturate, polar area medium (Figure 1E). Colporus medium, apices acute (Figures 1D, 2F), rarely rounded, membrane granulate; endoaperture very lalongate, terminals rounded (Figure 1D), lalongate only in [a]cus60.

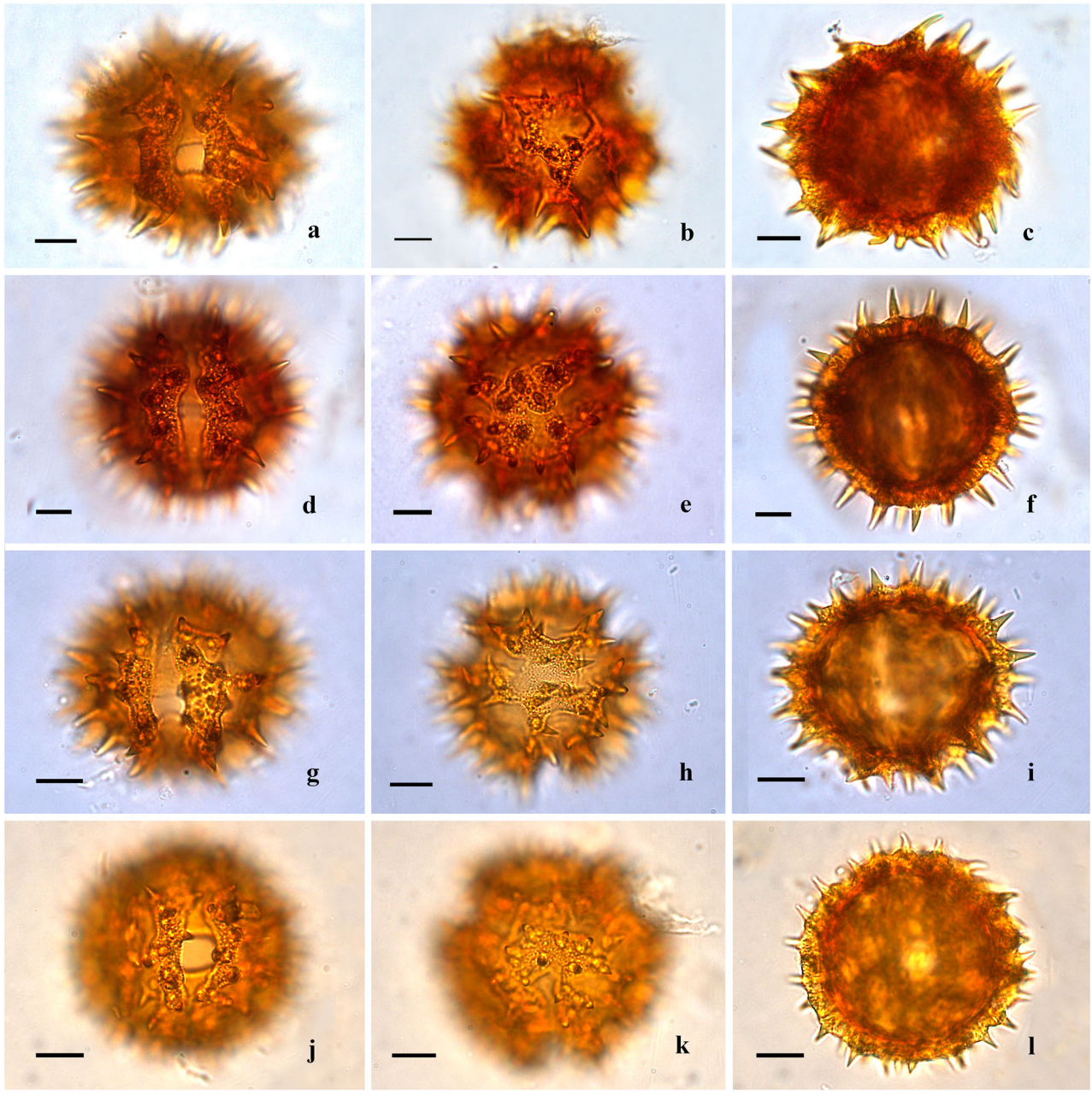

Figure 1. Light microscopy of *Centauropsis* Bojer ex DC. pollen grains. **A–C.** *Centauropsis antanossi* (Scott Elliot) Humbert. **A.** Detail of the colporus, endoaperture and surface, equatorial view. **B.** Apertures 3-colporate, long and acute apices and apocolpium surface. **C.** Optical section, equatorial view. **D–F.** *Centauropsis cuspidata* Humbert. **D.** Detail of the colporus, endoaperture and surface, equatorial view. **E.** Apertures 3-colporate, long and acute apices and apocolpium surface. **F.** Optical section, equatorial view. **G–H.** *Centauropsis decaryi* Humbert. **G.** Detail of the colporus, endoaperture and surface, equatorial view. **H.** Apertures 3-colporate, long and acute apices and apocolpium surface. **I.** Optical section, equatorial view. **J–L.** *Centauropsis fruticosa* Bojer ex DC. **J.** Detail of the colporus, endoaperture and surface, equatorial view. **K.** Apertures 3-colporate, long and acute apices and apocolpium surface. **L.** Optical section, equatorial view. Scale bars – 10 μm (A–C, G–L), 8 μm (D–F).

Exine 5.0–7.7 μm, sexine from 1.3–2.9 times thicker than nexine; spines 3.5 μm (Table II), base constricted, apices acute (Figures 1F, 2F); lophae and sublacunae nanoreticulate (Figure 2E–F).

Centauropsis decaryi *Humbert (Figure 1G–I, 2G–I).* — Pollen grains are medium-sized (46.3 [47.8 ± 0.7] 49.2 × 41.1 [42.5 ± 0.7] 43.9) × (44.9 [46.2 ± 0.6] 47.5), oblate spheroidal (Table I), amb

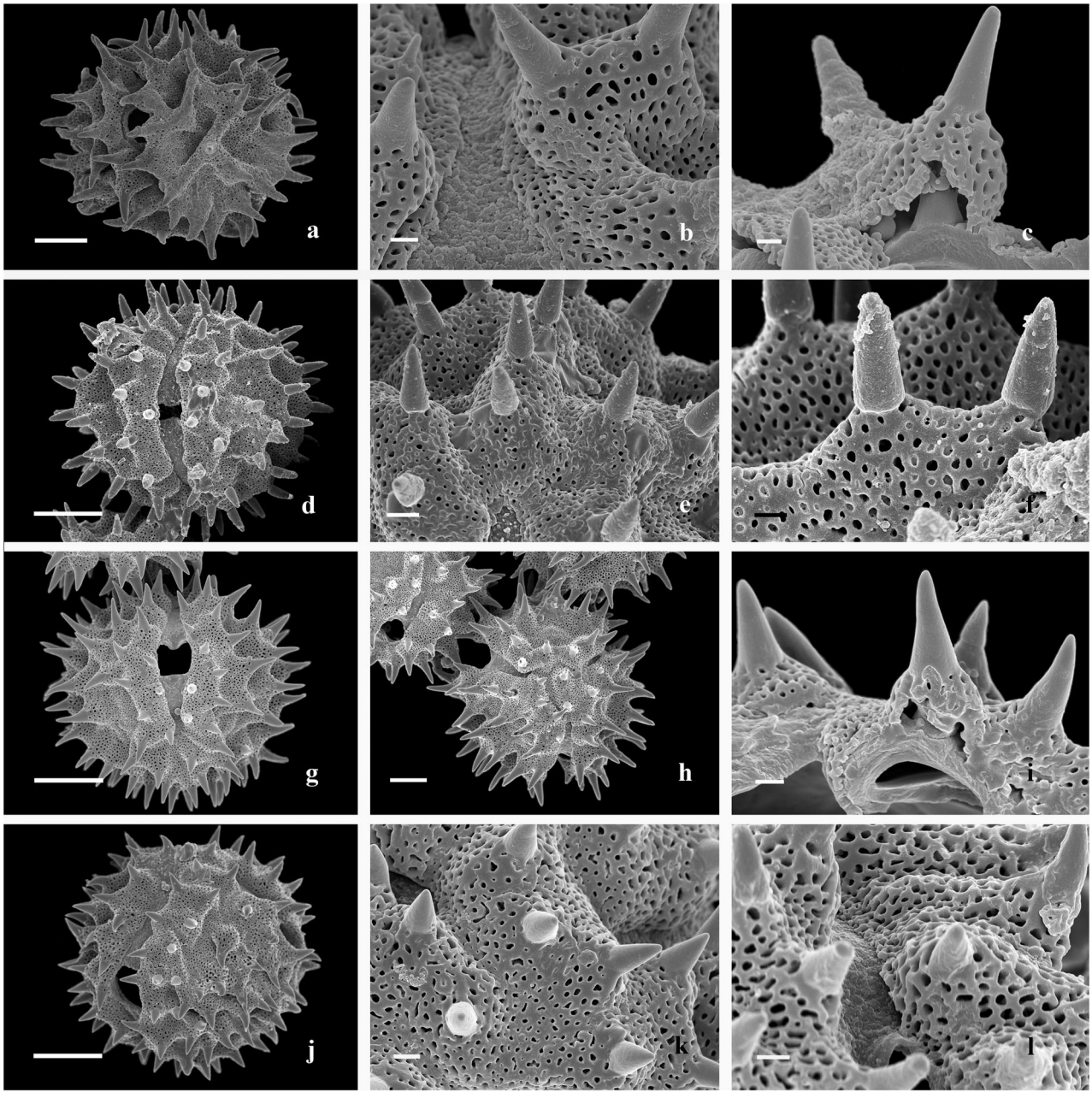

Figure 2. Scanning electron microscopy of *Centauropsis* Bojer ex DC. pollen grains. **A–C.** *Centauropsis antanossi* (Scott Elliot) Humbert. **A.** General view of the mesocolpium, equatorial view. **B.** Detail of apertural membrane of the colporus, margo, and mesocolpium. **C.** Fractured pollen grain, structure of exine layers. **D–F.** *Centauropsis cuspidata* Humbert. **D.** General view of the apertural area and mesocolpium, equatorial view. **E.** Detail of apocolpium, polar view. **F.** Detail of the spines and lophae on mesocolpium, equatorial view. **G–I.** *Centauropsis decaryi* Humbert. **G.** General view of the mesocolpium, equatorial view. **H.** General view of the apocolpium, polar view. **I.** Fractured pollen grain, structure of exine layers. **J–L.** *Centauropsis fruticosa* Bojer ex DC. **J.** General view of the apertural area and mesocolpium, equatorial view. **K.** Detail of apocolpium, polar view **L.** Detail of apertural membrane of the colporus, margo, and mesocolpium. Scale bars – 10 μm (A, D, G, H, J), 1 μm (B, C, E, F, I, K, L).

subtriangular to triangular (Figure 1H), ellipsoidal in equatorial view (Figures 1G, I, 2G), angulaperturate, polar area small (Figures 1H, 2H). Colporus long, apices rounded (Figures 1H, 2H), membrane granulate; endoaperture very lalongate, terminals acute (Figures 1H, 2G, H). Exine 5.2–8.4 μm, sexine from 1.3–2.6 times thicker than nexine; spines 5.0 μm (Table I), base non-constricted,

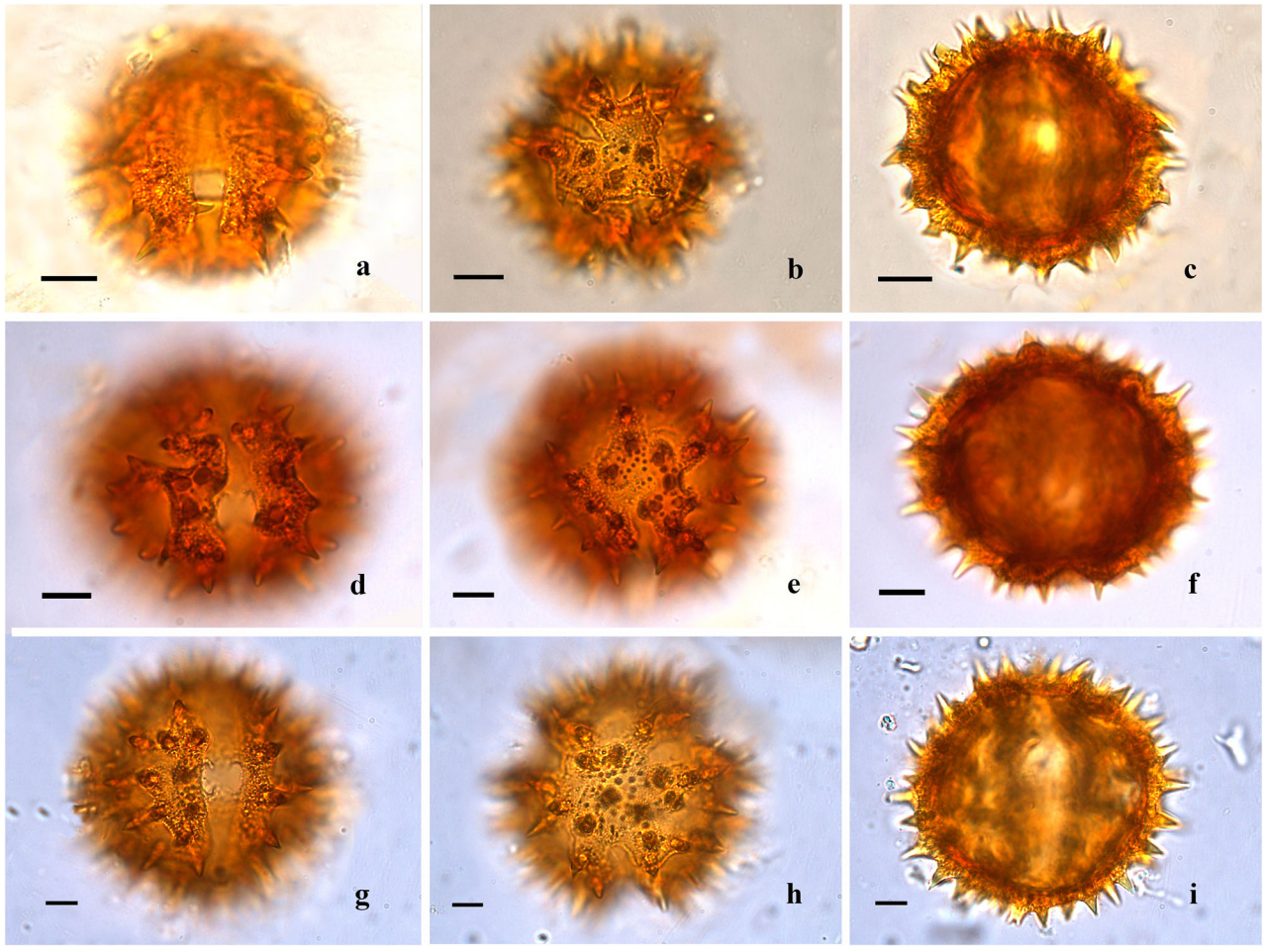

Figure 3. Light microscopy of *Centauropsis* Bojer ex DC. pollen grains. **A–C.** *Centauropsis fruticosa* var. *baronii* Humbert. **A.** Detail of the colporus, endoaperture and surface, equatorial view. **B.** Apertures 3-colporate, long and acute apices and apocolpium surface. **C.** Optical section, equatorial view. **D–F.** *Centauropsis perrieri* Humbert. **D.** Detail of the colporus, endoaperture and surface, equatorial view. **E.** Apertures 3-colporate, long and acute apices and apocolpium surface. **F.** Optical section, equatorial view. **G–I.** *Centauropsis rhaponticoides* Drake. **G.** Detail of the colporus, endoaperture and surface, equatorial view. **H.** Apertures 3-colporate, long and acute apices and apocolpium surface. **I.** Optical section, equatorial view. Scale bars – 10 μm (A–C), 8 μm (D–I).

apices acute (Figure 2I); lophae and sublacunae nanoreticulate (Figure 2H).

Centauropsis fruticosa *Bojer ex DC. var. fruticosa (Figure 1J–L, 2J–L)*. — Pollen grains are medium to large-sized (49.8 [50.9 ± 0.6] 52.1 × 43.5 [46.0 ± 0.3] 45.3) × (49.0 [50.5 ± 0.7] 52.0), oblate spheroidal (Table I), amb subtriangular, circular in equatorial view (Figure 1J), angulaperturate, polar area small (Figure 1K). Colporus long, apices rounded (Figures 1K, 2J, L), membrane granulate (Figure 2L); endoaperture very lalongate, terminals rounded (Figure 1J). Exine 2.3–7.1 μm, sexine from 1.8–3.2 times thicker than nexine; spines 4.1 μm (Table 1), base non-constricted, apices acute (Figure 2K, L); lophae and sublacunae nanoreticulate (Figure 2K).

Centauropsis fruticosa *var. baronii Humbert (Figures 3A–C, 4A–C, J, K)*. — Pollen grains are medium-sized (46.9 [47.6 ± 0.4] 48.4 × 42.3 [43.0 ± 0.3] 43.6) × (45.4 [46.3 ± 0.4] 47.3), oblate spheroidal (Table I), amb subtriangular, ellipsoidal in equatorial view (Figure 3A, C), angulaperturate, polar area small (Figure 3B). Colporus long, apices rounded, rarely acute (Figure 3B), membrane granulate (Figure 4A); endoaperture very lalongate, terminals acute (Figure 3B). Exine 5.0–7.5 μm, sexine from 1.8–3.1 times thicker than nexine; spines 4.3 μm (Table I), base non-constricted, apices acute (Figure 4B, C); lophae nanoreticulate-microechinate (Figure 4B, C) and sublacunae nanoreticulate (Figure 4B).

Centauropsis perrieri *Humbert (Figures 3D–F, 4D–F, L)*. — Pollen grains are medium-sized (46.8 [47.3

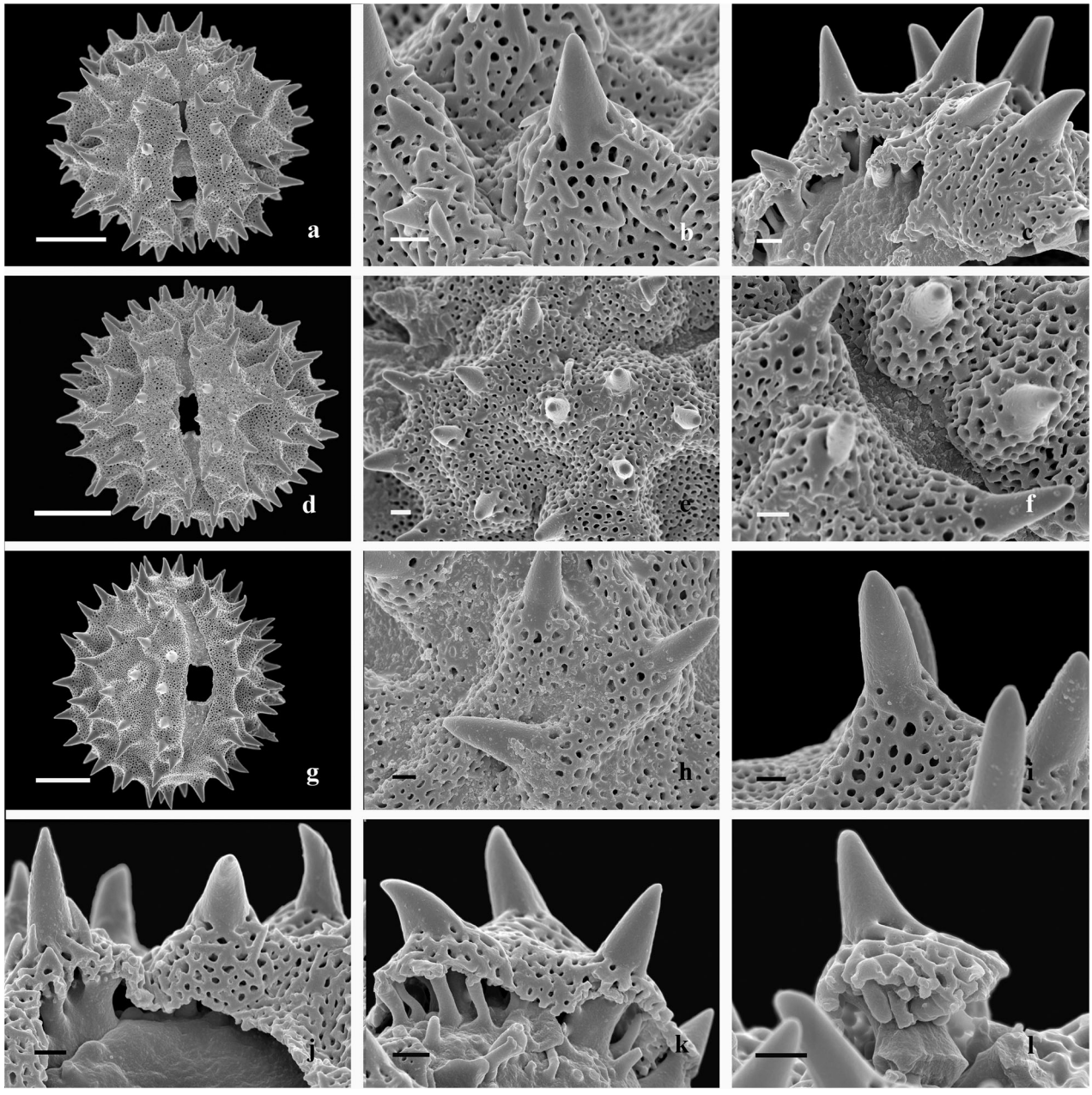

Figure 4. Scanning electron microscopy of *Centauropsis* Bojer ex DC. pollen grains. **A–C.** *Centauropsis fruticosa* var. *baronii* Humbert. **A.** General view of the apertural area, mesocolpium, and apocolpium, inclined equatorial view. **B.** Detail of the spines, lophae and microspines on mesocolpium, equatorial view. **C.** Fractured pollen grain, structure of exine layers. **D–F.** *Centauropsis perrieri* Humbert. **D.** General view of the apertural area, mesocolpium, equatorial view. **E.** Detail of the apocolpium, polar view. **F.** Detail of apertural membrane of the colporus, margo, and mesocolpium. **G–I.** *Centauropsis rhaponticoides* Drake. **G.** General view of the apertural area, mesocolpium, equatorial view. **H.** Detail of the apocolpium, polar view. **I.** Detail of the spines on mesocolpium, equatorial view. **J, K.** Fractured pollen grain, structure of exine layers of *Centauropsis fruticosa* var. *baronii* Humbert. **L.** Fractured pollen grain, structure of spine of *Centauropsis perrieri* Humbert. Scale bars – 10 μm (A, D, G), 1 μm (B, C, E, F, H–L).

± 0.3] 47.8 × 41.1 [41.8 ± 0.3] 42.4) × (45.1 [45.8 ± 0.4] 46.6), oblate spheroidal (Table 1), amb subtriangular (Figure 2E), ellipsoidal in equatorial view (Figure 3D, F), angulaperturate, polar area medium (Figures 3E, 4E). Colporus medium, apices rounded (Figures 3E, 4E) membrane granulate (Figure 4D); endoaperture very lalongate, terminals rounded (Figures 3D, 4E). Exine 5.4–7.8 μm, sexine from 1.8–3.3 times thicker than nexine; spines 3.2 μm (Table I), base

Table III. Morphology and ultrasculpture of *Centauropsis* pollen grains using light and scanning electron microscopy.

Specie	Size	Form	Colporus	Endoaperture class, terminals	Spine base, apices	Lophae
Centauropsis antanossi	L – M*	Oblate spheroidal	Medium, pointed	Lalongate, rounded	Non- constricted, acute	Nanoreticulate
Centauropsis cuspidata	M	Oblate spheroidal to prolate spheroidal	Medium, pointed	Very lalongate or lalongate* rounded	Constricted, acute	Nanoreticulate
Centauropsis decaryi	M	Oblate	Long, rounded	Very lalongate, pointed	Non- constricted, acute	Nanoreticulate
Centauropsis fruticosa	M–L	Oblate spheroidal	Long, rounded	Very lalongate, rounded	Non- constricted, acute	Nanoreticulate
Centauropsis fruticosa var. *baronii*	M	Oblate spheroidal	Long, rounded	Very lalongate, pointed	Non- constricted, acute	Nanoreticulate-microechinate
Centauropsis perrieri	M	Oblate spheroidal	Medium, rounded	Vary lalongate, rounded	Non- constricted, acute	Nanoreticulate
Centauropsis rhaponticoides	L	Oblate spheroidal	Long, rounded	Lalongate to very lalongate, rounded	Non- constricted, obtuse	Nanoreticulate

Notes: Large (L), medium M).

non-constricted, apices acute (Figure 4E, F); lophae and sublacunae nanoreticulate (Figure 4E).

Centauropsis rhaponticoides *Drake (Figures 3G–I, 4G–I)*. — Pollen grains are large-sized (56.4 [58.4 ± 1.0] 60.4 × 53.2 [54.8 ± 0.7] 56.3) × (56.1 [58.3 ± 0.9] 59.8), oblate spheroidal (Table I), amb triangular (Figure 3H), circular to ellipsoidal in equatorial view (Figures 3G, I, 4G), angulaperturate, polar area small (Figure 3H). Colporus long, apices rounded (Figures 3H, 4H), membrane granulate; endoaperture lalongate to very lalongate, terminals rounded (Figure 3H). Exine 7.4–9.8 µm, sexine from 1.1–2.0 times thicker than nexine; spines 5.4 µm (Table I), base non-constricted, apices acute (Figure 4I); lophae and sublacunae nanoreticulate (Figure 4H).

Principal component analysis (PCA)

The PCA explored the correlation between a suite of selected palynological characters, in a total of 11 metric variables and one class/index (Figure 5, Table IV). Correlation coefficients are shown in Table IV. The first two axes accounted for 88.76% of the total variance of the quantitative data analysed. The first axis explained 69.41% of the variance and was mainly associated with EL, CW, CL, PV, PA, EA and Se. The second axis summarised 19.35% of the total variance, with Sp, Ecl, EW, Ne, and Ex being the variables that contributed most to this axis.

Three individuals of *Centauropsis rhaponticoides* (rah07, rah10, rah11) represent the largest pollen grains, and the highest values relative to colporus and endoaperture dimensions were located on the upper left side of the PCA associated with high values of EA, PA, CL, CW, EL, EW. *Centauropsis antanossi* (and08, ant92), *C. fruticosa* var. *fruticosa* (fru36) and *C. cuspidata* (cus60) showed high Ex and the lowest Ecl representing the lalongate endoaperture, were located on the bottom left side of the PCA.

Due to their high Ecl values, *Centauropsis decaryi* (dec86, dec87) and *C. cuspidata* (cus63) were placed on the upper right side of the PCA with very lalongate endoaperture. Specimens with the smallest pollen grains and spines were located mainly on the bottom right side of the graph. These specimens also had the lowest EA, PA, PV, and Sp values: *C. fruticosa* var. *fruticosa* (fru94), *C. cuspidata* (cus34) and *C. perrieri* (per62).

Cluster analysis (UPGMA and Euclidean distance)

We identified two groups of specimens with 0% similarity (Figure 6). One group had similarity greater than 30% and comprised the species with the smallest pollen grains, colporus and endoaperture dimensions and the highest endoaperture class (*Centauropsis cuspidata* [cus34, cus63] *C. decaryi* [dec86, dec87], *C. fruticosa* var. *fruticosa* [fru94], *C. perrieri* [per32]). The other group had similarity greater than 45% and comprised the remaining species with the largest pollen grains, colporus and the endoaperture values and the smallest endoaperture class (*C. antanossi* [ant08, ant92], *C. fruticosa* [fru36], *C. rhaponticoides* [rah07, eah10, rah11] and *C. cuspidata* [cus60]). Two *C. rhaponticoides* specimens [rah07, rah70] showed 100% similarity and two *C. antanossi* almost 95% (ant08, ant92).

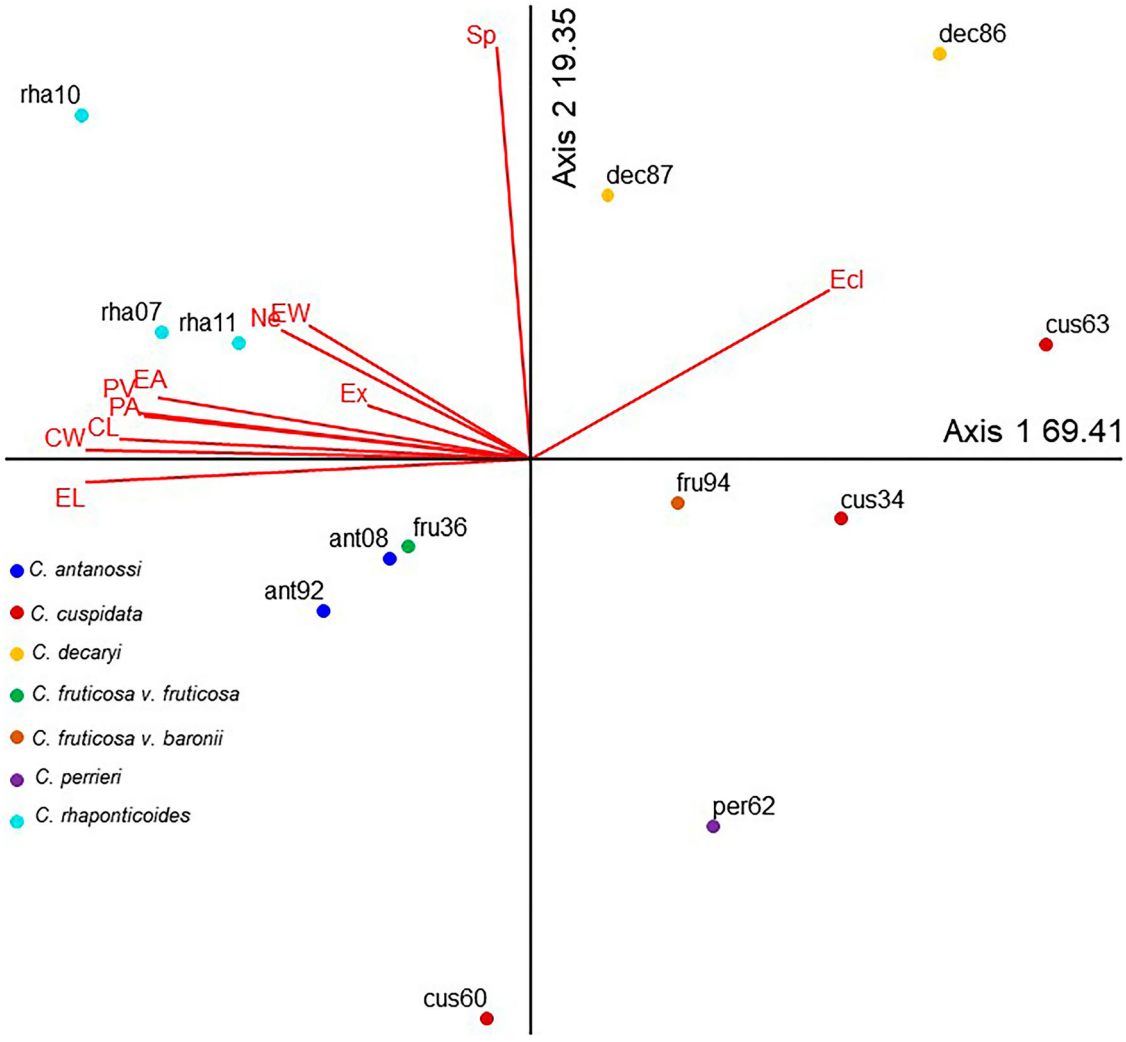

Figure 5. Principal component analysis biplot of the pollen grain metric variables and classes/indices of *Centauropsis* specimens.

Discussion

Pollen morphology of Centauropsis

The pollen of *Centauropsis* is characterised by 3-colporate aperture pattern, sublophate ornamentation, and nanoreticulate sexine. Our results also corroborate previous observations made for *Centauropsis fruticosa* by Kingham (1976), with exception of the spine length, which we have documented as slightly shorter (4.1 µm in our study versus 5.5–6.5 µm in Kingham's work). Pollen grain axis, colporus size, shape and size of spines, shape, and terminal of the endoaperture were useful characters to distinguish the species of *Centauropsis* (Table III).

In Compositae (and notably, several other genera in tribe Vernonieae), pollen grain size and morphology of the apertures (colporus and endoaperture), as well as of the spines, have been of important systematic value, such as in the genera *Chresta* Vell. ex DC., *Eremanthus* Less., *Graphistylis* B. Nord. *Paralychnophora* MacLeish, *Piptolepis* Sch.Bip. and *Verbesina* L. (Loeuille et al. 2012; Souza-Souza et al. 2015; Souza 2016; Siniscalchi et al. 2017; Moreira et al. 2019; Souza-Souza et al. 2022). Beyond these characters, other pollen morphological characters have been of importance for recognising species and groups of species, such as the shape of pollen grains (spheroidal, oblate spheroidal, prolate spheroidal), aperture number and position (3-colporate, 3-colpate, pantoporate), sexine ornamentation and reticulation (echinolophate, psilolophate, subechinolophate, echinate), muri ornamentation (psilate, perforate, reticulate, echinate), lacunae shape (hexagonal, pentagonal, circular) and ridge of the lacunae (interrupted, non-interrupted); these should be considered in future

Table IV. Pearson and Kendall coefficients of pollen grain metric variables and classes/indices from the first two ordination axes of the principal component analysis (PCA) of *Centoropsis* species.

Principal components		
Variables	Axis 1	Axis 2
EA	–1778	0.1366
PA	–0.1974	0.1288
PV	–0.1994	0.1266
CL	–0.2554	0.1070
CW	–0.4272	0.1156
EL	–0.6406	–0.2711
EW	–0.2153	0.3161
Se	–0.0547	0.0323
Ne	–0.1878	0.2537
Ex	–0.1191	0.1284
Ecl	0.3509	0.4973
Sp	0.0982	0.6491

EA, equatorial axis; PA, polar axis; PV, equatorial axis in polar view; CL, colpus length; CW, colpus width; EL, endoaperture length; EW, endoaperture width; Se, sexine thickness (*S* = infratectum + tectum); Ne, nexine thickness; Ex, exine thickness (Ex = *N* + *S*); Ecl, endoaperture class; Sp, spine length.

palynological and evolutionary studies in Vernonieae (Keeley & Jones 1979; Stanski et al. 2013; Souza et al. 2016; Siniscalchi et al. 2017; Souza-Souza et al. 2022).

Centauropsis fruticosa var. *baronii* showed acute endoaperture terminals (Figure 3A) (versus rounded endoaperture terminals in *C. fruticosa* var. *fruticosa*) (Figure 1J) and nanoreticulate-microechinate lophae (Figure 4B, C, J) (versus nanoreticulate lophae in *C. fruticosa* var. *fruticosa*) (Figure 2K) (Table III). These varieties also discretely differ in length of the pollen grain axes (i.e. they are different in shape), and in length of the colporus and endoaperture (Tables I, II). These morphometrical differences are confirmed by results from the PCA, in which *C. fruticosa* var. *baronii* is positioned on the bottom left corner of the plot (versus bottom right in *C. fruticosa* var. *fruticosa*) (Figure 5), and each specimen is represented in a different group, with 0% similarity (Figure 6). More studies are ongoing to re-assess their delimitation, based on other characters.

Palynotaxonomy

The 3-colporate pattern, presence of circular endoapertures, and smooth to perforate ornamentation are shared with the genera *Cabobanthus*, *Crystallopollen*, and *Cyanthillium*, belonging in the subtribes Erlangeinae and Centrapalinae, as well as with the South American genus *Pacourina* (subtribe Pacourininae) (Wortley et al. 2007; Blackmore et al. 2009).

Phylogenetic analyses of the total nuclear internal transcribed spacer (ITS) region, the chloroplast gene *ndhF*, and the non-coding spacer region *trnL-F* have placed *Centauropsis* in a combined Centrapalinae/Erlangeinae clade (Keeley et al. 2021). The palynological data available for this clade showed variation in the type of aperture (3-colporate, 3-porate, pantoporate) and type of ornamentation (lophate, sublophate) (Wodehouse 1928; Erdtman 1952; Jones 1981; Robinson 2007; Bunwong & Chantaranothai 2008; Robinson et al. 2014; Robinson et al. 2016). Both these characters have shown systematic value in different groups of angiosperms, including Compositae (Walker 1974;

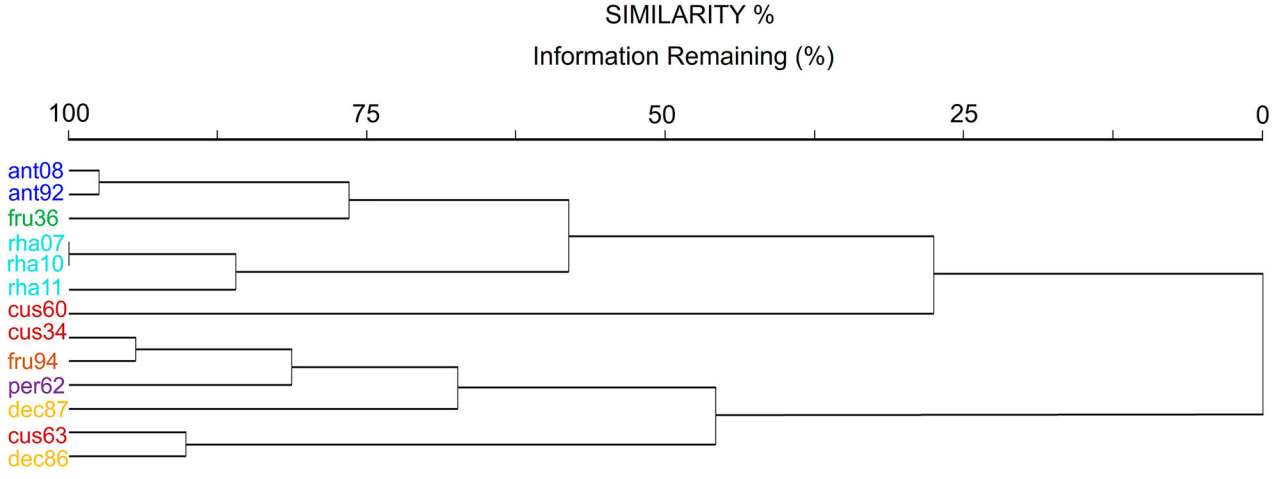

Figure 6. Dendrogram built from cluster analysis (Euclidean distance) for *Centauropsis* specimens, information remaining (%).

Feuer 1990; Banks 2003; Robinson et al. 2016; Oliveira et al. 2019). According to our observations and information from the literature, the 3-colporate pollen pattern (*Parapolydora*, *Hilliardella*, and *Centauropsis*) co-occurs with a sublophate exine pattern. Thus, all the porate pollen patterns (3-porate and pantoporate; *Crystallopollen*, *Cyanthillium*, *Pacourina* and *Cabobanthus*) share a lophate exine pattern.

However, palynological data from the remaining genera and species in the Centrapalinae/Erlangeinae clade is still lacking that would allow us to infer synapomorphic characters beyond *Centauropsis*. Thus, to date, only 15 of the 32 species (< 50%) in the clade that is sister to *Centauropsis* have had their pollen described (Wodehouse 1928; Erdtman 1952; Jones 1981; Robinson 2007; Bunwong & Chantaranothai 2008; Robinson et al. 2014; Robinson et al. 2016). In addition to a low number of species analysed, a complete, comprehensive, and standardised description of other pollen characters (i.e. pollen grain axes, colporus and endoaperture shape and size, spine shape and size) are not provided at species level, which would also help differentiate the species within the genera or perform a more robust pollen evolution analysis in future studies.

Previous studies have suggested the sublophate 3-colporate pattern, ('Pollen Type A' of Keeley and Jones (1979), found mostly in Africa, south-eastern Asia and some Neotropical taxa) is the ancestral condition (Keeley & Jones 1979) in Vernonieae but not all authors support this hypothesis (Robinson 1999). However, the model of pollen evolution in Vernonieae has not been tested in a molecular phylogenetic context at the tribal level, in part due to insufficient sampling breadth in previous studies especially for taxa from Africa and south-eastern Asia. The sampling limitations of previous studies are not surprising, as this is one of the most species-rich groups in Compositae, with *c.* 1500 species and a wide distribution (Keeley et al. 2021; Siniscalchi et al. 2017). Thanks to improved data accessibility from collections, as well as increasing scalability of molecular methods, these sampling limitations can be alleviated during the next decade, to help cast a broader picture of palynological diversity and pollen evolution in Vernonieae. As with the Centrapalinae-Erlangeinae combined clade, more extensive sampling at the species level (both molecular and palynological), and standardisation of pollen terminology, will be key to developing these resources in the future and more accurately test these hypotheses.

Conclusions

Centauropsis is a stenopalynous genus, with the 3-colporate aperture being conserved in all representatives of the genus studied to date. Further study that includes more comprehensive sampling of species in Vernonieae will be necessary to understand the diversity and evolution of pollen within this tribe, and to more confidently phylogenetically place *Centauropsis*, in relation to other genera in this tribe. Phylogenomic analyses are ongoing that will help understand the classification of the species of *Centauropsis* and elucidate the relationships within the genera of Vernonieae, for which the integration of these palynological data will be important.

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