

## **A contribution to the ultrastructural knowledge of the pollen exine in subtribe Inulinae (Inuleae, Asteraceae)**

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**Abstract.** To better understand the relationships within the Asteroideae and Inuleae, the structure of the pollen exine was investigated in seven genera and nine species of the subtribe Inulinae using LM, TEM and SEM. All taxa have a senecoid pattern of exine. The tectal complex consists of three main layers that differ in thickness and morphology: a tectum, a columellar layer, and a layer consisting of the basal region of the columellae. The absence or the vestigial condition of the foramina is considered as a plesiomorphy within the Asteroideae. All taxa have a complex apertural system that consists of an ecto-, a meso-, and an endoaperture. These apertures intersect respectively the tectal complex, the foot layer and the upper part of the endexine, and the inner layer of the endexine. A continuous transition among the different species of Inulinae was found for all the quantitative characters examined. This relative homogeneity of the pollen morphological characters enhances the naturalness of the subtribe Inulinae.

**Keywords:** Pollen; exine; Inulinae; Inuleae; taxonomy; cladistics; electron microscopy

The Inulinae sensu Anderberg et al. (2005) comprise 38 genera and 480 species mainly with a Eurasian and Mediterranean distribution. Roughly, it corresponds to the union of the subtribes Euinuleae and Buphtalmeae, created by Bentham in 1873, to the union of the subtribes Inulinae and Buphtalminae, created by Hoffmann in 1890, and to the union of the groups *Inula*, *Geigeria* and *Carpesium* of the subtribe Inulinae sensu amplo, proposed by Merxmüller et al. in 1977. The Inulinae (sensu Anderberg et al. 2005) were previously included in the Inuleae s. l., which also comprised the Gnaphalieae and the Plucheinae (as Plucheae), and proved to be polyphyletic (Bremer 1987, Anderberg 1988, Karis 1993, Bayer and Starr 1998, Panero and Funk 2002, Wagstaff and Breitwieser 2002, Goertzen et al. 2003).

The most modern concept of the Inuleae, proposed by Anderberg et al. (2005), consider the tribe as including the Inuleae (sensu Anderberg 1991a) and Plucheae (sensu Anderberg 1991b). Within the Asteroideae, the Inuleae

(sensu Anderberg et al. 2005) was placed on different positions in the various morphological and molecular cladograms of the Asteraceae. This tribe was considered as sister group to the rest of the Asteroideae taking into account the morphological studies performed by Bremer (1987, 1994) and Karis (1993) and the two non-coding chloroplast sequences investigation of Bayer and Starr (1998). In the *ndhF* tree made by Kim and Jansen (1995) the tribe Senecioneae was placed in a polytomy together with two other clades: one including the Inuleae and the Heliantheae s. l., and the other including the Anthemideae, Astereae, Calenduleae and Gnaphalieae. Based on comparative chloroplastidial DNA sequence data, Panero and Funk (2002) considered the Inuleae as sister group of a large clade comprising 13 different tribes and the Senecioneae as sister group of the other Asteroideae. ITS investigations carried out by Wagstaff and Breitwieser (2002) placed the Inuleae as the sister group of a clade comprising the Heliantheae s. l. and the *Athroisma* group, and the Calenduleae as sister group to the rest of the Asteroideae. Using the same cladogram approach, Goertzen et al. (2003) placed the Anthemideae and the Inuleae as sister groups, respectively, to the remaining of the Asteroideae and to a clade comprising the Senecioneae and the Calenduleae. Anderberg et al. (2005), in their cladograms supported by *ndhF* studies, placed the Senecioneae in a polytomy together with two large clades: one comprising the Inuleae-Inulinae, Inuleae-Plucheinae, Heliantheae, and the genera *Anisopappus*, *Blepharispermum*, *Athroisma*, *Callilepis* and *Zoutspansbergia*, and the other one including the Anthemideae, Astereae, Calenduleae and Gnaphalieae.

As demonstrated by several authors (Wagenitz 1955, 1976; Skvarla and Larson 1965; Skvarla and Turner 1966; Skvarla et al. 1977; Bolick 1978, 1991; Praglowsky and Grafström 1980; Blackmore 1982; Tormo Molina and Uebera Jiménez 1995; Breitwieser and Sampson 1997; Zavada and Villiers 2000; Ortiz and Pereira Coutinho 2001; Skvarla et al. 2005), the comprehension of the pollen exine

structure is of great importance to understand the evolution, systematic and ecology of the Asteraceae. Using light microscope (LM), Stix (1960) and Leins (1968, 1971) examined the structure of the pollen exine in, respectively, 5 genera and 11 species of Inuleae (which were grouped under 1 pollen type) and 32 genera and 137 species (which were grouped under 20 pollen types). So far, the only transmission electron microscopic (TEM) studies concerning the pollen exine of the Inuleae (sensu Anderberg et al. 2005) are those in *Inula britannica* (Skvarla and Turner 1966), *Alagopappus dichotomous* and *Blumea mollis* (Skvarla et al. 1977). The pollen wall architecture of the Inuleae was not investigated using the scanning electron microscope (SEM), making the pollen exine of this tribe one of the less studied within the Asteraceae.

In the present work we investigated the structure of the pollen exine in *Asteriscus aquaticus* (L.) Less., *Dittrichia viscosa* (L.) Greuter, *Inula salicina* L., *Jasonia tuberosa* L., *Limbarda crithmoides* (L.) Dumort., *Pallenis maritima* (L.) Greuter, *Pallenis spinosa* (L.) Cass., and *Pulicaria paludosa* Link using LM, TEM and SEM. The exine structure of *Pulicaria dysenterica* (L.) Gaertn. was also investigated using SEM. These taxa were chosen because they represent various clades in the cladograms of Eldenäs et al. (1998) (three clades), Francisco-Ortega et al. (2001) (five clades) and Anderberg et al. (2005) (three clades).

## Materials and methods

Pollen grains were collected from herbarium vouchers held at Coimbra (COI) and Oporto (PO) and then acetolysed according to Erdtman (1960). Specimens examined and voucher data are given at the end of the paper. The terminology for pollen descriptions follows Punt et al. (1994).

**Light microscopy.** Pollen grains were mounted in silicone oil and observed with a Leitz Laborlux LM using the X100 oil immersion objective. Each of the following characters: length of polar axis (P), length of equatorial diameter (E), spine length and spine width, was measured in 30 different pollen grains. P/E was then established.

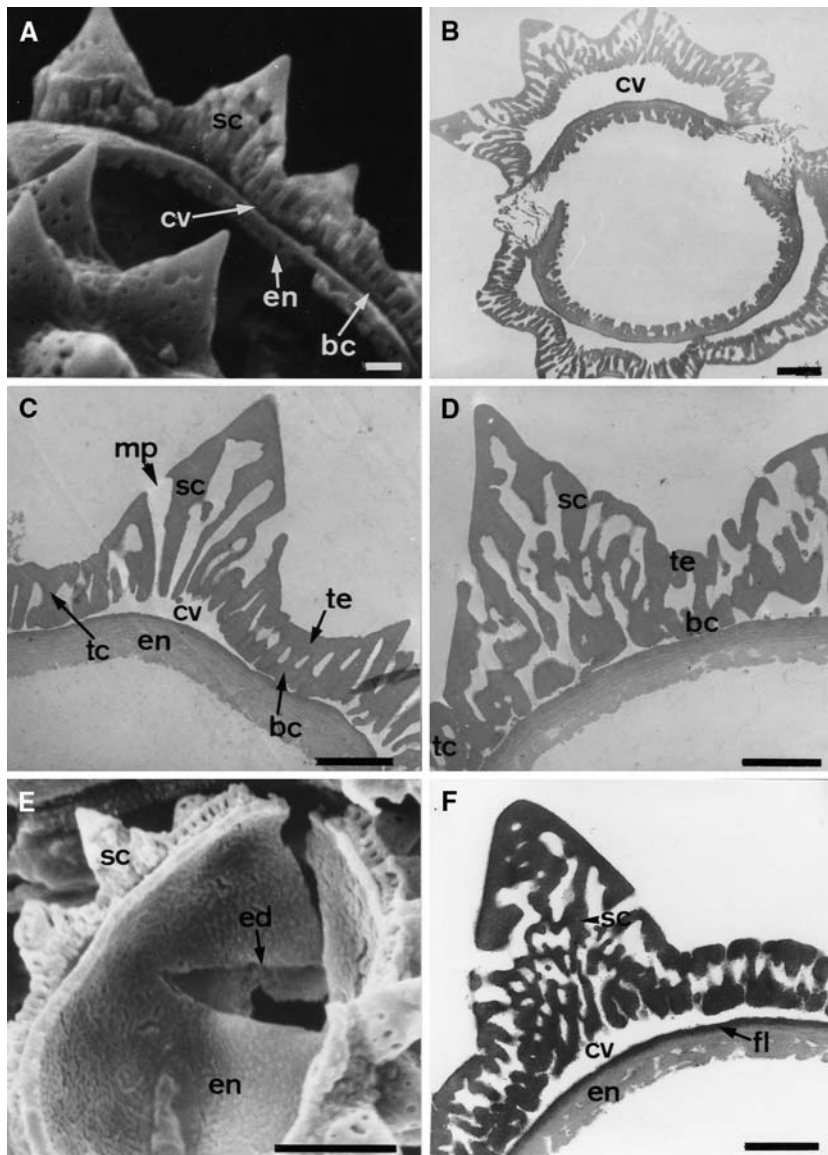
**Scanning electron microscopy.** Pollen grains were treated with ultra-sounds (35 kc/s, 1 h), dehydrated in a graded acetone series (70%–100%), and critical point dried. They were then mounted on aluminium stubs and sputter coated with a 30 nm layer of gold-palladium prior to examination with a JEOL JSM-5400 at 10 kV.

**Transmission electron microscopy.** Pollen grains were fixed in 2% osmium tetroxide in 0.1 M sodium cacodylate buffer, pH 7.2, for 24 h, dehydrated in a graded ethanol series (70%–100%), and embedded in Spurr's resin. Thin sections were obtained with a LKB Ultratome NOVA ultramicrotome equipped with a diamond knife, conventionally stained with uranyl acetate and lead citrate, and observed in a JEOL JEM-100 SX at 80 kV. Using micrographs of the exine of the species studied, 10 measurements were made for each of the following characters: thickness of the exine, endexine, foot layer, columellar layer, tectum and tectal complex, diameter of both the spinular microperforations and the interspinular microperforations, and distance from the base of each spine in which the spinular microperforations are located and the spinular columellae end. The values of the following ratios: E/exine thickness, endexine thickness/foot layer thickness, tectal complex thickness/foot layer thickness + endexine thickness, and spine height/spine width, were then calculated.

## Results

Pollen grains of all taxa examined are radially symmetrical, 3-zonocolporate, echinate, tectate, caveate, sub-oblate to sub-prolate, P/E = 0.80–1.17. P = 13.20–25.33  $\mu\text{m}$ , E = 12.45–28.46  $\mu\text{m}$ . E (average) /exine thickness (average) = 7.31–10.40. The tectal complex is structured. In the inter-spinular areas it is composed of three distinct main layers (Figs. 1–3): an external micro-perforated, (0.08–) 0.14–0.28 (–0.38)  $\mu\text{m}$  thick layer (tectum) in which the size of the micro-perforations varies between 0.03 and 0.12  $\mu\text{m}$ ; a median columellar layer (0.2–) 0.53–0.78 (–1.10)  $\mu\text{m}$  thick; and an internal micro-perforated layer (0.15–) 0.20–0.40 (–0.50)  $\mu\text{m}$  thick. The columellae give origin by proximal, medial and distal ramification, respectively to a more or less intermittently connected basal

region, internal tectum under the spines, and tectum (Figs. 1D, 2B, 3C). A large number of vestigial micro-foramina are seen in the tectal complex (Figs. 1C, 1D, 3C). The caveae are generally narrow (Figs. 1A, 1C, 2D), despite the fact that their size varies from species to species, from grain to grain, and even within the same pollen exine. In the latter case the caveae are usually larger under the spines than under the inter-spinular areas (Figs. 1B, 1C, 1F, 3B, 3C). The foot-layer is smooth (Figs. 1F, 2E, 3A) with a thickness of (0.04–) 0.05–0.10 (–0.13)  $\mu\text{m}$ . The endexine is (0.25–) 0.35–0.59 (–0.80)  $\mu\text{m}$  thick, i.e. (3.5–) 5–10 (–13)  $\times$  thicker than the foot layer in the inter-apertural areas becoming thicker towards the apertures (Figs. 1B, 2C, 3F). The upper surface of the endexine is smooth and lamellated while the inner surface is disrupted (Figs. 1A, 2D, 3E). The ectoapertures (colpi) are subterminal, broad in the equatorial area and acute at the ends. They intersect the tectal complex (Figs. 2C, 3F). The mesoapertures are lologate (Fig. 3D) and their limits are more or less covered by those of the ectoapertures making them difficult to observe under the SEM. They intersect the foot layer and the upper part of the endexine (Figs. 2C, 3F). The endoapertures are lalongate and either acute (Fig. 1E) or obtuse (Fig. 3D) at their ends. They intersect the inner layer of the endexine (Figs. 2C, 3F). The spines are conical [(1.57–) 2.00–5.5 (–6.00)  $\times$  (1.57–) 1.80–5.00 (–6.00)  $\mu\text{m}$ ] with convex (Fig. 1F) or, less frequently, straight or concave sides (Fig. 2D). All of them have a reasonably individualized apex and a sub-apical cavity that varies in shape and size (Figs. 2A, 2B, 2D, 3C). In *Limbarda crithmoides*, abnormal spines could also be seen in a few pollen grains (Fig. 2F). The structure of the spines is columellar (structured spines) and the connected basal region of the columellae always less individualized within the spines than in the inter-spinular areas (Figs. 1A, 1C, 2B, 3C). The spinular columellae are longer than the inter-spinular columellae, reaching (1/3–) 1/2–3/4 (–4/5) of the spinular height (Figs. 1A, 2D, 3C). They

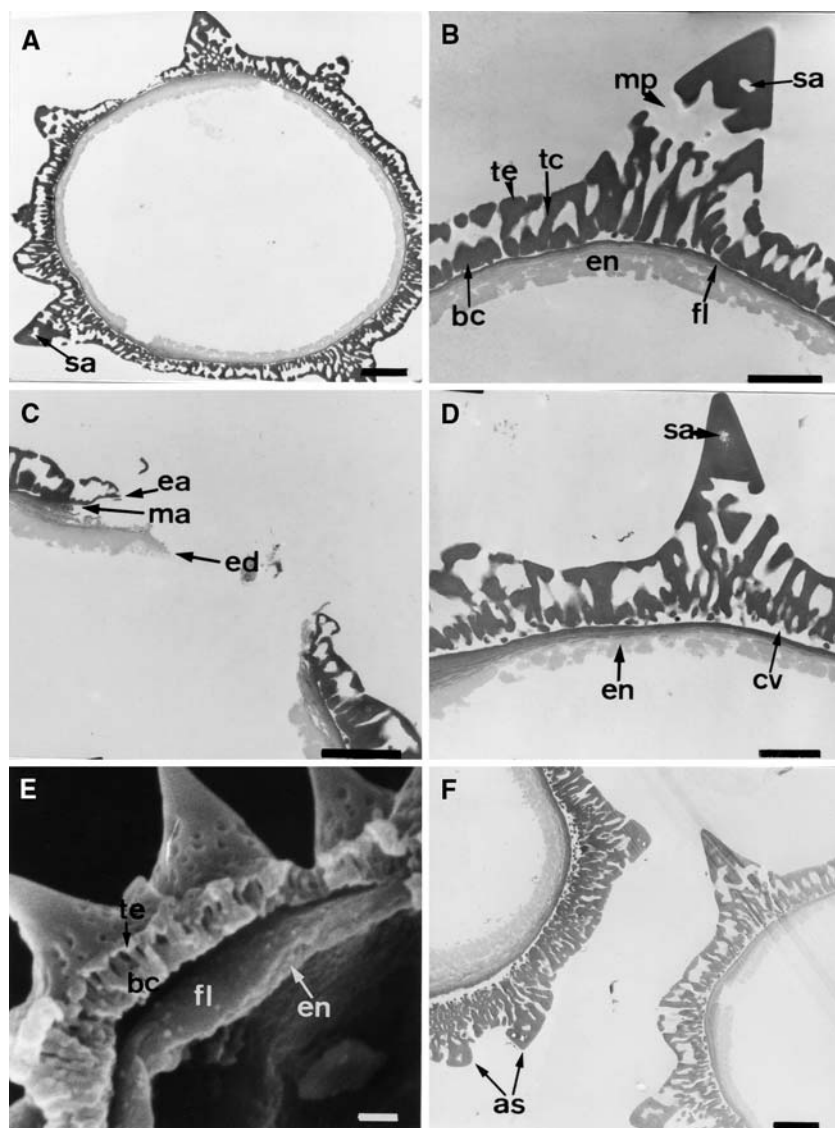


**Fig. 1.** **A, B** *Pallenis maritima* pollen grains. **A** SEM micrograph of a fractured exine. **B** TEM micrograph of a section at the equator. **C, D** *Dittrichia viscosa* pollen grains. **C** TEM micrograph of a section at the mesocolpium. **D** TEM micrograph of a section at the apocolpium. **E, F** *Inula salicina* pollen grains. **E** SEM micrograph of a fractured exine showing the endoaperture. **F** TEM micrograph showing detail of the exine. *en* endexine, *fl* foot layer, *cv* caveae, *bc* basal region of the columellae, *tc* tectal columellae, *te* tectum, *sp* spinular columellae, *ed* endoapertures, *mp* micro-perforations. **A, C, D, F** bars = 1  $\mu\text{m}$ ; **B** bar = 2  $\mu\text{m}$ ; **E** bar = 5  $\mu\text{m}$

are often branched making the structure of each spine complex (1D, 1F, 3A). Numerous micro-perforations are present in the lower (1/3–) 1/2–3/4 portion of each spine and their dimensions increase from the base [(0.04–) 0.10–0.28  $\mu\text{m}$ ] towards the apex [(0.30–)

0.40–0.75 (–1.00)  $\mu\text{m}$ ] of each spine (Figs. 1A, 2D, 3D).

Quantitative results concerning the pollen morphological characters for the seven genera and eight species studied with LM and TEM are shown in Table 1.

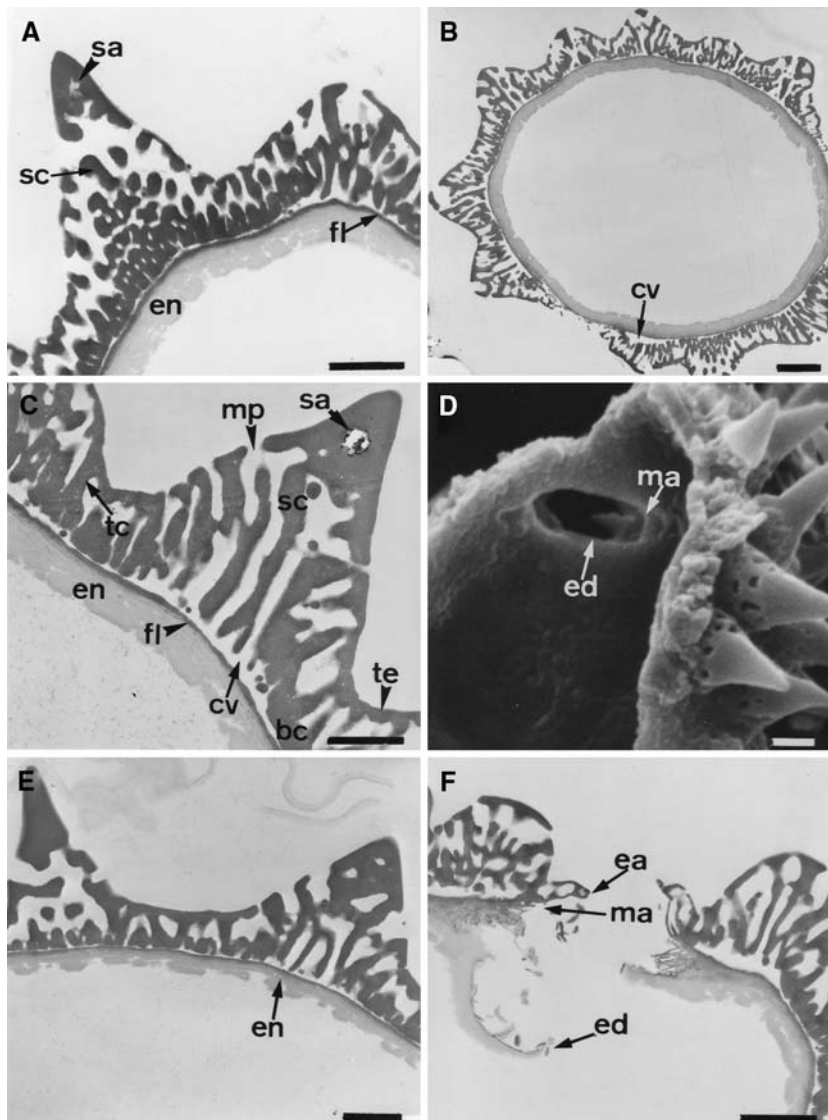


**Fig. 2.** A–C *Jasonia tuberosa* pollen grains. **A** TEM micrograph of a section at the meridian. **B** TEM micrograph showing the exine structure. **C** TEM micrograph of a section through an aperture. **D–F** *Limbarda crithmoides* pollen grains. **D** TEM micrograph showing detail of the exine. **E** SEM micrograph of a fractured exine showing separation between the endexine and the foot layer. **F** TEM micrograph of the exine structure in a normal (right side) and an abnormal (left side) pollen grain. *en* endexine, *fl* foot layer, *cv* caveae, *bc* basal region of the columellae, *ea* ectoapertures, *ma* mesoaperture, *ed* endoapertures, *as* abnormal spines, *sa* subapical cavity, *mp* micro-perforations. A bar = 2  $\mu\text{m}$ ; B–F bars = 1  $\mu\text{m}$

## Discussion

Albeit some authors (e.g. Breitwieser and Sampson 1997) have examined non-acetolysed pollen grains of Asteraceae using SEM or TEM, we opted to investigate acetolysed exines in order to allow an accurate compar-

ison between our data and those of previous similar works, including fossil pollen. We also aimed at examining the details of the central area of the apertural system using SEM. These details are usually obscured by the protrusions of the intine in non-acetolysed grains. In the



**Fig. 3.** **A** *Asteriscus aquaticus* pollen grain. TEM micrograph showing a detail of the exine. **B, C** *Pallenis spinosa* pollen grains. **B** TEM micrograph of a section at the meridian. **C** TEM micrograph showing the exine structure. **D** *Pulicaria dysenterica* pollen grain. SEM micrograph of a fractured exine showing the endoaperture and the mesoaperture. **E, F** *Pulicaria paludosa* pollen grains. **E** TEM micrograph showing detail of the exine; **F** TEM micrograph of a section through an aperture. *en* endexine, *fl* foot layer, *cv* caveae, *bc* basal region of the columellae, *tc* tectal columellae, *te* tectum, *ea* ectoaperture, *ma* mesoaperture, *ed* endoapertures, *sa* sub-apical cavity, *mp* micro-perforations. A, C–F bars = 1  $\mu\text{m}$ ; B bar = 2  $\mu\text{m}$

present work the action of ultrasounds increased effectively the frequency of fractures in the pollen exine, allowing for a deeper examination of the details of the apertures, especially concerning their internal area.

The presence of mesoapertures in the apertural system of the Inuleae pollen was

pointed out by Dimon (1971). In contrast, Diez (1987) referred that mesoapertures were not observable in the 5 genera and 9 species of Inuleae she studied. Our observations confirm the existence of an intermediate aperture in the pollen exine of this tribe. However, contrary to the statement of Dimon (1971) for the Aster-

**Table 1.** Summary of pollen morphological characters for the Inuleae species examined with LM and TEM

Characters	<i>Asteriscus aquaticus</i>	<i>Dittrichia viscosa</i>	<i>Inula salicina</i>	<i>Jasonia tuberosa</i>	<i>Limbarda crithmoides</i>	<i>Pallenis maritima</i>	<i>Pallenis spinosa</i>	<i>Pulicaria paludosa</i>
P ( $\mu\text{m}$ )	18.85 (16.58–21.28)	20.74 (15.72–25.13)	19.82 (16.06–22.28)	19.29 (15.15–22.57)	20.72 (17.49–25.33)	18.76 (15.66–21.54)	17.72 (13.20–21.10)	16.50 (14.24–21.30)
E ( $\mu\text{m}$ )	20.38 (17.36–22.84)	22.77 (16.50–28.26)	20.94 (17.58–25.03)	20.32 (17.08–23.35)	23.04 (18.66–28.46)	19.53 (16.84–23.50)	18.49 (12.45–21.86)	17.19 (15.81–20.12)
P/E	0.92 (0.84–1.05)	0.91 (0.84–1.03)	0.96 (0.85–1.10)	0.95 (0.89–1.17)	0.90 (0.86–1.07)	0.96 (0.89–1.07)	0.96 (0.80–1.11)	0.96 (0.87–1.17)
Exine thickness ( $\mu\text{m}$ )	2.29 (1.50–3.70)	2.19 (1.50–2.80)	2.33 (1.90–3.00)	2.23 (1.50–3.30)	3.10 (2.30–4.00)	2.67 (1.90–3.70)	2.07 (1.50–3.00)	1.82 (1.50–3.00)
E (average)/Exine thickness (average)	8.90	10.40	8.99	9.11	7.43	7.31	8.93	9.45
Endexine thickness ( $\mu\text{m}$ )	0.48 (0.35–0.60)	0.59 (0.50–0.80)	0.46 (0.40–0.60)	0.47 (0.40–0.60)	0.47 (0.30–0.65)	0.60 (0.50–0.90)	0.53 (0.40–0.65)	0.35 (0.25–0.45)
Foot layer thickness ( $\mu\text{m}$ )	0.10 (0.08–0.13)	0.08 (0.06–0.13)	0.10 (0.06–0.12)	0.07 (0.06–0.12)	0.10 (0.07–0.13)	0.08 (0.06–0.11)	0.07 (0.05–0.11)	0.05 (0.04–0.06)
Endexine/Foot layer thickness	4.9 (3.5–8)	9.6 (8.0–11.0)	6.0 (4.0–9.0)	8.3 (6.0–11.0)	5.6 (3.5–7.5)	10.0 (8.0–13.0)	7.1 (4.5–9.0)	6.0 (3.5–9.0)
Internal tectum thickness ( $\mu\text{m}$ )	0.33 (0.25–0.45)	0.34 (0.22–0.50)	0.38 (0.25–0.60)	0.42 (0.22–0.60)	0.54 (0.40–0.68)	0.45 (0.30–0.65)	0.34 (0.25–0.50)	0.39 (0.32–0.45)
Columellar layer thickness ( $\mu\text{m}$ )	0.53 (0.35–0.80)	0.58 (0.45–0.75)	0.74 (0.45–1.10)	0.56 (0.35–0.70)	0.78 (0.50–1.10)	0.74 (0.50–0.90)	0.61 (0.30–0.75)	0.58 (0.20–0.75)
External tectum thickness ( $\mu\text{m}$ )	0.14 (0.08–0.20)	0.28 (0.16–0.38)	0.19 (0.10–0.31)	0.20 (0.14–0.26)	0.19 (0.16–0.28)	0.16 (0.10–0.35)	0.20 (0.11–0.29)	0.15 (0.10–0.20)
Tectal complex thickness ( $\mu\text{m}$ )	0.95 (0.87–1.09)	0.99 (0.75–1.27)	1.06 (0.93–1.27)	0.99 (0.87–1.21)	1.34 (1.13–1.61)	1.05 (0.80–1.16)	1.03 (0.85–1.13)	0.81 (0.5–1.03)
Tectal complex/Foot layer + Endexine	1.95 (1.50–3.00)	1.69 (1.00–2.20)	2.17 (1.50–2.60)	2.00 (1.70–2.50)	2.30 (1.60–2.80)	1.73 (1.20–2.00)	1.85 (1.30–2.20)	2.24 (1.30–2.80)
Spine length ( $\mu\text{m}$ )	3.08 (2.30–3.90)	3.86 (3.00–4.90)	3.17 (2.20–4.00)	3.22 (2.30–5.50)	3.96 (2.74–5.10)	2.95 (2.35–3.53)	2.40 (1.50–3.60)	2.68 (1.40–3.80)
Spine width ( $\mu\text{m}$ )	3.46 (2.30–4.50)	3.49 (2.30–4.90)	3.31 (2.35–4.45)	3.10 (2.30–4.40)	3.94 (2.75–5.50)	3.56 (3.10–4.40)	2.94 (1.50–3.90)	2.60 (1.40–3.60)
Spine length/width	0.91 (0.60–1.17)	1.11 (1.00–1.42)	0.96 (0.85–1.10)	1.04 (0.81–1.56)	1.01 (0.75–1.42)	0.83 (0.64–1.00)	0.83 (0.67–1.00)	1.03 (0.57–1.50)
Structured part of the spines	(1/2–) 2/3–3/4	1/2–4/5	(1/3–) 1/2–3/4	1/2–2/3	(1/3–) 1/2–3/4	1/2–2/3	1/2–3/4	(1/3–) 1/2–3/4
Micro-perforated part of the spines	1/2–2/3 (–3/4)	(1/3–) 1/2–3/4	(1/3–) 1/2–3/4	(1/3–) 1/2–2/3	(1/3–) 1/2–3/4	1/2–2/3	(1/2–) 2/3–3/4	1/3–1/2 (–3/4)
Spinular micro-perforations ( $\mu\text{m}$ )	0.41 (0.28–0.63)	0.38 (0.20–0.55)	0.39 (0.28–0.56)	0.47 (0.24–0.72)	0.40 (0.29–0.55)	0.40 (0.04–0.70)	0.32 (0.14–0.60)	0.40 (0.21–1.00)

aceae species growing in the Mediterranean area analysed in her study, the mesoapertures do not intersect only the foot layer but they intersect “the foot layer and the distal layer of the endexine” as referred to Cardueae (Tormo Molina and Ubera Jiménez 1990). It must be noted that the boundary between foot layer and endexine is almost completely covered by the boundaries of the ectoapertures, a fact that undoubtedly makes difficult their observation with LM and SEM.

All taxa investigated in the present study showed an exine with Senecioid pattern, i.e., presence of caveae, internal foramina extremely reduced, one single layer of tectal columellae in the inter-spinular regions and a thin foot layer. According to Skvarla et al. (1977) the pollen exine of the Inulinae has both the Helianthoid and the Senecioid pattern. However, the authors reported the Helianthoid pattern only for the pollen exine of *Polycline proteiformis*, and this species is nowadays included in the Helenieae as *Athroisma proteiforme* (Humbert) Mattf. (Eriksson 1990). Therefore, taking into account that our present results and those of Skvarla represent more than 26% of the genera of Inulinae (sensu Anderberg et al. 2005), such a large sample indicates that, most likely, the Senecioid pattern is present in the pollen exine of all taxa of this subtribe.

Contrary to other authors (Skvarla and Turner 1966, Skvarla et al. 1977, Breitwieser and Sampson 1997), who considered the tectum of the pollen of Inuleae consisting of a single, discontinuous and more or less uniform layer, we consider the existence of a tectal complex composed of three distinct layers both morphologically and in thickness. This feature was reported for other taxa of Asteraceae (Dimon 1971, Praglowsky and Grafström 1980, Tormo Molina and Ubera Jimenez 1995, Zavada and Villiers 2000). Noteworthy, both the oldest pollen fossils of Asteraceae (Zavada and Villiers 2000) and those of Calyceraceae (Skvarla et al. 1977), the closest living relatives to Asteraceae, are acaveate, with the exine showing an Anthemoid pattern

in which the sub-TECTAL columellae support the structured tectum and the foramina are absent. Thus, the acaveate pollen is plesiomorphic, as stated by Wagenitz (1955, 1976), Skvarla et al. (1977) and Zavada and Villiers (2000).

As far as data obtained using LM and TEM may be compared, the qualitative features of the exine structure of the Inulinae investigated by us are generally close to those reported by Leins (1968, 1971). Also, our results agree with those of Skvarla et al. (1977) in that the internal tectum of Inuleae represents the dome-like thickenings referred by Leins (1971) for the bases of the spines. However, in most diagrams published by Leins (1968, 1971), the nexine 1 (foot layer) and the nexine 2 (endexine) are about the same thickness (e.g. in *Buphtalmum salicifolium* and *Inula graveolens*). In the taxa investigated in the present work, such observation is not verified since the endexine is at least 3.5 times thicker than the foot layer (Table 1). Also, the dimensions of the pollen grains of the entire species sample are smaller (3.6–5.3  $\mu\text{m}$ ) than those referred by Leins (1971) for the correspondent pollen types. Such divergent findings may be due to the different mounting media used for examining pollen grains. In fact, Leins (1971) employed glycerin jelly, which causes swelling of the exines with time contrarily to the silicone oil used in the present work.

The existence of a continuous transition among the quantitative pollen characters of the Inulinae species we have examined (Table 1), is demonstrative of a relative homogeneity of the pollen morphological characters in the Inulinae that emphasizes the fact that this subtribe is a natural taxon. In addition, it does not allow defining structural sub-types for the tectal complex of the taxa we have investigated, contrary to what Breitwieser and Sampson (1997) did for the Australian Gnaphalieae. Moreover, this relative homogeneity of the pollen characters somewhat weakens the pollen types system described by Leins (1971). According to this author, pollen grains of *Inula salicina* and *Jasonia tuberosa* belong to the pollen type *Inula parnassica*, those of *Dittrichia*



*viscosa* to the pollen type *Inula graveolens*, and those of *Asteriscus aquaticus*, *Pallenis maritima* and *Pallenis spinosa* to the pollen type *Buphtalmum salicifolium*. However, it must be noted that in the *Inula parnassica* and *Inula graveolens* pollen types, for instance, Leins (1971) pointed out, respectively, 1.2  $\mu\text{m}$  and 1.5–2.0  $\mu\text{m}$  for the sexine, whereas in the present study the thickness of the tectal complex (sexine) of *Inula salicina* and *Jasonia tuberosa* was found to be very close to that of *Dittrichia viscosa* (Table 1). It also must be noted that Leins (1971) considered the *Buphtalmum salicifolium* pollen type similar to the *Inula parnassica* pollen type.

In the majority of the cladistic studies, either the Inuleae (sensu Anderberg et al. 2005) either the Senecioneae or the Anthemidae, are considered sister groups of the other tribes of Asteroideae or belonging to a polytomy at the base of this subfamily. As referred above, all the Inuleae investigated in the present work have an exine with a Senecioid pattern. This is, by far, the most common exine pattern within the Senecioneae (Skvarla and Turner 1966, Skvarla et al. 1977, Bain et al. 1997). The Anthemidae is the only Asteroideae tribe that presents an acaveate (Anthemoid) exine pattern (Skvarla and Larson 1965, Skvarla and Turner 1966, Skvarla et al. 1977). Since the Senecioid pattern presents only vestigial foramina and the Anthemoid pattern lacks foramina, we consider that the presence of conspicuous foramina (Helianthoid and Gnaphalioid patterns) is an apomorphy within the Asteroideae. Concerning the pattern of the exine, the cladograms of most authors reveal that the Senecioid pattern is plesiomorphic within the Asteroideae. Then, the Anthemoid pattern of the Anthemidae should represent a character reversal. An alternative hypothesis, supported by the cladograms of Goertzen et al. (2003), is that the Anthemoid pattern is plesiomorphic and the caveate exines (including the Senecioid pattern) are apomorphic.

*Limbarda crithmoides* showed a great number of aborted pollen grains with altered shapes,

abnormal shaped spines, and a thicker and more compact exine than those of normal grains (see Fig. 2F). This condition is likely to be related to the existence of irregular meiosis caused by non-stabilized chromosomic numbers, a phenomenon that was demonstrated by authors such as Guinochet (1957) and Avetisian and Tonyan (1975) for the Asteraceae.

The conspicuousness of both the spines and the spinular microperforations in all taxa investigated in this work, identifies the pollen of the Inuleae as entomophilous. The rather thick columellae offer a good protection against compressive forces (Bolick 1978, 1981). In the spinular areas, the absence of the connected basal region of the columellae, the frequent presence of enlarged caveae, and the great development of the columellar interstices allow easy communication between the pollen surface and the caveae facilitating the exchanges of water and physiologically active substances. It must be noted that the height reached by the spinular columellae correspond quite well to the location of the spinular microperforations (Table 1), a condition that enhances the role of the spines as repositories and conductors of exine holding substances to the stigma surface (Blackmore 1982, Salgado-Labouriau 1982).

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## Appendix

### Studied specimens

#### *Asteriscus aquaticus*

Estremadura: Alcanena, Minde, 28. V. 1975, Alexandre, Serra e Bernardino s. n. (PO – 24597) (SEM, LM); Lisboa, Forte do Guincho, 25.V.1971, J. Matos e A. Matos 11472 (COI) (TEM, LM).

Ribatejo: Entre Fátima e Vila Nova de Ourém, 12. VI. 1972, Rozeira, Serra e Bernardino s. n. (PO – 24596).

*Dittrichia viscosa*

Douro Litoral: Gondomar, Marecos, 14. V. 1975, Alexandre, Serra e Bernardo s. n. (PO – 24733) (TEM, LM). Estremadura: Setúbal, Quinta do Cesteiro, 13. IX. 1980, Almeida e Carvalho s. n. (COI) (LM). Algarve: Lagos, junto ao porto, 12. IX. 1987, Francisco e Caldas s. n. (PO and 53065) (LM).

*Inula salicina*

Trás-os-Montes: Bragança, Moinho dos Padres, 18. VI. 1963, Pinto da Silva e Bento Rainha 7168 (PO – 24685) (SEM, LM). Douro Litoral, Porto Manso, Aregos, 28. IX. 1961, J. Paiva, J. Matos e Marques 8228 (COI) (LM); Vila Nova de Gaia, Oliveira do Douro, 17. VII. 1979 Felgueiras e Lousa s. n. (PO – 31517) (TEM, LM).

*Jasonia tuberosa*

Trás-os-Montes: Bragança, Monte de S. Bartolomeu, 27. VII. 1968, A. Fernandes, R. Fernandes e Matos 10750 (COI) (SEM, LM); Andados 3 km de Vinhais para Bragança, 05. VIII. 1967, J. Paiva, J. Matos e Alves 10032 (COI) (TEM, LM); Vinhais, próximo de Vila Verde, 02. VII. 1964, Pinto da Silva e Bento Rainha 7375 (PO – 24671) (LM).

*Limbarda crithmoides*

Beira Litoral: Aveiro, Vagos, Barra de Mira, 02. VIII. 1977, Marques e Pereira 406 (COI) (SEM, LM); Figueira da Foz, Fontela, 26. VIII. 1976, A. Matos e Alves 13819 (COI) (LM). Estremadura: Entre Samora Correia e Alcochete, Herdade de Camarate, 28. VIII. 1971, Rozeira, Barreto e Araújo s. n. (PO – 24879) (TEM, LM).

*Pallenis maritima*

Algarve: Cabo de S. Vicente, 28. IV. 1991, Armando, Serra e Loureiro s. n. (PO – 55410) (TEM, SEM, LM); Ponta de Sagres, 28. II. 1986, Moura 2876 (COI) (LM); Portimão, Praia da Rocha, 14.

V. 1959, Rozeira, Koepp e Costa s. n. (PO – 24588) (LM).

*Pallenis spinosa*

Beira Litoral: Coimbra, Cantanhede, 28.V. 1980, Marques 1958 (COI) (LM). Ribatejo: A 5 km de Mação, 17. VI. 1956, A. Fernandes, J. Matos e Santos 5819 (COI) (TEM, LM). Estremadura: Setúbal, Almada, 26. VI. 1980, Mendes s. n. (COI) (LM).

*Pulicaria dysenterica*

Beira Litoral: Aveiro, Ilhavo, 30. VII. 1993, Armando, Serra e Loureiro s. n. (PO – 55428) (SEM).

*Pulicaria paludosa*

Trás-os-Montes: Margens do Rio Sabor, na Estrada Moncorvo – Macedo de Cavaleiros, 06.VII. 1976, A. Matos, Pimenta e Forte 13707 (COI) (TEM, LM). Beira Litoral: Aveiro, entre a Ponte de Perrães e a Pateira de Fermentelos, 12.IX.1978, Marques e Pereira, 901 (COI) (TEM). Baixo Alentejo: Santiago do Cacém, Monte dos Alhos, 06.VI. 1968, Teles 1234 (LISE – 66878).

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