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MORPHOLOGY AND WALL ULTRASTRUCTURE OF THE DEVONIAN SPORE *ACINOSPORITES MACROSPINOSUS* RICHARDSON 1965 AND ITS BEARING ON THE ORIGIN OF THE MEGASPORE APICAL PROMINENCE

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The spore *Acinosporites macrospinosus* Richardson 1965 is common in exceptionally well preserved dispersed spore assemblages recovered from the Middle Devonian (Eifelian) 'Middle Old Red Sandstone' deposits from the Orcadian Basin, Scotland. This paper reports on a detailed light microscope (LM), scanning electron microscope (SEM) and transmission electron microscope (TEM) analysis of these spores. The spores are large and spinose with an apical prominence associated with the trilete mark. TEM analysis reveals that the wall consists of four layers, based on white-line-centred-lamellae (WLCL) that develop into laminae. The spore wall ultrastructure is most similar to that in extant and fossil lycopsids and a lycopsid affinity is proposed for the parent plant. Based on this interpretation a mechanism for spore wall development is presented. The nature and ecology of the parent lycopsid plant is unclear. However, based on similarities with younger bona fide megaspores, it is suggest that *A. macrospinosus* is probably an incipient megaspore produced by one of the first groups of lycopsids to experiment with heterosporous reproductive strategy.

This is the earliest report of an apical prominence on a lycopsid spore, a feature that went on to characterize many later lycopsid megaspores, leading to the development of extensive gulas and massas.

Key words: Mid Devonian, Middle Devonian, land plant, lycopsid, spores, gula, massa.

Introduction

This contribution forms part of a series of papers reporting on exquisitely preserved wall ultrastructure in dispersed spores from the Middle Devonian 'Middle Old Red Sandstone' deposits of Cromarty, Scotland (Wellman 2001, 2002, 2009). Based on material collected from this locality Lang (1925) published a groundbreaking paper that included what is possibly the first description of Devonian dispersed spores released by HF acid maceration. He described and illustrated nine types of dispersed spore ("Spore-type A" to "Spore-type I") and also a number of dispersed sporangia that contained some of these spores *in situ*. Subsequently, Richardson (1960, 1962, 1965) described the dispersed spore assemblages from this locality in great detail. One of the more interesting spores, *Acinosporites macrospinosus* Richardson 1965, is of particular interest because it bears a distinct apical prominence reminiscent of the gula/massa characteristic of many younger megaspores from the Late Devonian-Carboniferous (Hemsley et al. 1999). Consequently, it was decided to examine this dispersed spore taxon in more detail in anticipation that it might shed some light on the biological affinities of the spore and the evolutionary origins of the gula/massa. This contribution reports on a combined LM, SEM and TEM investigation of the morphology, gross structure and wall ultrastructure of Middle Devonian *A. macrospinosus*.

Material and Methods

Locality and geological setting

The material described herein was recovered from Middle Devonian “Middle Old Red Sandstone” deposits from Cromarty, the Black Isle, Scotland. These deposits were selected because of the abundance, excellent preservation and low thermal maturity of the preserved palynomorphs (Lang 1925; Richardson 1960, 1962, 1965). Samples of green siltstones and fine sandstones were collected from strata exposed in Coal Heugh (NH792672), a small stream draining into Miller’s Bay, near Cromarty. These strata belong with the Millbuie Sandstone Group (Horne and Hinxman 1914; Johnstone and Mykura 1989). This group comprises predominantly fluviatile deposits, but also contains a fish-bearing horizon that is equivalent to the Achanarras horizon. The Achanarras horizon represents a transgression of the Orcadian Lake that is equivalent to the Kacak Event (Marshall et al. 2007). These strata are considered to be of Eifelian (Mid Devonian) age based on biostratigraphical evidence from spore assemblages (Richardson 1960, 1962, 1965; Richardson and McGregor 1986; Marshall 1996; Marshall and Fletcher 2002) and fish (e.g. Blieck et al. 1988). Dispersed spore assemblages from the Achanarras horizon and its correlatives can be equated with the *devonicus-naumovii* Spore Assemblage Biozone of Richardson and McGregor (1986) and AD Oppel Zone of Streel et al. (1987) (Marshall and Fletcher 2002).

Preparation and techniques

Numerous samples of sediment were digested using standard HCl-HF-HCl acid maceration. Abundant organic residue was obtained that is dominated by dispersed spores and phytodebris (including entire sporangia). The dispersed spores and phytodebris are extremely well preserved and of low thermal maturity. The organic residue was subjected to heavy liquid separation using zinc chloride and then sieved using a 120 μm and/or 20 μm mesh. Strew mounted slides of the residue were prepared for LM analysis. In addition, individual spores were picked from the residue and mounted for SEM and TEM analysis. Material for SEM analysis was prepared by mounting individually picked spores on a double-sided sticky tab attached to a glass coverslip. When a suitable number of specimens had been picked, the coverslip was attached to an SEM stub using another double-sided sticky tab. The stub was then gold coated using a sputter coater, and ready for SEM analysis using a Philips 501B SEM. Material for TEM analysis was prepared by mounting individually picked spores on a block of freshly prepared agar. The specimen was sealed into the block by covering it with molten agar which solidifies on cooling. They were then dehydrated in ethanol and embedded in Spurr resin. Sections were cut on a microtome using a diamond knife, stained with uranyl acetate followed by Reynold's lead citrate, and examined using a Philips CM10 TEM. The embedded individual spores were cut, as near as possible, perpendicular to the plane of compression. Dimensions should therefore provide a reasonably accurate reflection of true thickness. All studies were conducted on unoxidised material. All rock, residue, slides, stubs, blocks and grids are curated at the Centre for Palynology of the University of Sheffield.

Descriptions

LM observations (Fig. 1)

The original description by Richardson (1965, p. 579) is: “Colour brown to reddish-brown. Size range, excluding spines, 80 to 160 μ (thirty-five specimens measured). Equatorial outline circular, subcircular, or triangular; hemispherical in lateral compression, flattened at the proximal pole. Exine thick covered by anastomosing ridges which are often convolute and closely packed but on some specimens the ridges form a loose, irregular, reticulate pattern; ridges bear spines 10 to 50 μ with stout often swollen or bulbous bases, and pointed apices. Ridges fused into tight ‘concertina’ folds around the contact areas. Triradiate mark with pronounced elevated, membranous ridges which form a distinct apical prominence; 21 to 52 μ high in lateral view; in polar compression the apical prominence forms contorted folds which reach the equatorial margin.”. I have little to add to this based on my LM observation of hundreds of specimens from the Coal Heugh locality (see also the taxonomic discussion in McGregor and Camfield 1982). Both of the specimens figured in Fig. 1 are in lateral compression illustrating the nature of the apical prominence.

SEM observations (Figs 2-3)

The high magnification SEM surface images of *A. macrospinosus* confirm the LM observations as outlined above, but also provide some supplementary information. Fig. 2 illustrates whole specimens in polar (2A), distal (2B) and lateral (2C) view. Figs 2A and 2C clarify the nature of the apical prominence and its relationship with the trilete mark. The apical prominence consists of a circular raised area with a surface of ‘lumpy’ appearance due to an ornament of irregular rugulae and verrucae. This is well seen in the top right of the high magnification image Fig. 3B. The membraneous ridges of the trilete mark arise from this circular raised area. Fig. 3 provides close up images of the ornament. It is clear from these images how the swollen bases of individual spines merge to form an irregular surface reticulum. It is also evident that the long spines have endings that are either pointed or slightly frayed, though they never form large, fully developed bifurcating anchor-tips or multifurcating grapnel-tips.

TEM observations (Figs 4-5)

The best sections are from a single specimen and all images and this description are based on this. It is clear from these TEM observations that the spore wall can be divided into four intergrading layers that are arranged concentrically around the lumen. These are termed Layers 1-4 herein, with Layer 1 bordering the lumen and Layer 4 at the outer margin off the spore (forming the ornament). Measurements of layer thicknesses are approximate due to the intergrading nature of the layers. The wall, excluding the spines but including the ridges on which the spines are mounted, is approximately 30 μm in thickness.

The innermost layer (Layer 1) is ca.1.0 μm in thickness. (Figs 4D-G, 5A-B) It is electron dense and appears dark. It is essentially homogeneous except for the presence of abundant white-line-centred-lamellae (WLCL) and occasional small voids (Figs 4F-G, 5A-B). The WLCL are concentrically-arranged and are wavy and often bifurcate and merge with one another. There are approximately eight throughout the thickness of this layer. The small voids tend to be elongate lying parallel to the wall layer (Figs 4F-G, 5A-B).

Layer 1 merges into Layer 2 that consists of concentrically-arranged laminae ca. 60 nm in thickness (Figs 4D-F, 5A-B). Elongated voids are developed along the junction between adjacent laminae. This layer measures ca. 0.5 μm in thickness and is less electron dense than Layer 1 appearing paler. It consists of approximately 6 to 8 laminae. The junction between Layers 1 and 2 can be taken as the last visible WLCL.

Layer 2 merges into Layer 3 (Figs 4A-B). It comprises laminae that are initially similar in size to those of Layer 2. However, moving towards the outside of the spore wall these become increasingly wider (up to 175 nm in thickness) (Figs 4H-I) and increasingly irregular and with more voids. This layer is considerably less electron dense than both Layers 1 and 2 and appears pale in comparison. Initially the laminae are straight, fairly continuous and concentrically arranged. Progressing towards the outside of the spore wall they become wider, increasingly wavy and less continuous with many more voids between them. Eventually this imparts a honeycomb appearance towards the outside of this layer (Figs 4A-B and 4H). Layer 3 comprises the bulk of the spore wall and is 24-28 μm in thickness.

Layer 4 forms the outer layer of the spore wall including the spines (Figs 4A-C). It is essentially a homogeneous layer that appears pale. Occasional lines, particularly on the inside of this layer, are more-or-less concentrically arranged suggesting remnant lamina (Fig. 4J). Elongate voids are present in the centre (core) of some of the spines (Fig. 4B).

Discussion

Biological affinities of Acinosporites macrospinosus

In terms of spore wall ultrastructure in extant plant groups, *A. macrospinosus* is most similar to that in lycopsids. Wall ultrastructure has been described in extant homosporous lycopsids (e.g. Pettitt 1966; Lugardon 1976; Uehara and Kurita 1991; Rowley 1995) and the heterosporous lycopsids *Isoetes* (e.g. Lugardon 1973; Robert et al. 1973; Brown and Lemmon 1991; Uehara et al. 1991; Taylor 1992) and *Selaginella* (e.g. Pettitt 1971; Robert 1971; Sievers and Buchen 1971; Lugardon 1972; Brown and

Lemmon 1985; Taylor 1989; Morbelli and Rowley 1993; Uehara et al. 1991; Hemsley et al. 1994; Gabarayeva 2000; Moore et al. 2006; Blackmore et al. 2012). In all of these the wall essentially develops centripetally, based on WLCL that are formed at the plasma membrane. Sporopollenin is accreted onto the WLCL forming laminae which may eventually merge into a homogeneous structure. Additional inner and outer layers may also be present, forming via underplating (often forming a granular layer below proximal regions) or additions of tapetal sporopollenin, respectively (reviewed in Lugardon 1990; Brown and Lemmon 1991; Tryon and Lugardon 1991; Wellman 2004; Wallace et al. 2011). However, neither additional outer or inner layers have been observed in *A. macrospinosus*.

A model for spore wall development

Based on interpretation of *A. macrospinosus* as a lycopsid, it is possible to suggest a likely developmental sequence for its spore wall. The first step most probably involved the folding of the plasma membrane into the final outer shape of the spore (see Uehara and Kurita 1991). From this point the wall developed centripetally based on the formation of WLCL on the plasma membrane. As more WLCL formed, sporopollenin (most likely produced by the tapetum) was progressively accreted onto older WLCL, and they developed into laminae. As development continued the first formed laminae (outermost) began to fold forming a honeycomb pattern. Laminae towards the outside of the spore wall may have been entirely swamped in sporopollenin to the extent that their original laminate structure was obscured. This process produces a four-layered wall: the innermost Layer 1 that consists of newly formed WLCL that are just beginning to accrete sporopollenin as they develop into laminae; Layer 2 that consists of newly formed laminae that are parallel and concentrically arranged around the lumen; Layer 3 consisting of laminae that are regular and parallel arranged toward the inside becoming more folded and forming a honeycomb pattern towards the outside; the outermost Layer 4 consisting of essentially homogeneous sporopollenin with only very rare and faint traces of laminae.

Further evidence from fossil in situ *spores*

The fossil record of *in situ* lycopsid spores has recently been reviewed by Bek (2017). There are relatively few records from the Middle Devonian and earlier. In terms of close relatives of the lycopsids from the Lower Devonian, simple retusoid trilete spores have been reported from a number of zosterophylls (e.g. Gensel et al. 2012) and the Rhynie chert plant *Asteroxylon mackiei* (Kerp et al. 2013). The dispersed spore taxon *Acinosporites lindlarensis* has been reported from the homosporous protolepidodendraceaen plant *Leclercqia* from a number of localities ranging in age from Early to Late Devonian (Richardson et al. 1993; Gensel and Albright 2006; Gensel and Kasper 2006; Xu et al. 2011). Poorly preserved spores of *Acinosporites*-type have also been reported *in situ* from Mid Devonian *Hoxtolgaya robusta* (Xu et al. 2012). Other Mid Devonian plants from which *in situ* spores have been reported are clearly heterosporous protolepidodendraceaens with a clear distinction between small microspores and large megaspores: *Longostachys latisporophyllus* (Cai and Chen 1996), *Minarodendron cathaysiense* (Liu et al. 2013); *Mixostrobus givetensis* (Senkevitsch et al. 1993).

A number of fossil spores have been ultrastructurally analysed that support the interpretation of *A. macrospinosus* as a lycopsid spore that developed in the postulated manner. *In situ* spores of *Acinosporites lindlarensis* have been examined ultrastructurally from the lycopsids *Leclercqia complexa* (Gensel and Albright 2006;

Wellman et al. 2009) and *Leclercqia andrewsii* (Gensel and Kasper 2006; Wellman et al. 2009) from the Lower Devonian of New Brunswick, Canada (Wellman 2018). Wall structure in *Leclercqia* differs from that in *A. macrospinosus* because the former has a distinct paraexospore that is separated from a laminar inner layer and is probably tapetally derived. However, it is clear that the inner laminar layer of *Leclercqia* is very similar to the wall of *A. macrospinosus*. Unfortunately, our sections of *A. macrospinosus* did not permit observation of the trilete mark and the potential presence of a proximal, interradial, multilaminate region that has been proposed as a synapomorphy of the ligulate lycopsid clade (Wellman et al. 2009).

Other Early-Middle Devonian spores interpreted as lycopsid that have been investigated ultrastructurally include *Ancyrospora* from the same horizon as the material considered herein (Wellman 2002) and megaspores of Mid Devonian age from Poland (Turnau et al. 2009; Zavialova and Turnau 2012), the USA (Taylor and Gullickson 2013) and Yunnan, China (Peng et al. 2016). The spores of *Ancyrospora* are interpreted as having developed in a similar way to those in *A. macrospinosus* (Wellman 2002), as was also demonstrated by Telnova (2017) in another species of *Ancyrospora* (*A. melvillensis*). Other Middle Devonian spores interpreted as lycopsid that have been ultrastructurally investigated are *Corystisporites acutispinosus*, *Coronospora variabilis*, *Grandispora ciliata* and *Pomeranisporites subtriangularis* (Zavialova and Turnau 2012) and *Longhuashanispora reticuloides*, *Ocksisporites maclarenii* and *Cereusisporites mirabilis* (Peng et al. 2016). All exhibit features of wall ultrastructure that are comparable with that in *A. macrospinosus*.

Coeval plant megafossils

Lycopsids were an important component of the Middle Devonian flora (Berry and Fairon-Demaret 2001) and by the early Late Devonian had begun to form forest ecosystems (Berry and Marshall 2015). They can be broadly subdivided into three groups: Pre-lycopsids (Drepanophycales), Protolepidodendrales and Early Arborescent Lycopsids. The terrestrial deposits of the Middle Devonian of the Orcadian Basin, Scotland yield well known plant megafossil assemblages (e.g. Miller 1841; Lang 1925, 1926; Perry 1989). However, few lycopsid taxa have been described. The common fossil *Thursophyton milleri* (Salter) Nathorst 1915 has long been considered to be of lycopsid affinity, although Perry (1989) suggested that it may have zosterophyll affinities based on its elliptical exarch xylem anatomy. It was probably a shrubby plant emerging from a rhizome. Unfortunately fertile parts are unknown. Regarding other potential lycopsid parent plants, rare examples of *Protolepidodendron* are also known from the Orcadian Basin (Lang 1926).

Implications regarding reproductive strategy

The size of *A. macrospinosus* is interesting with regard to the reproductive strategy adopted by the parent plant. Was it homosporous or heterosporous? There has been much debate concerning the evolution of heterospory in the Devonian (e.g. Bateman and DiMichele 1994). These authors demonstrated that a number of different heterosporous reproductive strategies exist and that heterospory evolved independently at least eleven times. Analysis of spore size distribution in the Devonian provides tantalizing clues regarding the evolution of heterospory. Chaloner (1967) and Richardson (1969) demonstrated that maximum spore size increases throughout the Silurian-Devonian. From the Llandovery (early Silurian) to Frasnian (Late Devonian) size distribution is right skewed suggesting that the majority of spores are small but increasingly larger forms are present. By the Famennian (Late Devonian) spore distribution is bimodal suggesting that there is a clear distinction between a population of smaller isospores/microspores and larger megaspores. Prior to this size distribution forms a continuum with no clear distinction between smaller isospores/microspores and larger megaspores.

A boundary of 200 μm is usually used to differentiate between isospores/microspores and megaspores. However, this boundary is arbitrary and there is little scientific reasoning for adopting it. Indeed Hemsley et al. (1999) suggest that the boundary should be at 115 μm based on a study of spore size and efficiency of subaerial dispersal. Spores that exceed the conventional 200 μm in diameter are known from the latest Pragian-earliest Emsian (Richardson and McGregor 1986) and it is likely that some forms of heterospory was practised by this time. However, the earliest evidence for heterospory from plant megafossils containing *in situ* spores is not until the late Emsian (Andrews et al. 1974; Bonacorsi et al. 2020). Heterospory almost certainly evolved independently in a number of plant lineages over the course of the Devonian, and this is reflected in the dispersed spore fossil record in the increasing maximum spore size and the increasing number of spores in this larger size range. Different heterosporous reproductive strategies were most likely practised, but it is impossible to identify these based only on evidence from fossil spores. However, by at least the Famennian there were almost certainly heterosporous plants that produced spores of two distinct size ranges that were no doubt very different in morphology. Prior to this it is likely that functional megaspores were present, but size ranges of microspores/megaspores overlapped, and morphology may not have been distinct.

During the Eifelian, there are many spore taxa whose size distibution straddles the 200 μm boundary. It is unclear whether these are: (i) large isospores of homosporous plants; (ii) large microspores of heterosporous plants with morphologically distinct microspores and megaspores; (iii) small megaspores of heterosporous plants with morphologically distinct microspores and megaspores; (iv) microspores and megaspores of heterosporous plants whose microspore/megaspore size ranges overlap and that are not morphologically distinct. *A. macrospinosus* measures 80-160 μm (excluding the spines). This size range is ambiguous (bearing in mind the suggestion of Hemsley et al. (1999) that microspore/megaspore segregation is around 115 μm) and any of the four cases outlined above is possible. However, based on similarities with younger bona fide megaspores, I suggest that *A. macrospinosus* is probably an incipient megaspore from among the first groups of lycopsids to experiment with heterosporous reproductive strategy.

On the origin and nature of the gula

One of the most interesting features of the spore *A. macrospinosus* is the proximal structure that bears the laesurae of the trilete mark. This structure is reminiscent of the apical prominence (often forming a gula or massa) developed on the proximal surface of large lycopsid megaspores, that is a particularly conspicuous feature of Late Devonian-Carboniferous megaspore assemblages. Hemsley et al. (1999) discuss the architecture and functional biology of apical prominences in megaspores. They suggest that extensions of the trilete laesurae and/or proximal contact areas may have acted like a shuttlecock, ensuring that the megaspore descended distal surface down, and in non-ornamented forms possibly also increased speed of descent. Hemsley et al. further suggest that the gula may have functioned in water dispersal, opening up so as to keep the megaspore afloat and aid the capture of microspores. Whatever the function(s) of megaspore apical prominences, it seems that megaspores (or incipient megaspores) were beginning to experiment with such features by the Mid Devonian. As the heterosporous lycopsids first began to appear, changes in reproductive strategy, megaspore function and associated megaspore morphology seem to have evolved hand-in-hand.

Conclusions

1/ The Middle Devonian dispersed spore *Acinosporites macrospinosus* Richardson 1965 has typical lycopsid spore wall ultrastructure.

2/ *A. macrospinosus* is probably an incipient megaspore from among the first groups of lycopsids to experiment with heterosporous reproductive strategy.

3/ The apical prominence of *A. macrospinosus* is a forerunner of the pronounced apical prominence (gulas and massas) of later megaspores.

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Literature cited

Andrews HN, PG Gensel, WH Forbes 1974 An apparently heterosporous plant from the Middle Devonian of New Brunswick. Palaeontol 17:387-408.

Bateman RM, WA Dimichele 1994 Heterospory: the most iterative key innovation in the evolutionary history of the plant kingdom. Biol Rev 69:345-417.

Bek J 2017 Paleozoic in situ spores and pollen. Lycopsida. Palaeontographica Abt. B 296:1-111.

Berry CM, M Fairon-Demaret 2001 The Middle Devonian flora revisited. Pages 120-139 *in* PG Gensel, D Edwards, eds. Plants invade the land: evolutionary and environmental perspectives. Columbia University Press, New York.

Berry CM, JEA Marshall 2015 Lycopsid forests in the early Late Devonian paleoequatorial zone of Svalbard. Geology 43:1043-1046.

Blackmore S, M Takahashi, K Uehara, AH Wortley 2012 Development of megaspores and microspores in *Isoetes japonica* A. Br. (Lycopodiophyta: Isoetaceae). Grana 51:84-96.

Blieck A, E Mark-Kurik, T Marss 1988 Biostratigraphical correlation between Siluro-Devonian invertebrate-dominated and vertebrate-dominated sequences: the East Baltic example. Pages 579-587 *in* NJ McMillan, AF Ambrey, DJ Glass, eds. Devonian of the world. Canadian Society of Petroleum Geology Memoir 14.

Bonacorsi NK, PG Gensel, FM Hueber, CH Wellman, AB Leslie 2020 A novel reproductive strategy in an Early Devonian plant. Curr Biol 30: R371-R392.

Brown RC, BE Lemmon 1985 A cytoskeletal system predicts division plane in *Selaginella*. Protoplasma 127:101-109.

Brown RC, BE Lemmon 1991 Sporogenesis in simple land plants. Pages 9-24 *in* S Blackmore, SH Barnes, eds Pollen and spores: patterns of diversification. Clarenden Press, Oxford.

Cai C, L Chen 1996 On a Chinese Givetian lycopod, *Longostachys latisporophyllus* Zhu, Hu and Feng emend.: its morphology, anatomy and reconstruction. Palaontographica Abt. B 238:1-43.

Chaloner WG 1967 Spores and land-plant evolution. Rev Palaeobot Palynol 1:83-93.

Gabarayeva NI 2000 Principles and current themes in sporoderm development. Pages 1-17 *in* MM Harley, CM Morton, S Blackmore, eds Pollen and spores: morphology and biology. Royal Botanic Gardens, Kew, UK.

Gensel PG, VM Albright 2006 *Leclercqia complexa* from the Early Devonian (Emsian) of northern New Brunswick, Canada. Rev Palaeobot Palynol 142:103-121.

Gensel PG, AE Kasper 2006 A new species of the Devonian lycopod genus, *Leclercqia*, from the Emsian of New Brunswick, Canada. Rev Palaeobot Palynol 137:105-123.

Gensel PG, CH Wellman, WA Taylor 2012 Spore wall ultrastructure in the Lower

Devonian zosterophylls *Renalia hueberi* and *Zosterophyllum divaricatum*. Int J Plant Sci 174:1302-1313.

Hemsley AR, ME Collinson, WL Kovach, B Vincent, T Williams 1994 The role of self-assembly in biological systems: evidence from iridescent colloidal sporopollenin in *Selaginella* megaspore walls. Phil Trans R Soc London B 345:163-173.

Hemsley, AR, AC Scott, ME Collinson 1999 The architecture and functional biology of freely dispersed megaspores. Pages 253-277 *in* MH Kurmann AR Hemsley, eds The evolution of plant architecture. Royal Botanic Gardens, Kew.

Horne J, LW Hinxman 1914 The geology of the area around Beauly and Inverness. Mem Geol Surv Great Britain 83:1-108.

Johnstone GS, W Mykura 1989 The northern highlands of Scotland (4th ed.). British Geological Survey, HMSO, London, 219 pp.

Kerp H, CH Wellman, M Krings, P Kearney, H Hass 2013 Reproductive organs and in situ spores of *Asteroxylon mackiei* Kidston & Lang, the most complex plant from the Lower Devonian Rhynie chert. Int J Plant Sci 174:293-308.

Lang WD 1925 Contributions to the study of the Old Red Sandstone flora of Scotland. I. On plant-remains from the fish-beds of Cromarty. II. On a sporangium-bearing branch-system from the Stromness Beds. Trans R Soc Edinburgh 54:253-279.

Lang WD 1926 Contributions to the study of the Old Red Sandstone flora of Scotland. III. On *Hostimella* (*Ptilophyton*) *thomsoni* and its inclusion in a new genus *Milleria*. Trans R Soc Edinburgh 54:785-790.

Liu L, DM Wang, JZ Xue, MC Meng, Y Guo 2013 Reinvestigation of the lycopod *Minarodendron cathaysiense* from the Middle Devonian of South China. N Jb Geol Pal Abh 268:325-339.

Lugardon B 1972 Sur la structure fine et la nomenclature des parois microsporales chez *Selaginella denticulate* (L.) Link et *Selaginella selagionoides* (L.) Link. Comptes Rendus de l’Academie des Sciences Paris 274:1256-1259.

Lugardon B 1973 Nomenclature et structure fine des parois acéto-résistantes des microspores d’*Isoetes*. Comptes Rendus de l’Academie des Sciences Paris 276:3017-3020.

Lugardon B 1976 Sur la structure fine de l’exospore dans les divers groupes de Ptéridophytes actuelles. Pages 231-250 *in* IK Ferguson, J Muller, eds The evolutionary significance of the exine. Linnean Society of London, London.

Lugardon B 1990 Pteridophyte sporogenesis: a survey of spore wall ontogeny and fine structure in a polyphyletic plant group. Pages 95-120 *in* S. Blackmore, RB Knox, eds Microspores: evolution and ontogeny. Academic Press, London.

Marshall JEA 1996 *Rhabdosporites langii*, *Geminospora lemurata* and *Contagisporites optivus*: an origin for heterospory within the progymnosperms. Rev Palaeobot Palynol 93:159-189.

Marshall JEA, TP Fletcher 2002 Devonian spores from a fluvial dominated lake margin in the Orcadian Basin, Scotland. Rev Palaeobot Palynol 118:195-209.

Marshall JEA, TR Astin, JF Brown, E Mark-Kurik, J Lazauskiene 2007 Recognising the Kacak Event in the Devonian terrestrial environment and its implications for understanding land-sea interactions. Pages 133-155 *in* RT Becker, WT Kirchgasser, eds Devonian events and correlations. Geological Society, London, Special Publicatioons, 278.

McGregor DC, M Camfield 1982. Middle Devonian miospores from the Cape de Bray, Weatherall, and Hecla Bay formations of northeastern Melville Island, Canadian Arctic. Geol Surv Can Bull 348:1-105.

Miller H 1841 The Old Red Sandstone; or, new walks in an old field. Thomas Constable & Co., Edinburgh.

Moore SEM, AR Hemsley, T Borsch 2006 Micromorphology of outer exospore coatings in *Selaginella* megaspores. Grana 45:9-21.

Morbelli MA, JR Rowley 1993 Megaspore development in *Selaginella.* I "Wicks", their presence, ultrastructure and presumed function. Sexual Plant Reproduction 6:98-107.

Peng H, F Liu, H Zhu 2016 Morphology and ultrastructure of Middle Devonian dispersed megaspores from Qujing, Yunnan, Southwest China. Rev Palaeobot Palynol 234:110-124.

Perry I 1989 The fossil flora of Shetland and adjacent areas. University of Bristol, Unpublished Ph.D. thesis, 239 pp.

Pettitt JM 1966 Exine structure in some fossil and recent spores and pollen as revealed by light and electron microscopy. Bull British Mus (Nat Hist) Geology 13:221-

257.

Pettitt JM 1971 Some ultrastructural aspects of sporoderm formation in pteridophytes. Pages 227-251 *in* G Erdtman, P Sorsa, eds An introduction to palynology. IV. Pollen and spore morphology/plant taxonomy, Pteridophyta. Almqvst & Wiksell, Stockholm.

Richardson JB 1960 Spores from the Middle Old Red Sandstone of Cromarty, Scotland. Palaeontol 3:45-63.

Richardson JB 1962 Spores with bifurcate-tipped processes from the Middle Old Red Sandstone of Scotland. Palaeontol 5:171-194.

Richardson JB 1965 Middle Old Red Sandstone spore assemblages from the Orcadian basin north-east Scotland. Palaeontol 7:559-605.

Richardson JB 1969 Devonian spores. Pages 193-221 *in* RH Tschudy, RA Scott, eds Aspects of palynology. Wiley, New York.

Richardson JB, DC McGregor 1986 Silurian and Devonian spore zones of the Old Red Sandstone continent and adjacent regions. Geol Surv Can Bull 364:1-79

Richardson JB, PM Bonamo, DC McGregor 1993 The spores of *Leclercqia* and the dispersed spore morphon *Acinosporites lindlarensis* Riegel: a case of gradualistic evolution. Bull Nat Hist Mus London (Geology) 49:121-155.

Robert D 1971 Étude, en microscopie électronique, des modalities des parois microsporales ches *Selaginella selaginoides* (L.). Mise en place du feuillet interne. Comptes Rendus de L'Academie des Sciences Paris 273:1933-1936.

Robert D, F Roland-Heydacker, J Denizot, J Laroche, P Fougeroux, L Davignon 1973 Étude de la paroi siliceuse chez la megaspore d'*Isoetes setacea*. Adansonia 13:313-332.

Rowley JR 1995 Are the endexines of pteridophytes, gymnosperms and angiosperms structurally equivalent? Rev Palaeobot Palynol 85:13-24.

Senkevitsch MA, AL Jurina, AD Arkhangelskaya 1993 On fructifications, morphology and anatomy of Givetian Lepidophytes in Kazakhstan. Palaontographica B 230:43-58.

Sievers, A, B Buchen 1971 Contrast between the spore cytoplasm and the growing sporoderm of *Selaginella* megaspores. Pages 654-659 in J Brooks, RR Grant, M Muir, R Van Gijzel, G Shaw, eds, Sporopollenin. Academic Press, London.

Streel M, K Higgs, S Loboziak, W Riegel, P Steemans 1987 Spore stratigraphy and correlation with faunas and floras in the type marine Devonian of the Ardenne-Rhenish regions. Rev Palaeobot Palynol 50:211-229.

Taylor WA 1989 Megaspore wall ultrastructure in *Selaginella*. Pollen et Spores 31: 251-288.

Taylor WA 1992 Megaspore wall development in *Isoetes melanopoda*: morphogenetic post-initiation changes accompanying spore enlargement. Rev Palaeobot Palynol 72:61-72.

Taylor WA, Gullickson KA 2013 Ultrastructure of transitional dispersed megaspores from the Middle Devonian of New York. Int J Plant Sci 174:309-316.

Telnova OP 2017 The morphology and ultrastructure of Devonian *Ancyrospora melvillensis* Owens. Byulleten' Moskovskogo Obshchestva Ispytatelei Prirody Otdel Biologicheskii 122:109-113.

Tryon AF, B Lugardon 1991 Spores of the Pteridophyta. Surface, wall structure, and diversity based on electron microscope studies. Springer-Verlag, New York, 648 pp.

Turnau E, N Zavialova, A Prejbisz 2009 Wall ultrastructure in some dispersed megaspores and seed-megaspores from the Middle Devonian of northern Poland. Rev Palaeobot Palynol 156:14-33.

Uehara KS, S Kurita 1991 Ultrastructural study on spore morphogenesis in *Lycopodium clavatum* (Lycopodiaceae). Am J Bot 78:24-36.

Uehara KS, S Kurita, N Sahashi, T Ohmoto 1991 Ultrastructural study of microspore wall morphogenesis in *Isoetes japonica* (Isoetaceae). Am J Bot 78:1182-1190.

Wallace S, A Fleming, CH Wellman, DJ Beerling 2011 Evolutionary development of the plant spore and pollen wall. AoB Plants 2011 plr027.

Wellman CH 2001 Morphology and ultrastructure of Devonian spores: *Samarisporites* (*Cristatisporites*) *orcadensis* (Richardson) Richardson, 1965. Rev Palaeobot Palynol 116:87-107.

Wellman CH 2002 Morphology and wall ultrastructure in Devonian spores with bifurcate-tipped processes. Int J Plant Sci 163:451-474.

Wellman CH 2004 Origin, function and development of the spore wall in early land plants. Pages 43-63 *in* AR Hemsley, I Poole, eds The evolution of plant physiology: from whole plants to ecosystems. Linnean Society Symposium Series No. 21, Elsevier Academic Press, London.

Wellman CH 2009 Ultrastructure of dispersed and *in situ* specimens of the Devonian spore *Rhabdosporites langii*: evidence for the evolutionary relationships of the progymnosperms. Palaeontol 52:139-167.

Wellman CH 2018 The classic Lower Devonian plant-bearing deposits of northern New Brunswick, eastern Canada: dispersed spore taxonomy and biostratigraphy. Rev Palaeobot Palynol 249:24-49.

Wellman CH, PG Gensel, WA Taylor 2009 Spore wall ultrastructure in the early lycopsid *Leclercqia* (Protolepidodendrales) from the Lower Devonian of North America: evidence for a fundamental division in the lycopsids. Am J Bot 96:1849-1860.

Xu HH, CM Berry, Y Wang, JEA Marshall 2011 A new species of *Leclercqia* Banks, Bonamo et Grierson (Lycopsida) from the Middle Devonian of North Xinjiang, China, with a possible climbing habit. Int J Plant Sci 172:836-846.

Xu HH, Y Wang, Q Wang 2012 A new homosporous, arborescent lycopsid from the Middle Devonian of Xinjiang, Northwest China. Palaeontol 55:957-966.

Zaviolova N, E Turnau 2012 Morphology and wall ultrastructure of some Middle Devonian dispersed megaspores from northern Poland. Rev Palaeobot Palynol 171:103-123.

EXPLANATION OF PLATE 1

Figs A-C. LM images of dispersed *Acinosporites macrospinosus* Richardson 1965 from the Millbuie Sandstone Group (Eifelian: Mid Devonian) from Coal Heugh, Miller's Bay, Cromarty, the Black Isle, Scotland. A, Sample ACH2, Slide 1, E.F.No. (P34/4). Lateral view. Note the apical prominence (AP). B-C. Sample ACH2, Slide 1, E.F.No. (J39). Lateral view. Specimen illustrated in different focal planes. Note the apical prominence (AP). Scale bar = 100 µm.

EXPLANATION OF PLATE 2

Figs A-C. SEM images of dispersed *Acinosporites macrospinosus* Richardson 1965 from the Millbuie Sandstone Group (Eifelian: Mid Devonian) from Coal Heugh, Miller's Bay, Cromarty, the Black Isle, Scotland. A, Stub CW006 (Image 0264/99). Proximal view. Note the prominent circular apical prominence (AP) bearing the trilete mark. B. Stub CW015 (Image 0771/99). Distal view. Note the nature of the ornament. C. Stub CW015 (Image 0781/99). Lateral view. Note the nature of the apical prominence (AP). Scale bar = 100 µm.

EXPLANATION OF PLATE 3

Figs A-B. SEM images of dispersed *Acinosporites macrospinosus* Richardson 1965 from the Millbuie Sandstone Group (Eifelian: Mid Devonian) from Coal Heugh, Miller's Bay, Cromarty, the Black Isle, Scotland. A, Stub CW015 (Image 0779/99). High magnification image of distal ornament from specimen illustrated in Pl. 2, fig. B. B. Stub CW015 (Image 0777/99). High magnification image of ornament (bottom left) below apical prominence (AP) (top right) from specimen illustrated in Pl. 2, fig. C. Scale bar = 50 μm (A) and 25 μm (B).

EXPLANATION OF PLATE 4

Figs A-J. TEM images of an individually picked specimen of the dispersed spore *Acinosporites macrospinosus* Richardson 1965 from the Millbuie Sandstone Group (Eifelian: Mid Devonian) from Coal Heugh, Miller's Bay, Cromarty, the Black Isle, Scotland. All images are from the same spore (one of three specimens embedded in Block CW023). A, Image R387, illustrating an almost entire spore. Note the lumen (L) near the centre of the section. The apical prominence and trilete mark are not present on this section; B, Image R374, a close up of (A) focussing in on the lumen (L); C, Image R375, a close up of (A) focussing on the ultrastructure of the ornament (Layer 5). V = void; D, Image R379, illustrating the ultrastructure around the lumen (L); E, Image R385, a close up of (D) focussing in on the ultrastructure around the lumen (L); F, Image R380, a close up of the ultrastructure immediately adjacent to the lumen (L) (the junctions between Layers 1-3 are marked with arrows); G, Image 391, a close up of the ultrastructure immediately adjacent to the lumen (L) (the junction between Layers 1-3 are marked with arrows); H, Image R388, a close up of the ultrastructure in Layer 3; I, Image R377, a close up of the ultrastructure in Layer 3; J, Image R376, a close up of ultrastructure in Layer 4. Scale bar = 54 μm (A) 32 μm (B) 17.6 μm (C) 3.4 μm (D) 2.1 μm (E) 1.0 μm (F) 0.9 μm (G) 1.4 μm (H) 0.7 μm (I) 2.9 μm (J).

EXPLANATION OF PLATE 5

Figs A-B. TEM images of an individually picked specimen of the dispersed spore *Acinosporites macrospinosus* Richardson 1965 from the Millbuie Sandstone Group (Eifelian: Mid Devonian) from Coal Heugh, Miller's Bay, Cromarty, the Black Isle, Scotland. All images are from the same spore (one of three specimens embedded in Block CW023). A, Image R381, a close up of the ultrastructure of Layers 1-2 surrounding the lumen (L) (the junctions between Layers 1-3 are marked with black arrows); B, Image R383, a close up of (A) showing the ultrastructure of Layers 1-2 surrounding the lumen (L) (the junctions between Layers 1-3 are marked with black arrows). White arrows indicate examples of White Line Centred Lamellae. Scale bar = 1000 nm (A) 560 nm (B).