

Spines vs. microspines: an overview of the sculpture exine in selected basal and derived Asteraceae with focus on Asteroideae

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Abstract This study presents a detailed examination of the echinate and microechinate sculpturing in relation to the size of pollen grains in 31 selected species of Asteraceae belonging to the subfamilies Barnadesioideae, Mutisioideae, Carduoideae and Asteroideae. The aims were to recognize sculpturing patterns, under LM and SEM, within large and small pollen of both basal and derived species and to explore the features that could have taxonomic value to apply in palynological disciplines. The detailed examination of the exine surface showed both the relevance and limits of sculptural patterns for taxonomy. Under LM, the microechinate sculpture gave little taxonomic information, whereas in the echinate sculpture, three exine types and two subtypes were recognized. Type I included microechinate exine, which is commonly present in large pollen grains of the basal lineages. Types II (subtypes IIa and IIb) and III included echinate and smaller pollen grains. In these types, spines were always regularly arranged and, were characterized by the length, shape, tip, perforations and distribution. Type IIa included more or less conical spines usually with a distended base, less than 4 μm in length, present in species of different tribes like Astereae, Eupatorieae, Helenieae, Gnaphalieae, Senecioideae and Heliantheae to a lesser extent. Type IIb includes slender spines with narrower bases, longer than 4 μm , present in species of Coreopsideae, Heliantheae, Tageteae and Eupatorieae to a lesser extent. Type III included spines with swollen base, blunt tip and perforations over their entire surface. This type was

present in only one of the basal species—*Carduus thomermeri*—and in one species of the derived tribe Helenieae, *Gaillardia megapotamica*. Probably, this is due to evolutionary convergence.

Keywords Asteraceae · Microspines · Pollen size · Spines

Introduction

In the family Asteraceae pollen grains display a broad variety of shapes, sizes, apertures and, sculpturing and structure of the exine. The significance of these morphological features in understanding phylogenetic and taxonomic relationships in the family has long been recognized (Blackmore et al. 2009; Skvarla et al. 1977). It has been suggested that the pollen morphology in the family might be related to some external or internal factor. For instance, a very thick exine has been related to dry environmental conditions (e.g. Dimon 1971; Hidalgo et al. 2008; Silkjak-Yakovlev 1990; Tellería and Katinas 2004), whereas the size or volume of pollen grains has been linked to reproductive aspects (Torres 2000). Based on the study of the pistil length/pollen volume ratio of 43 basal and derived species of Asteraceae, Torres (2000) confirmed the influence of the post-pollination processes on the evolution of pollen size. Thus, the general pattern of pollen size reduction in relation to pistil length present in many families of Angiosperms (Lee 1978) is also present in Asteraceae. The study of Torres (2000) prompted exploration of whether other morphological features, such as the sculpturing, are related to different pollen sizes within that group of species. Both echinate and microechinate pollen, i.e. with pointed elements longer and shorter than 1 μm respectively (Punt et al. 2007), are extremely widespread in Asteraceae. Blackmore

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et al. (2009) hypothesized that the echinate condition is a clear synapomorphy in the family, whereas the microechinate condition is plesiomorphic. Pollen of Asteraceae has long been known to be commonly present in honey, pollen loads, sediments, etc. Then, it is necessary to explore which are the significant features of the exine for morphological comparisons and thus, to obtain an accurate taxonomic identification of pollen types. Concerning this, pollen from basal and derived taxa presents different degrees of complexity. The structure in derived groups is relatively more homogenous than the diversity of exine types found in the basally branching lineages. The recognition of a number of morphotypes of Asteraceae in Patagonian sediments (e.g. Barreda et al. 2008; Palazzesi et al. 2009) illustrates the utility of exine types in basal taxa of this family. In contrast, in echinate pollen of derived taxa, information about the usefulness of exine features remains frequently unclear. The present study comprised the examination of the echinate and microechinate sculpturing of 31 basal and derived species belonging to the 26 genera of Asteraceae studied by Torres (2000). Most of these species which are phylogenetically derived according to Funk et al. (2009), have not been previously examined from a palynological point of view. Attention was focused on the shape, height and distribution patterns of both spines and microspines and, tectal perforation.

In this context, the aims of this study were: (1) to recognize patterns of sculpturing within the echinate and microechinate exine that might correspond with size categories of pollen grains and (2) to examine which are the features in the echinate pattern that commonly occur in derived species and, may thus have taxonomic value to apply in palynological analysis.

Materials and methods

Echinate and microechinate pollen from 41 specimens, representing 31 species of Asteraceae included in the study of Torres (2000) was studied. Nowadays, nomenclature of three of these species was changed: *Eupatorium clematideum* Griseb. is *Praxelis clematidea* (Griseb.) R. M. King & H. Rob., *Eupatorium inulaefolium* Kunth is *Austroeupatorium inulaefolium* (Kunth) R. H. King & H. Rob. and, *Grindelia discoidea* Hook. & Arn. is *G. pulchella* var. *discoidea* (Hook. et Arn.) Adr. Bartoli & Tortosa; whereas some species are placed in other tribes, *Bidens pilosa* and *Cosmos sulphureus* belong to Coreopsideae, *Achyrocline tomentosa* to Gnaphalieae and, *Porophyllum ruderale* and *Tagetes minuta* to Tageteae. Pollen was obtained from specimens deposited in the herbarium of the “División Plantas Vasculares del Museo de La Plata”, LP (Holmgren et al., <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>).

Additional published information on the pollen of both basal and derived species available in the literature is also discussed. Pollen grains were acetolyzed according to Erdtman (1960) at 100 °C restricted for 2 min; the slides were prepared by mounting the pollen in glycerol jelly and sealing with paraffin wax. Measurements of diameters of pollen grains and length of spines were taken on 20 and 15 pollen grains respectively from each specimen under LM Nikon Eclipse 200 microscope using 100× oil immersion objective, and a crossed micrometer eyepiece graticule. Measures of diameters exclude spines. Measurements of lengths of microspines were taken from 1 to 3 SEM images from each specimen. For SEM, acetolyzed pollen grains were suspended in 90% ethanol, mounted on stubs and examined using a JEOL JSM T-100 SEM. Means (\bar{X}) and standard deviations (SD) were calculated for all measures, i.e. equatorial and polar diameters, length of microspines and spines. In order to categorize pollen size, the classification proposed by Erdtman (1969) based on the largest diameter was followed, i.e. small pollen grains: 10–25 µm, medium 25–50 µm and large 50–100 µm.

Specimens studied: *Achyrocline tomentosa* Rusby: Hurrell 100 (LP); *Acmella decumbens* var. *affinis* (Sm.) R. K.: Jansen Frangi 297 (LP); *Austroeupatorium inulaefolium* (Kunth) King and Rob.: Sayago 2285 (LP); *Baccharis articulata* (Lam.) Pers.: Bottino 28 (LP); *Bidens pilosa* L.: Cabrera 10647 (LP); *Carduus thoermeri* Weinm.: Tellería 46 (LP), Torres Robles 501 (LP); *Centaurea solstitialis* L.: Cabrera 7511, 10082 (LP); *Chaptalia nutans* (L.) Polák: Venturi LP 006302; *Chuquiraga erinacea* D. Don: Soriano 1246 (LP), Cabrera 9035 (LP); *Cosmos sulphureus* Cav.: Delucchi 701 (LP); *Eupatorium arnotianum* Griseb.: Kiesling and Meglioli 6660 (LP); *E. hookerianum* Griseb.: Cabrera et al. 21976 (LP); *E. subhastatum* Hook. et Arn.: Cabrera and Fabris 3 (LP); *Flourensia campestris* Griseb.: Burkart 7501 (LP); *Gaillardia megapotamica* (Spreng.) Baker: Job 2947 (LP), Forcone 525 (CORD), Amat 320 (LP); *Grindelia pulchella* var. *discoidea* (Hook. et Arn.) Adr. Bartoli and Tortosa: Hunziker 3556 (LP); *Hyalis argentea* D. Don: Cabrera et al. 24298 (LP); *Mikania urticifolia* Hook. et Arn.: Rodrigo 28 (LP); *Mutisia decurrens* Cav.: Cabrera and Job 346 (LP), Grüner 132 (LP), Soriano 4294 (LP); *M. spinosa* Ruiz et Pav.: Landrum 4378 (LP), Hollermayer 725 (LP); *Porophyllum ruderale* (Jack.) Cass: Stuckert 6841 (LP), Montes 2234 (LP); *Praxelis clematidea* (Griseb.) R. M. King and H. Rob.: Fabris and Crisci (LP); *Senecio pampeanus* Cabrera: Boffa 1087 (LP); *Solidago chilensis* Meyen: Delucchi 3239 (LP); *Stevia satuireifolia* (Lam.) Lam.: Frangi 287 (LP); *Tagetes minuta* L.: Heringer 8385 (LP); *Trichocline reptans* (Wedd.) Hieron.: Cabrera et al. 24099 (LP); *Verbesina encelioides* (Cav.) Benth. & Hook.: Tolaba 3477 (LP); *Wedelia glauca* (Ortega) Hofm.: Cabrera 2112 (LP); Hurrell et al. 6865 (LP); *Zexmenia*

buphtalmiflora (Lorentz) Ariza: Delucchi 2134 (LP); *Zinnia peruviana* (L.) L.: Dawson 93 (LP).

Results and discussion

Microspines, spines, and size of pollen grains

Among the studied species, the exine surface and pollen size define three main morphological Types (I, II and III) and two subtypes (IIa and IIb) (Table 1; Fig. 1). Type I includes large pollen with microechinate exine of the most basal lineages represented by members of Mutisioideae: *Chuiraga erinacea*, *Chaptalia nutans*, *Mutisia decurrens*, *M. spinosa*, *Hyalis argentea*, *Trichocline reptans*, and Cardioideae such as *Centaurea solstitialis* (Fig. 3). This list can be enlarged with 104 species of basal Mutisioideae (Tellería and Katinas 2004, 2005, 2009) and 20 species of Gochnatioideae (Tellería et al. 2013) which also share large and medium pollen grains with microechinate sculpturing. Within the group studied by Torres (2000), the large pollen grains were correlated with the longest styles and with the phylogenetic position within the family (Torres 2000). In this first morphological group microspines are always unevenly arrangement, i.e. they do not appear to be in any particular pattern on the pollen surface, and the tectum is commonly little perforated. Under LM they are difficult to distinguish (Fig. 3a, b), but under SEM microspines appear as: (1) minute microspines on the surface as in *Chuiraga erinacea* (Fig. 2a) and most species in the genus (Urtubey and Tellería 1998); (2) sharp and uneven-sized, interspersed with minute granules as in *Chaptalia nutans* (Fig. 2b) and *Trichocline reptans* or; (3) uneven-sized with the base distended as in *Centaurea solstitialis* (Fig. 2c) and *Mutisia* species (Tellería and Katinas 2009). An exception in the basal species studied is the pollen of *Carduus thoermeri* which is echinate. Other basal taxa that also share echinate exine are *Gongylolepis* and *Wunderlichia* (Tellería 2008; Tellería et al. 2010; Ubierno et al. 2009) together with 42 members of the subfamily Gochnatioideae such as the monotypic genus *Cnicothamnus* and species of *Anastrophia*, *Gochnatia*, *Pentaphragus* and *Richterago* (Tellería et al. 2013). However, in all these cases the spines show a irregular arrangement whereas in *Carduus thoermeri* spines show a regular arrangement, appearing to be uniformly distributed over the pollen surface as in derived taxa.

The echinate exine characterizes Types II and III which are commonly present in derived species (Figs. 3c–l, 4, 5). In both Types spines usually follow a regular arrangement, i.e. it appears that they form a hexagonal, or pentagonal, pattern with one spine at each angle and one in the center of the hexagon with all spines equidistant from each other (Figs. 4a–d, f–h, 5a–b, d, f, h). The shape of the spine is

sometimes an elusive character to define but, in the studied group it can be roughly characterized by the shape of both base and tip, and tectal perforations; these features appear to be useful for recognizing distinct patterns. Type II includes small—and to a lesser extent—medium pollen grains, with more or less conical spines, with an acute tip and the tectum perforated only in the base. On the basis of spine length and shape of the spine base, two subtypes were recognized: IIa with spines commonly less than 4 μm in length, with distended base (Figs. 3d, f–h, 4) as in *Achyrocline tomentosa*, *Acmella decumbens* var. *affinis*, *Austro-eupatorium inulaefolium*, *Baccharis articulata*, *Eupatorium arnotianum*, *E. hookerianum*, *E. subastatum*, *Grindelia pulchella* var. *discoidea*, *Praxelis clematidea*, *Senecio pampeanus*, *Solidago chilensis*, *Stevia satuireifolia* and *Zinnia peruviana*; sometimes the distance among spines is very reduced giving the impression of a higher density of spines as in *Achyrocline*, *Baccharis*, *Eupatorium arnotianum*, *E. subastatum*, *Solidago* and *Stevia* (Fig. 4a–b, d, f), and IIb with spines longer than 4 μm , commonly with narrower bases, as they emerge abruptly from the exine surface (Figs. 3i, 5a–b, d) as in *Bidens pilosa*, *Cosmos sulphureus*, *Flourensia campestris*, *Mikania urticifolia*, *Porophyllum ruderalis*, *Tagetes minuta*, *Verbesina encelioides*, *Wedelia glauca* and *Xexmenia buphtalmiflora*. In some species, variability was detected; for instance in *Baccharis articulata* spines varied in length (Figs. 3d, 4b), whereas in *Eupatorium arnotianum* and *E. subastatum* the tip of the spines may be acute, rounded or truncate (Fig. 4d, e). This variability may lack taxonomic value because it may result from stress produced by insufficient nutrients and water (Skvarla et al. 2003). Type III includes spines with swollen base, blunt tip and perforations over its entire surface (microreticulate surface) as in *Gaillardia megapotamica* (Fig. 5h, i) and the basal species *Carduus thoermeri*, in this species bases are cushion-shaped (Fig. 5f, g). Additionally, the inner structure is also different in both spine types; in Type II, columellae only occupy the base of the spine in coincidence with tectum perforations as also observed—under TEM—in diverse Asteraceae (Skvarla et al. 1977) and in *Pulicaria* (Pereira Coutinho and Dinis 2007). In contrast, in Type III columellae occupy the whole interior of the spine, leaving a small space named the apical channel, toward the tip (Skvarla et al. 1977) (Fig. 6a, b). From a physiological point of view, tectal perforations act as repositories for active substances related to self incompatibility; they are connected with the cavea through the columellar interstices and the channels which occur below each spine (Blackmore 1982). Around apertures, spines generally follow a particular arrangement. Commonly, at each side of the ectoaperture there is a row of spines disposed in a manner that they are embedded or separate when the pollen grain is dehydrated or hydrated respectively, like a

Table 1 Microspines and spines length, polar and equatorial diameters of pollen grains, size categories according to Erdtman (1969) and pollen types and subtypes. Values show means \pm SD

Subfamily	Tribe	Species	Microspines and spines length (μm)	Polar diameter (μm)	Equatorial diameter (μm)	Size categories	Pollen type
Barnadesioideae		<i>Chuquiraga eri-nacea</i>	0.15 \pm 0.05	34.42 \pm 1.46	37.00 \pm 0.60	Medium	I
Mutisioideae		<i>Chaptalia nutans</i>	0.43 \pm 0.12	29.48 \pm 1.84	43.55 \pm 2.80	Large	I
		<i>Mutisia decurrens</i>	0.31 \pm 0.11	62.40 \pm 3.41	83.20 \pm 4.80	Large	I
		<i>M. spinosa</i>	0.47 \pm 0.14	40.50 \pm 2.84	61.19 \pm 3.90	Large	I
		<i>Hyalis argentea</i>	0.56 \pm 0.32	47.53 \pm 3.35	54.64 \pm 1.11	Large	I
		<i>Trichocline reptans</i>	0.19 \pm 0.07	41.93 \pm 4.53	55.84 \pm 4.39	Large	I
Carduoideae		<i>Carduus thoermeri</i>	6.08 \pm 0.54	50.80 \pm 2.81	49.30 \pm 2.07	Medium–large	III
		<i>Centaurea solstitialis</i>	0.65 \pm 0.30	30.76 \pm 1.44	37.34 \pm 2.016	Medium	I
Asteroideae	Astereae	<i>Baccharis articulata</i>	2.17 \pm 0.49	15.71 \pm 1.01	15.52 \pm 1.36	Small	IIa
		<i>Grindelia pulchella</i> var. <i>discoidea</i>	3.16 \pm 0.47	27.23 \pm 0.97	27.16 \pm 1.58	Medium	IIa
		<i>Solidago chilensis</i>	2.13 \pm 0.46	19.46 \pm 0.85	18.96 \pm 1.09	Small	IIa
	Coreopsideae	<i>Bidens pilosa</i>	4.75 \pm 0.71	25.98 \pm 1.15	25.54 \pm 1.26	Medium	IIb
		<i>Cosmos sulphureus</i>	6.88 \pm 0.75	25.30 \pm 1.10	24.90 \pm 0.70	Small–Medium	IIb
		<i>Austroeupatorium inulaefolium</i>	1.00 \pm 0.00	18.89 \pm 0.84	19.40 \pm 1.18	Small	IIa
	Eupatorieae	<i>Eupatorium arnotianum</i>	3.08 \pm 0.46	27.44 \pm 1.70	28.45 \pm 1.56	Medium	IIa
		<i>E. hookerianum</i>	2.00 \pm 0.00	23.10 \pm 1.65	26.55 \pm 1.49	Medium	IIa
		<i>E. subhastatum</i>	2.53 \pm 0.52	23.01 \pm 1.81	22.79 \pm 1.38	Small	IIa
		<i>Mikania urticifolia</i>	4.32 \pm 0.53	23.75 \pm 3.50	22.79 \pm 0.67	Small	IIb
		<i>Praxelis clematidea</i>	1.90 \pm 0.00	17.88 \pm 1.54	15.52 \pm 3.42	Small	IIa
		<i>Stevia satureiifolia</i>	3.00 \pm 0.00	21.15 \pm 1.04	22.17 \pm 1.41	Small	IIa
	Gnaphalieae	<i>Achylocline tomentosa</i>	1.8 \pm 0.33	18.72 \pm 0.92	18.43 \pm 0.86	Small	IIa
	Heliantheae	<i>Acmella decumbens</i> var. <i>affinis</i>	3.51 \pm 0.48	25.45 \pm 1.26	24.25 \pm 1.30	Small–Medium	IIa
		<i>Flourensia campestris</i>	5.93 \pm 0.58	28.18 \pm 1.65	28.13 \pm 1.17	Medium	IIb
		<i>Verbesina encelioides</i>	5.25 \pm 0.52	25.64 \pm 0.78	24.73 \pm 0.67	Small–Medium	IIb
		<i>Wedelia glauca</i>	5.01 \pm 0.49	28.08 \pm 2.21	30.07 \pm 1.16	Medium	IIb
		<i>Xexmenia buphtalmiflora</i>	4.75 \pm 0.00	30.36 \pm 0.95	20.37 \pm 1.00	Medium	IIb
		<i>Zinnia peruviana</i>	3.51 \pm 0.67	25.74 \pm 1.41	25.22 \pm 3.02	Small–Medium	IIa
		<i>Gaillardia megapotamica</i>	9.14 \pm 0.90	44.62 \pm 1.61	47.00 \pm 0.30	Medium	III
Senecioneae	<i>Senecio pampeanus</i>	2.85 \pm 0.00	27.52 \pm 2.30	28.13 \pm 1.13	Small	IIa	
Tageteae	<i>Porophyllum ruderale</i>	8.00 \pm 0.94	30.20 \pm 1.84	38.71 \pm 0.67	Medium	IIb	
	<i>Tagetes minuta</i>	4.75 \pm 0.00	30.23 \pm 2.48	29.68 \pm 3.54	Medium	IIb	

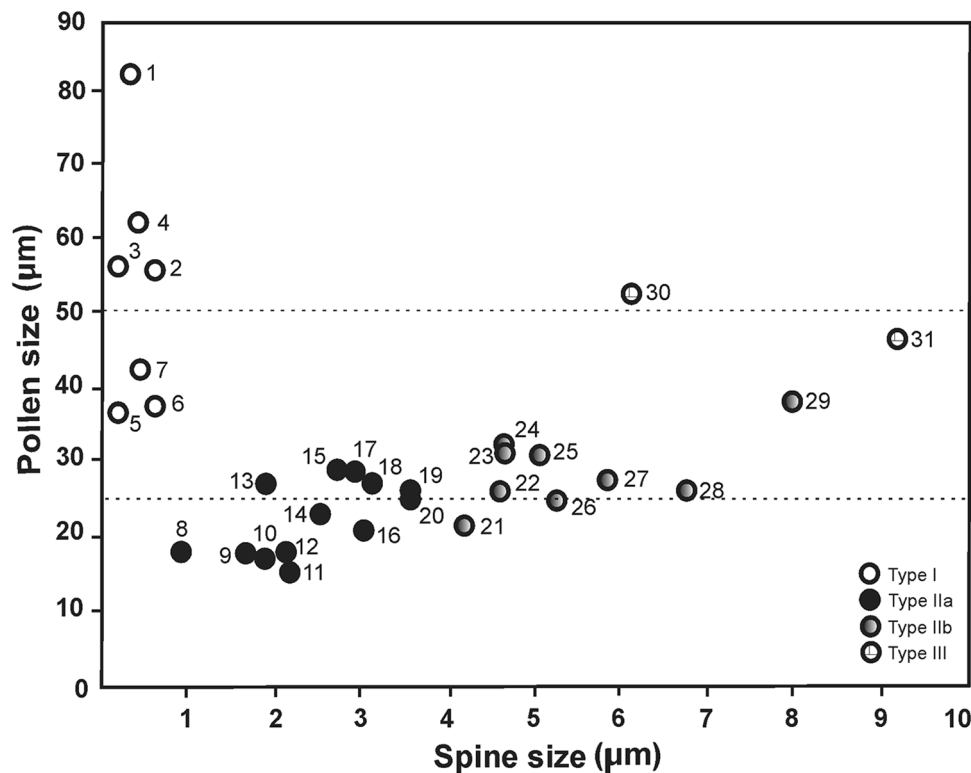


Fig. 1 Relationships between pollen grains size and spines length in 31 species of Asteraceae species. 1 *Mutisia decurrens*. 2 *Hyalis argentea*. 3 *Trichocline reptans*. 4 *Mutisia spinosa*. 5 *Chuquiraga erinacea*. 6 *Centaurea solstitialis*. 7 *Chaptalia nutans*. 8 *Austro eupatorium inulaefolium*. 9 *Achyrocline tomentosa*. 10 *Praxelis clematidea*. 11 *Baccharis articulata*. 12 *Solidago chilensis*. 13 *Eupatorium hookerianum*. 14 *Eupatorium subhastatum*. 15 *Senecio pampeanus*. 16 *Stevia satuireifolia*. 17 *Eupatorium arnotianum*. 18 *Grindelia*

pulchella var. *discoidea*. 19 *Zinnia peruviana*. 20 *Acmella decumbens* var. *affinis*. 21 *Mikania urticifolia*. 22 *Bidens pilosa*. 23 *Tagetes minuta*. 24 *Xexmenia buphtalmiflora*. 25 *Wedelia glauca*. 26 *Verbesina encelioides*. 27 *Flourensia campestris*. 28 *Cosmos sulphureus*. 29 *Porophyllum ruderale*. 30 *Carduus thoermeri*. 31 *Gaillardia megapota mica*. Dotted lines separate categories of pollen size according to Erdtman (1969)

zip (Fig. 5c, e). This zip could protect the pollen content against desiccation. According to Heslop-Harrison (1969), spines in pollen of Asteraceae are distributed maximizing the distance among them to prevent the hypertrophy of groups at less than a minimum distance. This author also mentioned that the position of spines is a consequence of epigenetic phenomena of pattern formation and it is not under direct genetic control. Interestingly, these phenomena appear to be absent in echinate pollen of basal species like *Wunderlichia mirabilis*, *Gongylolepis paniculata* and in members of Gochnatioideae (Tellería 2008; Tellería et al. 2013) but not in pollen of *Carduus thoermeri*. In the basal subfamily Carduoideae, both regular (as in *Carduus thoermeri* Fig. 5f) and irregular distribution patterns of spines or microspines (as in *Centaurea solstitialis* Fig. 2c) coexist (see also Tormo Molina and Uebera Jiménez 1995). According to the experimental study of Chaloner (1986) prominent exine ornamentation, as in the echinate pollen, could promote adherence of pollen grains to the stigma for a longer time, favoring effective pollination. In this context, the

microspines—common in basal species—and the spines regularly arranged—common in derived species—could have functional and evolutionary significance (Fig. 7).

Microspines, spines, and taxonomy

The analyzed features allowed the recognition of different sculpturing patterns but if no other features of exine are considered, their taxonomical utility is limited. Among basal taxa and under LM, the relative uniformity of microechinate pollen surface is compensated by a thick exine whose structural diversity contains taxonomic and phylogenetic significance (e.g. Tellería 2008). In contrast, in derived taxa the exine structure is quite uniform under LM, commonly with only one layer of thin columellae. Only two Types were established, and each subtype encompasses different tribes of the subfamily Asteroideae; in particular the Subtype IIa includes species of Astereae, Eupatorieae, Gnaphalieae, Heleniae, Heliantheae and Senecioideae (Table 1). This is in line with the results obtained

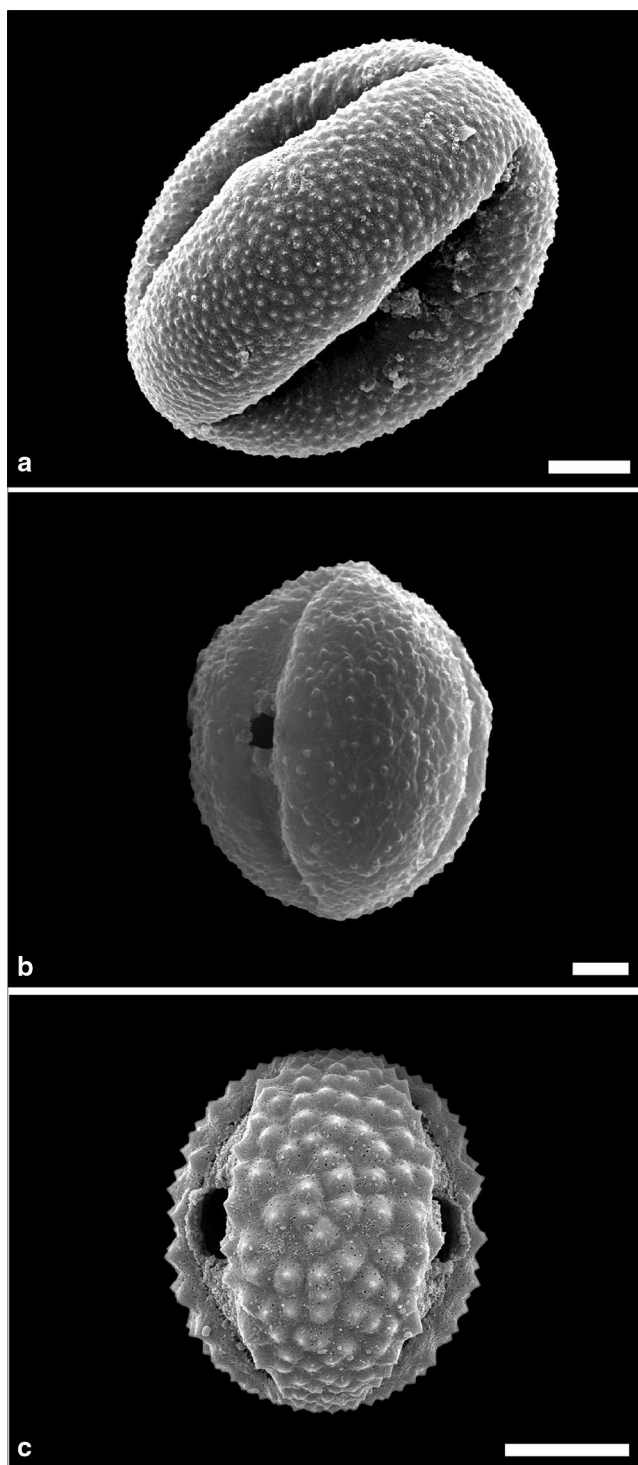


Fig. 2 SEM images of microechinate exine in selected Asteraceae species. Note the irregular arrangement of microspines that are shorter than 1 µm. **a** *Chuquiraga erinacea*. Note the minute microspines. **b** *Chaptalia nutans* showing microspines interspersed with granules. **c** *Centaurea solstitialis* showing microspines with distended bases. Scale bars are 5 µm

by Rodrigues Cancelli (2008) who defined different Types morphologically similar to subtype IIa. Those Types also include different tribes of Asteroideae reinforcing the overlap of pollen features among these tribes. For instance, the *Eupatorium* Type included diverse species of Eupatorieae [*Adenostemma brasilianum* (Pers.) Cass., *Austroeupatorium inulaefolium*, *Eupatorium pedunculatum* Hook. & Arn., *E. squamosum* D. Don, *E. purpureum* L., *Gymnocoronis spilanthoides* (D. Don) DC., *Kaunia rufescens* (DC.) King & Rob., and *Mikania viminea* DC.] and Senecioideae (*Erechtites hieraciifolia* (L.) Raf., *E. valerianifolia* (Link.) Less.) and, the *Baccharis* Type included species of Astereae (*Baccharis articulata*, *B. dentata* (Vell.) Barroso, *B. megapotamica* Spreng., *B. patens* Baker, *B. sagittalis* (Less.) DC., *B. spicata* (Lam.) Baill., *B. stenoccephala* Baker, *B. trimera* (Less.) DC., *B. usteri* Heering and *Erigeron blackei* Cabrera) and Eupatorieae (*Mikania cordifolia* (L.f.) Willd. and *M. micrantha* Kunth). Within Asteroideae, the precise identification of a particular species is uncommon but there are some exceptions as that of *Gaillardia megapotamica*. Curiously, spines of this derived species are similar in overall shape, tip morphology, tectum surface, distribution pattern and most structural features to those of the basal species *Carduus thoermeri* (Figs. 6, 5f, i). However, spines are notably longer in *G. megapotamica* and less numerous, which is consistent with its small size. Structurally, they are also similar although the exine in *G. megapotamica* is markedly caveate whereas in *C. thoermeri* the presence of cavea is uncertain; under LM, a very narrow separation between sexine and nexine was detected in some grains after acetolysis (Fig. 6a). Probably, this is the first example that shows convergence between basal and derived Asteraceae for this character. In the remaining species spines are only perforated at the base but differ in length and in the shape of the base. Pollen of species of the tribe Coreopsideae (*Bidens* and *Cosmos*), Heliantheae (*Flourensia*, *Verbesina*, *Wedelia* and *Xexmenia*), and Tageteae (*Porophyllum*), differed most due to their long and slender spines. By examining pollen grains of *Helenium*, *Schkhuria* and *Tagetes*, Stix (1960) considered that tribes Helenieae and Heliantheae were close. Those observations were later confirmed by exine ultrastructure (Skvarla et al. 1977) and relatively recently by molecular features (Funk et al. 2009). In examined species of Eupatorieae (*Austroeupatorium inulaefolium*, *Eupatorium arnotianum*, *E. hookerianum*, *E. subhastatum*, *Mikania urticifolia*, *Praxelis clematideum* and *Stevia satureifolia*), Astereae (*Baccharis articulata*, *Grindelia pulchella* var. *discoidea* and *Solidago chilensis*) and, Inuleae (*Achyrocline tomentosa*) features of pollen grains generally overlap (Fig. 1). However, some studies of pollen in different subtribes of Astereae, Gnaphalieae and Inuleae have considered diverse features of the sculpturing such as: the number of spines in polar optical section

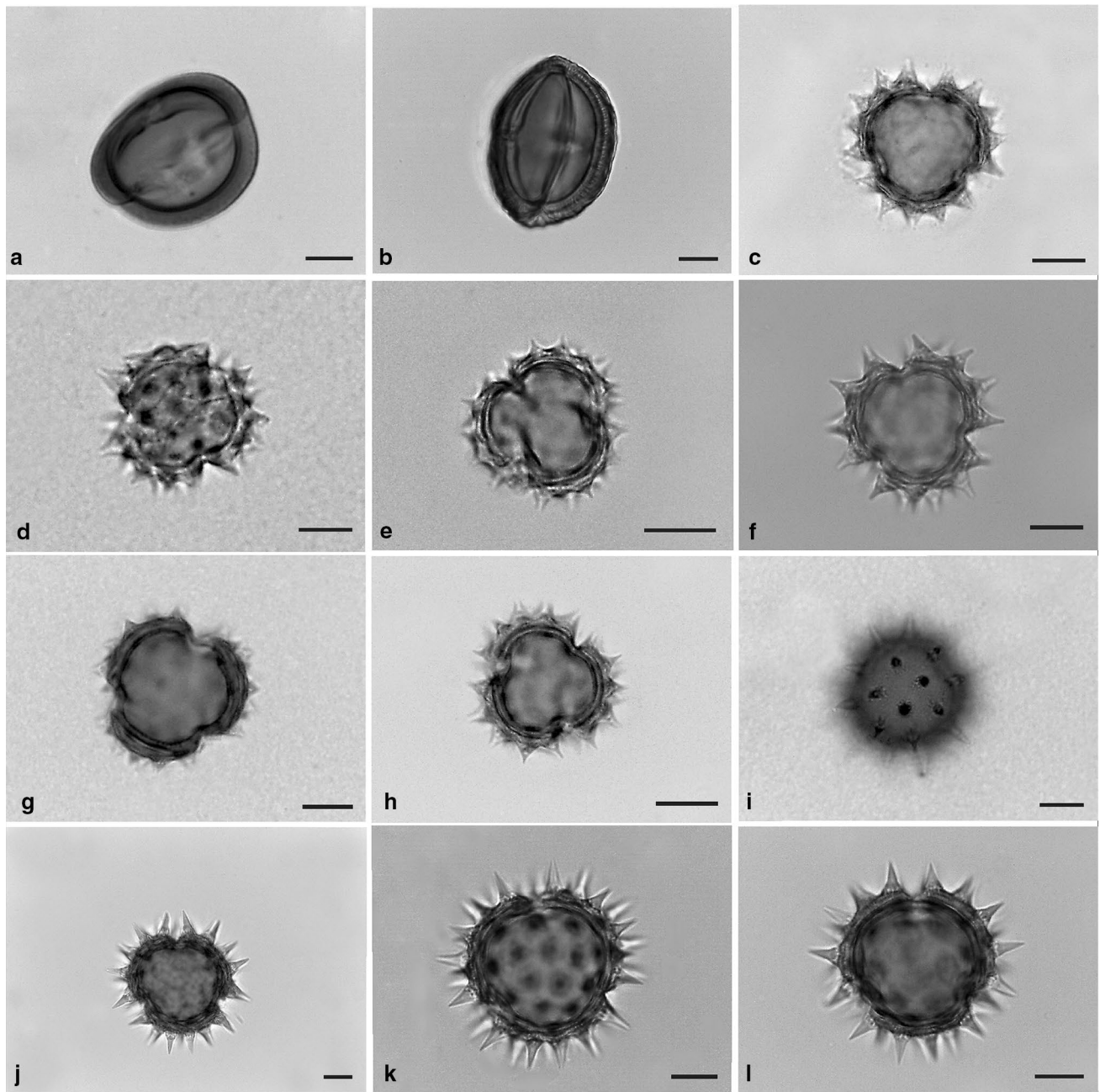


Fig. 3 Optical sections of whole grains in selected Asteraceae species. **a, b** Type I. **a** *Chuquiraga erinacea*. **b** *Chaptalia nutans*. Note that few microspines are distinguishable in optical section but the structure of exine is different among them. **c–l** echinate exine. Pollen grains in polar view for comparison. Type IIa. **c** *Acmella decumbens* var. *affinis*. **d** *Baccharis articulata*. Note the spines with differ-

ent length. **e** *Austro eupatorium inulaefolium*. **f** *Mikania urticifolia*. **g** *Senecio pampeanus*. **h** *Solidago chilensis*. Note the spines with distended bases. Type IIb. **i** *Cosmos sulphureus*. Note the narrow bases of the spines. **j** *Porophyllum ruderale*. **k** *Xexmenia buphtalmiflora*. **l** *Wedelia glauca*. Note columellae occupying the basis of spine. Scale bars are 10 μm

(e.g. Rodríguez Cancelli 2008); the shape and length/width ratio of spines together with their density (i.e. number of spines/ $100 \mu\text{m}^2$ of surface); the proportion of the spine that is perforated (expressed as a fraction); the diameter of tectum perforations (Pereira Coutinho and Dinis 2007, 2009); the apex shape of spines (Pereira Coutinho et al. 2011) and

the length and width of spines and distance among them (Righetti de Abreu et al. 2015). Although the pollen in these groups commonly presents appearance of uniformity, the taxonomic significance of spines features was showed in this study.

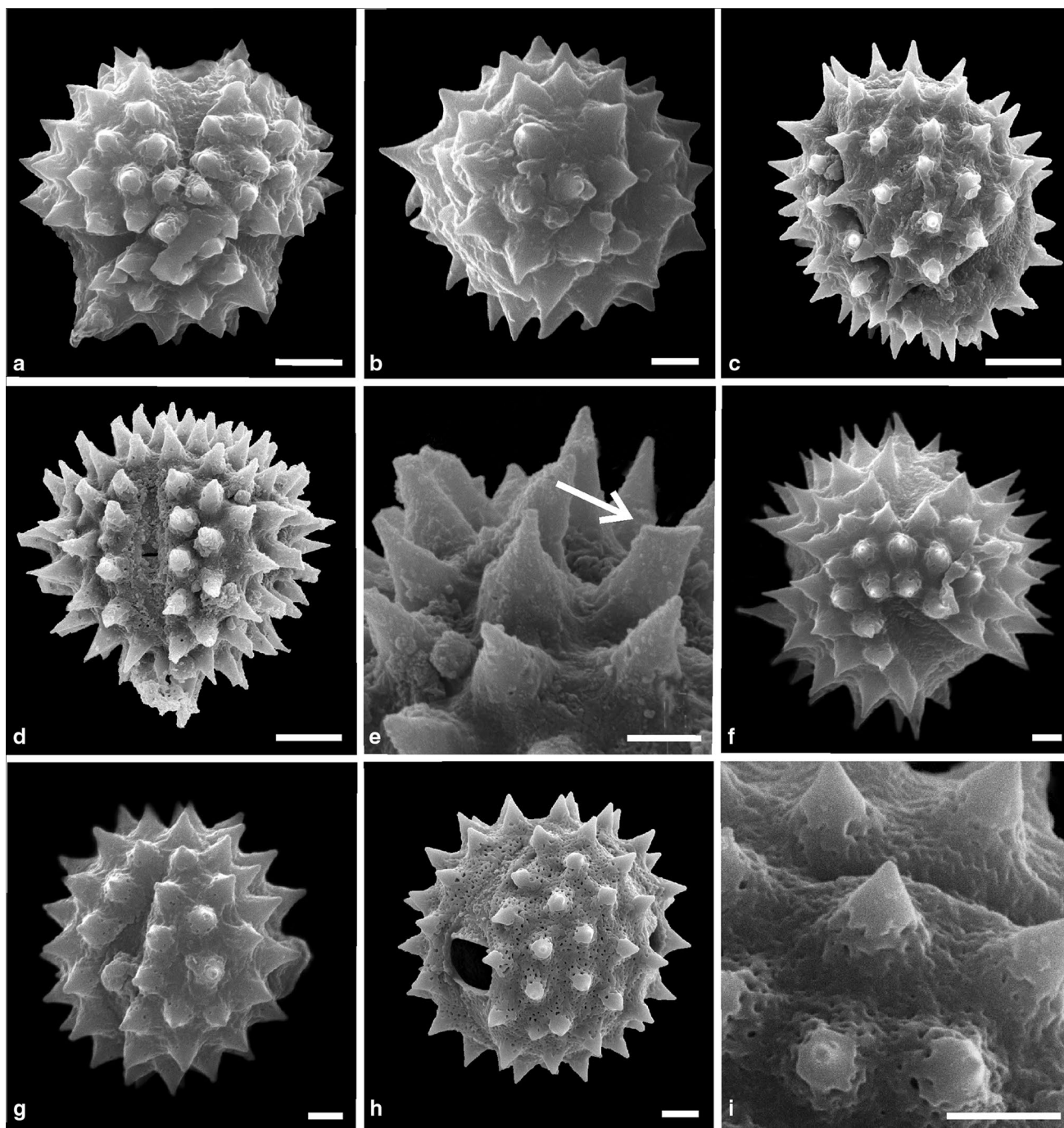


Fig. 4 SEM images of echinate exine in selected Asteraceae species with Type Ia. Spines are 1–4 μm long, conical, with distended base and showing regular pattern of distribution. **a** *Solidago chilensis*. **b** *Baccharis articulata*. Note the spines with different length. **c** *Austroepatorium inulaefolium*. **d, e** *E. subhastatum*. Detail of spines with

acute and truncate tips (arrow). **f** *Stevia satureiifolia*. **g** *Achyrocline tomentosa*. **h, i** *Senecio pampeanus*. **i** detail of spines showing perforated bases. Scale bars are 5 μm in **a, c, d, g, h**; 2.5 μm in **b**, 2 μm in **e, i**

Conclusions

In the studied species, pollen size and sculpturing vary in opposite ways: larger pollen grains of basal species are microechinate with uneven distribution of microspines

whereas, smaller pollen grains of derived species are echinate, with spines arranged in a clear pattern (Fig. 7). Detailed examination of microechinate and echinate pollen provides useful information on the distribution of sculpturing patterns and potential use as a diagnostic

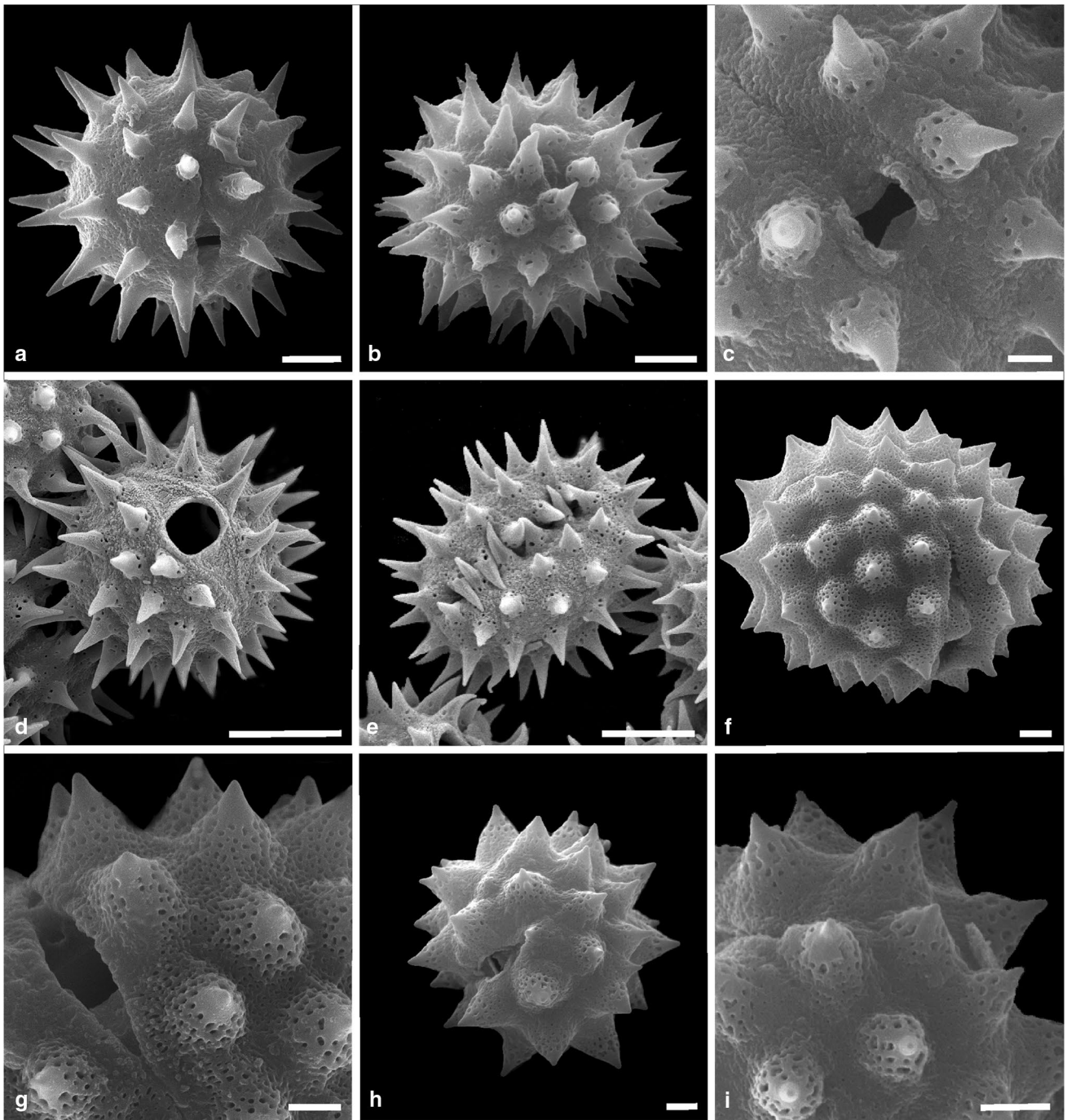


Fig. 5 SEM images of echinate exine in selected Asteraceae species with Type IIb or Type III. **a–e** Type IIb. Spines are longer than 4 μm , slender, with perforated narrow bases and showing regular distribution pattern. **a** *Cosmos sulphureus*. **b, c** *Porophyllum ruderale*. **c** apertural view, showing the arrangement of spines at both side of ectoap-

erture level. **d, e** *Xexmenia buphtalmiflora*. **e** spines are closed like a zip. **f–i** Type III. Spines with swollen base and completely perforated tectum. **f, g** *Carduus thoermeri*. **h, i** *Gaillardia megapotamica*. Scale bars are 5 μm in **a, b, f, h, i**, 10 μm in **c, g**

feature in palynological disciplines. Within basal species different microspine types were distinguished under SEM, whereas in derived species, features of spines such as length, shape, tip, perforations and distribution over the pollen surface allowed recognizing three

different types under LM. In agreement with other studies, these types commonly characterize groups of tribes. Type I characterizes Astereae, Eupatorieae, Gnaphalieae, Helenieae, Senecioideae and Heliantheae to a lesser extent; Type II characterizes species of Coreopsidae,

Fig. 6 LM micrographs of whole grains in optical section of Type III, showing spines with swollen bases and columellae occupying the whole interior of the spine. **a** *Carduus thoermeri*. Note the *cushion-shaped* spines. **b** *Gaillardia megapotamica*. Arrows indicate the cavea. Scale bars are 10 μm

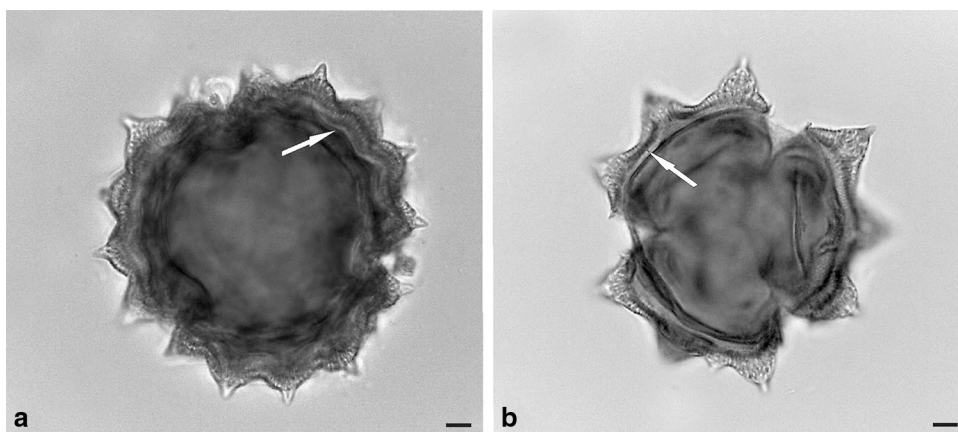
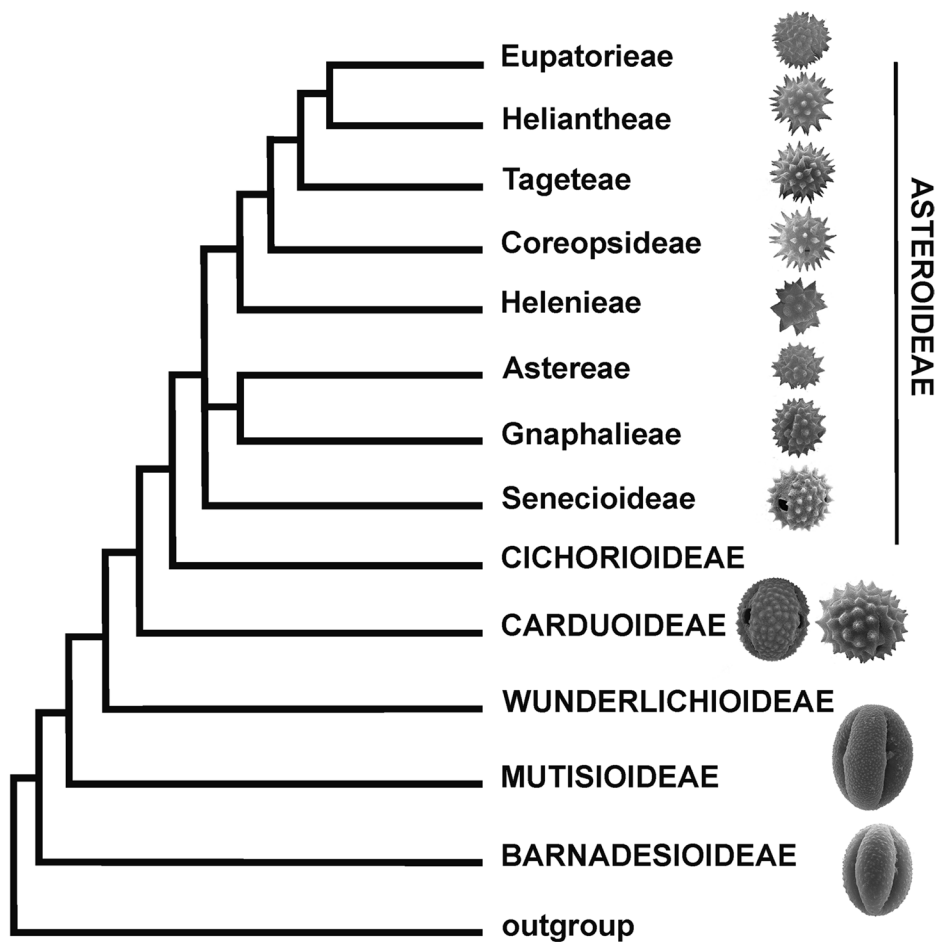


Fig. 7 Phylogeny of Asteraceae based on molecular data (modified from Funk et al. 2009) showing distribution of studied pollen types according to their size and sculpture



Heliantheae, Tageteae and Eupatorieae to a lesser extent; and Type III includes the basal species *Carduus thoermeri* and the derived *Gaillardia megapotamica*. Pollen from both species shares the sculpturing pattern and the features of spines, suggesting a probable evolutionary convergence. The inclusion of a larger number of species, including those of other tribes which were not considered in the present study, would reinforce these results

and would support the hypotheses about the probable functional significance of echinate sculpturing within Asteraceae.

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