

***Arcellites punctatus* sp. nov.: a new megaspore from the Early Cretaceous of Portugal studied using high resolution synchrotron radiation X-ray tomographic microscopy (SRXTM)**

ELSE MARIE FRIIS¹, KAJ RAUNSGAARD PEDERSEN² & FEDERICA MARONE³

¹Department of Palaeobiology, Swedish Museum of Natural History, Stockholm, Sweden, ²Department of Geoscience, University of Aarhus, Aarhus, Denmark, ³Swiss Light Source, Paul Scherrer Institute, Villigen PSI, Switzerland

Abstract

A new species of megaspores, *Arcellites punctatus* sp. nov. and associated microspores, are described from the Early Cretaceous Torres Vedras, Catefica and Buarcos localities in Portugal. The new taxon is most abundant in the Torres Vedras plant assemblages where it co-occurs with several other types of megaspores, remains of aquatic and terrestrial angiosperms as well as other plant fossils. The megaspore body is smooth without appendages, finely perforated and with a rugose surface pattern. High resolution synchrotron radiation X-ray tomographic microscopy (SRXTM) analysis of the megaspore wall shows a three-layered structure. The outer layer is punctate-perforate. Perforations are densely spaced in the valleys between the ridges, while they are more scattered or lacking on the top of the ridges. The middle layer is granular-fibrous and of uneven thickness causing the rugose surface pattern. The inner layer is evenly thin and solid. The outer layer of the megaspore body continues into the elongated, six-parted and inverted cone-shaped acrolamella or neck. Lateral margins of acrolamella segments are laminar and crenulate. Microspores occur attached to the acrolamella of all specimens studied in scanning electron microscopy (SEM) and SRXTM. In general morphology, the megaspores are closely similar to megaspores of *Arcellites rugosus* from Canada that also lack body appendages, but the Portuguese megaspores are distinguished by their distinct punctate-perforate megaspore wall structure.

Keywords: *Arcellites*, *Early Cretaceous*, *fossil megaspores*, *Marsileaceae*, *SRXTM*, *synchrotron radiation X-ray tomographic microscopy*, *water ferns*

The dramatic expansion of angiosperms during the Early Cretaceous was associated with major changes in the terrestrial ecosystem. There is also evidence for major changes in fresh water ecosystems during the same period. An early invasion of angiosperms into the aquatic realm is indicated by several whole plant occurrences (Sun et al. 2002; Friis et al. 2003; Mohr et al. 2008), isolated *Brasenia*-like floating leaves (Saporta 1894; Taylor et al. 2008), and flowers and seeds related to the Nymphaeales (Friis et al. 2009, 2011). Heterosporangiate ferns with inferred aquatic habit also became established and proliferated

markedly during the Early Cretaceous (e.g. Kovach & Batten 1989). This expansion undoubtedly also affected the production and diversity of fresh water ecosystems. Preliminary studies indicate that aquatic angiosperms were more abundant in the Early Cretaceous, decreased in importance during the Late Cretaceous and underwent a new radiation event in the earliest Cenozoic with the establishment of many modern taxa (Friis et al. 2011). Aquatic water ferns may have undergone a similar pattern with a major radiation in the Early Cretaceous, decrease in the Late Cretaceous and a turnover during the Paleocene

Correspondence: Else Marie Friis, Department of Palaeobiology, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, Sweden.
E-mail: else.marie.friis@nrm.se

(Received 5 November 2013; accepted 12 March 2014)

(Collinson et al. 2013). The full diversity and role of the Cretaceous aquatic flora in the changing ecosystems are, however, poorly understood.

In the Early Cretaceous mesofossil floras of Portugal, megaspores are sometimes abundant and diverse. Megaspores related to extant *Isoetes* L. and *Selaginella* P. Beauv. are particularly widespread, but there are also megaspores assignable to *Arcellites* Miner emend. Ellis et Tschudy and scattered occurrences of *Molaspora* Schmel emend. Hall, two extinct genera thought to be closely related to extant members of the heterosporous water fern family Marsileaceae (see references in Cúneo et al. 2013). Both genera are restricted to the Cretaceous (Kovach & Batten 1989) and *Arcellites* had its maximum diversification during the latter part of the Early Cretaceous. In the Portuguese mesofossil floras, typical *Arcellites* megaspores bearing a long cone-shaped acrolamella with six slightly twisted segments are currently known only from a single species from the Barremian–Aptian. This species is unusual among *Arcellites* megaspores in lacking body appendages. Other megaspores from Portugal that are or have been assigned to *Arcellites* are common in many of our mesofossil assemblages from the earliest Early Cretaceous (Berriasian–Hauterivian) of Portugal and include such taxa as *Arcellites vectis* (Hughes) Potter, *Arcellites medusus* (Dijkstra) Potter and *Bohemisporites pyriformis* (Dijkstra) Knobloch. They all lack the distinct acrolamellae and may not be closely related to *Arcellites* (for the systematic position of *Bohemisporites*, see Batten et al. 1996).

Synchrotron radiation X-ray tomographic microscopy (SRXTM) has been successfully used previously in the study of megaspores (Slater et al. 2011; Collinson et al. 2013). In the present study of the Barremian–Aptian *Arcellites* megaspores from Portugal, we apply SRXTM with increased resolution (pixel size 0.1625 µm), which gives better resolution of the stratification and three-dimensional (3D) distribution of structural elements of the megaspore wall, and thus, is a better basis for comparative and systematic analyses. The Portuguese megaspores are in gross-morphology, and in the lack of body appendages, closely similar to *Arcellites rugosus* Singh recorded from more or less contemporaneous (Barremian?–Aptian) sediments of Alberta, Canada (Singh 1964). The Portuguese megaspores are, however, distinguished from *Arcellites rugosus* in details of the wall ultrastructure and a new species, *Arcellites punctatus* sp. nov., is, therefore, established for the Portuguese fossils.

Material and methods

The *Arcellites* megaspores described here are mainly from samples collected at the Torres Vedras locality

(northeast of Forte de Forca, Portugal). At the time of collection, the locality was a large open clay pit close to the road from Torres Vedras to Sarge. The plant-bearing strata were later completely covered and destroyed by town development. Samples including the *Arcellites* megaspores are all from the lowermost part of the clay pit (Torres Vedras 38, 39, 40, 43, 46, 143, 144) and considered to be of Late Barremian–Early Aptian age (Friis et al. 2010, 2011). The samples were collected close to each other, but are very different in general composition. In sample Torres Vedras 38, *Arcellites* co-occurs with a variety of other megaspores as well as few remains of angiosperms including a male inflorescence of a *Hedyosmum*-like plant. Sample Torres Vedras 39 includes predominantly coprolites with pollen and spores and isolated angiosperm stamens. Sample Torres Vedras 43 comprises a rich assemblage of angiosperm fruits and seeds as well a diversity of seeds related to the Bennettitales–Erdtmanithecales–Gnetales complex such as seeds of *Tomcatia taylorii* Friis, Pedersen et Crane, *Quadrispermum parvum* Friis, Pedersen et Crane and *Ephedrispermum lusitanicum* Rydin, Pedersen, Crane et Friis (Rydin et al. 2006; Friis et al. 2013). Angiosperm remains include a number of nymphaealean seeds (Friis et al. 2010) as well as numerous other seeds, fruits and isolated stamens with pollen. Sample Torres Vedras 144 is dominated by a rich and abundant assemblage of megaspores, while other plant remains including angiosperms and conifers are subordinate. Rare oogonia of Characeae are also present and provide evidence of wetland environment. Single megaspores of *Arcellites punctatus* have also been recorded from the Catefica (sample Catefica 49) and Buarcos localities (sample Buarcos 157).

The fossils were extracted from the sediment samples by sieving in water. Adhering minerals were removed using 40% hydrogen fluoride (HF) and 10% hydrogen chloride (HCl). The acid treatment was followed by thoroughly rinsing in water and the organic residual was finally dried in air before being sorted under stereo-microscope.

Morphological details were studied using a Hitachi S-4300 field emission scanning electron microscope at 1–2 kV and internal details of the megaspore wall were studied by SRXTM at the TOMCAT beam line of the Swiss Light Source at the Paul Scherrer Institute, Villigen, Switzerland (Stampanoni et al. 2006). Imaging was made at 10 keV using a sCMOS detector. For overview scans, a 20× objective with a 20 µm thick lutetium aluminum garnet:cerium (LAG:Ce) scintillator screen was used, resulting in an isotropic pixel size of 0.325 µm. Higher resolution datasets of selected areas were acquired with a 40× objective coupled to a 5.9 µm thick Lu₂SiO₅:terbium (LSO:Tb) scintillator screen. The pixel size in this case was 0.1625 µm

(Friis et al. 2014). Typically, 1500 equiangular projections were recorded over 180° and then combined using dedicated reconstruction algorithms (Marone & Stampanoni 2012) to obtain a 3D volume. All datasets have been reconstructed uniquely exploiting absorption contrast and pure edge-enhancement. For few selected datasets, simple phase retrieval (Paganin et al. 2002) for boosting the contrast, e.g. between different layers has been performed. In this case, the high frequency component of the original data has been added to minimise loss in spatial resolution inherent in phase retrieval (Friis et al. In press). Slice data from the SRXTM volumes were analysed and manipulated using the Avizo 6.3 software package for computed tomography. Volume rendering (vortex) provides transparent reconstructions whereas isosurface rendering provides surface reconstructions; both types were used either separately or combined to enhance structures of the megaspore wall. Differences in density of the megaspore wall are enhanced by applying variation in colour intensity to virtual sections consisting of several consecutive slices.

Terminology used for describing *Arcellites* megaspores and wall structure varies considerably among authors particularly for the description of the layers of the megaspore wall. In this work, we have adapted neutral terms, but refer to terminology used in previous studies in the discussion section.

All specimens of *Arcellites punctatus* studied here are stored in the palaeobotanical collections of the Swedish Museum of Natural History, Stockholm, Sweden (S).

Systematic palynology

Order Salviniales
Incertae familiae

Genus *Arcellites* Miner emend. Ellis et Tschudy

Arcellites punctatus sp. nov.
(Figures 1–5)

Derivation of specific epithet. — From the distinctive punctate, perforated wall ultrastructure.

Specific diagnosis. — *Arcellites* megaspore with prominent six-parted acrolamella. Megaspore trilete. Megaspore body spherical with an open, coarsely rugulate surface formed from broad, rounded ridges; outer megaspore wall smooth lacking body appendages. Segments of acrolamella laminar with membranous, crenulate or serrate outer margin. Megaspore wall over the megaspore body three-layered; inner layer thin, of even thickness; middle layer granular-fibrous of uneven thickness causing the rugose surface

pattern; outer layer solid, of more or less even thickness, perforated by short, narrow canals that extend almost to the middle layer. Canals straight, of uneven length and distribution with most canals in the valleys between and along the flanks of the rounded ridges; canals absent or scattered over the ridges. Acrolamella extends from outer layer. Microspores adhering to the acrolamellae are trilete with raised proximal region, more or less circular outline, and a psilate-perforate, sometimes faintly rugulate surface.

Dimensions. — Total length of megaspore body and acrolamella ranges typically between 450–650 µm (mean = 556 µm); diameter of megaspore body ranges between 250–350 µm (mean = 284 µm) and length of acrolamella between 250–400 µm (mean = 300 µm); 15 specimens were measured. A single megaspore falls outside the typical size range with total length being 300 µm, diameter of megaspore 200 µm, and length of acrolamella 190 µm. Microspores are about 40–45 µm in diameter.

Holotype designated here. — S170173 (from sample Torres Vedras 43); illustrated here in Figure 1A, 6A, B.

Paratypes designated here. — S170218, S170219, S171531–S171533, S174135–S174140 (sample Torres Vedras 38); S174141 (sample Torres Vedras 39); S174142 (sample Torres Vedras 40); S170169–S170172, S174097 (sample Torres Vedras 43); S174134 (sample Torres Vedras 46); S174133 (sample Torres Vedras 143); S174123–S174132 (sample Torres Vedras 144).

Other specimens. — S174143 (sample Catefica 49); S174096 (sample Buarcos 157).

Type locality. — Torres Vedras, Portugal (39° 06' 13" N; 009° 06' 44" W).

Type horizon and age. — Fluvatile cross-bedded sands with intercalated clay beds and darker organic rich horizons (Almargem Formation); Early Cretaceous (Late Barremian–Aptian).

Description. — About 100 isolated megaspores were discovered from the Torres Vedras mesofossil flora. Two additional specimens were discovered from the Buarcos mesofossil flora (sample Buarcos 157) and one specimen from the Catefica mesofossil flora (sample Catefica 49). The megaspores from Torres Vedras are typically three-dimensionally preserved, rarely fragmented and probably produced close to the depositional basin. Four specimens (S171531–S171533, S174096) were studied using SRXTM. The specimens from Buarcos and Catefica are

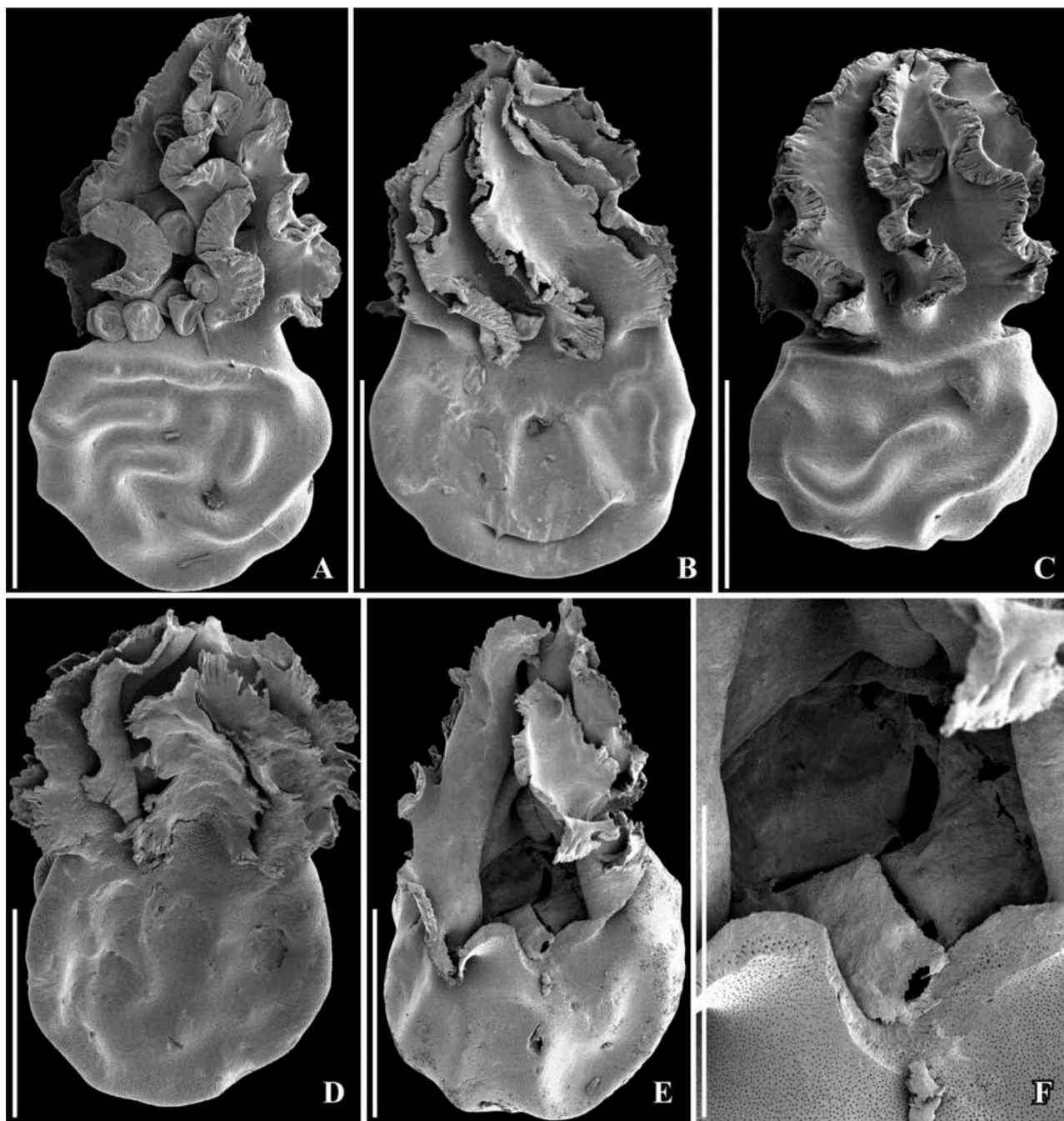


Figure 1. SEM images of *Arcellites punctatus* sp. nov. megaspores from the Torres Vedras locality, Portugal. **A–D**. Lateral view of megaspore with more or less spherical megaspore body and flame-shaped proximal acrolamella; megaspore body with broad, rounded ridges forming a rugulate pattern. **A**. Holotype (S170173, sample Torres Vedras 43). **B**. Megaspore with slightly twisted acrolamella (S170218, sample Torres Vedras 38). **C**. Megaspore with broad acrolamella and crenulate margins of acrolamella (S170172, sample Torres Vedras 43). **D**. Megaspore with indistinct ridges on the megaspore body and acrolamella with partly separate segments (S174128, sample Torres Vedras 144). **E**. Oblique view of fragmented megaspore with segments of acrolamella broken off showing top of megaspore with trilete mark (S174137, sample Torres Vedras 38). **F**. Detail of (E) showing trilete mark. Scale bars – 200 µm (A–E), 100 µm (F).

flattened and with indication of wear, which may indicate reworking.

The megaspores are trilete consisting of a spherical megaspore body and a prominent acrolamella that extends proximally from the megaspore body

covering the trilete mark (Figures 1, 2). In incident light, the megaspore body and inner folds of the acrolamella are dark brown and shiny while the lateral margins of the acrolamella segments are lighter, yellowish or brownish, and dull.

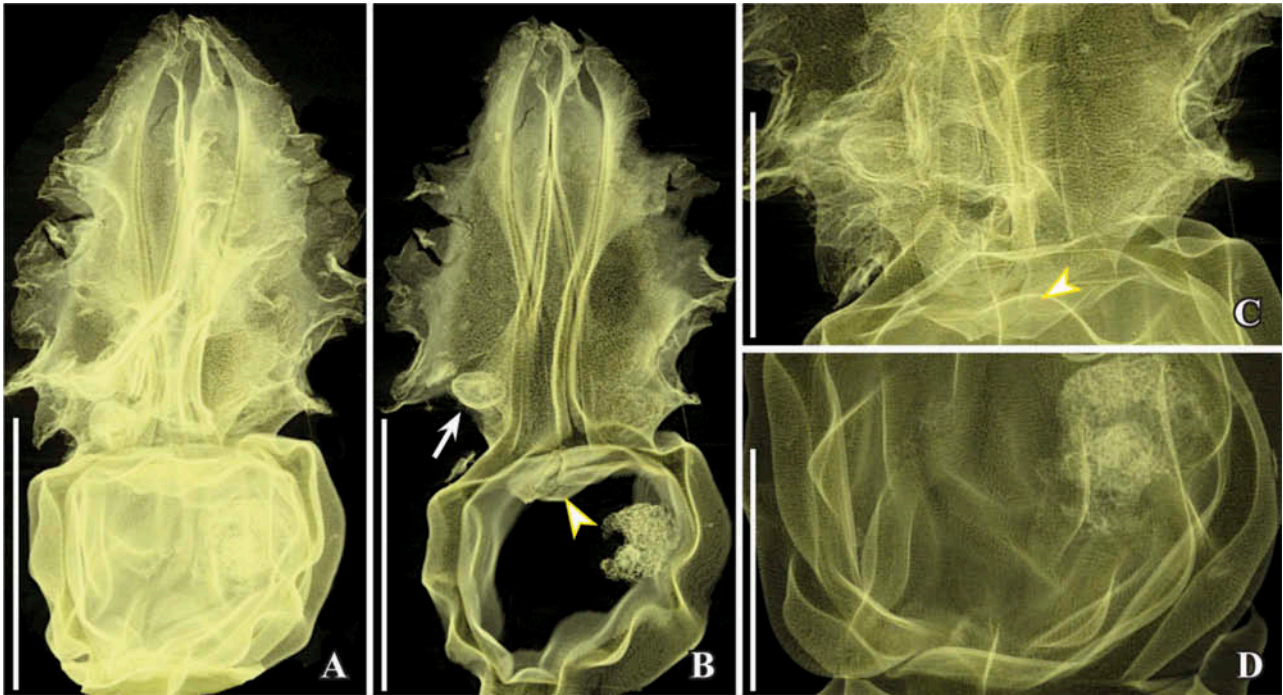


Figure 2. SRXTM reconstructions (voltex size 0.37) of *Arcellites punctatus* sp. nov. megaspores from the Torres Vedras locality, Portugal (S171531, sample Torres Vedras 38). **A.** Transparent voltex reconstruction of megaspore showing megaspore body and apical acrolamella. **B.** Longitudinal cut transparent voltex comprising several consecutive orthoslices showing microspore adhering to a segment of the acrolamella (arrow), the well-defined inner layer of the megaspore wall and trilete mark of the megaspore (arrowhead); note the finely perforate membranous margin of acrolamella segment. **C.** Transparent voltex reconstruction of transition zone between megaspore body and acrolamella showing the strongly pitted nature of the outer wall layer; trilete mark (arrowhead) seen at top of megaspore body. **D.** Transparent voltex reconstruction of megaspore body in lateral view showing the ridges and the pitted nature of the outer wall. Scale bars – 200 μm (A, B), 100 μm (C, D).

The megaspore body is smooth lacking body appendages, but has broad, rounded ridges that form an open rugulate pattern (Figures 1A–E, 2A, B, 3A, B). The acrolamella is elongate and flame-shaped formed from six segments that extend from the top of the megaspore body (Figures 1A–E, 2A, B). The acrolamella is star-shaped in transverse section (Figures 4A, 5A); each section is infolded, horseshoe-shaped in transverse section and with the two arms extending outwards. The segments are laminar with a membranous, crenulate or serrate outer margin that undulates longitudinally (Figure 1A–D) and sometimes slightly twisted (Figure 1B). The crenulations are about 30 μm deep. Neighbouring segments are fused near the base and loosely adhering further up. In some specimens, the segments are partly (Figure 1D) or completely (not shown) separated. The trilete mark is simple with short arms (Figures 1F, 2B).

The megaspore wall over the megaspore body is 16–26 μm thick consisting of three distinct layers (Figures 3C, 4B–D, 5B–D). The inner layer is of even thickness, about 4 μm thick over the whole of

the megaspore body (Figures 2B, 4B–D, 5B–D). It appears three-layered, but internal stratification is not clear. The middle layer is of uneven thickness, thicker under the ridges causing the rugose surface pattern (Figures 4B–D, 5B–D). It is about 4 μm thick in the valleys between the ridges and up to about 20 μm thick under the ridges. In SRXTM orthoslices, the middle layer appears densely granular (Figure 4B–D), but in 3D reconstructions combining voltex and isosurface rendering, the middle layer appears granular-fibrous (Figure 5B–D). The granular-fibrous nature of the middle layer is also observed with scanning electron microscopy (SEM) of fragmented specimens (Figure 3C, D). The outer layer is of more or less even thickness, up to about 10 μm thick. It is solid, but perforated by short, narrow canals that extend from the outer surface almost to the middle granular layer (Figures 3C, 4B, C, 5C, D). This perforation is seen as a dense punctation on the outer surface of the megaspore wall (Figures 1F, 2C, D, 3A, B). The canals are straight and of uneven length and distribution with a concentration in the valleys

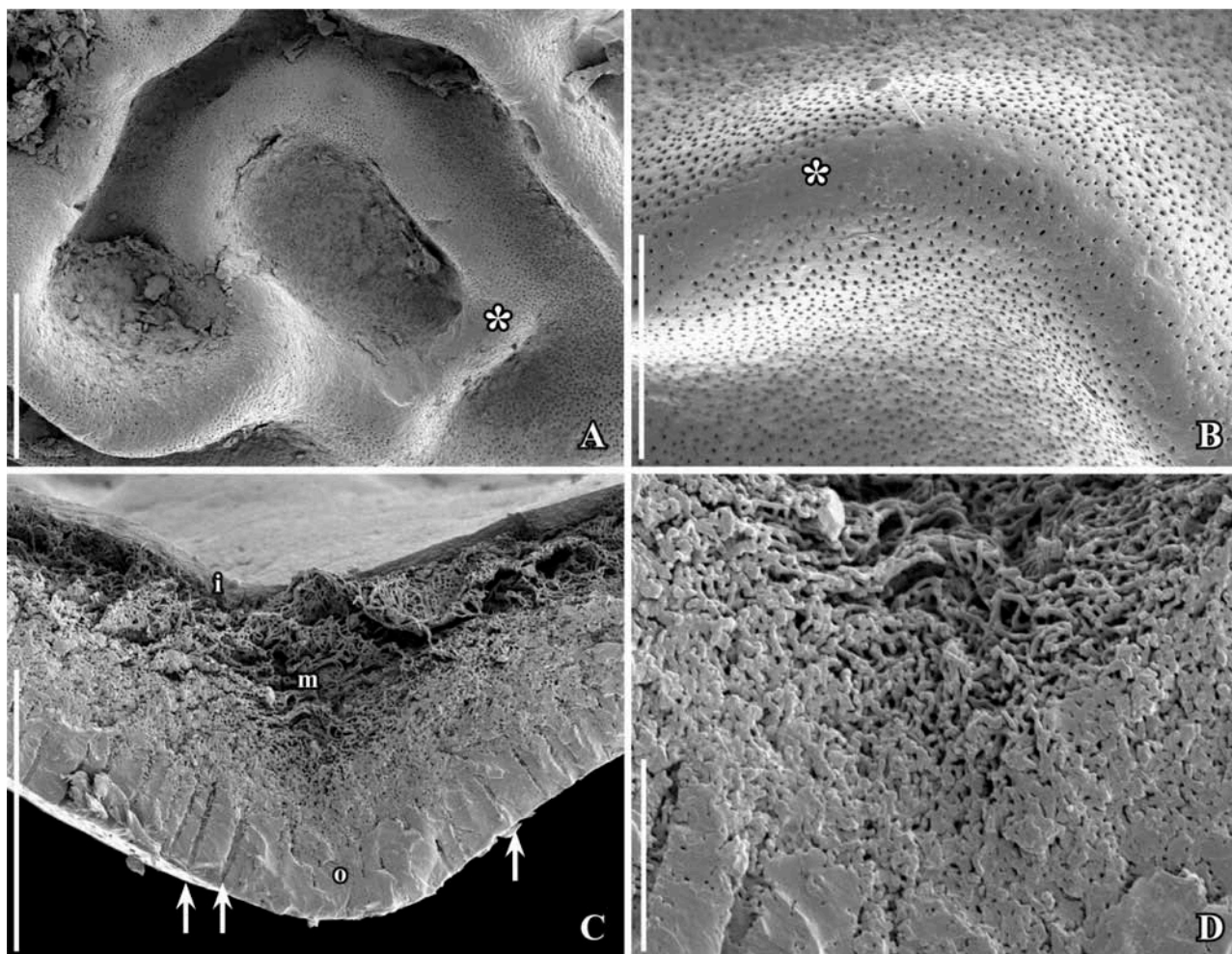


Figure 3. SEM images of *Arcellites punctatus* sp. nov. megaspores from the Torres Vedras locality, Portugal. **A.** Detail of outer surface of megaspore wall with rounded ridges forming the rugulate pattern; note perforation is less dense on top of ridges (asterisk) (S174138, sample Torres Vedras 38). **B.** Detail of outer surface of megaspore wall showing top of ridge with scattered perforations (asterisk) and valleys and flanks of ridges with dense perforation (S174129, Torres Vedras 144). **C.** Section through broken megaspore wall showing the three layers of the megaspore wall; the outer layer (o) with narrow canals extending from the surface (arrows) almost to the middle granular-fibrous layer (m); inner layer (i) thin and solid (S174136, sample Torres Vedras 38). **D.** Detail of (C) showing granular-fibrous nature of middle layer of megaspore wall. Scale bars – 50 μm (A), 20 μm (B, C), 5 μm (D).

between the ridges and along the flanks of the ridges while canals are more scattered or absent over the ridges. This pattern is very clear in the 3D SRXTM reconstructions (Figure 5B–E), but the more scattered occurrence of the perforations can also be observed in SEM images of the megaspore surface (Figures 3A, B).

The acrolamella extends from the outer perforated layer (Figures 2B, C, 4D), while the middle and inner layers are not present in the acrolamella. Perforation is also dense in the segments of the acrolamella (Figures 2B, C, 4A, 5A, 6A, B) where it is more or less evenly distributed in the inner parts, while finer or sometimes absent in the lateral, membranous margins (Figures 2B, 4A).

Microspores occur adhering to the acrolamellae in all specimens studied using SEM and SRXTM (Figures 1A–C, 2A, 4A, D, 5A, 6). The microspores are all of the same kind and most likely belong to the same species as the megaspores. They are typically flattened with a more or less circular outline, about 40–45 μm in diameter. The microspores are trilete and the trilete mark is raised in well-preserved specimens (Figure 6C, D), but in most microspores, it is collapsed (Figure 6B). The microspore wall adjacent to the trilete mark is somewhat crumpled or folded. The outer surface of the main microspore body is psilate-perforate, sometimes faintly rugulate, with scattered pores that are most conspicuous on the proximal face close to the trilete mark.

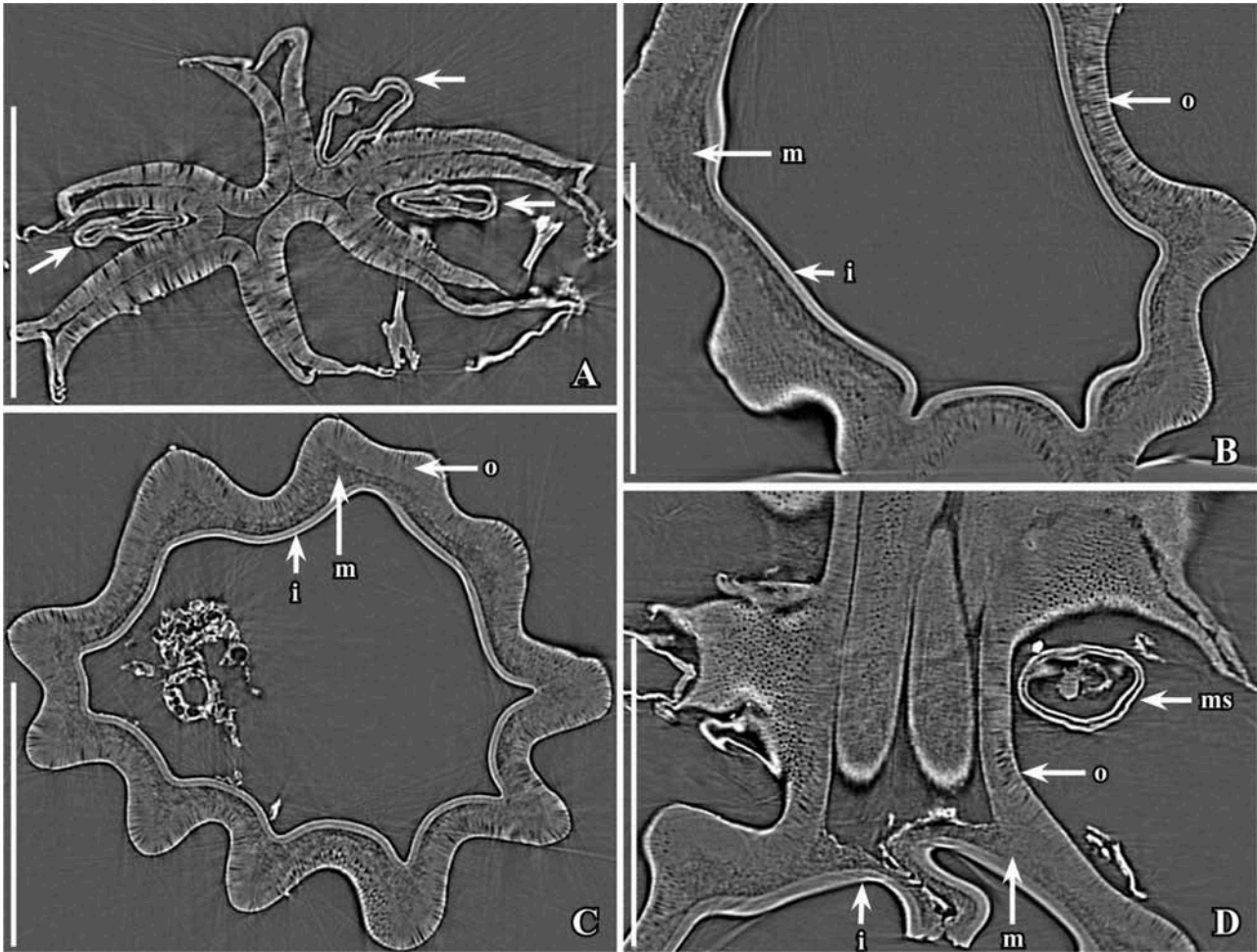


Figure 4. High resolution SRXTM (voxel size 0.1625) of *Arcellites punctatus* sp. nov. from the Torres Vedras locality, Portugal (S171531, sample Torres Vedras 38); transverse orthoslices (A, C), longitudinal orthoslices (B, D). A. Section in the proximal part of the acrolamella showing the six infolded segments forming a star-shaped pattern; note three adhering microspores (arrows) and the membranous margins of the segments. B, C. longitudinal (B) and transverse section (C) of megaspore body showing the three-layered nature of the megaspore wall with thin inner layer (i), middle, granular-fibrous layer (m) and outer, perforated layer (o); note that middle layer is thicker under the ridges causing the rugulate pattern. D. Longitudinal section through the transitional zone between megaspore body and acrolamella; note that inner (i) and middle (m) layer are only present in the megaspore body and that the acrolamella is built of the outer layer (o); note also adhering microspore (ms). Scale bars – 100 µm.

Discussion

Comparison with other fossil Arcellites megaspores

The Portuguese megaspores are clearly assignable to the extinct genus *Arcellites* first established by Miner (1935) based on megaspores from the Late Cretaceous of Greenland. The type material was fragmentary and emendation of the diagnosis was later given based on more complete megaspores (Potter 1963; Ellis & Tschudy 1964). The genus includes about 20 species characterised by their prominent, typically six-parted acrolamella, and most species have prominent appendages ornamenting the megaspore body (Li & Batten 1986; Batten & Kovach 1990; Batten et al. 1996; Nowak & Lupia 2004; Villar de Seoane & Archangelsky 2008). Li and Batten (1986)

grouped the species into four main types based on the presence or absence of body appendages and characters of the surface sculpture: 1, unsculptured; 2, rugulate; 3, with isolated or mainly isolated appendages; and 4, murornate to reticulate species with appendages at the junctions of the muri. The majority of species are of type 3 and 4, while type 1 and 2 include only a single species each, *Arcellites nudus* (Cookson et Dettmann) Potter and *Arcellites rugosus*, both species represented by only few specimens. *Arcellites nudus* is restricted to South Australia where it is reported from Albian deposits. It has an unsculptured megaspore body and is distinguished from the Portuguese megaspores in its slightly smaller size. Further, the megaspore wall is of even thickness over the entire spore body and lacks the broad ridges that

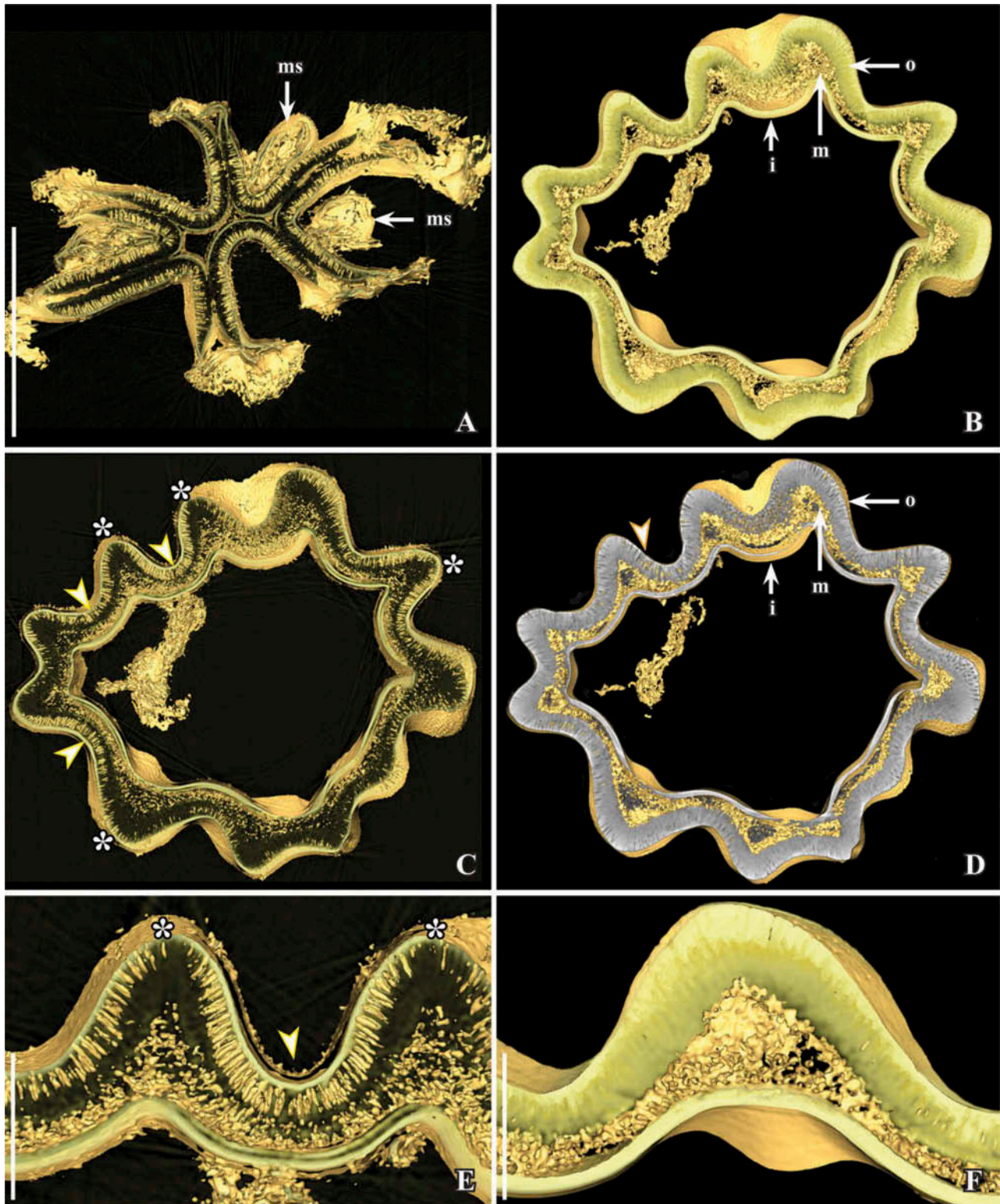


Figure 5. High resolution SRXTM (pixel size 0.1625) of *Arcellites punctatus* sp. nov. from the Torres Vedras locality, Portugal (S171531, sample Torres Vedras 38); transverse slices in combined voxels and isosurface reconstructions with superimposed orthoslice (D). Images in A, C and E have been reconstructed using absorption contrast and pure edge-enhancement; images in B, D and F have been reconstructed using simple phase retrieval with the addition of the high frequency component of the original data to minimise loss in spatial resolution inherent in phase retrieval. A. Slice through the proximal part of acrolamella in about same area as shown in Figure 4A showing the perforated zone of the acrolamella wall; note that perforations are more or less evenly distributed, but smaller in the membranous margins;

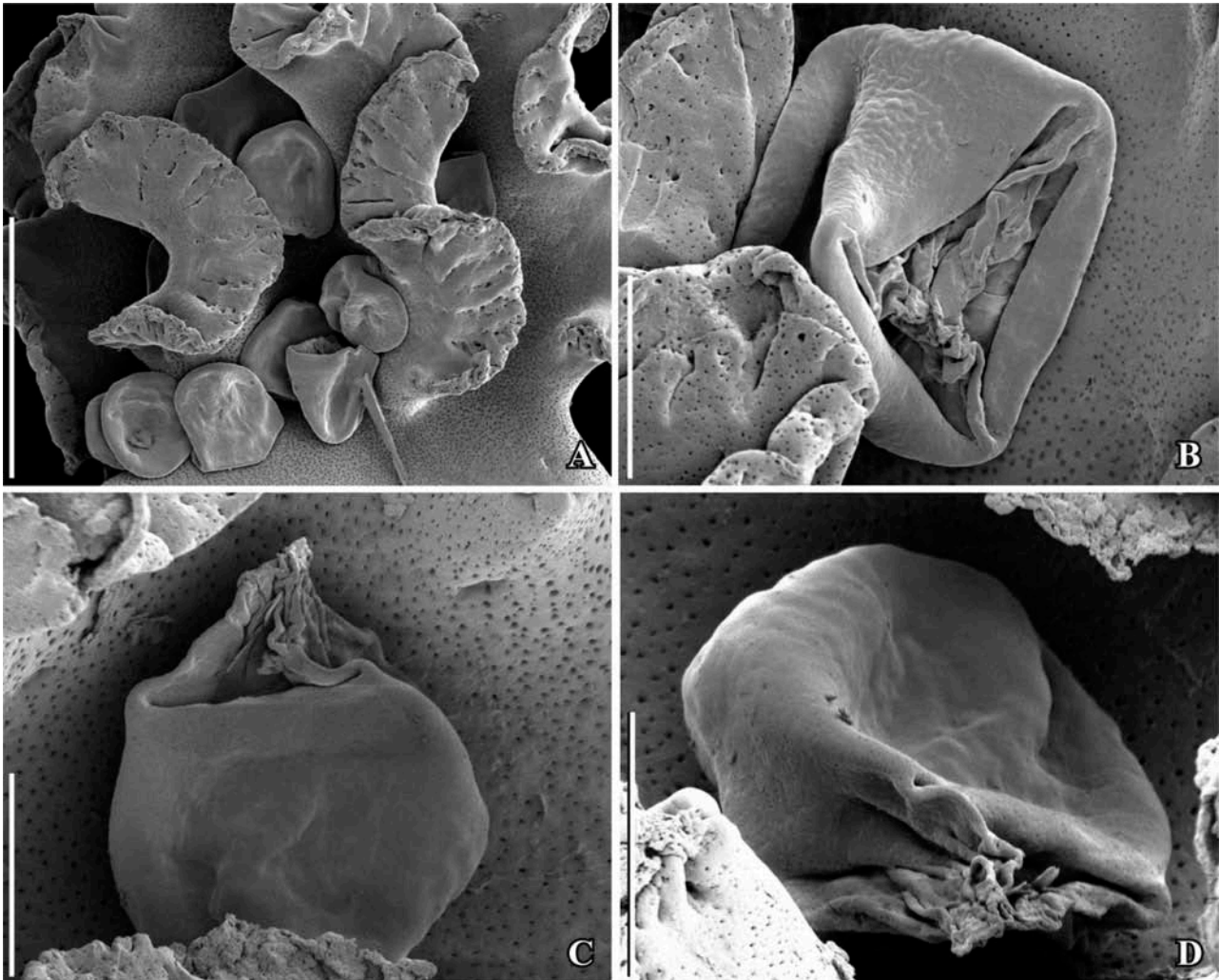


Figure 6. SEM images of microspores adhering to *Arcellites punctatus* sp. nov. megaspores from the Torres Vedras locality, Portugal. **A.** Detail of holotype showing group of microspores adhering to segments of acrolamella (S170173, sample Torres Vedras 43). **B.** microspore from holotype in proximal-lateral view showing compressed trilete mark (S170173, sample Torres Vedras 43). **C.** Microspore in lateral view showing raised trilete mark (S174129, sample Torres Vedras 144). **D.** Microspore in proximal view showing trilete mark and psilate-punctate surface. Scale bars – 100 μ m (A), 20 μ m (B–D).

characterise the Portuguese megaspores (Cookson & Dettmann 1958; Potter 1963). *Arcellites rugosus* was described from cored sections of the Mannville Group (Barremian?–Aptian) of East-Central Alberta, Canada (Singh 1964). It also has megaspores without body appendages and is characterised by broad irregular ridges that form a rugose pattern on

the surface of the megaspore body very similar to that of the Portuguese megaspores. The pattern of the ridges is controlled by thickenings of the megaspore wall in the same way as seen for the Portuguese specimens. Megaspores of *Arcellites rugosus* are also in general shape closely similar to the Portuguese megaspores, but *Arcellites rugosus* differs from the

← note also two adhering microspores (ms) (consecutive slices: 480–520). **B–F.** Slices through the megaspore wall, approximately in the middle of the megaspore body, cut in different thicknesses and with varying colour intensities to enhance differences in the wall stratification; inner layer (i) of megaspore wall thin, middle layer (m) thick under the ridges and thinner in the valleys; outer layer (o) more solid seen as outer light green zone in (B) and grey outer zone in (D); outer zone is strongly perforated; the canals are best seen in (C) and (E; enlarged section of C); note the uneven distribution of the canals that are more densely packed in the valleys (C, E: arrowheads) and only scattered under the ridges (C, E: asterisk); **B, F.** consecutive slices: 350–400; **C, E:** consecutive slices: 350–380; **D:** consecutive slices: 350–400. Scale bars – 100 μ m (A–D, shown in A), 50 μ m (E, F).

Portuguese specimens in size and certain ultrastructural features. Mean total length of *Arcellites rugosus* is 625 µm (556 µm for the Portuguese specimens) and the total length of the *Arcellites rugosus* holotype is 670 µm, while the longest Portuguese specimen is 650 µm. Singh (1964) noted that the absence of granular and pitted layers in the outer megaspore wall of *Arcellites rugosus* distinguished this species from *Arcellites nudus*. This is clear from the figured material and is a feature that also distinguishes *Arcellites rugosus* from the Portuguese megaspores that have a distinctly perforated outer wall. Singh's observations and figures are of high quality and since other *Arcellites* species figured by Singh (1964) do show distinct perforations, we believe that punctation, if present in *Arcellites rugosus*, is extremely fine. The Portuguese megaspores are, therefore, here ascribed to a new species, *Arcellites punctatus*, that in morphology is closely similar to *Arcellites rugosus*, but clearly separated in ultrastructural features and its slightly smaller size.

A single megaspore very similar to *Arcellites rugosus* and to the new *Arcellites punctatus* was described from the Barremian–Aptian of Northeast Inner Mongolia, China, as *Arcellites* sp. cf. *A. rugosus* Singh by Li and Batten (1986). *Arcellites* sp. cf. *A. disciformis* Miner emend. Ellis et Tschudy, recorded from the earliest Aptian of northern Germany by Batten (1995), is also very similar to *Arcellites rugosus*, but this single megaspore has rounded swellings rather than ridges.

Microspores associated with *Arcellites* megaspores are generally assigned to the genus *Crybelosporites* Dettmann. *Crybelosporites* spores are described as trilete and proximally cavate with a reticulate, rugulate or foveolate surface (Dettmann 1963). In only few cases are they psilate-punctate (Hueber 1982) as observed in the Portuguese fossils.

Wall structure in *Arcellites*

The wall structure of *Arcellites* has been variously described and interpreted, and there is no consensus regarding terminology (see Batten 2012). Earlier studies were based on light microscopy alone, while later studies have included SEM examinations as well as studies of ultrathin sections using transmission electron microscopy (TEM) (e.g. Batten et al. 1996; Nowak & Lupia 2004). Most studies show a three-layered wall of the megaspore body with a denser inner layer (intine of Batten et al. 1996; exine of Nowak & Lupia 2004), a granular or fibrous middle layer (inner exoexine of Batten et al. 1996; inner perine of Nowak & Lupia 2004) and a granular outer layer (outer exoexine of Batten et al. 1996; outer perine of Nowak & Lupia 2004). In *Arcellites stellatus* Nowak et Lupia, the inner layer is tripartite

with a middle layer of channels lined to the inside and outside by a solid zone (Nowak & Lupia 2004).

The resolution obtained using TEM is higher than that obtained with the high resolution SRXTM used here, but SRXTM does reveal the three-layered nature of the megaspore wall over the megaspore body and also shows the tripartite nature of the innermost layer. The SRXTM also reveals characters of the wall structure that are not easily detected by SEM and TEM. Most importantly, the distribution of the canals in the outer megaspore wall is clearly seen in SRXTM, both in the two-dimensional (2D) plan, but also the spatial distribution in 3D that cannot be observed in SEM or TEM. Our study clearly shows an uneven distribution of the canals with canals lacking almost completely under the ridges and densely packed in non-ridge areas. Also the length and density of the canals vary. This pattern is consistent in the four specimens that we have studied using SRXTM. It confirms that the ridges are a natural feature of the megaspore wall and not a result of crumpling due to preservation, but further studies of other species are needed to establish whether the distribution of the canals has further systematic implications.

Ecological implications

A prominent elongate acrolamella as known for the megaspores of *Arcellites* is unknown in extant megaspores. However, the *Arcellites* acrolamella is closely similar in general construction to the acrolamellae in the megaspores of extant *Regnellidium* Lindman and *Pillularia* L. In the megaspores of *Regnellidium*, *Pillularia* and the closely related extinct *Molaspora*, the acrolamella is also typically six-parted with more or less twisted segments, but the acrolamella is much shorter in these genera (e.g. Lupia et al. 2000; Cúneo et al. 2013). *Regnellidium* and *Pillularia* are heterosporous aquatic ferns in the family Marsileaceae. Extant members of the Marsileaceae grow in periodically flooded areas, temporary ponds and swamps (Kramer 1990). They are tiny plants with slender, often creeping rhizomes and leaves borne on slender petioles. *Regnellidium* and *Marsilea* L. have pinnate leaves with one or two pairs of closely spaced leaflets. The leaves may be completely submerged, floating or emerged. Very few vegetative parts of marsileaceous ferns have been recovered from the fossil record, but in a recent study, Cúneo et al. (2013) described *Regnellidium* rhizomes, roots, stems and leaves in association with megaspores of *Molaspora* and microspores of *Crybelosporites*. Marsileaceous leaves are also known from the Early Cretaceous of Australia (Nagalingum 2007) and Jordan (Hu et al. 2008). The morphology of these

fossils strongly suggests that Cretaceous marsileaceous ferns had a similar habit as their extant counterparts. There is no indication from the Early Cretaceous of a total floating habit and the aquatic ferns from this time interval may not have formed dense mats on lakes and streams as do the extant aquatic ferns of *Azolla* L. and *Salvinia* L.

In the Torres Vedras assemblages, *Arcellites punctatus* co-occur with other megaspores including those of *Molaspora lobata* (Dijkstra) Hall that are also thought to be marsileaceous as well as oogonia of aquatic algae (Characeae). The assemblages also include several different kinds of nymphaealean seeds and other plant remains indicating a diverse wetland and aquatic flora in an otherwise more dry vegetation with cholepidiacean conifers and other plants with xeromorphic features.

Conclusion

The new species of megaspores, *Arcellites punctatus* sp. nov., described here from the Early Cretaceous of Portugal, is distinguished from most other *Arcellites* megaspores in lacking appendages on the megaspore body. Rare *Arcellites* megaspores without body appendages that in gross morphology are similar to the Portuguese fossils are reported previously from widely separate localities in Canada, China and Europe. It is interesting that these occurrences are more or less contemporaneous (Barremian–Aptian) with the Portuguese fossil. High resolution SRXTM has provided a wealth of details on megaspore wall structure and has been particularly important in reconstructing the 3D distribution of canals in the outer megaspore wall. The method allows a better differentiation of the stratification and 3D distribution of structural elements of the megaspore wall and thus a better basis for comparative and systematic analysis.

Acknowledgements

The authors thank Marco Stampanoni (PSI, Villigen), Stefan Bengtson, Anna Lindström and Veneta Belivanova (NRM, Stockholm), for help with the SRXTM analyses, which were performed at the Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland. The authors are also grateful to David Batten and an anonymous reviewer for valuable comments. Support for this work was provided by the Swedish Natural Science Research Council (E. M. Friis) and European Union FP6 project 20100167 (P. C. J. Donoghue, S. Bengtson and E. M. Friis).

References

- Batten DJ. 1995. Megaspores from lowermost Aptian beds in Northern Germany. *Geologisches Jahrbuch, Reihe A* 141: 403–443.
- Batten DJ. 2012. Taxonomic implications of exospore structure in selected Mesozoic lycopsid megaspores. *Palynology* 36: 144–160. doi:10.1080/01916122.2011.642122.
- Batten DJ, Dutta RJ, Knobloch E. 1996. Differentiation, affinities and palaeoenvironmental significance of the megaspores *Arcellites* and *Bohemisporites* in Wealden and other Cretaceous successions. *Cretaceous Research* 17: 39–65. doi:10.1006/cres.1996.0004.
- Batten DJ, Kovach WL. 1990. Catalog of Mesozoic and Tertiary megaspores. American Association of Stratigraphic Palynologists, Contributions Series 24: 1–227.
- Collinson ME, Smith SY, Van Konijnenburg-van Cittert JHA, Batten DJ, Van der Burgh J, Barke J, Marone F. 2013. New observations and synthesis of Paleogene heterosporous water ferns. *International Journal of Plant Sciences* 174: 350–363. doi:10.1086/668249.
- Cookson C, Dettmann ME. 1958. Cretaceous “megaspores” and a closely associated microspore from the Australian region. *Micropaleontology* 4: 39–49. doi:10.2307/1484250.
- Cúneo NR, Hermsen EJ, Gandolfo MA. 2013. *Regnellidium* (Salviniales, Marsileaceae). Macrofossils and associated spores from the Late Cretaceous of South America. *International Journal of Plant Sciences* 174: 340–349. doi:10.1086/668811.
- Dettmann ME. 1963. Upper Mesozoic microfloras from southeastern Australia. *Proceedings of the Royal Society of Victoria, New Series* 77: 1–148.
- Ellis CH, Tschudy RH. 1964. The Cretaceous megaspore genus *Arcellites* Miner. *Micropaleontology* 10: 73–77. doi:10.2307/1484626.
- Friis EM, Crane PR, Pedersen KR. 2011. Early flowers and angiosperm evolution. Cambridge: Cambridge University Press.
- Friis EM, Doyle JA, Endress PK, Leng Q. 2003. *Archaeofructus* – angiosperm precursor or specialized early angiosperm? *Trends in Plant Science* 8: 369–373.
- Friis EM, Marone F, Pedersen KR, Crane PR, Stampanoni M. In press. Three-dimensional visualisation of fossil flowers, fruits, seeds and other plant remains using synchrotron radiation X-ray tomographic microscopy (SRXTM): New insights into Cretaceous plant diversity. *Journal of Paleontology* 88. doi:10.1666/13-099.
- Friis EM, Pedersen KR, Crane PR. 2010. Cretaceous diversification of angiosperms in the western part of the Iberian Peninsula. *Review of Palaeobotany and Palynology* 162: 341–361. doi:10.1016/j.revpalbo.2009.11.009.
- Friis EM, Pedersen KR, Crane PR. 2013. New diversity among chlamydospermous seeds from the Early Cretaceous of Portugal and North America. *International Journal of Plant Sciences* 174: 530–558. doi:10.1086/668250.
- Friis EM, Pedersen KR, von Balthazar M, Grimm GW, Crane PR. 2009. *Monetianthus mirus* gen. et sp. nov., a nymphaealean flower from the Early Cretaceous of Portugal. *International Journal of Plant Sciences* 170: 1086–1101. doi:10.1086/605120.
- Hu S, Taylor DW, Brenner GJ, Basha SH. 2008. A new marsilealean fern species from the Early Cretaceous of Jordan. *Palaeoworld* 17: 235–245. doi:10.1016/j.palwor.2008.02.004.
- Hueber FM. 1982. Megaspores and a palynomorph from the lower Potomac Group in Virginia. *Smithsonian Contributions to Paleobiology* 49: 1–69. doi:10.5479/si.00810266.49.1.
- Kovach WL, Batten DJ. 1989. Worldwide stratigraphic occurrences of Mesozoic and Tertiary megaspores. *Palynology* 13: 247–277. doi:10.1080/01916122.1989.9989362.

- Kramer KU. 1990. Marsileaceae. In: Kramer KU, Green PS, eds. The families and genera of vascular plants. Pteridophytes and Gymnosperms, 180–183. Berlin: Springer.
- Li W-B, Batten DJ. 1986. The early cretaceous megaspore *Arcellites* and closely associated *Crybelosporites* microspores from northeast Inner Mongolia, P.R. China. Review of Palaeobotany and Palynology 46: 189–208. doi:10.1016/0034-6667(86)90014-X.
- Lupia R, Schneider H, Moeser GM, Pryer KM, Crane PR. 2000. Marsileaceae sporocarps and spores from the Late Cretaceous of Georgia, U.S.A.. International Journal of Plant Sciences 161: 975–988. doi:10.1086/317567.
- Marone F, Stampanoni M. 2012. Regridding reconstruction algorithm for real-time tomographic imaging. Journal of Synchrotron Radiation 19: 1029–1037.
- Miner EL. 1935. Paleobotanical examinations of Cretaceous and Tertiary coals. American Midland Naturalist 16: 585–625. doi:10.2307/2419854.
- Mohr BR, Bernardes de Oliveira MEC, Taylor DW. 2008. *Pluricarpellatia*, a nymphaealean angiosperm from the Lower Cretaceous of northern Gondwana (Crato Formation, Brazil). Taxon 57: 1147–1158.
- Nagalingum N. 2007. *Marsileaceaphyllum*, a new genus for marsileaceous macrofossils: Leaf remains from the Early Cretaceous (Albian) of southern Gondwana. Plant Systematics and Evolution 264: 41–55. doi:10.1007/s00606-006-0497-7.
- Nowak MD, Lupia R. 2004. *Arcellites stellatus* new species, a new megaspore from the Lower Cretaceous of Maryland, USA. Journal of Paleontology 78: 1207–1213. doi:10.1666/0022-3360(2004)078<1207:ASNSAN>2.0.CO;2.
- Paganin D, Mayo SC, Gureyev TE, Miller PR, Wilkins SW. 2002. Simultaneous phase and amplitude extraction from a single defocused image of a homogeneous object. Journal of Microscopy–Oxford 206: 33–40. doi:10.1046/j.1365-2818.2002.01010.x.
- Potter DR. 1963. An emendation of the sporomorph *Arcellites* Miner, 1935. Oklahoma Geology Notes 23: 227–230.
- Rydin C, Pedersen KR, Crane PR, Friis EM. 2006. Former diversity of *Ephedra* (Gnetales): Evidence from Early Cretaceous seeds from Portugal and North America. Annals of Botany 98: 123–140. doi:10.1093/aob/mcl078.
- Saporta GD. 1894. Flore fossile du Portugal. Nouvelles contributions à la flore Mésozoïque. Accompagnées d'une notice stratigraphique par Paul Choffat. Lisbon: Imprimerie de l'Académie Royale des Sciences.
- Singh C. 1964. Microflora of the Lower Cretaceous Mannville Group, East-Central Alberta. Research Council of Alberta Bulletin 15: 1–239.
- Slater BJ, McLoughlin S, Hilton J. 2011. Guadalupian (Middle Permian) megaspores from a permineralised peat in the Bainmedart Coal Measures, Prince Charles Mountains, Antarctica. Review of Palaeobotany and Palynology 167: 140–155. doi:10.1016/j.revpalbo.2011.07.007.
- Stampanoni M, Groso A, Isenegger A, Mikuljan G, Chen Q, Bertrand A, Henein S, Betemps R, Frommherz U, Bohler P, Meister D, Lange M, Abela R. 2006. Trends in synchrotron-based tomographic imaging: The SLS experience. In: Bonse U, ed. Developments in X-ray tomography V. San Diego: Proceedings of SPIE—The International Society for Optical Engineering.
- Sun G, Ji Q, Dilcher DL, Zheng S, Nixon KC, Wang X. 2002. Archaeofractaceae, a new basal angiosperm family. Science 296: 899–904. doi:10.1126/science.1069439.
- Taylor DW, Brenner GJ, Basha SH. 2008. *Scutifolium jordanicum* gen. et sp. nov. (Cabombaceae), an aquatic fossil plant from the Lower Cretaceous of Jordan, and the relationships of related leaf fossils to living genera. American Journal of Botany 95: 340–352. doi:10.3732/ajb.95.3.340.
- Villar de Seoane L, Archangelsky S. 2008. Taxonomy and biostratigraphy of Cretaceous megaspores from Patagonia, Argentina. Cretaceous Research 29: 354–372. doi:10.1016/j.cretres.2007.07.002.