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# Evolutionary significance of exine ultrastructure in the subfamily Barnadesioideae (Asteraceae) in the light of molecular phylogenetics

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ABSTRACT

Barnadesioideae (94 species) is the sister subfamily to the rest of the Asteraceae (23,000 species). Pollen grains in this subfamily are structurally and sculpturally distinctive and diverse. Although pollen morphology has contributed to the taxonomy of the subfamily, there is a gap of knowledge concerning the evolution of the exine structure. This study aims at exploring the systematic and phylogenetic significance of optimizing selected pollen characters of Barnadesioideae on the latest molecular phylogenetic tree. Transmission electron microscope (TEM) observations on pollen of selected species, some of them never explored so far, show that the exine probably evolved from a thin pattern (ca. 1–3 μm), with a well-developed foot layer and solid and free columellae, present in sister family Calyceraceae, towards a thicker (>6–11 μm) and a more complex columellate-granulate bilayered exine in Barnadesioideae (with very delicate columellae). The particular exine structure observed in the monotypic *Schlechtendalia luzulaefolia*, which combines compact and independent columellae (common in more derived Asteraceae) with a granular internal tectum as the inner ectexine layer (as in Barnadesioideae), reinforces its distant phylogenetic position within Barnadesioideae. More derived lineages within Asteraceae (e.g. Mutisioideae) retained some ancestral exine features although evolved an even thicker exine and a columellate trilayered exine (with robust columellae), rare in the angiosperm pollen grains.

## 1. Introduction

The first comprehensive pollen studies on Asteraceae were based on light microscope (LM) and conducted by Wodehouse (1935), Erdtman (1952), and Stix (1960), revealing important variations in morphology, particularly in the exine sculpture (e.g. psilate, echinate, lophate). The later advent of the electron microscopy (SEM and TEM) enabled Skvarla and collaborators (Skvarla and Larson, 1965; Skvarla and Turner, 1966; Skvarla et al., 1977) to describe the major patterns of the Asteraceae exine structure. Wagenitz (1976) and later authors (e.g. Blackmore et al., 2009) recognized the main evolutionary trends in pollen of Asteraceae supporting a fairly uniform exine structure in the latest diverging members of the family (currently comprised in Asteroideae) (e.g. cavea always present, single layer of thin columellae, and perforated tectum), and a more variable exine structure in early diverging groups, such as in Mutisioideae and Barnadesioideae. In these subfamilies, the exine is more variable (e.g. cavea uncommon, double layers of thin or thick columellae, tectum imperforated or slightly perforated, Katinas et al., 2008; Tellería et al., 2013; Zao et al., 2006). This particular morphological variability in the pollen of the earliest lineages offers an outstanding opportunity for exploring the systematic and phylogenetic significance of some groups of taxa. Molecular phylogenetic studies conducted on ancestral group of Asteraceae, such as Barnadesioideae (Gustafsson et al., 2001; Gruenstaeudl et al., 2009), provide a framework to hypothesize on the evolution of the exine structure in this subfamily.

The concurrent advances in palynology and phylogenetic analyses have allowed Banks and Rico (1999), Doyle (2005), and Mark et al. (2012), for example, to optimize pollen characters of several angiosperm families onto their molecular trees. In particular, Blackmore et al. (2009) optimized for the first time pollen characters of Asteraceae on a molecular supertree inferring a number of trends and evolutionary events. However, due to the broad taxonomic scale of their study, they only optimized pollen characters of the major clades, with scarce details, at genus level, of the earliest branches of the family (i.e. Barnadesioideae).

Barnadesioideae is monophyletic and sister to the rest of Asteraceae according to morphological and molecular evidence (e.g. Panero and Funk, 2008; Ortíz et al., 2009; Stuessy et al., 2009). It comprises nine genera and 91 species entirely restricted to South America (Stuessy et al., 2009). Pollen features within Barnadesioideae are highly distinctive, and several morphological types have been described (Urtubey and Tellería, 1998; Zao et al., 2000). However, exine features

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have never been accurately scored in a phylogenetic context, and hence their relationship with either the rest of Asteraceae or the sister family Calyceraceae remains obscure. This lack of knowledge has led to the general believe that pollen of the basally branching Asteraceae differs very little from that of Calyceraceae (Blackmore et al., 2009).

Here we conduct a detailed ultrastructural exine analysis of selected species of Barnadesioideae in the context of the evolution of this clade with the aim of exploring the systematic and phylogenetic significance of selected pollen characters on the basis of the latest molecular phylogenetic tree.

## 2. Materials and methods

Most of ultrastructural exine data of all species included by Gruenstaeudl et al. (2009) were obtained in the present study, except for most species of Calyceraceae which have been extensively documented by De Vore et al. (2007) (Table 1). Data of all voucher specimens examined with TEM are listed in Appendix A. The analysis of acaveate exine of *Dasyphyllum velutinum* was included for comparison with other species of *Dasyphyllum* which commonly have caveate exine (Urtubey and Tellería, 1998).

In the context of taxonomical groups here considered, *Picris* (Subfam. Cichorioideae) is a phylogenetically distant species (Funk et al., 2009). However, we include its exine data in order to follow the scheme proposed by the molecular study (Gruenstaeudl et al., 2009), but it was excluded from the phylogenetic analysis of characters. SEM photographs were included to illustrate the appearance of the pollen studied. Pollen samples were taken from specimens deposited in the following herbaria: HAO, HUT, LP, MCNS, TRU (Holmgren et al., http://sciweb.nybg.org/science2/IndexHerbariorum.asp). Some exine data were taken from Skvarla et al. (1977), Gustafsson et al. (1997), Zao et al. (2000), Tellería et al. (2003), Tellería and Katinas (2009), and De Vore et al. (2007) (Table 1).

To prepare the material for subsequent examinations, anthers were broken and opened in distilled water to release the pollen grains. The fragmented anthers and pollen were washed into centrifuge tubes with distilled water, centrifuged and decanted. After the second wash, samples were passed through mesh sieve (100 μm) to remove fragments of the flower. The prepared samples were then divided to provide material for SEM and TEM.

**Table 1**
Record of TEM-studies on pollen of Barnadesiodeae and Mutisioideae.

| TAXA | AUTHOR |
|---|---|
| BARNADESIOIDEAE | |
| *Arnaldoa weberbaueri*[a] | Zao et al. (2000), this work |
| *B. lehmanii* | Skvarla et al. (1977) |
| *B. odorata*[a] | This work |
| *Chuquiraga erinacea*[a] | This work |
| *C. rotundifolia* | Zao et al. (2000) |
| *Dasyphyllum excelsum*[a] | Skvarla et al. (1977) |
| *D. lepthacanthum*[a] | This work |
| *D. velutinum*[a] | This work |
| *Doniophyton anomalum* | This work |
| *D. patagonicum*[a] | Skvarla et al. (1977); Zao et al. (2000); Blackmore et al. (2010) |
| *Duseniella patagonica*[a] | Zao et al. (2000) |
| *Fulcaldea laurifolia*[a] | Zao et al. (2000) |
| *Huarpea andina* | Zao et al. (2000) |
| *Schlechtendalia luzulaefolia*[a] | Skvarla et al. (1977); Zao et al. (2000); this work |
| MUTISIOIDEAE | |
| *Mutisia decurrens*[a] | This work |
| *M. kurtzii* | Tellería and Katinas (2009) |
| *Mutisia spinosa* | Tellería and Katinas (2009) |
| *Nassauvia looseri*[a] | This work |
| *Perezia wrightii*[a] | Skvarla et al. (1977); Blackmore et al. (2010) |
| *Proustia cuneifolia* | This work |
| CICHORIOIDEAE | |
| *Picris echioides* | This work |
| CALYCERACEAE | |
| *Boopis anthemoides*[a] | De Vore et al. (2007); this work |
| *Calycera spinulosa* | De Vore et al. (2007) |
| *Nastanthus spathulatus* | De Vore et al. (2007) |

[a] Species included in this study.

For SEM, some of obtained pollen grains were dehydrated with acetic acid and then acetolyzed using standard technique (Erdtman, 1960). Later, pollen grains were suspended in 90% ethanol, pipetted onto stubs, dried at room temperature for c. 24 hours, coated with gold palladium and examined in a JEOL JSM T-100 SEM.

For TEM, fresh pollen grains were fixed in 1.5% glutaraldehyde and then buffered 2% Os $O_4$ for 2 hours at room temperature. After that, they were washed for 30 min in distilled water, dehydrated in an ethanol series, and finally embedded in Spurr's low viscosity epoxy resin (Spurr, 1979). Ultrathin sections were cut using a diamond knife fitted into a Sorvall Porter-Blum MT2-B ultramicrotome. Sections were mounted on single grids and stained with lead citrate (1 min) and uranyl acetate for 10 min. Sections were examined with a transmission electron microscope Jeol JEM 1200 EX II at the Servicio Central de Microscopía Electrónica of the Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. This study considers characters of the exine ultrastructure observed at the equatorial mid-mesocolpium (i.e. number of layers and their constituent elements). Concerning the terminology, this paper follows that of Skvarla et al. (1977), Bolick, (1991), and Blackmore et al. (1984, 2010).

We selected six characters of exine structure that were found to be variable among Barnadesioideae taxa: exine thickness, columellae, internal tectum, ectexine sublayers, cavea, and foot layer. Definition and states of each character are given in Appendix B together with the data matrix (Table 2). Characters' evolution was reconstructed by optimizing states on the tree topology of Gruenstaeudl et al. (2009) using Mesquite program version 2.75 (Maddison and Maddison, 2011).

## 3. Results and discussion

Pollen of Barnadesioideae shows variation in exine thickness and exine structure (internal organization of columellae, internal tectum, ectexine sub-layers, and cavea). These exine variations are diagrammatically illustrated in Fig. 1.

**Table 2**
Data matrix of exine characters.

| TAXA | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Barnadesioideae | | | | | | |
| *A.weberbaueri* | 1 | 1 | 1 | 0 | 2 | 0 |
| *B.odorata* | 2 | 1 | 0 | 0 | 1 | 0 |
| *Chuquiraga erinacea* | 1 | 1 | 1 | 0 | 0 | 0 |
| *C. rotundifolia* | 1 | 1 | 1 | 0 | 0 | 0 |
| *C. oppositifolia* | 1 | 1 | 1 | 0 | 0 | 0 |
| *Dasyphyllum excelsum* | 1 | 1 | 1 | 0 | 2 | 0 |
| *D. lepthacanthum* | 1 | 1 | 1 | 0 | 2 | 0 |
| *Duseniella patagonica* | 1 | 1 | 1 | 0 | 0 | 0 |
| *Doniophyton anomalum* | 1–2 | 1 | 1 | 0 | 0 | 0 |
| *Fulcaldea laurifolia* | 1 | 1 | 1 | 0 | 0 | 0 |
| *Schlechtendhalia luzulaefolia* | 1 | 0 | 2 | 1 | 0 | 0 |
| Mutisioideae | | | | | | |
| *Mutisia decurrens* | 1–2 | 0 | 2 | 2 | 0 | 1 |
| *Nassauvia looseri* | 1 | 0 | 2 | 1 | 0 | 1 |
| *Perezia wrightii* | 1 | 0 | 2 | 1 | 0 | 1 |
| *Proustia cuneifolia* | 1 | 0 | 2 | 1 | 0 | 1 |
| Calyceraceae | | | | | | |
| *Boopis anthemoides* | 0 | 0 | 0 | 0 | 0 | 1 |
| *Acicarpha tribuloides* | 0 | 0 | 0 | 0 | 0 | 1 |
| *Nastanthus spathulatus* | 0 | 0 | 0 | 0 | 0 | 1 |
| *Calycera spinulosa* | 0 | 0 | 0 | 0 | 0 | 1 |
| Cichorioideae | | | | | | |
| *Picris echioides* | 2 | 1 | 1 | 0 | 1 | 1 |

### 3.1. Exine thickness

Three broad categories are here distinguished: Thin (1-ca. 3 μm), Medium ~~exine~~ (>3–6 μm), and Thick (>6-11 μm) exine. The Thin exine is observed in the sister clade Calyceraceae (Plate IV, 6), the Medium exine in *Arnaldoa* (Plate I, 2), *Chuquiraga*, *Dasyphyllum excelsum*, *D. lepthacantum*, *Duseniella*, *Fulcaldea*, *Nassauvia looseri* (Plate IV, 2), *Proustia*, and *Schlechtendalia*, and the Thick exine is recorded in *Barnadesia* and *Mutisia*.

### 3.2. Exine structure

#### 3.2.1. Structural patterns and columellae types

We recognized two of the exine patterns established for the family (Blackmore et al., 2010): columellate–granulate and columellate. The first one comprises columellae with more or less sinuous outline as they were formed of packed granules. In turn, these columellae are interspersed with granules as in all Barnadesioideae (Plate I, 2, 4, 6;

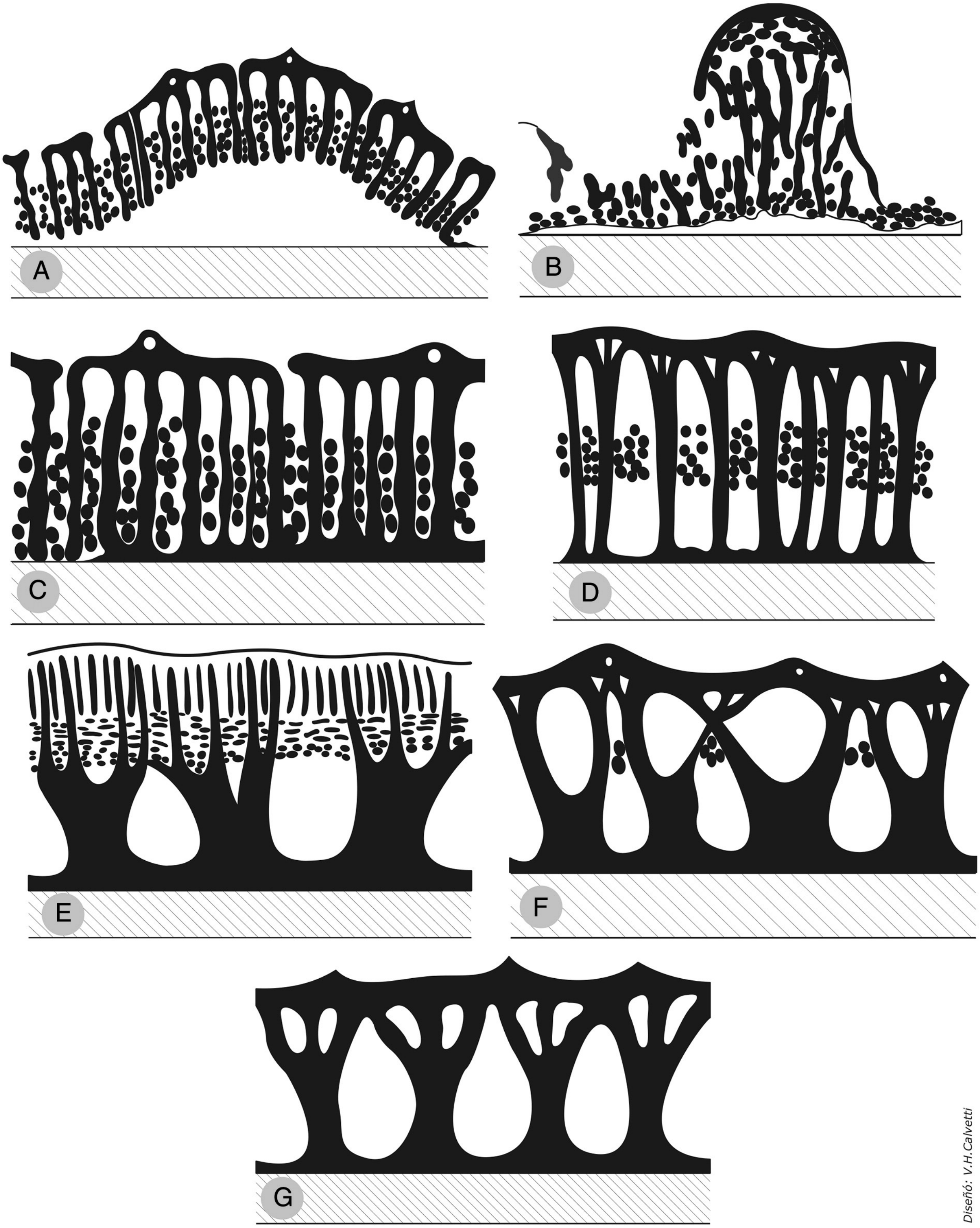

**Fig. 1.** Diagrammatic interpretations of the pollen wall of Barnadesioideae (A, *Arnaldoa*; B, *Barnadesia*; C, *Dasyphyllum*; D, *Schlechtedhalia*), Mutisioideae (E, *Mutisia*; F, *Nassauvia*) and Calyceraceae (G, *Boopis*).

Plate II, 2,3; Plate III, 2; Fig. 1, A–C), with exception of *Schlechtendalia* which has rather compact columellae (Plate III, IV; Fig. 1, D). The columellate pattern only comprises compact and independent columellae. It was observed in all Mutisioideae: *Mutisia* (Plate III, 6; Fig. 1, E), *Nassauvia* (Plate IV, 2; Fig. 1, F), *Perezia* (as seen in Skvarla et al., 1977, Fig. 22 D), *Proustia* (Plate IV, 4); and in Calyceraceae (Plate IV, 6; Fig. 1, G).

### 3.2.2. Internal tectum

*Schlechtendalia* is the only member of Barnadesioideae with an internal-like tectum, formed by tightly packed granules crossed by columellae (Plate III, 4; Fig. 1, D). An internal-like tectum is also present in *Mutisia*, formed by two layers which differ in density and staining (Plate III, 6). The typical internal tectum as defined by Skvarla et al. (1977) is present in *Nassauvia*, formed by the lateral fusion of branching columellae giving a "zigzag"appearance (Plate IV, 2; Fig. 1, F), even recognized at LM (Parra and Marticorena, 1972; Tellería et al., 2003).

The internal tectum is usually crossed by ramifications of columellae coming from the innermost ectexine layer (Plate III, 6; Plate IV, 2, 4; Fig. 1, E) as was also observed in pollen of *Gerbera* (Southworth, 1966). However, in *Schlechtendalia* columellae are branched after crossing the internal tectum (Plate III, 4; Fig. 1, D).

### 3.2.3. Ectexine sub-layers

We recognized one, two, or three sublayers in the ectexine of Barnadesioideae and Mutisioideae. In Barnadesioideae, it is commonly columellate–granulate, but within this pattern, we recognized two intergrading conditions. On the one hand, a single-layered exine with columellae and granules loosely structured, giving—under LM—a spongy aspect as occurs in *Barnadesia* (Plate I, 4; Fig. 1, B) which is in agreement with previous contributions (Urtubey, 1997; Zao et al., 2000). On the other hand, a bilayered exine (i.e. with both columellae and granules densely packed in the lower ¾ of the exine; under the tectum granules disappear and only columellae remain), as in *Arnaldoa*, *Chuquiraga* (Plate I, 2, 4; Fig. 1, A), *Dasyphyllum* (Plate II, 2, 3; Fig. 1, C), *Fulcaldea*, and *Doniophyton* (Plate III, 2).

### 3.2.4. Cavea

The occurrence of a cavea (i.e. space between exine layers above the foot layer, Blackmore et al., 1984) within basal Asteraceae is limited to some genera of the subfamily Barnadesioideae (Urtubey and Tellería, 1998; Zao et al., 2000). We observed full cavea (i.e., without any columellae spanning the space above the foot layer) between apertures or between depressions and apertures in *Arnaldoa* (Plate I, 2, Fig. 1, A) and *Dasyphyllum lepthacanthum* (Plate II, 2) and *D. excelsum*, and partial cavea in *Barnadesia odorata* (Plate I, 4; Fig. 1, B).

### 3.2.5. Foot layer

From TEM sections, Skvarla et al. (1977) claimed that the foot layer, or inner layer of ectexine, is universally present in Asteraceae exines, but it was found to be highly reduced and discontinuous in Barnadesioideae, as was previously suggested by Parra and Marticorena (1972) from LM study. The foot layer is continuous and well developed in pollen of Mutisioideae (*Mutisia*, *Nassauvia*, and *Proustia*) and Calyceraceae (*Boopis*) (Plate III, 6; Plate IV, 2, 4, 6; Fig. 1, E–G).

## 3.3. Phylogenetic significance of exine characters

The results of optimizing exine structure characters on the molecular phylogeny of Barnadesioideae showed a variety of patterns. Regarding exine thickness (Fig. 2), the Thin exine (ca. 1–3 μm) is regarded as an ancestral (plesiomorphic) character state present in the outgroup (Calyceraceae).

Barnadesioideae retained the Thin exine pattern in the earliest divergent lineages (e.g. *Dasyphyllum*). A Thicker exine (>6–17 μm) arose in ancestral non-Barnadesioideae Asteraceae (e.g. Mutisioideae).

Regarding columellae type (Fig. 3), compact columellae present in the outgroup, is the ancestral state from which the granulate type developed. Usually, compact columellae are independent of each other. The ancestral state for Barnadesioideae is the granulate columellae; commonly, they are interspersed with granules in the lower portion of the ectexine. This structural pattern characterizes the subfamily. The compact columellae of the exine of *Schlechtendalia* are unique within Barnadesioideae and appears as reversal to the ancestral state present in Calyceraceae. Based on a developmental perspective on the sporoderm, it has been recently suggested that the structural differences in exine organization in Asteraceae might have resulted from changes in the developmental program of self-assembly (Blackmore et al., 2010). From an adaptive point of view, Bolick (1991) suggested

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**Plate I.** Pollen of Barnadesioideae.

1–2. *Arnaldoa weberbaueri* (Sagástegui 7410). 1. SEM, equatorial view of shrunken grain showing intercolpal depressions (arrows). 2. TEM, exine section of mesocolpium showing the columellate–granulate structure and the full cavea.

2–3. *Barnadesia odorata* Fabris 4679 (LP). 3. SEM, tilted equatorial view. Note the continuous and psilate tectum on the ridges (arrow) and the incomplete reticuloid tectum covering lateral faces of ridges (asterisk). 3. TEM, exine section, showing columellae and granules forming a loose structure that becomes more compact under ridge (asterisk). Note the sinuous outline of columellae as they were formed from compacted granules (partially framed by a circle), the reduced foot layer (arrow), and the partial cavea.

4–5. *Chuquiraga erinaceae* (Soriano 1246 LP). 4. SEM, equatorial view. 5. TEM, exine section showing the barely perforated tectum (arrows) and delicate columellae interspersed with fine granules in the inner portion.

Abbreviations: E: endexine; Cg: columellate–granulate layer; C: columellate layer: Cv: cavea.

Scale bars of 1, 3, and 5:10 μm; of 2:1 μm; of 4:2 μm, and 6: 0.5 μm.

**Plate II.** Pollen of Barnadesioideae (*cont.*) (see on page 6)

1–2. *Dasyphyllum leptacanthum* (Cabrera 12256 LP). 1. SEM, tilted polar view showing depressions (arrow). 2. TEM, exine section between a depression (arrow) and one of the apertural areas (double arrow) showing the full cavea and the columellate–granulate structure.

3. *D. velutinum* (Cabrera 12241 LP). TEM, acaveate exine section showing the barely perforated tectum (black arrow); two layers of ectexine; reduced foot layer (white arrow) and endexine.

Abbreviations: E: endexine; Cg: columellate-granulate layer; C: columellate layer.

Scale bars of 1: 10 μm; of 2 and 3: 0.5 μm.

**Plate III.** Pollen of Barnadesioideae and Mutisioideae. (see on page 7)

1–2. *Doniophyton anomalum* (*Bonifacino et al. 96 LP*). 1. SEM, equatorial view. 2. TEM, exine section showing columellate–granulate lower ectexine layer and the upper columellate layer, note thin columellae.

3–4. *Schlechtendalia luzulaefolia* (Cabrera 21873). 3. SEM, equatorial view showing equatorial depression (arrow). 4. TEM, exine section next to depression (arrow) showing the columellae intercepted by a thick internal tectum. Note that columellae are continuous from the reduced foot layer to the external tectum (white arrows) and they branch under the external tectum.

5–6. *Mutisia decurrens* (Cabrera et Job 346). 5. SEM, equatorial view. 6. TEM of exine section showing the columellate ectexine intercepted by the internal tectum delimiting two layers of columellae. Note the thick foot layer and that columellae of the upper layer appear as a continuation of those of the inner layer (arrow).

Abbreviations: E: endexine; Cg: columellate–granulate layer; C: columellate layer; It: internal tectum; F: foot layer; E: endexine.

Scale bars of 1 and 3:5 μm; of 2:0.5 μm; of 4 and 6: 1 μm and 5: 10 μm.

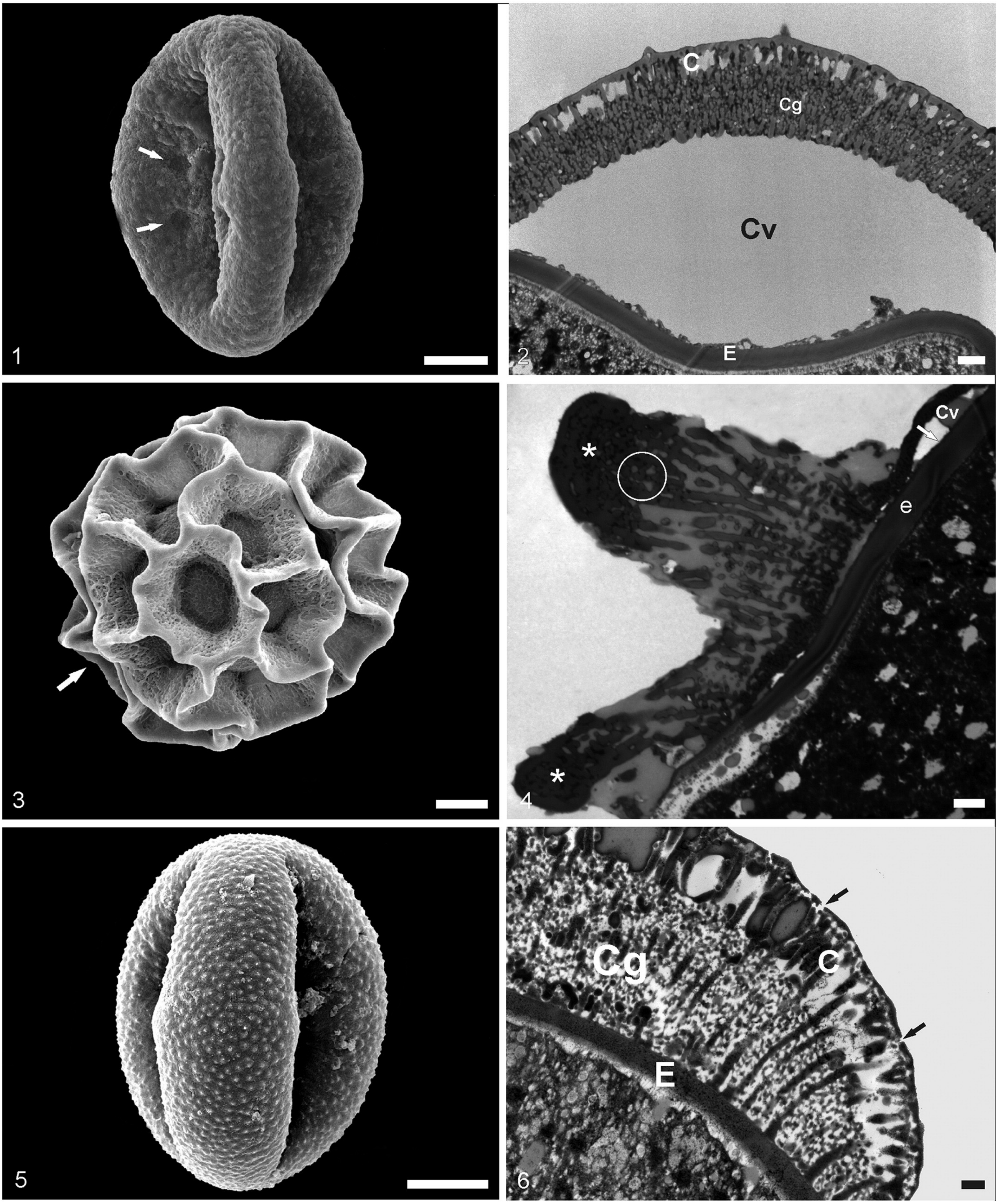

Plate I

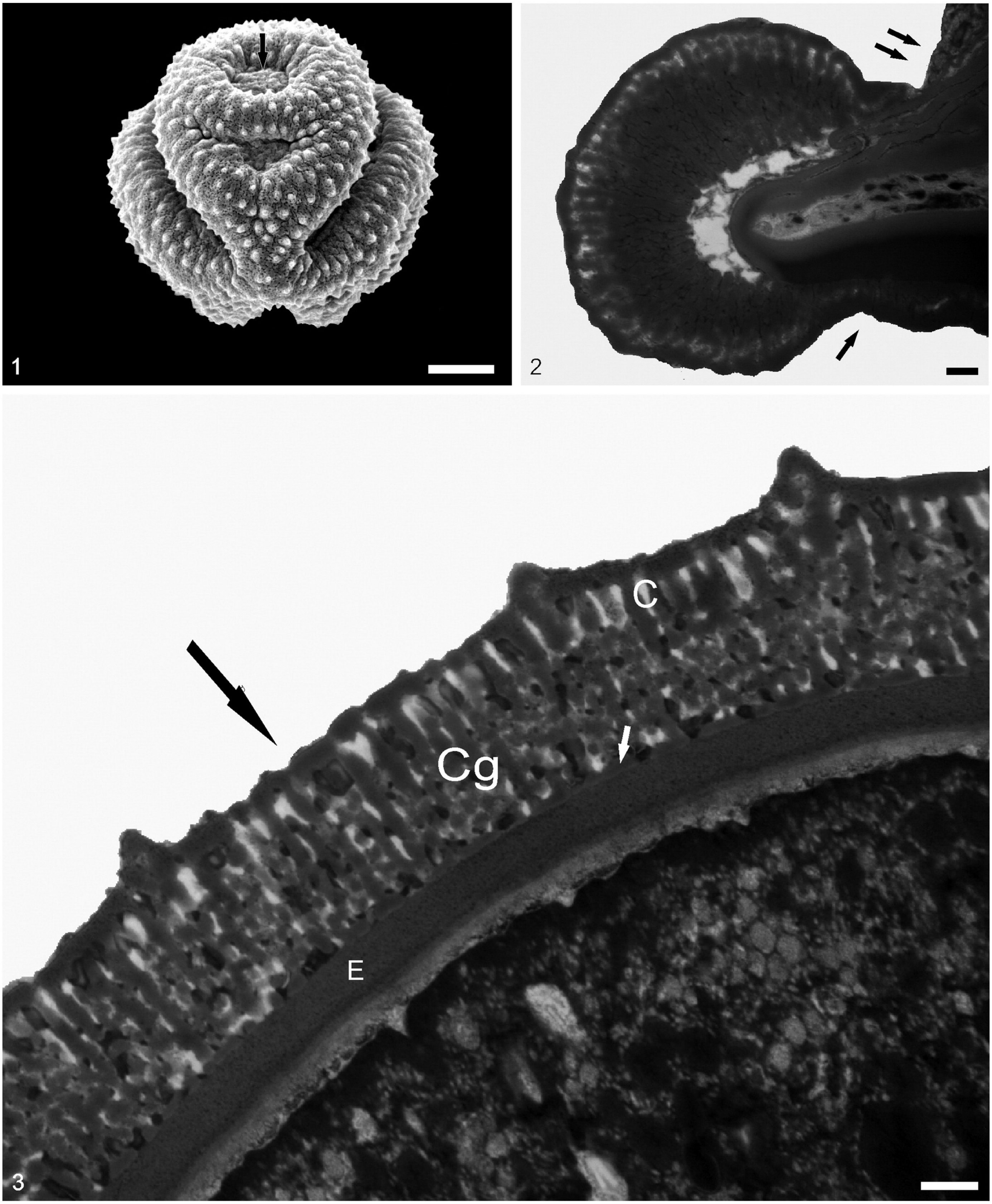

**Plate II** *(caption on page 4)*.

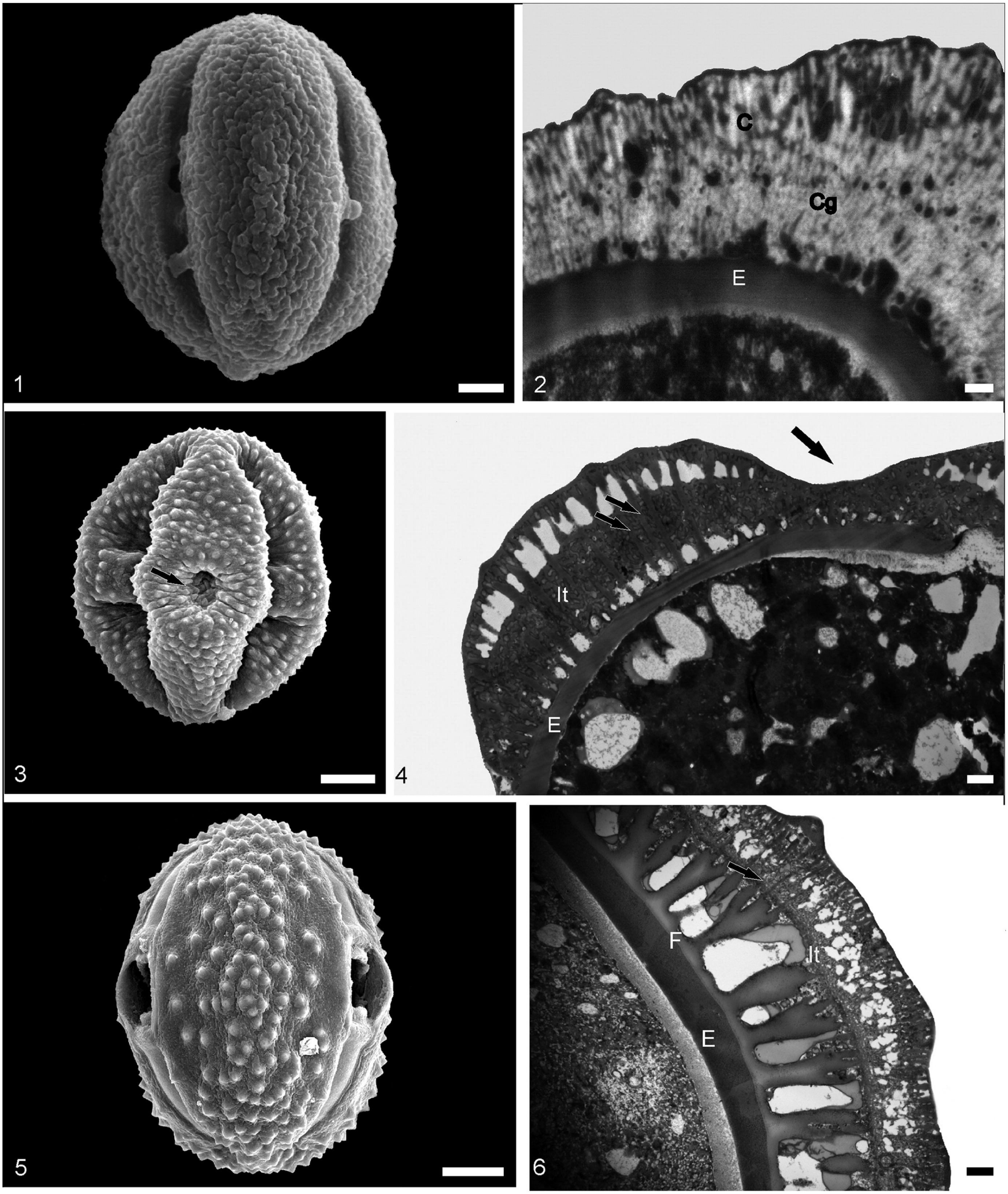

**Plate III** *(caption on page 4)*.

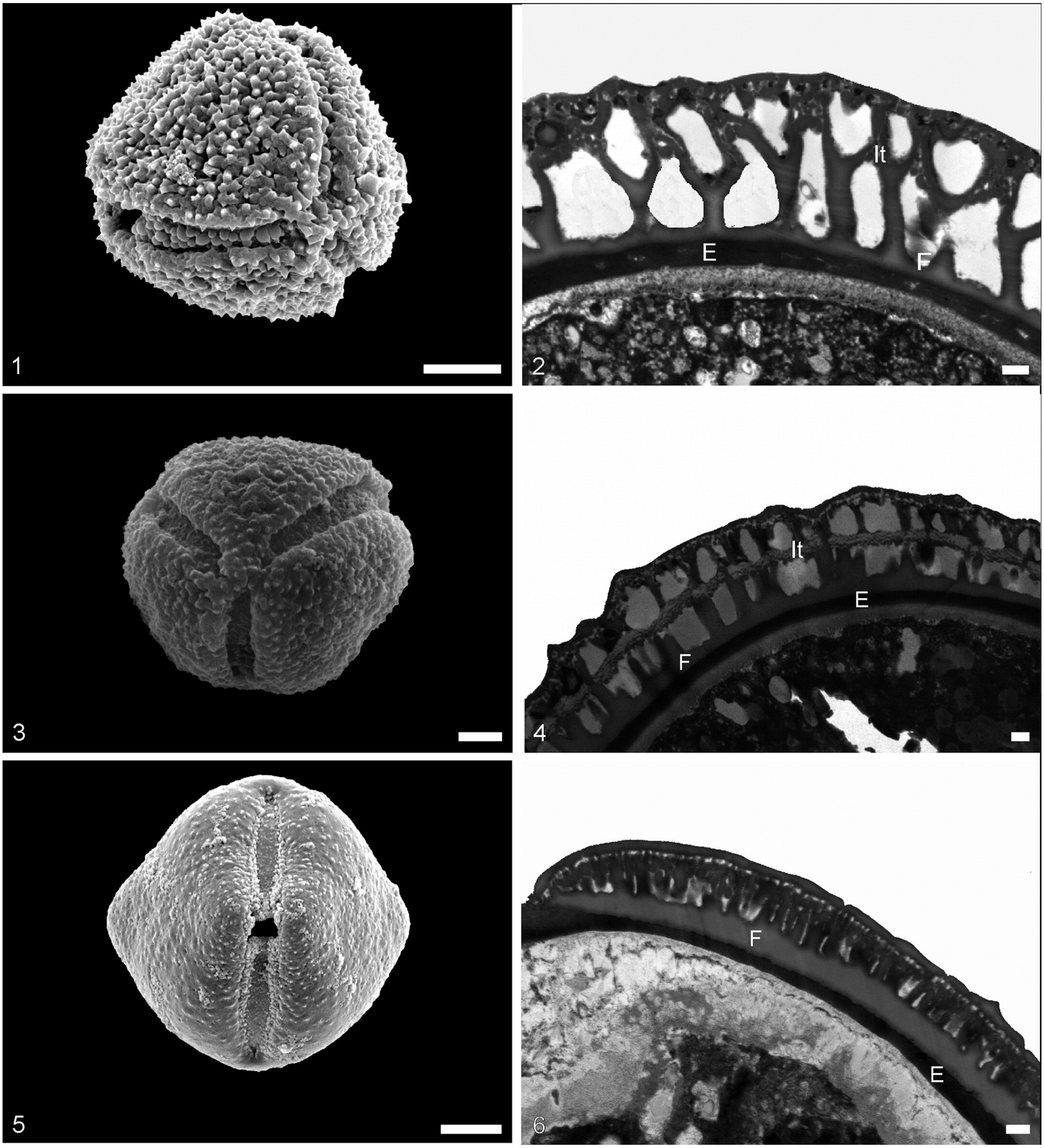

**Plate IV.** Pollen of Mutisioideae and Calyceraceae.

1–2. *Nassauvia looseri* (Looser 5755 LP). 1. SEM, tilted polar view. 2. TEM, exine section showing two layers of columellae with branches laterally united forming a "zigzag" internal tectum.
3–4. *Proustia cuneifolia* (Fabris et Zuloaga 8466). 3. SEM, polar view. 4. TEM, exine section showing two layers of ramified columellae separates by an internal tectum.
5–6. *Boopis anthemoides* (Bottino 437). 5. SEM, equatorial view. 6. TEM, exine section showing a thick and little perforated tectum and a thick foot layer.
Abbreviations: E: endexine; It: internal tectum; F: foot layer; E: endexine.
Scale bars of 1:5 μm; of 2:0.5 μm; of 4:1 μm; of 3 and 5: 5 μm and 6: 0.4 μm.

that thicker columellae in pollen of Compositae could resist large compressive forces. In this respect, it would be interesting to explore the forcing factors that might have triggered the shift from a columellate to columellate-granulate structure. It could be possible that columellae interspersed with granules give greater rigidity to the pollen wall. Perhaps the depressions present in a large number of species could compensate

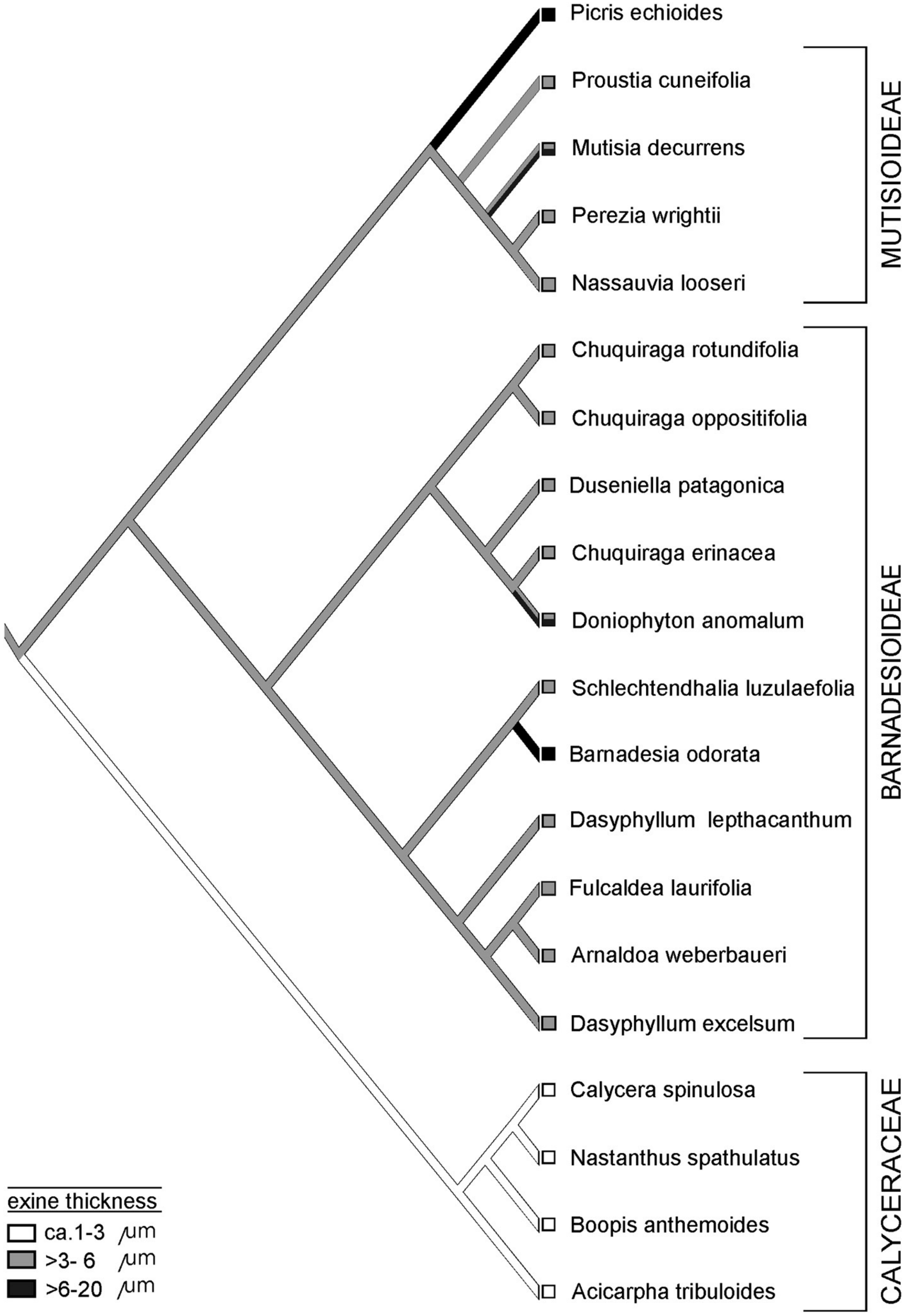

**Fig. 2.** Exine thickness optimized onto the molecular phylogeny of Gruenstaeudl et al. (2009) using parsimony reconstruction in Mesquite (Maddison and Maddison, 2011).

for such rigidness by providing areas of greater flexibility. In this respect, it would be interesting to explore if these patterns have some adaptive significance. Columellate pattern characterizes the clade of Mutisioideae, though only four species of this large subfamily were included in the phylogeny; it is worth mentioning that pollen is uniformly columellate (Zao et al., 2006; Katinas et al., 2008). Columellate pattern is shared not only by Calyceraceae but also by the *Scaevola-Goodenia* pollen type which belongs to the Goodeniaceae family (Gustafsson et al., 2001). This family was also regarded as outgroup of Asteraceae due to morphological and molecular characters (Bremer and Guftafsson, 1997; Lundberg, 2009).

The number of sublayers of the ectexine has evolved in a way that is widely coincident with the molecular phylogeny (Fig. 4). The ancestral condition is the single layer which is present in Calyceraceae. The bilayered ectexine is present in two well-supported monophyletic groups of Barnadesioideae. These groups are formed by *Chuquiraga rotundifolia*, *C. oppositifolia*, *Duseniella patagonica*, *Chuquiraga erinacea*, and *Doniophyton anomalum* on the one hand and by *Dasyphyllum lepthacanthum*, *Fulcaldea laurifolia*, *Arnaldoa weberbaueri*, and *Dasyphyllum excelsum* on the other hand. In *Schlechtendalia*, the trilayered ectexine appears as parallelism. In *Barnadesia odorata*, the columellate–granulate exine with loosely structured elements forming a single-layered ectexine occurs as an autapomorphy and is shared with that of *Huarpea andina* (Gamerro, 1985). In Mutisioideae clade, the ectexine is trilayered due to compact columellae and are intercepted with an internal tectum.

The lack of an internal tectum is the ancestral state retained in Barnadesioideae, except in *Schlechtendalia*, in which the internal tectum

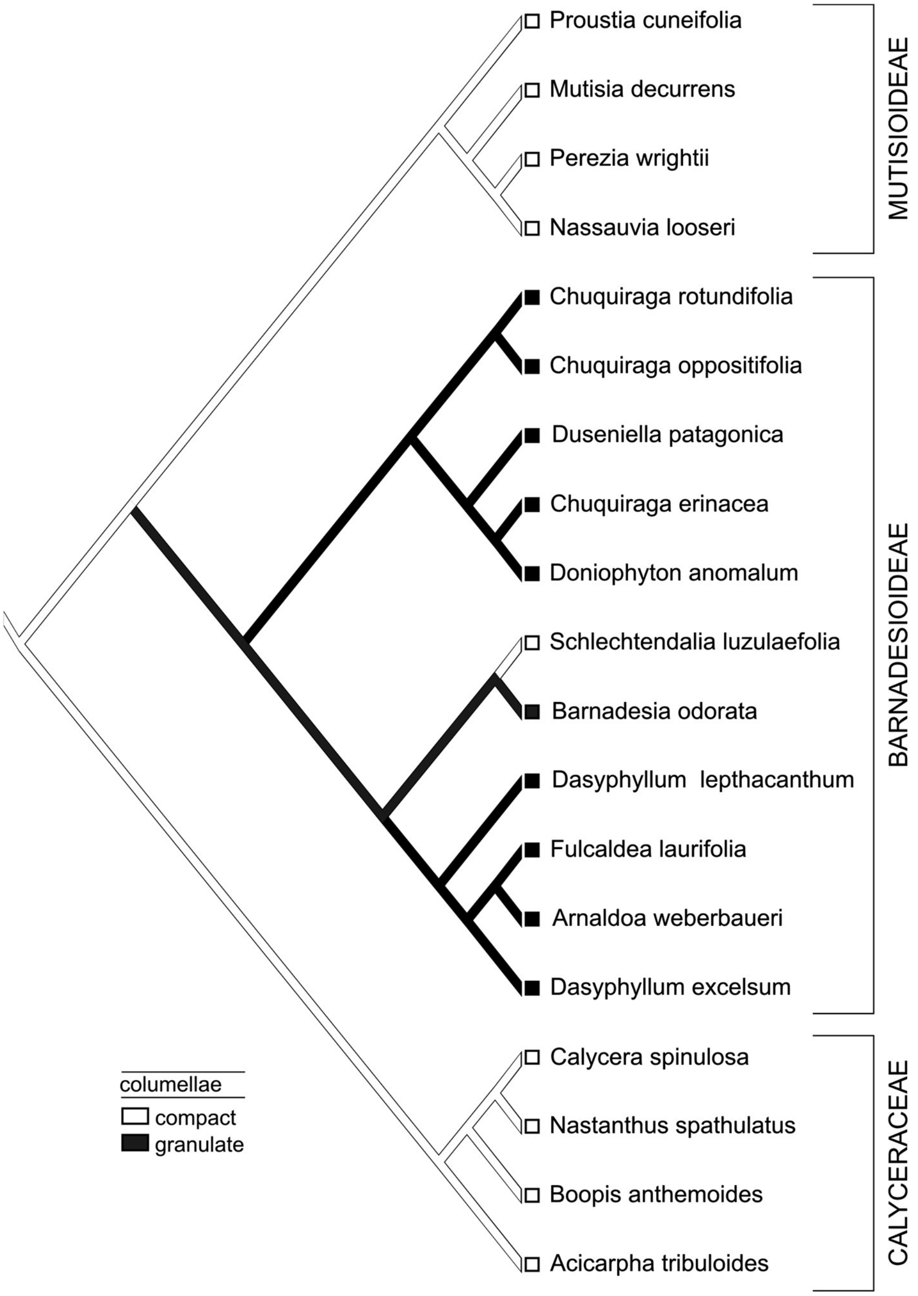

**Fig. 3.** Columellae type optimized onto the molecular phylogeny of Gruenstaeudl et al. (2009) using parsimony reconstruction in Mesquite (Maddison and Maddison, 2011).

appears as parallelism (Fig. 5). The internal tectum is a derived state that appears in pollen grains of Mutisioideae.

The lack of cavea is the ancestral state shared with Calyceraceae and retained in Mutisioideae (Fig. 6). The presence of cavea is exclusive of one of the two monophyletic groups of Barnadesioideae; is partial in *Barnadesia odorata*, and full in *Arnaldoa weberbaueri* and two species of *Dasyphyllum* (of the section *Dasyphyllum*, Cabrera, 1959), *D. lepthacanthum* and *D. excelsum*. In *Fulcadea laurifolia*, the acaveate state occurs as reversion. Cavea was regarded being potentially present in all Asteraceae exine by Blackmore et al. (1984). These authors suggested that the selection in favor of the differing functional attributes of caveate and acaveate pollen has probably led to the occurrence of both, or intermediate, forms during the course of evolution. This feature, which is derived in Barnadesioideae, is retained in most of advanced Asteraceae mainly as full caveae (as seen in Blackmore et al., 2009).

The well-developed foot layer is the ancestral state (Fig. 7). It occurs in Calyceraceae and is present in all Mutisioideae. A partial and barely distinguishable foot layer is the derived state and a synapomorphy in Barnadesioideae.

## 4. Conclusions

TEM observations conducted in the present study, some on pollen of unexplored species, revealed significant features to the phylogenetic understanding of Barnadesioideae. These features concern mainly the columellae, foot layer, and ectexine layers which are distinctive in the context of Asteraceae. Tracing selected ultrastructural characters on the molecular tree allowed us to define several groups that have been previously recognized by DNA-based studies (Gruenstaeudl et al., 2009). The exine probably evolved from a thin exine with a well-

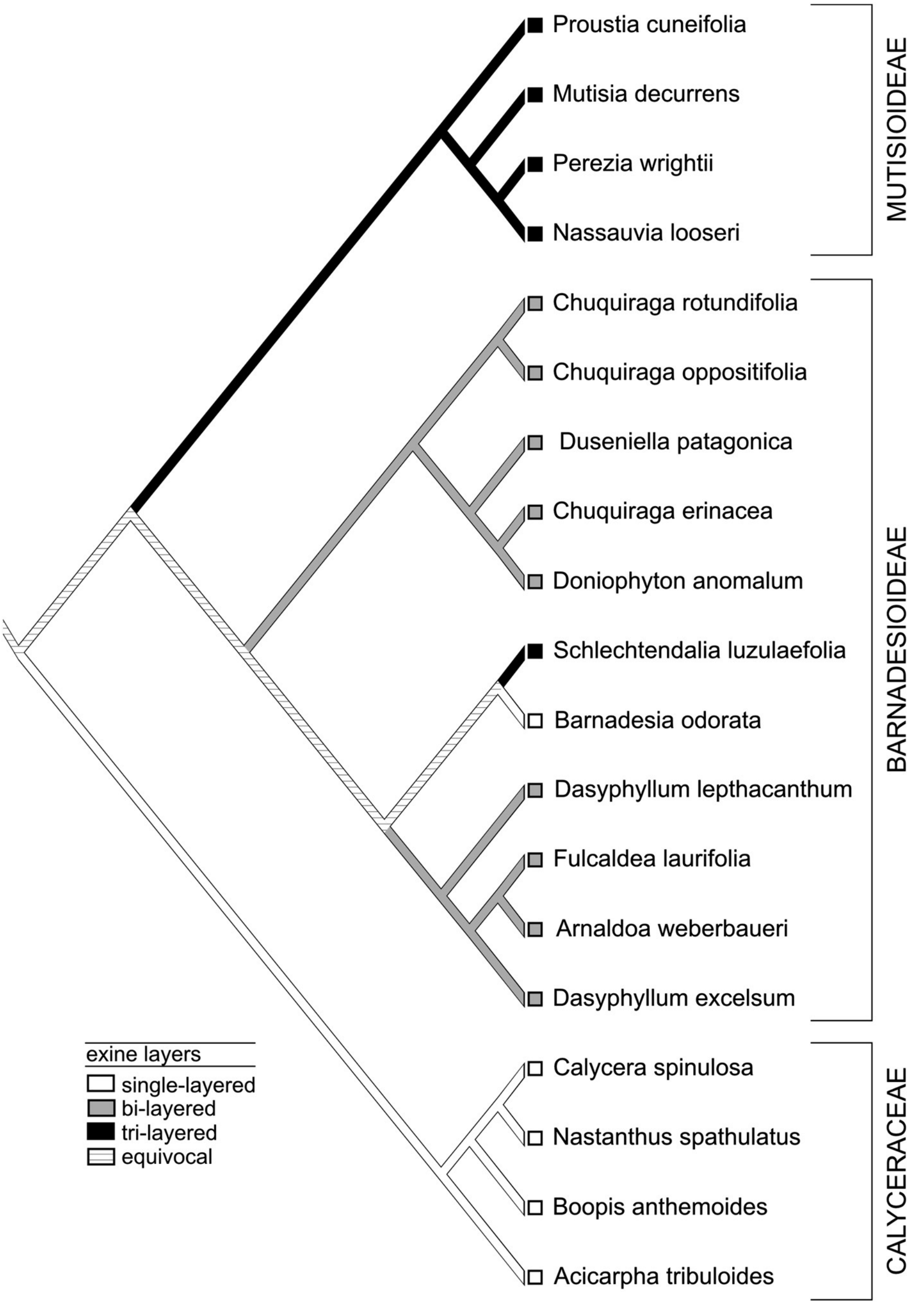

**Fig. 4.** Exine layers optimized onto the molecular phylogeny of Gruenstaeudl et al. (2009) using parsimony reconstruction in Mesquite (Maddison and Maddison, 2011).

developed foot layer and solid and independent columellae through three general pathways: i) to a columellate–granulate bilayered exine in Barnadesioideae (with very delicate columellae), and a columellate trilayered exine in Mutisioideae (with robust columellae); ii) the strong reduction of the foot layer and the acquisition of cavea in some species of Barnadesioideae; iii) the change to a thicker exine involved the acquisition of the internal tectum formed by the linking of lateral branches of columellae in Mutisioideae. The single layer of columellae, the reduced foot layer, the cavea, and the lack of internal tectum are retained in advanced Asteraceae (Blackmore et al., 2009). Unlike the other members of Barnadesioideae, the monotypic genus *Schlechtendalia luzulaefolia* showed a number of reversions and parallelisms. Its exine structure combines the compact columellae and the internal tectum of Mutisioideae, with densely granulate internal tectum like the inner ectexine layer of Barnadesioideae. Interestingly, *Schlechtendalia* has been considered as a cryptic taxon, its extra-palynological characters are unique within Barnadesioideae (Stuessy et al., 2009). This genus, regarded as isolated and specialized by Bremer et al. (1994), during late Miocene inhabited in Patagonia (Southern Argentina) together other related members of Barnadesioideae (Palazzesi et al., 2009; Barreda et al., 2010), but today it grows in northeastern Argentina, Uruguay, and southern Brazil. Ultrastructural exine characters reinforce the unique position of *Schlechtendalia.* Additional ultrastructural studies, in particular of the closest families of the Asterales clade, will probably shed new light on the early evolution of the Asteraceae.

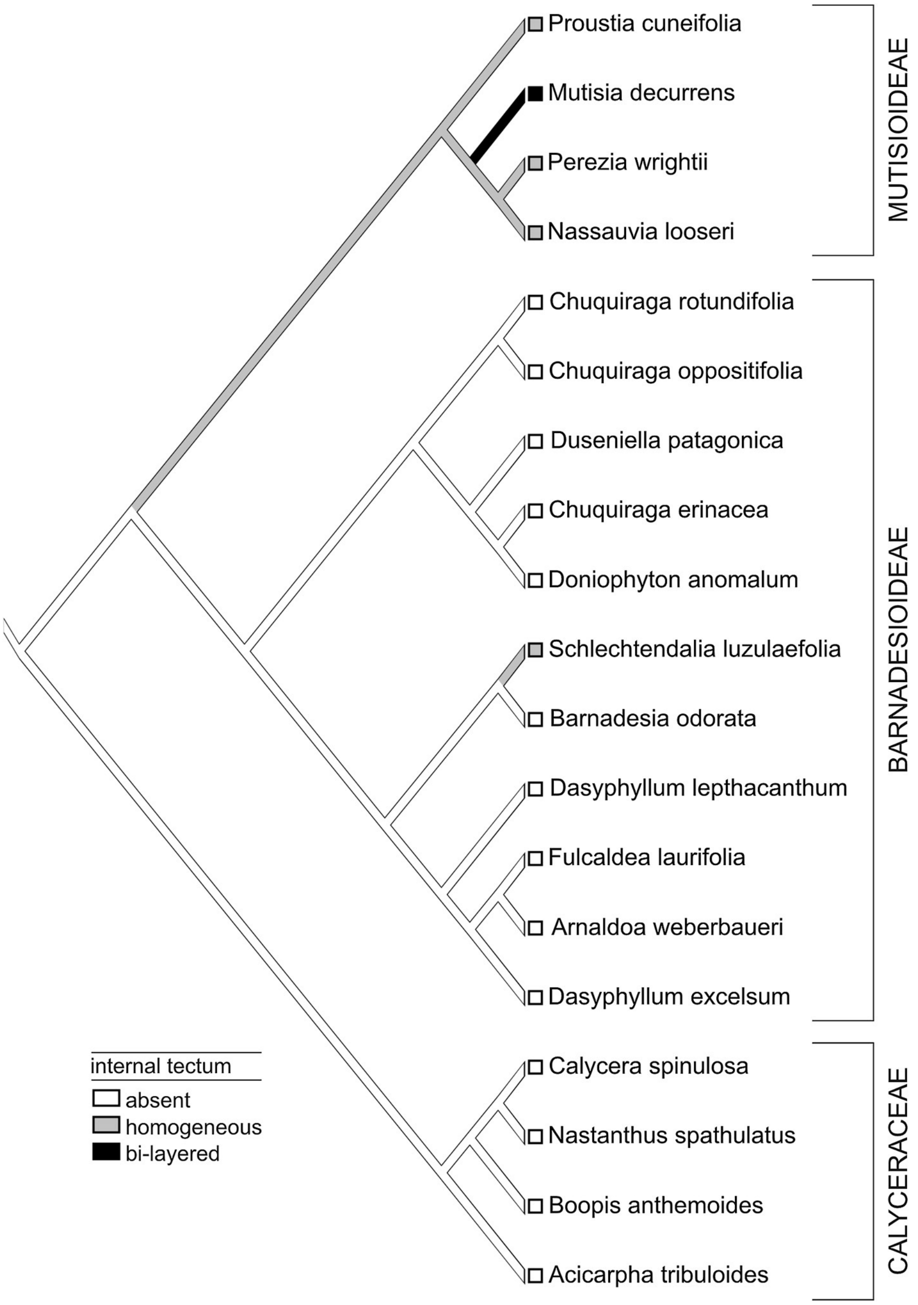

**Fig. 5.** Internal tectum optimized onto the molecular phylogeny of Gruenstaeudl et al. (2009) using parsimony reconstruction in Mesquite (Maddison and Maddison, 2011).

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## Appendix A. Specimens investigated with TEM

*Arnaldoa weberbaueri*(Muschl.) Ferreira: Sagástegui 7410 (LP), López et Sagástegui 8159, 8218 (HUT). *Barnadesia odorata* Griseb.: Fabris 4679 (LP). *Boopis anthemoides*Juss.: Bottino 437 (LP). *Chuquiraga erinacea* D. Don: Soriano 1246 (LP), Herbario General 111 (MCNS), Cabrera 9035 (LP). *Dasyphyllum excelsum* (Don) Cabrera: Garavente 4146 (LP). *Dasyphyllum lepthacanthum* (Gardner) Cabrera: Cabrera 12256 (LP), Occhioni 1023 (LP). *D. velutinum* (Baker) Cabrera: Cabrera 12241 (LP), Melo Barreto 10884 (LP), Duarte 2906 (LP). *Doniophyton anomalum* (D. Don) Kurtz: Bonifacino et al. 96 (LP), Buenanueva s/n° (LP), King 648 (LP), Kiesling 4587 (LP), Maldonado 1448 (LP), Ameghino s/n° (LP). *Schlechtendalia luzulaefolia* Less.: Cabrera 21873, 32308 (LP), Pareira 8490 (LP), Gamerro 1364 (LP); Hatschbach 35458 (LP). *Mutisia decurrens* Cav. Cabrera et Job 346 (LP), Grüner 132 (LP),

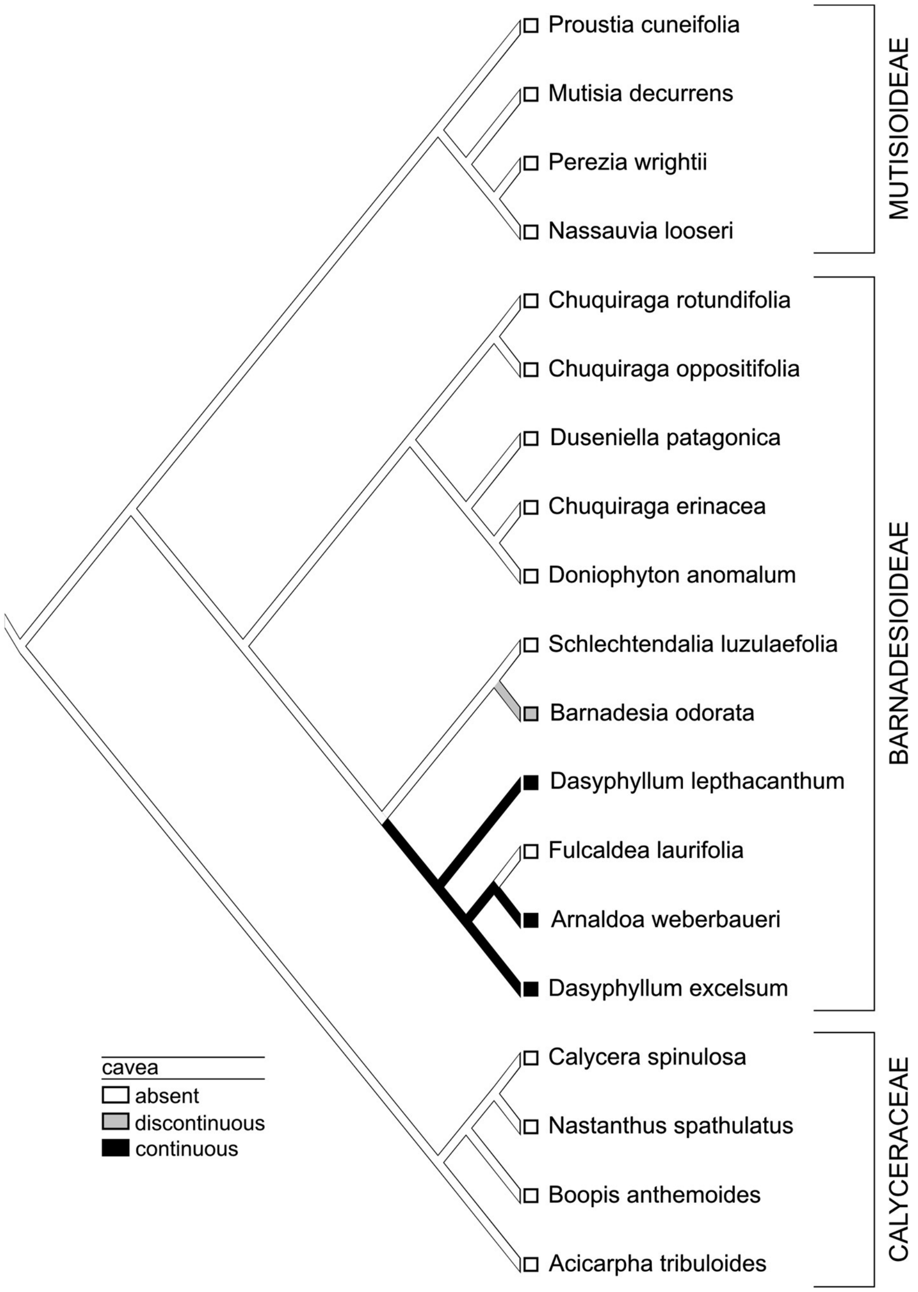

**Fig. 6.** Cavea optimized onto the molecular phylogeny using parsimony of Gruenstaeudl et al. (2009) reconstruction in Mesquite (Maddison and Maddison, 2011).

Soriano 4294 (LP). *Nassauvia looseri* Cabrera: Looser 5755 (LP). *Picris echioides* L.: Torres Robles et al. 1749 (LP). *Proustia cuneifolia* D. Don: Fabris et Zuloaga 8466 (LP).

## Appendix B. Exine characters used in this analysis

1- Exine thickness. Exine thickness in the mesocolpial areas ranges between ca. 1–3 μm Thin (scored as 0), >3–6 μm Medium (scored as 1), and >6–11 μm Thick (scored as 2).
2- Columellae types. Columellae may have compact appearance and lack interspersed granules (scored as 0), as in *Mutisia*, *Nassauvia*, *Perezia*, *Proustia*, *Schlechtendalia*, and Calyceraceae, or they may be thin, with more or less sinuous outline, as it they were formed from packed granules (scored as 1), as in *Arnaldoa*, *Barnadesia*, *Chuquiraga*, *Doniophyton*, *Fulcaldea*, and *Dasyphyllum*.
3- Ectexine layers. Ectexine is single-layered (scored as 0) in pollen of *Barnadesia* and Calyceraceae, bilayered, with granules lower ¾ parts of the thickness giving a bilayered structure (scored as 1), in *Arnaldo*a, *Chuquiraga*, *Dasyphyllum*, *Doniophyton*, *Fulcaldea*, and *Picris*. In *Nassauvia*, *Proustia*, *Mutisia*, and *Schlechtendalia luzulaefolia* the ectexine is trilayered (scored as 2).
4- Internal tectum (middle layer of the exine). Internal tectum absent (scored as 0) or present consisting in one homogeneous layer (scored as 1), or consisting in two distinct layers (scored as 2).
5- Cavea. It may be absent (scored as 0), discontinuous (foot layer and endexine are partially linked as occurs in *Barnadesia*) (scored as 1), or continuous (between apertures or between depressions and apertures) as occurs in *Arnaldoa* and *Dasyphyllum* (scored as 2).
6- Foot layer. The foot layer is present in pollen of all studied species, but it may be continuous and well developed as in *Mutisia*, *Proustia*,

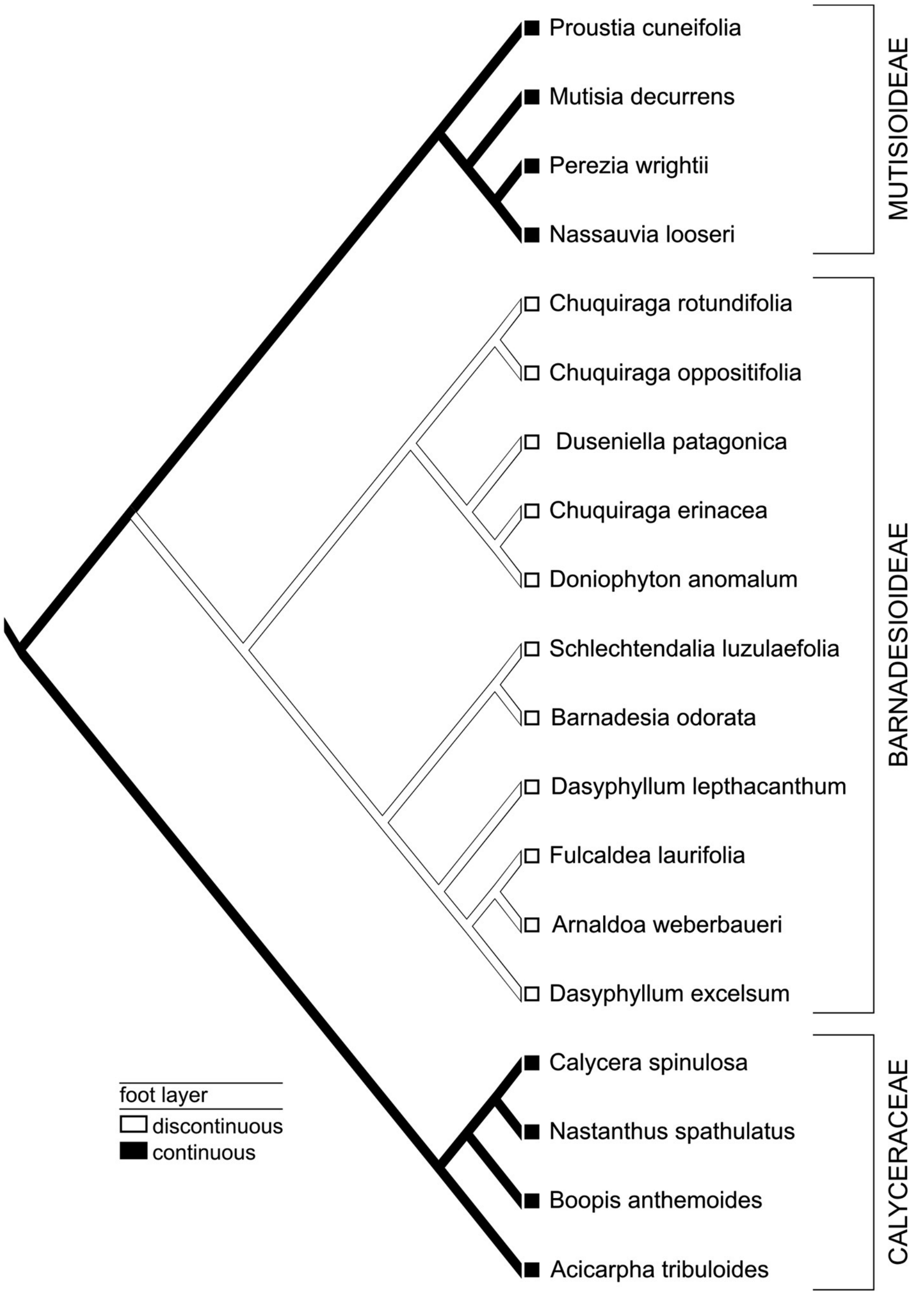

**Fig. 7.** Foot layer optimized onto the molecular phylogeny of Gruenstaeudl et al. (2009) using parsimony reconstruction in Mesquite (Maddison and Maddison, 2011).

*Nassauvia*, and *Boopis* (scored as 1) or discontinuous and little distinguishable as in species of Barnadesioideae (scored as 0).

## References

Banks, H., Rico, L., 1999. Pollen morphology and phylogenetic analysis of *Eperua* Aublet (Detarieae: Caesalpinioideae: Leguminosae). Grana 38, 261–276.

Barreda, V., Palazzesi, L., Tellería, M.C., Katinas, L., Crisci, J.V., 2010. Fossil pollen indicates an explosive radiation of basal Asteracean lineages and allied families during Oligocene and Miocene times in the Southern Hemisphere. Rev. Palaeobot. Palynol. 160, 102–110.

Blackmore, S., van Ham, Helvoort, Punt, W., 1984. On the terminology, origins and functions of caveate pollen in Compositae. Rev. Palaeobot. Palynol. 43, 293–301.

Blackmore, S., Wortley, A.H., Skvarla, J.J., Robinson, H., 2009. Evolution of pollen in Compositae. In: Funk, V.A., Susanna, A., Stuessy, T., Bayer, R. (Eds.), Systematics, evolution and biogeography of the Compositae, IAPT, Viena, Austria, pp. 101–130.

Blackmore, S., Wortley, A.H., Skvarla, J.J., Gabarayeba, N.I., Rowley, J., 2010. Developmental origins of structural diversity in pollen walls of Compositae. Plant Syst. Evol. Plant Syst. Evol. 284, 17–32.

Bolick, M., 1991. Pollen diameter, exine thickness, and ultrastructure in the tribes of Compositae. Compositae Newsl. 19, 17–21.

Bremer, K., Guftafsson, M.H.G., 1997. East Gondwana ancestry of the sunflower alliance of families. Proc. Natl. Acad. Sci. U. S. A. 94, 9188–9190.

Bremer, K., Anderberg, A.A., Karis, P.O., Nordenstam, B., Lundberg, J., Ryding, O., 1994. Cladistics and classification. Tinber Press, pp. 56–60.

Cabrera, A.L., 1959. Revisión del género *Dasyphyllum*. Rev. Mus. La Plata 38, 22–99.

De Vore, M., Zao, Z., Cansen, R.K., Skvarla, J.J., 2007. Pollen morphology and ultrastructure of calyceraceae. Lundellia 10, 32–48.

Doyle, J., 2005. Early evolution of angiosperm pollen as inferred from molecular and morphological phylogenetic analyses. Grana 44, 227–251.

Erdtman, G., 1952. Pollen morphology and plant taxonomy: I. Angiosperms. An introduction to palynology vol. 1. Almquist and Wiksell, Stockholm.

Erdtman, G., 1960. The acetolysis method. A revised description. Sven. Bot. Tidskr. 54, 561–564.

Funk, V.A., Susanna, A., Stuessy, T.F., Robinson, H., 2009. Classification of Compositae. In: Funk, V.A., Susanna, A., Stuessy, T., Bayer, R. (Eds.), Systematics, evolution and biogeography of the Compositae. IAPT, Viena, Austria, pp. 171–176.

Gamerro, J.C., 1985. Morfología del polen de *Huarpea* y su relación con *Barnadesia* (Mutisieae, Compositae). Darwiniana 26, 43–51.

Gruenstaeudl, M., Urtubey, E., Jansen, R.K., Samuel, R., Barfuss, M.H.J., Stuessy, T.H., 2009. Phylogeny of Barnadesioideae (Asteraceae) inferred from DNA sequence data and morphology. Mol. Phylogenet. Evol. 51, 72–587.

Gustafsson, M.H.G., Grafström, E., Nilsson, N., 1997. Pollen morphology of the Goodeniaceae and comparisons with related families. Grana 36, 185–207.

Gustafsson, M.H.G., Pepper, A.S.R., Albert, V.A., Källersjö, M., 2001. Molecular phylogeny of the Barnadesioideae (Asteraceae). Nord. J. Bot. 21, 149–160.

Katinas, L., Prusky, J., Sancho, G., Tellería, M.C., 2008. The subfamily Mutisioideae (Asteraceae). Bot. Rev. 74, 469–716.

Lundberg, J., 2009. Asteraceae and relationships within Asterales. In: Funk, V.A., Susanna, A., Stuessy, T., Bayer, R. (Eds.), Systematics, evolution and biogeography of the Compositae. IAPT, Viena, Austria, pp. 157–169.

Maddison, W.P., Maddison, D.R., 2011. Mesquite: a modular system for evolutionary analysis. Version 2.75 (http://mesquiteproject.org.).

Mark, P.J.L., Wortley, A.H., Furness, C.A., 2012. Not a shrinking violet: pollen morphology of Violaceae (Malpighiales). Grana 5, 181–193.

Ortíz, S., Bonifacino, M., Crisci, J.V., Funk, V., Hansen, H.V., Hind, D.J.N., Katinas, L., Roque, N., Sancho, G., Susana, A., Tellería, M.C., 2009. The basal grade of the Compositae: the fate of Mutisieae (sensu Cabrera) and the Carduoideae. In: Funk, V.A., Susanna, A., Stuessy, T., Bayer, R. (Eds.), Systematics, evolution and biogeography of the Compositae. IAPT, Viena, Austria, pp. 193–213.

Palazzesi, L., Barreda, V., Tellería, M.C., 2009. Fossil pollen grains of Asteraceae from the Miocene of Patagonia: Barnadesioideae affinity. Rev. Palaeobot. Palynol. 155, 83–88.

Panero, J.L., Funk, V.A., 2008. The value of sampling anomalous taxa in phylogenetic studies: major clades of the Asteraceae revealed. Mol. Phylogenet. Evol. 47, 757–782.

Parra, O., Marticorena, C., 1972. Granos de polen de plantas chilenas. II. Compositae-Mutisieae. Gayana 21 (107 pp.).

Skvarla, J.J., Larson, D.A., 1965. An electron microscopic study of pollen morphology in the Compositae with special reference to the Ambrosiinae. Grana Palynol. 6, 210–269.

Skvarla, J.J., Turner, B.L., 1966. Systematics implications from electron microscopic studies of Compositae pollen—a review. Ann. Mo. Bot. Gard. 53, 200–256.

Skvarla, J.J., Turner, B.L., Patel, V.C., Tomb, A.S., 1977. Pollen morphology in the Compositae and in morphologically related families. In: Heywood, V.H., Harborne, J.B., Turner, B.L. (Eds.), The biology and chemistry of the Compositae. Academic Press, pp. 141–248.

Southworth, D., 1966. Ultrastructure of *Gerbera jamiesonii* pollen. Grana Palynol. 6, 324–337.

Spurr, A.R., 1979. A low-viscosity epoxy resin embedding for electron microscopy. J. Ultrastruct. Res. 26, 31–43.

Stix, E., 1960. Pollenmorphologie und Systematik an Compositen. Grana Palynol. 2, 41–104.

Stuessy, T.F., Urtubey, E., Gruenstaeudl, M., 2009. Barnadesieae (Barnadesioideae). In: Funk, V.A., Susanna, A., Stuessy, T., Bayer, R. (Eds.), Systematics, evolution and biogeography of the Compositae. IAPT, Viena, Austria, pp. 215–228.

Tellería, M.C., Katinas, L., 2009. New insights into the pollen morphology of *Mutisia* (Asteraceae, Mutisieae). Plant Syst. Evol. 280, 229–241.

Tellería, M.C., Urtubey, E., Katinas, L., 2003. *Proustia* and *Lophopappus* (Asteraceae, Mutisieae): generic and subtribal relationships based on pollen morphology. Rev. Palaeobot. Palynol. 123, 237–246.

Tellería, M.C., Sancho, G., Funk, V.A., Ventosa, I., Roque, N., 2013. Pollen morphology and its taxonomic significance in the tribe Gochnatieae (Compositae, Gochnatioideae). Plant Syst. Evol. 299, 935–948.

Urtubey, E., 1997. Morfología del polen de *Barnadesia* (Asteraceae, Barnadesioideae). Bol. Soc. Argent. Bot. 33, 69–75.

Urtubey, E., Tellería, M.C., 1998. Pollen morphology of the subfamily Barnadesioideae (Asteraceae) and its phylogenetic and taxonomic significance. Rev. Palaeobot. Palynol. 104, 19–37.

Wagenitz, G., 1976. Systematics and phylogeny of the Compositae (Asteraceae). Plant Syst. Evol. 125, 29–46.

Wodehouse, R.P., 1935. Pollen grains. McGraw-Hill, New York and London.

Zao, Z., Skvarla, J.J., Jansen, R.K., DeVore, M., 2000. Phylogenetic implications of pollen morphology and ultrastructure in the Barnadesioideae (Asteraceae). Lundellia 3, 26–40.

Zao, Z., Skvarla, J.J., Jansen, R.K., 2006. Mutisieae (Asteraceae) pollen ultrastructure atlas. Lundellia 9, 51–76.