

# Morphology and structure of the pollen cone and pollen grain of the *Araucaria* species from Argentina

GEORGINA M. DEL FUEYO<sup>1</sup>, MARTA A. CACCAVARI<sup>1,2</sup> AND ELIZABETH A. DOME<sup>1</sup>

<sup>1</sup> CONICET. Museo Argentino de Ciencias Naturales "Bernardino Rivadavia". Av. Ángel Gallardo 470, C1405DJR Buenos Aires, Argentina.

<sup>2</sup> CONICET. Centro de Investigaciones Científicas y de Transferencia de Tecnología a la Producción. Dr. Materi y España s/n. (3105) Diamante, Entre Ríos, Argentina.

**Key words:** Araucariaceae, *Araucaria*, pollen cones, pollen ultrastructure.

**ABSTRACT:** The pollen cone and the pollen grain of the two Argentinean species of *Araucaria* are described with LM, SEM and TEM. Primordia of pollen cones are formed in April and May and reach maturity by mid-October in *A. angustifolia* (Bert.) O. Kuntze and by mid-November in *A. araucana* (Mol.) K. Koch. Characters of the mature pollen cones and microsporophylls between both taxa are clearly differentiated. Pollen grains are spheroidal-sub-spheroidal, inaperturate, and asaccate with granulate exine and a subequatorial annular area that corresponds to the sexine thickness. Sculpturing consists of irregularly dispersed granules that are sometimes fused to each other (*A. angustifolia*) or forming microrugulae (*A. araucana*). Microgranules and microspinules are also present. The pollen wall ultrastructure is formed by a granular ectexine and lamellated endexine. Granular elements in *A. angustifolia* are more loosely disposed, form more interstices, and are gradually smaller towards the endexine than in *A. araucana*. To assess the probable relationships within the family, we compared the pollen grains of the two *Araucaria* species with those of other extant genera (*Agathis*, *Wollemia*) and also with fossil pollen (*Araucariacites*, *Balmeiopsis*, *Cyclusphaera*, *Dilwynites*) attributed to Araucariaceae.

## Introduction

The family Araucariaceae in Argentina is represented by the genus *Araucaria* Juss., Section *Araucaria* (Wilde and Eames, 1952), with two species, which have a very restricted and disjunct distribution as well as different ecological constraints. *Araucaria angustifolia* (Bert.) O. Kuntze, known as Pino Paraná, is found in the subtropical to temperate rainforests (500 to 1800

m s.l.) of NE Misiones Province, between 25° to 26° latitude S. *A. araucana* (Mol.) K. Koch, also known as the monkey puzzle tree, grows in cool temperate forests (900 to 1800 m s.l.) of SW Neuquén Province, between 37° and 40° latitude S (Veblen *et al.*, 1995).

The three genera of Araucariaceae contain 41 species; 19 spp. of *Araucaria* Juss., 21 spp. of *Agathis* Salisbury, and 1 spp. of *Wollemia* Jones, Hill and Allen. Few studies address the morphology of the pollen cones and the structure of the pollen grains. Chamberlain (1935) and later Sporne (1965) provided brief reports on the pollen cone morphology in the family. Burlingame (1913) provided a detailed description of the pollen cone development in *Araucaria*. Wodehouse (1935) and Ueno (1960) generally described the characters of the pollen grain for the family, and Ueno

Address correspondence to: Dr. Georgina M. Del Fueyo. Museo Argentino de Ciencias Naturales "Bernardino Rivadavia". Av. Ángel Gallardo 470, C1405DJR Buenos Aires, ARGENTINA.

E-mail: [gdelfueyo@macn.gov.ar](mailto:gdelfueyo@macn.gov.ar) / [georgidf@yahoo.com.ar](mailto:georgidf@yahoo.com.ar)  
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(1959), Barth (1962) and Heusser (1971) described them for *Araucaria*. Pocknall (1981), used LM and SEM to study the pollen grain of *Agathis australis* (D. Don) Salisbury. Chambers *et al.* (1998) used LM and SEM to study the pollen of the recently erected genus of the family *Wollemia*. Studies on the exine stratification on the araucarian grains are scarce: Ueno (1959) briefly described *Araucaria angustifolia*, Van Campo and Lugardon (1973) studied *Agathis*, *A. alba* Foxworthy, Kurmann (1992) studied *Agathis australis*, and Dettmann and Jarzen (2000) studied the exine structure in *Wollemia nobilis*.

*Araucariacites* Cookson ex Couper, *Balmeiopsis* Archangelsky, *Cyclusphaera* Elsik, and *Dilwynites* Harris are fossil pollen grains with araucarian affinities that have been recorded at least since the Jurassic (210 million years; Dettmann, 1963; Archangelsky *et al.*, 1984). *Araucariacites*, *Balmeiopsis*, and *Cyclusphaera* have mostly a Gondwanan distribution (South America, Antarctica, South Africa, India, Australia), even though the first two taxa have also been recorded from the Early Cretaceous of Canada and the Jurassic and Triassic of Europe (Van Konijnenburg-Van Cittert, 1971; Archangelsky, 1994; Chambers *et al.*, 1998). In contrast, *Cyclusphaera* has been mostly found in the Cretaceous of South America and South Africa (Del Fueyo and Archangelsky, 2005) while *Dilwynites* has only been recorded from the Late Cretaceous sediments of Australia, New Zealand and Antarctica (Dettmann and Jarzen, 2000). In Argentina, especially in Patagonia, *Araucariacites* was reported from the Jurassic (Volkheimer, 1989) throughout the Eocene-Miocene (Guerstein and Quattrocchio, 1991), whereas *Balmeiopsis* is only known from the Barremian-Aptian until probably the Albian (Archangelsky, 1977) and *Cyclusphaera* from the Valanginian to the Maastrichtian (Volkheimer, 1980). However, araucarian seed cones (Stockey, 1978; 1982), ovuliferous scales (Archangelsky, 1966), and pollen cones (Del Fueyo, 1991), as well as vegetative remains (Del Fueyo and Archangelsky, 2002), were also described from the Mesozoic of Patagonia. All these records confirm the long history of Araucariaceae in Argentina, and today it remains an important group.

We described and compared the morphology of the pollen cone and the structure and ultrastructure of the pollen grain of *Araucaria angustifolia* and *A. Araucana*. To assess the probable relationships within the family, we compared these features with those of the living and fossil representatives of Araucariaceae.

## Material and Methods

The material of the two species of *Araucaria* was collected from trees growing in their natural habitats. *A. angustifolia* in the province of Misiones, Argentina and Brazil and *A. araucana* in the province of Neuquén.

For the study of the phenology, early, young, and mature pollen cones were fixed in formalin-acetic alcohol (FAA), dehydrated in a graded ethanol series, embedded in Paramat, serially sectioned at 10 µm, and stained in safranin-fast green (O'Brien and Mc Culley, 1981).

For light microscopy (LM), acetolyzed (Nilsson and Praglowski, 1992) and non-acetolyzed pollen was embedded in glycerin-phenol and observed with a Leitz Laborlux 12 microscope. A minimum of twenty pollen grains were observed for each sample. Measurements in brackets correspond to the mean of the recorded values. Greater sexine detachments and deformed grains were observed in acetolyzed pollen than in non-acetolyzed pollen.

For scanning electron microscopy (SEM), microsporophylls and mature and non-acetolyzed pollen grains were dehydrated in an ethyl alcohol series and coated with gold-palladium. Observations were made with a JEOL-T 100 SEM at La Plata Natural History Museum and with a Leitz AMR 1200 at Morón University.

For transmission electron microscopy (TEM), mature and non-acetolyzed pollen grains were fixed in glutaraldehyde, post-fixed in osmium tetroxide, dehydrated in an alcohol series, infiltrated with Spurr resin, placed in Eppendorf moulds, and dried in a vacuum. Ultrathin sections were cut with a diamond knife and stained with potassium permanganate (5-10 min) and uranylacetate (5 min). Specimens were viewed with a Jeol JEM 100C at the Electronic Microscopy Laboratory of CICV-INTA Castelar.

Microscope slides and samples for SEM and TEM are housed in the Actuopalinotheca and at the herbarium of the Buenos Aires Natural Sciences Museum "Bernardino Rivadavia" under the prefix BAPa and BA, respectively. The specimens examined are: *A. angustifolia*: Brasil, Río Grande, BA 73354, BAPa 3138. Argentina, Misiones, San Antonio, INTA Experimental Station (fresh material): Tree 41, BAPa 3139; Tree NN, BAPa 3140 (MEB, MET). *A. araucana*: Argentina, Neuquén; N. Huapi, BA 34924, BAPa 3135; Srta. Mamuil, BA 34927, BAPa 3136; La Meliquina, BA 21852, BAPa 3137; Primeros Pinos, (fresh material), BAPa 3155 (MEB, MET).



**FIGURES 1-4.** Figs. 1-2. *Araucaria araucana*. **Fig. 1.** Mature pollen cone. Bar: 2 cm. **Fig. 2.** Microsporophyll in lateral view observed with SEM. Bar: 2, 5 mm. Figs.3-4. *Araucaria angustifolia*. **Fig. 3.** Microsporophyll in abaxial view seen with SEM. Bar: 2, 5 mm. **Fig. 4.** Mature pollen cone. Bar: 2 cm.

The pollen grain terminology follows Punt *et al.* (2007).

## Results

### Phenology

The phenology of *A. angustifolia* and *A. araucana* shows few variations. The primordia of the pollen cones are already formed during April and May, and the archesporium is differentiated in June and remains in this stage until the mid-September. At the end of June the microspores are formed and begin to develop into the characteristic multinucleate microgametophyte with numerous starch grains. In *A. angustifolia*, pollen grains are mature by the mid-October, when pollination takes place. In *A. araucana*, the development is slower and the pollen grains are mature by the mid-November.

### Morphology of the pollen cones

The two Argentinean species of *Araucaria* are dioecious. The pollen cones of both species are single and occur laterally on the tips of the branches. The mature cones in *A. araucana* are sub-cylindrical, slightly curved, reddish and up to 13 cm long and up to 6 cm wide (Fig. 1). In *A. angustifolia*, the mature cones are cylindrical, brown, straight, longer (up to 20 cm long) and narrower (up to 3 cm wide; Fig. 4).

The microsporophylls are disposed in a dense and imbricate spiral on the cone axis. They are peltate, and numerous cylindrical microsporangia, which are parallel to and free from the stalk, hang on their abaxial face. The sporophylls in *A. araucana* have a distal portion that end in a long flattened and obtuse apex. They are strongly curved and overlap the adjacent sporophyll (Fig. 2). *A. angustifolia* have a rhomboidal expanded distal portion (Fig. 3). The number of sporangia is up to 20 per sporophyll in *A. araucana* and up to 22 in *A. angustifolia*. The dehiscence in both species is longitudinal.

### Characters of the pollen grain

#### *Araucaria angustifolia*

**Observation with LM** (Figs. 5-7, 10-12).

Pollen of *A. angustifolia* is asaccate, inaperturate, and 60 (87) 95 x 52 (70) 84 µm in diameter. The pollen is spheroidal in outline in polar view (Figs. 10, 11), while it is ellipsoidal in lateral view (Figs. 5-7). The pollen also has a subequatorial annular area (Figs. 7, 11). Broad

exine depressions are seen in dehydrated specimens. The exine is 2-4 µm thick and atectate with a nexine that is equal to or thinner than the sexine. In the annular area, the exine shows a thickening of the sexine (3 µm thick), in which it is observed sexine detachments (Fig. 7). The sexine has a conspicuously granulate sculpture (Figs. 5, 6) of pilate elements, as seen in the optical section (Fig. 5).

**Observation with SEM** (Figs. 11, 12).

The granulate elements (less than 1 µm in diameter) are irregularly dispersed, showing different degrees of coalescence and forming granules of different sizes. Each granule has microgranule-like and isolated microspinule-like elements on its surface (Figs. 11, 12).

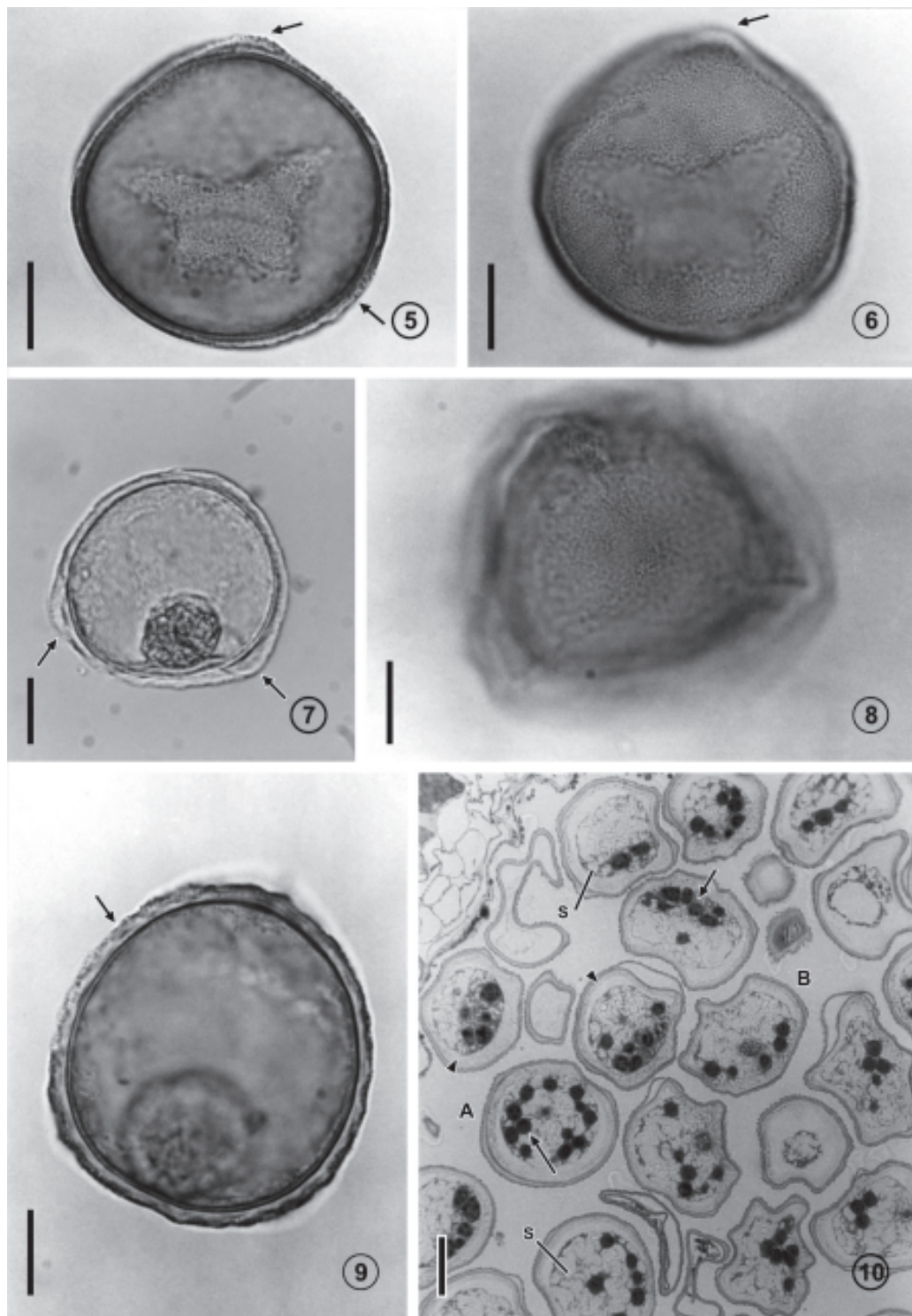
**Observation with TEM** (Figs. 16, 18, 19)

The exine structure consists of an outer granular ectexine, a lamellated endexine, and an innermost fibrillar intine (Fig. 18). The ectexine is defined by granules, which decrease gradually in diameter towards the endexine: the most external are large, dark, electron dense, and spherical- to irregular-shaped because of the microspinule-like and the microgranule-like elements. The inner granules are smaller, less electron dense, spherical, and loosely disposed with numerous interstices (Fig. 19). The endexine is formed of two layers: endexine I and endexine II. Endexine I has from 10 to 12 parallel lamellae, where the innermost are compactly arranged and the outermost are sinuous, loosely disposed, and closely related to the innermost granules of the ectexine (Fig. 19). Each lamella shows a central, less electron-dense area surrounded by darker sporopollenin (Fig. 18). Endexine II is a thin and amorphous layer contiguous to the intine. This layer is 2.0 µm thick and is composed of irregularly disposed microfibrillae. At the contact with the endexine, the intine appears as a regular, darker layer (Fig. 18). Figure 16 illustrates the Ubisch bodies and their relationship to the tapetal membrane and the pollen wall. They have a spheroidal to irregular shape with an microgranule-like and microspinule-like sculptures. These orbicules have a single electron transparent core and a homogeneous wall.

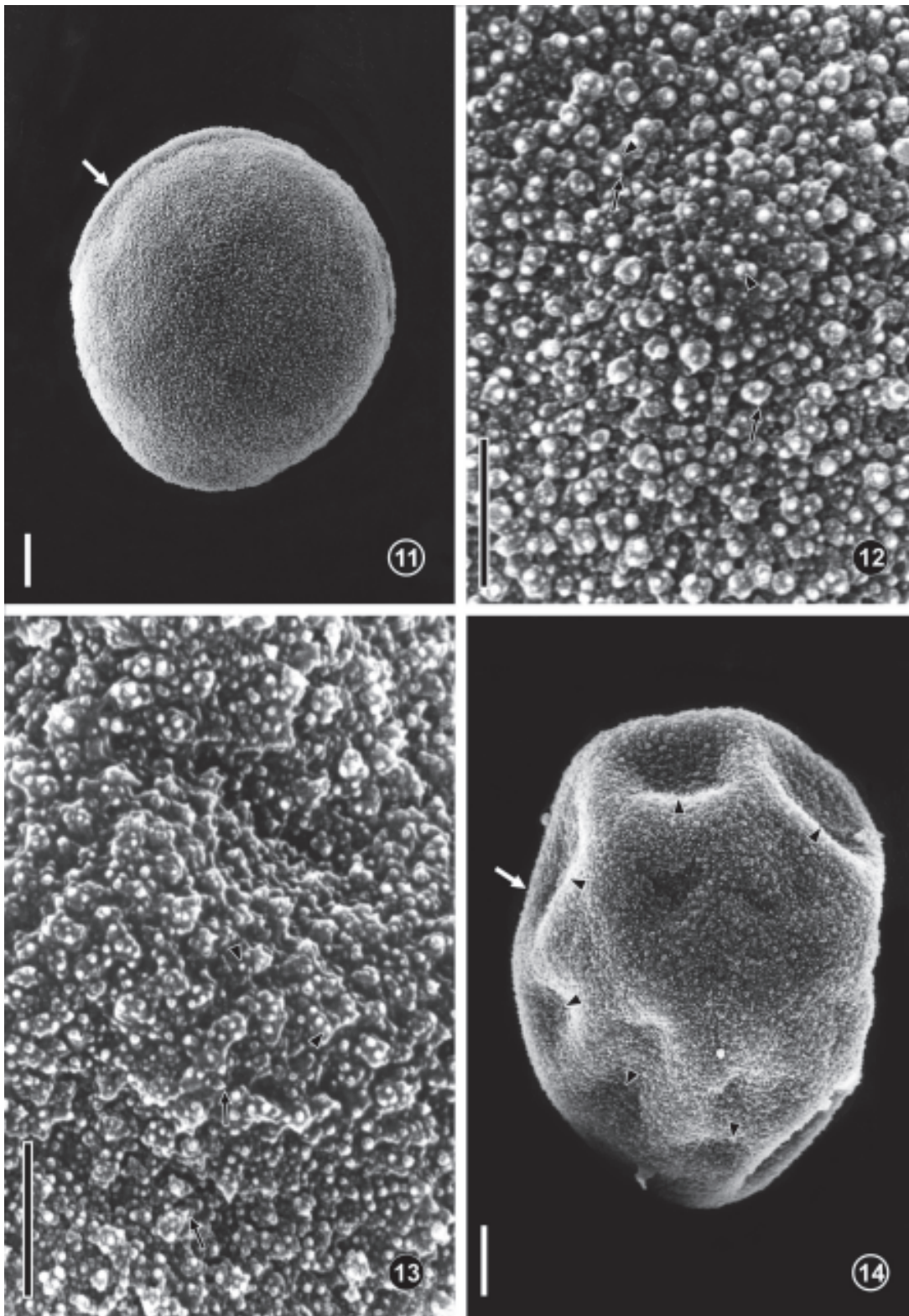
#### *Araucaria araucana*

**Observation with LM** (Figs. 8, 9).

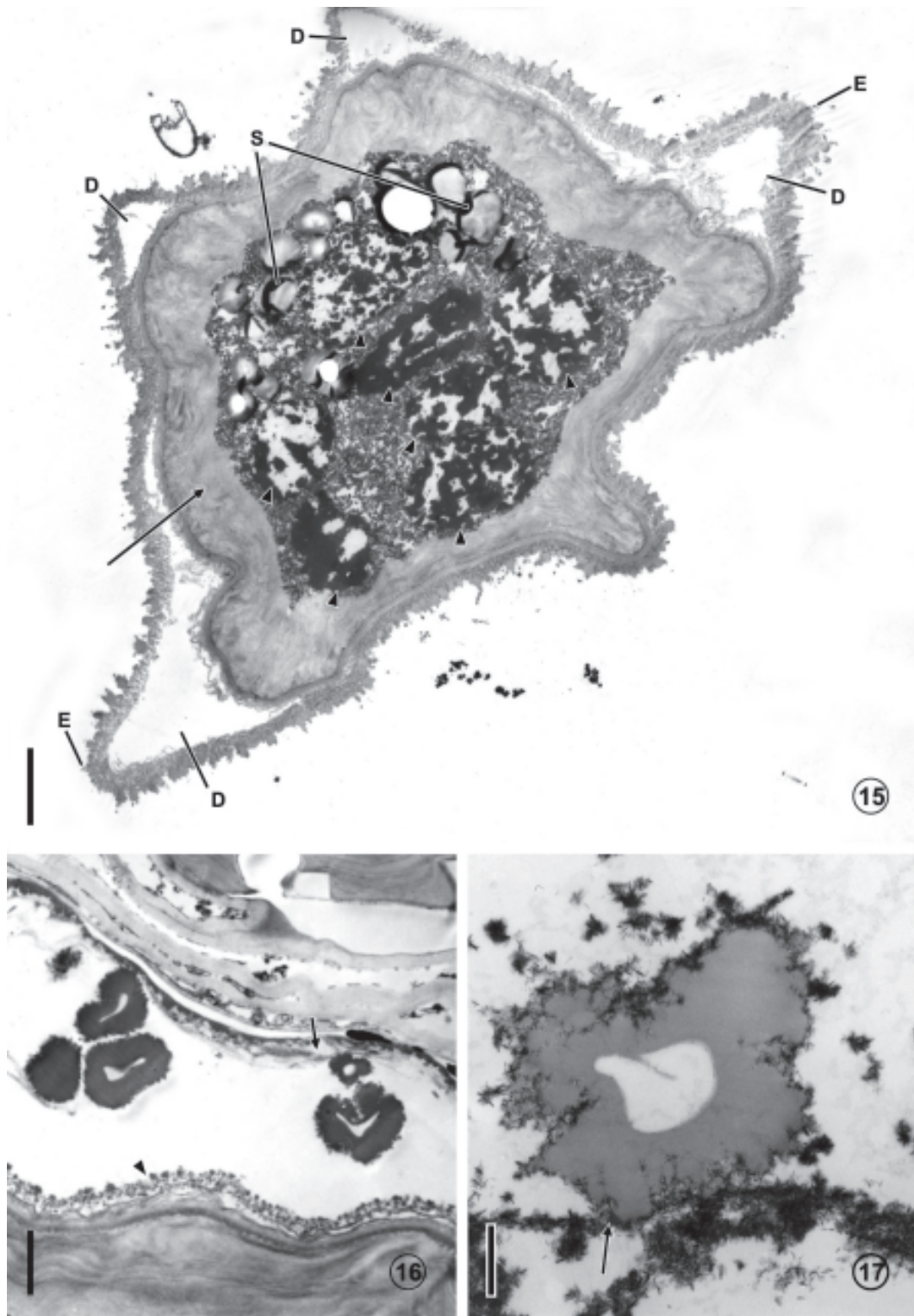
The pollen of *A. araucana* is asaccate, inaperturate, and 65 (74) 85 x 52 (57) 76 µm in diameter. The pollen is spheroidal to subspheroidal (Fig. 9). The exine is 3-4 µm thick, atectate, with a granulate sculpture, and the



**FIGURES 5-10.** Light micrographs of pollen grains. **Figs. 5-7, 10.** *Araucaria angustifolia*. **Figs. 8, 9.** *Araucaria araucana*. **Fig. 5.** Optical cross section. Arrows show the subequatorial annular thickening with a thicker and slightly detached sexine. Note the pilate likeness of the sexine with spinules. Bar: 20  $\mu\text{m}$ . **Fig. 6.** Exine surface focus showing granulate sculpturing. Arrow indicates subequatorial annular thickening. Bar: 20  $\mu\text{m}$ . **Fig. 7.** Lateral view. Arrows show subequatorial position of the annular thickening. Bar: 20  $\mu\text{m}$ . **Fig. 8.** Exine surface focus showing granulate sculpturing. Bar: 20  $\mu\text{m}$ . **Fig. 9.** Optical cross section. Arrow indicates spinules. Bar: 20  $\mu\text{m}$ . **Fig. 10.** Dehiscing microsporangium in cross section with mature pollen grains. A: in polar view, B: in lateral equatorial view. Note tapetal membrane with Übisch bodies, the thick intine (arrowheads), nucleus (arrows) and starch grains (s). Bar: 30  $\mu\text{m}$ .



**FIGURES 11-14.** Scanning electron micrographs of pollen grains. Figs. 11-12. *Araucaria angustifolia*. **Fig. 11.** Polar view showing subequatorial annular area (arrow). Bar: 10  $\mu\text{m}$ . **Fig. 12.** Detail of exine sculpturing. Note coalescence of microgranules at different degrees (arrowheads) and microspines (arrows). Bar: 5  $\mu\text{m}$ . Figs. 13-14. *Araucaria araucana*. **Fig. 13.** granulate-rugulate sculpturing showing microgranules (arrowheads) and microspines (arrows). Bar: 5  $\mu\text{m}$ . **Fig. 14.** Polar view showing subequatorial annular area (arrow) and depressions (arrowheads). Bar: 10  $\mu\text{m}$ .



**FIGURES 15-17.** Transmission electron micrographs of pollen grains. Figs. 15-17. *Araucaria araucana*. Fig. 16. *Araucaria angustifolia*. **Fig. 15.** Longitudinal section through the entire unacetolysed pollen grain. Note the thick intine (arrow), numerous nucleus (arrowhead) at the proximal polar area and starch grains (s). Also, the subequatorial annular area with thicker ectexine (E) and detachments of the ectexine (D). Bar: 7  $\mu$ m. **Fig. 16.** Orbicules in the tapetal membrane (arrow). Arrowhead indicates the pollen wall. Bar: 2  $\mu$ m. **Fig. 17.** Ubisch body attached to the tapetal membrane (arrow). Dark crystals are potassium permanganate precipitate. Bar: 500 nm.

nexine is equal or thinner than the sexine (Fig. 8). The pollen has a subequatorial annular area with a sexine thickening (3  $\mu\text{m}$  thick). Mostly, this character is difficult to observe in *A. araucana* because usually the sexine detaches from the nexine as a complete layer (Fig. 8).

#### Observation with SEM (Figs. 13, 14).

Observed with SEM (Fig. 14), the granular elements (less than 1  $\mu\text{m}$  in diameter) appear irregularly fused and form microrugulae that have microgranules and microspinules on their surface (Fig. 13). In dehydrated grains exine depressions are very common and easily recognizable (Fig. 14).

#### Observation with TEM (Fig. 15, 17, 20)

Ultrastructurally, the pollen wall of *A. araucana* is composed of a granular ectexine, a lamellated endexine, and a fibrillar intine. The ectexine is also easily detachable from the endexine (Fig. 15). Ectexine granules are uniformly electron dense, spherical to irregular in shape, and decrease abruptly in size towards the endexine (Fig. 20). The outermost granules are closely tight appressed, sometimes fused to each other, and have sporadic microgranule-like and microspinule-like projections on their surfaces. In contrast, the inner granules are loosely disposed and only a few are fused (Fig. 20). The endexine has two layers: endexine I and II. Endexine I has 8–12 parallel lamellae—the innermost tightly compacted, and the outermost more loosely disposed and related to the ectexine granules. In this species, the lamellae clearly show a central area that is less electron-dense and the darker sporopollenin elements on both sides. Endexine II appears as an amorphous more electron-dense layer. Finally, the 5- $\mu\text{m}$ -thick intine has inner microfibrillae that are randomly arranged and outer microfibrillae that form a compact darker irregular layer (Fig. 20). Ubisch bodies are present and associated with the tapetal membrane. They are spherical to irregular in outline with microgranules and microspinules on their surface and a less electron-dense center (Fig. 17).

## Discussion

**Pollen cones. Comparison with living *Araucaria* species.** The mature pollen cones of the two Argentinean species of *Araucaria* can be clearly differentiated. In *A. angustifolia*, pollen cones are longer, more narrow, brown, and the microsporophylls are distally rhomboidal. In *A. araucana*, the cones are shorter, thicker, reddish, and the microsporophylls have a long, obtuse dis-

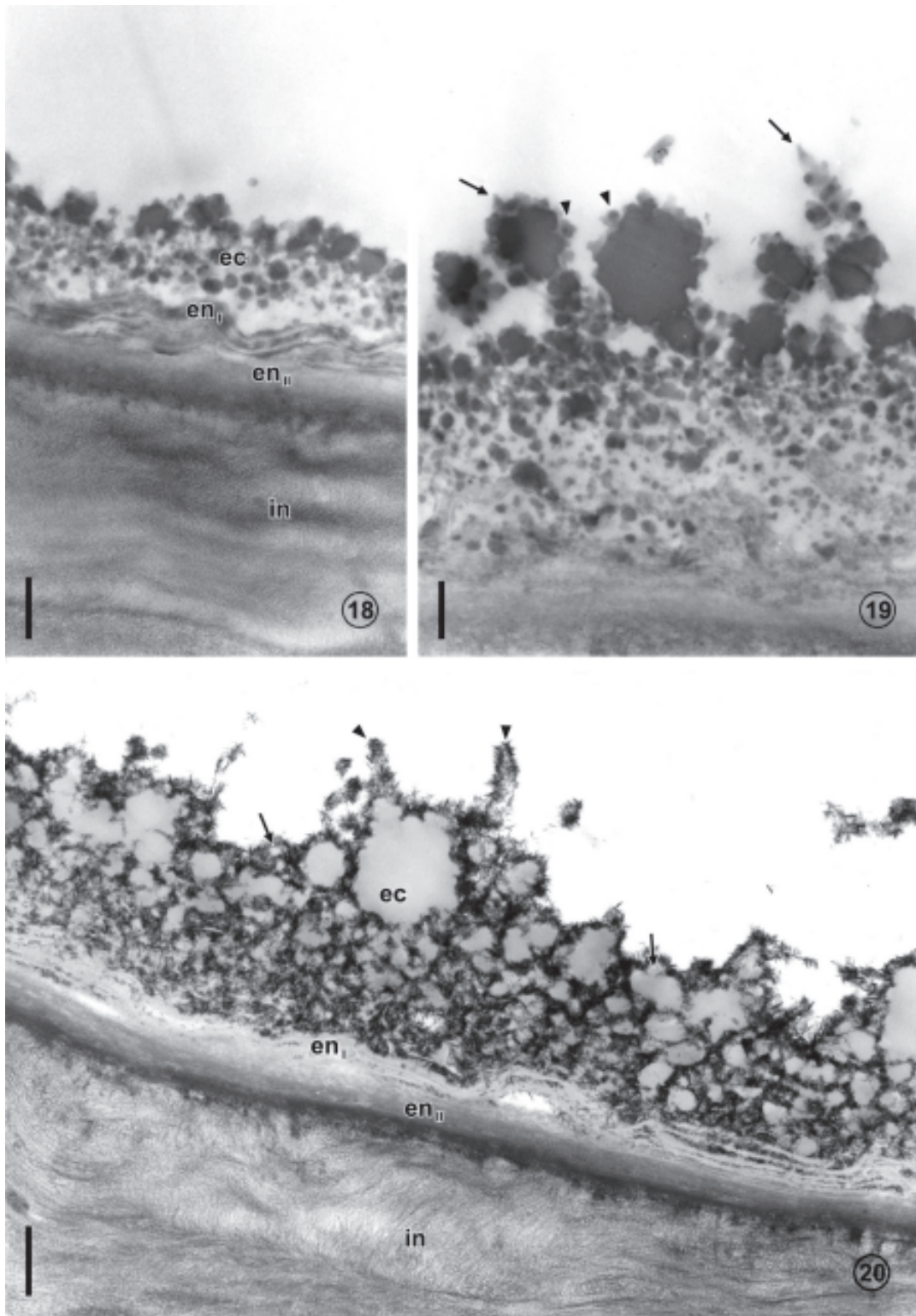
tal apex. Comparisons with pollen cones of other *Araucaria* species were not possible because data on their morphological features is unavailable (Krüsmann, 1985).

**Pollen grain. Comparison with living *Araucaria* species.** The morphology of the pollen grains of the two Argentinean species are similar. However, the pollen grains show some differences with respect to the pollen wall thickness and exine sculpturing. The exine and intine in *A. angustifolia* are thinner than in *A. araucana*, granules are fused to each other forming bigger granules and rugulae in *A. angustifolia* than in *A. araucana*. At an ultrastructural level, we observed a distinct difference in the ectexine: in *A. angustifolia* the granular elements are smaller towards the endexine, are more loosely disposed, and form more interstices than in *A. araucana*. The endexine in both taxa is similar; the only difference is the greater number of lamellae in *A. angustifolia*.

Within the genus *Araucaria*, the pollen grain characters of both Argentinean taxa are consistent with what has been described for other species. *Araucaria laubenfelsii* Corbeson, as illustrated by Dettmann and Jarzen (2000), is similar in size and gross morphology to *A. angustifolia* and *A. araucana*. Both species share with *A. columnaris* Hook. (Cookson and Duigan, 1951) a sexine composed of closely placed granules of 1  $\mu\text{m}$  in diameter, which terminate in short spines. These authors also observed that the sexine can be detached from the lamellate nexine as a complete layer, the same as in *A. araucana*. The sexine detachment seems to be a feature that is shared by most of the *Araucaria* pollen grains studied. This is probably because the sexine granular elements are loosely connected. Also, as it was shown by Courtinat (1987) for *A. excelsa* R. Br., the aggressiveness of the chemical treatment (acetolysis, in our study) produces sexine detachments.

The subequatorial annular area described in *A. angustifolia* and *A. araucana* was better observed in non-acetolyzed material where it can be recognized by a thicker sexine, which mostly appears detached. The presence of this feature was mentioned by Wodehouse (1935) and by Ueno (1959) in non-acetolyzed specimens and illustrated by Heusser (1971, Plate 8-55) in acetolyzed grains of *A. araucana*. More recently, Caccavari (2003, Figs. 3, 1, 5) illustrated the annular thickening in non-acetolyzed grains of *A. angustifolia*. In the opinion of Wodehouse (1935), this subequatorial annular area corresponds morphologically in position to the germinal furrow present in the pollen grains of primitive gymno-





**FIGURES 18-20.** Transmission electron micrographs of the pollen wall. Figs. 18-19. *Araucaria angustifolia*. **Fig. 18.** Exine section showing granulate ectexine (ec), lamellate endexine I (en<sub>I</sub>), amorphous endexine II (en<sub>II</sub>) and thick fibrillate intine (in). Bar: 500 nm. **Fig. 19.** Detail. Note microspines (arrows), microgranules (arrowhead) and a less electron dense amorphous material within granules interstices. Bar: 500 nm. **Fig. 20.** *Araucaria araucana*. Exine section illustrating granulate ectexine (ec), endexine I lamellar (en<sub>I</sub>), endexine II amorphous (en<sub>II</sub>) and fibrillate intine (in). Arrows indicate microgranules and arrowhead microspines. Dark crystals are potassium permanganate precipitate Bar: 500 nm.

sperms like *Cycas*. Wodehouse (1935) also pointed out that the trace of a vestigial furrow in *Araucaria* may be indicative of a grain in which sacchi have been suppressed. This finding does not agree with the idea that the araucarian form may lead to the winged podocarp or pine, as was suggested by Stiles (1908).

**Pollen grain. Comparison with living genera of Araucariaceae.** In comparing the pollen grain of *A. angustifolia* and *A. araucana* with *Agathis* and *Wollemia* of the Araucariaceae, we found that they are similar in gross morphology (inaperturate, nonsaccate, spheroidal, and granulate exine). However, *Agathis* is smaller in average diameter (40-56  $\mu\text{m}$ ; Cookson and Duigan, 1951) and the exine is thicker (3  $\mu\text{m}$ ; Pocknall, 1981). The *Wollemia* pollen grain is considerably smaller in average diameter (15-60  $\mu\text{m}$ ; Chambers *et al.*, 1998) and the exine sculpturing is coarser. However, the exine of *A. angustifolia* under SEM shows a certain resemblance. A feature that is common among the three taxa is the detachment of the sexine; however, in *Wollemia*, the detachment is in irregular patches. In contrast, Chambers *et al.* (1998) and Dettmann and Jarzen (2000) have noted, in dehydrated grains of *Wollemia*, the formation of broad depressions, which are somewhat similar to those in dehydrated material of *A. angustifolia* (Caccavari, 2003). According to this last author, these depressions are involved in the harmomegathy mechanisms during pollination (Blackmore and Barnes, 1986).

Comparison of the pollen wall ultrastructure of the species described here with other araucarian taxa demonstrate that they are slightly different. In *Agathis*, the granular ectexine elements are densely packed leaving few interstices (Van Campo and Lugardon, 1973; Kurmann, 1992). In this respect, *Araucaria araucana* is more closely related to *Agathis* than *A. angustifolia*. The pollen wall of *Wollemia*, which consists of large granules embedded in a matrix of small granules with large interstices between them (Dettmann and Jarzen, 2000), is similar to that of *A. angustifolia*.

Even though additional studies of the pollen grain morphology and ultrastructure of the remaining species of Araucariaceae still need to be conducted, the observations of the Argentinean taxa are consistent with the observations by Setoguchi *et al.* (1998) of molecular phylogeny of the family. In this analysis, the homology of *rbcL* sequences between *A. angustifolia* and *A. araucana* (both belonging to Section *Araucaria* Wilde and Eames) were identical. Wilde and Eames (1952), who also recognized the other three sections of *Araucaria* (*Eutacta*, *Bunya*, and *Intermedia*), and Setoguchi

*et al.* (1998) have concluded that each section forms a monophyletic group. Also, these last authors found that the monophyly of *Agathis* and *Araucaria* is well supported by high bootstrap values of 100% and 89%, respectively, and that *Wollemia* was derived first and then *Agathis* and *Araucaria*.

**Pollen grain. Comparison with other nonsaccate conifers.** *Saxegothaea* is a monospecific genus that has a similar pollen grain morphology to *Araucaria*. The taxonomic affinity of *Saxegothaea* is still uncertain. Taking into consideration the wood anatomy, the seed cone, the pollen grain morphology, and the DNA sequences from *rbcL* gene in plastids, some authors have related *Saxegothaea* to Araucariaceae (Stiles, 1908; Ueno, 1960; Médus *et al.*, 1989; Gamero, 1995), but also to Podocarpaceae (Page, 1990; Price *et al.*, 1993), and a few authors have considered it a monogeneric family (Gaussen, 1974). The pollen of *Saxegothaea* is somewhat different from that of *A. angustifolia* and of *A. araucana* by having a smaller size (34-56  $\mu\text{m}$  in diameter) and 3-4 leptomes (Gamero, 1995). The resemblance among these three taxa can be observed at the level of the exine ultrastructure. The granular elements in *Saxegothaea* are also of different size and shape, irregularly distributed, and form interstices between them. The presence of a foot layer and a tectum-like structure formed by fused granules overlying other granules (Médus *et al.*, 1989) are features that are not present in either *A. angustifolia* or in *A. araucana*.

Pollen grains that are nonsaccate, spheroidal, inaperturate, and granulate also occur in several other conifer genera. The differences with the Argentinean *Araucaria* species differ from other genera in the pollen size and the pollen wall ultrastructure. The ectexine of *Pseudotsuga* and *Larix* of the Pinaceae has a granular layer and a narrow footlayer (Kurmann, 1990). Pollen grains of the Cupressaceae *s. l.* (Gadek *et al.*, 2000; Farjon, 2005) have a small diameter (22-53  $\mu\text{m}$ ), a tectate ectexine (in subfamily Cupressoideae, according to Pocknall, 1981 and Lugardon, 1995), and a footlayer and a germinal zone with a papilla (Heusser, 1971; Ho and Sziklai, 1973; Kurmann, 1990; Uehara and Sahashi, 2000). Taxaceae and Cephalotaxaceae are clearly distinguished by their small diameter: 19-20  $\mu\text{m}$  and 30-33  $\mu\text{m}$ , respectively (Ueno, 1959; Kurmann, 1990).

**Comparison with fossil araucarian pollen grains and pollen cones. Dispersed pollen grains.** Among the fossil pollen grains assigned to Araucariaceae, *A. angustifolia* and *A. araucana* show a more striking

resemblance to *Araucariacites* Cookson ex Couper than to *Dilwynites* Harris, *Balmeiopsis* Archangelsky and *Cyclusphaera* Elsik. The large diameter (40-100  $\mu\text{m}$ ) and fine granulate sculpture with spinae of *Araucariacites* (Dettmann and Jarzen, 2000) are morphological features shared with *Araucaria*. Also, the pollen wall ultrastructure is similar. *Araucariacites* has a mostly granular to vermiform ectexine, where the sporopollenin units are loose in the interior and become more compact towards the surface (Archangelsky, 1994). The differences are more marked with *Dilwynites*; even though it has the same pollen morphology, it differs in the thin granular ectexine sculpturing, which consists of less crowded verrucae that are irregularly distributed and overlay a thick endexine (Dettmann and Jarzen, 2000). *Balmeiopsis* and *Cyclusphaera* are both discoidal with a granulate sculpture, but the features that distinguish them from the *Araucaria* species are a well developed equatorial sexine thickening with one polar aperture in *Balmeiopsis* (Archangelsky, 1977) and with two polar apertures in *Cyclusphaera* (Elsik, 1966). In addition, observation with TEM of the exine stratification of these two fossil taxa, showed that the ectexine is composed of granular to elongate-vermicular sporopollenin units that are compact in the apertural side and with few spaces in the non-aperturate side (Archangelsky, 1994; Del Fueyo and Archangelsky, 2005).

Our results are consistent with what it is widely accepted; *Araucaria* and *Agathis* are similar to fossil *sporae dispersae* referred to *Araucariacites*, whereas *Dilwynites* is closely related to *Wollemia* (Chambers *et al.*, 1998; Dettmann and Jarzen, 2000; Cantrill and Raine, 2006), and *Balmeiopsis* along with *Cyclusphaera* have no extant representatives (Archangelsky, 1977; Elsik, 1966).

**Pollen cones.** Araucarian fossil pollen cones with pollen grains preserved *in situ* have been found in several Mesozoic localities. Notably, the size of these pollen cones are smaller than those seen on living species (Sporne, 1965). Archangelsky and Gamero (1967) found *Balmeiopsis* in cones (7 mm long, 2 mm wide) organically connected to *Brachyphyllum irregulare* Archang from the Lower Cretaceous strata of Patagonia.

*Araucariacites* has been identified in pollen cones (12 mm long, 6 mm wide) attached to *Brachyphyllum mamillare* Lindley and Hutton (Kendall, 1949) from the Jurassic of Yorkshire. This pollen type was also recovered from cones (2 mm long, 1.8 mm wide) organically attached to *Nothopheuen brevis* (Del Fueyo, 1991) from the Early Cretaceous of Patagonia.

*Alkastrobis peltatus* was recently created based on araucarian pollen cones (10 mm long, 5 mm wide) of the same age and provenance, containing *Cyclusphaera* (Del Fueyo and Archangelsky, 2005). Fossilized *Wollemia*-like branches have been described with associated pollen cones (40 mm long, 10 mm wide) from the Cenomanian of Australia (Chambers *et al.*, 1998). Pollen grains have not been found *in situ*, but the authors believe that these remains are comparable to disintegrating *Wollemia* cones.

The araucarian pollen grain records (as *sporae dispersae* and *in situ* remains) demonstrate that the Araucariaceae were more diversified, especially in Patagonia during the Early Cretaceous. *Balmeiopsis*-type and *Cyclusphaera*-type pollen became extinct while *Araucariacites*-type was the only one that survived and is represented by the two extant forms *Araucaria angustifolia* and *Araucaria araucana*.

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