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The ultrastructure of *in situ* araucarian pollen from the male cone *Callialastrobus sousai*, Lower Cretaceous of Catefica, Lusitanian Basin, western Portugal

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ABSTRACT

Pollen morphology and ultrastructure are described for fossil pollen of the *Araucariacites* and *Callialasporites* types extracted from a *Callialastrobus sousai* pollen cone previously reported from the Lower Cretaceous Almargem Formation near the village of Catefica, in the Estremadura region, western Portugal. Pollen grains were studied with transmitted light and scanning and transmission electron microscopy. The pollen grains are medium-sized, being smaller and with a thicker exine in the *Callialasporites* type. Both pollen types appear inaperturate, although both polar regions are represented by a thinner exine. The exine sculpture (granulate/microechinate) and ultrastructure (granular sexine and lamellate/homogeneous endexine) are generally the same in *Araucariacites*- and *Callialasporites*-type grains. Saccus-like structures in the *Callialasporites*-type pollen are formed by loosely arranged endexine lamellae, while *Araucariacites*-type pollen is asaccate. The exine structure of the *Araucariacites*- and *Callialasporites*-type grains also agrees well with that of known araucariaceous pollen.

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1. Introduction

Araucariaceae are an important family of ancient conifers that had an extensive distribution in both hemispheres during the Mesozoic. According to the fossil record, the group first appeared in the Late Triassic, proliferated during the Jurassic and Early Cretaceous, and gradually declined in number of taxa and geographic distribution from the Late Cretaceous to the present (Stockey, 1982, 1994; Stockey and Ko, 1986; Vakhrameev, 1991; Hill, 1995; Del Fueyo and Archangelsky, 2002; Kunzmann, 2007a, 2007b; Axsmith et al., 2008). Currently, members of the Araucariaceae are confined to South America and Australasia, comprising the genera *Araucaria* Jussieu and *Agathis* Salisbury (known since the eighteenth and nineteenth centuries, respectively) and the monotypic genus *Wollemia* W.G.Jones, K.D.Hill et J.M.Allen, more recently discovered in New South Wales, Australia (Jones et al., 1995; Del Fueyo et al., 2008; Farjon, 2008; Taylor et al., 2009; Escapa and Catalano, 2013).

Several palynological studies have documented the common occurrence of araucarian pollen grains assigned to the genera *Araucariacites* Cookson and *Callialasporites* Sukh Dev in Early Cretaceous palynofloras from the western Portuguese Basin (Groot and Groot, 1962; Mohr, 1989; Trincão, 1990; Heimhofer et al., 2008; Mendes et al., 2011, 2014, 2018, 2019, 2022), emphasizing the importance of Araucariaceae in communities of this region.

Recently, Kvaček et Mendes (2020) described a new araucarian pollen cone, *Callialastrobus sousai* J.Kvaček et Mendes, that produced both *Araucariacites*- and *Callialasporites*-type pollen grains based on material from the late Aptian-early Albian Catefica mesofossil flora in the Lusitanian Basin, western Portugal. This discovery unequivocally confirmed the araucarian origin of the pollen cone consisting of about 30 microsporophylls, each bearing three pollen sacs containing pollen of *Araucariacites*- and *Callialasporites*-type.

Five pollen fossil-genera have been proposed to have affinities with Araucariaceae: *Araucariacites*, *Cyclusphaera* Elsik, *Balmeiopsis* Archangelsky (= *Inaperturopollenites limbatus* Balme), *Callialasporites* (= *Zonalapollenites dampieri* Balme, *Z. trilobatus* Balme, *Applanopsis* Döring), and *Dilwynites* W.K. Harris. The differences among the genera concern pollen size, exine sculpture, aperture condition, presence/

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absence of equatorial thickening, and saccate/asaccate condition (Archangelsky, 1977, 1994; Chambers et al., 1998; Del Fueyo et al., 2008, 2012; Dettmann and Jarzen, 2000). The exine ultrastructure has been studied in a number of dispersed pollen taxa (Kedves and Pardutz, 1974; Taylor et al., 1987; Zavada, 1992; Archangelsky, 1994; Batten and Dutta, 1997; Del Fueyo et al., 2012; Dettmann and Jarzen, 2000) and in pollen found *in situ* in araucarian cones (Del Fueyo, 1991; Del Fueyo and Archangelsky, 2005); the *in situ* data have until now included only pollen corresponding to *Cyclusphaera* and (with immature pollen) to *Araucariacites*.

In this paper we describe the pollen morphology and ultrastructure of pollen grains from a pollen cone of *Callialastrobus sousai* and make comparisons with previously published ultrastructural research.

2. Material and methods

The fossil material documented here was collected by M.M. Mendes in a road cut between the villages of Catefica and Mugideira, about 4 km south of Torres Vedras, in the Estremadura region of western Portugal (39° 3' 16" N; 09° 14' 24" W). The pollen cone was extracted from a dark gray mudstone layer rich in plant remains, belonging to the Almargem Formation (Rey, 1993), previously assigned to the "Grès de Torres Vedras" unit (Carta Geológica de Portugal, Folha 30-D Alenquer; Zbyszewski and Assunção, 1965), considered to be late Aptian–early Albian in age (Fig. 1). The exact stratigraphic position of the Catefica deposits is not certain. However, some authors have argued that Catefica locality can be dated more precisely as late early Albian, based on comparison with pollen in better-dated coastal marine sequences (Heimhofer et al., 2007; Doyle and Endress, 2014; Tanrikulu et al., 2018). For a more detailed account of the geological context and stratigraphy of the Catefica locality, see Mendes et al. (2017) and Kvaček and Mendes (2020).

The sedimentary rocks exposed at the Catefica locality are rich in plant mesofossils. The mesofossil flora is clearly dominated by angiosperms, represented by flowers, fruits, and seeds, but it also includes many twigs and seeds of coniferous plants, selaginellaceous shoots and megaspores, and numerous fragments of thalloid liverworts.

A single pollen cone (specimen P0655) with *in situ* pollen grains was extracted from the rock sample (Catefica sample 360) by sieving in water through a 125 µm net mesh, followed by treatment with hydrofluoric (40% HF) and hydrochloric (10% HCl) acids and thorough rinsing in water, using standard methods previously described in earlier

studies (Friis et al. 1988, 2009). The fossil specimen was mounted on an aluminum stub, coated with gold for 60 s in a sputter coater, and the *in situ* pollen grains were examined under a Hitachi S-3700 N SEM at 5 kV, at the National Museum, Prague.

A part of the pollen cone was carefully removed with a scalpel and used for observations of the pollen. Glycerin slides of individual pollen grains were prepared, and these grains were photographed with a Carl Zeiss Axioplan 2 light microscope equipped with a × 100 oil immersion objective and an AxioCam 105 digital camera. Individual pollen grains and pollen clumps were washed in ethanol and transferred to a piece of photographic film for SEM studies. The film was glued to an aluminum stub with nail polish and sputter-coated with gold for 8 min. The pollen grains were observed and photographed with a Tescan Vega3 XMU SEM with an accelerating voltage of 10 and 20 kV at the A. A. Borissiak Paleontological Institute, Russian Academy of Sciences.

For TEM analyses, individual pollen grains were removed from the SEM stub and embedded in epoxy resin in the proportions described by Zavalova et al. (2018). The pollen grains were sectioned with a Leica EM UC6 ultramicrotome equipped with a diamond knife. The ultrathin sections were not stained, as they already showed satisfactory contrast with the microscope used. The sections were examined with a Jeol 1011 TEM with an accelerating voltage of 80 kV at the Laboratory of Electronic Microscopy, Faculty of Biology, Lomonosov Moscow State University. The Jeol 1011 TEM was equipped with a side-mounted Orius SC1000W digital camera (11 megapixels, effective 8.5 megapixels); Digital-Micrograph version 2.0 (Gatan) software was used for image processing.

Remnants of polymerized resins with embedded fossil pollen grains, grids with ultrathin sections, and digital photos are kept at the Laboratory of Paleobotany, A. A. Borissiak Paleontological Institute of the Russian Academy of Sciences (PIN), in Moscow. The araucariaceous male cone with *in situ* pollen grains used for the present study is housed in the palaeobotanical collections of the Geological Museum of Lisbon, Portugal (P).

3. Results

Two types of pollen grains were recognized (Plates I–VI). Most grains belong to Pollen type I (Plates I, II, IV, V), while four grains are included in Pollen type II (Plates III, VI). The exine surface and ultrastructure of the two pollen types are generally the same. Of type I, 26 pollen grains were studied under LM, 22 with SEM, and four with TEM. Four

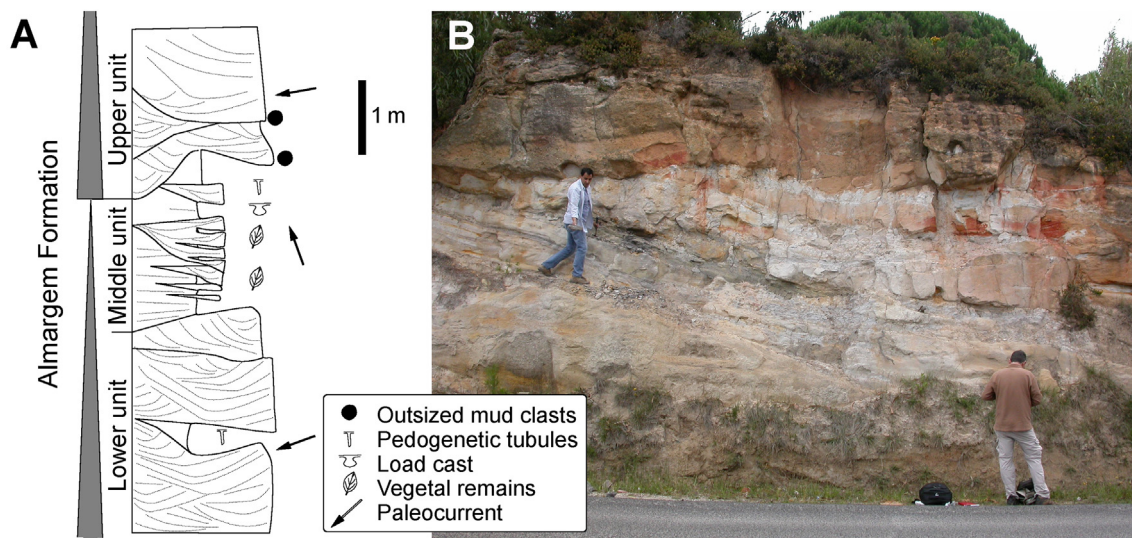


Fig. 1. The Catefica locality. (A) Stratigraphic log. (B) General view of Catefica outcrop section showing fine-grained organic-rich fossil beds between sandstones.

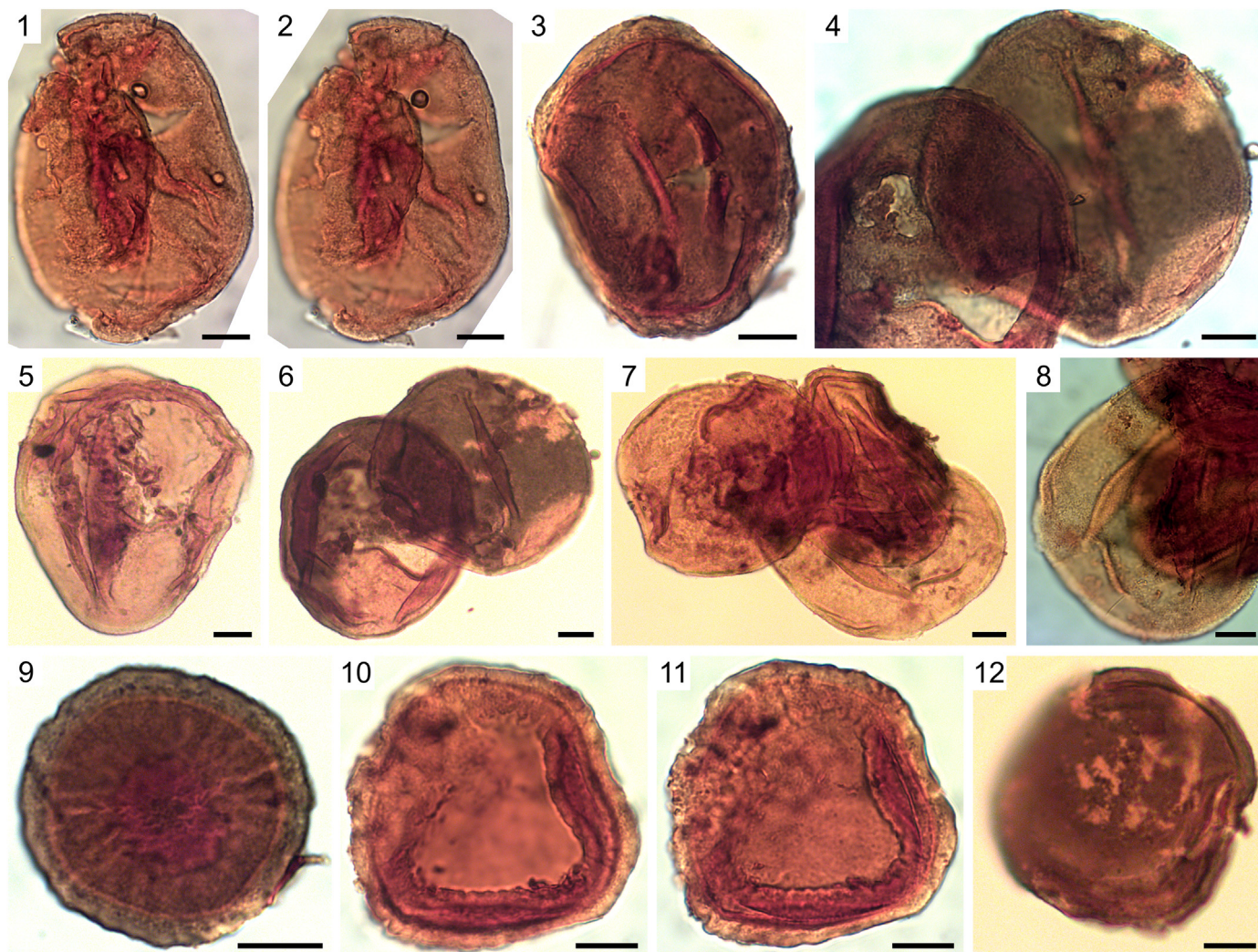


Plate I. *Callialastrobus sousai*, LM. 1–8. Pollen type I (= *Araucariacites* type). 1, 2. Same pollen, different focal levels. 4. Close-up of pollen in 6. 9–12. Pollen type II (= *Callialasporites* type). 10, 11. Same pollen, different focal levels. Scale bars: 10 µm.

pollen grains of type II were studied with LM and SEM, and three of these were studied with TEM.

3.1. Pollen type I (Plate I, 1–8, Plate II, Plate IV, Plate V)

LM. Pollen grains are spheroidal, asaccate, often slightly crumpled, $67.2 (61.7\text{--}80.0) \mu\text{m} \times 55.3 (42.5\text{--}65.0) \mu\text{m}$. The pollen grains appear inaperturate. The exine surface is granulate; the pollen wall is $2.4 (2\text{--}3) \mu\text{m}$ thick, two-layered, with an outer layer two or three times thicker than the inner one; the exine thickness appears to differ a bit around the pollen perimeter (Plate I, 1–8).

SEM. Pollen grains are $64 (56.7\text{--}72.2) \mu\text{m} \times 56 (37.1\text{--}70.5) \mu\text{m}$, microechinate (Plate II, 1–5). The sculpture elements are densely distributed, conical, $0.55 (0.31\text{--}0.72) \mu\text{m}$ high (Plate IV, 1–6). In the presumed equatorial area where the exine (according to LM) is often slightly thicker, folds are observed (Plate II, 2–4, Plate IV, 1, 4).

TEM. The exine consists of a granular layer (ectexine), a lamellate layer (endexine I), and an innermost homogeneous layer (endexine II) (Plate V, 1–6). The layers interpreted as ectexine and endexine I are of the same electron density; the innermost layer is more electron dense. The exine is slightly thicker in the presumed equatorial area, with the ectexine $1.31 (0.91\text{--}2.0) \mu\text{m}$ and endexine $1.52 (1.08\text{--}2.55) \mu\text{m}$ thick; in the polar areas the ectexine is $0.57 (0.43\text{--}0.78) \mu\text{m}$ thick, and the endexine is $0.87 (0.67\text{--}1.08) \mu\text{m}$ thick (Plate V, 1). The ectexine consists of

differently sized small granules; towards the outside they are more densely compacted, forming tectum-like layer; sometimes the ectexine elements appear oval or rod-like in ultrathin section in this area (Plate V, 2, 4, 6). In the polar areas, the ectexine is mostly composed of this “tectal” layer (Plate V, 2, 3, 6); both polar areas are characterized by thinner ectexine than that in the equatorial area. One of the polar areas appears thinner than the other; however, the difference in the thickness is not considerable. The endexine I is lamellated, $1.31 (0.86\text{--}1.44) \mu\text{m}$ thick in the equatorial area, and about $0.5\text{--}0.6 \mu\text{m}$ in the polar ones. Lamellae differ in thickness, with the outer ones being thicker; 8–10 lamellae can be distinguished within the endexine I (Plate V, 5). The lamellae often become loosely spaced in the equatorial area and tightly adpressed at the poles (Plate V, 2–6). The endexine II is irregular in thickness, homogeneous, $0.3 (0.05\text{--}0.54) \mu\text{m}$; it can be uniformly thin or form small discrete thickenings (Plate V, 2, 4, 6).

3.2. Pollen type II (Plate I, 9–12, Plate III, Plate VI)

LM. Pollen grains are spheroidal and keep their shape well, in contrast to Pollen type I; also, this type seems to have a thicker exine than Pollen type I (Plate I, 9–12). The pollen grains appear inaperturate. The pollen size is $48.8 (35.5\text{--}56.0) \mu\text{m} \times 44.4 (35.5\text{--}51.0) \mu\text{m}$. The exine surface is granulate; the pollen wall is $3.9 (3\text{--}5) \mu\text{m}$ thick, two-layered, with an outer layer three or four times thicker than the inner one.

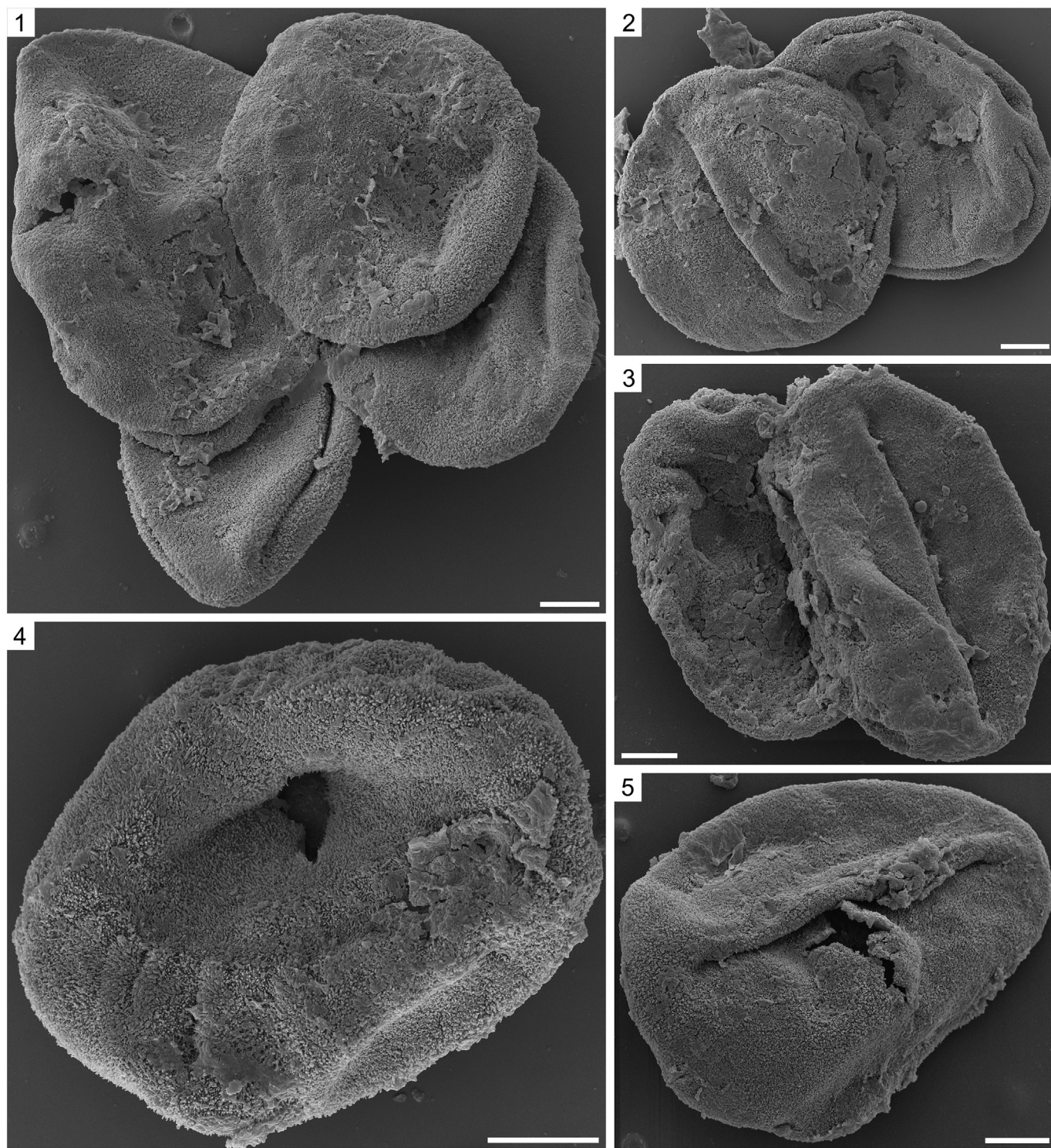


Plate II. *Callialastrobus sousai*, SEM, pollen type I (= *Araucariacites* type), general view. 1–3. Several pollen grains. 4, 5. Polar view, exine folds are seen in 4. Scale bars: 10 μm .

SEM. Pollen grains are 46.4 (35.7 – 54.4) $\mu\text{m} \times 42.5$ (34.0 – 48.5) μm , microechinate (Plate III, 1–6). The sculpture elements are densely distributed, conical, 0.55 (0.31 – 0.72) μm high. In the presumed equatorial area, an encircling band and folds are observed (Plate III, 1–5).

TEM. The exine stratification and layer structure are nearly the same as in Pollen type I. The differences are in the thicker ect- and endexine, probably a better-expressed “tectal” area, and smaller

ectexinal granules (Plate VI, 1–6). Regions with extremely loosely spaced lamellae of endexine I occasionally occur (Plate VI, 1, 4, 5). The ectexine in the equatorial area is 1.41 (1.0 – 1.85) μm thick, in the polar area, 0.93 (0.71 – 1.07) μm . The endexine is 1.7 (1.35 – 1.93) μm thick in the equatorial area, and about 1.21 μm in the polar one. The endexine I is 1.25 (0.8 – 1.46) μm thick in the equatorial area, and about 0.71 μm thick in the polar one. The endexine II is 0.5 (0.36 – 0.74) μm thick.

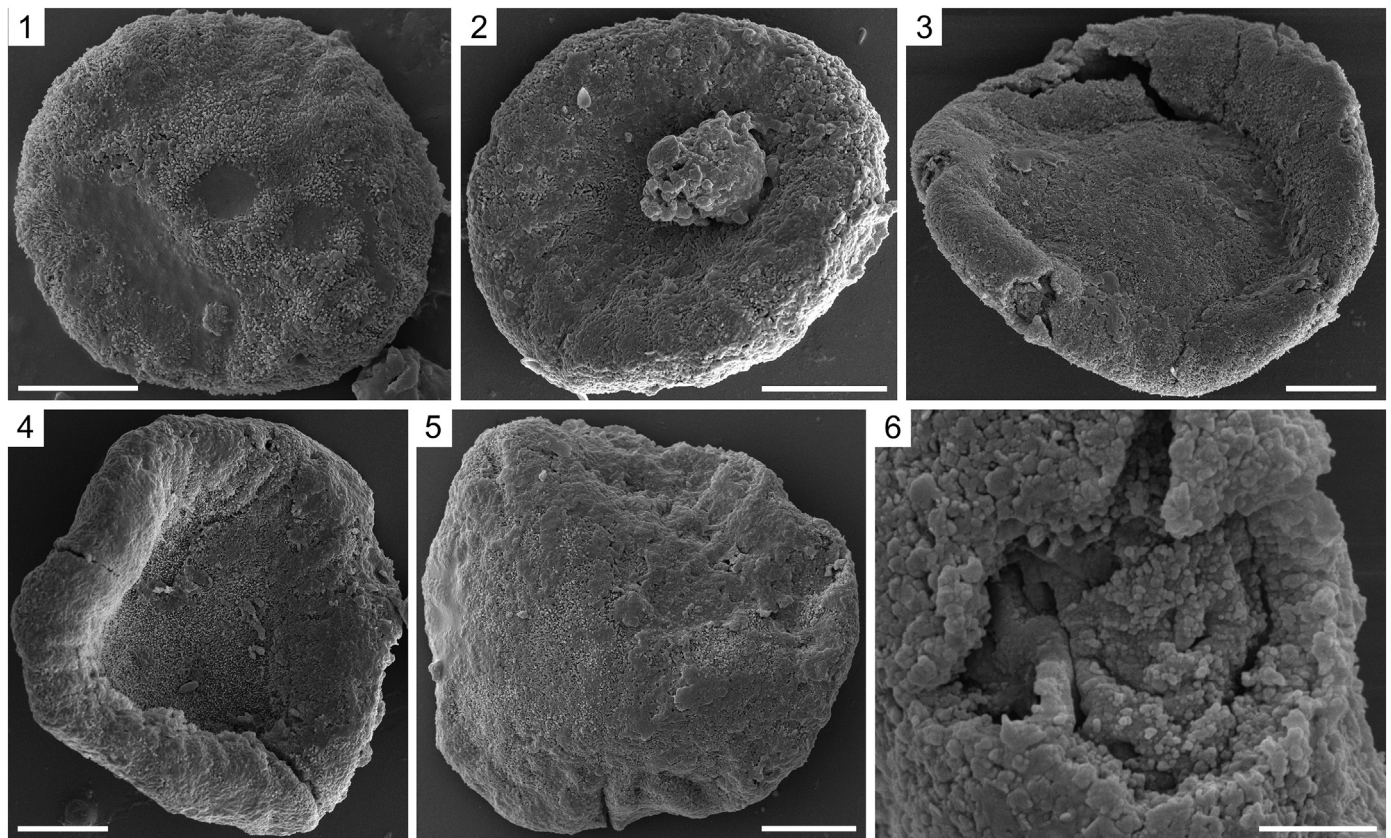


Plate III. *Callialastrobus sousai*, SEM, pollen type II (= *Callialasporites* type). 1–5. General view. 1, 2. Same pollen, opposite polar views. 4, 5. Same pollen, opposite polar views. 6. Close-up of a pollen grain with an exine rupture, showing endexine. Scale bars: 10 µm (1–5), 2 µm (6).

4. Discussion

4.1. *In situ* araucarian pollen with a focus on pollen cones containing more than one pollen type

So far, quite a few araucarian male cones with *in situ* pollen are known (e.g., see review in Balme, 1995; Del Fueyo and Archangelsky, 2005; Kvaček et al., 2018; Kvaček and Mendes, 2020), with the majority of them from Jurassic and Lower Cretaceous deposits. Most of these *in situ* pollen grains correspond to the *Araucariacites* type.

In situ *Cylusphaera*-type pollen grains measuring 50–58 µm are known from *Alkastrobos peltatus* Del Fueyo et Archangelsky from the Lower Cretaceous (Aptian) of Argentina (Del Fueyo and Archangelsky, 2005).

Balmeiopsis-type pollen measuring 55–92 µm was found in *Brachyphyllum irregulare* Archangelsky from the Lower Cretaceous of Argentina (Archangelsky, 1977; Archangelsky and Gamarro, 1967).

Pollen grains of the *Callialasporites* type measuring 50–70 µm with three rudimentary sacchi were observed in *Masculostrobus dorchensis* Barale from the Jurassic of France (Barale, 1981).

Leslie et al. (2009) described the pollen from *Upatoia barnardii* Leslie, Herendeen et Crane from the Upper Cretaceous (Santonian) of Georgia, USA. The *in situ* pollen grains are 52–75 µm in diameter; the exine is up to 3.5 µm thick and often strongly folded around the equator, giving the grain a characteristic wavy outline. There is no clear aperture, but the polar exine thins to 0.7 µm. The authors did not relate their pollen to any formal pollen taxon, but it is similar to *Araucariacites* or *Callialasporites* grains with a poorly developed monosaccus. The variation in the pollen morphology in *Upatoia barnardii* appears quite similar to that in *Callialastrobus sousai*.

Several other taxa show more than one pollen type within the same pollen cone, as in *Callialastrobus sousai*. Male cones attached to or associated with *Brachyphyllum mamillare* Brongniart from the Middle Jurassic of England were reported to contain pollen grains of two types (59% versus 41%) (van Konijnenburg-van Cittert, 1971). Both pollen types are of the same size (about 70 µm); according to the description, *Callialasporites*-type pollen grains show some variation in the degree of “saccus” development. Those pollen types resemble the pollen grains under study, although the percentage of the *Callialasporites* type is lower in our material, and the *Callialasporites* type is usually smaller than the *Araucariacites* type, in contrast to the nearly uniform size of both pollen types in the material of van Konijnenburg-van Cittert (1971).

Raab et al. (1986) described dispersed *Araucariacites australis* Cookson (about 90%) and *Callialasporites trilobatus* Balme (about 10%) in beds containing shoots of *Brachyphyllum lorchii* Raab, Horowitz et Conway from the Upper Jurassic of Israel; the *Araucariacites* pollen grains are about 80 µm in size versus about 70–75 µm for the *Callialasporites* pollen.

Brachyphyllum mirandai Archangelsky from the Lower Cretaceous (Aptian) of Argentina was found associated with male cones of morphology similar to that of *Callialastrobus* (Archangelsky, 1963). Their anatomy, e.g., the number of pollen sacs, is not completely known, but they also contain two types of pollen grains: dominant grains of the *Araucariacites* type and rare pollen grains comparable to *Callialasporites* (Archangelsky, 1963).

Pollen grains of the *Araucariacites* type measuring 61 (54–68) µm in diameter were observed in male cones *Masculostrobus graiterensis* Allenbach et van Konijnenburg-van Cittert from the Jurassic (middle to upper Oxfordian) of the Jura Mountains, Switzerland (Altenbach

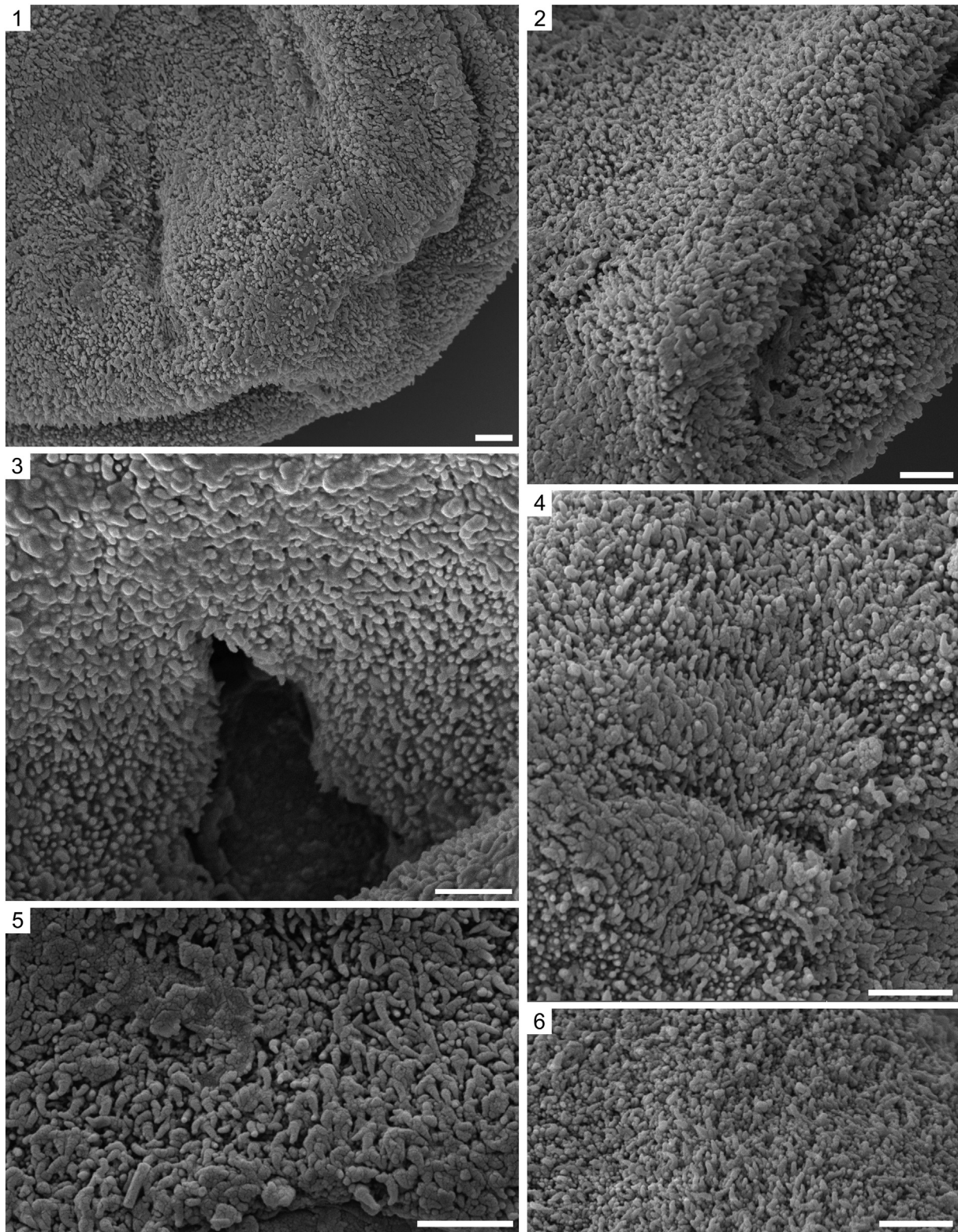
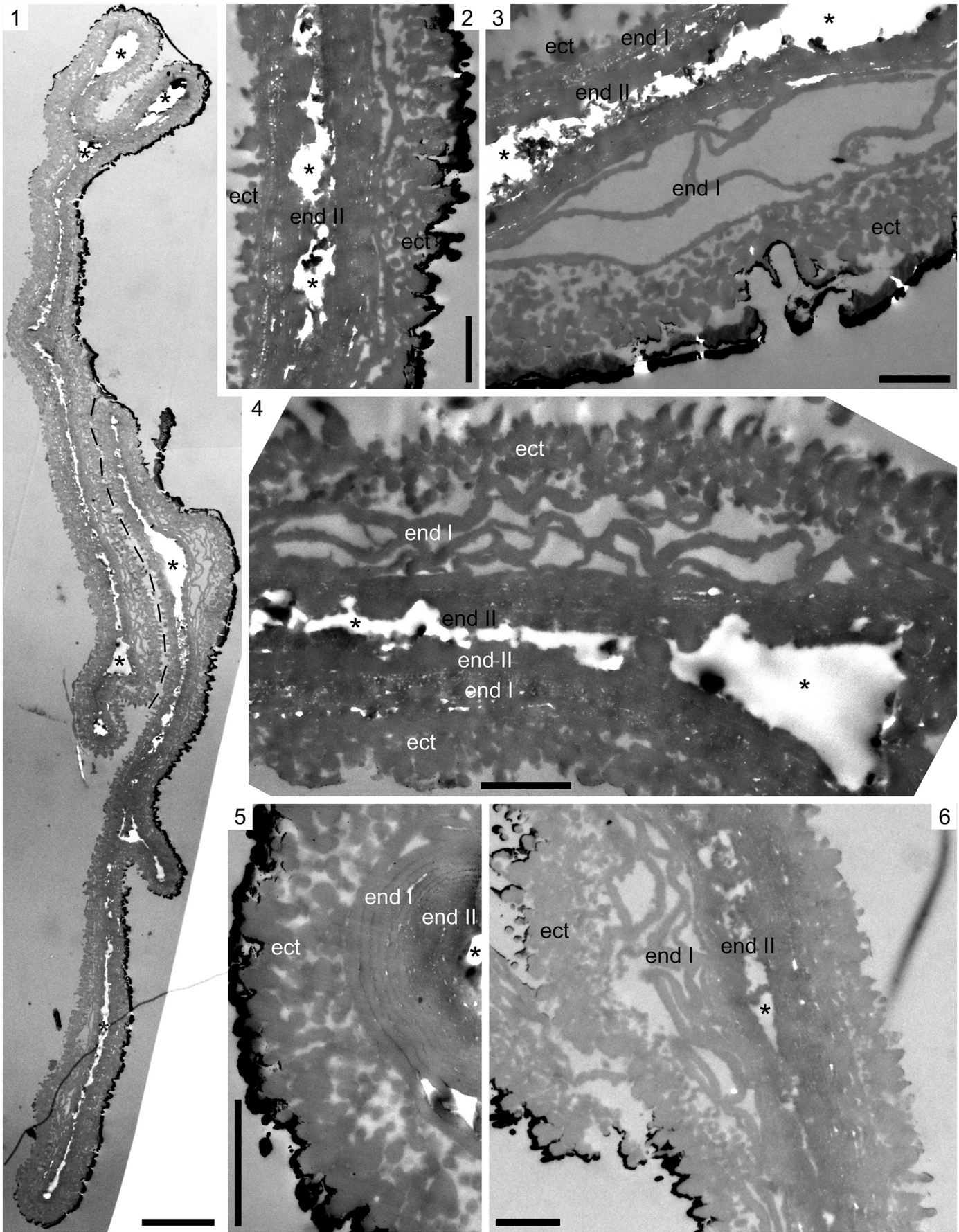


Plate IV. *Callialastrobus sousai*, SEM, pollen type 1 (= *Araucariacites* type), close-ups, microechinate sculpture. 1, 2, 4. Equatorial area; 1, 4: exine folds are seen. 3, 5, 6. Polar area. Scale bars: 2 μm .

and Van Konijnenburg-van Cittert, 1997); however, some pollen grains showed an “inner cavity” resulting from separation of the nexine from the sexine (the authors assigned this pollen type to *Callialasporites*, and such pollen variability is similar to what we observed in this study).

Apterocladus lanceolatus Archangelsky from the Lower Cretaceous (Aptian) of Argentina bears pollen grains with three incipient sacchi, like grains of *Callialasporites dampieri* and *Callialasporites trilobatus*; some pollen cones show smaller pollen with poorly delimited sacchi, probably representing immature grains (Gamerro, 1965; Archangelsky, 1966).



The pollen size is about 40–64 µm. *Apterocladus lanceolatus* was assigned to Podocarpaceae based on its leaf morphology and cuticular structure. However, the known exine ultrastructure of dispersed *Callialasporites* pollen shows a probable araucariaceous affinity. Most pollen grains of Podocarpaceae have quite different exine ultrastructure (alveolate) and saccus morphology. Nevertheless, extant *Saxegothaea* Lindl. is characterized by asaccate pollen with a granular ectexine and lamellate endexine (Médus et al., 1989).

For modern *Araucaria*, van Konijnenburg-van Cittert (1971), Courtinat (1987), and Del Fueyo et al. (2008) reported some variation in pollen of the same species. Van Konijnenburg-van Cittert (1971) studied pollen grains of *Araucaria araucana* (Molina) K.Koch in LM and found that in a considerable number of pollen grains the nexine is shrunken to various degrees; she therefore suggested that there might be a trend to a separation of the nexine from the sexine in Araucariaceae, such that the pollen grains appear monosaccate. Del Fueyo et al. (2008) observed exine depressions and detachments of the sexine from the nexine in the studied species; the detachments are reflected in small cavities between the sexine and nexine. Del Fueyo et al. (2008) also reported a loose organization of both the outer lamellae of endexine I and the inner sexine granules. Courtinat (1987) showed pollen morphology similar to that of the *Araucariacites* and *Callialasporites* types in the pollen cone of the same species, *Araucaria excelsa* (Lamb.) W.T. Aiton. He also showed that apparent variation in pollen morphology (exine detachments, depressions, cavities) increased after chemical treatment. Exine detachments have also been observed in modern *Wollemia* pollen (Chambers et al., 1998).

Accumulating data show that some araucarian taxa are characterized by a continuous range of pollen variation, with the extreme variants assignable to the *Araucariacites* and *Callialasporites* morphotypes, whereas others are known to yield either *Cyclusphaera*- or *Balmeiopsis*-type pollen. The two latter pollen types seem to show a lower level of variability. We suppose that it is the presence of more rigid structural features, such as clearly delimited aperture(s) and distinct equatorial thickenings, that may tend to prevent morphological modifications in such pollen. It appears that the presence of two pollen types or some variability in the pollen morphology may be a common phenomenon in araucarian taxa with pollen of the *Araucariacites* and *Callialasporites* types. Additional studies of *in situ* pollen grains are needed, with particular focus on their variability.

4.2. Interpretation of the studied pollen structure

Most of the studied pollen grains (type I) resemble the pollen fossil-genus *Araucariacites* in pollen size, wall thickness, and absence of a clear aperture. Four pollen grains (type II) are smaller, with a thicker exine and a well-defined spheroidal shape; these grains correspond to the pollen fossil-genus *Callialasporites*, although we observed no pronounced sacci. In the ultrathin sections there are regions of local thickening of the exine, which Del Fueyo et al. (2012) described as “a labile connexion” between the ectexine and endexine I, mostly because of the loose organization of lamellae of the endexine I (Plate VI, 1, 4, 5), and no cavity between the exine layers was observed.

The exine sculpture appears uniform throughout the pollen surface. Often there are encircling bands and/or folds in the presumed equatorial area. These bands likely result from the difference in ectexine thickness in the equatorial and polar areas. The ectexine is thinner in both polar areas; however, in one area the ectexine is usually slightly thinner than in the other. Thus, the aperture is not visible in LM and SEM and

is not expressed in morphology, but ultrastructure clearly reveals it as a rather large, rounded area at one pole.

The ectexine and endexine I have the same electron density, but we interpreted them as such because of the lamellate structure of the endexine I and for consistency with the usage of researchers who previously studied the exine ultrastructure of araucarian pollen. In contrast to previously studied material, pollen grains of *Callialastrobus sousai* have endexine lamellae of differing thickness; the outer lamellae are thicker, and thickness gradually decreases in the inner ones. The outermost lamellae also appear thicker when they are loosely organized.

4.3. Comparison with the morphology and exine ultrastructure of previously studied araucarian pollen

Pollen grains of *Araucariacites*, *Callialasporites*, and *Dilwynites* appear to be inaperturate, but *Balmeiopsis* was described as having one polar aperture, and *Cyclusphaera* is characterized by two polar apertures (Archangelsky, 1994; Dettmann and Jarzen, 2000; Del Fueyo et al., 2008, 2012). There is an equatorial thickening in *Cyclusphaera*, *Balmeiopsis*, and *Callialasporites*, in contrast to *Araucariacites* and *Dilwynites*. Pollen grains of extant Araucariaceae (*Araucaria*, *Agathis*, and *Wollemia*) are of the *Araucariacites* type, whereas *Wollemia* pollen is more like *Dilwynites* in its exine thickness and sculpture. The exine sculpture is granular, microverrucate or microechinate, with the sculpture elements being somewhat coarser in *Araucariacites* (Archangelsky, 1977; Chambers et al., 1998). *Dilwynites* differs from *Araucariacites* in having more variable sculpture and a thicker exine (Chambers et al., 1998).

Exine ultrastructure has been studied for all five fossil-genera on the basis of *in situ* or dispersed pollen. Most authors who have studied araucarian pollen used the terms “sexine” and “nexine” in describing the sporoderm ultrastructure; here we have followed their terminology in discussing earlier works, despite the fact that these terms have often been applied for light microscopy only. Below we consider only studies that described exine ultrastructure.

Araucariacites has been studied as dispersed (Kedves and Pardutz, 1974; Zavada, 1992; Archangelsky, 1994; Batten and Dutta, 1997) and *in situ* (Del Fueyo, 1991) pollen. It is characterized by a rather thin exine, with a granular sexine and a two-layered nexine. The outer nexine layer is lamellate, whereas the inner one is usually more electron dense and homogeneous. Archangelsky (1994) and Zavada (1992) reported a somewhat thinner exine in the polar region, but Batten and Dutta (1997) did not mention any thickening or thinning in the pollen wall, and Del Fueyo (1991) studied immature pollen in which there were no clear differences or stratification in the exine. Zavada (1992) described *Araucariacites* as having a tectum, whereas Batten and Dutta (1997) reported larger granules towards the outer part of the sexine, and Archangelsky (1994) also indicated that the sexine elements became more compact in the outer part. Sometimes ill-defined columellae or irregularly shaped rods were observed (Zavada, 1992; Archangelsky, 1994). Thus, previous data on sporoderm ultrastructure of *Araucariacites* correspond well with our results.

Balmeiopsis (Kedves and Pardutz, 1974; Zavada, 1992; Archangelsky, 1994) has been studied as dispersed pollen only. Archangelsky (1994) revealed a spongy sexine, greatly thickened in the equatorial area, and a two-layered nexine, with a lamellate outer layer and thin, more electron dense inner layer. No rods or columella-like elements were observed. Zavada (1992) found no equatorial thickening and no aperture in *Balmeiopsis* pollen; however, for this reason it is unclear why he

Plate V. *Callialastrobus sousai*, TEM, pollen type I (= *Araucariacites* type). 1. Two pollen grains, section of the whole grain, showing thinner polar and thicker equatorial areas. 2–4. 6. Part of the exine, with polar (thinner) and equatorial (thicker) areas on the same photo. 3, 4, 6. Loosely organized lamellae are seen. 5. Part of the exine in the equatorial area, showing lamellae of differing thickness, with the outermost being the thickest. ect – ectexine, end I – endexine I, end II – endexine II; asterisk indicates gametophyte cavity, dashed line indicates the border between the two pollen grains. 1–4. Sections of pollen figured in Plate I, 4, 6, Plate II, 2, Plate IV, 1, 5. Section of pollen figured in Plate I, 3, Plate II, 4, Plate IV, 3, 4, 6. Section of pollen figured in Plate I, 1, 2, Plate IV, 5. Scale bars: 5 µm (1), 1 µm (2–6).

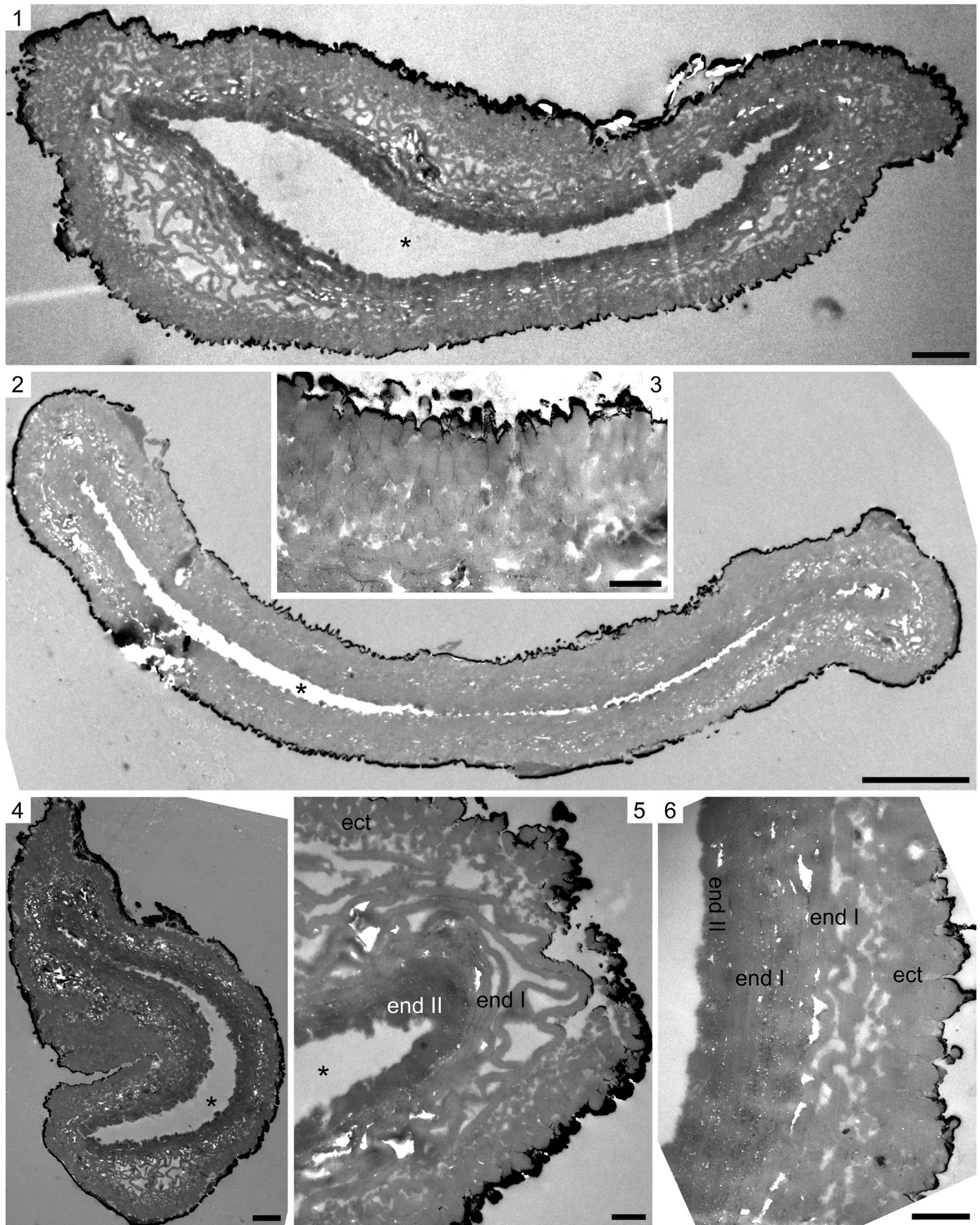


Plate VI. *Callialastrobus sousai*, TEM, Pollen type II (= *Callialasporites* type). 1, 2, 4. Sections of whole pollen grain; section in 4 is somewhat oblique. 3. Ectexine with infractectal elements that appear to be rod-like. 5. Part of exine in equatorial area. 6. Part of exine in polar area. 1, 5, 6. ect – ectexine, end I – endexine I, end II – endexine II; asterisk indicates gametophyte cavity. Sections of pollen figured in Plate I, 12, Plate III, 3, 6. 2, 4. Sections of pollen figured in Plate I, 10, 11, Plate III, 4, 5. 3. Section of pollen figured in Plate I, 9, Plate III, 1, 2. Scale bars: 2 μ m (1, 4), 5 μ m (2), 0.5 μ m (3, 5, 6).

referred this pollen to the genus *Balmeiopsis* and not to *Araucariacites*. He reported a tectum, a granular infratectum and a thin lamellate nexine with granules on the inner surface of the lamellae.

Cyclusphaera has been studied as both dispersed (Taylor et al., 1987; Zavada, 1992; del Fueyo et al., 2012) and *in situ* (Del Fueyo and Archangelsky, 2005) pollen. It has a granular sexine and a two-layered nexine. The sexine is thickened in the equatorial area; the granules often have a columella-like appearance. The outer nexine layer is lamellate, the inner one is amorphous and more electron dense. No “tectum” (or compact granular layer) is distinguished in the sexine, except in material studied by Zavada (1992), who reported an “imperforate” tectum for pollen he identified as *Cyclusphaera psilata* Volkheimer et Sepulveda. However, Zavada included no TEM illustrations for *Cyclusphaera* pollen, so we cannot make a definite comparison.

Callialasporites has been studied as dispersed pollen only (Archangelsky, 1994; Batten and Dutta, 1997). It shows a granular sexine and a lamellate nexine; Archangelsky (1994) reported an inner nexine layer that was more electron dense and homogeneous, and an outer lamellate one. Batten and Dutta (1997) did not mention any thinning or thickening within the pollen wall, but Archangelsky (1994) indicated a slightly thinned sexine at one pole and a somewhat thickened sexine in the equatorial area. Batten and Dutta (1997) observed loosely spaced nexine lamellae near the boundary with the sexine in *C. dampieri*, like those we observed in our *Callialasporites* pollen.

Dilwynites ultrastructure has been studied in dispersed *D. granulatus* Harris and *D. tuberculatus* Harris (Dettmann and Jarzen, 2000). The sporoderm consists of a granular ectexine and lamellate endexine; the ectexine is thinner than in other fossil araucarian pollen, while the endexine lamellae are loosely arranged and the outer endexine looks spongy.

Among extant species, *Araucaria* (*A. araucana* (Mol.) K. Koch., *A. angustifolia* (Bert.) O. Kuntze, Del Fueyo et al., 2008) shows a granular ectexine and two-layered endexine, the latter with an outer lamellate layer and an inner amorphous, more electron dense layer. There is also an annular area formed by thickening of the sexine. *Agathis* (Van Campo and Lugardon, 1973; Kurmann, 1992) is characterized by a granular sexine, with an outer zone of more compact granules, and a lamellate nexine. Pollen grains of *Wollemia* (Dettmann and Jarzen, 2000) have a granular ectexine and lamellate endexine, with the lamellae tightly appressed in the inner part of the endexine and loosely separated in the outer part. The ectexine granules are larger in the outer part. No difference in the exine thickness around the grain is mentioned in the description or observed in the illustrations.

In general, known araucarian pollen grains, including our material, exhibit similar ultrastructure, with a granular sexine and two-layered (lamellated and homogeneous/amorphous) nexine. In most studied species, the sexine has larger granules in its outer part and is reduced in thickness in one or both polar regions, even in inaperturate *Araucariacites*. Although Batten and Dutta (1997, pl. II, 1, 7, 12; pl. III, 5) did not report a difference in exine thickness for their *Araucariacites* and *Callialasporites* species, several illustrations give an impression of possible minor equatorial thickening. The exception among studied fossil araucarian pollen types is *Dilwynites*, for which no difference in exine thickness is mentioned, and the endexine of the two studied species appears somewhat different from the endexine of other araucarian pollen. The apparently constant thickness of the exine and the thin ectexine with large granules forming the sculpture make *Dilwynites* and pollen of modern *Araucaria*, *Wollemia*, and *Agathis* more similar to each other than they are to *Araucariacites*, *Balmeiopsis*, *Cyclusphaera*, and *Callialasporites*.

Another difference concerns whether the equatorial thickening of the pollen wall occurs because of the thickening of the ectexine (*Balmeiopsis*, *Cyclusphaera*, and *Callialasporites* by Archangelsky, 1994) or the loose organization of the endexine lamellae (our material, *Callialasporites* according to Batten and Dutta, 1997); there are also

equatorial spaces/cavities between the exine layers in *Callialasporites* (Archangelsky, 1994; Batten and Dutta, 1997). In the *Callialasporites*-type pollen under study here, the exine thickening at the equator occurs because of the loosely organized endexine I (lamellate outer nexine layer). Sukh Dev (1961) in the diagnosis of *Callialasporites* described it as having a “body encircled by an equatorial bladder, which may be incomplete”; he indicated that the new genus included pollen grains with many variations in the form of the body and saccus (bladder) number, and that different species of *Callialasporites* show transitions from one condition to the other. This was also supported by later studies, with findings of different forms even in the same microsporangium (Gamerro, 1965). The true nature of *Callialasporites* sacchi should be documented by future ultrastructural studies. While our data have indicated an unusual structure of the saccus, represented by loosely organized lamellae of the endexine I, Archangelsky (1994) and Batten and Dutta (1997) reported a cavity between the sexine and nexine in the equatorial area. This cavity might result from fossilization and the “labile” connection between the nexine and sexine, or it might represent a eusaccate condition. Probably this structure does not represent a “true” saccus and may be more comparable to the saccus-like structures (velum, puffy frill, flange-like sacchi, etc.) reported for some *Tsuga* species, *Baisianthus* pollen, and several dispersed Cretaceous pollen types (Zavada and Dilcher, 1988; Kurmann, 1990; Tekleva and Krassilov, 2004).

Del Fueyo et al. (2008) reported an annular area in extant *Araucaria araucana* and *A. angustifolia*, represented by an equatorial thickening of the sexine, although it is not visible in their published illustrations. In our material there is wrinkling and folding in the presumed equatorial area that may be comparable to this annular area.

There is tectum-like layer formed by more compact granules in the outer part of the sexine in some fossil araucarian pollen, including *Araucariacites* pollen (except for that studied by Batten and Dutta, 1997), *Balmeiopsis* studied by Zavada (1992), and *Cyclusphaera* studied by Del Fueyo et al. (2012), but there is relatively uniform stratification of the sexine in others.

Among fossil araucarian pollen types, *Balmeiopsis* shows more similarity to *Cyclusphaera* in its considerable equatorial thickening, presence of one or two apertures, and “spongy” sexine. *Araucariacites* shares more characters with *Callialasporites* and often shows transitional forms from one fossil-genus to the other, sometimes within the same pollen cone. Pollen grains of extant *Araucaria* species show similar variability in their morphology. Despite having the same general morphology and ultrastructure, *Dilwynites* appears more distinct from other fossil araucarian pollen types, being closer to *Araucariacites* among those, but instead it shows more similarity to extant araucarian pollen, of *Wollemia*, *Araucaria*, and *Agathis*. This might, however, result from the fact that the ultrastructure of *Dilwynites* has been studied in fossils from Tertiary deposits, whereas the exine ultrastructure of other fossil araucarian pollen is known for taxa from the Jurassic and Cretaceous. More data on the exine ultrastructure from both Mesozoic and Tertiary deposits are needed for an accurate comparison.

5. Conclusions

Variation in pollen size, morphology and ultrastructure was found between two types of pollen grains extracted from a cone of *Callialastrobus sousai*. The studied pollen grains can be clearly identified with the pollen fossil-genera *Araucariacites* and *Callialasporites*. The exine sculpture and sporoderm structure are almost the same, differing in a somewhat thicker exine and more pronounced looseness of the endexine lamellae in pollen of the *Callialasporites* type. The general morphology and ultrastructure of the studied pollen agree well with previous data on araucariaceous pollen as being asaccate or (sub)saccate (in the case of *Callialasporites*) and granulate/microechinate, with a granular sexine and two-layered (lamellate and homogeneous/amorphous) endexine. An apparently unique feature revealed for the

Callialasporites-type of pollen is its saccus ultrastructure, which is characterized by a very loose organization of the endexine lamellae with no cavity between the exine layers. The observed ultrastructure of both the *Araucariacites* and *Callialasporites* pollen types corroborate earlier interpretations of the fossil-genus *Callialastrobus* as produced by members of the Araucariaceae.

Data availability

Data will be made available on request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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