Pollen and the Evolution of Arctotideae (Compositae)

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Abstract Recent molecular studies have elucidated the phylogeny of Compositae tribe Arctotideae, and found it to contain two, well supported, monophyletic subtribes, Arctotidineae and Gorteriinae, as well as some polyphyletic and problematic genera. On the basis of this new information, it may now be possible to identify diagnostic characters and synapomorphies to support the groupings defined within Arctotideae. Pollen characters have been shown to be particularly variable in Compositae. This paper aims to investigate the utility of those characters in the context of recent molecular phylogenies, in order to determine synapomorphic and diagnostic characters in Arctotideae. The pollen of each genus is described, illustrated with scanning electron micrographs, and optimised on a phylogeny of the tribe. Many pollen characters were found to be very informative when considered in the context of the current best estimate of phylogenetic relationships. Pollen morphology provides synapomorphies for clades at a number of hierarchical levels within Arctotideae, including the two subtribes, Arctotidinae and Gorteriinae, the grouping of *Eremothamnus* and *Hoplophyllum*, and smaller clades. It also supports the exclusion of *Platycarpha* from the tribe. The plesiomorphic palynological state for the tribe is discussed. Particular attention is paid to the evolution of different patterns of lophae (surface ridges). A single origin for the lophate condition is proposed as the most parsimonious mode of evolution in Arctotideae.

Keywords Arctoteae · Arctotidinae · Asteraceae · Characters · Gorteriinae · Palynology · Phylogeny

Introduction

In recent years, great advances have been made in phylogeny reconstruction, classification, morphological and anatomical study in flowering plants as a whole and Compositae in particular (Jansen et al., 1991; Kim & Jansen, 1995; Funk et al., 2005). One group that has received considerable attention, both through morpho-

logical (McKenzie et al., 2005; Karis, 2006) and molecular (*trnL-F*, *ndhF* and ITS sequences; Funk et al., 2004, 2007) means, is the tribe Arctotideae (previously Arctoteae; the African daisies). As with many new or re-defined tribes, the next step is to identify diagnostic characters and synapomorphies to support the new groupings defined within Arctotideae.

Arctotideae Phylogenetics

Previous studies have shown robust support for the fact that Arctotideae is a member of subfamily Cichorioideae, which also includes the larger tribes Cichorieae (Lactuceae), Vernonieae and Liabeae (Panero & Funk, 2002; Funk et al., 2004, 2005), and smaller tribes Gundelieae, Moquineae, and Eremothamneae (Robinson, 1994). Arctotideae has also consistently been shown to comprise two well-supported monophyletic subtribes, Arctotidineae and Gorteriinae (Funk et al., 2004, 2007; Karis, 2006), illustrated in Figs. 1 and 2. Arctotidineae (Fig. 1) comprises a single well-defined clade. Within Gorteriinae (Fig. 2), molecular data indicate that there are three well-supported clades: the *Gazania* (GAZ) clade, comprising *Gazania*, *Gorteria* and *Hirpicium*; *Didelta* (DID) clade, containing *Didelta* and *Berkheya spinosissima*; and *Berkheya* (BER) clade, comprising *Cullumia*, *Cuspidia*, *Heterorachis* and the remaining species of *Berkheya*.

The two largest genera in Gorteriinae, *Berkheya* and *Hirpicium*, are clearly non-monophyletic in molecular phylogenies. For *Berkheya*, only 22 species were included in the most comprehensive molecular phylogeny (and so not all species are discussed below), but seven of the eight series described by Roessler (1959) are

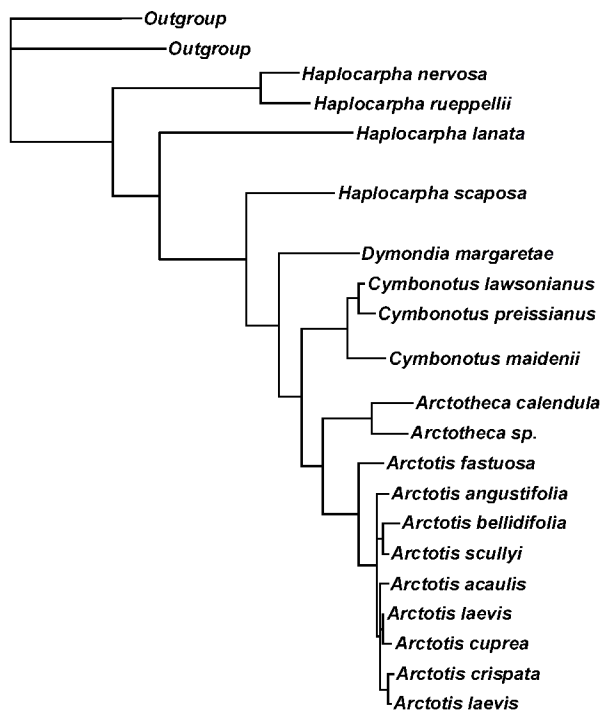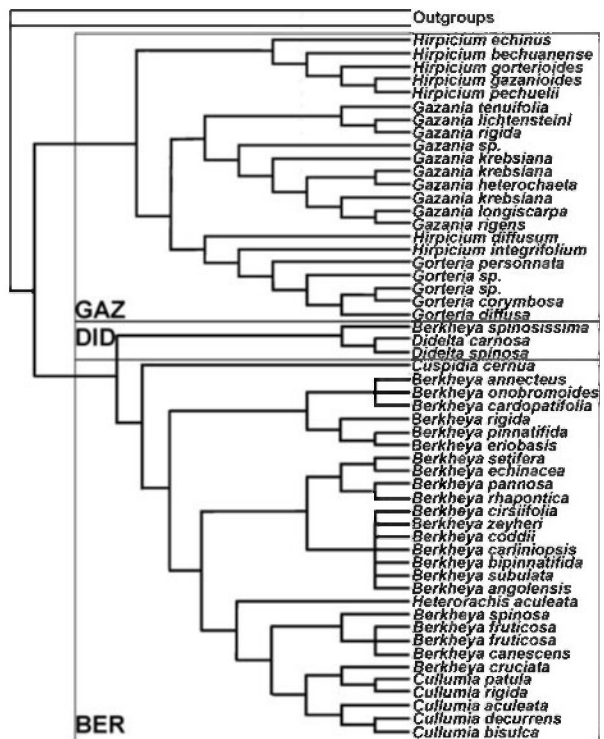Fig. 1 Phylogeny of Arctotidineae (redrawn from Funk et al., 2007)

Fig. 2 Phylogeny of Gorteriinae (redrawn from Funk & Chan, 2008)

covered. Molecular phylogenetic analyses have identified a number of monophyletic clusters within *Berkheya*: *Ber 1* (in the DID clade) so far contains only *B. spinosissima*; *Ber 2* contains *Berkheya annecteus*, *Berkheya cardopatifolia*, *Berkheya eriobasis*, *Berkheya onobromoides*, *Berkheya pinnatifida* and *Berkheya rigida*; *Ber 3* contains *Berkheya angolensis*, *Berkheya bipinnatifida*, *Berkheya carliniopsis*, *Berkheya cirsiifolia*, *Berkheya coddii*, *Berkheya congolensis*, *Berkheya echinacea*, *Berkheya onopordifolia*, *Berkheya pannosa*, *Berkheya radula*, *Berkheya rhapontica*, *Berkheya setifera*, *Berkheya subulata* and *Berkheya zeyheri*; *Ber 4* contains *Berkheya canescens*, *Berkheya cuneata*, *Berkheya fruticosa* and *Berkheya spinosa*; and *Ber 5* contains only *Berkheya cruciata*.

The second non-monophyletic genus is *Hirpicium*, which also divides into two clades: *Hir 1*, containing *Hirpicium bechuanense*, *Hirpicium echinus*, *Hirpicium gazanioides*, *Hirpicium gorterioides*, and *Hirpicium pechuelii* and *Hir 2*, containing *Hirpicium diffusum* and *Hirpicium integrifolium*.

The genus *Cullumia*, found in the BER clade with Ber 2–Ber 5, is paraphyletic comprising two groups: *Cul 1*, containing *Cullumia aculeata*, *Cullumia bisulca* and *Cullumia decurrens* and *Cul 2*, *Cullumia patula* and *Cullumia rigida*. The latter clade also contains *B. cruciata* (Ber 5) in molecular analyses.

Also evident from molecular analyses is the problem of the relationship between Eremothamneae (*Eremothamnus* and *Hoplophyllum*) and the tribe Arctotideae as a whole. Sometimes molecular data show Eremothamneae to be sister group to Gorteriinae; at other times it is sister to Arctotidinae (Funk et al., 2004). In either of these cases, if Arctotideae itself is to be monophyletic it must be defined to include

Eremothamneae (Funk et al., 2004). However, there is a possibility that the Eremothamneae, and also the genus *Heterolepis*, do not belong in Arctotideae at all (i.e., that Arctotideae as currently circumscribed is not monophyletic). Both Eremothamneae and *Heterolepis* are at present unstable in terms of their position in the group using molecular data. Additional molecular sequences are being gathered in order to address this question (Funk & Chan, unpublished data). Thus, a number of outstanding systematic questions remain in Arctotideae phylogenetics, including the relationships among the various *Berkheya* groups, the placement of Eremothamneae and *Heterolepis*, and consequently the monophyly of Arctotideae itself (Funk et al., 2004).

Palynological Characters

Pollen characters have been shown to be particularly variable in Compositae (Blackmore et al., 2008). However they have previously been considered to conflict with relationships based on evolutionary history and described as contributing "substantially to the problem of generic and tribal groupings." (Turner, 1977: 1115). This was largely due to pollen characters being viewed in the light of what are now known to have been inaccurate representations of relationship. Although the phylogeny of Compositae has recently been radically altered, the view that pollen characters are highly homoplastic continues to prevail. However, homoplasy need not preclude a set of characters from being informative, instead merely limiting the level of the taxonomic hierarchy at which they are most useful (Blackmore, 2000). As is the case with other classes of morphological characters, palynological characters may be most useful when subjected to critical evaluation as synapomorphies or diagnostic characters in the context of robust molecular hypotheses of relationship (Scotland et al., 2003). The purpose of this paper is to investigate the utility of pollen characters in Compositae tribe Arctotideae in the context of the molecular phylogenies recently presented (Funk et al., 2004, 2007; Funk & Chan, 2008). Our aim is to assess whether pollen characters can now be considered synapomorphic and diagnostic, fitting and supporting the current hypotheses of relationship within Arctotideae.

Materials and Methods

Pollen Preparation

Taxa were selected to represent all genera presently or potentially assigned to Arctotideae, including each of the eight series of *Berkheya* defined by Roessler (1959), with two exceptions: the monotypic series Angustae and series Armatae. For the first of these we have been unable to find individuals in the field and there were no recent herbarium collections; for the second, specimens were collected but have not yielded useable material. Collection data is detailed in Table 1.

All pollen samples were prepared for viewing with the scanning electron microscope (SEM). Pollen was obtained from herbarium sheets and acetolysed (Erdtman, 1960). The only exception was *Gazania krebsiana* (Fig. 6g), for which

Table 1 Collections of Arctotideae Pollen Examined

Species	Location	Collector	Herbarium	Figures
Arctotheca calendula (L.) Levyns	Australia	Funk 12,213	AUS, US	4f, g
Arctotheca calendula (L.) Levyns	South Africa	Funk & Koekemoer 12,418	PRE, US	4e, h
Arctotis fastuosa Jacq.	South Africa	T.H. Trinder–Smith 238	US	4i–l
Arctotis glandulosa Thunb.	South Africa	Goldblatt 7490	US	5b
Arctotis scullyi Dummer	South Africa	Trinder–Smith 205	US	5a, c, d
Berkheya angustifolia (Houtt.) Merr.	South Africa	Koekemoer 1426	PRE, US	12i–l
Berkheya canescens DC.	South Africa	Trinder–Smith 347	US	10e–h
Berkheya cruciata Willd.	South Africa	Rycoft 2975	US	11a–c
Berkheya cruciata Willd.	South Africa	Koekemoer 2002	US	11d
Berkheya fruticosa Ehrh.	South Africa	B. Maguire 201	US	10i–l
Berkheya onopordiflora (DC.) Burtt–Davy	South Africa	Funk & Koekemoer 12,424	PRE, US	9e–h
Berkheya pannosa Hilliard	South Africa	Funk & Koekemoer 12,423	PRE, US	9c,d
Berkheya radula (Harv.) Burtt–Davy	South Africa	Funk & Koekemoer 12,427	PRE, US	9i–l
Berkheya rigida Thunb.	South Africa	Funk & Koekemoer 12,403	PRE, US	8i–l
Berkheya spinosissima Willd.	South Africa	Trinder–Smith 346	US	7i–l
Berkheya subulata Harv.	South Africa	Sidey 3371	US	9a,b
Cullumia aculeata (Houtt.) Roessler	South Africa	Koekmoer 2021	PRE, US	11e–h
Cullumia bisulca (Thunb.) Less.	South Africa	Funk & Koekemoer 1935	PRE, US	11j, l
Cullumia bisulca (Thunb.) Less.	South Africa	Koekemoer 1392	PRE, US	11i, k
Cullumia decurrens Less.	South Africa	Fourcade 806	US	12a–d
Cullumia rigida DC.	South Africa	T. Trinder–Smith 182	US	12e–h
Cuspidia cernua (L.f.) B.L. Gurtt	South Africa	Brink 233	PRE	8e–h
Cymbonotus maidenii (G. Beauv.) A. Holland & V.A. Funk	Australia	Holland & Fechner 1339	QLD, US	5e–h
Didelta carnosa Ait.	South Africa	Funk & Koekemoer 12,533	PRE, US	8b, d
Didelta spinosa (L.f.) Ait.	South Africa	Trinder–Smith 142	US	8a, c
Dymondia margaretae Compton	South Africa	Brynard 101	PRE	5i–l
Eremothamnus marlothamnus O. Hoffm.	South Africa (Luderitzbucht)	W.Giess & D. Vay Vauuren 710	PRE (?)	3e–h
Gazania krebsiana Less.	Natal-Africa	Rudatis 398	KEW	6f1
Gazania krebsiana Less.	Oklahoma	Greenhouse	OKL	6g
Gazania krebsiana Less.	South Africa	Funk & Koekemoer 12561	US	6h
Gazania longiflora Less.	South Africa	E. G. Bryant 514	KEW	6f2
Gazania rigens (L.) Gaertn.	Australia	V. A. Funk 12230	US	6e
Gorteria diffusa Thunb.	South Africa	Trinder–Smith 103	US	6i, k
Gorteria personata L.	South Africa	Bayliss 4025	US	6j, l
Haplocarpha nervosa (Thunb.) Beauverd	Lesotho	Funk & Koekemoer 12,417	PRE, US	6c

Table 1 (continued)

Species	Location	Collector	Herbarium	Figures
Haplocarpha scaposa Harv.	South Africa	Funk & Koekemoer 12401	PRE, US	6a, b, d
Heterolepis aliena (L.f.) Druce	South Africa	Goldblatt & Manning 8599	US	4a–d
Heterorachis aculeata (Burm.f.) Roessler	South Africa	Compton 13441	PRE	10b
Heterorachis aculeata (Burm.f.) Roessler	South Africa	Oliver 3867	PRE	10a, c, d
Hirpicium bechuarense (S. Moore) Roessler	S. Rhodesia	C. R. Drummond 5735	K	7a, c, d
Hirpicium echinus Less.	South Africa	Koekemoer & Funk 1966	PRE, US	7b
Hirpicium integrifolium Less.	South Africa	Koekemoer & Funk 1956	PRE, US	7e–h
Hoplophyllum spinosum DC	South Africa	Koekemoer 2045	PRE, US	3i–l
Platycarpha carlinoides Oliver & Hiern.	Namibia	Bergland 3549	US	3d
Platycarpha parvifolia S. Moore	South Africa	Funk & Koekemoer 12,564	US	3a–c

pollen was removed from cultivated plants in the University of Oklahoma Greenhouse. Following acetolysis, the herbarium samples were cleaned and concentrated through sucrose (Chissoe & Skvarla, 1974), made conductive with solutions of osmium and thiocarbohydrazide (Chissoe et al., 1995), dried through hexamethyldisilazane (Chissoe et al., 1994), pulse sputter coated with a gold/palladium (60/40) target in a Hummer VI sputter coater (Chissoe & Skvarla, 1996) and mounted on double stick carbon tape attached to TEMSCAN 100-CX rectangular copper specimen boats. Freshly collected pollen was directly shaken into a solution of 2% glutaraldehyde in 0.1 M sodium cacodylate buffer at 40°C, pH 7.4, for 24 h. The glutaraldehyde was then removed by centrifugation and the pollen was rinsed through four buffer and distilled water washes. Drying and other processing steps followed the procedure as described above for acetolysis-treated samples.

Fractured pollen grains often occurred as a normal result of sample processing. However, when necessary, fractures were induced by mild sonication (c. 15 s) of the pollen/distilled water residue concentrated in tapered test tubes immediately after acetolysis treatment.

SEM examination was accomplished with a JEOL 880 SEM equipped with a lanthanum hexaboride gun operating at 15 kV. Images were digitally processed and the final plates prepared using Adobe PhotoShop 7. SEM images are displayed in vertical columns, one column per genus, and characterise four views: polar, apertural, equatorial, and fractured sections, sometimes taken from different species in a genus, often from different collections of the same species, in order to document as much variation and/or similarity within groups as possible. Showing this range of views is especially important for taxa with lophate structure because more than one view is needed to differentiate their complex surface morphologies. Furthermore, the pollen of many Arctotideae is poorly known and for some taxa SEMs are shown here for the first time.

Character Optimisation

Pollen characters were optimised on a phylogeny of Arctotideae (Funk et al., 2004) as unordered multistate characters using MacClade (Maddison & Maddison, 2005). Equivocal character changes were optimised using the "show all most parsimonious character states at each node" option.

Results

Terminology

Due to the complex nature of palynological characters, and the many derivations of lophae and spines in Arctotidae, we use a terminology (also followed in Blackmore et al., 2008) which partitions the macro-ornamentation of pollen grains into two separate characters, rather than blending them in the widespread but ambiguous terms "echinate," "lophate" and "echinolophate" (e.g. Punt et al., 1994). In the following discussion, echinate means having surface spines of at least 1 μm in height, regardless of whether there are surface ridges or not; the alternative, psilate, means without surface spines of at least 1 μm in height (a distinction traditionally applied because such grains appear effectively smooth under LM). Lophate means having ridges dividing the surface into lacunae; and non-lophate describes the absence of these ridges, regardless of the presence of spines.

Pollen Descriptions

Below, we describe the general pollen characteristics seen in Arctotideae, including *Eremothamnus* and *Hoplophyllum* which must be included if the tribe is mono-phyletic (Funk et al., 2004). This is followed by a specific pollen description for each genus in the tribe (for non-monophyletic genera, sub-generic grouping). Finally we describe the pollen of *Platycarpha* (Fig. 3a–d), which has now been excluded from Arctotideae following molecular phylogenetic analysis (Funk et al., 2004).

The pollen of all Arctotideae genera studied is oblate-spheroidal and tricolporate (sometimes tetracolporate in *Eremothamnus* and *Hoplophyllum*), with lalongate endoapertures and no mesoapertures (which are present in some related taxa including Gundelieae, Lactuceae and Vernonieae). The surface of Arctotideae pollen comprises a perforated tectum, and the infratectum is made up of one or two layers of solid, relatively narrow columellae, separated by a clear or fragmented internal tectum. The columellae are usually aggregated under the spines. The infratectum and endexine-foot layer are sometimes separated by a shallow cavea, which can be seen in our SEMs but is also clearly visible using the transmission electron microscope (TEM; Skvarla & Turner, 1966; Skvarla et al., 1977); in some genera (e.g. *Haplocarpha*) this space is spanned by thread-like columellae. Arctotideae pollen may be psilate or echinate, with spines of varying number and size but always having perforations towards the base. In addition the grains may be lophate (having a conspicuous pattern of ridges enclosing polygonal lacunae), sub-lophate (having shallow ridges, or spines distributed in a lophate pattern) or entirely non-lophate. For

those taxa that are lophate, Arctotidae are unique in Compositae in having six equatorial and six polar lacunae. In the descriptions that follow, only those characters that vary between genera within the tribe are discussed. Additional notes may be found in the plate legends.

The pollen of *Eremothamnus* (Fig. 3e–h) is 30–40 μm in diameter, oblate-spheroidal, sub-triangular in polar view, elliptic in equatorial view and tri- or tetra-colporate. The colpi are separate and of narrow-medium thickness with obtuse ends, the ora lalongate. The infratectum comprises two indistinct layers, the outer layer columellate, the inner supporting layer spongy, which might indicate the presence of internal foramina (TEM study is needed to determine this with certainty). The grains are caveate, with a few narrow columellae rising from the foot layer. They are non-lophate and echinate, the spines many, dense, conical with a long tapering point, up to 8 μm long, the bases slightly swollen.

The pollen of *Hoplophyllum* (Fig. 3i–l) is very similar to that of *Eremothamnus*. It is 30–40 μm in diameter, spheroidal (round in equatorial view and round or sub-triangular in polar view) and tri- or tetra-colporate. The colpi are of medium width, with obtuse ends. The infratectum is c. 0.8 μm thick, single-layered and spongy. The grains are partially or fully caveate, the cavea shallow to medium, sometimes with thread-like columellae across the cavea. The inside of the endexine is dimpled. The grains are echinate, with more than 100 spines distributed densely and evenly across the grain. The spines are 5–8 μm high and 2–2.5 μm in diameter at the base, with perforations and a solid apex, slightly swollen at the base, and lack columellae inside. The pollen surface is smooth and not raised between the spines; it is non-lophate.

The pollen of *Heterolepis* (Fig. 4a–d) is 25–30 μm in diameter, oblate-spheroidal (round in equatorial view and round or sub-triangular in polar view) and tricolporate. The colpi are narrow with acute ends. The infratectum is 1.2–1.5 μm thick and comprises two distinct columellae layers separated by a single or multiple, spongy internal tectum. The outer layer is 0.8–1 μm thick and the inner 0.4–0.5 μm. The columellae of the inner layer are evenly distributed and not swollen at the base. The inside of the endexine is smooth. The grains are partially caveate, the cavea is of medium depth, spanned by thread-like columellae. The grains are echinate, with c. 70 spines distributed unevenly across the grain. The spines are 3–4 μm high and 4.5–5 μm in diameter at the base, with perforations throughout, including at the apex, slightly swollen at the base, with pendant columellae inside. The pollen surface is reticulate and slightly raised between the spines; it is non-lophate.

Pollen grains of the sister taxa *Arctotheca* (Fig. 4e–h) and *Arctotis* (Figs. 4i–l; 5a–d) appear very similar to one another. In both genera the grains are 16–20 μm in diameter, oblate-spheroidal (round in equatorial view, round or sub-triangular in polar view) and tricolporate. The colpi are of narrow-medium width with acute ends. The infratectum is 0.8–1.2 μm thick and comprises two distinct columellae layers separated by multiple, compact, internal tecta. The outer layer is 0.42–0.44 μm thick and the inner 0.5–0.52 μm. The columellae of the inner layer are aggregated under the spines and not swollen at the base. The inside of the endexine is smooth. The grains are partially caveate, the cavea shallow-medium. The grains are echinate, with 48–63 spines, distributed evenly across the grain. The spines are 2.5–4 μm high and c. 3 μm in diameter at the base, with perforations throughout, including at the apex,
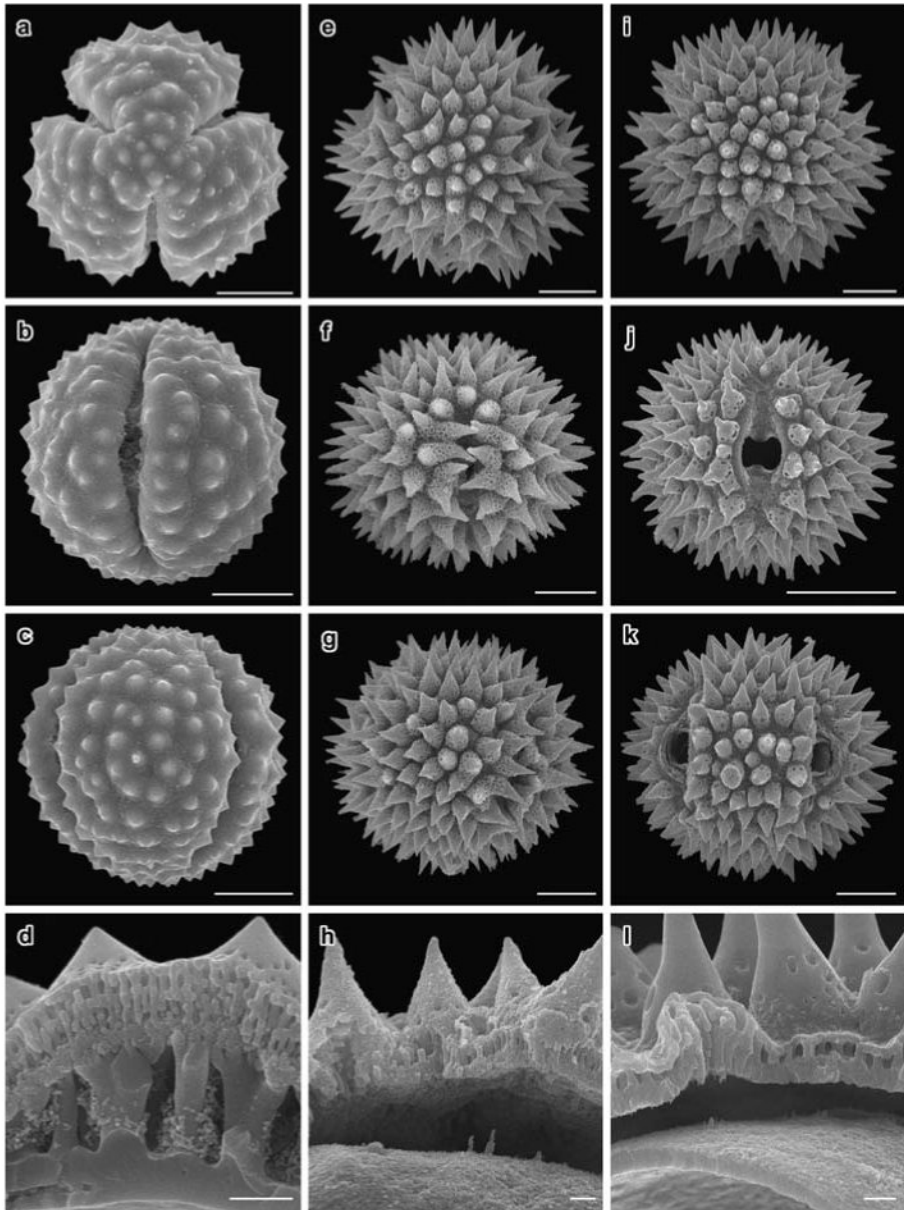
Fig. 3 SEM views of pollen of Arctotideae and excluded genera. **a–c** *Platycarpha parvifolia.* **a** Polar; **b** apertural; **c** equatorial. **d** *Platycarpha carlinioides*, fractured grain, showing thick inner layer of columellae supporting an upper layer of narrow columellae divided by a horizontal internal tectum. **e–h** *Eremothamnus marlothianus.* **e** Polar; **f** apertural; **g** equatorial; **h** fractured grain, showing single layer of columellae showing basal thickening and with occasional incomplete narrow columellae crossing the cavea. **i–l** *Hoplophyllum spinosum.* **i** Polar; **j** apertural; **k** equatorial; **l** fractured grain. Note great similarity in all views to that of *Eremothamnus. Scale bars*: whole grains, 10 μm; fractured grains, 1 μm
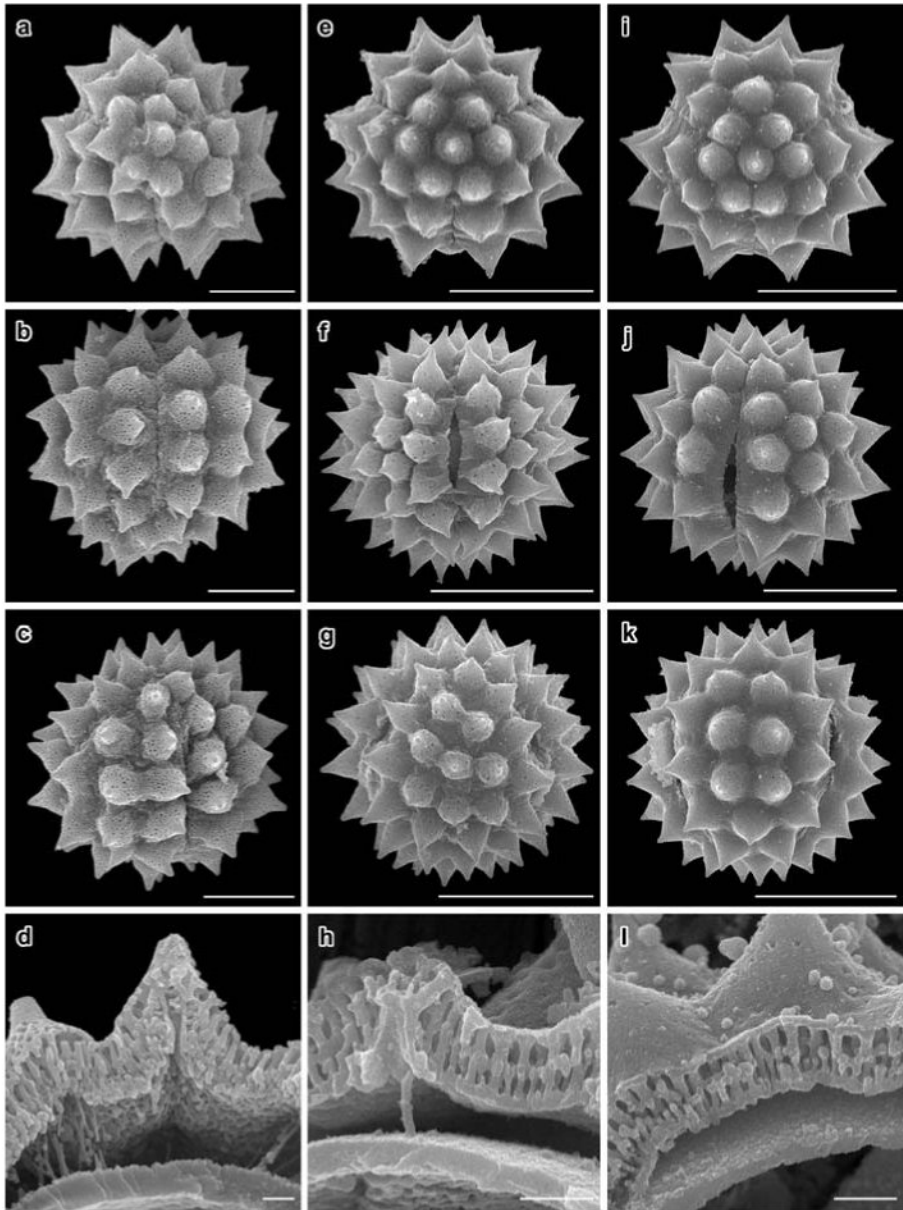
Fig. 4 SEM views of Arctotideae pollen. **a–d** *Heterolepis aliena*. **a** Polar; **b** apertural; **c** equatorial; **d** fractured grain, showing region of columellae only intermittently divided by horizontal internal tectum and cavea spanned by numerous narrow columellae under the spines connected to foot layer. **e–h** *Arctotheca calendula*. **e** Polar; **f** apertural; **g** equatorial; **h** fractured grain, showing well developed horizontal internal tectum, cavea and thick columellae under spine. **i–l** *Arctotis fastuosa*. **i** Polar; **j** apertural; **k** equatorial; **l** fractured grain. Note similarity of surface morphology in these three taxa. *Scale bars*: whole grains, 10 μm; fractured grains, 1 μm
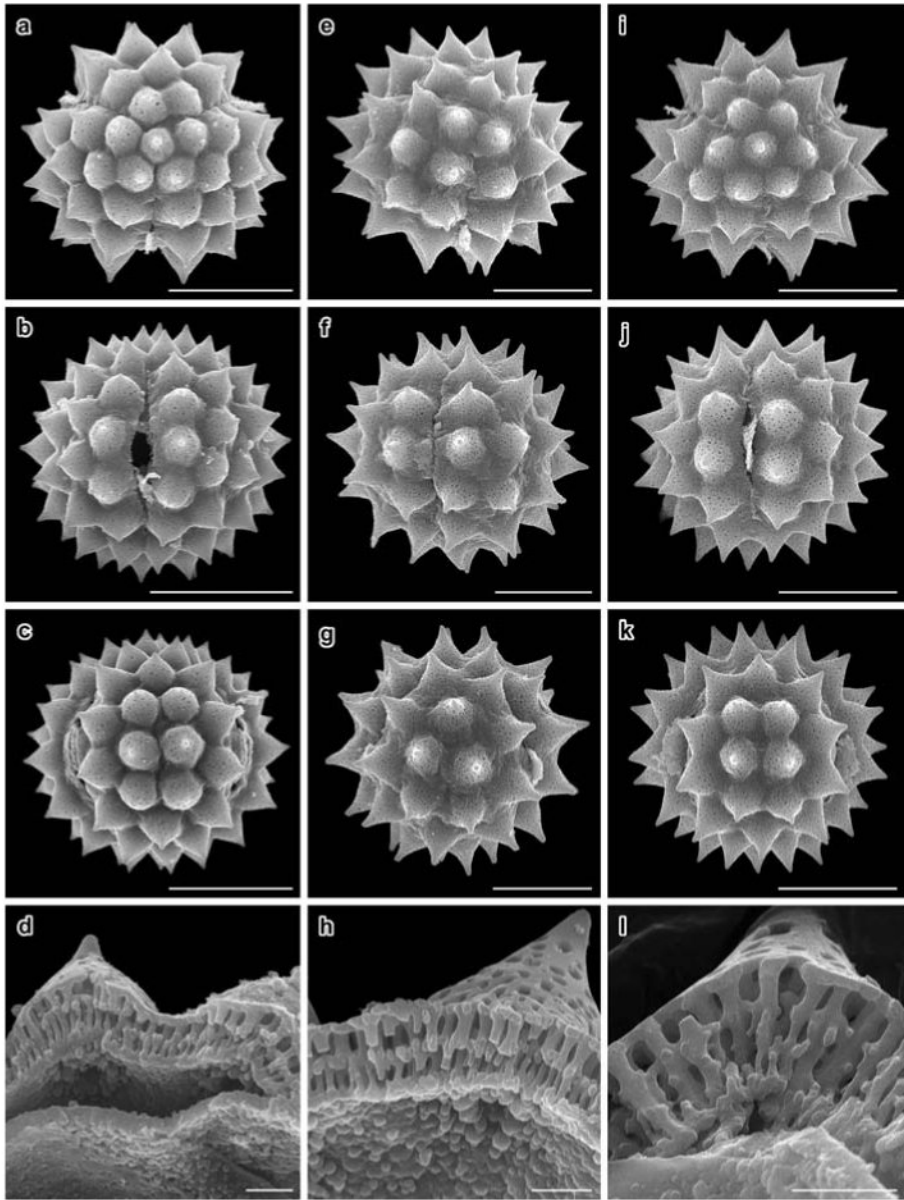
Fig. 5 SEM views of Arctotideae pollen. **a**, **c**, **d** *Arctotis scullyi*. b. *Arctotis glandulosa*. **a** Polar; **b** apertural; **c** lateral, d, fractured grain, showing well-developed horizontal internal tectum and cavea. **e–h.** *Cymbonotus maidenii* **e** Polar; **f** apertural; **g** equatorial; **h** fractured grain, showing prominent horizontal tectum equally dividing columellae. **i–l** *Dymondia margaretae*. **i** Polar; **j** apertural; **k** equatorial; **l** fractured grain, view almost directly under a spine. Note similarity in morphology between pollen grains of these four species. *Scale bars*: whole grains, 10 μm; fractured grains, 1 μm

arising smoothly or abruptly from the surface, with pendant or fully-attached columellae inside. The pollen surface is smooth and it may be slightly raised between the spines; it is non-lophate.

The pollen of *Cymbonotus*, the only non-African member of the tribe (Fig. 5e–h), also shows strong similarity to that of *Arctotis* and *Arctotheca*. It is 25–27 μm in diameter, spheroidal and tricolporate. The colpi are narrow with acute ends. The infratectum is 1–1.3 μm thick and comprises two distinct columellate layers separated by multiple, compact internal tecta. The outer layer is c. 0.6 μm thick and the inner is c. 0.7 μm. The columellae of the inner layer are aggregated under the spines and not swollen at the base. The inside of the endexine is dimpled. The grains are partially caveate, the cavea shallow-medium. The grains are echinate, with 42–48 spines, distributed evenly across the grain. The spines are 3–4 μm high and 3.4–3.8 μm in diameter at the base, with perforations throughout, including at the apex, slightly swollen at the base, without columellae inside. The pollen surface is smooth and it is not raised between the spines; it is non-lophate.

The pollen of *Dymondia* (Fig. 5i–l) is 18.5–23.5 μm in diameter, spheroidal (round in equatorial view and round or sub-triangular in polar view) and tricolporate. The colpi are narrow with acute ends. The infratectum is c. 1 μm thick and comprises two indistinct columellate layers, separated by multiple, compact, internal tecta. The outer layer is 0.4–0.5 μm thick and the inner is 0.5–0.6 μm. The columellae of the inner layer are evenly-distributed across the grain and not swollen at the base. The inside of the endexine is smooth. The grains are caveate, the cavea shallow. The grains are echinate, with 68–78 spines unevenly and sparsely distributed across the grain. The spines are 2–3 μm high and 3–4 μm in diameter at the base, with perforations throughout, including at the apex, slightly swollen at the base, with pendant columellae inside. The pollen surface is smooth, not raised between the spines; it is non-lophate.

The pollen of *Haplocarpha* (Fig. 6a–d) is 18–26 μm in diameter, oblate-spheroidal and tricolporate. The colpi are narrow and have acute ends. The infratectum is 0.93 μm thick and comprises two distinct columellate layers, separated by multiple spongy internal tecta. The outer layer is c. 0.43 μm thick and the inner c. 0.48 μm. The columellae of the inner layer are aggregated under the spines and not swollen at the vase. The inside of the endexine is smooth. The grains are partially caveate, the cavea medium in depth, with thread-like columellae spanning it at intervals. The grains are echinate, with 50–57 spines distributed evenly across the grain. The spines are 2.5–3 μm high and 3–4 μm in diameter at the base, with perforations throughout, including at the apex, arising smoothly from the surface of the grain, with pendant or fully-attached columellae inside. The pollen surface is smooth and sometimes raised between the spines, which is characteristic of the genus; it is non-lophate.

Within Gorteriinae, the pollen grains of *Gazania* (Fig. 6e–h) and *Gorteria* (Fig. 6i–l) are very similar to one another. They are 22–24 μm in diameter, oblate-spheroidal and tricolporate. The colpi are broad with acute ends. The infratectum is 0.45–0.6 μm thick, comprising a single columellate layer subtended by single or multiple, compact internal tecta. The grains are caveate, the cavea shallow. The grains are psilate. The pollen surface is reticulate and lophate with characteristic lophae flapping over one another, left above right, at the colpi. There are no abporal
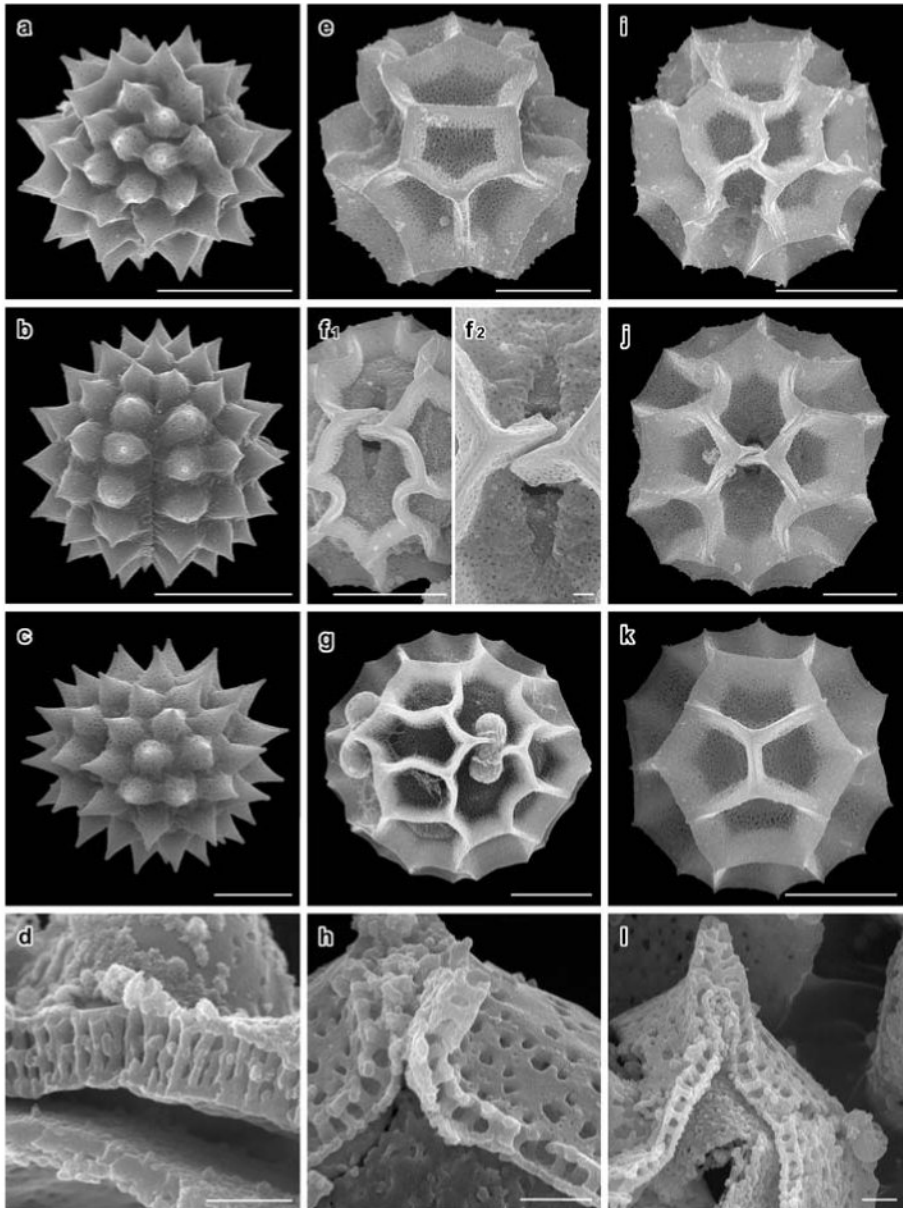
Fig. 6 SEM views of Arctotideae pollen. **a, b, d** *Haplocarapha scaposa.* **c** *Haplocarpha nervosa.* **a** Polar; **b** apertural; **c** equatorial; **d** fractured grain, showing well developed horizontal internal tectum and cavea. **e** *Gazania rigens.* **f1, g, h** *G. krebsiana.* **f2** *Gazania longiflora.* **e** Polar view, showing large lacunae with highly perforated surface. **f1** Apertural view, showing unequal arms of lophae directly over pore opening with left portion of ridge always over the right portion (compare with unacetolysed equivalent, **g**); **f2**, higher magnification of lophae at aperture (scale bar 1 μm); **g** equatorial view of unacetolysed grain, showing non-resistant aperture plugs between the lophae at aperture; **h** fractured grain showing single columellae layer and portion of cavea. Note striking difference in columellae structure from previously fractured grains. **i, k** *Gorteria diffusa.* **j, l** *Gorteria personata.* **i** Polar view; **j** aperture, showing left lopha over right in pore region; **k** lateral view showing aperture with left-over-right arrangement of lophae; **l** fractured grain. *Scale bars*: whole grains, 10 μm (except **f2**, see above); fractured grains, 1 μm

or interporal lacunae, six paraporal lacunae, six polar lacunae and three or six poral lacunae. The lacunae are 12–18 μm in diameter.

The pollen of *Hirpicium* (Fig. 7a–h) varies greatly in size, from 25–40 μm. It is oblate-spheroidal (elliptic in equatorial view, round or sub-triangular in polar view) and tricolporate. The colpi are broad with acute ends. The infratectum comprises a single columellate layer subtended by a single, compact internal tectum. The columellae are aggregated under lophae and not swollen at the base. The inside of the endexine is smooth. The grains are partially caveate, the cavea shallow. The grains are psilate. The pollen surface is reticulate and lophate with the lophae sometimes overlapping one another, left over right or right over left, or just meeting at the colpi. As in *Gazania* and *Gorteria* there are no abporal or interporal lacunae, six paraporal lacunae, six polar lacunae and three or six poral lacunae. The lacunae are 6–18 μm in diameter. There are no consistent differences between the pollen of the two groups of *Hirpicium* found in molecular analyses (Hir 1 and Hir 2), based on the three species examined here (Fig. 7a–h).

The pollen of *B. spinosissima* (Ber 1; Fig. 7i–l) is 40–45 μm in diameter, oblate-spheroidal (round or elliptic in equatorial view, sub-triangular in polar view) and tricolporate. The colpi are medium width with acute ends. The infratectum is 0.8–1.2 μm thick, comprising a single columellate layer subtended by a single, compact internal tectum. The columellae are evenly distributed and not swollen at the base. The grains are caveate, the cavea of medium depth. The grains are echinate, with c. 77 spines, sparsely clumped on lophae. The spines are distinctively pointed, c. 5 μm high and 3–4.5 μm in diameter at base, with perforations throughout, including at the apex, slightly swollen at base, with columellae inside fully attached to the foot layer. The pollen surface is reticulate and lophate.

The pollen of both *Didelta* species, *Didelta carnosa* (Fig. 8b, d) and *Didelta spinosa* (Fig. 8a, c) is very similar. They are 30 μm in diameter, oblate-spheroidal (round in equatorial view, round or sub-triangular in polar view) and tricolporate. The colpi are narrow with obtuse ends. The infratectum is 1.2–1.5 μm thick, comprising two distinct, columellae layers separated by a single compact or spongy tectum. The outer layer is 0.6–0.8 μm thick, the inner 0.4–0.6 μm. The columellae of the inner layer are aggregated under lophae and not swollen at the base. The inside of the endexine is smooth. The grains are partially caveate, the cavea shallow or medium depth. The grains are echinate, with 72–80 spines, clumped in a lophate-type pattern. The spines are 1–4.5 μm high and 5–6 μm in diameter at base, with perforations throughout, including at the apex, arising smoothly from the surface, with very characteristic hanging columellae inside. The pollen surface is smooth and sub-lophate.

The pollen of *Cuspidia* (Fig. 8e–h) is 28–30.5 μm in diameter, oblate-spheroidal (round or elliptic in equatorial view, sub-triangular in polar view) and tricolporate. The colpi are narrow with acute ends. The infratectum is 1–1.2 μm thick, comprising a single columellae layer subtended by a single spongy internal tectum. The columellae are aggregated under lophae and not swollen at their base. The inside of the endexine is smooth. The grains are caveate, the cavea shallow. The grains are echinate, with c. 74 spines sparsely clumped on lophae. The spines are 2–5 μm high and 2.5–4 μm in diameter at base, with perforations throughout, including at the apex, and hanging columellae inside. The pollen surface is smooth and sub-lophate,
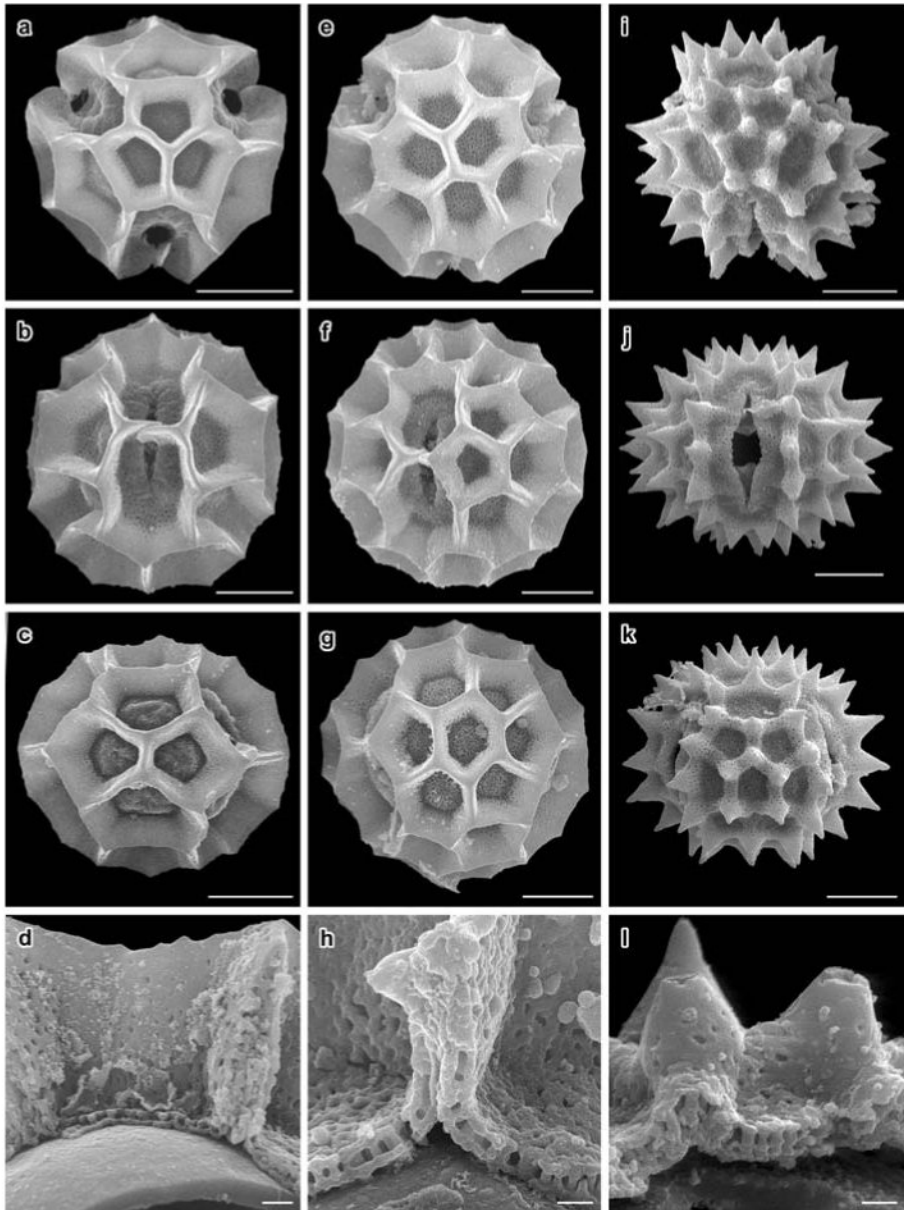
Fig. 7 SEM views of Arctotideae pollen. **a, c, d** *Hirpicium bechuarense*. **b** *H. echinus*. **a** Polar; **b** aperture, showing left lopha over right in pore region; **c** lateral, showing lophae on left and right margins of pollen grain; **d** fractured grain. **e–h** *H. integrifolium*. **e** Polar, note greater number of lacunae than in previous taxa; **f** aperture, showing left lopha over right in pore region; **g** equatorial; **h** fractured grain. **i–l** *B. spinosissima*. **i** Polar; **j** apertural; **k** equatorial; **l** fractured grain, showing single columellae layer with weakly developed horizontal internal tectum above a cavea. *Scale bars*: whole grains, 10 μm; fractured grains, 1 μm
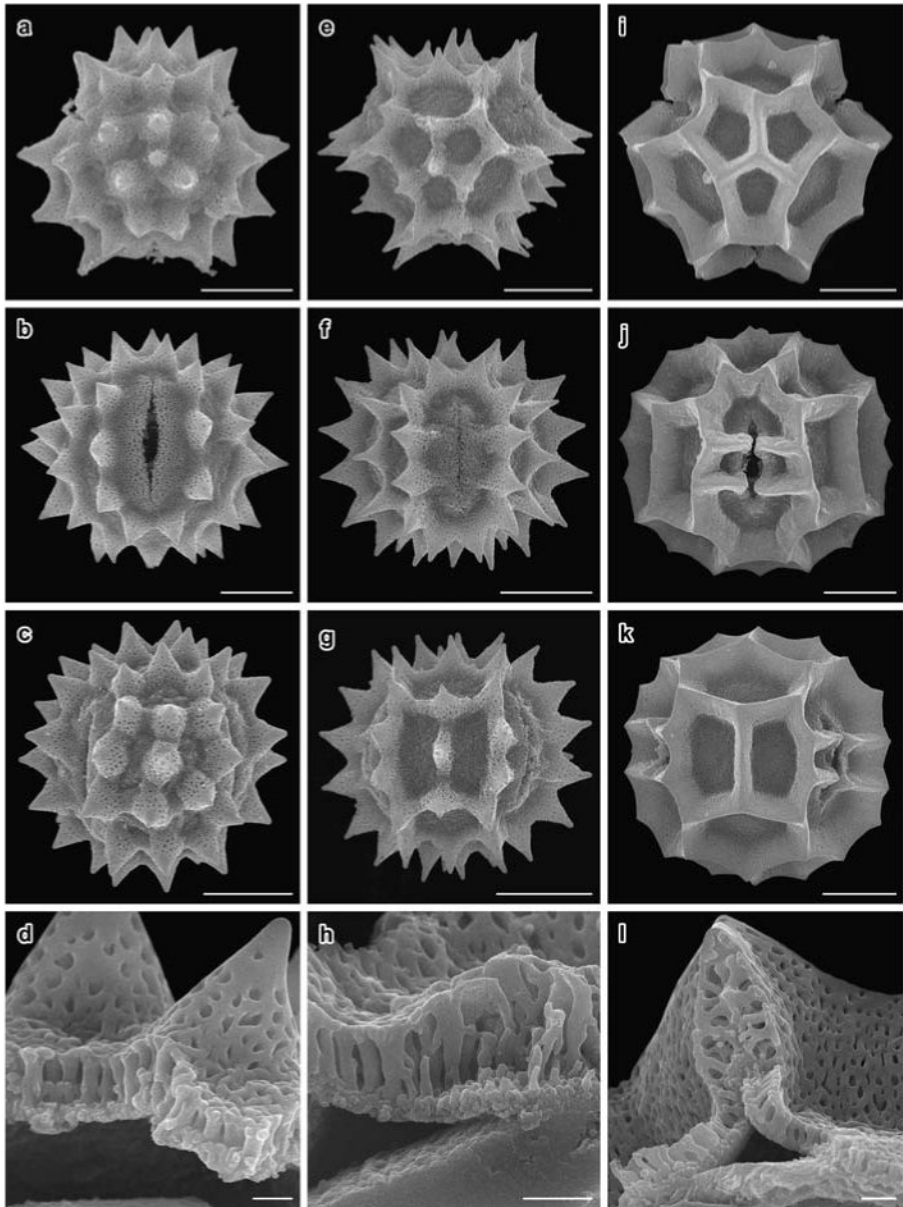
Fig. 8 SEM views of Arctotideae pollen. **a, c** *D. spinosa*. **b, d** *D. carnosa*. **a** Polar; **b** apertural; **c** equatorial; **d** fractured grain. **e–h** *Cuspidia cernua*. **e** Polar; **f** apertural; **g** equatorial; **h** fractured grain. **i–l** *B. rigida*. **i** Polar; **j** apertural, note two lophae in aperture area; **k** equatorial; **l** fractured grain, note similarity to Gorteriinae such as *Gazania* (Fig. 6h) and *Gorteria* (Fig. 6l.). *Scale bars*: whole grains, 10 μm; fractured grains, 1 μm

the lophae all connected and never crossing the apertures. There are six aboral lacunae, no interporal lacunae, six paraporal lacunae, no polar lacunae and three poral lacunae. The lacunae are 4–14 μm in diameter and the lophae bear characteristic small ridges across them.

The pollen of the Ber 2 and Ber 3 groups (Figs. 8i–l, 9a–l) is very similar and highly variable, at 27–33 μm in diameter, oblate-spheroidal (round or elliptic in equatorial view and round or sub-triangular in polar view) and tricolporate. The colpi are narrow with obtuse ends. The infratectum is 0.68–0.9 μm in diameter and
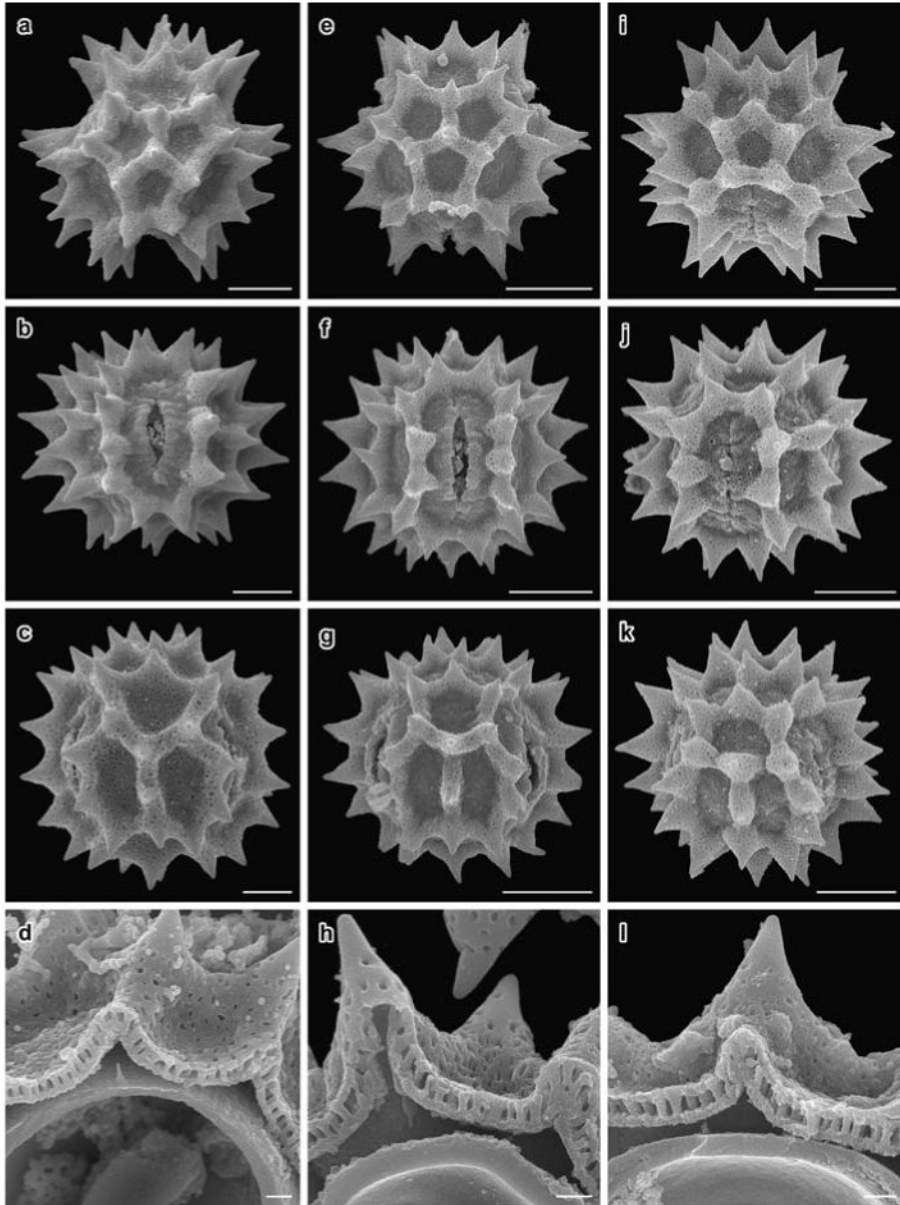

Fig. 9 SEM views of Arctotideae pollen. **a, b** *B. subulata*. **c, d** *B. pannosa*. **a** Polar; **b** apertural; **c** equatorial; **d** fractured grain similar to *B. rigida* (Fig. 8l). **e–h** *B. onopordifolia*. **e** Polar; **f** apertural; **g** equatorial; **h** fractured grain. **i–l** *B. radula*. **i** Polar; **j** apertural; **k** equatorial; **l** fractured grain. *Scale bars*: whole grains, 10 μm; fractured grains, 1 μm

comprises a single columellate layer subtended by a single, compact internal tectum. The columellae of this layer are aggregated or evenly distributed and not swollen at the base. The inside of the endexine is smooth. The grains are partially caveate, the cavea shallow, with thread-like columellae spanning it at intervals. The grains may be psilate or echinate, in which case having 60–72 spines, sparsely grouped on lophae. The spines are c. 1 μm high and 2–6 μm in diameter at the base, with perforations throughout, including at the apex, arising smoothly from the surface, with columellae fully attached to the foot layer inside. The pollen surface is reticulate and (sub-)lophate, the lophae all connected and never crossing the apertures. There are six or no abporal lacunae, four, six, eight or no interporal lacunae, four or six paraporal lacunae, six polar lacunae and three or six poral lacunae. The lacunae are very variable in size and shape, from 4–17 μm in diameter, which is characteristic of these groups. *B. angolensis* and *B. rigida* (Fig. 8i–l) differ significantly from the rest of the group, being strongly lophate and non-echinate, reminiscent of the grains of some *Cullumia* species—we consider these to represent the extreme of evolution in this group, from non- or sub-lophate, strongly echinate grains, to those that are lophate and psilate.

The pollen of *Heterorachis* (Fig. 10a–d) is 30–35 μm in diameter, oblate-spheroidal (round or elliptic in equatorial view, sub-triangular in polar view) and tricolporate. The colpi are medium width with obtuse ends. The infratectum is c. 1.5 μm thick, comprising two indistinct columellae layers separated by multiple compact internal tecta. The outer layer is 0.7–0.9 μm thick and the inner 0.7–1.2 μm. The columellae of the inner layer are evenly-distributed and not swollen at the base. The inside of the endexine is smooth. The grains are caveate, the cavea medium depth. The grains are echinate, with 74–80 spines clumped on lophae. The spines are 1–4.25 μm high and 3–4.5 μm in diameter at base, with perforations but solid at the apex (unusual for this group), arising smoothly from the surface, without columellae inside. The pollen surface is reticulate and sub-lophate.

The pollen of Ber 4 (Fig. 10e–l) is coherent and very different from the previous *Berkheya* groups, being much larger and non-lophate with a different exine infrastructure. It is c. 60 μm in diameter, oblate-spheroidal and tricolporate. The colpi are of medium width with acute ends. The infratectum is 1.6–2.5 μm thick, comprising two indistinct columellae layers separated by a single, compact internal tectum. Both columellae layers are 1–1.5 μm thick. The columellae of the inner layer are evenly-distributed and not swollen at the base. The inside of the endexine is smooth. The grains are caveate, the cavea of medium depth. The grains are echinate, with c. 79 spines, clumped in a pattern as if on lophae. The spines are 4.75 μm high and 4–7 μm in diameter at the base, with perforations throughout, including at the apex, slightly swollen at the base, with columellae fully-attached to the foot layer. The pollen surface is reticulate and raised between the spines but not lophate.

The pollen of *B. cruciata* (Ber 5; Fig. 11a–d) is also very different from the preceding *Berkheya* species, being psilate and heavily lophate—similar to that of *B. rigida* in Ber 2 (Fig. 8i–l). It is c. 40 μm in diameter, oblate (elliptic in equatorial view and sub-triangular in polar view) and tricolporate. The colpi are of medium width with obtuse ends. The infratectum is c. 1 μm thick and comprises a single columellate layer subtended by a single, compact internal tectum. The columellae are evenly-distributed and not swollen at the base. The inside of the endexine is smooth.
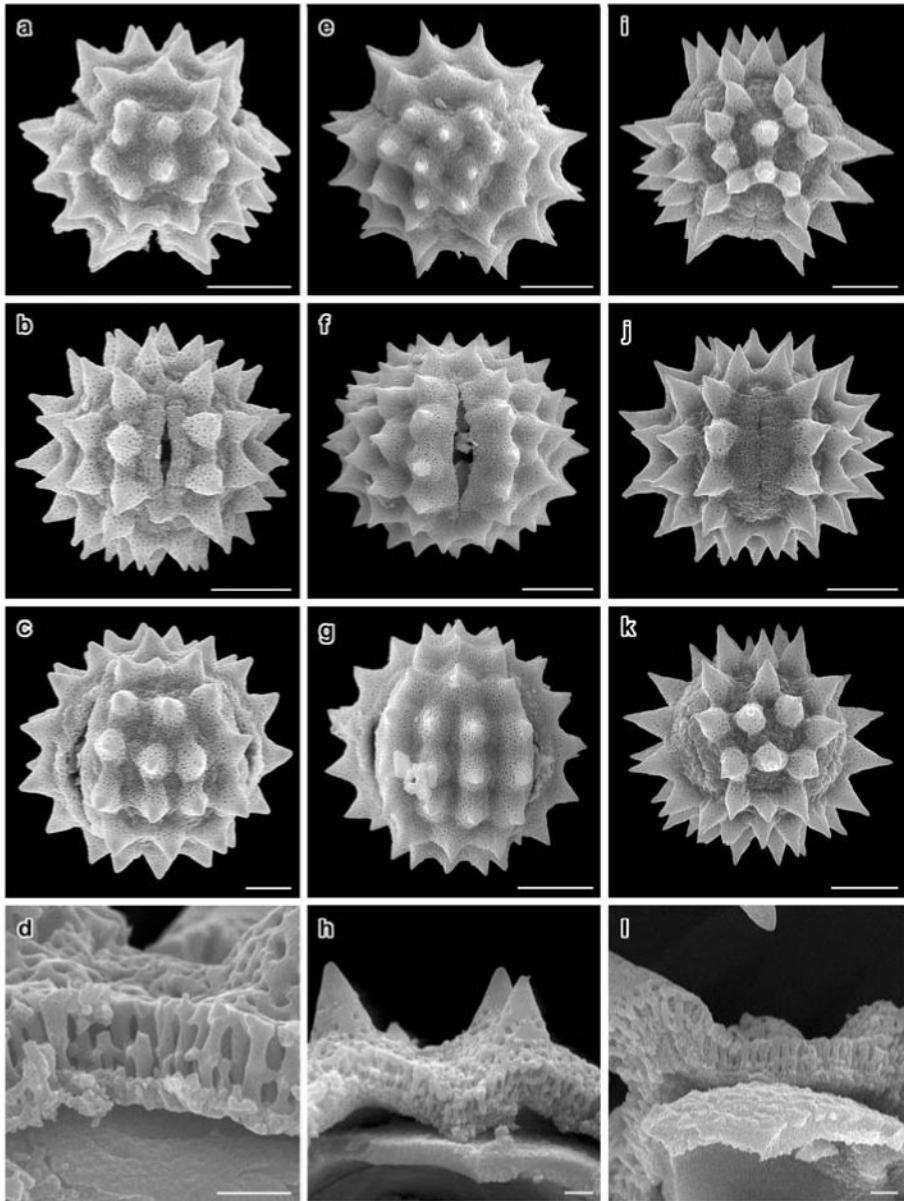
Fig. 10 SEM views of Arctotideae pollen. **a–d** *H. aculeata*. **a** Polar; **b** apertural; **c** equatorial; **d** fractured grain, showing internal tectum. **e–h** *B. canescens*. **e** Polar; **f** apertural; **g** equatorial; **h** fractured grain. **i–l** *B. fruticosa*. **i** Polar; **j** apertural; **k** lateral; **l** fractured grain, showing absence of internal tectum dividing columellae. *Scale bars*: whole grains, 10 μm; fractured grains, 1 μm

The grains are caveate, the cavea shallow. The grains are psilate. The pollen surface is reticulate and lophate, the lophae reaching to the apertures but failing to fuse across them. There are six abporal lacunae, six interporal lacunae, no paraporal lacunae, six polar lacunae and no poral lacunae. The lacunae are 10–20 μm in
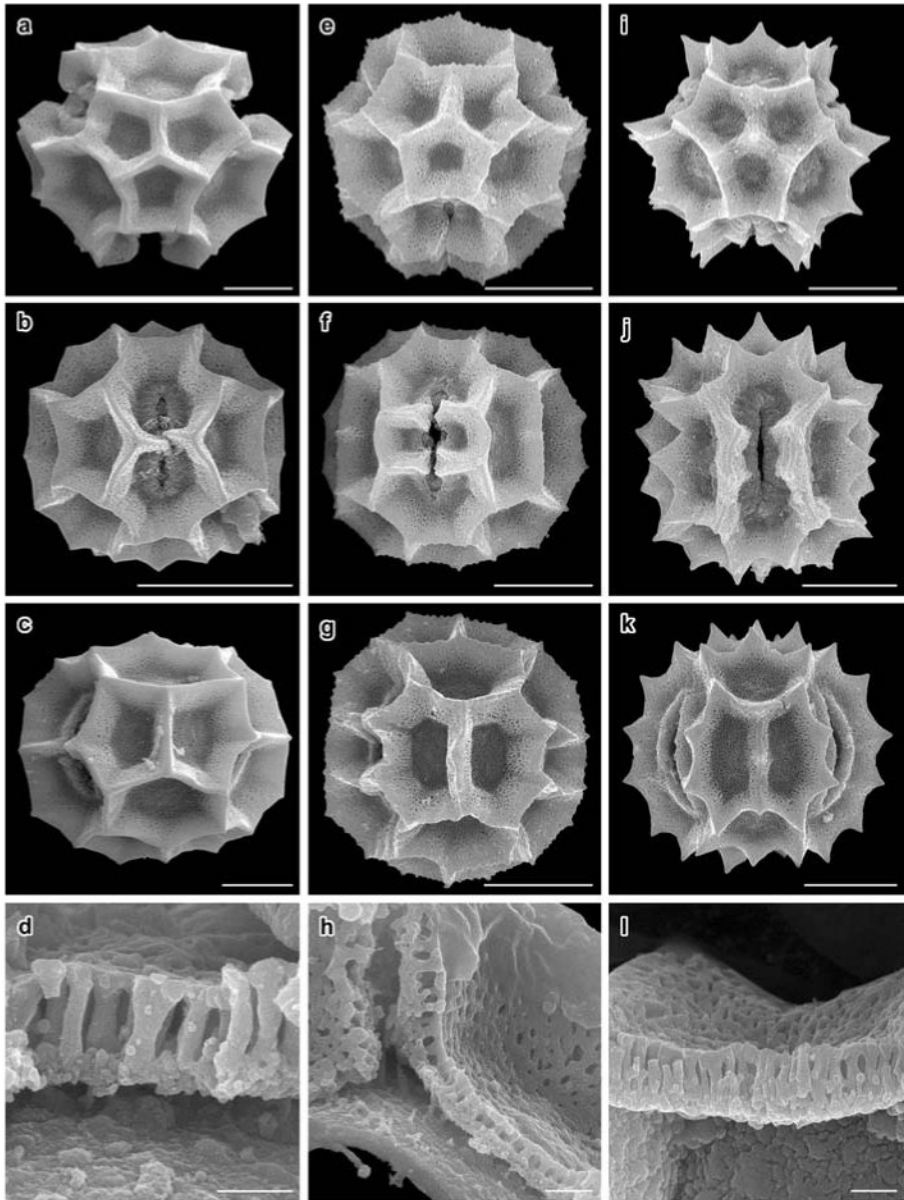
Fig. 11 SEM views of Arctotideae pollen. **a–d** *B. cruciata*. **a** Polar; **b** apertural, note that lophae at apertures lie *right* over *left*; **c** equatorial; **d** fractured grain. **e–h** *C. aculeata*. **e** Polar; **f** apertural; **g** equatorial; **h** fractured grain. **i–l** *C. bisulca*. **i** Polar; **j** apertural; **k** equatorial; **l** fractured grain. *Scale bars*: whole grains, 10 μm; fractured grains, 1 μm

diameter. It was noticed that in some grains of this species (e.g. Fig. 11b), as in some grains of *H. integrifolium* (not shown), the lophae at the apertures overlap with the lophae on the right side above that on the left. This is contrary to all other grains seen, in which the left lopha lies above the right. It was also noticed that such grains tend to be substantially smaller than normal (see scale on Fig. 11b).

The pollen of the Cul 1 group (Figs. 11e–l, 12a–d) is 24–26 µm in diameter, oblate-spheroidal (round or elliptic in equatorial view, round or sub-triangular in polar view) and tricolporate. The colpi are narrow and obtuse or acute at ends. The infratectum is 0.9–1.1 µm thick and comprises two distinct columellate layers
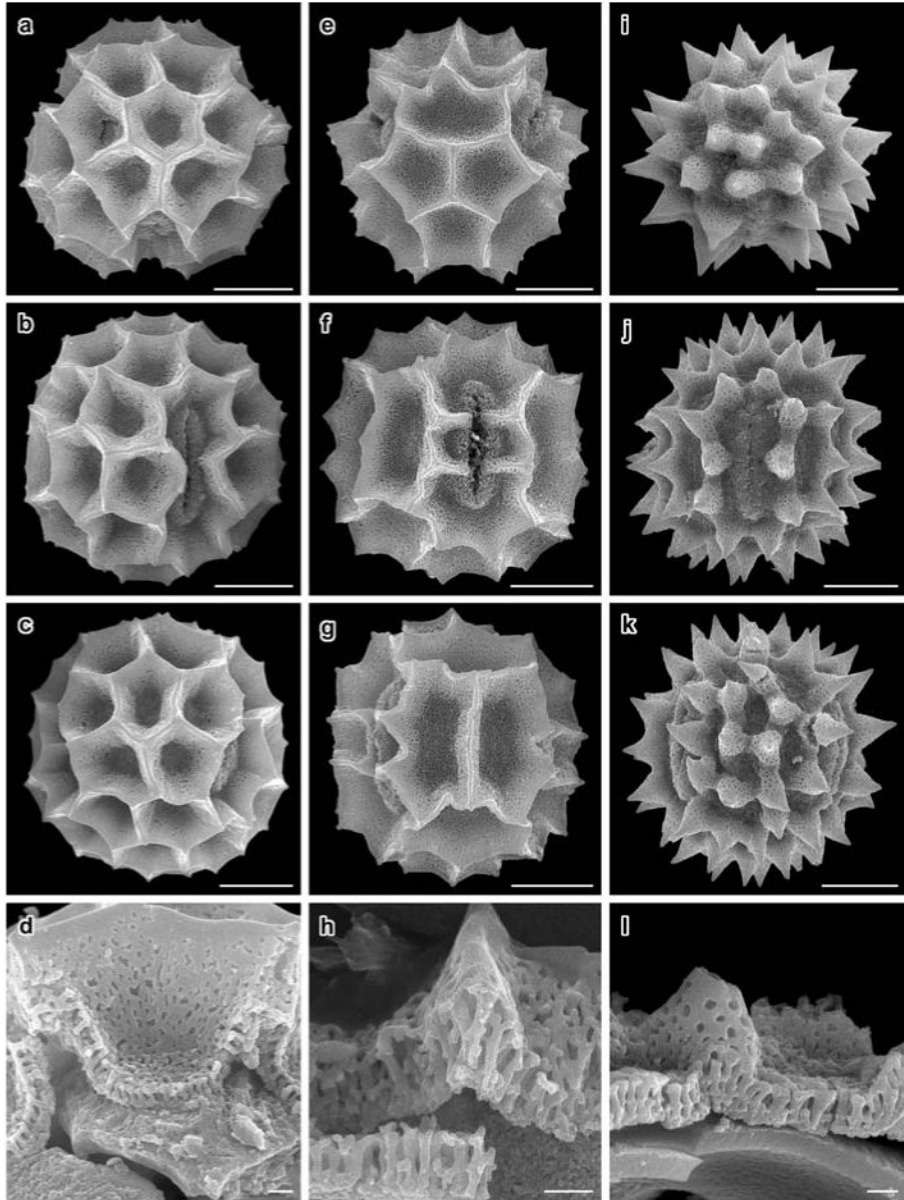

Fig. 12 SEM views of Arctotideae pollen. **a–d** *C. decurrens*. Note the greater number of lacunae compared to other *Cullumia* (Fig. 11e–l). **a** Polar; **b** apertural; **c** equatorial; **d** fractured grain, showing highly perforated and web-like nature of the vertical surface of the lophae. **e–h** *C. rigida*. **e** Polar; **f** apertural; **g** equatorial; **h** fractured grain. **i–l** *B. angustifolia*. **i** Polar; **j** apertural; **k** equatorial; **l** fractured grain. *Scale bars*: whole grains, 10 µm; fractured grains, 1 µm

separated by a single, compact internal tectum. The outer layer is 0.5–0.6 μm thick and the inner 0.4–0.5 μm. The columellae of the inner layer are aggregated under lophae and not swollen at the base. The inside of the endexine is smooth. The grains are partially caveate, the cavea shallow. The grains may be psilate or echinate, in which case having c. 50 spines, sparsely distributed on lophae. The spines are c. 1 μm high and c. 4 μm in diameter at the base, with perforations throughout, including at the apex, arising abruptly from the surface of the grain and with hanging columellae inside. The pollen surface is highly perforate, reticulate and lophate, with sharp points at the intersections of the lophae, the characteristically sharp lophae reaching to the apertures and meeting but failing to fuse across them. There are varying numbers of lacunae, 5–10 μm in diameter. *C. decurrens* is notable within this group for having the greatest number of lacunae, which do not meet at the apertures but alternate in a "zig-zag" fashion.

Although similar to other *Cullumia* species in terms of surface pattern, the pollen of *C. rigida* (Cul 2, Fig. 12e–h) differs significantly in exine ultrastructure from the previous group. It is 25–30 μm in diameter, oblate-spheroidal (round or elliptic in equatorial view, sub-triangular in polar view) and tricolporate. The colpi are medium width with acute ends. The infratectum is c. 0.4 μm thick, comprising a single columellae layer subtended by a single spongy internal tectum. The columellae of this layer are aggregated beneath lophae. The inside of the pollen surface is smooth. The grains are partially caveate, the cavea shallow. The grains may be psilate or echinate, in which case there are c. 54 spines sparsely distributed upon lophae. The spines are c. 1 μm high and 1.5–2 μm in diameter at the base, with perforations throughout, including at the apex, arising smoothly from the surface, with hanging columellae inside. The pollen surface is highly perforate, reticulate and lophate, the lophae reaching the apertures but not meeting or fusing across them. There are six abporal lacunae, three interporal lacunae, no paraporal lacunae, six polar lacunae and three poral lacunae. The lacunae are 5–8 μm in diameter.

The pollen of *Berkheya angustifolia* (Fig. 12i–l), which has not yet been included in molecular phylogenetic analyses, is quite different from other species, especially in exine infrastructure. It is c. 30 μm in diameter, spheroidal (round in equatorial view, round or sub-triangular in polar view) and tricolporate. The colpi are narrow with acute ends. The infratectum is 1.3–1.7 μm thick, comprising two indistinct columellae layers separated by a single, compact, internal tectum. The outer layer is 1–1.2 μm thick and the inner 0.5–0.7 μm. The columellae of the inner layer are evenly-distributed and not swollen at the base. The inside of the endexine is smooth. The grains are caveate, the cavea shallow. The grains are echinate, with c. 79 spines, sparsely and irregularly clumped in a lophate-type pattern. The spines are 4–6 μm high and 4–5 μm in diameter at the base, with perforations throughout, including at the apex, slightly swollen at base, without columellae inside (unusual for this group). The pollen surface is reticulate and sub-lophate.

The pollen of *Platycarpha* (Fig. 3a–d), is 15–25 μm in diameter, prolate-spheroidal (round in equatorial view and round or sub-triangular in polar view) and tricolporate. The colpi are narrow with acute ends. The infratectum is c. 3 μm thick and comprises two distinct columellate layers, separated by compact, multilayered internal tecta. The outer layer of fine columellae is 1–1.2 μm thick, the inner 1.5–2 μm thick, comprising very thick columellae which are highly branched at the distal

end but not swollen at the base, and are evenly distributed around the grain. The grains are ecaveate. The inside of the endexine is smooth. The grains are echinate, with more than 100 rounded spines distributed unevenly across the grain. The spines are 1 μm high and 2.5–3 μm in diameter at the base, with perforations and a solid apex, emerging smoothly from the surface, without a spine-channel, and containing columellae, fully attached to the foot layer. The pollen surface is smooth and not raised between spines; it is non-lophate.

Character Optimisation

The results of optimising pollen characters, as described above, on a phylogeny of Arctotidae (Funk et al., 2004) are shown in Fig. 13. The two well supported subtribes of the molecular phylogeny, Arctotidinae and Gorteriinae, can both be distinguished by well defined suites of pollen characters. The pollen of Arctotidinae (A) is characterised by a smooth-surfaced tectum and by a ratio of outer infratectum to inner infratectum thickness less than unity. Gorteriinae (G) pollen is almost always lophate or sub-lophate (the one exception is the highly-nested group represented by *B. cuneata*, not shown) and the grains tend to be more oblate than those of Arctotidinae. In addition, the well-supported grouping of *Eremothamnus* and *Hoplophyllum* is support by the pollen characters round-ended colpi and many, small, dense spines.

Palynological characters also distinguish some smaller clades within the subtribes. The genera *Gazania*, *Gorteria*, *and Hirpicium* (GAZ) can clearly be recognised by psilate, lophate grains with flap-like overlap of lophae across the colpi and a certain configuration of lacunae, broad, acute-ended colpi and an infratectum never greater than 0.5 μm thick. The *Didelta* clade is defined by a ratio of outer infratectum to inner infratectum thickness greater than unity, which is not found in its sister taxon, *B. spinosissima* (Ber 1).

The third major group, the BER clade, has no synapomorphic pollen characters. However, the DID and BER clades together share echinate pollen grains where the spines are arranged along (or, where absent, as if on) lophae. Excluding *Cuspidia*, the members of the BER clade share a reticulate pollen grain surface, and the smaller grouping of Ber 4, Ber 5 and *Cullumia* shares a tendency to revert to psilate grains.

Arctotidinae and *Heterolepis* share a two-layered infratectum, colpi with pointed ends and a fragmented internal tectum. This group plus *Eremothamnus* and *Hoplophyllum* share round, non-lophate, echinate grains. However these last characters are all plesiomorphies shared with out-group taxa, and therefore not useful in defining groups.

Discussion

As shown above, pollen characters provide synapomorphies for various levels of groupings within Arctotideae, including the two subtribes, Arctotidinae and Gorteriinae, the grouping of *Eremothamnus* and *Hoplophyllum*, the smaller GAZ and DID clades, the BER clade excluding *Cuspidia*, and the DID-BER clade (Fig. 13). The GAZ clade in particular is subtended by a large number of
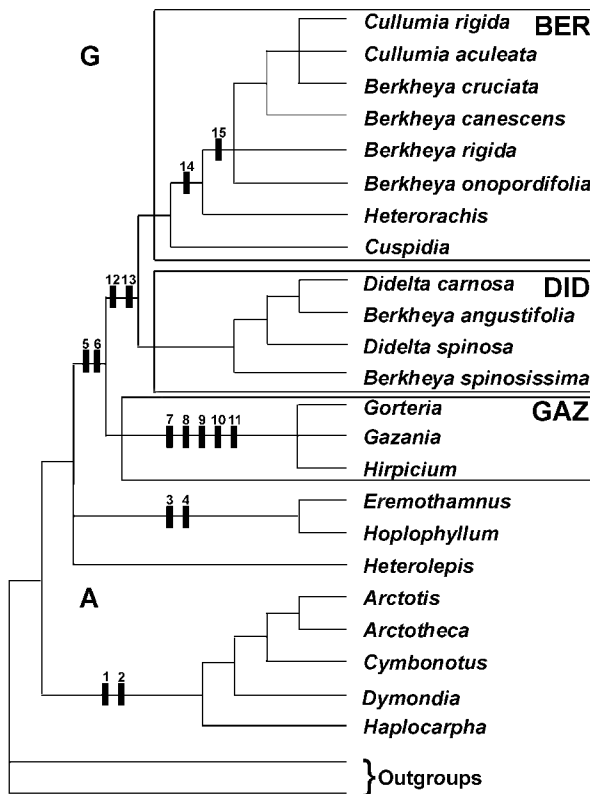
Fig. 13 Phylogeny of Arctotideae based on molecular data (modified from Funk et al., 2004) showing distribution of unambiguously synapormorphic characters. *1* Tectum surface smooth; *2* ratio of outer infratectum to inner infratectum thickness less than 1; *3* colpi ends obtuse; *4* spines many, small, dense; *5* tendency to produce elliptic grains; *6* grains (sub-)lophate (character reversed in *B. cuneata*); *7* lophae flap-like over colpi; *8* colpi broad; *9* colpi ends acute; *10* grains psilate; *11* infratectum less than 0.5 μm thick; *12* spines clumped on ridges; *13* grains echinate (reversed in *Cullumia* clade); *14* tectum surface reticulate; *15* tendency to produce psilate grains. *Grey boxes* indicate subtribes: *A* Arctotidinae; *G* Gorteriinae. *Outline boxes* indicate informally-named clades *sensu* Funk et al. (2004): *BER Berkheya–Cullumia* clade (no unambiguous synapomorphies); *DID Didelta* clade (no unambiguous synapomorphies); *GAZ Gazania–Gorteria–Hirpicium* clade

synapomorphic pollen characters (psilate, lophate grains with flap-like lophae overlaps, broad, acute-ended colpi and an infratectum less than 0.5 μm thick). This is consistent with the relatively long branch leading to this clade in the molecular phylogeny, implying ample time for characteristic pollen features to evolve, and coincident with a transition from shrubs to annual or perennial herbs along this branch.

The pollen of *Platycarpha* is distinguished from the rest of the tribe by a number of autapomorphies: its inner layer of columellae is much broader than those of the outer layers and distally branched, and it has rounded spines lacking spine channels. These features corroborate its previous exclusion from Arctotideae on molecular and macro-morphological grounds. It is not yet clear in which existing tribe the genus should be placed—it lies on a long branch within Cichorioideae and may be closest to Liabeae–Vernonieae or to Arctotideae (Funk et al., 2004; Funk & Chan, 2008).

However, it is interesting to note that the outer layers of the exine in *Platycarpha* (Fig. 3d), which contain columellae divided at the mid-point by a rough internal tectum, are similar in structure to the entire exine of some other Arctotideae such as *Arctotheca calendua* (Fig. 4h) and *B. canescens* (Fig. 10h). Such a pattern seems to repeat frequently throughout the family, and may represent convergent adaptation, or an underlying tendency for columellae to form such a ramified structure.

Apart from *Platycarpha*, the other "problem genera" also differ from the rest of the tribe in pollen morphological characters. *Eremothamnus* and *Hoplophyllum* are remarkably similar in pollen morphology: both genera exhibit many, dense, long, conical spines and a very similar, caveate infratectum. Both have a tendency to produce tetra-colporate grains. The members of Eremothamneae are divergent in pollen morphology from the remainder of Arctotideae, which is congruent with other divergent aspects of their morphology (Funk et al., 2004). We suggest that the pollen type, which is also similar to that of *Catananche* in tribe Cichorieae (Wortley et al., 2007), may represent something close to the plesiomorphic pollen condition for Cichorioideae as a whole.

It remains unclear whether Arctotideae are monophyletic and where the group should be rooted relative to other taxa in Cichorioideae. The only palynological feature that is unique to the tribe is the presence of six equatorial and six polar lacunae, but this is confined only to the lophate taxa. However, the features of the pollen in the grade comprising what are thought to be the most basal branches of the tribe (Arctotidinae, *Heterolepis*, *Hoplophyllum* and *Eremothamnus*) suggest that plesiomorphic palynological characters for Arctotideae may be round, non-lophate and echinate pollen. The presence of these character states in other tribes of Cichorioideae suggests that they may also be the plesiomorphic condition for the subfamily. This pattern remains common in Arctotidinae, but many taxa within Gorteriinae also retain this basic shape—for instance *Cuspidia*, one of the first-branching taxa in the BER clade.

Lophate pollen is considered to be a derived and effective adaptation for economic use of pollen wall materials while allowing harmomegathy (Blackmore, 1982). In contrast to the situation in Cichorieae (Blackmore, 1986), where lophate pollen is considered to have evolved independently a number of times, in Arctotideae it is most parsimonious to assume a single origin of the lophate condition, with a single reversal to non-lophate in *B. cuneata* (not shown). This is coincident with the fact that, although the pattern of lophae and lacunae may vary between species, all lophate Arctotideae share six equatorial and six polar lacunae. The transition to fully lophate pollen appears to have occurred gradually and can be hypothesised to have occurred through the clumping and merging of spines. Echinate, non-lophate pollen is the plesiomorphic condition present in Arctotidinae and Eremothamneae; in this scenario the spines become swollen at the base and clumped in a lophate-like pattern (sub-lophate grains) along the branch leading to Gorteriinae, and the spine bases become fully united into a ridge in many members of this group. In the GAZ clade, the merger is complete to the tips of the spines, giving the pollen grains a psilate appearance. The development of lophae is associated with a tendency to lose the lower layer of infratectum columellae, from the two-layered state found in Arctotidinae and *Heterolepis*. This trend can be seen in the Gorteriinae DID and BER clades (Fig. 14), where the majority of taxa (including most species of the polyphyletic

Fig. 14 Phylogeny of DID and BER clades of Gorteriinae based on molecular data (modified from Funk et al., 2004) showing distribution of sub-lophate and lophate pollen grains
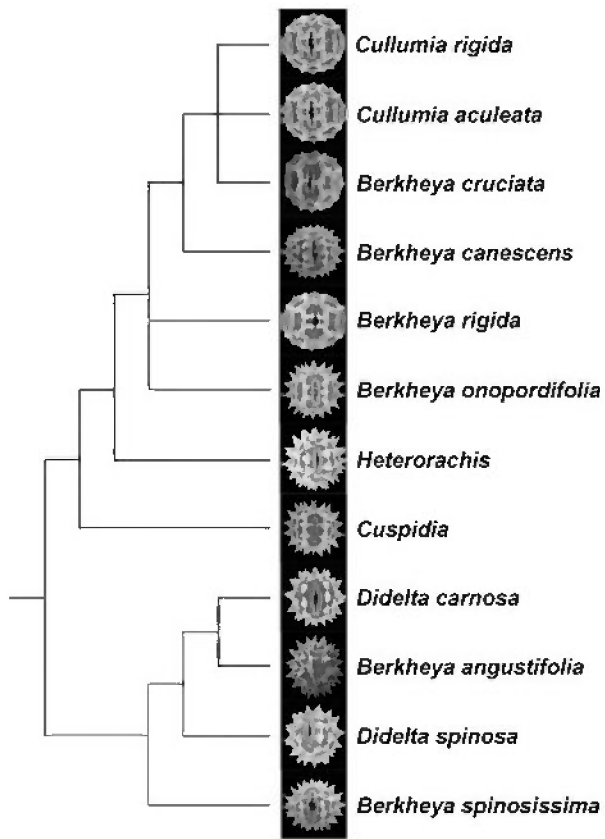
Berkheya) are sub-lophate. More pronounced ridged connections between spines are seen independently in *B. spinosissima*, *B. angolensis*, *B. rigida*, *B. onopordifolia* and *Cuspidia*, all of which have a reduced (single-layered infratectum). The most strongly lophate, psilate grains, are seen in the derived clade comprising *B. cruciata* and *Cullumia*. Within this, one branch (Cul 1) has a double-layered infratectum while its sister-group (*B. cruciata* and *C. patula*) have a single-layered one. The two groups of *Cullumia* may, however, be linked by a highly perforated tectum and the presence of two lophae ridges over each colpus, features they do not share with the closely-related *B. cruciata*.

The number of lophae is a variable character within Arctotideae, particularly in the monophyletic group containing Cul 1 and Ber 5. *B. cruciata* represents one extreme of this group, having just 26 large lacunae, and *C. decurrens* the other, with around 50, much smaller lacunae. The precise orientation of the lophae, particularly at the apertures, may be worthy of further investigation: as noted above, in some grains of *B. cruciata* and *H. integrifolium*, apertural views show the right-hand lopha overlapping above the left (Fig. 11b). This is contrary to the most common pattern, which is left over right (e.g. *Gazania*, Fig. 6e–g). In *B. cruciata*, the "right over left" pattern is associated with very small grains, approximately half the normal diameter. This leads us to hypothesise that such, rare, pollen grains are developmentally

abnormal in some way. A "right-over-left" pattern is also illustrated, but not discussed, in *Gazania pinnata* by Ueno (1972:104), but has not to our knowledge been documented elsewhere. Whether these abnormal grains are limited to these three species or are found in other lophate species, we have as yet been unable to determine, but their presence in three genera, not closely related within Gorteriinae, suggests the latter may be the case. It is also unclear whether the orientation of the lophae is important to normal (size) development or if both abnormal lophae orientation and reduced size are symptoms of some underlying developmental problem. This also merits further investigation.

In some species, such as *B. rigida* and *C. rigida* (Figs. 8j, 12f respectively), there are two lophae lying in the aperture region but in this case the lophae never overlap. This difference between overlapping and non-overlapping apertural lophae appears to be real and not an effect of acetolysis, since the apertures in the overlapping type are often more, rather than less, broad than the non-overlapping. If it were due to acetolysis we would expect overlapping to coincide with somewhat narrowed apertures. Furthermore, there is a qualitative difference in structure, whereby the overlapping lophae are based on extensions of the pollen wall which cross the aperture (e.g. Fig. 6f), whereas there is no such extension of the pollen wall in the non-overlapping ones (e.g. Fig. 12f). From the small sample studied here, it is not possible to say whether single ridges are always of the overlapping type and double ones always of the non-overlapping type, but we highlight this as something that may also be worthy of further study.

At subgeneric level, pollen characters cannot easily be used to characterise the five distinct clades of *Berkheya*. For instance, the pollen of Ber 1, which is in an entirely different clade to the rest of *Berkheya*, is hard to distinguish from that of some species of in Ber 2 and Ber 3, e.g. *B. onopordifolia* or *B. subulata*. Furthermore, the pollen of some taxa within Ber groups—such *B. rigida* and *B. pinnatifida* which are closely related within Ber 2, is quite different—*B. rigida* being strongly lophate and *B. pinnatifida* sub-lophate and strongly echinate—although they share the same basic pattern of lacunae, and could fit equally well into any of the clades within the BER clade.

In contrast, the pollen of Ber 4 (Fig. 4a–d) may be useful for distinguishing this group, being consistently different in size and structure from the other *Berkheya* groups studied. That of Ber 5 (Fig. 11a–d) is very different from those *Berkheya* species close to it in the phylogeny, but similar to that of *B. rigida* in Ber 2. The pollen of *B. angustifolia* (Fig. 12i–l) is quite different from other species, especially in exine infrastructure, and indicates it would be useful to include this, so far unsequenced species, in future molecular systematic studies to determine its relationships to other members of *Berkheya*.

The placement of *Heterorachis* remains uncertain (Funk et al., 2004). Its pollen retains many of the plesiomorphic characters of the group and as such is very similar to that of *Cuspidia*, *Arctotis*, etc. The continual recurrence of plesiomorphic characters, as seen in all the members of the DID clade, and repeated evolution of more derived ones, means that the use of pollen morphology to indicate relationships within higher Arctotideae remains problematic. At deeper levels, however, such as defining Arctotidineae, the GAZ clade, and the relationship of *Eremothamnus* to *Hoplophyllum*, pollen morphology can be highly informative.

Pollen morphology in Arctotideae has been shown to be highly variable. A number of characters vary at too low a level to be useful (are highly homoplastic at tribal level), for instance pollen grain size and shape, number of infractectum layers and their thicknesses, extent and depth of cavea, spine shape and internal structure, number and size of abporal, interporal, paraporal and poral lacunae, and internal endexine surface. However, other characters can be very informative when considered in the context of the current best estimate of phylogenetic relationships. These include ectoaperture width and shape at ends, mean infractectum thickness, ratio of infratectum layer thicknesses, internal tectum type, macro-ornamentation, spine number, density, distribution and size, and the form of lophae overlap at colpi. A consideration of these characters has led us to uncover several synapomorphies and diagnostic characters for use within Arctotideae (see Fig. 13). Detailed study of a wide range of carefully-conceptualised characters can enable those that are informative to be identified and used in classification and to corroborate molecular phylogenies. This approach should prove to be very useful in other groups, particularly in Compositae tribes outside the Asteroideae, where pollen morphological variation is similar to that seen in Arctotideae.

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