



Palynotaxonomy of the genus *Gladiopappus* (*Dicomeae*, *Asteraceae*) with special emphasis on the exine ultrastructure and mesoapertures

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Key words

Dicomeae
exine
LM
Mutisieae
pollen
SEM
TEM

Abstract The pollen morphology of *Gladiopappus vernonioides* was studied with transmission (TEM) and scanning (SEM) electron microscopy and with light microscopy (LM). An Anthemoid pattern of exine ultrastructure was found. The pollen morphology of *Gladiopappus* supports the inclusion of this genus in the tribe *Dicomeae* and subtribe *Dicominiae* but not in the *Mutisieae* s.str. The apertural system of *G. vernonioides* includes a mesoaperture that intersects the foot layer and the upper layer of the endexine, a condition already pointed out for several tribes of *Asteroideae* (*Helenieae*, *Gnaphaliinae*, *Heliantheae*, *Inuleae*, *Senecioneae*) and *Carduoideae* (*Cardueae*, *Dicomeae*). It is suggested that the existence of an intermediate aperture could characterize the apertural system of the *Asteraceae* as a synapomorphy.

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INTRODUCTION

In 1947, when travelling in the south of Madagascar, the French botanist Jean-Henri Humbert discovered a new endemic species and genus of *Asteraceae* at the cape Sainte Marie, on the edge of a limestone plateau. In the following year he described *Gladiopappus* and its only species (*G. vernonioides* Humb.), and included it in the *Mutisieae* s.lat. (Humbert 1948). This tribe, in its classic concept, was described, for the first time, by Cassini (1817) and later accepted by the generality of botanists (e.g., Bentham 1873, Hoffmann 1894). One of the most important was Cabrera (1951, 1965, 1971, 1977), who published several of the most relevant works related to the tribe taxonomy. The *Mutisieae* s.lat. were later considered by many authors (e.g., Bremer 1987, Panero & Funk 2002, Katinas et al. 2009, Tiangang et al. 2011) as an artificial group which they divided in several tribes. One of these taxa was the *Dicomeae*, a small tribe that Panero & Funk (2002) proposed for the first time. Although several botanists (e.g., Hind 2007) followed Humbert's (1948) taxonomic treatment and considered *Gladiopappus* as belonging to the *Mutisieae*, Ortiz et al. (2009) included it in the *Dicomeae*. Other authors accepted the inclusion of *Gladiopappus* in this tribe (e.g., Ortiz et al. 2013), sometimes stressing that they did so on a provisional basis (e.g., Tree of Life Web Project 2009). Therefore, the tribal position of the genus is still controversial.

Many authors have studied the pollen morphology of the *Mutisieae* s.lat. with light microscopy (LM) (e.g., Wodehouse 1929a, b, Stix 1960, Dimon 1971, Parra & Marticorena 1972, Pastana 1989) or scanning electron microscopy (SEM) (with

or without LM, as did Hansen 1990, Lin et al. 2005, Zhao et al. 2006, Tellería & Katinas 2004, 2009 and Wortley et al. 2012). Skvarla & Turner (1966), Southworth (1966) and Tellería & Katinas (2009) investigated, with transmission electron microscopy (TEM), the exine ultrastructure of, respectively *Mutisia campanulata*, *Gerbera jamesonii* and *Mutisia spinosa*, three taxa that belong to the *Mutisieae* s.str. Although Skvarla et al. (1977) employed TEM to study the exine ultrastructure of four genera belonging to the *Mutisieae* s.lat. (*Dasyphyllum*, *Doniophyton*, *Glossarion* and *Schlechtendalia*), further studies (e.g., Katinas et al. 2009) have shown that none of these taxa correspond to the modern concept of the tribe. In fact, *Dasyphyllum*, *Doniophyton* and *Schlechtendalia* belong to the *Barnadesieae* (*Barnadesioideae*), and even *Glossarion*, a genus that Cabrera (1977) and Katinas et al. (2008) included in the *Mutisiinae* was not considered as belonging to the modern *Mutisieae* (Katinas et al. 2009). For their part, Ortiz & Pereira Coutinho (2001) and Pereira Coutinho et al. (2012) studied, with LM, SEM, and, in two cases (*Erythrocephalum* and *Pleiotaxis*), also with TEM, the palynology of seven genera of *Dicomeae* (*Cloiselia*, *Dicoma*, *Dicomopsis*, *Erythrocephalum*, *Macledium*, *Pasaccardoa* and *Pleiotaxis*).

Zhao et al. (2006) published the only observations on the pollen morphology of *G. vernonioides*. They employed LM and SEM – but not TEM – to study the exine of *G. vernonioides*, basing their descriptions on the pollen grains of a single specimen (*Humbert 20326*, isotype) in the Kew Herbarium. Only five measurements of the exine characters were taken, and the study lacked a statistical analysis of the data. For these reasons, we consider Zhao et al.'s (2006) pollen description of *G. vernonioides* as incomplete. In addition, considering the uncertainties in the taxonomic placement of the taxon and the well-known importance of pollen morphology for the taxonomy and ecology of the *Asteraceae* (e.g., Wodehouse 1935, Wagenitz 1976, Skvarla et al. 1977, Bolick 1978, Blackmore 1982, Wang et al. 2009, Blackmore et al. 2010, Wortley et al. 2012, Tellería et al. 2013), we have decided to study the exine of that rare genus in greater detail (TEM, SEM and LM).

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Table 1 Palynological studies on the subfamilies of the *Asteraceae* and the presence of mesoapertures.

Subfamilies	Mesoapertures	Authors
<i>Barnadesioideae</i>	Not referred	Urtubey & Tellería (1998), Stuessy et al. (2009)
<i>Famatinanthoideae</i>	Yes	Freire et al. (2014)
<i>Mutisioideae</i>	Yes	Tellería & Katinas (2009), Freire et al. (2014)
<i>Stifftioideae</i>	Yes	Tellería & Katinas (2004)
<i>Wunderlichioideae</i>	Not referred	Zhao et al. (2006), Tellería (2007)
<i>Gochnatioideae</i>	Yes	Tellería et al. (2013)
<i>Hecastocleidoideae</i>	No	Tellería & Katinas (2005)
<i>Carduoideae</i>	Yes	Leonardis et al. (1983), Tormo-Molina & Uberta-Jiménez (1990, 1995), Duistermaat (1996), Wortley et al. (2008), Pereira Coutinho et al. (2012)
<i>Pertyoideae</i>	Not referred	Tellería & Katinas (2005), Katinas et al. (2008)
<i>Gymnarrhenioideae</i>	Not referred	Zhao et al. (2006)
<i>Cichorioideae</i>	Yes	El Ghazaly (1980), Blackmore (1982), Wang et al. (2009)
<i>Corymbioideae</i>	Not referred	Wortley et al. (2007)
<i>Asteroideae</i>	Yes	Dimon (1971), Pereira Coutinho (2002), Pereira Coutinho & Paiva (2003), Jaramillo & Trigo (2006), Pereira Coutinho & Dinis (2007, 2009), Osman (2011), Montes & Murray (2014), Pereira Coutinho et al. (2014, 2016)

Table 2 Exine layers involved in the mesoapertures.

Subfamily	Tribe	Exine layers	Microscopies	Authors
<i>Mutisioideae</i>	<i>Mutisieae</i>	Inner layer of the sexine + outer layer of the nexine	LM, SEM	Tellería & Katinas (2009)
<i>Carduoideae</i>	<i>Athroismeae</i>	Foot layer + outer layer of the endexine	TEM	Pereira Coutinho et al. (2012)
	<i>Cynareae</i>	Foot layer + outer layer of the endexine Tectum	SEM, TEM LM	Tormo-Molina & Uberta-Jiménez (1990, 1995) Leonardis et al. (1983)
<i>Cichorioideae</i>	<i>Arctoteae</i>	Foot layer	LM	Dimon (1971)
	<i>Cichorieae</i>	Outer layer of the endexine	LM, SEM	El Ghazaly (1980)
		Foot layer	SEM, TEM	Blackmore (1982)
<i>Vernonieae</i>	Foot layer	LM	Dimon (1971)	
<i>Asteroideae</i>	<i>Anthemideae</i>	Foot layer	LM	Dimon (1971)
	<i>Gnaphalieae</i>	Foot layer	LM	Dimon (1971)
		Foot layer + outer layer of the endexine	TEM	Pereira Coutinho & Dinis (2009)
	<i>Helenieae</i>	Foot layer + outer layer of the endexine	TEM	Pereira Coutinho (2002)
	<i>Heliantheae</i>	Foot layer	LM	Dimon (1971)
		Foot layer + outer layer of the endexine	TEM	Pereira Coutinho (2002), Pereira Coutinho et al. (2016)
	<i>Inuleae</i>	Foot layer	LM	Dimon (1971)
		Foot layer + outer layer of the endexine	TEM	Pereira Coutinho & Dinis (2007)
	<i>Eupatorieae</i>	Foot layer	LM	Dimon (1971)
	<i>Millerieae</i>	Foot layer + outer layer of the endexine	TEM	Pereira Coutinho (2002), Pereira Coutinho & Paiva (2003)
	<i>Senecioneae</i>	Foot layer + outer layer of the endexine	TEM	Montes & Murray (2014)

A general point to be also considered is the question of the presence of mesoapertures, i.e., 'the middle part of a compound aperture in which there is also an ectoaperture and an endoaperture' (Punt et al. 2007). This is a rare condition in the angiosperms. It occurs in the *Polygonaceae* (Punt et al. 2007), some *Boraginaceae* (Saad-Limam et al. 2002) and the great majority of the *Asteraceae*. A considerable number of authors discussed the questions of the presence of mesoapertures (see Table 1) and which exine layers it involved (see Table 2) in the apertural system of the *Asteraceae*. It is worth noting that the structure was recorded in all the main subfamilies (*Helianthoideae*, *Carduoideae*, *Cichorioideae*, *Mutisioideae*).

Our main objectives were to characterize the pollen morphology of *Gladiopappus* and to clarify its tribal position. We also intended to provide some data about the existence and structural morphology of a mesoaperture in its pollen apertural system and to help to clarify its taxonomic significance for the *Asteraceae*.

MATERIALS AND METHODS

Specimens seen

Gladiopappus vernonioides Humb.

MADAGASCAR, Cap Sainte Marie, 11 July 1948, *Jean de Dieu 1428-RN* (P); Falaise terminale du Cap Sainte Marie, 23 Sept. 1958, *M.G. Cours s.n.* (P).

General treatment

The pollen grains of two specimens belonging to the Herbarium of the Muséum National d'Histoire Naturelle, Paris (see above) were collected and acetolyzed according to Erdtman (1960). The terminology for exine descriptions followed, in general, Punt et al. (2007) and, for some details of the exine structure (columellae nomenclature), Blackmore et al. (2009).

LM

The pollen material was included in silicone oil (Andersen 1960) and then observed and photographed with a Motic BA 310 light microscope equipped with a digital camera. Thirty measurements of the following characters were taken: polar axis (P), equatorial diameter (E), exine thickness in the polar areas, ectoaperture length, mesoaperture length and width,

endoaperture width, spines length and basal width. The ratios P/E and spine length/basal width were then calculated.

SEM

After dehydration in an increasing ethanol gradient, the pollen grains were mounted on aluminium stubs, covered with gold-palladium with an ion sputter coater JEOL JFC-1100 (1200 V, 6 mA, 10 minutes) and observed with a Hitachi SU-70 scanning electron microscope operating at 4 kV. Twenty measurements of the diameter of the spine and inter-spines perforations were taken.

TEM

The pollen grains were fixed with osmium tetroxide 2 % in 0.1 M sodium cacodylate buffer (pH 7.2, 24 h), dehydrated in an increasing ethanol gradient (70–100 %) and embedded in Spurr's resin. Ultra-thin sections were made with a Leica EM UC6 EMFC6 ultramicrotome fitted with a diamond knife, and contrasted with uranyl acetate and lead citrate. Then they were observed with a FEI-Tecnaï G2 Spirit Biotwin transmission electron microscope operating at 100 kV. Twenty measurements of the following characters were taken: tectum, internal tecta, foot layer and endexine thickness (these two characters in non-apertural areas); inter-spines middle and outer columellae width.

Statistics

The maximum and minimum values, arithmetic mean and standard deviation are reported for all observed measures.

RESULTS

Pollen grains description

Pollen in monads, isopolar (Fig. 1a, b), with radiate symmetry, 3-zono-colporate, elliptic in meridian optical section (Fig. 1a, b), subcircular in equatorial optical section, oblate-spheroidal to subprolate, P/E = 0.96–1.31 (1.10 ± 0.09). P = 32.50–49.20 (40.70 ± 4.08) μm , E = 32.50–40.80 (37.00 ± 1.97) μm . Ectoapertures: colpi, acute at the ends (Fig. 1c, d, 2a), 23.00–28.00 (25.20 ± 2.16) μm long; mesoapertures lalongate, elliptic (Fig. 1c, d), length = 4.50–12.40 (8.60 ± 2.74) μm , width = 6.80–18.60 (9.73 ± 3.02) μm ; endoapertures lalongate, constricted at the centre, more or less acute at the ends (Fig. 1c, d), width = 13.30–20.00 (16.20 ± 1.81) μm ; costae present. Exine with an Anthemoid pattern, i.e., acaveate (Fig. 1a, b, 2c, e, 3a–f), without internal foramina (Fig. 3a–f), with a large series of supporting columellae bearing shorter levels of outer columellae that alternate with internal tecta; exine 5.00–9.20 (7.25 ± 1.20) μm thick at the poles; tectum perforate (Fig. 2a–e, 3a–f), 0.16–0.27 (0.21 ± 0.03) μm thick; outer internal tectum 0.08–0.20 (0.14 ± 0.03) μm thick, perforate (Fig. 3c–f); inner internal tectum 0.29–1.00 (0.70 ± 0.19) μm thick, with an interlaced morphology (Fig. 2c–e, 3a–f); inter-spines supporting columellae longer and thicker than the inter-spines middle and outer layers columellae (Fig. 3a–f), frequently distally ramified (Fig. 2c–e, 3b–e); inter-spines middle layer columellae 0.14–0.29 (0.23 ± 0.05) μm thick, inter-spines outer layer columellae 0.07–0.23 (0.13 ± 0.05) μm thick; foot layer thicker than the endexine (Fig. 2c, 3a–f) except at the apertural areas (Fig. 3a, b). Sculpture echinate, spines acute to obtuse (Fig. 1a, b, 2a–e), 2.10–4.20 (3.10 ± 0.52) μm long, 4.20–6.70 (5.36 ± 0.68) μm wide at the base, spine length/basal width = 0.42–0.71 (0.58 ± 0.08), spines supporting columellae longer than

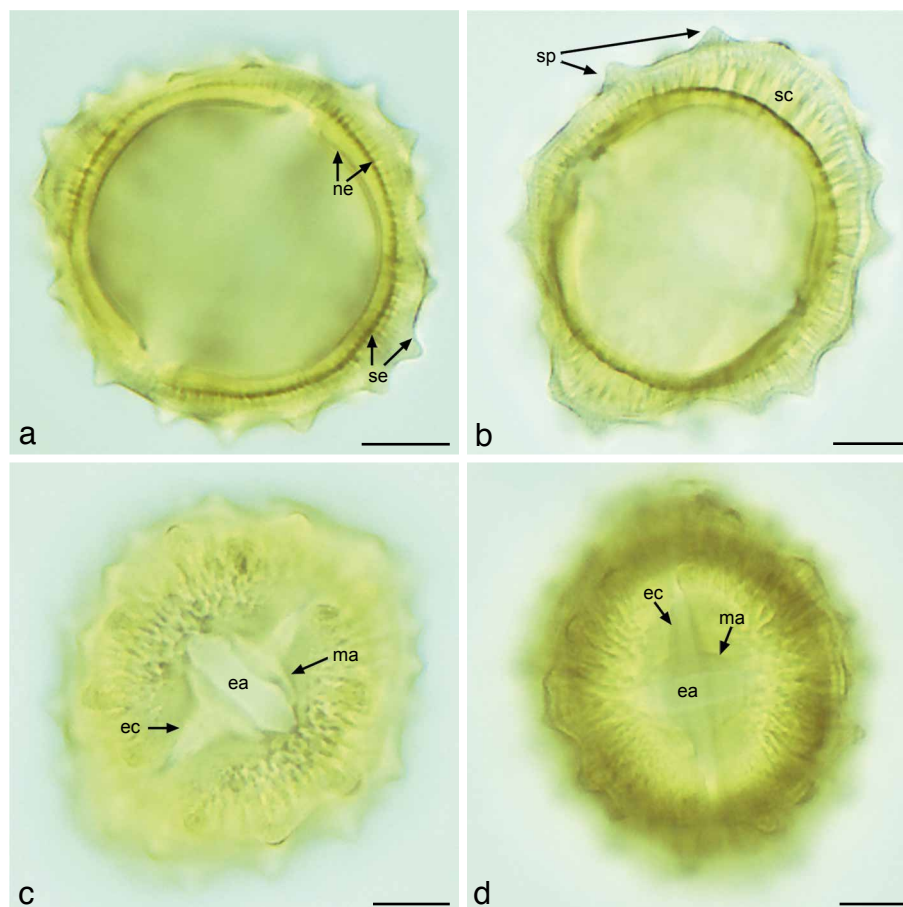


Fig. 1 LM micrographs. a, b. Oblique view of a meridional optical section; c, d. meridional superficial view of an aperture. — ea – endoaperture; ec – ectoaperture; ma – mesoaperture; ne – nexine; sc – supporting columellae; se – sexine; sp – spines. — Scale bars: 10 μm .

the inter-spines supporting columellae (Fig. 3b–d, f), reaching 1/3–1/2 of the spine length (Fig. 3b–d, f); perforations reaching 1/3–1/2 of the spine length (Fig. 2a–e), increasing in dimensions to the apex (Fig. 2a–e), diameter = 0.09–0.57 (0.29 ± 0.14) μm ; inter-spines sculpture scabrate-perforate (Fig. 2a, b, d, e), perforations diameter = 0.05–0.19 (0.11 ± 0.04) μm .

DISCUSSION

Palynotaxonomy

Our results agree, in general, with Zhao et al. (2006). Nevertheless, the values of P/E we found indicate that the shape of the pollen grains of *Gladiopappus* is more variable than they reported. They are oblate-spheroidal to prolate, and not simply prolate as Zhao et al. (2006) indicated. These authors postulated, but could not prove, the existence of 'possibly more than one columellae layer above proximal (basal) columellae'. The use of TEM allowed us to securely observe two levels of columellae and two internal tecta above the supporting columellae.

It must be stressed that Skvarla et al. (1977) suspected that the quantification of columellae levels and internal tecta could be useful from a taxonomic perspective.

The pollen sculpture of the *Mutisieae* s.str. is generally microechinate or microgranulate (Katinas et al. 2009), macrogranulate (Lin et al. 2005), or, more rarely (as in some species of *Mutisia*), microechinate-rugulate or rugulate (Tellería & Katinas 2009). Parra & Marticorena (1972) pointed out heights of 0.5–1.5 (1.8) μm for the exine of 61 taxa of the five genera (*Brachyclados*, *Chaetanthera*, *Chaptalia*, *Mutisia*, *Trichocline*) of *Mutisieae* s.str. they studied. Conversely, the *Dicomeae* present a clearly echinate sculpture, with a spine average length of about 3 μm and reaching a maximum of 8 μm (Ortiz & Pereira Coutinho 2001, Zhao et al. 2006, Pereira Coutinho et al. 2012). This is also a feature of the pollen grains of *Gladiopappus* (see Results), the type of sculpture and the spine size approaching this taxon to the *Dicomeae*, but not to the *Mutisieae* s.str. Another pollen feature that, as a trend, separates *Gladiopappus* from the *Mutisieae* s.str. is the ratio E/exine thickness, which Bolick (1991) described as useful for the taxonomy and phylo-

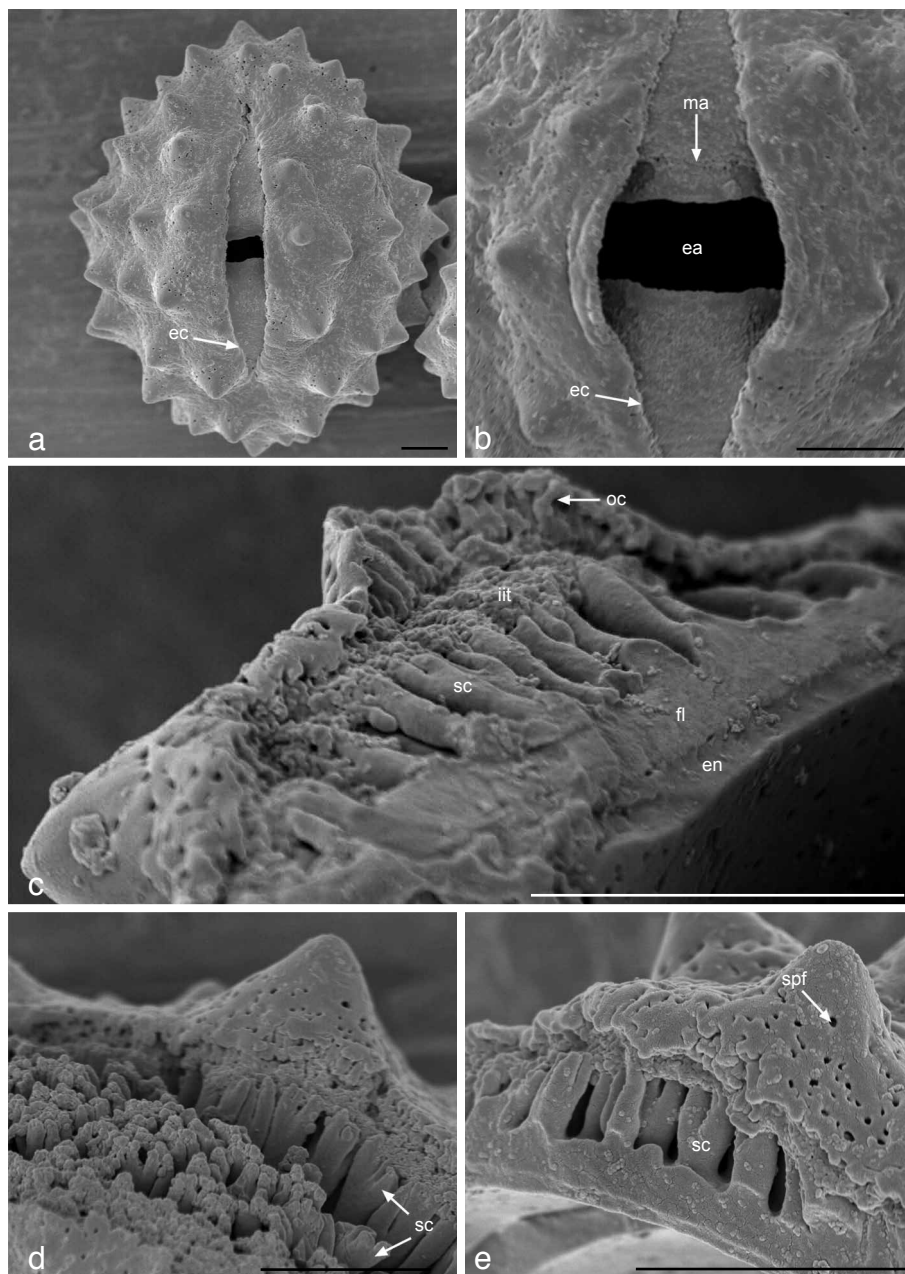


Fig. 2 SEM micrographs. a. Meridional view, showing an aperture; b. detail of the same view; c–e. details of fractured exines. — ea – endoaperture; ec – ectoaperture; en – endexine; fl – foot layer; iit – inner internal tectum; ma – mesoaperture; oc – outer columellae; sc – supporting columellae; spf – spine perforations. — Scale bars: 5 μm .

Table 3 Average ratio E/exine thickness of the genera of *Mutisieae* s.str.

Genera	E/exine thickness (average)	Authors
<i>Brachyclados</i>	7.7	Tellería & Katinas (2004)
<i>Chaetanthera</i>	7.6	Tellería & Katinas (2004)
<i>Chaptalia</i>	6.8	Parra & Marticorena (1972)
<i>Mutisia</i>	7.5	Tellería & Katinas (2009)
<i>Pachylaena</i>	6.3	Parra & Marticorena (1972)
<i>Trichocline</i>	6.6	Parra & Marticorena (1972)

geny of the *Asteraceae*. In fact, the average ratio for *G. vernonioides* is 5.1, a value that is somewhat lower than the ratio found by Pereira Coutinho et al. (2012) for the *Dicomeae* (6.1), but, even so, closer to it than to the average values of all the genera of *Mutisieae* s.str. that we have calculated based on other authors' data (see Table 3). It can be noted that even

Bolick's (1991) average value (6.6) of the mentioned ratio for the *Mutisieae* s.lat. (which comprised several taxa that are currently included in other tribes) is far from that of *G. vernonioides*. Also, the mesoapertures involve different exine layers in the *Dicomeae* and *Mutisieae* s.str. (see Table 2 and the subsection Mesoapertures of the Discussion).

Skvarla et al. (1977) described the Anthemoid pattern of ultrastructure for the *Anthemideae* and *Barnadesieae*. It was also reported for most of the *Cardueae* (Skvarla et al. 1977, Tormo-Molina & Ubeira-Jiménez 1995), the *Mutisieae* (Tellería & Katinas 2009) and the *Dicomeae* (Ortiz & Pereira Coutinho 2001, Pereira Coutinho et al. 2011). This pattern also characterizes the exine of *Gladiopappus*, and it is relevant to compare its details with the two subtypes that Pereira Coutinho et al. (2011) reported for the *Dicomeae*. They described the existence of 'supporting columellae thick, densely distributed and more or less straight' for the *Dicominiae* and 'at least some of the sup-

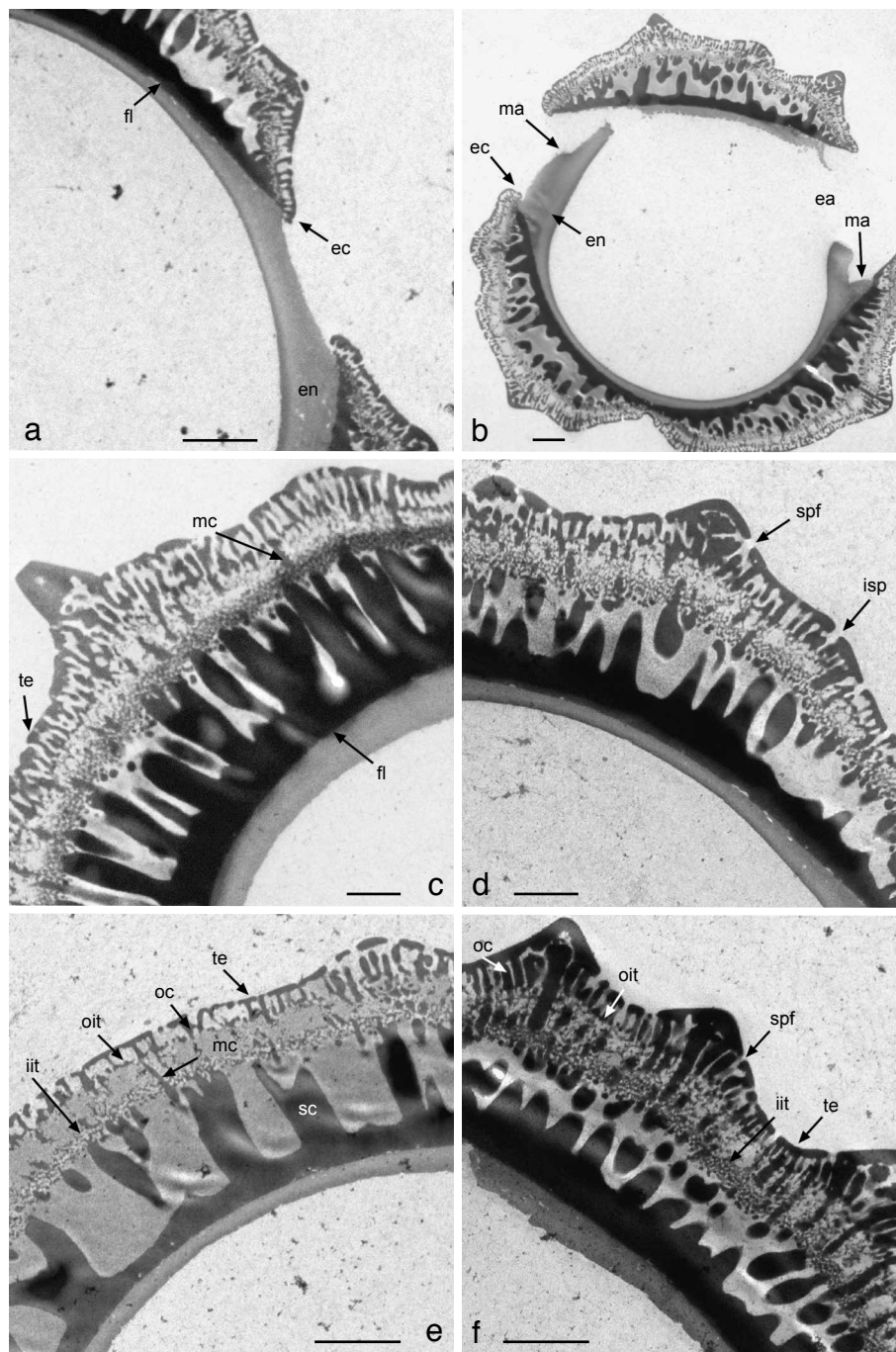


Fig. 3 TEM micrographs. a. Detail of an aperture; b. general equatorial section; c–f. details of exine sections. — ea – endoaperture; ec – ectoaperture; en – endexine; fl – foot layer; iit – inner internal tectum; isp – inter-spine perforations; ma – mesoaperture; ma – mesoaperture; oc – outer columellae; oit – outer internal tectum; mc – middle columellae; sc – supporting columellae; spf – spine perforations; te – tectum. — Scale bars: 2 μ m.

porting columellae thin, more or less loosely distributed and more or less curved' for the *Pleiotaxinae*. Clearly, the exine of *Gladiopappus* belongs to the first subpattern, which supports the inclusion of this genus in the subtribe *Dicominae*.

Mesoapertures

Our results agree with those described by Tormo-Molina & Ubera-Jiménez (1990, 1995) and Pereira Coutinho et al. (2012) for the *Carduoideae* and by Pereira Coutinho (2002), Pereira Coutinho & Paiva (2003), Pereira Coutinho & Dinis (2007, 2009), Pereira Coutinho et al. (2011, 2016) and Montes & Murray (2014) for the *Asterioideae*. Nevertheless, they only partially agree with the data reported by El Ghazaly (1980) and Blackmore (1982) for the *Cichorioideae*. In fact, these authors considered the mesoaperture as involving, respectively, the outer layer of the endexine and the foot layer, but all our observations indicate that it intersects the foot layer and the upper layer of the endexine. Tellería & Katinas (2009) described the mesoaperture of *Mutisia* (*Mutisioideae*) as involving 'the inner layer of the sexine and the outer layer of the nexine'. Although they employed LM, SEM and TEM, it is not clear which of these techniques they specifically used to observe these structures. Bearing in mind Tellería & Katinas' (2009) description, we think that they did not observe the details of the apertures with TEM. This implies that, in *Mutisia*, the columellae, the foot layer and the distal part of the endexine or the columellae and the foot layer are involved in the mesoaperture, in any case a different situation from that we observed in *Gladiopappus* and other taxa. In the case of *Gladiopappus* it was relatively easy to observe the mesoapertures limits with LM and SEM, but this is not always the case because, sometimes, they are either diffuse (Tellería & Katinas 2004) or more or less covered by those of the endoapertures (Dimon 1971, Tellería & Katinas 2004) or ectoapertures (Pereira Coutinho & Dinis 2007, 2009). In the last case, the internal view, with SEM, of fractured exines, can reveal the presence of the mesoapertures (Pereira Coutinho & Dinis 2007), and we suspect that, in some cases, the authors did not observe them because they did not employ TEM or SEM to study the exine ultrastructure and/or the endexine in fractured pollen grains. In conclusion: our data and other authors' (see Table 2) reveal that the presence of mesoapertures characterizes not only all the most important subfamilies of the *Asteraceae* (*Asterioideae*, *Cichorioideae*, *Carduoideae*, *Mutisioideae*) but also many of the smaller ones (*Famatinanthoideae*, *Stiffioideae*, *Gochnatioideae*), although some variation can occur in the exine layers that are intersected by these pollen structures. It is our conviction that, at least as a trend, the existence of a mesoaperture characterizes the apertural system of the *Asteraceae* as a synapomorphy, and that future careful examinations with TEM and SEM will reveal its presence in more subfamilies of *Asteraceae*.

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