

Palynotaxonomy of the subtribe Anisopappinae (Athroismeae, Asteraceae) and the genus Duhaldea (Inuleae, Asteraceae) based on their exine ultrastructure

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3 **Palynotaxonomy of the subtribe Anisopappinae (Athroismeae, Asteraceae) and**
4 **the genus *Duhaldea* (Inuleae, Asteraceae) based on their exine ultrastructure**
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27 **Keywords**

28 *Anisopappus*, Anisopappinae, *Duhaldea*, exine, mesoapertures, palynotaxonomy,
29 ultrastructure
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34 **Abstract**

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36 The exine ultrastructure of eighteen species of *Anisopappus* (Anisopappinae,
37 Athroismeae, Asteraceae) and three species of *Duhaldea* (Inuleae, Asteraceae) was
38 studied using Transmission Electron Microscopy (TEM). Two ultrastructural patterns
39 were identified: Senecioid and Helianthoid. The Senecioid pattern is characteristic of
40 the species included in the basal clades of the phylogenetic tree of *Anisopappus*, as
41 published in earlier studies. The Helianthoid pattern is restricted to the taxa placed in
42 the derived clades, including the core group of species of *Anisopappus* where *A.*
43 *chinensis*, the type species, is included. The observed ultrastructural differences seem
44 to suggest that a future splitting of *Anisopappus* into several different genera should
45 not be disregarded and that the Helianthoid pattern represents, within this genus, the
46 derived condition. The ultrastructure of the exine supports the inclusion, suggested by
47 previous authors, of the species *Duhaldea stuhlmannii*, but not that of *Philyrophyllum*
48 *schinzii*, in *Anisopappus*. In contrast, the three studied species of *Duhaldea* (*D. cappa*,
49 *D. nervosa*, *D. revoluta*) present an exine with a Senecioid pattern, which is
50 characteristic of the tribe Inuleae, in which they are included. The ectoaperture,
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3 mesoaperture, and endoaperture of all studied Anisopappinae and of *Duhaldea*,
4 respectively intersect the tectum plus columellae, the foot layer and the outer part of
5 the endexine, and the inner part of the endexine. This feature seems to characterize, in
6 a consistent way, the “Heliantheae alliance”, and is also present in all the studied
7 Asteroideae (excl. Anthemideae), Dicomioideae, and Carduoideae.
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13 14 1. Introduction

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17 The genus *Anisopappus* Hook. & Arn. was first described by Hooker and
18 Arnott (1837). At the end of the 20th century, Ortiz et al. (1996) and Eldenäs and
19 Anderberg (1996) respectively published their taxonomic and cladistics analyses of
20 this taxon, respectively. More recently, based on nuclear (ETS, ITS) and plastid
21 (*trnL-trnF*) molecular data, Bentley et al. (2015) concluded that *Philyrophyllum*
22 *schintzii* O. Hoffm., [formerly placed in the Gnaphalieae by Anderberg (1991)] fell
23 into the same clades as the species of *Anisopappus*. Based on further molecular data
24 (ETS, ITS, *ndhF*, *trnH-psbA*, *trnL-trnF*), Bengtson et al. (2017) published the first
25 phylogenetic study of Athroismeae (including 18 species of *Anisopappus*). The first
26 molecular phylogenetic analysis of the genus *Anisopappus*, was published by
27 Bengtson et al. (2021) using nuclear (ETS, ITS) and plastid (*ndhF*, *trnL-trnF*, *trnQ-*
28 *rps16*) data.
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38 *Duhaldea* DC., a small genus comprising 12-15 species of Asian perennial
39 herbs and subshrubs, was first described by De Candolle (1836) as belonging to the
40 tribe Inuleae. Although, several authors (e.g., Ling 1979) included this taxon in *Inula*
41 L., but, later, Anderberg (1991) reinstated it (Yang et al. 2022). In recent years, the
42 analyses carried out by Bengtson et al. (2017, 2021) and mentioned above
43 demonstrated that *D. stuhlmannii* (O. Hoffm) Anderb. should be transferred to
44 *Anisopappus*. Comparing its exine with the exines of other species of *Anisopappus*
45 and *Duhaldea*, including the type species of this genus, *D. cappa* (Buch.-Ham ex D.
46 Don) Pruski & Anderb., might provide further data to test this classification.
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53 Regarding Anisopappinae, Pereira Coutinho et al. (2014) published the only
54 study on the exine ultrastructure of the Anisopappinae, namely that of *Anisopappus*
55 *chinensis* Hook and *Cardosoa athanasioides* (Paiva & S. Ortiz) S. Ortiz & Paiva and
56 described a Helianthoid pattern (see definition below) for these two taxa.
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The tribe Athroismeae presents several taxonomic problems (Bengtson et al. 2017) including uncertainties in its subtribe Anisopappinae [e.g., the delimitation of boundaries among different species (Ortiz et al. 1996), including the Malagasy taxa (Bengtson et al. 2021)], although the delimitation of species has been partly clarified by the phylogenetic study (Bengtson et al. 2021), the result of which, to a large extent, agrees with the classification of Wild (1964). The present work intended to contribute to the elucidation of some of the above-mentioned questions, using exine ultrastructure. In many cases, the pollen morphology of Asteraceae shows good congruence with the topology of the supertrees of the family (Blackmore et al. 2009). Besides, palynological data have been useful to understand the taxonomy and phylogenetics of Asteraceae as demonstrated by many authors, such as Skvarla and Larson (1965), Skvarla et al. (1977), Blackmore et al. (2009), Pereira Coutinho and Dinis (2007, 2009), Pereira Coutinho et al. (2021), Tellería et al. (2013) and Noedoost et al. (2021).

Subsidiarily to the main objective, the present work also addresses the presence and morphology of mesoapertures in the exine of Anisopappinae and in *Duhaldea*, an issue we have been studying, in reference to the Asteroideae, the Dicomioideae, and the Heliantheae alliance, since 2007. Mesoapertures are “the middle part of a compound aperture in which there is also an ectoaperture and an endoaperture” (Punt et al. 2007). They are rare in pollen grains, existing only in very few families (e.g., Asteraceae and Polygonaceae) (Punt et al. 2007; Pereira Coutinho et al. 2016, 2018). In the Asteraceae, their morphology, which is of taxonomic interest, has been studied by several authors (e.g., Tormo-Molina and Ubera-Jiménez 1990; Tellería and Katinas 2009; Montes and Murray 2014; Pereira Coutinho et al. 2016, 2018).

The exine ultrastructure of the Asteraceae belongs to two groups: caveate [i.e., with caveae: “a cavity between two layers of the exine extending to the colpus margin where the layers meet” (Punt et al. 2007, p. 14)] and acaveate (without those structures). Within the caveate type, two patterns are considered: with or without internal foramina [i.e., small cavities situated in the sexine (Punt 2007); Fig. 1], which are respectively called Helianthoid and Senecioid [Skvarla & Turner (1966a, 1966b); Skvarla et al. (1977)].

2. Material and methods

2.1. *Pollen material*

The pollen was extracted from mature capitula from specimens deposited at the herbaria COI, K, P, PRE, SANT (see Appendix). We have studied the exine ultrastructure of a wide sampling of *Anisopappus* [at least one species from 10 of the 14 clades published by Bengtson et al. (2021); see this article and the Appendix].

2.2. *Transmission Electron Microscopy*

Pollen grains were acetolised according to Erdtman (1960). The fixation of the pollen grains was carried out with 2% osmium tetroxide in sodium cacodylate buffer (0.1 M, pH 7.2) for 24 h. Then, the material was included in agar (2%), dehydrated with a graded ethanol series (70%-100%), and embedded using an epoxy embedding kit (Fluka Analytical, Sigma Aldrich, Germany). Ultra-thin sections were obtained with a Leica EM UC6 (Leica Co; Austria) ultramicrotome, double contrasted with uranyl acetate (2%, 5 min) and lead citrate (0.2%, 7 min), and further observed with a FEI-Tecnaï G2 Spirit Biotwin electron microscope at 100 kV. Images were obtained using AnalySIS 3.2.

2.3. *Terminology*

The palynological terminology follows that of Punt et al. (2007) and Skvarla et al. (1977).

3. Results and Discussion

3.1. *Exine ultrastructure*

All the taxa examined have pollen with a caveate exine. This is the rule within the Asteroideae, the Anthemideae (the exine of which is acaveate) representing the only exception (Skvarla and Larson 1965; Skvarla et al. 1977). The found ultrastructural patterns are summarized in Table 1 and depicted below:

- a) Helianthoid: (Figs. 1A; 2B-D, F; 3A, C, D, F; 4A, D, F; 5A, B).
- b) Senecioid: (Figs. 1B; 2A, E; 3B, E; 4B, C, E, G-I; 5C-E).

Table 1 – Ultrastructural patterns of exine in the Anisopappinae and *Duhaldea*

Species	Ultrastructural pattern of exine
<i>Anisopappus africanus</i> Oliv. & Hiern	Helianthoid

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3	<i>A. alternifolius</i> (Less.) Bengtson &	Senecioid
4	Anderb.	
5		
6	<i>A. angolensis</i> O. Hoffm.	Helianthoid
7		
8	<i>A. athanasioides</i> Paiva & S. Ortiz	Helianthoid
9		
10	<i>A. chinensis</i> Hook. & Arn.	Helianthoid
11		
12	<i>A. corymbosus</i> Wild	Senecioid
13		
14	<i>A. grangeoides</i> (Vatkne & Höpfner)	Helianthoid
15	Merxm.	
16		
17	<i>A. holstii</i> (O. Hoffm.) Wild	Helianthoid
18		
19	<i>A. junodii</i> Hutch.	Senecioid
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21	<i>A. kirkii</i> (Oliv.) Brenan	Helianthoid
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23	<i>A. lastii</i> (O. Hoffm.) Wild	Helianthoid
24		
25	<i>A. latifolius</i> (S. Moore) B. L. Burtt	Senecioid
26		
27	<i>A. oliveranus</i> Wild	Helianthoid
28		
29	<i>A. pumilus</i> (Hiern) Wild	Helianthoid
30		
31	<i>A. schinzii</i> (O. Hoffm.) Bengtson, M.	Senecioid
32	Englund, Pruski & Anderb.	
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34	<i>A. smutsii</i> Hutch.	Senecioid
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36	<i>A. stuhlmannii</i> (O. Hoffm.) Bengtson,	Helianthoid
37	M. Englund, Pruski & Anderb.	
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39	<i>A. sylvaticus</i> (Humbert) Wild	Senecioid
40		
41	<i>Duhaldea cappa</i> (Buch.-Ham. ex D.	Senecioid
42	Don) Pruski & Anderb.	
43		
44	<i>D. nervosa</i> (Wall. ex DC.) Anderb.	Senecioid
45		
46	<i>D. revoluta</i> Anderb.	Senecioid
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3.2. *Mesoapertures*

In all the studied species a mesoaperture was present in the apertural system, intersecting the foot layer and the outer part of the endexine (Fig. 5). The ectoaperture and endoaperture intersect, respectively, the tectum and columellae, and the inner part of the endexine, respectively (Fig. 5).

In the context of this section, some points are to be highlighted:

3.3. *The existence of two ultrastructural patterns of exine*

3.3.1. *In the tribe Athroismeae*

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3 Although not common in the Asteroideae, the presence of different patterns of
4 exine cannot be considered a rare condition, as the Senecioneae (Skvarla et al. 1977),
5 Gnaphalieae (El-Ghazali and Anderberg 1995) and Heliantheae (Skvarla and Larson
6 1965; Skvarla and Turner 1966a; Pereira Coutinho 2002) present both Helianthoid
7 and Senecioid patterns. However, in these last two tribes only a reduced number of
8 genera - two in the Gnaphalieae [*Aliella* Qaiser & Lack and *Phagnalon* Cass. (El-
9 Ghazali and Anderberg 1995)], five in the Heliantheae [*Ambrosia* L., *Dicoria* Torr. &
10 A. Gray, *Iva* L., *Parthenice* A. Gray (Payne and Skvarla 1972) and *Xanthium* L.
11 (Skvarla and Larson 1965; Pereira Coutinho et al. 2021)] have the Senecioid pattern.
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20 3.3.2. *In the subtribe Anisopappinae*

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22 This is a rare situation within the Asteroideae. Even so, both the Helianthoid
23 and the Senecioid patterns are present in other subtribes, such as Ambrosiinae
24 (Heliantheae) (Skvarla and Larson 1965; Payne and Skvarla 1970), Senecioninae and
25 Tussilaginatae (Senecioneae) (Skvarla et al. 1977). Nevertheless, only one of these
26 patterns characterizes the exine of all the Astereae, Calenduleae, Coreopsideae,
27 Helenieae, Heliantheae (excl. Ambrosiinae), Inuleae and Millerieae (Payne and
28 Skvarla 1970; Pragłowski and Grafström 1980; Breitwieser and Sampson 1997;
29 Pereira Coutinho 2002; Pereira Coutinho and Dinis 2007, 2009; Pereira Coutinho et
30 al. 2014, 2016).
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39 3.3.3. *In the genus Anisopappus*

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41 The presence of two structural patterns of exine in a single genus
42 (*Anisopappus*) would be, to our knowledge, a unique occurrence within the
43 Asteraceae. In fact, in all the literature we have consulted related to the exine
44 ultrastructure of this family (Skvarla and Larson 1965; Skvarla and Turner 1966a,
45 1966b; Skvarla et al. 1977; Pragłowski and Grafström 1980; Vincent and Norris 1989;
46 Bain and Walker 1995; El-Ghazaly and Anderberg 1995; Breitwieser and Sampson
47 1997; Ortiz and Pereira Coutinho 2001; Pereira Coutinho 2002; Pereira Coutinho and
48 Paiva 2003; Pereira Coutinho and Dinis 2007, 2009; Tellería and Katinas 2009;
49 Pereira Coutinho et al. 2011, 2012, 2016, 2018, 2021), we could not find a single
50 example of a genus in which the exine presented more than one ultrastructural pattern.
51 In fact, species from the same genus presenting distinct patterns have been separated
52 into different genera. A good example of this condition, among many others, was
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3 demonstrated by Bain and Walker (1995) in their study on the exine ultrastructure of
4 *Senecio* L. In fact, these authors found that all the studied aureoid species shared the
5 Helianthoid pattern, while the non-aureoid species consistently presented the
6 Senecioid pattern. Based on this, they suggested that the aureoid taxa should be
7 placed in another genus (*Packeria* Á. Löve & D. Löve), as, in fact, it was done later
8 (Bain and Golden 2000). Another example is the New Zealand species *Anaphalis*
9 *rupestris* C. J. Webb, whose type of columellae bases layer differs significantly from
10 that of *Anaphalis margaritacea* (L.) Benth. & Hook. f. This led Breitwieser and
11 Sampson (1997) to conclude that *Anaphalis rupestris* should belong to a different
12 genus. And, in fact, Anderberg (1991) transferred it to *Anaphalioides* Kirpichnikov,
13 where it was synonymised.

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15 The data we obtained seem to indicate that splitting *Anisopappus* into some
16 genera should not be ruled out. It is worth noting that, apparently, the Senecioid
17 pattern characterizes exclusively the basal clades, being the Helianthoid pattern fully
18 restricted to derived clades [see Bengtson et al. (2021, p. 354), and the Appendix]. Of
19 course, for this separation to be made, other characters besides the exine ultrastructure
20 would have to be considered.

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22 The same can be pointed out for *Duhaldea* DC. In fact, unlike the species we
23 studied of this genus [*D. cappa* (Buch.-Ham. ex D. Don) Pruski & Anderb., *D.*
24 *nervosa* (Wall. ex DC.) Anderb., *D. revoluta* Anderb.], *D. stuhlmanii* (O. Hoffm.)
25 Anderb. (= *Anisopappus stuhlmannii*) presents a clear Helianthoid structural pattern.
26 Moreover, this taxon also differs from the other species of *Duhaldea* by a number of
27 additional features such as the presence of crystals in the cypsela epidermis and the
28 morphology of the anther appendages and thecae (Bengtson et al. 2017; 2021) leading
29 these authors to suggest that *D. stuhlmanii* should be included in the Athroismeae and
30 in *Anisopappus*. The pollen data supports this transference since most of the species
31 of *Anisopappus* studied (including the type species, *A. chinensis*) show a Helianthoid
32 structure. In contrast, all the other three *Duhaldea* herein studied show an
33 ultrastructural pattern of the Senecioid type, which is typical of the tribe Inuleae, to
34 which the genus belongs.

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3.4. The case of *Anisopappus schinzii*

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3 Bentley et al. (2015) proposed that *Philyrophyllum* should be integrated into
4 the Athroismeae and, more precisely, into *Anisopappus*. The exine of eleven of the
5 species of *Anisopappus* studied [*A. africanus* Oliv. & Hiern, *A. angolensis* O. Hoffm.,
6 *A. athanasioides* Paiva & Ortiz, *A. chinensis* Hook & Arn., *A. grangeoides* (Vatkne &
7 Höpfner) Merxm., *A. holstii* (O. Hoffm.) Wild, *A. kirkii* (Oliv.) Brenan, *A. lastii* (O.
8 Hoffm.) Wild, *A. oliveranus* Wild, *A. pumilus* (Hiern) Wild, *A. stuhlmannii* (O.
9 Hoffm.) Bengtson, M. Englund, Pruski & Anderb.], including the type species (*A.*
10 *chinensis*), exhibited a different pattern (Helianthoid). However, *A. alternifolius*
11 (Less.) Bengtson & Anderb., *A. corymbosus* Wild, *A. junodii* Hutch., *A. latifolius* (S.
12 Moore) B. L. Burt, *A. smutsii* Hutch., and *A. sylvaticus* (Humbert) Wild shared the
13 Senecioid pattern with *Anisopappus schinzii*. It is important to stress that all of these
14 species belong to the basal clades of the maximum clade credibility tree published by
15 Bengtson et al. (2021). As the two ultrastructural patterns are distinctly different, we
16 consider that these pollen data do not support the inclusion of *Philyrophyllum* in
17 *Anisopappus*. Furthermore, *Anisopappus* may eventually be divided into several
18 genera, considering that *A. schinzii* does not have same the exine ultrastructure as the
19 type species.

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22 Based on the work of Leins (1973) carried out with light microscopy, Anderberg
23 (1991) stated that the exine of *Philyrophyllum schinzii* (= *Anisopappus schinzii*)
24 differs from that of *Anisopappus* by having a double layer. However, our TEM
25 observations of the exine ultrastructure from both genera did not confirm these
26 differences. In fact, both exines showed a well-defined tectum, a single layer of
27 columellae, and an inner layer constituted by the fused base of the columellae. This
28 layer often showed a somewhat more complicated ultrastructure than the tectum, but
29 neither *Anisopappus* nor *Philyrophyllum* present the true internal interlaced sub-
30 columellar layer that characterizes the Gnaphalieae (Breitwieser and Sampson 1997;
31 Pereira Coutinho and Dinis 2009).

32 33 34 3.5. The mesoapertures of the *Anisopappinae* and *Duhaldea*

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37 The question of the presence of an intermediate aperture in the exine of
38 Asteraceae and especially in Asteroideae, Heliantheae alliance and Carduoideae has
39 been addressed by several authors (Tormo-Molina and Ubera-Jiménez, 1990, 1995;
40 Pereira Coutinho 2002; Pereira Coutinho and Paiva 2003; Pereira Coutinho and Dinis
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2007, 2009; Montes and Murray 2015; Pereira Coutinho et al. 2012, 2014, 2016, 2018, 2021). Concerning the Asteroideae and the Heliantheae alliance, in all the subtribes studied so far [Asteroideae: Gnaphaliinae (Pereira Coutinho and Dinis 2009), Inulinae (Pereira Coutinho and Dinis 2007), Senecioniinae (Montes and Murray 2015); Heliantheae alliance: Ambrosiinae (Pereira Coutinho et al., 2021), Coreopsidinae (Pereira Coutinho 2002), Gaillardiiinae (Pereira Coutinho 2002), Galinsoginae (Pereira Coutinho 2002, Pereira Coutinho and Paiva 2003), Helianthinae (Pereira Coutinho 2002), Marshaliinae, Psathyrotinae (Pereira Coutinho 2002)], the mesoapertures intersect the foot layer and the outer part of the endexine (and the ecto and endoapertures the tectum plus columellae and the inner part of the endexine, respectively). In accordance, the same was reported for Carduoideae [subtribes Carduinae, Centaureinae, Carlininae (Tormo-Molina and Uberta-Jiménez 1990) and tribe Dicomeae (Pereira Coutinho et al. (2018))]. This is also the case for Anisopappinae and *Duhaldea*, two taxa in which all the studied species presented the same features in their apertures, being in agreement with the data previously pointed out for the tribes Athroismeae (Pereira Coutinho et al. 2014) and Inuleae (Pereira Coutinho and Diniz 2007), to which they belong. This condition is common to many taxa of Asteroideae and to all species and genera of the Heliantheae alliance that were studied.

4. Conclusions

The genus *Anisopappus* includes two types of exine ultrastructural pattern: the basal clades have a Senecioid pattern and the derived clades have a Helianthoid pattern. The pollen data support the transference of *Duhaldea stuhlmannii* to *Anisopappus*, as already suggested by previous authors. Pollen data do not support transferring *Philyrophyllum schinzii* to *Anisopappus*. In the exines of all the taxa of *Anisopappus* and *Duhaldea* studied, the ectoaperture, mesoaperture and endoaperture, respectively, intersected the tectum and columellae, the foot layer and upper part of the endexine, and the inner part of the endexine. This characteristic was previously described in many subtribes of the Asteroideae and the Heliantheae alliance. These palynological results, complemented with other morphological information and taking into account the topology of the recent phylogenetic analyses (Bengtson et al. 2017, 2021) support the recognition of several different genera in the subtribe Anisopappinae in addition to *Anisopappus*.

Conflict of interest

The authors declare that there is no conflict of interest among them.

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36 Appendix

37 Herbarium specimens studied

38 *Anisopappus africanus* Oliv. & Hiern

39 Angola: Reise nach Südwest-Africa, Kunene-Sambesi Expedition, 16.IV.1900, H.
40 Baum 823 (COI); Lunda, Nova Chaves, Rio Labège, 14.VIII.1927, Carrisso &
41 Mendonça 257 (COI); Moxico, Vila Luso, pr. Rio Luena, V.1937, Gossweiler 11171
42 (COI) [Fig. 2B].
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51 *Anisopappus alternifolius* (Less.) Bengtson & Anderb. [*A. salviifolius* (DC.) Wild]
52 (P), P-02804572 [Fig. 2A].
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57 *Anisopappus angolensis* O. Hoffm.

58 Angola: Humpata, III.1883, F. Newton 66 (COI) [Fig. 2C].
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5 *Anisopappus athanasioides* Paiva & S. Ortiz [*Cardosoa athanasioides* (Paiva &
6 S.Ortiz) S.Ortiz & Paiva]

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8 Angola: Huambo: Cachihugo, 23 Jun 2007, S. Ortiz, Paiva, Rodriguez-Oubiña,
9 Carballal, Serrano & Soares 847 (SANT) [Fig. 4F]; Chianga, Nova Lisboa, 10 May
10 1965, Hélder Cardoso s.n. (COI [holotypus]).
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15 *Anisopappus chinensis* (L.) Hook. & Arm subsp. *chinensis*

16
17 Angola: Huíla, Ungueria, pr. Rio Chacuto, 2.VI.1937, Gossweiler 11137 (COI); Sá da
18 Bandeira, entre o Rio Nene e Huíla, 14.V.1966, C. Henriques 975 (COI) [Fig. 1A,
19 2D].
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24 *Anisopappus corymbosus* Wild.

25
26 Angola: Benguela, entre Sousa Lara e o Lobito, 19.VII.1969, Grandvaux Barbosa
27 11724 (COI) [Fig. 2E]; Mossâmedes, Chopia, Serra da Lua, 20.V.1937, Gossweiler
28 10845 (COI); Mossâmedes, rio Mucumgo, 27.V.1937, A. W. Excell & F. A.
29 Mendonça 2259 (COI).
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34 *Anisopappus grangeoides* (Vatke & Höpfner ex Klatt) Merxm.

35
36 Angola: Huila, M. Koekemoer 3684 (PRE) [Fig. 2F].
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40 *Anisopappus holstii* (O. Hoffm.) Wild

41
42 Tanzania: Iringa District, Great North road, 5 ml. S. of Crossing Great Ruaha River,
43 18.IV.1962, R. Polhill & S. Paulo 2063 (P), P-0063814 [Fig. 3A].
44
45

46 *Anisopappus junodii* Hutch.

47
48 South Africa: Mpumalanga, Blydepoort Nat. Reserve, 14.VI.2008, Koekemoer 3606
49 (PRE) [Fig. 3B]; N. Province, above Klaserie waterfall, Mariepskop, 3.IX.2000,
50 Burrows 7005 (PRE); Transvaal, Lekgalameetse Nat. Reserve, 17.VII.1985, Stalmans
51 574 (PRE); Transvaal, Haenertsburg, lot 366, foot of Iron Crown Mountain,
52 13.VIII.1985, Venter 10857 (PRE).
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58 *Anisopappus kirkii* (Oliv.) Brenan
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3 Mozambique: Zambezia, Gurué, lugares secos, nos montes, 29.VI.1943, A. R. Torre
4 5625 (P); Koekemoer 4253 (PRE) [Fig. 3C].
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8 *Anisopappus lastii* (O. Hoffm.) Wild

9
10 Angola: Cuanza Norte, Serra de N'Gama, Dalutando-Cazengo, 16.VII.1935,
11 Gossweiler 10275 (COI); Huíla, Ungueria, pr. Rio Chacuto, 2.VI.1937, Gossweiler
12 11137 (COI). Moçambique: António Enes, na Costa, 20.X.1965, A. Gomes & Sousa
13 4883 (COI) [Fig. 3D].
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18 *Anisopappus latifolius* (S. Moore) B. L. Burt

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20 South Africa: Transvaal, Pilgrim's Rest, 22.XII.1932, Smuts & Gillett 2322 (PRE);
21 Transvaal, Pilgrim's Rest, 22.XI.1951, Codd 6731 (PRE) [Fig. 3E].
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23
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25 *Anisopappus oliveranus* Wild

26
27 Kenya: Monts Boura, Teita, VII.1897, R. P. Sacleux s. n. (P), P00063824 [Fig. 3F].
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29
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31 *Anisopappus pumilus* (Hiern) Wild

32
33 Angola: Huambo, Nova Lisboa, 6.IX.1964, Chianga, B. Teixeira & Figueira 7884
34 (COI) [Fig. 4A].
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38 *Anisopappus schinzii* (O. Hoffm.) Bengtson, M. Englund, Pruski & Anderberg
39 (*Philyrophyllum schinzii* O. Hoffm.)

40
41 Namibia: Lianshulu restcamp, SE Katima Mulilo, 20.V.1981, DS Hardy 5596 (PRE)
42 [Fig. 4B].
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44
45

46 *Anisopappus smutsii* Hutch.

47
48 South Africa: Mpumalanga, Mpakeni Beacon, 20.VII.1996, Deall 3936 (PRE);
49 Mpumalanga, Buffelksloof Natural Reserve, 14.V.2008, Kokemoer 3604 (PRE) [Fig.
50 4C]; Transvaal, Starvation Kreek, portion of Kaapsche Hoop Hiking Trail,
51 30.VII.1987, Onderstall 1353 (PRE). Swaziland: Shiselweni, 21.VIII.2002, Dlamini
52 A2415 (PRE).
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58 *Anisopappus stuhlmannii* (O. Hoffm.) Bengtson, M. Englund, Pruski & Anderb.
59 (*Duhaldea stuhlmannii* O. Hoffm.)
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3 Tanzania: Magaza, Shagayu Forest Reserve, Lushoto Distr., Tonga Region, 5.X.1964,
4 C. D. Mgaza 628 (K) [Fig. 4D].
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8 *Anisopappus sylvaticus* (Humbert) Wild
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10 Madagascar: Bassin inférieur du Mangoro, forêt orientale, 300 m alt. X.1927, H.
11 Perrier de la Bâthie 18.131 (P), P-02804558 [Fig. 4E].
12
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14

15 *Duhaldea cappa* (Buch.-Ham ex D. Don) Pruski & Anderb.
16

17 Nepal: Near Sisagri. Dry forests. 23.XI.1948. E. K. Janaki Ammal 1450 (K) [Fig. 1B,
18 4G].
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22 *Duhaldea nervosa* (Wall. ex DC.) Anderb.
23

24 China: Yunan, 1898, Henry 11683 (K).
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26 Thailand: B. Hansen, G. Seidenfansen & T. Smitinand 10790 (K) [Fig. 4H].
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29 *Duhaldea revoluta* Anderb.
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31 Siam: A. F. G. Kerr, s/d, A. F. G. Kerr 1502 (K) [Fig. 4I].
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Table 1 – Ultrastructural patterns of exine in the Anisopappinae and *Duhaldea*

Species	Ultrastructural pattern of exine
<i>Anisopappus africanus</i> Oliv. & Hiern	Helianthoid
<i>A. alternifolius</i> (Less.) Bengtson & Anderb.	Senecioid
<i>A. angolensis</i> O. Hoffm.	Helianthoid
<i>A. athanasioides</i> Paiva & S. Ortiz	Helianthoid
<i>A. chinensis</i> Hook. & Arn.	Helianthoid
<i>A. corymbosus</i> Wild	Senecioid
<i>A. grangeoides</i> (Vatkne & Höpfner) Merxm.	Helianthoid
<i>A. holstii</i> (O. Hoffm.) Wild	Helianthoid
<i>A. junodii</i> Hutch.	Senecioid
<i>A. kirkii</i> (Oliv.) Brenan	Helianthoid
<i>A. lastii</i> (O. Hoffm.) Wild	Helianthoid
<i>A. latifolius</i> (S. Moore) B. L. Burt	Senecioid
<i>A. oliveranus</i> Wild	Helianthoid
<i>A. pumilus</i> (Hiern) Wild	Helianthoid
<i>A. schinzii</i> (O. Hoffm.) Bengtson, M. Englund, Pruski & Anderb.	Senecioid
<i>A. smutsii</i> Hutch.	Senecioid
<i>A. stuhlmannii</i> (O. Hoffm.) Bengtson, M. Englund, Pruski & Anderb.	Helianthoid
<i>A. sylvaticus</i> (Humbert) Wild	Senecioid
<i>Duhaldea cappa</i> (Buch.-Ham. ex D. Don) Pruski & Anderb.	Senecioid
<i>D. nervosa</i> (Wall. ex DC.) Anderb.	Senecioid
<i>D. revoluta</i> Anderb.	Senecioid

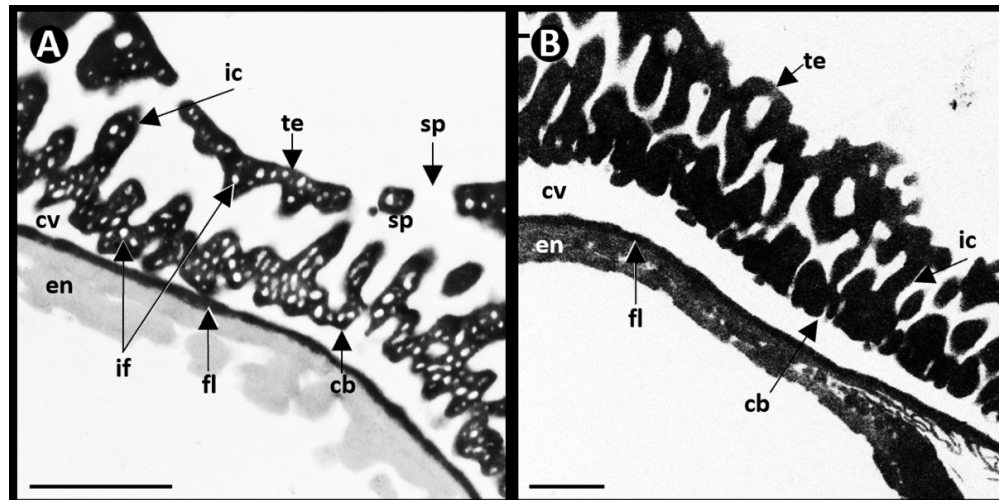


Figure 1. TEM micrographs of a Helianthoid and Senecioid patterns of the exine. A) *Anisopappus chinensis* (Helianthoid pattern); B) *Duhaldea cappa* (Senecioid pattern). cb – columellae base; cv – caveae; en – endexine; fl – foot layer; ic – inter-spinular columellae; if – internal foramina; ip – inter-spinular perforation; sa – sub-apical cavity; sc – spinular columellae; sp – spinular perforation; te – tectum. Bars = 1 μ m.

102x50mm (300 x 300 DPI)

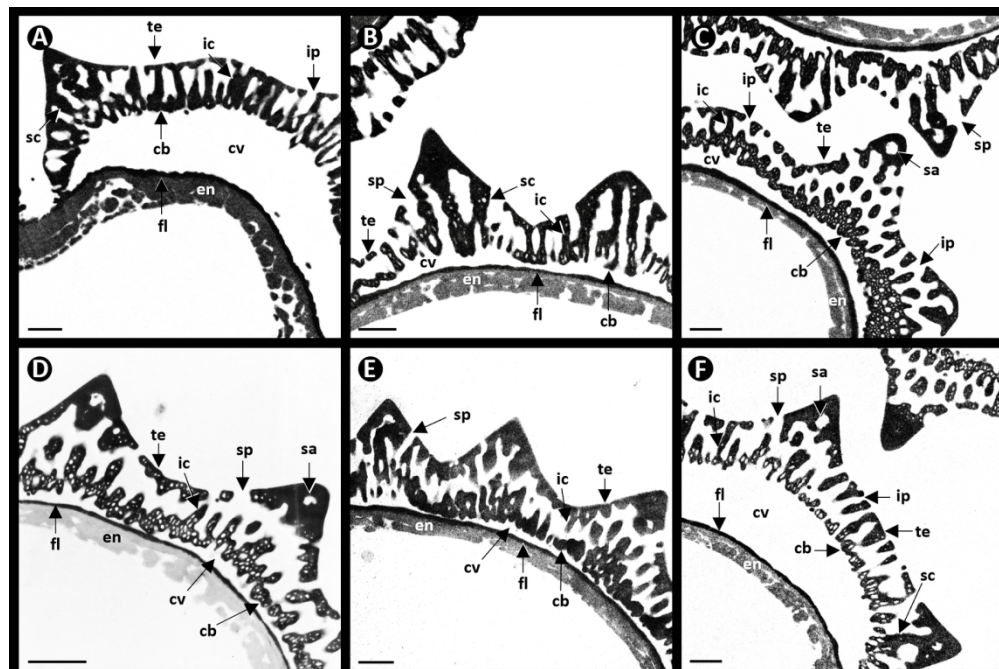


Figure 2. TEM micrographs of the exine of Anisopappinae species. A) *Anisopappus alternifolius* (Senecioid pattern); B) *A. africanus* (Helianthoid pattern); C) *A. angolensis* (Helianthoid pattern); D) *A. chinensis* (Helianthoid pattern); E) *A. corymbosus* (Senecioid pattern); F) *A. grangeoides* (Helianthoid pattern). cb – columellae base; cv – caveae; en – endexine; fl – foot layer; ic – inter-spinular columellae; ip – inter-spinular perforation; sa – sub-apical cavity; sc – spinular columellae; sp – spinular perforation; te – tectum. Bars = 1 μ m.

155x103mm (300 x 300 DPI)

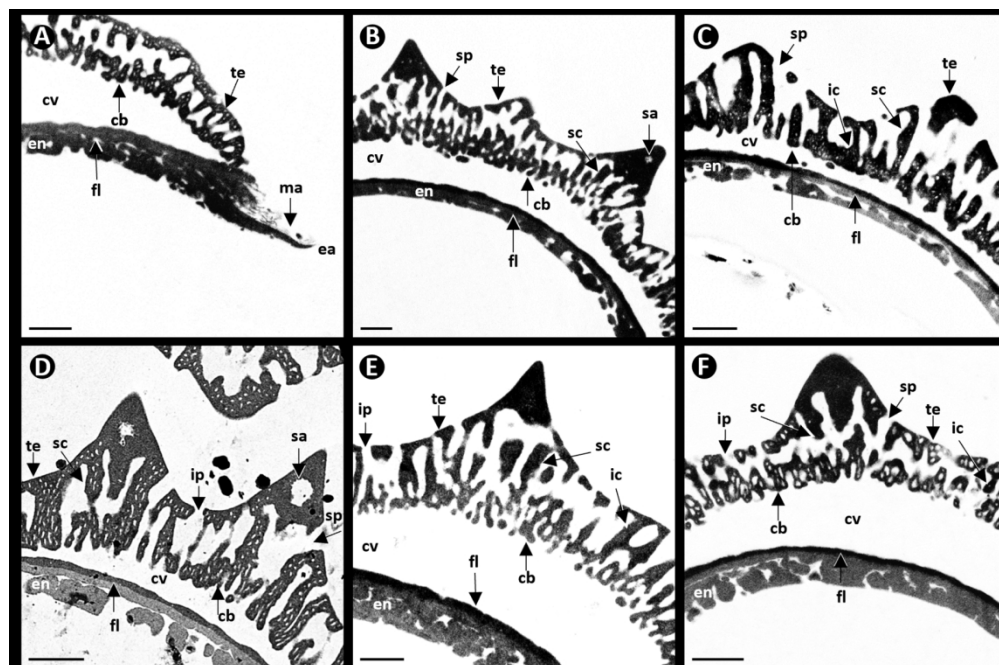


Figure 3. TEM micrographs of the exine of Anisopappinae species. A) *Anisopappus holstii* (Helianthoid pattern); B) *A. junodii* (Senecioid pattern); C) *A. kirkii* (Helianthoid pattern); D) *A. lastii* (Helianthoid pattern); E) *A. latifolius* (Senecioid pattern); F) *A. oliveranus* (Helianthoid pattern). cb – columellae base; cv – caveae; en – endexine; fl – foot layer; ic – inter-spinular columellae; ip – inter-spinular perforation; sa – sub-apical cavity; sp – spinular perforation; te – tectum. Bars = 1 μ m.

155x102mm (300 x 300 DPI)

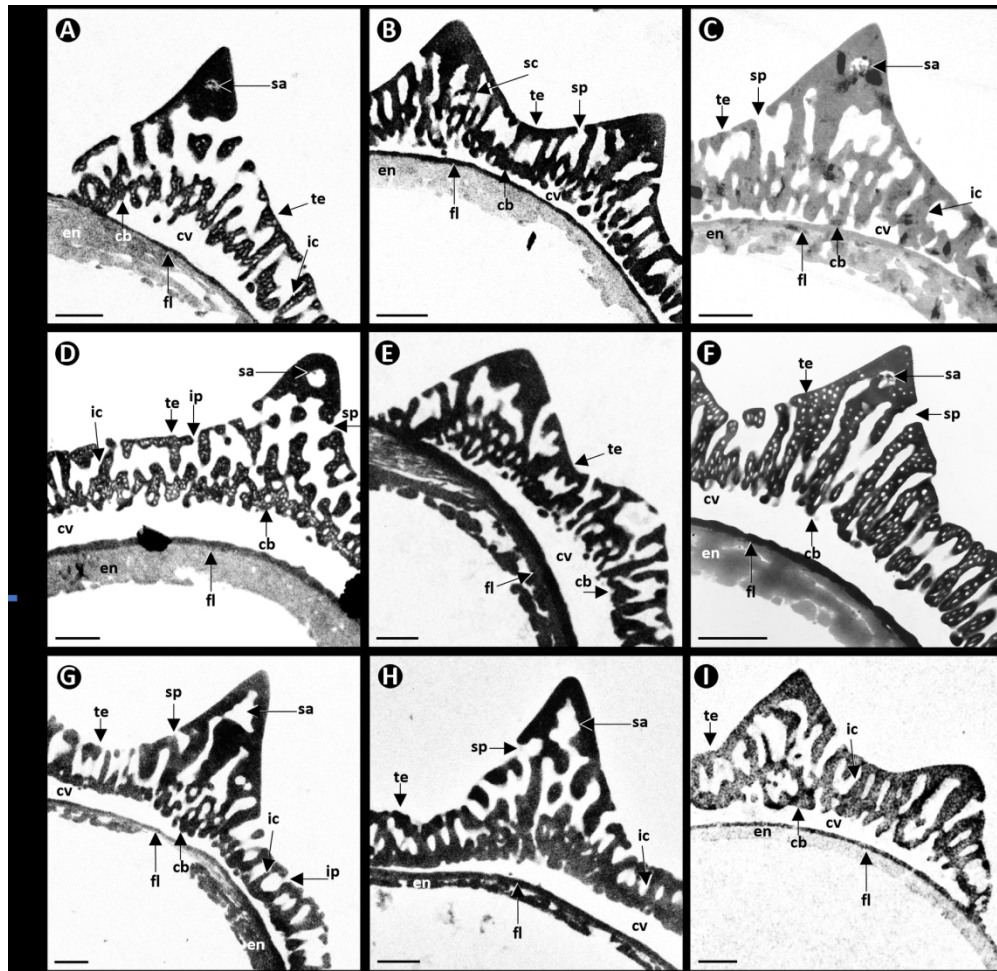


Figure 4. TEM micrographs of the exine of Anisopappinae and Duhaldea (Inuleae) species. A) *Anisopappus pumilus* (Helianthoid pattern); B) *A. schinzii* (Senecioid pattern); C) *Anisopappus smutsii* (Senecioid pattern); D) *A. stuhlmannii* (Helianthoid pattern); E) *A. sylvaticus* (Senecioid pattern); F) *A. athanasioides* (Helianthoid pattern); G) *Duhaldea cappa* (Senecioid pattern); H) *D. nervosa* (Senecioid pattern); I) *D. revoluta* (Senecioid pattern). cb – columellae base; cv – caveae; en – endexine; fl – foot layer; ic – inter-spinular columellae; ip – inter-spinular perforation; sa – sub-apical cavity; sp – spinular perforation; te – tectum. Bars = 1 μ m.

159x153mm (300 x 300 DPI)

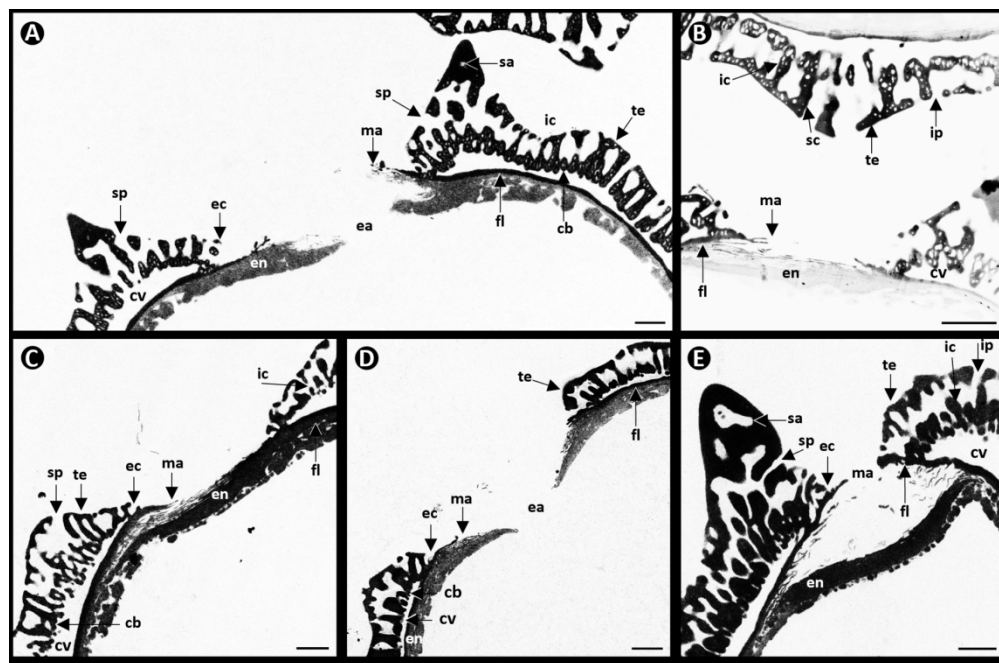


Figure 5. TEM micrographs of apertural areas of the exine of Anisopappinae. A) *Anisopappus angolensis* (Helianthoid pattern); B) *A. chinensis* (Helianthoid pattern); C) *A. latifolius* (Senecioid pattern); D) *A. schinzii* (Senecioid pattern); E) *Duhaldea cappa* (Senecioid pattern). cv – caveae; ea – endoaperture; ec – ectoaperture; en – endexine; ic – inter-spinular columellae; ip – inter-spinular perforation; ma – mesoaperture; sp – spinular perforation te – tectum. Bars = 1 μ m.

166x109mm (300 x 300 DPI)