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Species of the water-fern megaspore genus *Molaspora* from a Cenomanian deposit in western France: occurrence, sporoderm ultrastructure and evolutionary relationships

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Abstract
The partially reticulate sculpture of *Molaspora aspera* sp. nov., a marsileaceous megaspore from a Cenomanian deposit in western France, distinguishes it from other species of *Molaspora*. An acrolamella entirely surrounds and obscures a small tetrad scar, a feature that has been demonstrated hitherto within members of the genus only in *M. fibrosa*. It was also encountered for the first time in *M. lobata*, with which the new species is associated in the same French mesofossil assemblage. The ultrastructure of the sporoderm of *M. aspera* is similar to that of *M. lobata*, but differs particularly in that the inner epispore is markedly thicker and may also contain numerous large, homogeneous spherules or, alternatively, holes of comparable dimensions and only a few small spherules. It is possible that these are a response to some hostile bacterial or other activity when the developing sporoderm was partially permeable. The cavity replacing part of the epispore in one of the specimens, and in the specimen of *M. lobata* examined, may be a preservational feature or have served to increase buoyancy of the spore in water. *Molaspora lobata* is very similar to megaspores of fossil and extant *Regnellidium*, but *M. aspera* bears some resemblance to other members of extant Marsileaceae and certain species of Cretaceous *Arcellites*, although there are significant differences between them. This suggests that *Molaspora* is a heterogeneous taxon embracing megaspores produced by water ferns of more than one natural genus, of which only *Regnellidium* has survived to the present day.

Keywords: *Late Cretaceous, Cenomanian, Marsileaceae, megaspores, sporoderm ultrastructure, trilete suture*

The water-fern family Marsileaceae contains three extant genera, *Marsilea, Pilularia* and *Regnellidium*. All are heterosporous. The first two genera are cosmopolitan but *Regnellidium* is found only in a relatively small part of South America (Tryon & Tryon 1982; Batten et al. 2011a; Cúneo et al. 2013; and others). Phylogenetic analyses have indicated that *Pilularia* and *Regnellidium* are sister taxa, and that *Marsilea* is sister to this clade (Pryer 1999; Nagalingum et al. 2008).

Fossil representatives of the family in the form of macrofossil remains and reproductive bodies have been reported with increasing frequency during the past 25 years. Of the former, the earliest record may be as old as latest Jurassic (Yamada & Kato 2002). However, most have been encountered in Albian and younger Cretaceous deposits (e.g. Skog & Dilcher 1992; Nagalingum 2007; Hu et al. 2008; Cúneo et al. 2013; Hermsen et al. 2014), a period during which the heterosporous ferns diversified (Collinson 1991; Kovach & Batten 1993; Pryer 1999). Reproductive organs, especially the dispersed megaspore species *Molaspora lobata*, (Dijkstra 1949) Hall, in Hall et Peake 1968 have been more commonly encountered than vegetative remains. Apart from a couple of Aptian records, *M. lobata* has been recorded most often from deposits ranging in age from Albian to Paleocene, with very similar forms being attributable to species of *Regnellidium* (Kovach & Batten 1989; Batten & Kovach 1990; Lupia et al.

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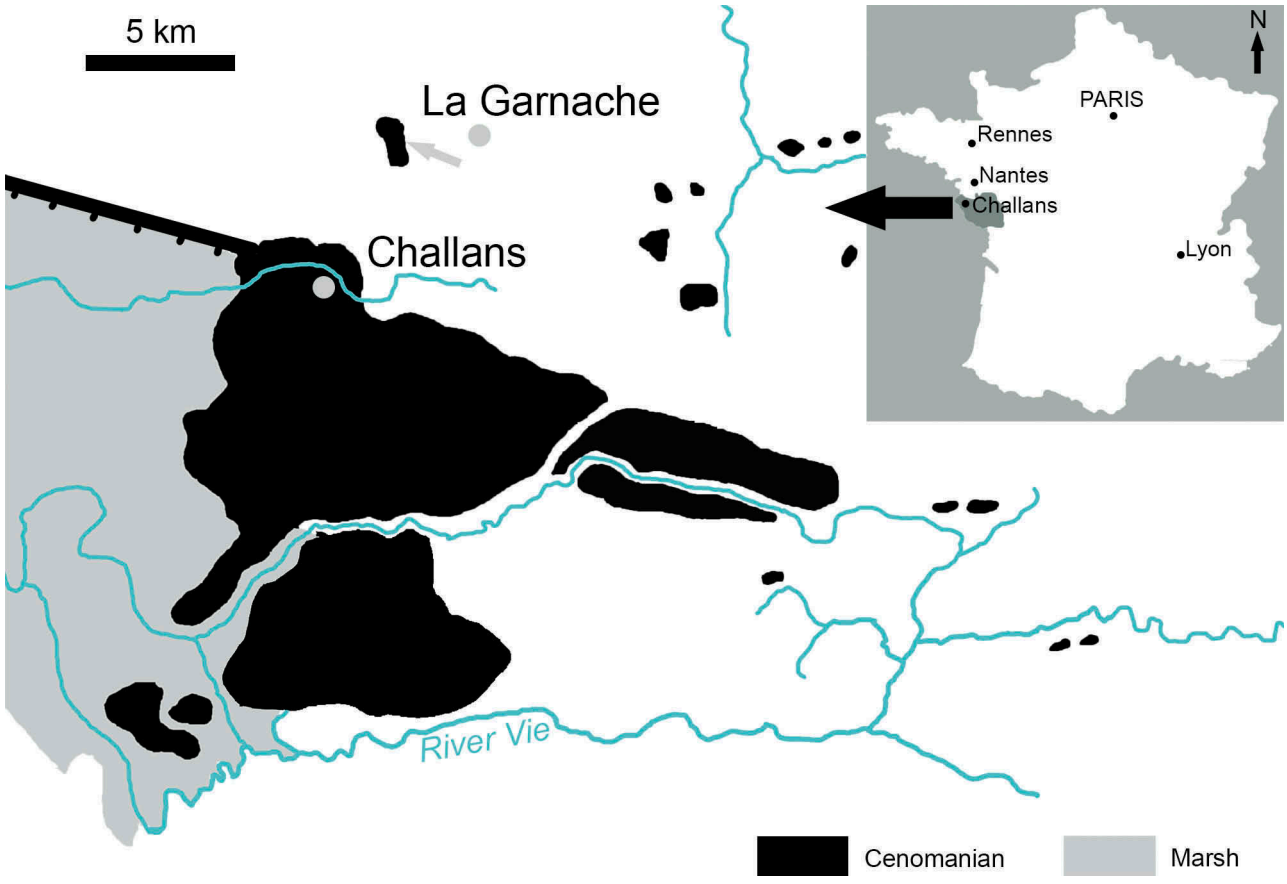

Figure 1. Simplified geological map of part of the Challans-Commequiers Basin showing Cenomanian outcrops and the location of the La Garnache site.

2000; Batten et al. 2010, 2011a, 2011b; Lupia 2011; Cúneo et al. 2013; Friis et al. 2014; Hermsen et al. 2014). It has also been associated with, and found in sporocarps of, Late Cretaceous plant remains identified as species of this genus (Lupia et al. 2000; Cúneo et al. 2013). The ecological significance of sporocarps, which are unique to the Marsileaceae, has been considered by Nagalingum et al. (2006).

Other species of *Molaspora* have been reported only rarely. Of these, none has been found *in situ*, and only *M. salinum* Kovach et Dilcher 1988 has been indirectly associated with marsileaceous remains (see Discussion section). The lack of a close association with marsileaceous macrofossils also applies to the main subject of this article. Specimens of two species of *Molaspora* were recovered along with a variety of other megaspores from a temporary exposure of lignitic clay at a locality known as La Garnache 2 in Vendée, western France (Néraudeau et al. 2017). This locality and another in close proximity (Figure 1) have been investigated previously for their palynological content and abundant faunal and other inclusions in amber (Néraudeau et al. 2017). One of the species was identified as *M. lobata*, the other was recognised as new. Emphasis is placed here on the

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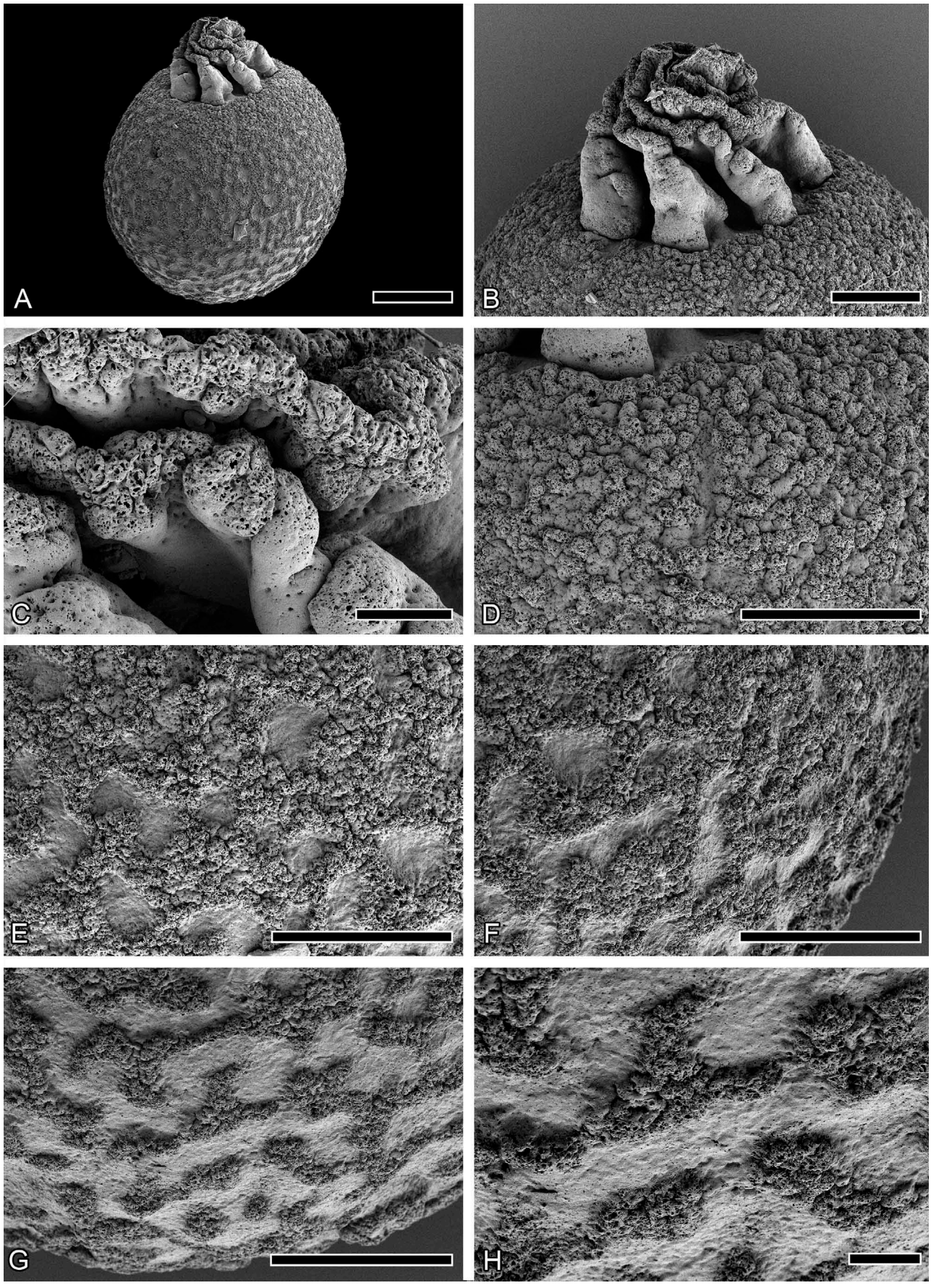
Figure 2. Gross morphology and surface sculpture of *Molaspora aspera* sp. nov., holotype, SEM, stub DJB2014/4, specimen 11, IGR-PAL-5754. **A.** Whole specimen; background rendered black using Adobe Photoshop CC. **B.** Close-up of acrolamella and surrounding sculpture of outer epispore, which consists mainly of closely packed bulbous elements, but also includes a few scattered lumina of irregular shape. **C.** Detail of the tops of lobes of the acrolamella close to the proximal pole showing their open, vacuolated appearance. **D.** Close-up of the sculpture near the acrolamella; the closely packed bulbous elements have a strongly perforated, vacuolate appearance. **E.** Close-up of sculpture on the proximal face towards the equator showing lumina of irregular shape between clusters of perforated bulbous elements. **F.** Close-up of sculpture of distal face just below the equator. **G.** The reticulation has broken down close to and around the distal pole; instead the sculpture consists of irregular masses of similarly perforated, loosely structured bulbous elements. **H.** Detail of part of (**G**). Scale bars – 100 μm (A), 50 μm (B, D–G), 10 μm (C, H).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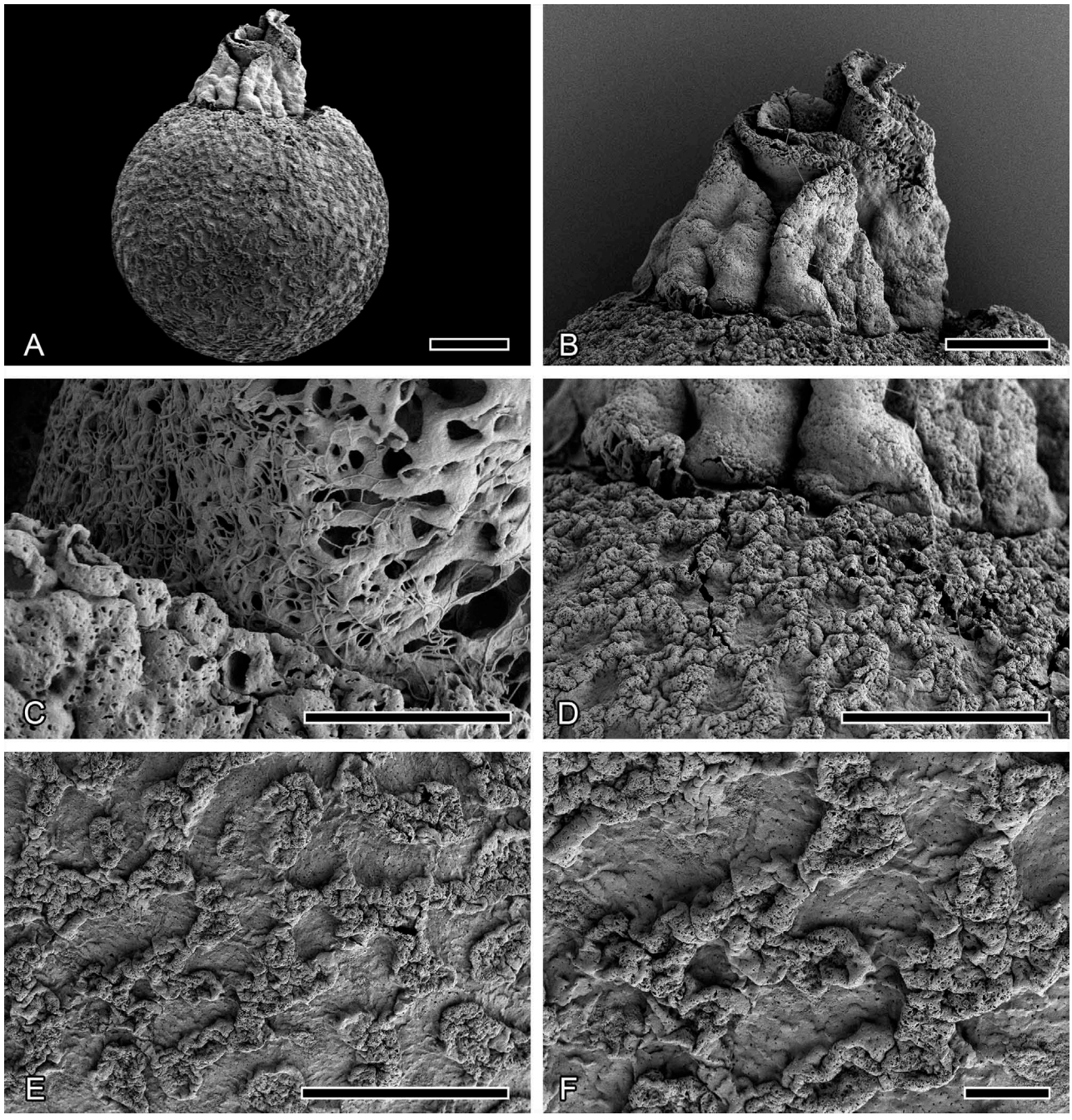


Figure 3. Gross morphology and surface sculpture of *Molaspora aspera* sp. nov., SEM, stub DJB2014/4, specimen 25, IGR-PAL-5755. **A.** Whole specimen; background rendered black using Adobe Photoshop CC. **B.** Close-up of acrolamella and surrounding sculpture of outer epispore. **C.** Detail of the upper part of two folds of the acrolamella showing very open construction and many narrow threads of sporopollenin on the inside of one of the folds. **D.** Sculpture adjacent to the acrolamella of this specimen consists of not only rounded and irregularly shaped bulbous elements but also small lumina. **E.** Detail of irregularly reticulate sculpture in equatorial region. **F.** Part of the same area at higher magnification. Scale bars – 100 μm (A), 50 μm (B, D, E), 10 μm (C, F).

latter. We describe not only its gross morphology but also its ultrastructure. Use of both a scanning electron microscope (SEM) and a transmission electron microscope (TEM) is invaluable in morphological analyses of megaspore wall layers. Hence, six specimens of the new species were examined in this way along with a single specimen of *M. lobata* for comparison.

Material and methods

The megaspore assemblage was extracted from the sedimentary matrix in stages, the first of which was initial soaking of rock fragments in a flask of warm water on a hot plate followed by repeated immersion in warm *c*. 5% $Na_4P_2O_7$ (sodium pyrophosphate) and washing on a sieve with a mesh size of 85 μm over a period of several days to reduce the bulk of the sample. The residue was then subjected to the standard palynological procedure of brief treatment in 10% hydrochloric acid (HCl) to remove any carbonates and more prolonged digestion in hydrofluoric acid (HF) to remove silicates before being washed and sieved again. The megaspores and other mesofossils in the organic matter recovered were picked out individually from the aqueous residue using a fine paint brush. All were stored dry in micropalaeontological slides for examination under a stereo-microscope. Two specimens of the new species were selected for examination under an SEM (Jeol JSM 840 SEM) in Aberystwyth University. These were mounted on adhesive carbon tabs fixed to stubs and coated with gold-palladium. Four specimens along with several representatives of *Molaspora lobata* were dispatched to Moscow for ultrastructural analysis.

The inner sporoderm structure of these four specimens was studied and compared with that of one specimen of *Molaspora lobata*. Observations were accomplished in halves of spores in reflected light (Leica MZ16 stereomicroscope in the Palaeontological Institute of the Russian Academy of Sciences: PIN), in semithin sections in transmitted light (Axioplan 2 Zeiss microscope: PIN) and under an SEM (TESCAN SEM: PIN), and in ultrathin sections under a TEM (Jeol JEM–1011 TEM, Laboratory of Electron Microscopy, Lomonosov Moscow State University: MSU). All specimens were examined under transmitted and reflect light microscopes (LMs), one under the SEM, and three under the TEM. The methods are after Zavialova and Karasev (2017) with one modification: a Pipetman micropipette was used to handle epoxy-free semithin sections instead of an eyelash attached to a toothpick.

One representative of the new species was cut more or less perpendicular to its polar axis at several levels: at the acrolamella, at the acrolamella/spore body junction, and within the body of the megaspore. Another was also cut perpendicular to the polar axis in the distal-equatorial area. Two, along with a single specimen of *Molaspora lobata*, were cut in half with a razor blade, and the two halves embedded separately. Blocks enclosing the proximal hemispheres were oriented so that the plane of sectioning was parallel to the polar axis and the sections passed through the apertural area. A portion of the distal sporoderm was also cut more or less perpendicular to the polar axis.

The specimens of *Molaspora aspera* that were examined under the SEM in Aberystwyth are housed in the Geological Institute of the University of Rennes 1. Remains of polymerized resins with embedded megaspore remains, grids with ultrathin sections, files of LM photographs, and SEM and TEM micrographs are retained in the Laboratory of Palaeobotany, Palaeontological Institute, Moscow. Copies of all figured materials are also deposited in the collections of the Geological Institute of the University of Rennes 1.

Systematic palynology

Terminology

The terminology used here for the sporopollenin wall layers is after Tryon and Lugardon (1990). Their exospore and epispore is equivalent to exine and perine, respectively, which were used by Batten et al. (2011a, 2011b), and to the terminology of Schneider and Pryer (2002), who referred to exine and inner and outer sublayers of solid perine (equivalent to our inner and outer epispore), these perine sublayers being distinguished from the inner and outer sublayers of gelatinous perine in fresh specimens, which are not fossilised. In common with all of these authors, we regard an acrolamella as an apical aggregation of leaf-like or lobed, commonly twisted, segments that enclose the germinal area, which may or may not show a triradiate suture (for a discussion of the inconsistent application of the term acrolamella, see Batten et al. 2011b).

Genus *Molaspora* Schemel 1950, emend. Hall 1963

Type species. — *Molaspora lobata* (Dijkstra 1949) Hall, in Hall et Peake 1968

Molaspora aspera sp. nov.

Material. — Mesofossil preparation MFP508, La Garnache 2 exposure, Vendée, western France.

Derivation of name. — L. *asper*, rough; after the slightly rough surface of the body of the megaspore as seen under a reflected LM.

Holotype. — SEM stub DJB2014/4, specimen 11, Figure 2.

Repository. — The figured material has been deposited in the Geological Institute of the University of Rennes 1 under collection numbers IGR-PAL-5754 and IGR-PAL-5755.

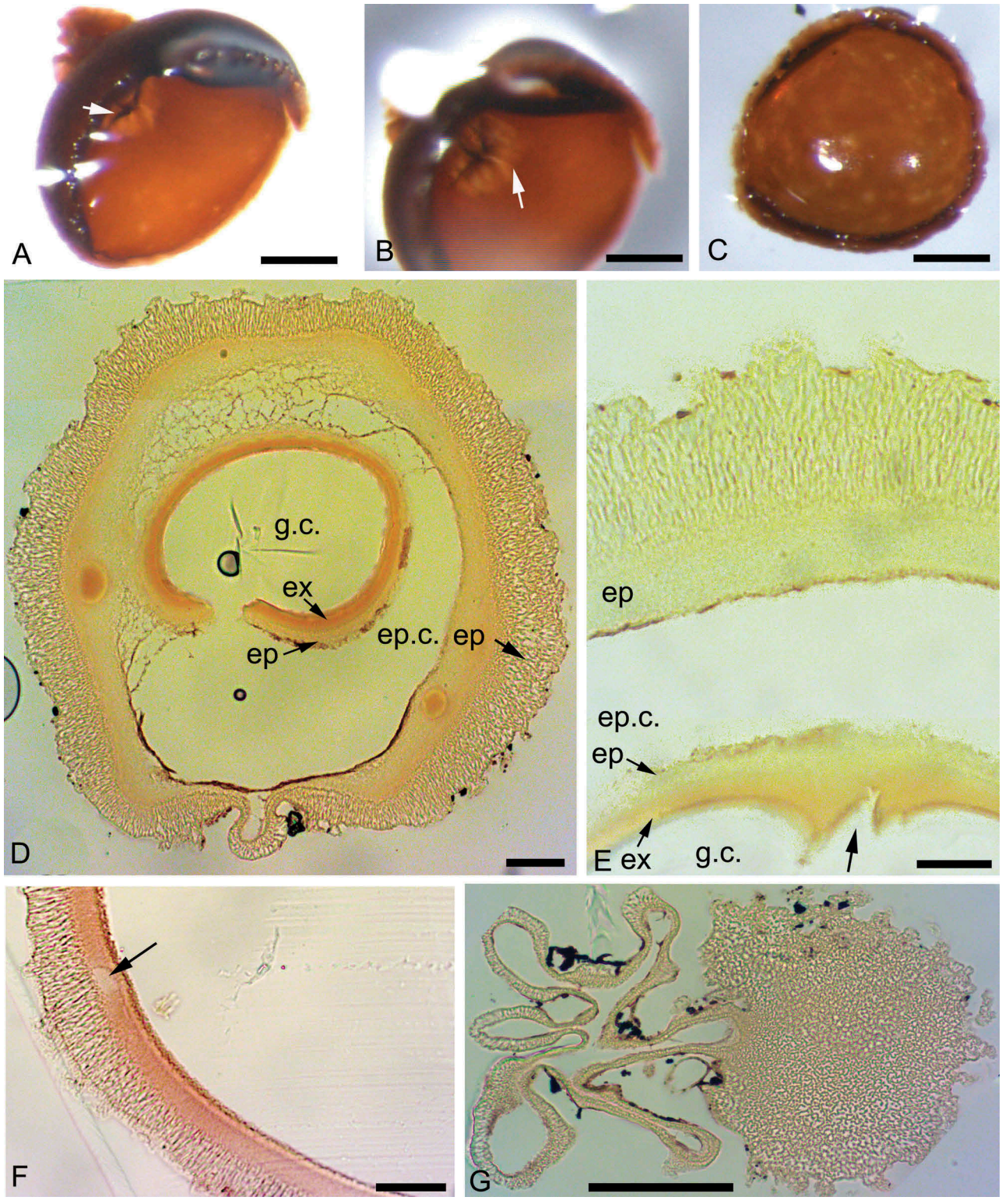


Figure 4. *Molaspora aspera* sp. nov., LM. **A.** Specimen #40, proximal half of the megaspore, interior view; a trilete scar is visible (arrow), reflected light. **B.** Same specimen, tilted, two of the arms of the proximal scar are in shadow and appear dark whereas the third arm is not and appears light (arrow), reflected light. **C.** Same specimen, distal half of the megaspore, interior view; holes in the epispore are visible as light spots, reflected light. **D.** Specimen #30, semithin section; note gametophyte cavity (g.c.), exospore (ex), a cavity within the epispore (ep.c.), and spherules in the epispore (ep), transmitted light. **E.** Specimen #30, an area of a semithin section, a slit (arrow) in the exospore (ex) may represent an obliquely cut arm of the proximal scar; gametophyte cavity (g.c.) and a cavity (ep.c.) within the epispore (ep) are visible, transmitted light. **F.** Specimen #40, an area of a semithin section in the distal hemisphere; note hole (arrow) in the epispore, transmitted light. **G.** Specimen #30, semithin section, acrolamella and proximal part of the megaspore, transmitted light. Scale bars – 100 μm (A–C), 20 μm (D, E), 10 μm (F), 50 μm (G).

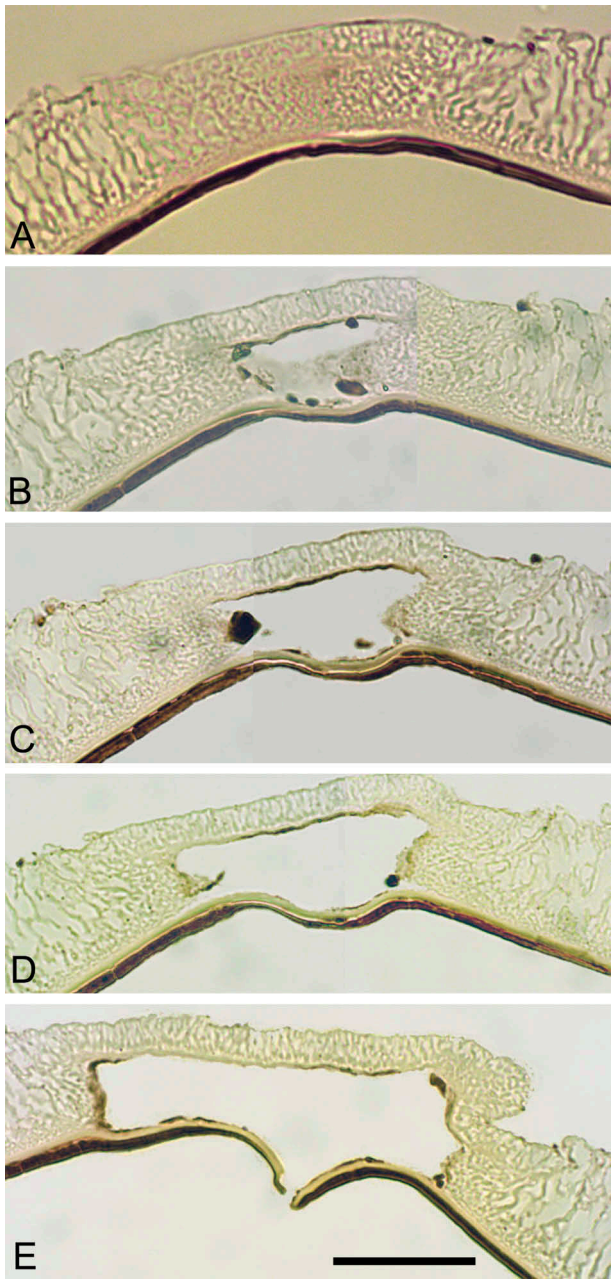


Figure 5. *Molaspora lobata*, specimen #32, semithin sections, LM. **A–E.** Sequence of sections towards and through the proximal scar. Scale bar – 20 μm.

Type locality. — La Garnache 2, near Challans, Vendée, north-western France: for details, see Néraudeau et al. (2017).

Type stratum and age. — Lignitic clay series, Cenomanian.

Diagnosis. — A species of *Molaspora* with a prominent acrolamella, the twisted lobes of which become more open in structure towards its apex: it completely surrounds and hides a small trilete suture. Sculpture of spore body consists of irregularly subcircular to somewhat meandering bulbous elements that are usually tightly packed close to acrolamella but arranged to form a reticulate pattern with increasingly wide lumina towards equator and onto distal face. This reticulation commonly breaks down close to and around distal pole where instead sculpture consists of irregular masses of similarly bulbous aggregations. Both individual and aggregations of sculptural elements have an open structure that gives them a perforated to vacuolated appearance. They are formed from elevations of outermost epispore over an alveolate outer epispore.

Description. — Body of spore spherical to subspherical (Figures 2A, 3A), 330–508 μm in diameter (18 specimens). Acrolamella (Figures 2B, 3B) 100–160 μm in basal diameter, 100–130 μm high; comprises six lobes that become narrower and have an increasingly perforate, more open structure upwards (Figures 2C, 3C); base of lobes *c.* 40–60 μm wide. Bulbous sculptural elements consist of irregular perforated to vacuolated mounds *c.* 7–10 μm in diameter and *c.* 5–8 μm high (Figures 2D–H, 3D–F); when forming muri of irregular reticulum, muri typically *c.* 7–10 μm wide and lumina up to 50 μm in maximum diameter (Figures 2E, F, 3D–F); when not forming a reticulum the bulbous elements form irregular aggregations that vary considerably in shape and extent (Figure 2G, H).

Remarks. — The acrolamella has a twisted, lobate rather than the more leaf-like appearance in, for example, many specimens of *Molaspora lobata* (e.g. in Batten et al. 2011a, figure 4C, D and Cúneo et al. 2013, figure 4A, B), but not all (e.g. Tosolini et al. 2002, figure 9R, S). The exospore aperture is completely hidden by the acrolamella, but we observed a proximal scar in reflected light during the course of embedding the megaspores that were cut in half. It is visible from the inside of the proximal hemisphere: the arms are about 50 μm long (Figure 4A, B). Its presence was confirmed in sections that were oriented parallel to the polar axis of the spores. Hitherto, morphological data in the literature provide unequivocal evidence of the presence of an exosporal aperture in only one species of *Molaspora* (*M. fibrosa* Singh 1983), and it has yet to be demonstrated in megaspores of closely similar extant and fossil *Regnellidium*. Our study illustrates clearly the exospore aperture not only in this new species but also, for the first time, in *M. lobata* (Figure 5A–E), all sections of which fortunately passed through one of the rays of the scar. We were not lucky enough to have two of the rays cut by one section.

Five species of *Molaspora* megaspores have been erected previously. *Molaspora lobata*, the type species of

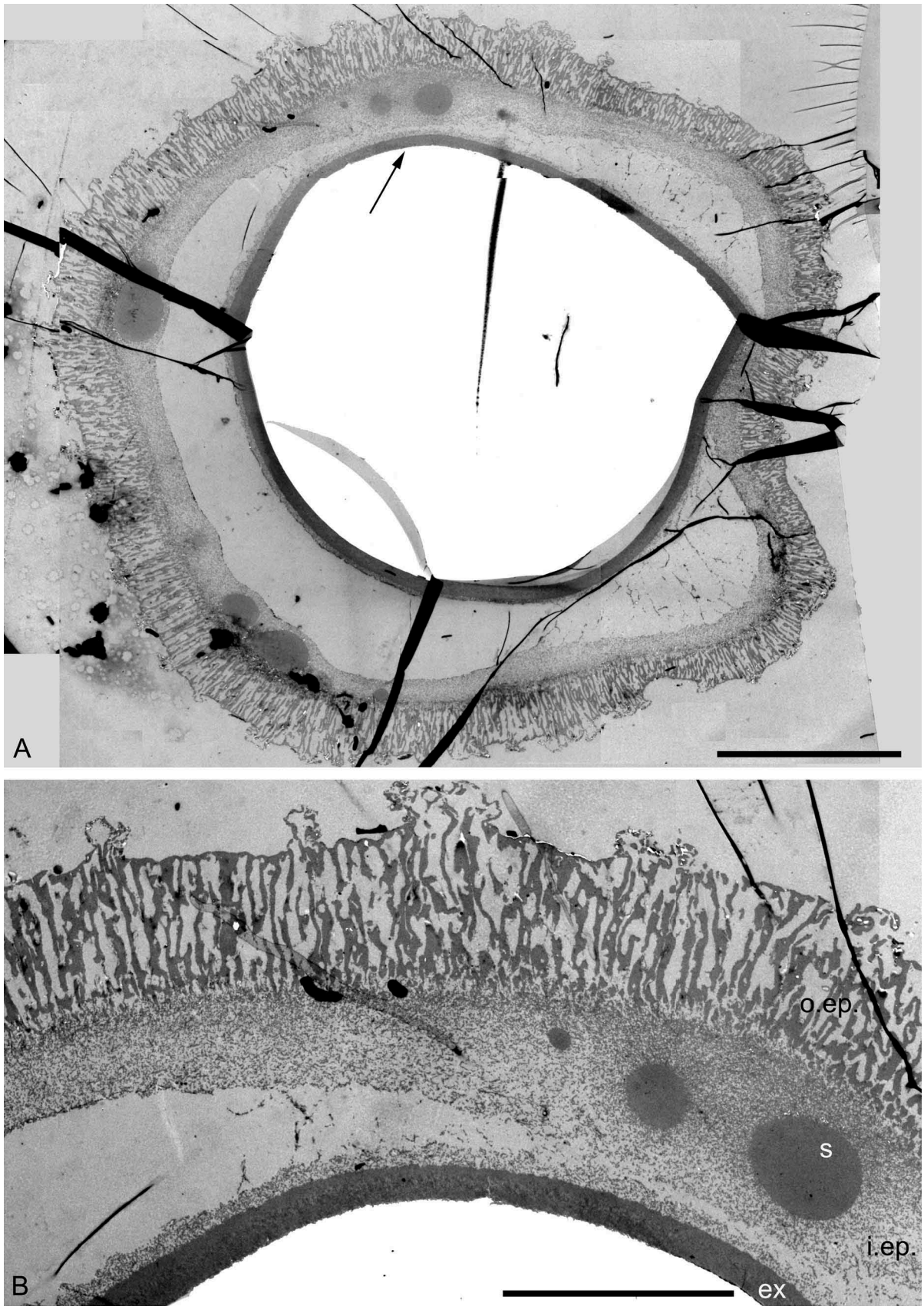


Figure 6. *Molaspora aspera* sp. nov., specimen #30, ultrathin sections, TEM. **A.** Montage of an ultrathin section in the proximal-equatorial area of the megaspore; arrow indicates the position of the enlarged area. **B.** Enlargement of (**A**): ex, exospore; i.ep., inner epispore; s, spherule; o.ep., outer epispore. Scale bars – 50 μm (A), 20 μm (B).

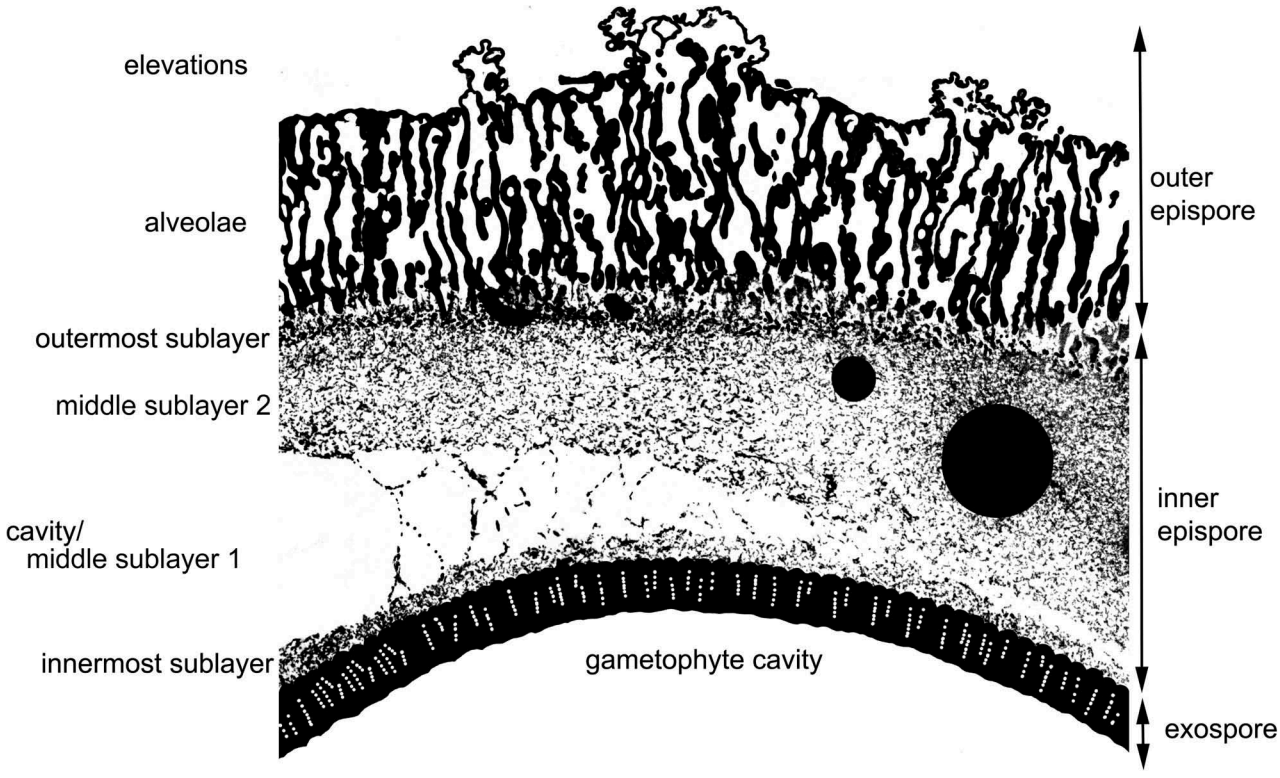


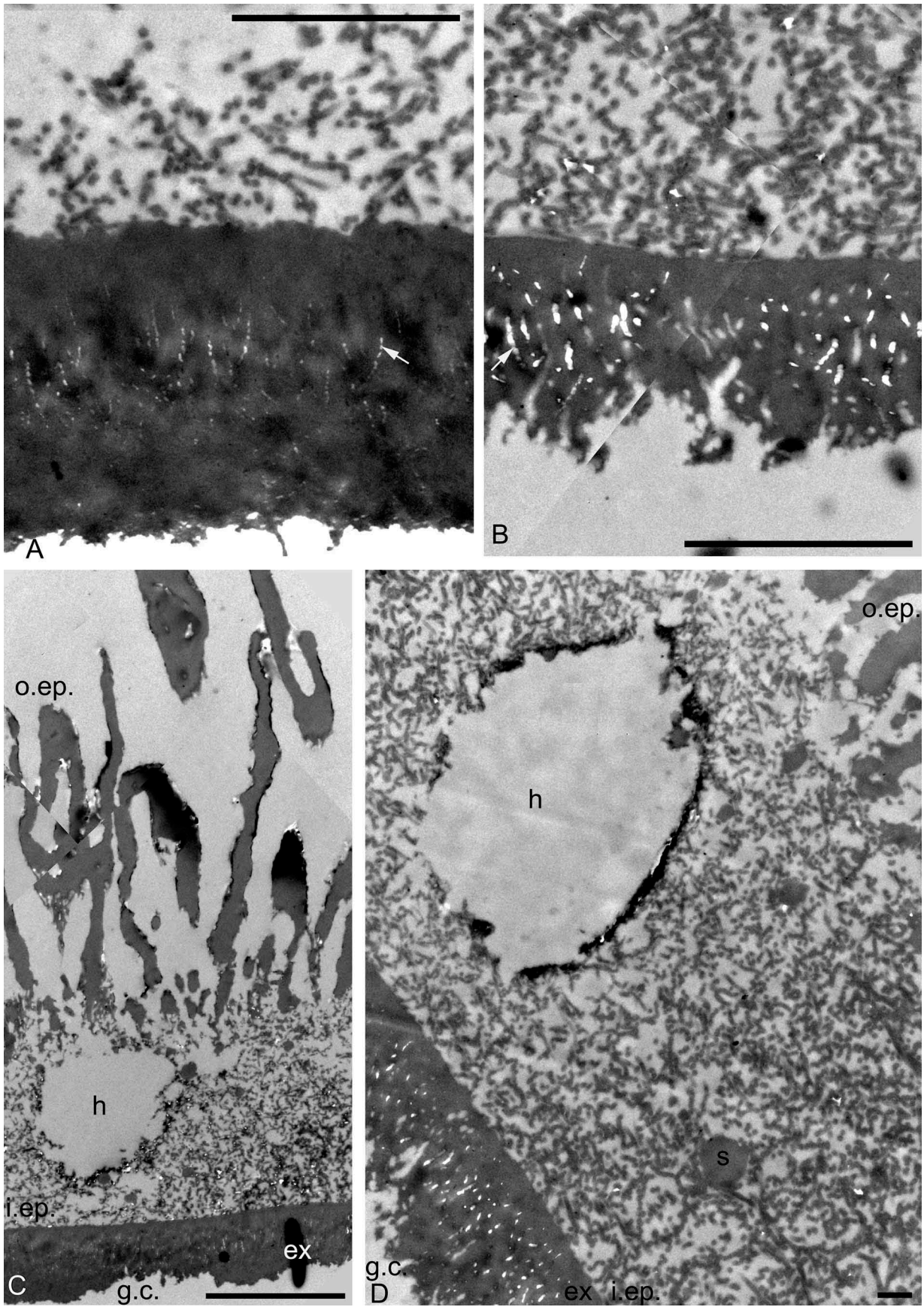
Figure 7. *Molaspora aspera* sp. nov., schematic representation of the sporoderm ultrastructure.

the genus, has been most widely reported, and its morphology and similarity to modern *Regnellidium* spores have been discussed in some detail (Batten 1988; Lupia et al. 2000; Takahashi et al. 2001; Batten et al. 2011a, 2011b; Cúneo et al. 2014). All have shown that beneath the sculpture of irregularly shaped granules and verrucate to papillate elements there is a thick prismatic or alveolate zone, the majority of the sporopollenin elements being oriented normal to the surface. This extends upwards from a more compact meshwork of sporopollenin elements, and is clearly visible in thick sections and damaged specimens under the SEM. Beneath this outer wall (epispore/perine) is a thin inner layer (exospore/exine) that may sometimes be partly detached (see Batten et al. 2011b). The ultrastructure of *M. aspera* is similar, though not identical, to that of *M. lobata* (see later) in that the inner epispore is thicker: the inner epispore/total epispore thickness ratio is about 0.46 in *M. aspera* whereas it is about 0.19 in *M. lobata*. *Molaspora aspera* also differs from *M. lobata* in having a partially reticulate sculpture.

Other, previously published, species of *Molaspora* differ from *M. aspera* in the following ways: *M. fibrosa* was described as having finely fibrous perine and sculpture of dense fibrous matting (Singh 1983, p. 95). Although not clearly discernible in his photographs, it is clear that the sculpture differs from that of *M. aspera*. *Molaspora hallii* (Lachkar, in Floquet et Lachkar 1979) Batten 1988 lacks a surface ornament, although as noted by Batten (1988), this may be a result of degradation of the body wall, because the specimens appear to be rather poorly preserved. The spore body of *M. reticulata* Campbell et Untergasser 1972 has a reticulate sculpture, but the muri are uniformly very high and narrow (up to 28 μm and 1–2 μm, respectively according to Campbell & Untergasser 1972) and surround lumina 10–15 μm in diameter. *Molaspora salinum* is also reticulate, but unlike *M. aspera* the reticulum extends over the whole of the spore body, the muri are shorter, more pronounced, of very different construction, and surround smaller lumina. Furthermore, its sculpture is developed from either the entire outer epispore, which does not appear to be alveolate, or the entire epispore (cf. Kovach & Dilcher 1988, plate 2, figure 11). The sculpture of all of the other species of *Molaspora*, as well as the megaspore of *Regnellidium*, is developed from the outermost zone of the epispore.

Ultrastructure

The sporoderm consists of an exospore (exine) and an epispore (perine) (Figures 4D, E, 6A, B, 7, 11B). The thickness of the exospore is relatively constant in each section, but it is thicker proximally than distally (2.5–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2.7 μm and 1.6–1.9 μm, respectively) and can reach 3.1–4.5 μm in sections made relatively close to the proximal pole. It appears homogeneous in semithin sections under the SEM (Figure 9E, G) and under low magnification in ultrathin sections in the TEM (Figure 6A), but highly magnified TEM ultramicrographs show that the middle of this layer contains narrow interrupted channels mostly 0.02–0.03 μm, but occasionally up to 0.05 μm wide, which are directed more or less perpendicular to the surface of the sporoderm and occupy about 1.2–1.7 μm of the thickness of this layer (Figure 8A, B). The inner surface of the exospore is slightly uneven and in two specimens it appears torn, with more distinct channels that extend to this surface (Figure 8B).

The epispore is at least four times as thick as the exospore, and often much thicker (Figures 4D, E, 6A, B, 7). The innermost portion is firmly attached to the outer surface of the exospore (Figures 4D, E, 6B, 7, 9E, G). The inner layer consists of a complex of short, fine, variously directed threads that are more densely packed adjacent to the exospore than away from it (Figures 7, 9F–H). The threads are about 0.06 μm in diameter and often appear as circular elements in ultrathin sections when cut transversely (Figure 8A, B). Semithin sections prove that they are threads (Figure 10F). This is also evident from the co-occurrence of circular and variously elongated sections of these elements in ultrathin sections (Figure 8A, B). The inner layer is *c.* 7 μm thick and not clearly layered in regions where the epispore is thinnest, but elsewhere it is divisible into four sublayers (Figure 7). The innermost of these is *c.* 1.5–2.9 μm thick, above which is a sublayer 2.4–29.8 μm thick that consists of the same structural elements but very loosely arranged (middle sublayer 1: Figures 7, 9E, H, 10D): in one specimen, it is partly replaced by a cavity of comparable thickness over a considerable part of the sporoderm (Figure 9D).

The overlying sublayer (middle sublayer 2: Figure 7) is similar to the inner sublayer in its construction but more variable in thickness (5.8–14.7 μm), and in one specimen it contains numerous large spherules (Figures 6B, 7, 9C, 10E) that occasionally reach 14 μm in diameter. At least seven (and up to 12) spherules were observed in each section of this specimen. We are sure that they are spherules because they vary greatly in diameter, but always have circular outlines. Their ultrastructure seems to be entirely homogeneous even under very high magnifications (Figure 6B).

In the same sublayer of two of the other specimens studied there are holes of comparable dimensions (Figure 4C, F), but they are much less numerous than spherules, and their outlines are more irregular (Figure 8C). A rim that demarcates the holes can be present (Figure 8C). A few small spherules 0.4–1 μm in diameter were found in these specimens (Figure 8D). The fourth specimen examined lacks both spherules and holes in this layer.

The outermost sublayer is thin (*c.* 1 μm). It also comprises threads, but these are more densely packed than those of the underlying sublayer.

The outer epispore is 6–14.3 μm thick and regularly alveolate (Figures 4E, 7, 10B). The alveolae are directed more or less perpendicular to the sporoderm surface, although they can be partly fused together and branched (Figures 6B, 9E, 10D). The partitions are 0.2–0.7 μm thick and lined with thin threads (Figure 10A). There are elevations over this layer that consist of slightly thinner partitions (Figures 6B, 7, 10A, B). These form the surface sculpture of the spore.

With the exception of its outermost sublayer, the inner epispore wedges out at the proximal pole, and the outer epispore and the outermost sublayer of the inner epispore become elevated to form an acrolamella (Figures 10C, 11C). This surrounds an exosporal tetrad (trilete) scar. The exospore ruptures along the arms of this scar (Figure 11A). The presence of the outermost sublayer of the inner epispore on the acrolamella is evident in semithin sections observed under the SEM: threads of this sublayer line the inner surface of the acrolamella (Figure 9A, B). The outermost sublayer of the inner epispore disappears in peripheral areas of the lobes of the acrolamella lobes (Figure 11C, D). There are six lobes (Figures 4G, 9A, 11D), the thinnest portions of which are situated close to the centre of the acrolamella, where they are about 1.4 μm thick. The lobes become thicker towards their periphery, reaching a thickness of about 5.5–6 μm (as measured in areas of the lobes that were cut more or less perpendicularly).

Discussion

The exospore aperture

Many descriptions of *Molaspora* in the literature do not indicate whether an exosporal aperture is present or absent. Instead it is usually simply reported as being

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Figure 8. *Molaspora aspera* sp. nov., ultrathin sections, TEM. **A.** Exospore and the innermost layer of the epispore; arrow indicates one of the channels in the exospore, specimen #30. **B.** Exospore, which appears torn in this specimen, and the innermost layer of the epispore; arrow indicates one of the channels in the exospore, specimen #40. **C.** Area of a section with a hole (h) in the inner epispore (i.ep.); gametophyte cavity (g.c.) and outer epispore (o.ep.) are partly visible, specimen #38. **D.** Area of a section showing a hole (h) and two small spherules (s) in the inner epispore (i.ep.); gametophyte cavity (g.c.); exospore (ex); and outer epispore (o.ep.) are also partly visible, specimen #38. Scale bars – 2 μm (A, B), 5 μm (C), 0.5 μm (D).

surrounded by a short, convoluted neck or other similar descriptions of the acrolamella (e.g. neck-like elevation in Dijkstra 1961). Takahashi et al. (2001, p. 432) noted that the aperture is 'circular in outline and surrounded by a terminal acrolamella', the whole of the apertural region 'forming a pore-like structure'. Singh (1983, p. 94) considered *Molaspora* to bear a small trilete germinal aperture, but did not show this in his photographs of *M. lobata*. In his description of this species Batten (1988) mentioned the presence of a triradiate suture, but again did not illustrate it, nor did, for example, Tosolini et al. (2002) or Lupia (2011). This is because it is usually completely hidden by the acrolamella. In his brief description of *Molaspora* cf. *lobata*, Colin (1975) noted, somewhat surprisingly, that a monolete mark is barely visible, but the single specimen he encountered and figured (Colin 1975, plate 1, figure 8) does not seem to show this. The proximal pole of the specimen he identified as *Hughesisporites galericulatus* (Dijkstra 1951) Potonié 1956, but which in fact is referable to *Molaspora*, is surmounted by an acrolamella, so no germinal is seen.

Lupia et al. (2000, figure 3.17) showed a transmitted light image of *Molaspora lobata* in the vicinity of the acrolamella. The sporoderm is greatly thinned in this area, but no trace of a trilete scar is visible. However, they noted (p. 981) that 'Viewed from the outside, the trilete mark is apparently hidden by the acrolamella.' Batten et al. (2010, plate 4, figure 8) figured a specimen of this species that is missing its acrolamella. The exospore beneath is crumpled, but no trilete mark is discernible.

Hence, hitherto a trilete suture has not been demonstrated in this species or in closely related fossil or extant *Regnellidium*. The proximal germinal structure in *Regnellidium* has been referred to as an apical papilla (Chrysler & Johnson 1939), and described as short and papilla-like (e.g. Tryon & Lugardon 1990, p. 359). Chrysler and Johnson (1939, figures 29, 33) published a drawing of a longitudinal section of a megaspore of modern *R. diphyllum* Lindm.: a thinned exospore (they named it the inner layer of the epispore) is visible in the area of the proximal pole, but no arms of a triradiate scar are indicated. They wrote that this layer does not exist in the area of the 'papilla'. Schneider and Pryer (2002, figure 8f) showed an SEM micrograph of a thick section of a *R. diphyllum* megaspore in a similar area. This indicates that the exospore is present over the pole, but no arms of a triradiate mark are visible. Nevertheless, although not demonstrated, they stated that the megaspores of this and other marsileacean species are trilete (Schneider & Pryer 2002, p. 499).

The lack of any reference to a trilete suture in *Molaspora hallii* (apex of spore consisting of several membranous layers in the form of a fan more or less surrounding or twisted around an orifice: loosely translated from Floquet & Lachkar 1979, p. 144), *M. reticulata* ('proximal pole surmounted by prominent small convoluted acrolamellar structure or neck': Campbell & Untergasser 1972, p. 2555) and *M. salinum* ('Aperture surrounded by a short, convoluted neck': Kovach & Dilcher 1988, p. 96) suggests that it was not seen because either it was obscured by the acrolamella or no evidence of its presence could be detected. However, Singh (1983, plate 26, figure 7) illustrated a trilete mark beneath the acrolamella of a specimen of *M. fibrosa*.

The orientation of the sections of the first specimen of *Molaspora aspera* to be examined ultrastructurally was not strictly perpendicular to the polar axis. Hence, sections show some lobes of the acrolamella and the epispore, but no exospore or gametophyte cavity (Figure 9A). Some sections show an interrupted exospore relatively close to the proximal pole (e.g. Figures 4E, 9G). This gap may represent a section of an opened arm of the proximal scar; however, it is also likely that the interruption of the exospore, which is partly detached from the bulk of the epispore and folded, is because the top of a fold was cut. Tryon and Lugardon (1990, figure 221.12) showed a similar slit in the outer exospore of a megaspore of *Marsilea strigosa* Willd. (*Marsilea pubescens* Ten. in Lugardon & Husson 1982, figure 6). These observations demonstrate the difficulty of recognising a trilete suture in marsileaceous megaspores. Our later sections of *Molaspora aspera* and *Molaspora lobata* were more successful in that we were able to prove its existence, as described earlier.

Lugardon and Husson (1982) stated that the apertural organisation of exospores of megaspores of water ferns is always similar to that of microspores. However, the only member of the Marsileaceae they examined in their paper was identified as *Marsilea pubescens*. Tryon

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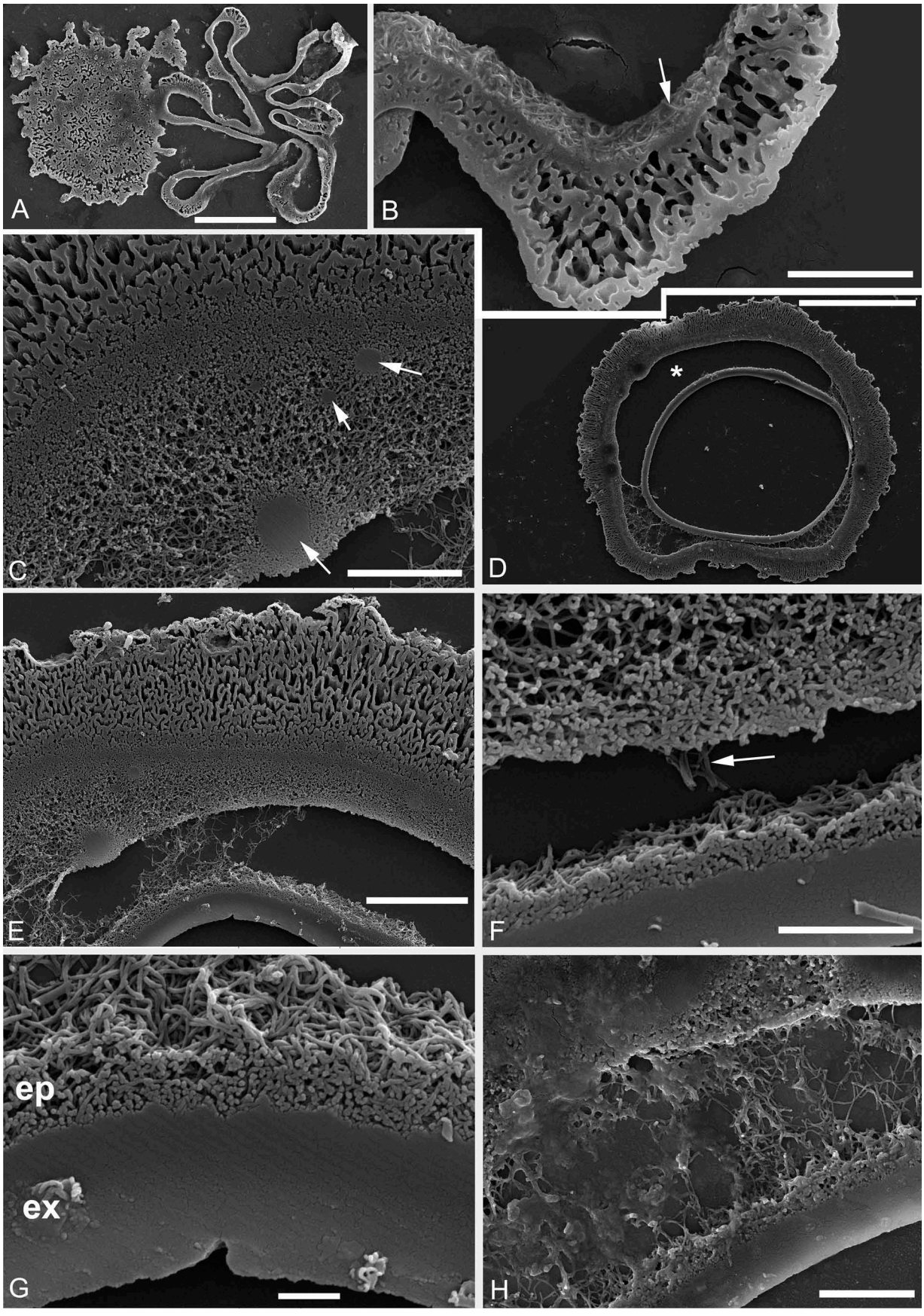
Figure 9. *Molaspora aspera* sp. nov., specimen #30. Epoxy-free semithin sections, SEM. **A.** Section in the area of the junction between the acrolamella and body of the spore; six lobes of the acrolamella are cut. **B.** Lobe of acrolamella; alveolate infrastructure is visible as well as the internal surface formed by threads (arrow). **C.** Enlargement of (**E**) in the area of the epispore, larger and smaller spherules are visible (arrows). **D.** Body of the spore sectioned; a cavity is present within the epispore (asterisk). **E.** Section of the spore through the exospore and epispore. **F.** Splitting within the epispore; threads are evident as structural elements (arrow). **G.** Enlargement of (**E**) showing the exospore (ex) and the innermost sublayer of the epispore (ep). **H.** Very open sublayer (middle sublayer 1) of the epispore. Scale bars – 50 μm (A), 10 μm (B, C, H), 100 μm (D), 20 μm (E), 5 μm (F), 2 μm (G).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and Lugardon (1990) defined the aperture of megaspores of not only *Regnellidium* (see earlier) but also *Marsilea* and *Pilularia* as papilla-like.

Chrysler and Johnson (1939) reported that a tetrad of megaspores is formed during the ontogenesis of *Regnellidium diphyllum*, three members of which then abort, and the mature megaspore does not bear a proximal scar. Turnau et al. (2009) studied seed-megaspores of *Granditetraspora zharkovae* Arkhangelskaya et Turnau emend. Turnau et Prejbisz, which can be preserved as permanent tetrads with one functional and three underdeveloped spores, and as monads with one functional spore. TEM data show that the functional megaspore retains a proximal scar (Turnau et al. 2009, plate 7, figure 1). These two examples show that a proximal scar is sometimes retained and is sometimes missing from mature megaspores when a solitary megaspore becomes mature in the sporangium.

Significance of spherules and cavity within the epispore

Spherules have never been reported from megaspores of related or any other taxa. Their presence in one specimen of *Molaspora aspera*, their presumed analogues, holes, in two specimens that contain only a few, much smaller spherules, and the absence of both from the fourth specimen, suggest that these structures are secondary developments that took place either when the plants were alive or on fossilisation. One possibility is that they are a result of local secondary homogenisation of the sporoderm, although such a modification has not been reported previously in any megaspore morphotype. Framboidal pyrite (cf. Batten 1985, plate 1) could explain the irregular holes, but there is no evidence of pyrite crystals or their relict structures in the specimens.

When alive, the spherules could have been structures that armoured or strengthened the relatively loose sporoderm, but they are not present in all of the specimens examined. The rims around the holes might suggest a protective reaction of the sporoderm to some hostile activity, such as bacterial or fungal infection. Since both the spherules and the holes are present in the middle layer of the sporoderm rather than on its surface, we suggest that the modifications of the wall took place when the developing sporoderm was partially permeable.

The cavity replacing the middle part of the epispore in one of the four specimens of *Molaspora aspera* sectioned (Figure 7), and also observed in the specimen of *M. lobata* examined, may be a preservational feature or have served to increase buoyancy of the spore in water.

Significance of ultrastructural characters for comparison with other species of the genus and morphologically close taxa

Our data on the sporoderm ultrastructure of *Molaspora aspera* and *M. lobata*, as well as available data in the literature on the inner structure of most members of *Molaspora* and of fossil and extant *Regnellidium* (e.g. Tryon & Lugardon 1990; Batten et al. 2011a), show that it is similar in these taxa. The epispore in all cases can be subdivided into outer and inner layers. The outer epispore consists of alveolae, most of which are directed normal to the surface. They are much less densely packed than the elements of the inner epispore, which is composed of much thinner, irregularly arranged threads.

The outer epispore usually clearly shows regular, elongated alveolae in both ultrathin sections under the TEM and semithin and thick sections under the SEM (e.g. *Molaspora hallii*, Floquet & Lachkar 1979, plate 3, figures 11, 12; *M. lobata*, Takahashi et al. 2001, figure 1F; *Regnellidium upatoensis*, Lupia et al. 2000, figure 2.8; *R. diphyllum*, Schneider & Pryer 2002, figure 8F). This character was not detectable when the sections were oblique and passed through the distal portion of the megaspore (e.g. *R. diphyllum*, Tryon & Lugardon 1990, figure 222.6; *M. salinum*, Kovach & Dilcher 1988, plate 2, figures 11, 12).

As a rule, the surface sculpture is formed by the outermost portion of the outer epispore. The only exception appears to be *Molaspora salinum*: as noted earlier, its coarse sculpture seems to be formed by the outer, or even the entire, epispore (Kovach & Dilcher 1988, plate 2, figure 11).

The inner epispore is developed to varying degrees in several of these taxa. It is thicker in *Molaspora aspera* than in *M. lobata*, *Molaspora* sp. cf. *M. fibrosa* Singh 1983 and species of *Regnellidium*. The inner epispore/total epispore thickness ratio (as calculated from relevant figures and/or descriptions) is about 0.46 in *M. aspera*, 0.20 in *Molaspora* sp. cf. *M. fibrosa* Singh 1983;

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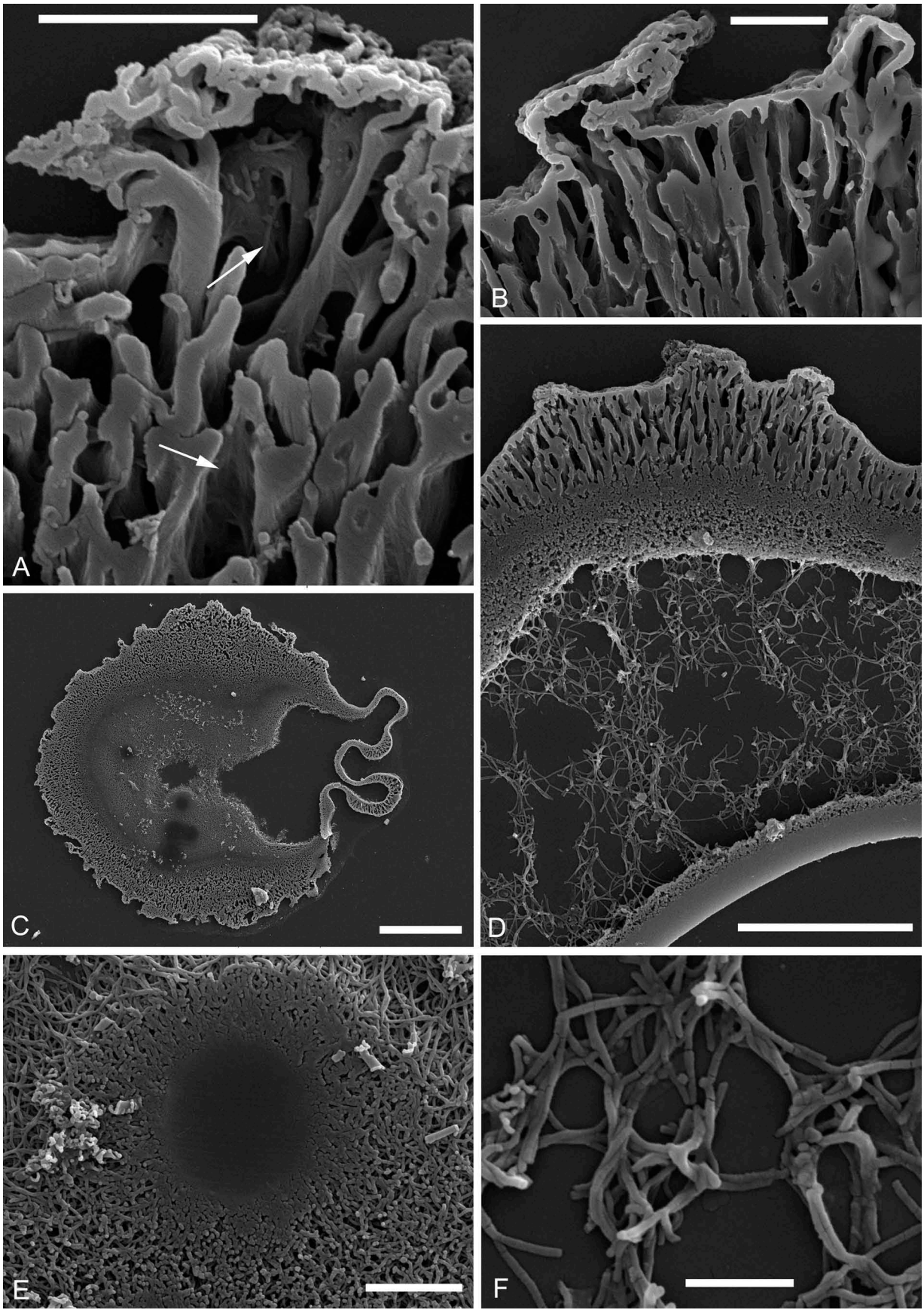
Figure 10. *Molaspora aspera* sp. nov., specimen #30. Epoxy-free semithin sections, SEM. **A.** Alveolae and elevations of the outer epispore; note that the inner surface of the partitions is lined with threads (arrows). **B.** Alveolae and elevations of the outer epispore. **C.** Section in the area of the junction between the acrolamella and the body of the spore. **D.** Section through the sporoderm in the area of well-developed, very open sublayer (middle sublayer 1) of the epispore. **E.** Enlargement of (**C**) showing a spherule. **F.** Threads of the very open sublayer (middle sublayer 1) of the epispore. Scale bars – 5 μm (A, B, E), 50 μm (C), 30 μm (D), 2 μm (F).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(Lupia 2015, p. 498), 0.19 in *M. lobata* (Batten et al. 2011a, figure 7; and present study), 0.15 in *R. sibiricum* Dorofeev, *R. turgaicum* (Dorofeev) Dorofeev (Batten et al. 2011a, figure 2) and *R. diphyllum* Lindm. (Tryon & Lugardon 1990, figure 222.6), and 0.06 in *R. pusillum* (Batten et al. 2011a, figure 3). Available depictions of thick sections of other species of *Molaspora* (i.e. *M. hallii* in Floquet & Lachkar 1979 and *M. salinum* in Kovach & Dilcher 1988) do not allow us to evaluate this character.

Of interest is that this ratio in megaspores of modern *Pilularia globulifera* L. and *P. minuta* Dur. is about 0.76 and 0.68, respectively (Tryon & Lugardon 1990; figures 223.6, 223.7). On this basis, *Molaspora aspera* is not very close to the megaspores of other members of *Molaspora* or of modern *Regnellidium*, but quite similar to those of modern *Pilularia.*

By contrast, unlike *Molaspora aspera*, the ultrastructure of *Molaspora lobata* is more similar to that of modern *Marsilea* and fossil and modern *Regnellidium* megaspores. Figures in Pettitt (1979a, figure 18a, b) show the outer epispore of *Marsilea drummondii* A. Braun megaspores is palisade-like, in common with *Molaspora lobata* and *Regnellidium* (see also Pettitt 1979b, figure 1A, Southworth & Myles 1985, figure 1; Gardenal et al. 2007, figure 4C, H, for similar illustrations). An exception to this typical structure is the megaspore wall of *Marsilea strigosa* (Tryon & Lugardon 1990, figure 221.10), which consists of three tiers of alveolae composed of short, variously directed elements.

Hence, *Molaspora lobata* is very similar to megaspores of *Regnellidium* and has characters in common with megaspores of species of *Marsilea*, whereas *Molaspora aspera* resembles to a degree some other members of the family, in particular *Pilularia*. Furthermore, the ultrastructure of *Molaspora salinum* is quite different from that of other members of *Molaspora*. It is, therefore, possible that *Molaspora* is a heterogeneous taxon that embraces megaspores produced by water ferns of more than one natural genus, of which only *Regnellidium* has survived until now.

Comparison of gross morphology and ultrastructural characters with species of Arcellites

Until recently, there were no records of *Molaspora* older than Albian, but it has now been encountered in deposits dated as Barremian–Aptian in Portugal (Friis & Pedersen 2014; Friis et al. 2014). This is still later than the first, Barremian, records of six species of *Arcellites*, the gross morphology of which, in common with *Molaspora*, suggests an adaptation to dispersal over water. It also suggests that *Arcellites* is referable to the Order Marsileales, but not necessarily to the Marsileaceae; it could represent an extinct family instead (Collinson 1991; Batten et al. 1996). The fact that the microspore *Crybelosporites* is commonly associated with it supports not only a marsilealean relationship but also evolutionary links with *Molaspora*.

A seventh species of *Arcellites*, *A. medusus* (Dijkstra 1951) Potter 1963, first appears in Valanginian deposits. Batten et al. (1996, p. 62) suggested that its parent plants might have been different from those producing the younger representatives of the genus. Spores referred in the past to *A. pyriformis* (Dijstra 1951) Potter 1963, which appeared even earlier, in the Berriasian, are now placed in *Bohemisporites* because it is not a water-fern megaspore, its sporoderm architecture having a selaginellalean aspect (Batten et al. 1996).

In common with *Molaspora*, the triradiate suture of species of *Arcellites* is obscured by a prominent 'neck' in undamaged specimens. This consists of leaf-like segments with infolded central parts that are commonly twisted against each other and fused at their margins usually to form six ridges. In those species that are represented by specimens that have been damaged around the proximal pole or in other ways, in common with the larger size of the 'neck', the triradiate scar is seen to be somewhat, but sometimes only marginally, larger than in species of *Molaspora*: e.g. in *A. vectis* (Hughes) Potter (Batten et al. 1996, figure 9e), *A. stellatus* Nowak et Lupia (Nowak & Lupia 2004, figures 1.6, 2.8) and *A. punctatus* Friis et al. (Friis et al. 2014, figure 1E, F).

Comparison between the ultrastructure of the sporoderm of *Molaspora aspera* with that of species of *Arcellites* reveals some similarities but also differences. TEM sections of the exospore of *A. hexapartitus* (Dijkstra) Potter in Batten et al. (1996, figure 4b, referred to as intexine) and of *A. hexapartitus* and *A. stellatus* in Nowak and Lupia (2004, figures 2.3 and 2.4, respectively) is channelled in a manner similar to that in *M. aspera*. The inner epispore of *Arcellites* is also comparable to that of *Molaspora* in 'representing a generally loosely structured, three-dimensional meshwork of sporopollenin

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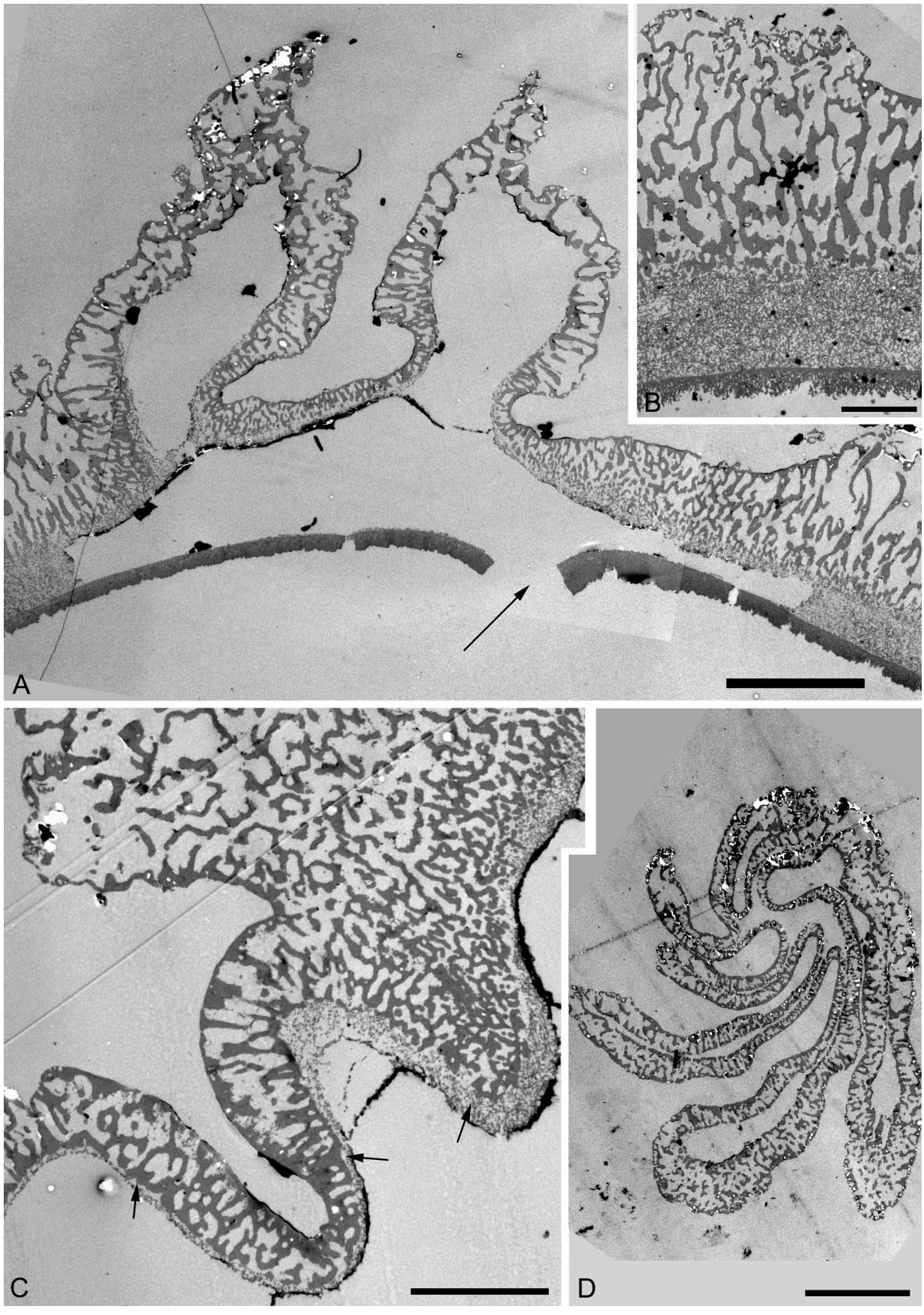
Figure 11. *Molaspora aspera* sp. nov., ultrathin sections, TEM. **A.** Apertural area; the exospore ruptures along an arm of the scar (arrow), specimen #38. **B.** Section in the distal area of the megaspore, specimen #40. **C.** A lobe of the acrolamella; inner epispore wedges out (arrows), specimen #40. **D.** Transverse section of the acrolamella with six lobes, specimen #30. Scale bars – 20 μm (A, D), 5 μm (B), 10 μm (C).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threads' (Batten et al. 1996, p. 59). The cavities within the epispore of *M. aspera* bear some resemblance to those beneath the appendages of the specimen of *Arcellites* sp. A. in Lupia (2004, figure 3B; recorded as *A. stellatus* in Nowak & Lupia 2004). However, the outer epispore of *M. aspera* is totally different from that of species of *Arcellites*. Hence, the fact that the ratio of inner epispore to total epispore in *Arcellites* is similar to that of *M. aspera* is of little consequence.

Overall, although there are some ultrastructural similarities between *Molaspora aspera* and certain species of *Arcellites*, there are significant differences between them, which serves to emphasise that *Molaspora* is a paraphyletic assemblage of marsileaceous megaspores consisting of a mixture of ancestral and newly evolved characters, i.e. with characters in between those of *Arcellites* and the extant 'crown' group of marsileaceous genera, most notably *Regnellidium*. This is also suggested by the fact that *Molaspora lobata* and *Molaspora salinum* were the only marsileaceous megaspores isolated from the Dakota Formation from which Skog and Dilcher (1992) described *Marsilea johnhallii* Skog et Dilcher (now *Marsileaceaephyllum johnhallii* [Skog et Dilcher] Nagalingum 2007), and there are other records of occurrences of *Molaspora lobata* in close proximity to beds that have yielded *Marsileaceaephyllum* (e.g. in Vallati et al. 2017). Megaspores identified as *Molaspora lobata* were also recovered by Cúneo et al. (2013) from deposits that yielded *R. thomas-taylorii* Cúneo, Gandolfo et Hermsen 2013. Likewise, Hermsen et al. (2014) found *Molaspora lobata* in Late Cretaceous beds yielding *Mirasolita* and *Lugiomarsiglia*.

So far, however, *Arcellites* has not been associated with macrofossil remains. It is conceivable that the older marsileaceous macrofossils reported by Yamada and Kato (2002) and Sender et al. (2014) might eventually prove to be connected with it.

Associated microspores

Lupia et al. (2000) recovered megaspores and associated microspores in sporocarps which they described as *Regnellidium upatoiensis*. They regarded the dispersed megaspores in the same samples as identical to *Molaspora lobata*, and the microspores to be attributable to *Crybelosporites*. Batten et al. (2011a, 2011b) described for the first time a few specimens of dispersed *M. lobata* with adhering microspores that they also referred to *Crybelosporites*. The papillate surface sculpture of the megaspores and deeply irregular reticulation of the surface of the microspores, possibly together with mucilage initially, may well have aided this adhesion (Batten et al. 2011a, 2011b). Lupia (2015) figured *C. pannuceus* (Brenner) Srivastava 1977-like microspores in a broken specimen of *Molaspora* sp. cf. *M. fibrosa* from the Cenomanian of Maryland. Unfortunately, no specimens of *M. aspera* have been found with attached microspores. Spores referred to *Crybelosporites* sp. in a palynological preparation of the same sample are rather large (*c.* 70–80 μm in diameter: Néraudeau et al. 2017, figure 6D) and unlikely to be the associated microspore: those associated with *M. lobata* are within the range 25–58 μm (Lupia et al. 2000; Batten et al. 2011a; Cúneo et al. 2013).

Although varying in morphology at species level, microspores associated with *Arcellites* are also commonly attributable to *Crybelosporites* (e.g. Cookson & Dettmann 1958; Hueber 1982; Li & Batten 1986; Tosolini et al. 2002; Lupia 2004, 2015; Friis et al. 2014), which further emphasises the mixed evolutionary links between the parent plants of *Arcellites* and *Molaspora*.

Biostratigraphic and palaeoenvironmental significance

Most of the records of *Molaspora* pertain to *M. lobata*, which is also the most long-ranging species (Kovach & Batten 1989; Batten & Kovach 1990; Batten et al. 2011a; Collinson et al. 2013; Friis et al. 2014). Of the other species that have been described, almost all have been reported only from Cenomanian deposits: the single exception is *M. reticulata*, which was encountered in deposits in Alberta (Canada) dated as mid-Campanian and mid-Maastrichtian. The occurrence of *M. aspera* in western France adds another Cenomanian representative of *Molaspora* to this record.

Despite differences in the inner epispore/total epispore thickness ratios mentioned earlier, the structure of the wall of *Molaspora aspera* is broadly consistent with that of *Molaspora lobata* and the megaspores of the three extant genera, *Marsilea*, *Pilularia* and *Regnellidium* within the family Marsileaceae, the last of these being the closest morphologically (e.g. Batten 1988; Collinson 1991; Hemsley et al. 1999; Lupia et al. 2000; Takahashi et al. 2001; Schneider & Pryer 2002; Batten et al. 2011a, 2011b; Cúneo et al. 2014). This structure was clearly beneficial to dispersal in water rather than air: see Schneider and Pryer (2002) for a discussion of functional aspects of spores of heterosporous ferns. Hence the morphology of *Molaspora aspera* and its association with *Molaspora lobata* in the La Garnache assemblage are entirely consistent with a seasonally wet or aquatic habitat for the parent plants of this species.

Conclusions

The ultrastructure of the sporoderm of *Molaspora aspera* is similar, but not identical, to that of the most common representative of the genus, *M. lobata*, and to modern *Regnellidium*. Although there are

similarities between *M. aspera* and certain species of *Arcellites*, there are also significant differences. It seems clear that *Molaspora* encompasses a paraphyletic assemblage of marsileaceous megaspores with characters in between those of *Arcellites* and the extant marsileaceous genera. Data on the ontogenesis of the protective wall of megaspores of the latter, particularly on the formation of the outer epispore, would be pertinent to the evaluation of differences between the sporoderm ultrastructure of *Arcellites* and *Molaspora*.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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