



This is a repository copy of *Wall ultrastructure of the Permian pollen grain Lueckisporites virkkiae Potonié et Klaus 1954 emend. Clarke : Evidence for botanical affinity.*

White Rose Research Online URL for this paper:  
<https://eprints.whiterose.ac.uk/155648/>

Version: Accepted Version

---

**Article:**

Gibson, M.E., Taylor, W.A. and Wellman, C.H. (2020) Wall ultrastructure of the Permian pollen grain *Lueckisporites virkkiae* Potonié et Klaus 1954 emend. Clarke : Evidence for botanical affinity. *Review of Palaeobotany and Palynology*, 275. 104169. ISSN 0034-6667

<https://doi.org/10.1016/j.revpalbo.2020.104169>

---

Article available under the terms of the CC-BY-NC-ND licence  
(<https://creativecommons.org/licenses/by-nc-nd/4.0/>).

**Reuse**

This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs (CC BY-NC-ND) licence. This licence only allows you to download this work and share it with others as long as you credit the authors, but you can't change the article in any way or use it commercially. More information and the full terms of the licence here: <https://creativecommons.org/licenses/>

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.



[eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk)  
<https://eprints.whiterose.ac.uk/>

Wall ultrastructure of the Permian pollen grain *Lueckisporites virkkiae* Potonié et Klaus 1954 emend. Clarke : Evidence for botanical affinity

Martha E. Gibson, Wilson A. Taylor, Charles H. Wellman



PII: S0034-6667(19)30262-3

DOI: <https://doi.org/10.1016/j.revpalbo.2020.104169>

Reference: PALBO 104169

To appear in: *Review of Palaeobotany and Palynology*

Received date: 22 August 2019

Revised date: 7 January 2020

Accepted date: 12 January 2020

Please cite this article as: M.E. Gibson, W.A. Taylor and C.H. Wellman, Wall ultrastructure of the Permian pollen grain *Lueckisporites virkkiae* Potonié et Klaus 1954 emend. Clarke : Evidence for botanical affinity, *Review of Palaeobotany and Palynology*(2019), <https://doi.org/10.1016/j.revpalbo.2020.104169>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Wall ultrastructure of the Permian pollen grain *Lueckisporites virkkiae* Potonié et Klaus 1954  
emend. Clarke 1965: evidence for botanical affinity

Martha E. Gibson<sup>a</sup>, Wilson A. Taylor<sup>b</sup>, Charles H. Wellman<sup>a</sup>

<sup>a</sup>*Department of Animal & Plant Sciences, University of Sheffield, Alfred Denny Building,  
Western Bank, Sheffield S10 2TN, UK*

<sup>b</sup>*Department of Biology, University of Wisconsin-Eau Claire, 105 Garfield Avenue, Eau  
Claire, WI 54701, USA*

## ABSTRACT

Permian spore-pollen assemblages are dominated by striate bisaccate pollen grains. The botanical affinity of many of these is unknown, which limits their value in ecological reconstruction and biostratigraphy. *Lueckisporites virkkiae* Potonié et Klaus 1954 emend. Clarke 1965 is a bitaeniate bisaccate pollen grain of uncertain botanical affinity that dominates late Permian Euramerican assemblages, but also bridges phytogeographical provinces. It is classified into three ‘variants’, which capture a spectrum of morphological variability, but further complicates matters by questioning whether it comprises a natural taxon. Here we report on a TEM analysis of the gross structure and wall ultrastructure of *L. virkkiae* variants from the upper Permian (Lopingian) of Kimberley, Nottinghamshire, UK. The variants have the same gross structure and wall ultrastructure. The exine consists of a three-layered ectexine composed of a thin tectum, an alveolate infratectum and a thin foot layer, subtended by a solid endexine. Alveolae are either small and rounded, columellate-like, or irregularly shaped, depending on whether the exine is viewed in transverse or longitudinal section. Sacci are protosaccate, with narrow and irregular, or large and voluminous, endoreticulations. Similarities in gross structure and wall ultrastructure suggest that the current taxonomic grouping is correct with the variants representing the range of variation within a single species. We suggest that the differences in morphology are subtle and attributable to harmomegathy. Comparisons with fossil and modern conifer pollen grains suggest an affinity with conifers. Co-occurrence with the Zechstein conifer macrofossil *Pseudovoltzia liebeana* (Geinitz) Florin 1880 (Majonicaceae) indicates this as a possible source.

**Key words:** Late Permian, *Lueckisporites virkkiae*, Gymnosperm, Saccate pollen, Exine ultrastructure, Alveolar infratectum

## 1. Introduction

Late Permian spore-pollen assemblages worldwide are characterized, and often dominated, by striate bisaccate pollen. This is despite the high levels of floral provincialism shown by plant megafossils at this time and the fact that at least three distantly related plant groups are known to produce such pollen (glossopterids, peltasperms and conifers) (Chaloner, 2013). Late Permian Euramerican spore-pollen assemblages, such as those recovered from the Zechstein Basin, are often dominated by an example of this pollen type: *Lueckisporites virkkiae* Potonié et Klaus 1954 emend Clarke 1965 (e.g. Clarke, 1965; Visscher, 1971). Being a ‘bridging taxon’ that occurs across phytogeographical provinces, this taxon is not only important ecologically, but it also has biostratigraphical significance (Stephenson, 2016). However, as yet, the botanical affinity of *L. virkkiae* has not been confidently assigned. Matters are complicated because it exhibits a high degree of morphological variability that has confounded pollen taxonomists and led to questions regarding whether the taxon represents a natural grouping. The lack of understanding regarding the taxonomic status and botanical affinity of *L. virkkiae* hinders its biostratigraphical utility and use in vegetational reconstruction of the floras of Euramerica and elsewhere. In this paper we report on a detailed Transmission Electron Microscopy (TEM) analysis of the morphological variants of *L. virkkiae* in an attempt to clarify its taxonomic status and to shed light on the botanical affinities of this pollen grain.

## 2. Materials and methods

## **2.1. Locality and geology**

Samples were collected from an upper Permian sequence from a railway cutting at Kimberley, Nottinghamshire, UK [NZ 503 453]. This exposure has long been known (Wilson, 1876; Howard et al., 2009) and its palaeobotany (Stoneley, 1958; Cleal and Thomas, 1995) and palynology (Clarke, 1965) are well documented. A 10 m sequence of the Cadeby Formation rests unconformably on Upper Carboniferous Coal Measures. It consists of a 1.5 m thick basal layer of Permian Breccia, overlain by 7 m of 'Lower Marl', which is in turn overlain by several meters of 'Lower Magnesian Limestone' (Howard et al., 2009). At Kimberley the 'Lower Marl' consists of red and yellow, medium grained, calcareous sandstone containing thin bands of pale grey shale. It is from these thin shale bands that the palynomorphs used in this study were recovered. This sequence corresponds to sample K5 of Clarke (1965). The Cadeby Formation represents marginal shallow marine deposits of the first major transgressive phase of the first depositional cycle of the Zechstein Sea (Smith and Taylor, 1992). The precise age of the Magnesian Limestone is uncertain, but is generally considered to be late Permian (Lopingian).

## **2.2. Preparation and techniques**

Collected rock samples were subjected to standard palynological HCl-HF-HCl acid maceration techniques. The residue was sieved using a 20 µm sieve and subjected to heavy liquid separation using zinc chloride dissolved in 10% HCl. Recovered pollen grains are extremely well preserved and of very low thermal maturity with no artificial oxidation required. The organic residue was divided with some stew mounted onto glass slides for light-microscopic (LM) analysis and some retained for picking. Three specimens of each of the three variants of Clarke (1965) were individually picked for TEM analysis. Picking was

achieved by transferring the palynological residue into ethanol and strew mounting this onto glass slides. The ethanol was left to evaporate naturally before slides were examined under a light microscope attached to a micromanipulator. Selected pollen grains were then individually picked. Picked specimens were encased in agar and dehydrated in 100% ethanol, impregnated with Spurr resin, and cut into ultrathin sections using a diamond knife. Two perpendicular sections were cut; one along the transverse axis and the other along the longitudinal of the pollen grain (Fig. 2). Sections were examined and photographed under a FEI Technai Spirit TEM at 80 kV. Composite TEM images made from individual micrographs were compiled using the GIMP 2.0 software package. Materials are curated in the Centre for Palynology of the University of Sheffield (rock, remaining organic residue, LM slides) and the Department of Biology at the University of Wisconsin Eau Claire (TEM blocks and sections).

### 3. Taxonomic history of *Lueckisporites virkkiae*

The genus *Lueckisporites* was established by Potonié et Klaus (1954) with the type species *L. virkkiae*. The original generic concept was rather broad and allowed for multiple taeniae, although the description of the type species made it clear that it possessed only two. Subsequently numerous authors discussed the genus and some proposed formal emendations. Various authors considered the value of expanding the genus to include forms with multiple taeniae (Potonié, 1958, 1966; Grebe and Schweitzer, 1962; Mädler, 1964a; Singh, 1965). However, this was eventually deemed inappropriate due to the already complex taxonomy of taeniate pollen grains and established practical applications of *L. virkkiae* in biostratigraphy. Therefore a bitaeniate concept for *Lueckisporites* was justified (Leschik, 1965b, 1959; Jansonius, 1962; Orłowska-Zwolińska, 1962; Klaus, 1963; Hart, 1964, 1965; Ullrich, 1964,

Clarke, 1965; Efremova, 1966; Mosler, 1966; Stephenson, 2008). Both Jansonius (1962) and Klaus (1963) formally emended the generic diagnosis specifying that it had only two taeniae, although Bharadwaj (1974)'s emendation allowed for more.

By this time, it had become apparent that *Lueckisporites* is characteristic of, and often dominant in, late Permian palynological assemblages of the Euramerican floral province. Many authors included all of their specimens in the type species *L. virkkiae*, whilst accepting that there was a great deal of morphological variation within this 'species concept'. However, some authors recognized a wider range of species (e.g. Klaus, 1963; Schaarschmidt, 1963; Singh, 1965), while others encompassed the continuum of morphological variations in the form of 'variants' (Clarke, 1965) or 'palynodemes' (Visscher, 1971).

Clarke (1965) used the emended diagnosis of Klaus (1963) but emended the diagnosis of the type species *Lueckisporites virkkiae*. He suggested that *L. virkkiae* showed great morphological variation with intergradation between three extremes he termed Variants A – C. According to Clarke (1965) Variant A is the largest and is described as having well-developed, distinctly separate proximal thickenings and well-developed sacci, and is most similar to the holotype and *L. microgranulatus* Klaus 1963. Variant B has sacci that are less well-developed and a small saccus offlap and is most similar to *L. parvus* Klaus 1963. Variant C has a weakly developed proximal cap that is not completely separated into two halves, and is also generally smaller with a more elongate corpus, and is most similar to *L. microgranulatus* "kleinere variante" Klaus 1963.

Visscher (1971) introduced a 'palynodeme concept'. He suggested the taxon could be subdivided into 11 forms (Norm A: Aa, Ab', Ab'', Ac; Norm B: Ba, Bb, Bc', Bc''; Norm C; Norm D, Norm E), based on variation among four characters: (i) the structure of the sexine of



the cappa; (ii) the shape of the sacci; (iii) the structure of the sexine of the sacci; (iv) the presence of teratological variations.

In both the schemes of Clarke (1965) and Visscher (1971) the variants/palynodeme norms do seem to differ in size. However, size as a means of differentiation is known to be problematic because it may reflect morphological variation, but can also be influenced by taphonomic effects (e.g. compression), ontogeny or even hydration and harmomegathic effects.

Ultimately, it is unclear whether the morphological variation observed in *Lueckisporites virkkiae* represents natural intraspecific variation or taphonomic, taphonomic, ecological, or some other factor. This lack of understanding obviously hinders our ability to use *L. virkkiae* as an interpretive tool.

This study uses the three “variant” described by Clarke (1965) rather than the palynodeme concept of Visscher (1971) for ease of discrimination. Clarke’s three variants are easily recognized and provide a good starting point for assessing wall ultrastructure among the morphological variation exhibited by *L. virkkiae*.

#### **4. Previous interpretations of the botanical affinity of *Lueckisporites***

The botanical affinity of the genus *Lueckisporites* has been considered based on association with plant megafossil remains (e.g. Visscher, 1971) or interpretation of in situ pollen (Klaus, 1966; Clement-Westerhof, 1987).

Visscher (1971) proposed an affinity with the conifer *Ullmannia bronnii* Göppert 1944 based on a strong correlation in abundance between the dispersed pollen grain *Lueckisporites virkkiae* and the plant megafossil *Hiltonia rivuli* Stoneley 1956 (a synonym of

*U. brononii*) in the Hilton Plant Beds. However, in situ pollen reported from *Ullmannia frumentaria* (Sclotheim) Göppert 1850 show extensive morphological variation and can be placed with the genera *Jugasporites* Leschik 1956, *Limitisporites* Leschik 1956, *Triadispora* Klaus 1964 and *Illemites* Potonié et Kremp 1954 (Florin, 1944b; Potonié and Schweitzer, 1960; Potonié, 1962; Visscher 1971; Schweitzer, 1986; Balme, 1995). Importantly, none of these taxa are striate. In situ pollen associated with *Ullmannia* cf. *brononii* were similarly reported as belonging to the non-striate taxon *Alisporites* Daugherty 1941 emend. Nilsson 1958 (although Visscher (1971) questioned the relationship between the examined cone and *U. brononii*).

Klaus (1966) reported (but did not illustrate) in situ pollen, which he compared to *Lueckisporites junior* Klaus 1963, from the enigmatic plant *Pramelreuthia haberfelneri* Krasser 1916 from the Triassic of Austria. Unfortunately, the biological affinity of this plant is far from clear. *Lueckisporites* has also been associated with the conifer family Majonicaceae after *Lueckisporites*-like pollen was extracted from a cone of *Majonica alpina* Clement-Westerhof 1987 from the upper Permian of Italy (Clement-Westerhof, 1987). However, only a few inconclusive light microscope images of the in situ pollen were published that were subsequently interpreted as *Lueckisporites* by authors such as Balme (1995). Nonetheless, these pollen grains do appear to be bisaccate and bitaeniate.

## 5. Descriptions

LM images of all three variants of *Lueckisporites virkkiae* are provided in Plate I. TEM images and interpretive drawings are provided for each of the variants: Variant A (Plate II, 1-5); Variant B (Plate III, 1-5); Variant C (Plate IV, 1-4). Descriptive terminology is based

on Osborn and Taylor (1994)'s descriptions of gymnosperm pollen ultrastructure. Sections are of unbroken pollen grains and exhibit limited compression.

### **5.1 Variant A**

Pollen grains of Variant A possess two well-developed, distinctly separate taeniae on the proximal surface oriented parallel to the transverse axis. The corpus is elliptical in outline, with the transverse axis exceeding the longitudinal axis. The well-developed semicircular, or more than semi-circular, sacchi give grains of Variant A a diploxytonoid outline (Fig. 1, a-c).

In transmitted light the surface of the corpus and taeniae appears infrapunctate or infrabaculate. The sacchi are composed of radially arranged anastomosing muri that become smaller and more frequent towards the periphery of the sacchi (Plate I, 1).

Under TEM the pollen grains have a bilayered exine composed of an outer three part ektexine and an inner endexine, and varies between 1.40  $\mu\text{m}$  and 3.55  $\mu\text{m}$  in thickness (Plate II, 2, 5).

The ektexine is composed of a thin outer tectum (0.20  $\mu\text{m}$  to 0.49  $\mu\text{m}$ ) with a contour that is smooth and gently undulating. The infratectum is the thickest layer (0.69  $\mu\text{m}$  to 1.67  $\mu\text{m}$ ) and appears as an alveolar layer beneath the tectum. The alveolae are small and rounded, or long and elongated in transverse section (Plate II, 1) and are separated by substantial partitions. In longitudinal section (Plate II, 3) the alveolae appear more elongate and irregular. The size and density of the alveolae vary across the ektexine. Alveolae become progressively smaller and denser towards the outer edge of the ektexine. This pattern is observed in both planes of section and within individual pollen grains. The foot layer is thin (0.08  $\mu\text{m}$  to 0.23  $\mu\text{m}$ ) and can be difficult to distinguish from the overlying infratectum. The

proximal face is covered by the taeniae, however, the distal face of the corpus displays a different exine stratification. It consists only of endexine (Plate II, 3). Taeniae have an ultrastructure consistent with the ektexine.

The endexine, when visible, is a prominent homogeneous layer that varies in thickness between 0.43  $\mu\text{m}$  and 1.16  $\mu\text{m}$ , possibly due to differential compression across pollen grains. No lamellations are visible. The endexine was not present in all specimens, but this was caused by the endexine either being too compressed or specimens fractured during sectioning. The only region of the exine experiencing thinning is in the region of where the taeniae meet on the cappa (Plate II, 3). However, it is unclear whether this is a fracture in the exine or whether it represents a true aperture.

The sacci are semicircular and well-developed, appearing protosaccate with endoreticulations that are continuous with the foot layer and span the entire width of the sacci. Endoreticulation morphology is independent of sectioning plane, appearing consistent in both transverse and longitudinal section. Alveolae vary in size and density within the saccus, with alveolae becoming smaller towards the perimeter of the sacci. Partitions between the alveolae are thick and substantial, creating column-like structures in transverse section (Plate II, 4).

## **5.2 Variant B**

Pollen grains of Variant B have two well-developed, distinctly separate taeniae on the proximal surface. The corpus is subcircular-circular in outline, with the transverse and longitudinal axes being more-or-less equivalent relative to Variants A and C. The overall grain appears more haploxytonoid than diploxytonoid due to the small saccus offlap and smaller germinal furrow (Fig. 1, d-f).

In transmitted light the corpus and taeniae appears infrapunctate or infrabaculate. Sacci are composed of radially arranged anastomosing muri that become smaller and more frequent towards the perimeter (Plate I, 2).

In thin section the exine is bilayered consisting of a three part outer ectexine and an inner endexine (Plate III, 2, 3). It varies in thickness between 1.69  $\mu\text{m}$  to 2.06  $\mu\text{m}$ . The ectexine is composed of an outer tectum (0.18  $\mu\text{m}$  to 0.50  $\mu\text{m}$ ) with a smooth and gently undulating contour. An intermediate infratectum (1.25  $\mu\text{m}$  to 1.48  $\mu\text{m}$ ) has an ultrastructure that is dependent on the sectioning plane. In transverse section the infratectum appears alveolar with elongated alveolae close to the foot layer; the alveolae become progressively smaller and more rounded towards the outer edge of the infratectum. In longitudinal section the infratectum appears to be composed of larger columellate-like partitions, with smaller, more rounded alveolae towards the outer edge (Plate III, 3). The foot layer is very thin (0.06  $\mu\text{m}$  to 0.12  $\mu\text{m}$ ) (Plate III, 3) and is present in all specimens. The distal surface of the grain is composed only of endexine, or possibly a very thin layer of ectexine (Plate III, 4). The ultrastructure of the taeniae is composed of ectexine and endexine, with alveolae resembling those of the ectexine (Plate III, 5).

The endexine forms a prominent, homogeneous, and continuous layer underneath the foot layer (0.63  $\mu\text{m}$  to 1.52  $\mu\text{m}$ ). No lamellations are visible and in some specimens the endexine is thick (Plate III, 3, 4) while in others it is more reduced, possibly due to compression or an artefact of the sectioning process. A rough layer is present on the inside of one specimen (Plate III, 5) – this was observed in only one instance, but likely represents an artefact of the sectioning process. No thinning of the exine is observed in these sections meaning the location of the aperture is unclear, although it could be located between the taeniae on the proximal surface as in Variants A and C.

The sacci are less well-developed than in Variant A and C, appearing greatly reduced in section (Plate III, 2). The sacci appear protosaccate but internal structure varies with the sectioning plane. In longitudinal section sacci are composed of large endoreticulations with smaller and more frequent endoreticulations towards the perimeter of the saccus (Plate III, 5). In transverse section the endoreticulations appear continuous with the foot layer, and are reduced in volume, forming a less extensive network of alveolae (Plate III, 4).

### 5.3 Variant C

Pollen grains of Variant C possess two taeniae on the proximal surface, that are often less distinct than in Variants A and B. The sacci are semi-circular but less so than Variant A, and the corpus is noticeably elongated along the transverse axis. The overall outline is haploxytonoid to diploxytonoid (Fig. 1, c-i).

In transmitted light pollen grains appears infrapunctate or infrabaculate, with no visible pattern on the proximal taeniae. The sacci are composed of radially arranged anastomosing muri that increase in frequency but become smaller towards the periphery of the sacci (Plate I, 3)

In section the exine is bilayered with an outer three part ectexine and an inner endexine. The exine varies in thickness between 2.48  $\mu\text{m}$  and 5.68  $\mu\text{m}$  (Plate IV, 2).

The ectexine is composed of an outer tectum (0.14  $\mu\text{m}$  to 0.20  $\mu\text{m}$ ) with a thin and irregularly undulating contour. Some specimens display more undulation than others (Plate IV, 2b, 5). The underlying infratectum is alveolar and thick (0.64  $\mu\text{m}$  to 2.40  $\mu\text{m}$ ). The foot layer is thin (0.90  $\mu\text{m}$  to 1.54  $\mu\text{m}$ ), and forms a continuous layer along the base of the ectexine (Plate IV, 3).

The endexine is either relatively thick (0.87  $\mu\text{m}$  to 1.54  $\mu\text{m}$ ), or it appears as a solid layer of constant thickness beneath the ectexine. No lamellations are observed. There is a slight thinning of the endexine center of the distal face (Plate IV, 1).

The proximal and distal surface of the exine of the corpus differ. The distal face is composed only of an endexine, while the proximal face is composed of both ectexine and endexine (Plate IV, 3). The exine between the taeniae appears to be composed only of endexine (Plate IV, 1, 2).

Saccus structure varies within single specimens, however, it is consistent between transverse and longitudinal sections. Sections taken further into the saccus reveal a longer and more differentiated, voluminous protosaccate endoreticulation pattern that is more consistent with other specimens of Variant C (Plate IV, 2a). Sections bisecting the saccus offlap show this region to be composed of many small, rounded alveolae. Towards both the inner and outer peripheries of the sacci the alveolae increase in volume forming a more complex network. Partitions between alveolae are long and thin (Plate IV, 2b).

Taeniae were not visible in these sections of Variant C, however, they are possibly captured on the proximal surface of PP10 visible as a dramatic reduction in the thickness of the ectexine in the upper left hand quarter of the micrograph (Plate IV, 2b)

The proximal and distal exines of the corpus differ with the distal face consisting only of endexine while the proximal face also has an ectexine. The area on the proximal face where only the endexine is developed possibly represents an aperture.

## 6. Discussion

### 6.1 *The bisaccate condition (phylogeny and functional morphology)*

Among the extant flora bisaccate pollen occur only in gymnosperms where they are produced by some Pinaceae and Podocarpaceae (Coniferales) (see Kurmann, 1992). In the Phyllocladaceae (*Phyllocladus*) the 'bisaccate' pollen grains appear to be vestigial (Tomlinson, 2000). Fossil bisaccate pollen grains have been reported in situ from various extinct and extant groups of Coniferales (extinct Voltziales and extant Pinaceae and Podocarpaceae) in addition to the extinct pteridosperm groups: Glossopteridales, Crystospermales, Peltaspermales, Caytoniales and Callistophytales (Balme, 1995; Traverse, 2007). The phylogenetic significance of bisaccate pollen is debated. For example, Tomlinson (2000) notes that bisaccate pollen is exclusive to gymnosperms and suggests that it may be ancestral in the conifers but with frequent losses. Others suggest that it may have arisen numerous times independently due to convergence (Leslie, 2008).

The actual function and adaptive significance of the bisaccate condition is also highly debated with suggestions that it may: (i) aid long distance wind dispersal and hence outbreeding (e.g. Schwendeman et al. 2007); (ii) aid discharge from the sporangium (e.g. Niklas, 1985); (iii) have a homeostatic effect in limiting water loss through the sulcus (e.g. Wodehouse, 1935); (iv) facilitate pollination by orientating the pollen grain in the pollination drop (e.g. Doyle, 1945); (v) increase the likelihood of capture by pollen scavenging whilst on the pollen drop (e.g. Tomlinson et al., 1991). As noted by Tomlinson (2000) these proposed functions are not mutually exclusive.

### 6.2 *Gross structure and wall ultrastructure of bisaccate pollen grains*

Analysis of gross structure and ultrastructure of both extant and fossil bisaccate pollen grains has the potential to address some of the problems outlined above. Most importantly,



the identification of homologies among wall layers may inform us of their phylogenetic significance in bisaccate pollen grains, i.e. whether their phylogenetic distribution is most likely a function of evolutionary inheritance (synapomorphy) or convergence (homoplasy). Most recently the wall ultrastructure of extant bisaccate pollen producers has been reviewed in Kurmann (1990, 1992) and Kurmann and Zavada (1994) and that of fossil producers by Taylor and Taylor (1987), Kurmann and Zavada (1994) and Osborn and Taylor (1994). Wall ultrastructure in the bisaccate pollen grains of extant coniferales (Pinaceae and Podocarpaceae) is similar and based on a four layered wall system consisting of lamellated endexine and an ectexine comprising a foot layer, infratectum and tectum (Kurmann, 1992). Wall layering in fossil plants is similarly organized. As reviewed by Osborn and Taylor (1994) fossil gymnosperm pollen grains are composed of a four layered wall with a homogenous endexine in mature grains, and a three part ectexine composed of a perforated or undulating outer tectum, an inner alveolar or granular infratectum, and a thin foot layer. The main distinction between extant and many fossil bisaccate pollen is the lack of proximal taeniae that were such a prominent feature during the late Palaeozoic (Chaloner, 2013).

### 6.3 Gross structure and wall ultrastructure of *Lueckisporites virkkiae*

The analysis of gross structure and wall ultrastructure in the three variants of *Lueckisporites virkkiae*, as reported in this paper, has a number of implications. Regarding ontogeny and taxonomy, the overall similarity in pollen wall ultrastructure suggests that variation in gross structure/morphology that defines the three variants most likely represents subtle structural differences due to ontogeny and developmental plasticity and/or preservation (e.g. pollen hydration prior to discharge) rather than major taxonomic differences. It seems highly likely that all three variants examined represent mature pollen grains naturally

dispersed, as suggested by their unlamellated endexine, and as would be expected from dispersed pollen assemblages. Minor variations in wall ultrastructure probably reflect deviations in the angle of sectioning that can alter the shape of the alveolae or perceived thickness of layers within the exine.

Similarly, a number of studies of in situ bisaccate pollen have demonstrated significant intraspecific morphological variation among bisaccate pollen grains both from the fossil record (e.g. Schweitzer, 1986; Lindström et al., 1997) and among extant plants (Kurmann and Zavada, 1994; Tomlinson, 1994; Owens et al., 1998; Leslie, 2008). This obviously has serious implications for the taxonomy of dispersed pollen grains and suggests there may be a degree of taxon inflation. It is also relevant to the current debate concerning mutagenesis among bisaccate pollen caused by anoxic stress (Foster and Afonin, 2005; Benca et al., 2018) as it suggests that a degree of natural variation in morphology might be expected.

If the variants of *Lueckisporites virkkiae* belong to the same species then it is likely that they represent continuous, rather than discrete, forms. It is possible that the differences are a result of the relative elasticity of the exine that allows it to accommodate changing osmotic pressure of the cytoplasm, as during hydration or dehydration, causing considerable modification to the structure and appearance of the pollen grain wall. Due to the dispersed nature of this material, there can be no definitive proof of what order the variants may align regarding least to most hydrated. However, based on the appearance of modern bisaccate pollen grains in their dehydrated state, with the sacci folded over the corpus giving the pollen grains a more spherical outline (Osborn and Taylor, 1994; Pacini and Hesse, 2012), it is possible that Variant B with its large saccus overlap and reduced sacci represents a relatively dehydrated state. If pollen grains of *L. virkkiae* hydrate similarly to modern bisaccate pollen grains then it is possible that the order is Variant B – Variant C – Variant A. Variant A is also

the most abundant within dispersed assemblages (e.g. Visscher, 1971), suggesting that it may be the final, most inflated, form.

#### 6.4 The phylogenetic significance of the protosaccate versus eusaccate condition

The phylogenetic utility of saccus condition is limited because of the common compression of sacci that can make pollen grains appear superficially protosaccate. Direction of sectioning plane is also important because grains can appear protosaccate or eusaccate dependent on whether sections are longitudinal or transverse (Osborn and Taylor, 1994), as a result of sacci tapering off laterally where they attach to the corpus. Ontogenetic stage can also contribute to a protosaccate appearance. It is possible for under-developed pollen grains to appear superficially protosaccate if the sacci are not yet fully expanded (Osborn and Taylor, 1994). The robustness of endoreticulations also factors into determining whether a grain is protosaccate or eusaccate as delicate endoreticulations may be easily ruptured or separated from the underlying endexine during saccus expansion.

The pollen grains in this study are relatively uncompressed which rules out wall ultrastructure appearing superficially protosaccate. The endoreticulations observed in the specimens are not discontinuous endoreticulations that have been preservationally compressed. All variants have been sectioned both transversely and longitudinally to account for saccus tapering, and no distinct difference in saccus structure was observed. Ontogenetically, these specimens are surmised to be mature due to their unlamellated endexine (even following staining) and the endoreticulations observed in all specimens are robust and intact. All this suggests strongly that the endoreticulations in *Lueckisporites virkkiae* are protosaccate, rather than eusaccate.

Nonetheless, proving that *Lueckisporites virkkiae* is protosaccate is not very helpful regarding determining a biological affinity for this pollen grain. This is because it is unclear which of the protosaccate or eusaccate conditions is the more primitive, as both have been described from different groups of gymnosperms creating debate about whether or not they are homologous (Crane, 1990).

### 6.5 Phylogenetic affinities of *Lueckisporites virkkiae*

Regarding the biological affinities of the parent plant of *Lueckisporites virkkiae*, pollen wall ultrastructure is again potentially more important than gross structure. Bisaccate pollen have been reported from diverse plant groups (summarized above) and is not particularly informative as the phylogenetic pattern represents either substantial losses or numerous independent origins. However, the distinctive four-layered wall and characteristic ultrastructure, coupled with the bisaccate condition, would seem to point to affinities with the conifers as this is a feature recognized in both extant and fossil conifer groups that produce bisaccate pollen.

Other pollen grains are taeniate, such as those produced by the Glossopteridales, but they display a different ultrastructure, largely in that they are eusaccate with a complete separation of the sexine and nexine in the region of the saccus. Glossopterid pollen grains, extracted from *Arberiella*-type sporangia, have a corpus exine with an infrastructural layer composed of irregular shaped rods or partitions (Zavada, 1991); a different infratectum organization than the alveolar infratectum and protosaccate sacchi observed in *L. virkkiae*. Furthermore, predispersal expansion of the corpus has been observed in the sporangia of *Arbereilla* sp. cf. *A. africana* Pant and Neutiyal (Lindström et al., 1997). Predispersal expansion is another possible explanation for the differences in gross morphology observed.

Thus it seems likely that *L. virkkiae* was produced by a conifer based on a consideration of pollen morphology, gross structure and wall ultrastructure. Considering the composition of the flora described from the late Permian Zechstein Basin a possibility is *Pseudovoltzia liebeana* (Geintz), a member of the extinct conifer family Majonicaceae (Clement-Westerhof, 1987). Another member of the Majonicaceae, *Majonica alpina* Clement-Westerhof, is known to produce bitaeniate bisaccate pollen that bears a distinct resemblance to *Lueckisporites* (Clement-Westerhof 1987). An Angaran basal conifer group contemporary *Sashinia* Meyen 1968 is also known to produce the morphologically similar protobisaccate pollen grain *Scutasporites* Klaus 1963 (Gomankov et al., 1998; Gomankov, 2009). The lack of in situ reproductive material for *Pseudovoltzia* from the British Zechstein makes this association tentative and we are aware that palaeobotanical/palynological co-occurrence is a complicated issue and basing affinities on such data may be problematic. However, the elimination of other known macroflora-pollen association from the source assemblage does provide some support for this interpretation; *Ullmannia frumentaria* (Schlotheim) Göppert 1850 is known to produce *Illenites* sp. (Schweitzer 1986) as well as *Jugasporites*, *Limitisporites* and *Triadispora* (Florin, 1944b; Potonié and Schweitzer, 1960; Potonié, 1962; Visscher 1971; Schweitzer, 1986; Balme, 1995), and *Alisporites* sp. is produced by a voltziaceous conifer (Grauvogel-Stamm, 1978).

## 7. Conclusions

The three variants of the bisaccate pollen grain *Lueckisporites virkkiae* all exhibit similar wall ultrastructure characterized by an alveolar infratectum and homogenous endexine. We conclude that the variants belong to a single taxon and that the morphological differences between them are a result of the effects of harmomegathy with the variants

representing different levels of pollen hydration prior to discharge. A comparison with known fossil and extant bisaccate pollen has shown that the pollen grains share a number of gross structure and wall ultrastructure characteristics with coniferous pollen. However, comparisons are hindered by the absence of extant taeniate bisaccate pollen grains from which natural variation in striate features could be assessed and the development of taeniae understood. Co-occurrence data suggests *Pseudovoltzia liebeana* (family Majonicaceae) as a possible parent plant, which is supported by reports of in situ pollen in other members of Majonicaceae in the fossil record.

### Acknowledgements

MEG was funded by a NERC studentship through the ACCE (Adapting to the Challenges of a Changing Environment) Doctoral Training Partnership (Award Reference 1807541). We thank G. Warrington for taxonomic advice.

### Declaration of interests

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

### References

- Balme, B.E., 1995. Fossil in situ spores and pollen grains: an annotated catalogue. Review of Palaeobotany and Palynology 87, 81-323.
- Bharadwaj, D.C., 1974. On the classification of gymnospermous spores dispersae; in Symposium on structure, nomenclature and classification of pollen and spores. Birbal Sahni Institute of Palaeobotany, Lucknow, Special Publication 4, 7-52.

Chaloner, W.G., 2013. Three Palynological Puzzles. *International Journal of Plant Sciences* 174 (3), 602-607.

Clarke, R.F.A., 1965. British Permian saccate and monosulcate miospores. *Palaeontology* 8 (2), 322-54.

Cleal, C.J., Thomas, B.A., 1995. *Palaeozoic Palaeobotany of Great Britain*. Geological Conservation Review Series No. 9, Chapman and Hall, London.

Clement-Westerhof, J.A., 1987. Aspects of Permian palaeobotany and palynology, VII. The Majonicaceae, a new family of Late Permian conifers. *Review of Palaeobotany and Palynology* 52, 375-402.

Crane, P.R., 1990. The phylogenetic context of microsporogenesis. In: S. Blackmore and R. B. Knox (editors). *Microspores: Evolution and Ontogeny*, pp. 11-41, Academic Press, London.

Doyle, J., 1945. Development lines in pollination mechanisms in the Coniferales. *Scientific Proceedings of the Royal Dublin Society A*. 24, 43-62.

Efremova, G.D., 1966. On classification of dispersed pollen. *Striatiti Pant* 1954; *In: Zavialova, N.E., Meyer-Mankian, N.R., and Gomankov, A.V.* 2001. Ultrastructure of some Permian pollen grains from the Russian Platform; *In: Goodman, D.K., and Clarke, R.T.* (eds.), *Proceedings of the IX international Palynological Congress, Houston, Texas, U.S.A., 1996*; American Association of Stratigraphic Palynologists Foundation, 99-114.

Florin, R., 1944b. Die Koniferen des Oberkarbons und des unteren Perms. VII. *Palaeontographica B* 85, 459-654.

Foster, C.B., Afonin, S., 2005. Abnormal pollen grains: an outcome of deteriorating atmospheric conditions around the Permian-Triassic boundary. *Journal of the Geological Society* 162, 653-659.

Gomankov, A.V., Balme, B.E., Foster, C.B., 1998. Tatarian palynology of the Russian Platform: a review. *Proceedings of the Royal Society of Victoria* 110, 115-135.

Gomankov, A.V., 2009. Pollen Evolution in Cordaites and Early Conifers. *Palaeontological Journal* 43, 1245-1252.

Grauvogel-Stamm, L., 1978. La flore du Grès à Voltzia (Buntsandstein Supérieur) des Vosges du Nord (France), morphologie, anatomie, interprétations phylogénique et paléogéographique. *Sciences Géologiques, Université Louis Pasteur de Strasbourg, Institute de Géologie, Memoir* 50, 1-225.

Grebe, H., Schweitzer, H.J., 1962. Die Sporae dispersae des niederrheinischen Zechsteins. *Fortschritte in der Geologie von Rheinland und Westfalen* 12, 201-224.

Hart, G.F., 1964. A review of the classification and distribution of the Permian Miospore; Disaccate Striatiti. *C.R. 5<sup>th</sup> Congr. Internat. Carbonif. Strat. Geol.* 3, 1171-1199.

Hart, G.F., 1965. The Systematics and Distribution of Permian Miospores. *Witwatersrand University Press*, pp. 252

Howard, A.S., Warrington, G., Carney, J.N., Ambrose, K., Young, S.R., Pharoah, T.C., 2009. Geology of the country around Nottingham. *Memoir of the British Geological Survey, Sheet* 126 (England and Wales).

Jansonius, J. 1962., Palynology of the Permian and Triassic sediments, Peace River Area, Western Canada. *Palaeontographica B* 110, 35-98.



Klaus, W., 1963. Spores aus dem südalpin Perm. Jahrbuch der Geologischen Bundesanstalt 106, 229-363.

Klaus, W., 1966. Zwei Pflanzenreste der alpinen Trias mit ihren Sporen (*Lueckisporites* und *Decussatisporites*). Verhandlungen der Geologischen Bundesanstalt 1, 172-177.

Kurmann, M.H., 1990. Exine formation in *Cunninghamia lanceolata* (Taxodiaceae). Review of Palaeobotany and Palynology 64, 175–179.

Kurmann, M.H., 1991. Pollen ultrastructure in *Elatides williamseni* (Taxodiaceae) from the Jurassic of North Yorkshire. Review of Palaeobotany and Palynology 69, 291-298.

Kurmann, M.H., 1992. Exine stratification in extant gymnosperms: a review of published transmission electron micrographs. Kew Bulletin 47, 25–39.

Kurmann, M.H., Zavada, M.S., 1994. Pollen morphological diversity in extant and fossil gymnosperms. In: M.H. Kurmann, J.A. Doyle and M.S. Zavada (Eds.), Ultrastructure of Fossil Spores and Pollen and its Bearing on Relationships among Fossil and Living Groups. The Royal Botanic Gardens, Kew, England, pp. 221.

Leschik, B., 1956. Die Keimflora von Neuwelt bei Basel, 2. Die Iso- und Mikrosporen. Schweiz. Palaeontol. Abhandl. 72, 1-70.

Leschik, B., 1956. Sporen aus dem Salzton des Zechsteins von Neuhof (bei Fulda). Palaeontographica B 100, 122-142.

Leslie, A., 2008. Interpreting the Function of Saccate Pollen in Ancient Conifers and Other Seed Plants. Intl. J. Plant Sci. 169, 1038-1045.

Lindström, S., McLoughlin, S., Drinnan, A.N., 1997. Intraspecific variation of taeniate pollen within Permian glossopterid sporangia, from the Prince Charles Mountains, Antarctica. International Journal of Plant Sciences 158, 673–684.

Mädler, K., 1964. Die geologische Verbreitung von Sporen und Pollen in der deutschen Trias. Geol. Jahrb., Beih. 65, 1-147.

Mosler, H., 1966. Der Stoffbestand und Fossilinhalt des Grauen Salztone im südlichen Bereich der Deutschen Demokratischen Republik. Freiberg Forschungshefte, C 201, 95-128.

Niklas, K. J., 1985. The aerodynamics of wind pollination. The Botanical Review 51, 328-386.

Orłowska-Zwolińska, T., 1962. A first finding of Zechstein spore-morphs in Poland. Kwart. Geol. 6, 283-297.

Osborn, J.M., Taylor, T.N., 1994. Comparative ultrastructure of fossil gymnosperm pollen and its phylogenetic implications; *In*: M.H. Kurmann, J.A. Doyle and M.S. Zavada (Eds.), Ultrastructure of Fossil Spores and Pollen and its Bearing on Relationships among Fossil and Living Groups. The Royal Botanic Gardens, Kew, England, pp. 221.

Owens, N.J., Takaso, T., Runions, J., 1998. Pollination in conifers. Trends in Plant Science 3, 479-485.

Potonié, R., 1958. Synopsis der Gattungen der Sporae Dispersae. II. Sporites (Nachträge), Saccites, Aletes, Praecolpates, Polyplicates, Monocolpates. Beihefte zum Geologischen Jahrbuch, Hefte 31, pp. 114.

Potonié, R., 1962. Synopsis der Sporae in situ. Beihefte zum Geologischen Jahrbuch, Hefte 52: 1-204.

Potonié, R., 1966, Nachtrage zu allen Gruppen (Turmae), Pt. 4; *In*: Synopsis der Gattungen der Sporae dispersae. Beihefte zum Geologischen Jahrbuch, Hefte 72, pp. 244.

Potonié, R., Klaus, W., 1954. Einige Sporengattungen des alpinen Salzgebirges. Beihefte zum Geologischen Jahrbuch 68, 517-546.

Potonié, R., Schweitzer, H.J., 1960. Der Pollen von *Ullmannia frumentaria*. Paläontologische Zeitschrift 34, 27–39.

Schaarschmidt, F., 1963. Sporen und Hystricosphaerideen aus dem Zechstein von Büdingen in der Wetterau. Palaeontographica B 113, 38-91.

Schweitzer, H.J., 1986. The land flora of the English and German Zechstein sequences. Geological Society, London, Special Publication 22, 31-54.

Schwendemann, A.B., Wang, G, Mertz, M.L., McWilliams, R.T., Thatcher, S.L., Osborn, J.M., 2007. Aerodynamics of saccate pollen and its implications for wind pollination. American Journal of Botany 94, 1371–1381.

Singh, H.P., 1965. Saccate pollen grains from the Lower Triassic of Hallstatt, Austria. Palaeobotanist 13, 74-81.

Smith, D.B., Taylor, J.M.C., Arthurton, P.S., Brookfield, M.E., Glennie, K.W., 1991. Permian. Geological Society, London, Memoirs 13, 87-96.

Stephenson, M.H. 2008, Spores and Pollen from the Middle and Upper Gharif members (Permian) of Oman. Palynology 32, 157-183.

Stephenson, M.H., 2010. Permian palynostratigraphy: a global overview; *In*: Lucas, S.G. and Shen, S.Z. (Eds.) The Permian Timescale. Geological Society, London, Special Publications, pp. 1-450.

Stoneley, H.M.M., 1958. The Upper Permian Flora of England. British Museum Natural History Geology Bulletin 3, 295-337.

Taylor, T.N., Taylor, E.L., 1987. The ultrastructure of fossil gymnosperm pollen. Bulletin de la Société Botanique de France 134, 121-140.

- Tomlinson, P.B., Braggins, J.E., Rattanbury, J.A., 1991. Pollination drop in relation to cone morphology in Podocarpaceae: a novel reproductive mechanism. *American Journal of Botany* 78, 1289-1303.
- Tomlinson, P.B., 1994. Functional morphology of saccate pollen in conifers with special reference to Podocarpaceae. *International Journal of Plant Sciences* 155, 699– 715.
- Tomlinson, P.B., 2000. Structural features of saccate pollen types in relation to their functions. Pages 147–162; *In*: Harley, M.M., Morton, C.M., Plackwell, S., (Eds.) *Pollen and spores: morphology and biology*. Royal Botanic Gardens, Kew, England, pp. 254.
- Traverse, A., 2007. *Paleopalynology: Second Edition*. Volume 28 of *Topics in Geobiology*, Springer Science & Business Media, pp. 813.
- Ullrich, H., 1964. Zur Stratigraphie und Paläontologie der marin beeinflussten Randfazies des Zechsteinbeckens in Ostthüringen und Sachsen. *Freiberger Forschungsh., C, 169*, pp. 163.
- Visscher, H., 1971. The Permian and Triassic of the Kingscourt Outlier, Ireland. *Geological Survey of Ireland, Special Paper, No. 1*, pp. 114.
- Wilson, E., 1876. Geology of Nottingham. *Quarterly Journal of the Geological Society of London* 32, 1-535.
- Wodehouse, R.P., 1935. *Pollen Grains. Their Structure, Identification and Significance in Science and Medicine*. McGraw-Hill, New York, pp. 574.

### Plate Descriptions

Figure 1. Diagrammatic reconstruction of the three variants of *Lueckisporites virkkiae* Potonié et Klaus 1954 emend. Clarke 1965. a-c Variant A, d-f Variant B, g-i Variant C. a, d,

g pollen grains in proximal polar view; b, e, h pollen grains in lateral view; c, f, i pollen grains in terminal polar section. Figure modelled after Text-Fig 8 in Clarke (1965).

Figure 2. Schematic for the orientation of perpendicular sections taken through *Lueckisporites virkkiae* Potonié et Klaus 1954 emend. Clarke 1965. Longitudinal sections pass through each saccus and parts of the proximal taeniae. Transverse sections pass through a single saccus and both proximal taeniae.

Plate I. Three variants of *Lueckisporites virkkiae* Potonié et Klaus 1954 emend. Clarke 1965 from the Cadeby Formation, Kimberley, Nottinghamshire, U.K., LM. England Finder coordinates included. 1) Variant A (P45) 2) Variant B (B33/1) 3) Variant C (K48/1). Scale bar (1-3) 50 µm. Images taken using a QImaging (Model No. 01-MP3.3-RTV-R-CLR-10) camera mounted on an Olympus BH-2 transmitted light microscope in conjunction with QCapture Pro software.

Plate II. *Lueckisporites virkkiae* Potonié et Klaus 1954 emend. Clarke 1965. The ultrastructure of Variant A specimens. TEM.

1.A section through the transverse axis of a whole pollen grain, capturing the taeniae in transverse section. Arrow indicates saccus endoreticulations which are continuous with the foot layer. Specimen 9. TEM.

2.Variant A whole pollen grain ultrastructure schematic modelled after Specimen 9.

3.A section through the longitudinal axis of a whole pollen grain. Arrow indicates thinning of the exine between the taeniae on the proximal surface. Specimen 3. TEM.

4.Detail of the saccus region. Arrow indicates alveolar structure and protosaccate endoreticulations of the saccus. Specimen 5. TEM.

5.Enlargement of the exine of the taeniae showing alveolar infratectum of the ektexine of the proximal taeniae. Specimen 5. TEM.

d – distal, p – proximal, s – saccus, pt – proximal taeniae, gc – germinal cavity

Scale bars (1-4) 4  $\mu\text{m}$ , (5) 800 nm

Plate III. *Lueckisporites virkkiae* Potonié et Klaus 1957, emend. Clarke 1965. The ultrastructure of Variant B. TEM.

1.A section through the longitudinal axis of a whole pollen grain. Specimen 13. TEM.

2.Variant B whole pollen grain ultrastructure schematic modelled after Specimen 13.

3.Enlargement of the exine of the taeniae. Arrow indicates the narrow foot layer. Specimen 1. TEM.

4.Saccus detail. Endoreticulation structure visible. Specimen 1. TEM.

5.Enlargement of distal exine. Arrow indicates a protosaccate endoreticulation inside the saccus. Note how alveolae are smaller and more rounded on the distal face towards the inside of the ektexine. Specimen 13. TEM

d – distal, p – proximal, s – saccus, pt – proximal taeniae, gc – germinal cavity

Scale bars (1 a, b, 2 a, b) 4  $\mu\text{m}$ , (3) 400 nm (4, 5) 2  $\mu\text{m}$

Plate IV. *Lueckisporites virkkiae* Potonié et Klaus 1954 emend. Clarke 1965. The ultrastructure of Variant C. TEM.

1a. A section through the transverse axis of a whole pollen grain. Arrow indicates endoreticulations within the saccus. Specimen 11. TEM.

1b. Variant C whole pollen grain schematic in transverse section. Specimen 11.

2a. A section through the longitudinal axis of a whole pollen grain. Arrow indicates the germinal cavity. Specimen 10. TEM.

2b. Variant C whole pollen grain schematic in longitudinal section. Arrow indicates the germinal cavity. Specimen 10.

3. Enlargement of exine in transverse section. Arrow indicates the thin foot layer. Specimen 11. TEM.

4. Enlargement of exine in longitudinal section. Specimen 11. TEM.

5. Enlargement of saccus showing internal network of endoreticulations. Specimen 10. TEM.

d – distal, p – proximal, s – saccus, gc – germinal cavity

Scale bars (2 a, b) 4  $\mu\text{m}$ , (3-5) 2  $\mu\text{m}$

## Highlights

- *Lueckisporites virkkiae* ultrastructure revealed as alveolar and protosaccate
- *Lueckisporites virkkiae* variants A-C exhibit homologous ultrastructure
- Subtle structural differences between variants due to ontogeny or harmomegathy
- Distinctive four-layered wall and bisaccate condition point to conifer affinity
- Combined evidence suggests affinity to *Pseudovoltzia liebeana* (Majonicaceae)



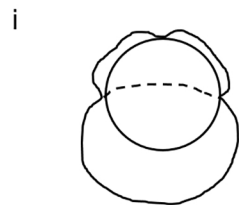
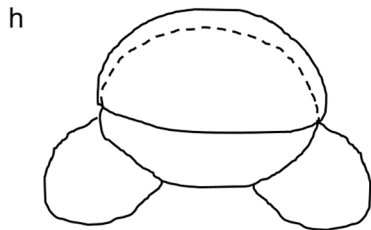
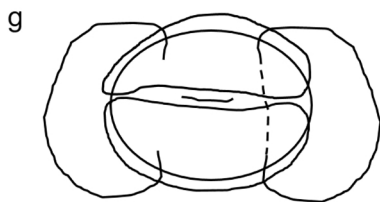
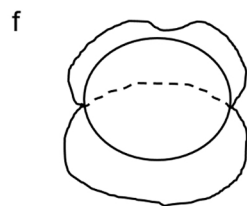
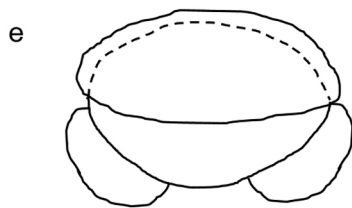
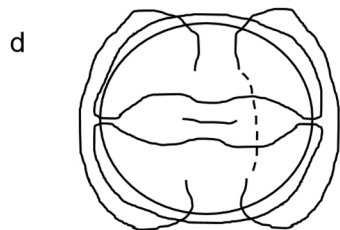
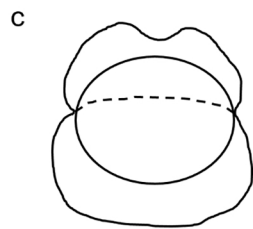
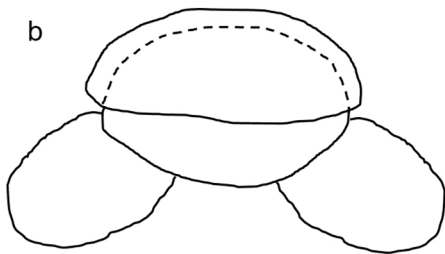
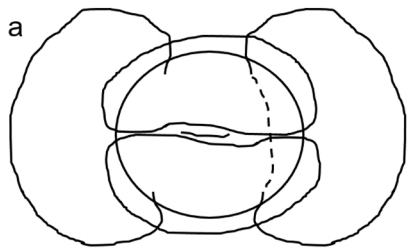


Figure 1

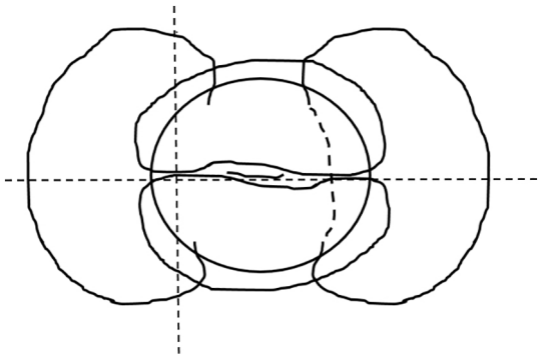


Figure 2