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Pollen morphology of the least known families of the order Asterales

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Pentaphragmataceae; Phellinaceae; Roussaceae

## 1. Introduction

The order Asterales comprises ca. 26000 species distributed in 11 families unevenly sized (Table 1). The MGCA core of Asterales (Menyanthaceae, Goodeniaceae, Calyceraceae and Asteraceae) along with Campanulaceae, represents about 98% of the total diversity of the order (Lundberg, 2009). The remaining families of the Asterales include Roussaceae, the smallest family (four genera and six species), Phellinaceae (one genus and 10 species), Alseuosmiaceae (five genera and 11 species), Argophyllaceae (two genera and 21 species), and Pentaphragmataceae (one genus and 30 species) (Lundberg, 2009; APG, 2016). Most

of these small families comprise shrubs or trees with the exception of Pentaphragmataceae which include mostly herbs. They inhabit the Southern Hemisphere: Australia, New Guinea, New Zealand, New Caledonia and Solomon Islands, except the subfamily Rousseoideae (Roussaceae) endemic to Mauritius (Lundberg, 2009). Lundberg & Bremer (2003) reconstructed a reliable phylogeny of the order Asterales on the basis of both, molecular and morphological data. These authors, however, found a weak to moderate support in the circumscription of some basal clades, in particular the named APA clade formed by the families Alseuosmiaceae, Phellinaceae and Argophyllaceae. The necessity of additional data to define new potential synapomorphies within Asterales was later stressed by Lundberg (2009). The knowledge about pollen morphology of these small families within Asterales is relatively scarce comparing with the great amount of morphological pollen data coming from the MGCA clade. Only 13 out of 49 species were studied (Dunbar, 1978; Pragłowski and Grafstrom, 1985 and; Polevova, 2006). Here we explore the pollen morphology of 18 species of the smallest Asteralean families: Alseuosmiaceae, Argophyllaceae, Pentaphragmataceae, Phellinaceae and Roussaceae, on the basis of detailed microscopy analysis (light microscope, scanning electron microscope and transmission electron microscope), providing additional characters, some of them overlooked in previous analysis. Our new data will provide novel structures for future taxonomic and phylogenetic analysis. These characters can be potentially useful for accurate identification of dispersed fossil pollen grains found in sediments.

## **2. Materials and methods**

Pollen from 29 specimens, belonging to 18 species of five families of order Asterales was examined. The specimens studied are deposited in the herbaria CANB, CBG and K (Holmgren *et al.*, <http://sciweb.nybg.org/science2/IndexHerbariorum.asp>). For light microscopy (LM) pollen was acetolyzed and chlorinated following Erdtman (1960); the slides were prepared by mounting the pollen in glycerol jelly and sealing with paraffin. Diameters were measured on 20 grains and exine thickness on 10 grains under LM Nikon Eclipse 200 microscope using 100 X oil immersion objective and a crossed micrometer eyepiece graticule. Photomicrographs were taken using a Leica DM 2500 microscope and a Leica DFC 7000T camera. For scanning electron microscopy (SEM), acetolyzed pollen grains were suspended in 90 % ethanol, mounted on stubs and examined using a JEOL JSM T-100 SEM. For TEM, the fresh pollen grains were fixed in 1.5 % glutaraldehyde and then buffered 2 % Os O<sub>4</sub> for 2 hours at room temperature. After that, they were washed for 30 min in distilled water and dehydrated in an ethanol series and finally embedded in acetone-Spurr 3:1 for 6 h and twice in Spurr for 24 h. Ultrathin sections were cut using a diamond knife fitted into a Sorvall Porter-Blum MT2-B ultramicrotome. Sections were mounted in single grids and stained with lead citrate (1 min) and uranyl acetate for ten minutes. The examinations were made with a transmission electron microscopy Jeol JEM 1200 EX II from the Servicio Central de Microscopía Electrónica of the Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata. Due to the scarce availability of herbaria specimens from most of the species, TEM examination on ultrastructure of exine could only be conducted on *Rousseia simplex* and *Cuttsia viburnea*. However, examinations made by other authors (Dunbar, 1978; Praglowski and Grafstrom, 1985 and; Polevova, 2006) were considered for discussion.

Terminology used to describe general morphology is consistent with Punt *et al.* (2007). Images were digitally processed and the final plates prepared using Adobe PhotoShop 7.

Specimens studied: **Alseuosmiaceae:** *Alseuosmia macrophylla* Cunn.: Chapman 258560 (K), s.n. 301803 (CANB), *A. quercifolia* Cunn.: Melville (K); *Crispiloba disperma* (Moore) Steenis: Arboretum 1501 (K), Gray 1904 (K), Gray 63672 (QRS); *Periomphale balansae* Baill.: Gardner 7979 (CANB), Jobson 3993 (CANB); **Argophyllaceae:** *Argophyllum brevistylum* Guillaumin: Mackee 7974 (CANB); *A. ellipticum* Labill.: Schode 5260 (CANB), *A. lejourdanii* Muell.: Fogster 9478 (K), *A. montanum* Schltr.: Arboretum 2185 (K), *A. nitidum* Forst. & Forst.: Mackee 8051 (CANB), *A. nullumense* Baker: Pedersen 43 (CBG); *Corokia carpodetoides* (Muell.) Sm.: Hutton 98 (CBG), *C. buddleioides* Cunn.: Andreos s.n. (K), Gardner 247 (CBG), *C. cotoneaster* Raoul: Webb 82/29 (CANB), *C. whiteana* Sm.: Williams NRAC5 (CANB); **Pentaphragmataceae:** *Pentaphragma decurrens* Airy Shaw: Christensen 1055 (K), *P. horsfieldii* (Miq.) Airy Shaw: 114003 (QRS); *P. spatulisepalum* Airy Shaw Wong 1107 (K); **Phellinaceae:** *Phelline macrophylla* Baill: Chickee 2686 (K), Mc Kee 2686 (K); **Roussaceae:** *Abrophyllum ornans* (Muell.) Hook: Gilmour 7513 (CBG), Arboretum 1064 (K); *C. serratus* Forst. & Forst.: Melville 5432 (K), Thompson 222 (CBG); *Cuttsia viburnea* Muell.: Telford 2623 (CBG), *Roussea simplex* Sm. : Botana 1483 (K). The pollen descriptions of the selected species within Asterales were provided by family and genus listed alphabetically. Pollen grains from different species belonging to the same genus were grouped in a single description when they shared the morphology. Measurements for each species as well as sexine/nexine ratio were given in Table 2 with the exception of those of tetrads of *Carpodetus* sp., which were included in the description. In this study all genera were documented with exception of

*Periomphale* due to collapsed pollen grains were found in the two examined specimens. A key to distinguish pollen types within selected species was provided.

### 3. Systematics descriptions

Fam. ALSEUOSMIACEAE

**General description:** pollen grains are radially symmetrical, oblate-spheroidal, spheroidal or subprolate, rhomboidal-subrhomboidal or circular in equatorial view, subangular in polar view. The polar diameter ranges 24-104  $\mu\text{m}$ , and equatorial diameter between 25-102  $\mu\text{m}$ . The apertures are triporate or tricolporate, with long or short ectoaperture but always with rounded ends and microgranulate-granulate membrane; the endoaperture is diffuse. The exine thickness ranges between 2-5  $\mu\text{m}$  thick, is tectate with thick fossulate or verrucate tectum. The structure is indistinguishable under LM in most of species.

*Note:* under LM the structure of exine could only be distinguished in pollen of *Crispiloba disperma* which is granulate. *Comparison with previous studies:* according to TEM studies the structure is also granular in species of *Alseuosmia* (Polevova, 2006).

*Alseuosmia macrophylla*, *A. quercifolia* (Plate I, 1-2; Plate II, 1-2)

Pollen oblate-spheroidal or subprolate, rhomboidal-subrhomboidal in equatorial view.

Tricolporate, with ectoaperture long forming bridge at endoapertural level; apertural membrane scabrate, sometimes with solitary conspicuous granules. Exine 2-3  $\mu\text{m}$  thick, fossulate. SEM: exine psilate at the poles.

*Crispiloba disperma* (Plate I, 3-4; Plate II, 3-4)

Pollen spheroidal, circular in equatorial and polar view. Triporate, pori elliptic or subrectangular shaped, 10-23  $\mu\text{m}$  diameter, with compact annulus of 5  $\mu\text{m}$  thick and scabrate membrane. Exine tectate, irregularly thickened, granulate structure, sexine thick, foveolate-fossulate in polar areas and psilate or slightly punctate at mesoporia. In optical section the inner outline of the exine appears uneven as it has large granules. *Note:* infratectal granules can be observed in optical section, mainly close to the apertures.

*Periomphale balansae.*

Pollen oblate-spheroidal, rhomboidal-subrhomboidal in equatorial and subcircular in polar view. Tricolpate, ectoaperture long, endoaperture very reduced, with microgranulate membrane. Exine with compact aspect, verrucate. In optical section the inner face of the exine appears uneven. *Note.* Specimens examined presented collapsed pollen grains.

Fam. ARGOPHYLLACEAE

**General description:** pollen grains are radially symmetrical, oblate-suboblate, spheroidal or spheroidal-suboblate, circular or subrhomboidal in equatorial view, circular, sub-circular in polar view. The apertures are tricolpate, ectoapertures are long with rounded ends, forming bridge on the endoaperture, with scabrate- microgranulate membrane; the endoapertures are lalongate, diffuse. The polar diameter ranges 14-30  $\mu\text{m}$ , and the equatorial diameter between 16-29  $\mu\text{m}$ . The exine is *ca.* 2  $\mu\text{m}$  thick, it is tectate, fossulate, fossulate-microechinate or microechinate-microgranulate. The structure is columellate. The sexine is separated from the nexine toward the endoaperture forming a distinct bridge on the external surface. The ratio sexine/nexine ranges between 0.5:1, 1:1 and 1:2. *Note.*

Pollen grains of species of Argophyllaceae are similar to those of some species of

Calyceraceae due to: small size, suboblate shape, sexine bridges on the endoaperture and tectum little sculpturate.

*Argophyllum lejourdanii*. *A. ellipticum*. *A. montanum*. *A. nullumense* (Plate I, 5-6; Plate II, 5-6; Plate III, 1)

Pollen oblate-suboblate, subrhomboidal in equatorial view, sub-circular. Exine fossulate.

Nexine commonly thickened at colpus level.

*Corokia* (Plate I, 5-8; Plate III, 2-6)

Pollen oblate-suboblate or spheroidal-suboblate, circular to subrhomboidal in equatorial view, circular or angular to sub-angular in polar view. The exine is fossulate (*C.*

*buddleioides*), fossulate-microechinate (*C. carpodetoides*, *C. cotoneaster*).

#### PENTAPHRAGMATACEAE

*Pentaphragma decurrens*, *P. spatulisepalum*, and *P. horsfieldii* ( Plate I, 11-12; Plate IV, 1)

**General description:** pollen grains radially symmetrically, pleurotreme. The apertures are tri- or bicolporate. The polar diameter ranges 8-21  $\mu\text{m}$ , and the equatorial diameter between 16-32  $\mu\text{m}$ . The exine is psilate. The structure is indistinguishable. The sexine is separate from the nexine at apertural level. *Note:* Due to the scarce thickness of exine pollen grains commonly collapse; tectum surface is psilate with sparse microgranules under SEM. Pollen grains of *P. horsfieldii* are similar to the other two species examined, but it only has two apertures developed and elliptic outline in polar view (Plate IV, 1). *Comparison with previous studies:* general morphology is similar to those species studied by Dunbar (1978).

Fam. PHELLINACEAE



*Phelline macrophylla* Baill. (Plate I, 11-12; Plate IV, 2-4)

Pollen grains are radially symmetrically, spheroidal, circular to elliptic in equatorial view and circular-subcircular in polar view. The polar diameter ranges 18-25  $\mu\text{m}$ , the equatorial diameter between 15-21  $\mu\text{m}$ . The aperture is tricolporate with long ectoaperture with rounded ends; the endoaperture is very reduced only distinguishable in optical section (Plate I, 13). The exine is echinate, scabrate among spines; spines 3-4  $\mu\text{m}$  length, unevenly arranged, in lower focus of L.O. the base of spines differ in size and shape. SEM: the surface exine is scabrate among spines. The spines are formed by baculiform elements which are clearly distinguishable in the lower half of the spine, toward the upper half these elements converge in a solid tip (Plate IV, 4). Baculiform elements are commonly isolated on the edges of apertures. *Note:* TEM studies showed that the structure is columellate with thick nexine (Polevova, 2006).

Fam. ROUSSACEAE

**General description:** pollen grains are dispersed in monads or tetrads (only *Carpodetus*). The monads are radially symmetrical, suboblate, suboblate-spheroidal or spheroidal; the tetrads are tetrahedral. The polar diameter ranges 14-39  $\mu\text{m}$ , the equatorial diameter ranges between 15-44  $\mu\text{m}$ . The apertures are hexaporate or tricolporate, with ectoaperture long, with rounded ends, the endoaperture is lalongate, protruding or cingulated, with scabrate membrane. The exine thickness is 2-2.5  $\mu\text{m}$ , tectate, scabrate or rugulate. The structure is columellate, granulate, or with compact aspect. Ratio sexine/nexine: 1:1, 1:2, 1:3.

*Abrophyllum ornans* (Plate I, 15-16; Plate IV, 5-6)

Pollen suboblate, subangular in polar view. Tricolporate, the endoaperture is lalongate, protruding. The exine is scabrate, columellate. Nexine thickened toward the endoaperture.

*Carpodetus serratus* (Plate V, 1-2)

Pollen grains arranged in permanent tetrahedral tetrads of 85-105  $\mu\text{m}$  x 30-45  $\mu\text{m}$ . Equatorial diameter of single grains ranges between 21-28  $\mu\text{m}$ . The aperture is tricolporate, with colpi arranged in pairs at six points of the tetrads (Garside's law, Punt *et al.* 2007), the ectoaperture is thin, the endoaperture is lalongate, disposed close to the contact area of adjacent grains. The exine has compact aspect, *ca.* 2.5-3  $\mu\text{m}$  thick, scabrate. Sexine/ nexine ratio: 1:3, 1:2. Pollen grains remain together after acetolysis. SEM: the exine surface is scabrate perforate, more or less psilate around ectoaperture (Plate V, 2) and in the poles (Plate V, 1). *Comparison with previous studies:* the genus *Carpodetus* includes other eight species from which the five species studied by Pragłowski & Grafström (1984) also present pollen in tetrahedral tetrads.

*Cuttsia viburnea* (Plate I, 17-18; Plate V, 3-6)

Pollen suboblate-spheroidal. Tricolporate, the endoaperture is lalongate, *ca.* 2 x 8  $\mu\text{m}$ . Exine rugulate, columellate.

*Roussea simplex* (Plate I, 19-20; Plate VI, 1-4)

Pollen spheroidal, zono-hexaporate, pores arranged in three pairs in the equatorial zone, each one slightly displaced towards different poles. Diameter of pore 8-19  $\mu\text{m}$ , covered with scabrate membrane. Exine psilate. SEM: the exine surface has sparse puncta. TEM: the structure has thick tectum and infratectal granulate layer (Plate VI, 4). *Comparison with*

*previous studies*: pollen of *R. simplex* was regarded similar to that of *Ribes*, due to the complete tectum and the “polyporate” aperture (Hideux and Ferguson, 1976). Both pollen types share the complete tectum, but pollen of *Ribes* is pantoporate with pori operculate and microgranulate annulus (Forcone, A., 2014).

Key to pollen of Alseuosmiaceae, Argophyllaceae, Pentaphragmataceae, Phellinaceae and Roussaceae

- |  |  |   |
|--|--|---|
| 1. Pollen sheds in tetrads   | <i>Carpodetus serratus</i>                           |   |
| 1'. Pollen sheds in monads   |  | 2 |
| 2. Pollen porate   |  | 3 |
| 3. Triporate   | <i>Crispiloba disperma</i>                           |   |
| 3'. Hexaporate   | <i>Roussea simplex</i>                               |   |
| 2'. Pollen colporate   |  | 4 |
| 4. Exine strongly sculpturated   |  | 5 |
| 5. Echinata  | <i>Phelline macrophylla</i>                          |   |
| 5'. Verrucate  | <i>Periomphale balansae</i>                          |   |
| 4'. Exine slightly sculpturated  |  | 6 |
| 6. Psilate or scabrate   |  | 7 |
| 7. Psilate. Oblate-peroblate grains                                    | <i>Pentaphragma</i> sp                               |   |
| 7'. Scabrate. Suboblate grains   | <i>Abrophyllum ornans</i>                            |   |
| 6'. Exine rugulate or fossulate  |  | 8 |
| 8. Rugulate  | <i>Cuttsia viburnea</i>                              |   |
| 8'. Fossulate with or without suprategal elements                      |  | 9 |
| 9. With minute suprategal microspines commonly granules or microspines | <i>Corokia carpodetoides</i> , <i>C. cotoneaster</i> |   |
| 9'. Without suprategal elements  | <i>Alseuosmia</i> sp., <i>Argophyllum</i>            |   |

#### 4. Discussion

The lack of fossil pollen records of these phylogenetically related families may be explained, in part, by the poor knowledge about their morphology. Our analyses show a considerable variation in pollen morphological characters among Alseuosmiaceae, Argophyllaceae, Pentaphragmataceae, Phellinaceae and Roussaceae. Dispersal unit and aperture type are significant in delimiting some monotypic genera. Both exine sculpture and structure have strong significance in characterizing species or groups of species, and also in suggesting relationships among them, while pollen size and shape commonly show little variation. In the following paragraphs, we briefly discuss the most important morphological attributes useful to characterize these families.

##### 4.1. Dispersal unit

At anthesis pollen grains are commonly shed in monads; only *Carpodetus serratus* sheds its pollen grains in permanent tetrahedral tetrads. Within Asterales permanent tetrads also occur in the genus *Lechenaultia* (Gustafsson *et al.*, 1997), which belong to the family Goodeniaceae, but unlike those of *Carpodetus* they are uniplanar, with porate grains and reticulate exine.

##### 4.2. Size and shape

Shape is commonly spheroidal to oblate-suboblate. Size of pollen grains commonly ranged between 10 to 30  $\mu\text{m}$ , *i.e.* they fall into the classes small and medium pollen sized (Erdtman, 1954), with exception of those of *Crispiloba disperma* and species of *Pentaphragma* which are very large and very small respectively (Table 2).

#### 4.3. Aperture type

Apertures are tricolporate, triporate or hexaporate. The tricolporate aperture is the most generalized type as occurs in most of the large families of the order, such as Asteraceae, Calyceraceae and Goodeniaceae. It is present in Argophyllaceae, the monotypic Pentaphragmataceae and Phellinaceae, and most species of Alseuosmiaceae and Roussaceae. Ectoaperture is commonly long with rounded ends; in species of Argophyllaceae they form a conspicuous bridge on endoaperture similar to that of Calyceraceae (De vore *et al.*, 2007). Endoapertures may be minute, distinguishable only in optical section in Phellinaceae (Plate I, 13) or diffuse and lalongate in species of *Alseuosmia* and Argophyllaceae (Plate I, 5 and 9). Apertural membrane in tricolporate grains is commonly scabrate (Plate II, 1-2 Plate III, 1), but in some species may presents a few but remarkable granules as in *Alseuosmia macrophylla* (Plate II, 1-2), or bacula commonly topped in an acute tip as in *Phelline macrophylla* (Plate IV, 4). Triporate aperture is exclusive of *Crispiloba disperma* (Plate I, 3-4; Plate II, 3-4) and zono-hexaporate of *Roussea simplex* (Plate I, 19; Plate VI, 1). As far as we know aperture of *Roussea*, consisting of six pores arranged in pairs on the equatorial plane of pollen grains, is unique in Angiosperms.

#### 4.4. Exine sculpture and structure

Tectum occurs in all species, it may be psilate or commonly little sculpturate including regulate or fossulate patterns; elaborated echinate exine is exclusive of Phellinaceae. Infratectal elements are represented by columellae or granules; is granulate in species of Alseuosmiaceae, Pentaphragmataceae and Phellinaceae and columellate in species of

Argophyllaceae, while both granulate and columellate structure are present in Roussaceae. Columellate structure is commonly distinguished in optical section under LM. Conversely, granulate structure only can be evidenced with TEM, with exception of *Crispiloba disperma*. In this species the exine is unevenly thickened, as can be seen in optical section (Plate I, 3, 4), infractectal granules forms a thin layer that can be clearly distinguish under LM, mainly close to the apertural areas. In pollen of *Rousseia simplex* granulate structure was evidenced with TEM (Plate V, 4), as in species of *Alseuosmia* (Polevova, 2006), *Carpodetus* (Praglowsky & Grafström, 1984) and *Pentaphragma* (Dunbar, 1978). Granular structure also occurs in other taxa belonging to different angiosperms groups as Annonaceae and Fabaceae (Guinet & Le Thomas, 1990), Apocynaceae (Van der Ham *et al.*, 2001) and Nymphaeaceae (Coiro and Barone Lumaga, 2013). Although in the species here studied differences of thickness between sexine and nexine are elusive to be measured, they are quite apparent to the eye in most of species examined. Commonly, the nexine is comparatively equal or thicker than sexine (Table 2). Similar observations were made at ultrastructural level in pollen of Leguminosae (Guinet, 1981). This author suggested that the difference of development between ectexine (sexine plus foot layer) and endexine (nexine), depend on the quantity of sporopollenin involved in its building. Thus, a great amount used to build the endexine is at the expense of a less development of the ectexine. This might be occur in these small families given that in the context of the Asterales, in pollen of the largest families as Asteraceae and Goodeniaceae the ectexine exhibits comparatively greater development than the endexine.

#### 4.5. Taxonomic significance

The morphological features of pollen have different significance in the characterization of studied families. The exine sculpture is distinctive in the two monotypic families, echinate in Phellinaceae and psilate, with sparse microgranules, in Pentaphragmataceae. Pollen features show no marked diversity in Argophyllaceae where the six species studied share tricolporate aperture, tectum little sculpturate commonly fossulate with or without supratectal elements, columellate exine structure, small to medium size and shape typically oblate to suboblate. These features are similar to those of some species of Calyceraceae studied by DeVore *et al.*, (2007). Further studies are necessary to strength the differences between pollen from both families. Alseuosmiaceae presents comparatively less consistency, most of species have tricolporate aperture and granulate exine structure. However, *Crispiloba disperma* has an isolate position in the family by its triporate apertures, psilate-foveolate tectum and large size. Roussaceae, with four very distinct types, shows the greatest pollen diversity within the families studied. Pollen of both *Carpodetus serranus* and *Roussea simplex* are very distinguishable from each other; *C. serranus* is released in tetrahedral tetrads whereas *R. simplex* in zono-hexaporate monads. The other two species of Roussaceae differ in sculpture exine, scabrate in *Abrophyllum ornans* and rugulate in *Cuttsia viburnea*. Pollen of *Roussea* is poorly known, it possesses a unique type of aperture consisting of six pores arranged in pairs in the equatorial zone and smooth exine surface with some dispersed puncta (Plate I, VI). This peculiar pollen type is consistent with its particular biogeography and pollination. This enigmatic species is the sole member of Roussaceae, endemic from Mauritius whereas the remaining members inhabit Eastern Australia, New Guinea and New Zealand (Lundberg, 2001). At anthesis, pollen grains of *Roussea* are embedded in a sticky substance and dispersed by geckos which have been recognized as its exclusive pollinators (Hansen & Müller, 2009).

## 5. Conclusions

Our study reveals a highly variable pollen morphology of these Asteralean families. The tricolporate character of *Phelline macrophylla* and the zono-hexaporate aperture of *Roussea simplex* are here described for the first time. Roussaceae is the more diverse family at level of dispersion unit, aperture type, sculpture and structure of exine. The occurrence of granular structure of exine in other families as Annonaceae, Apocynaceae, Fabaceae and Nymphaeaceae in which the structure is commonly columellar is other example of convergent evolution in Angiospermae. According to the molecular phylogeny of Asterales proposed by Lundberg & Bremer (2003), pollen morphology is consistent with the moderate molecular support of the APA clade. Conversely, pollen features are not consistent in Roussaceae; this family is well supported from molecular and reproductive features but is palynologically diverse.

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Plate I. Pollen of Alseuosmiaceae (LO). 1-2. *Alseuosmia macrophylla* (Chapman 258560 K). 1. Equatorial view in optical section. 2. Polar view in upper focus. Note rounded ends of ectoapertures. 3-4. *Crispiloba disperma* (Arboretum 1501 K). 3. Tilted polar view showing the thick annulus (arrow) of the aperture. 4. Equatorial view in optical section. Note the irregular inner outline of exine. 5-6. *Argophyllum lejourdanii* (Fogster 9478 (K)). 5. Equatorial view in optical section. 6. Polar view in optical section. 7-8. *Corokia buddleioides* (Gardner 247 CBG). 7. Equatorial view in optical section. 8. Polar view in optical section. 9-10. *C. cotoneaster* (Webb 82/29 CANB). 9. Equatorial view, intermediate focus. 10. Polar view in optical section. 11-12. *Pentaphragma decurrens* (Christensen 1055 K). 11. Equatorial view in optical section. 12. Polar view in optical section. 13-14. *Phelline macrophylla* (Chickee 2686 K). 13. Equatorial view in optical section. Note the endoaperture 14. Polar view in optical section. 15-16. *Abrophyllum ornans* (Gilmour 7513 CBG). 15. Equatorial view in optical section. 16. Polar view in intermediate focus 17-18. *Cuttsia viburnea* (Telford 2623 CBG). 17. Equatorial view in optical section. 18. Polar view in upper focus. Note rounded ends of ectoapertures. 19-20. *Roussea simplex* (Botana 1483 K). 19. Equatorial view in upper focus showing one pair of apertures on the equator (arrows). 20. Polar view in upper focus. Scale bars: 10  $\mu\text{m}$ .

Plate II. Pollen of Alseuosmiaceae (SEM). 1-2. *Alseuosmia macrophylla* (Chapman 258560 K) 1. Polar view showing rounded ends of apertures and conspicuous granules on the apertural membrane (arrow). 2. Detail of psilate tectum in the polar area and weakly fossulate sculpture on the mesocolpium. 3-4. *Crispiloba disperma* (Arboretum 1501 K). 3. Equatorial view showing psilate sculpture on the mesopodium, foveolate at the poles, and conspicuous porus with irregular thickened annulus and sculpturated membrane. 4. Detail

of the foveolate-fossulate tectum in the polar area. Pollen of Argophyllaceae (SEM). 5-6. *Argophyllum nullumense* (Pedersen 43 CBG). 5. Tilted equatorial view showing weakly sculpturated tectum and bridge on the endoaperture. 6. Polar view showing colpi with rounded ends. Scale bars of 1, 5 and 6: 5  $\mu\text{m}$ ; of 2 and 4: 2  $\mu\text{m}$ ; of 3: 10  $\mu\text{m}$

Plate III. Pollen of Argophyllaceae (*cont.*). 1. *Argophyllum lejourdanii* (Fogster 9478 (K). Equatorial view showing fossulate exine and bridge on the endoaperture (arrow). 2-4. *Corokia carpodetoides* (Hutton 98 CBG). 2. Equatorial view. 3. Polar view showing rounded ends of endoapertures. 4. Detail of the fossulate-microechinate sculpture. 5-6. *C. cotoneaster* (Webb 82/29 CANB). 5. Equatorial view showing bridge on the endoaperture 6. Detail of exine surface showing microgranulate-microechinate surface and sculpturated aperture membrane. Scale bars of 1, 2 and 3: 5  $\mu\text{m}$ ; of 4, 5 and 6: 2  $\mu\text{m}$

Plate IV. Pollen of Pentaphragmataceae (SEM). 1. *Pentaphragma horsfieldii* (Airy Shaw 114003 QRS). Collapsed grain in polar view showing two apertures and microgranulate tectum surface. Pollen of Phellinaceae (SEM). 2-4. *Phelline macrophylla* (Chickee 2686 K). 2. Tilted equatorial view. 3. Polar view showing rounded ends of ectoapertures. 4. Detail of exine showing spines formed by baculiform elements (arrows) united at the tip and scabrate tectum among spines. Note the single baculiform elements on apertural membrane (asterisks). Pollen of Roussaceae (SEM). 5-6. *Abrophyllum ornans* (Gilmour 7513 CBG). 5. Equatorial view showing weakly sculpturated exine and bridge on the endoaperture (arrow). 6. Polar view showing rounded ends of ectoapertures. Scale bars of 1, 5 and 6: 5  $\mu\text{m}$ ; of 2 and 3: 2  $\mu\text{m}$ ; of 4: 1  $\mu\text{m}$ .

Plate V. Pollen of Roussaceae (*cont.* SEM and TEM). 1-2. *Carpodetus major* (Regdado 1145 K). 1. Polar view of one pollen grain of the tetrahedral tetrad. 2. 3-6 *Cuttsia viburnea* (Telford 2623 CBG) 3. Equatorial view showing weakly sculpturated tectum. 4. Polar view showing rounded ends of ectoapertures 5. Detail of rugulate sculpture. 6. TEM exine section showing with thick endexine (En), conspicuous columellae (c) and tectum (T). Scale bars of 1, 2, 3, 4 and 5: 5  $\mu\text{m}$ ; of 6: 1  $\mu\text{m}$ .

Plate VI. Pollen of Roussaceae (*cont.* SEM and TEM). 1-4. *Rousseia simplex* (Botana 1483 K). 1. Equatorial view showing two pairs of pores. 2. Detail of scabrate apertural membrane and psilate tectum with sparsed puncta. 3. Polar view showing three pores. 4. TEM exine section showing thick tectum (T), layer of reduced columellae (arrows) and thin endexine (En). Scale bars of 1 and 3: 5  $\mu\text{m}$ ; of 2 and 4: 1  $\mu\text{m}$ .

Table 1. Number of genera, species and distribution of Asterales (from Lundberg, 2009). In

bold families studied.

<b>TAXA</b>	<b>GENERA</b>	<b>SPECIES</b>	<b>DISTRIBUTION</b>
Asteraceae	>1600	ca. 23000	Cosmopolitan
<b>Alseuosmiaceae</b>	<b>5</b>	<b>10</b>	<b>E Australia, New Guinea, New Zealand</b>
<b>Argophyllaceae</b>	<b>2</b>	<b>ca. 20</b>	<b>E Australia, Lord Howe Is., New Caledonia, New Zealand, Rapa Is.</b>
Calyceraceae	4	ca. 60	Southern South America
Campanulaceae	84	ca. 2400	Cosmopolitan
Goodeniaceae	11	ca.440	Mainly Australia
Menyanthaceae	5	ca. 60	Almost cosmopolitan
<b>Pentaphragmataceae</b>	<b>1</b>	<b>30</b>	<b>SE Asia to New Guinea</b>
<b>Phellinaceae</b>	<b>1</b>	<b>11</b>	<b>New Caledonia</b>
<b>Rousseaceae</b>	<b>4</b>	<b>6</b>	<b>Mauritius, E Australia, New Guinea, New Zealand, Solomon Is.</b>
Stylidiaceae	6	ca. 245	Australia, New Zealand, South America, SE Asia

Table 2. Pollen grains measurements. \* only four grains were measured.

TAXA	Dp ( $\mu\text{m}$ )	$\bar{x}\pm\delta$	De ( $\mu\text{m}$ )	$\bar{x}\pm\delta$	P/E	Exine thickness ( $\mu\text{m}$ )	Sexine/nexine ratio
<b>Alseuosmiaceae</b>							
<i>Alseuosmia macrophylla</i>	38-52	45 $\pm$ 4	51-52	51 $\pm$ 3	Oblate-subprolate	ca. 2	0.5:1
<i>A. quercifolia</i>	44-55	50 $\pm$ 4	44-55	50 $\pm$ 4	Subprolate	ca.2-3	1:2
<i>Crispiloba disperma</i>	75-104	91 $\pm$ 11	72-102	88 $\pm$ 15	Spheroidal	ca.4-5	2-4:1
<i>Periomphale balansae</i>	24-29	26 $\pm$ 2	25-32	30 $\pm$ 4	Oblate-spheroidal	ca.4	indistinguishable
<b>Argophyllaceae</b>							
<i>Argophyllum ellipticum</i>	14-18	17 $\pm$ 2	18-24	21 $\pm$ 2	Oblate-suboblate	ca.2	1:1
<i>A. lejourdanii</i>	18-26	22 $\pm$ 3	23-27	24 $\pm$ 2	Oblate-suboblate	ca.2	1:1; 0.5-1
<i>A. montanum</i>	20-26	22 $\pm$ 2	21-26	22 $\pm$ 2	Spheroidal	ca.2	1:1; 0.5-1
<i>A. nullumense</i>	17-21	19 $\pm$ 1	16-20	19 $\pm$ 1	Spheroidal	ca.2	0.5:1
<i>Corokia buddleioides</i>	23-30	27 $\pm$ 1	23-28	26 $\pm$ 1	Oblate-suboblate	ca.2	1:1.5
<i>C. carpodetoides</i>	22-25	23 $\pm$ 1	20-27	25 $\pm$ 2	Spheroidal-suboblate	ca.2	1:1
<i>C. cotoneaster</i>	22-30	25 $\pm$ 2	25-29	27 $\pm$ 1	Spheroidal-suboblate	ca.2	1:1
<b>Pentaphragmataceae</b>							
<i>Pentaphragma decurrens</i>	8-10	9 $\pm$ 1	16-20	17 $\pm$ 3	Oblate-peroblate	<1	indistinguishable
<i>P. horsfieldii</i> *	21		32		Oblate-peroblate	<1	indistinguishable
<i>P. spatulisepalum</i>	13-18	15 $\pm$ 2	23-24	24 $\pm$ 2	Oblate-peroblate	<1	indistinguishable
<b>Phellinaceae</b>							
<i>Phelline macrophylla</i>	18-25	22 $\pm$ 2	15-21	20 $\pm$ 1	Spheroidal	1.5-2	1:2
<b>Rousseaceae</b>							
<i>Abrophyllum ornans</i>	14-15	15 $\pm$ 0.5	15-18	17 $\pm$ 1	Suboblate	ca.2	1:1
<i>Cuttsia viburnea</i>	19-24	22 $\pm$ 2	21-24	22 $\pm$ 2	Suboblate-spheroidal	ca.2-2.5	1:1.5
<i>Roussea simplex</i>	34-39	37 $\pm$ 1	39-44	41 $\pm$ 2	Spheroidal	ca.2	1:3

### Highlights

- Pollen in poorly explored families of Asterales was studied with LM, SEM and TEM.
- There is a highly variation in pollen morphological characters.
- Pollen is highly diverse in Roussaceae but less variable in Argophyllaceae.
- Results are significant for taxonomic and palaeontological purposes.

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