

Pollen studies in the genus *Echinops* L. and *Xeranthemum* group (Asteraceae).

T. Garnatje¹ and J. Martín²

¹*Institut Botànic de Barcelona (CSIC-Ajuntament de Barcelona), Passeig del Migdia
s.n., Parc de Montjuïc, 08038 Barcelona, Catalonia, Spain.*

²*Laboratori de Botànica, Facultat de Farmàcia, Universitat de Barcelona, Av. Joan
XXIII, s.n., 08028 Barcelona, Catalonia, Spain.*

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Corresponding author: Teresa Garnatje. Institut Botànic de Barcelona (CSIC-Ajuntament de Barcelona). Passeig del Migdia s.n. Parc de Montjuïc. 08038 Barcelona. Catalonia. Spain. Phone: 34-932890611. Fax: 34-932890614. E-mail: laboratori@ibb.csic.es

Abstract:

The genus *Echinops* and the *Xeranthemum* complex have been related in our first molecular study of the tribe Cardueae. A survey of pollen morphology and ultrastructure with electron microscopy was carried out in the present paper in all the genera belonging of these groups, allowing to identify four pollen types. The exine pattern indicates that there are two pollen types in the genus *Echinops* (including *Acantholepis*), although no differences have been found between sections in *Echinops*. On the one hand, the annual species of *Echinops* and *Acantholepis orientalis* show a pollen type that differs from that of perennial species of the former genus and on the other hand, the genera *Chardinia* and *Xeranthemum* exhibit a distinct type of pollen to that of *Amphoricarpos* and *Siebera*. The internal structure of pollen is the same for all the genera. Only slight differences were observed in the tectum, but in all cases these concerned the anthemoid type. Statistically significant correlations between pollen size and the life cycle were found.

Key words: *Acantholepis*, *Amphoricarpos*, *Chardinia*, Compositae, electron microscopy, palynology, *Siebera*.

Introduction

The genus *Echinops* appears closely related with the genera of the *Xeranthemum* complex in our phylogenetic preliminary study about the Cardueae (Garcia-Jacas *et al.*, 2002). This is the main reason for which we undertook a palynological survey of all this group.

The genus *Echinops* L. belongs to the tribe Cardueae Cass. and has traditionally been considered the basis for the subtribe Echinopsidinae O. Hoffm., one of the four into which the tribe has been divided. The genus *Acantholepis* Less. is classified in the same tribe (Bentham, 1873; Hoffmann, 1894; Dittrich, 1977; Bremer, 1994; Susanna & Garcia-Jacas, 2005).

The genus *Echinops* occupies semi-humid zones in tropical Africa, semi-arid lands in North Africa, the Mediterranean basin, and temperate regions in Eurasia up to Central Asia (Jäger, 1987). *Echinops* comprises between 125 and 130 species, which are classified in eight sections: *Echinops*, *Chamaechinops* Bunge, *Nanechinops* Bunge, *Oligolepis* Bunge, *Pleiacme* Bornm., *Ritro* Endl., *Ritrodes* Bunge and *Terma* Endl. (Shishkin & Bobrov, 1997). *Nanechinops* differs from the others, because it includes three annual species, while all the remaining ones are biennial or perennial.

Rechinger (1979) postulated a distinct sectional organization, and divided *Echinops* into five sections: *Oligolepis* Bunge, *Ritropsis* Greuter and Rech., *Echinops* s. l., *Hololeuce* Rech. and *Nanechinops* Bunge. Fries (1923) classified the *Echinops* species from East of Africa in four sections: *Cenchrolepis* Hochst. (which includes *E. giganteus* A. Rich.), *Hamolepis* R. E. Fr., *Phaeochaete* Bunge and *Pterolepis* O. Hoffm. The section *Phaeochaete* includes *E. mildbraedii* Matff. *Echinops hispidus* Fresen., has been placed in the sections *Terma* and *Oligolepis* (Mattfeld, 1924). *Echinops hispidus* and *E.*

giganteus occur in the Eastern tropical Africa whereas the distribution of *E. mildbraedii* is restricted to Cameroun, Nigeria and Central African Republic (Jeffrey, 1968). We could not follow only one sectional classification for two reasons: i) the sections proposed by the different authors do not agree; ii) some African sections are not represented in Eurasia (and viceversa) and every author studied only a geographical area.

Acantholepis is a monotypic annual genus which includes *A. orientalis* Less., a species that occurs in the near East and in West Asia as far as Pakistan and Afghanistan (Tomšovic, 1997). This species was previously described within *Echinops* as *E. acantholepis* Jaub. and Spach. Most authors (Dittrich, 1977, 1996; Rechinger, 1979; Petit, 1988; Bremer, 1994; Petit, Mathez & Qaid, 1996; Petit, 1997; Shishkin & Bobrov, 1997) consider *Acantholepis* an independent genus that is closely related to *Echinops*. Kruse & Meusel (1972) state that *Acantholepis* is very similar to annual *Echinops*. Using the ITS region and the gene *matK* (Garcia-Jacas *et al.*, 2002), a tribal and subtribal delimitation in Cardueae, including only four *Echinops* species, groups *Echinops* and *Acantholepis* in a well-supported clade. Similar results were obtained in preliminary studies on the molecular phylogeny of *Echinops* and *Acantholepis* by means of ITS region analyses (Garnatje *et al.*, 2005).

Amphoricarpos Vis. is a perennial genus that comprises five species that are distributed in Southeast Europe, Turkey and the Caucasus. According to molecular data (unpublished results) this genus is the most primitive in the group. *Amphoricarpos autariatus* Blečić & E. Meyer occurs in the Balkan Peninsula (Siljak-Yakovlev, 1968a), whereas *A. exsul* O. Schwarz is an endemic species of the eastern Mediterranean region (Grierson, 1975).

Siebera J. Gay is an annual genus. According to Bremer (1994) it comprises two species, which are treated as only one, *S. pungens* J. Gay, in the Flora of the USSR (Shishkin & Bobrov, 1997). This species grows in Western and Central Asia. Morphologically, it is very close to the genus *Xeranthemum* and the molecular phylogeny shows that the two taxa are strongly related.

The genus *Xeranthemum* L. belongs to the tribe Cardueae, subtribe Carduinae (Susanna & Garcia-Jacas, in press) and comprises five annual species (Bremer, 1994) distributed throughout Southern Europe, Southwest Asia and North Africa, and frequently cultivated as an ornamental flower. *Xeranthemum inapertum* (L.) Mill. grows on the steppes and the low mountains of Southern Europe and Asia Minor and frequently shows a ruderal behaviour. *Xeranthemum annuum* L. occurs in Central Europe, the western Mediterranean, and Asia Minor. It grows on the steppes, sands, dry slopes, among shrubs in low mountains, sometimes as a ruderal species (Shishkin & Bobrov, 1997). Morphological and cytogenetic characters (Garnatje *et al.*, 2004) show that *Xeranthemum* is closely related to *Siebera*.

Chardinia Desf. is a monotypic annual genus with only the species, *Ch. orientalis* Kuntze spread in some low and medium altitude mountains of Central and Western Asia, where it occupies the fields and shows a ruderal behaviour.

Several palynological studies have tested the usefulness of pollen morphology to support Compositae taxonomy (Skvarla & Turner, 1966; Dimon, 1971; Carlquist, 1976; Wagenitz, 1976; Perveen, 1999; Torrell *et al.*, 1999; Martín & Garcia-Jacas, 2000; Martín, Torrell & Vallès, 2001; Vilatersana *et al.*, 2001; Martín *et al.*, 2003).

There are two basic patterns of exine ultrastructure in the Compositae, the caveate helianthoid pattern and the non-caveate anthemoid pattern. The pollen type of the

Cardueae is predominantly anthemoid, tricolporate, echinate and covered with micropores (Bolick, 1978).

The objectives of this work were to study the pollen of the *Acantholepis*, *Echinops* and *Xeranthemum* group. More specifically, our aims were: A) To check the systematic validity of the exine ornamentation and other external characters, on the one hand, and the ultrastructural features in this group of species on the other. B) To test the relationships between the pollen size and the systematic and/or cytogenetic parameters. These purposes are particularly addressed to find up i) the relationships (if any) between the pollen of the *Echinops-Acantholepis* group and that of the *Xeranthemum* group and ii) the palynological differences between the sections of *Echinops*.

Materials and Methods

Plant material

Table 1 shows the origin of all species studied. A study using scanning electron microscopy (SEM) was performed on 12 species of *Echinops*, two species of *Amphoricarpos* and one species from each of the following genera: *Acantholepis*, *Chardinia*, *Siebera* and *Xeranthemum*. These 12 *Echinops* species represent as many sections as possible following the three classifications stated in the introduction, of some of them with more than one species. Section *Pleiacme* (Shishkin & Bobrov, 1997) is not represented; it is a monospecific section including *Echinops tournefortii* Ldb. ex Trautv., which Rechinger (1979) included in sect. *Echinops*. Section *Hololeuce* Rechinger, 1979), also not represented, contains only *E. hololeucus* Rech. f., an Iranian endemic. In addition, two species of the African sections *Cenchrolepis* and *Phaeochaete*

(Fries, 1923) have also been considered to enlarge the geographical range of the research. Transmission electron microscopy (TEM) studies were also carried out on one perennial species of *Echinops* (*E. galaticus*) and one species from all the other genera, except *Chardinia* –because of insufficient material. Finally, pollen from all the species was measured under an optic apparatus.

Methods

Pollen was obtained from dried herbarium specimens. The anthers were dehydrated with a drop of 96% ethanol and dissected with a lancet. A part of free pollen grains were acetolyzed following the micro-method described by Avetissian (1950). For SEM, the acetolyzed pollen was gold-coated with diode sputtering and observed under a Hitachi 52300 at 15 kv. For TEM, non-acetolyzed pollen was fixed in 2% w:v paraformaldehyde/2.5% v:v glutaraldehyde in 0.1 M phosphate buffer pH= 7.4. Postfixation was then performed in OsO₄ 1% w:v + K₃Fe (CN)₆ 0.8% w:v in the same buffer. Samples were dehydrated in acetone, embedded in Spurr's resin and cut to 600 Å in a microtome Ultracut-E. Slices were observed under a Hitachi H-800 MT. All microscopy procedures were performed at the Scientific and Technical Services at the University of Barcelona.

For biometrical measures, pollen samples were acetolyzed following Avetissian, mounted on glycerogelatine and sealed with nail polish. Measurements were made using a Visopan apparatus (Reichert, Austria). For each specimen, 15 fully developed pollen grains were measured. Following Edrtman (1969) and Reitsma (1970), the parameters considered were: polar diameter (P), equatorial diameter (E) and sphericity (P/E). For each parameter, the arithmetic mean and standard deviation are given. In some cases a mean was not obtained because of lack of material.

Statistical analysis

Pearson's correlations between pollen size (P and E axes) and DNA content and chromosome number were calculated. A one-way ANOVA was performed to evaluate whether the differences between annual and perennial species were significant.

Statgraphics Plus 5.0 (Statistical Graphics Corp.) was used for the statistical analysis.

Results and discussion

We distinguish 4 types of pollen on the basis of morphology and ornamentation (Table 2).

Perennial *Echinops* type

This is a trizonocolpate isopolar pollen with radial symmetry, with a P/E between 1.06 and 1.46 (prolate-spheroidal to prolate) with subprolate dominance; microechinate/echinate-verrucate, with suprategal processes from 1.5 to 3 μm in diameter in the same individual, finishing in a rounded point. The intercolpal zone raised, producing a pronounced bridge (Figs. 1A, B). Pollen grains are large or very large (Table 2). The intercolpal zone of perennial *Echinops* pollen (in our case, *E. galaticus*) showed a discontinuous tectum, with 3 to 5 strata of columellae which tended to show progressive fragmentation, and a columellar infrategalium with branched columellae that was never totally developed (Fig. 2A). Infrategalium columellae show thicknesses from 0.5 to 1.3 μm and are separated by spaces. The external ornamentation in the shape of spinules was observed only for the first stratum of the tectum, as expected. The structure of this pollen is similar to that of *Carduncellus*, but more larger: *Carduncellus* presents only 3 tectal strata (Vilatersana *et al.*, 2001). The intercolpal

bridge results in an increase in the number of strata of tectal columellae and an external projection of the whole exine. On the cut carried out in the above-mentioned bridge, the tectum measures about 8-8.5 μm , the infratectum 6.5 μm and the whole exine 15.5 to 16 μm .

Tomšovic (1997) considers that all the *Echinops* species have a similar pollen type except *E. strigosus* L., to which he attributes some 40 μm of diameter (relatively small for the genus). The structure this author named "ectosexine" (i.e., the tectum) is simple and columellate. Stix (1971), Blackmore (1990) and Tomšovic (1997) agree in general the pollen of *Echinops* is "very biggest" and with very different structure that in the other perennial Asteraceae.

Blackmore (1990) reported that the pollen grains of *Echinops* are tricolporate with short, scattered spines and elaborate ectexine that comprises a microperforate tectum overlying an outer zone of slender, anastomosing columellae and an inner zone of large, distally digitate columellae. We agree with this description when referring to perennial species.

The sections of perennial species cannot be separated on the basis of pollen type, since they all show the same pattern of ornamentation and external and internal characteristics. Regarding dimensions, we did not have enough data to perform an adequate statistic analysis. Nevertheless, the pollen of section *Echinops* presents generally smaller diameters than some pollen from other sections.

Annual *Echinops* type (includes *Acantholepis*)

The second pollen type is common to the annual and biennial species of *Echinops* and to the genus *Acantholepis*.

Echinops strigosus presents a trizonocolpate, isopolar pollen, with radial symmetry, microechinate ornamentation with suprategal pointed elements from 0.10 to 0.15 μm , which are regularly distributed; spheroidal to subprolate, with P/E between 1.08 and 1.21 (Fig. 1D). P has a mean of 34.55 μm and E 31.77 μm . Our results differ from those of Valdés *et al.* (1987) and Tomšovic (1997), who considered this pollen larger.

This discrepancy could be because of our samples contracted during acetolysis (Table 2). The tectum has three layers; it is continuous in the superior and columellate in the median layer. The inferior layer has shorter columellae and a granulate rather than reticulate appearance, although this inferior layer is also formed by modified columellae. Infrategum is equally columellate with columellae from 0.6 to 1.3 μm of thick, separated by spaces (Fig. 2B). *Echinops nanus* (Fig. 1C) has the same pollen type but smaller (Table 2). A P/E relation of 1.13 implies that it is a prolate-spheroidal pollen. This is the only pollen type described by Tormo & Uberta (1995) in *Echinops*.

In *A. orientalis*, the pollen presents a type like that of *Echinops nanus* (Fig. 1E). The P/E is 1.25, so it is a subprolate pollen. Tomšovic (1997) states that *Acantholepis* pollen is tricolporate, prolate, with a thin nexine and columellae-structured endosexine (infrategum) (2.5 μm thick; we found thicknesses of 2 to 2.5 μm) as is usual in the tribe Cardueae, and psilate; we agree with the general description, although we think that this pollen is not psilate, but microechinate.

Acantholepis also presents a three-layered tectum (Fig. 2C), continuous in the superior layer, columellate in the median, with branched columellae, and with an

inferior layer which is also columellate, here with very modified columellae that resemble a reticle. The infratectum presents equally branched or ramified columellae from 0.5 to 0.6 μm thick, and separated by wide spaces.

The pollen of *Acantholepis* is similar to but larger than that of *E. strigosus*. Conversely, it is very distant from that of *E. nanus*. The sizes of *E. strigosus* pollen are also consistent with those of the perennial species *E. albicaulis*. This observation supports the section *Ritrodes*; however, the morphology differs greatly between both species. Our results show that in the annual or biennial species of this genus the pollen grains are smaller.

Amphoricarpos/Siebera type.

Amphoricarpos autariatus presents a trizonocolporate isopolar pollen with radial symmetry, prolate-spheroidal ($P/E=1.10$), spiny, with spines c. 3.5 μm and small perforations of 0.2 μm between them (Fig. 1F). It belongs to the anthemoid type, already reported in this taxon by Siljak-Yakovlev (1986a, 1986b), who described large infratectal columellae and not a continuous endocolpus, and who stated that “it exhibits an endexine with endosculpturing around and between the endoapertures (which contributes to harmomegathy)”. We found a three-layered tectum with two levels of columellae; the upper one is discontinuous (Fig. 2D). The spiny structures on the surface belong to the tectum, and appear as structured elevations of the same. The infratectum is columellar, with ramified discontinuous columellae from 0.6 to 1 μm thick, with are widely spaced. *Amphoricarpos exsul* has a pollen like that of *A. autariatus* with few variations in measures (Table 2).

Siebera pungens shows a trizonocolporate isopolar pollen with radial symmetry, prolate-spheroidal ($P/E= 1.08$), spiny, with spines of c. $4 \mu\text{m}$ and small perforations of c. $0.2 \mu\text{m}$ between them (Fig. 1G).

The structure of *Siebera* pollen is almost undistinguishable from that of *Amphoricarpos*: partial tectum of three layers, with columellae apparently less clearly formed than in the latter genus, although this may be an artifact. The columellae of infratectum are equal, ramified, discontinuous and with spaces between them, and with thickness from 0.6 to $1 \mu\text{m}$ (Fig. 2E). Pollen dimensions are also very similar; but slightly smaller in *Siebera* (annual).

Xeranthemum/Chardinia type

Xeranthemum inapertum presents a trizonocolporate isopolar pollen with radial symmetry, prolate-spheroidal ($P/E=1.09$), with verrucoid ornamentation (structural elements of c. $0.9 \mu\text{m}$, with an orifice in the apex) which has few perforations (Fig. 1H). Our measurements ($25 \times 23 \mu\text{m}$, Table 2) differ from those reported by Valdés, Díez & Fernández (1987) ($33 \times 34 \mu\text{m}$). *Xeranthemum annuum* shows a structure similar to that of *Amphoricarpos* and *Siebera*; three-layered tectum, with a discontinuous first layer, the second columellar with irregular columellae and the third even more irregular, with fragmented or absent columellae; infratectal columellae from 0.6 to $1 \mu\text{m}$ of thick, ramified and with spaces between them. As in the two other genera, the supraexinic elements (here verrucoid formations) have the same columellar ultrastructure (Fig. 2F).

Chardinia orientalis has a pollen with the same ornamentation as that of *Xeranthemum*, but more spheroidal ($P/E = 1.03$) in our samples (Fig. 1I). Pollen dimensions are similar, but smaller in *Xeranthemum*.

On the basis of our observation, we conclude that the internal structure of our pollen samples coincides with that named anthemoid by Bolick (1978) and that described by Nordenstam & El-Ghazaly (1978) in the genus *Amberboa* (Asteraceae, Centaureinae). However, their external appearance differs greatly.

We found the same structure in distinct genera, which contrasts with the findings of Pehlivan (1995), who reported anthemoid and caveate structures within *Centaurea*.

The results of ANOVA show significant differences in pollen size between annual and perennial species for the polar axis length ($P=0.0023$), the equatorial axis ($P=0.0022$) and the P/E ratio ($P=0.0160$). The average polar axis length is $30.59 \mu\text{m}$ for the annual species and $54.01 \mu\text{m}$ for the perennial ones and the averages for the equatorial axis length are $27.10 \mu\text{m}$ and $42.29 \mu\text{m}$ for the annuals and perennials, respectively. The P/E ratio is 1.12 for the annual species and 1.25 for the perennials, is always about the prolate pollen grains.

Petit (1996) proposed that, on the basis of palynological data, *Echinops* and *Xeranthemum* groups are related. He also concluded that this connection is not correlated with any phytomorphological character. Our results support a relation between *Echinops* and *Xeranthemum* only regarding internal pollen structure, since in external morphology patterns were perfectly differentiated in the two groups. Using palynological results, Petit (1996) considers that *Amphoricarpus* is not connected to *Xeranthemum*. Here we observed that the external appearance of *Amphoricarpus* pollen differs greatly from that of *Xeranthemum* and *Chardinia*. Although morphological and molecular data perfectly include *Amphoricarpus* within the *Xeranthemum* group,

Amphoricarpos shares the pollen characters with *Siebera*, this pattern belonging to a more primitive type than the others.

Concluding remarks

The genus *Echinops* presents two pollen types, which correspond to annual and perennial species. The external morphology of annual *Echinops* pollen is not similar to the genera considered relatives, such as *Xeranthemum/Chardinia* or *Amphoricarpos/Siebera*. However, in our study *Echinops* pollen was always anthemoid. Perennial species of *Echinops* presented a subprolate microechinate/echinate verrucoid pollen, with very prominent intercolpia in the shape of a bridge.

Annual species of *Echinops* presented a subprolate microechinate pollen without a prominent intercolpia, a characteristic shared with *Acantholepis orientalis*. This observation supports the hypothesis that *Acantholepis* is an annual *Echinops* (Garnatje *et al.*, 2005). Perennial species of *Echinops* have a significantly larger pollen grain than the annual ones. The internal structure differed in the tectum, being discontinuous in perennials and continuous in annuals (which brings the “psilate” aspect- in fact, microechinate- cited by Tomšovic, 1997). This notwithstanding, it is similar at the rest, because it is not caveate and belongs to an anthemoid type. The difference between both structures consists in the number of layers in the tectum and the length of the infratectal columellae, but, although spectacular, it is in itself only quantitative. All the annual and perennial species of the genus *Echinops* presented the same internal structure, except for the tectum.

Amphoricarpos and *Siebera* presented the same external type of pollen, echinate, prolate-spheroidal, and anthemoid according the internal structure.

Xeranthemum and *Chardinia* presented the same external appearance of pollen grains: verrucoid and prolate-spheroidal; *Xeranthemum*, the only which we were able to cut, has the same internal structure than the *Amphoricarpos/Siebera* complex, anthemoid as that of genus *Echinops* itself.

Aknowledgements

We thank N. Garcia-Jacas, O. Hidalgo, A. Susanna and J. Vallès for theirs suggestions on the paper. We also thank N. Cortadellas, A. Rivera, R. Fontarnau and A. Domínguez for their assistance and R. Rycroft for correcting the English manuscript. Comments by S. Blackmore have been useful to improve the manuscript. This work was subsidized by DGICYT (Spanish government; projects BOS2001-3041-C02-01 and BOS2001-3041-C02-02).

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Table 1 Provenance of the species studied.

Taxon	Section	Location, collector(s) and date
<i>Acantholepis orientalis</i> Less.		Uzbekistan, Bukhara: Kyzylkum, 30 km N of Bukhara, <i>Khassanov</i> , IX.1999 (BC).
<i>Amphoricarpus autariatus</i> Blečić and E. Mayer		Serbia and Montenegro: Mt. Durmitor, cliffs, c. 1500 m, <i>V. Stevanovic, D. Lakusic, M. Niketic</i> , 4.X.1991 (BC)
<i>Amphoricarpus exsul</i> Schwarz		Turkey, Mugla: road from Ballik to Ören, <i>Ertuğrul, Garcia-Jacas and Susanna</i> 2256, 29.VII.2002 (BC).
<i>Chardinia orientalis</i> Kuntze		Iran, Kurdistan: 45 km before Kermanshah to Sanandaj, 1400 m, <i>Garcia-Jacas, Mozaffarian, Susanna 1715 and Vallès</i> , 11.VIII.1996 (BC).
<i>Echinops albicaulis</i> Kar. et Kir.	<i>Ritrodes</i>	Kazakhstan, Almatinskaya oblast: On the road of Kapchigai to Bakanaz, <i>Ivaschenko, Susanna 2144 and Vallès</i> , 26.VIII.2000 (BC).
<i>Echinops fontqueri</i> Pau in Font Quer	<i>Oligolepis</i>	Morocco : 20 Km SE of Tanger, 100 m, <i>Gómiz</i> , 19.V.2002 (BC).
<i>Echinops galaticus</i> Freyn	<i>Echinops</i>	Turkey, Sivas: 11 Km North of Zara to Serefiyeh, <i>Ertuğrul, Garcia-Jacas and Susanna</i> 2416, 8.VIII.2002 (BC).
<i>Echinops giganteus</i> A. Rich.	<i>Cenchrolepis</i>	Cameroun: Lake Monoun, NNW of Foubot; 1200m. 1972 (P).
<i>Echinops hispidus</i> Fresen.	<i>Terma</i> or <i>Oligolepis</i>	Ethiopia: 100 Km S. of Addis Abeba. 1977 (P).
<i>Echinops microcephalus</i> Sibth. et Sm.	<i>Echinops</i>	Greece, Halkidiki : 1 Km from Nea Moudania to Thessaloniki. 40° 11'N, 23° 16'E. <i>Husain, Jury and Rutherford</i> 172, 18.IX.1981 (BC).
<i>Echinops mildbraedii</i> Mattf.	<i>Phaeochaete</i>	Cameroun: Lake Monoun, NNW of Foubot; 1200m. 1987 (P).

<i>Echinops nanus</i> Bunge	<i>Nanechinops</i>	Kazakhstan, Almatinskaya oblast: Kurtagai canyon, on the road to Narienkul near the bridge over Charin river, <i>Ivaschenko, Susanna 2132 and Vallès, 24.VIII.2000 (BC)</i> .
<i>Echinops ritro</i> L.	<i>Echinops</i>	Spain, Valencia: Montgó, near Denia. <i>A. et O. de Bolós, 18.IX.1957 (BC)</i>
<i>Echinops saissanicus</i> (B. Keller) Bobrov	<i>Chamaechinops</i>	Kazakhstan: Turaigir Mountains near Charin canyon, 1000 m. <i>Ivaschenko, Susanna 2124 and Vallès, 23.VIII.2000 (BC)</i> .
<i>Echinops sphaerocephalus</i> L.	<i>Echinops</i>	Spain, Barcelona: Alt Berguedà, La Clusa. 1250 m. Rosell, 3.VIII.1976 (BC)
<i>Echinops strigosus</i> L.	<i>Ritrodes</i>	Spain, Murcia: Lorca, El Conde de San Julián. <i>Hno Jerónimo, 21.VI.1923 (BC)</i> .
<i>Siebera pungens</i> J. Gay		Turkey, Gaziantep: fallow fields at the entry of the town, <i>Ertuğrul, Garcia-Jacas, Susanna 2316 and Uysal, 4.VIII. 2002 (BC)</i> .
<i>Xeranthemum annuum</i> L.	<i>Xeranthemum</i>	Turkey, Kahraman Maraş: 2 Km from Narh, <i>Ertuğrul, Garcia-Jacas, Susanna 2323 and Uysal, 4.VIII. 2002 (BC)</i> .
<i>Xeranthemum inapertum</i> (L.) Mill.	<i>Xeranthemum</i>	Spain, Madrid: Torrelaguna, <i>Garcia-Jacas and Susanna 1456, 21.VI.1994 (BC)</i> .

Table 2 Measurements of pollen grains [P=polar length (μm); E=equatorial length (μm); SD=standard deviation; n=15 measurements]

Taxon	P (mean \pm SD)	rang	E (mean \pm SD)	rang	P/E
<i>Acantholepis orientalis</i>	41.87 \pm 2.68	37.10-44.00	33.36 \pm 2.24	29.70-36.00	1.25
<i>Amphoricarpus autariatus</i>	35.55 \pm 1.89	30.00-36.60	32.10 \pm 2.88	26.60-36.60	1.10
<i>Amphoricarpus exsul</i>	33.80 \pm 3.69	26.60-36.60	29.75 \pm 2.44	26.60-33.30	1.13
<i>Chardinia orientalis</i>	33.33 \pm 4.17	29.16-37.50	30.41 \pm 3.48	25.00-33.33	1.09
<i>Echinops albicaulis</i>	34.66 \pm 1.82	33.30-36.60	32.66 \pm 5.47	23.30-36.60	1.06
<i>Echinops fontqueri</i>	82.88 \pm 8.03	66.60-95.00	56.44 \pm 7.42	46.60-70.00	1.46
<i>Echinops galaticus</i>	64.71 \pm 5.77	56.60-72.30	51.43 \pm 3.62	48.02-57.80	1.25
<i>Echinops giganteus</i>	-	60.52-66.44	-	37.82-41.44	-
<i>Echinops hispidus</i>	75.30 \pm 4.42	70.00-86.20	57.44 \pm 3.68	50.00-65.50	1.31
<i>Echinops microcephalus</i>	51.48 \pm 5.32	43.20-57.20	39.05 \pm 6.90	33.30-51.03	1.31
<i>Echinops mildbraedii</i>	-	46.66-60.43	-	33.33-42.10	-
<i>Echinops nanus</i>	14.50 \pm 0.91	13.77-16.00	12.75 \pm 1.02	12.00-13.94	1.13
<i>Echinops ritro</i>	52.21 \pm 4.33	45.83-58.33	41.80 \pm 3.60	37.50-45.83	1.24
<i>Echinops saissanicus</i>	54.44 \pm 3.47	51.60-60.00	43.10 \pm 2.77	40.00-50.00	1.26
<i>Echinops sphaerocephalus</i>	55.10 \pm 2.54	51.60-60.00	39.10 \pm 2.18	36.60-43.30	1.40
<i>Echinops strigosus</i>	34.55 \pm 1.87	30.00-36.00	31.77 \pm 2.39	28.30-35.00	1.08
<i>Siebera pungens</i>	34.04 \pm 3.83	30.00-40.00	31.23 \pm 6.05	23.30-38.68	1.08
<i>Xeranthemum inapertum</i>	25.26 \pm 2.66	23.33-30.00	23.07 \pm 2.21	20.00-26.60	1.09

Figure 1. Scanning electron micrographs of (A) *Echinops hispidus*; (B) *Echinops galaticus*; (C) *Echinops nanus*; (D) *Echinops strigosus*; (E) *Acantholepis orientalis*; (F) *Amphoricarpos autariatus*; (G) *Siebera pungens*; (H) *Xeranthemum inapertum*; (I) *Chardinia orientalis*.

Figure 2. Transmission electron micrographs of (A) *Echinops galaticus*; (B) *Echinops strigosus*; (C) *Acantholepis orientalis*; (D) *Amphoricarpos autariatus*; (E) *Siebera pungens*; (F) *Xeranthemum annuum*.



