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Fragments of the earliest land plants

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The earliest fossil evidence for land plants comes from microscopic dispersed spores[1–3]. These microfossils are abundant and widely distributed in sediments, and the earliest generally accepted reports are from rocks of mid-Ordovician age (Llanvirn, 475 million years ago)[4]. Although distribution, morphology and ultrastructure of the spores indicate that they are derived from terrestrial plants, possibly early relatives of the bryophytes, this interpretation remains controversial[5] as there is little in the way of direct evidence for the parent plants. An additional complicating factor is that there is a significant hiatus between the appearance of the first dispersed spores and fossils of relatively complete land plants (megafossils)[6]: spores predate the earliest megafossils (Late Silurian, 425 million year ago) by some 50 million years[7]. Here we report the description of spore-containing plant fragments from Ordovician rocks of Oman. These fossils provide direct evidence for the nature of the spore-producing plants. They confirm that the earliest spores developed in large numbers within sporangia, providing strong evidence that they are the fossilized remains of bona fide land plants. Furthermore, analysis of spore wall ultrastructure supports liverwort affinities.

During the Ordovician period Oman lay on the northwest margin of the continent Gondwana, at mid-latitudes to the south of the palaeoequator. Borehole Ghaba-1 (northern Oman) penetrates a thick Ordovician sequence, consisting primarily of terrestrial and marginal marine deposits, but punctuated by conspicuous shale units representing rare but significant marine incursions[8]. The fossils described herein were recovered from a monotonous sequence of grey-green sandy siltstones and fine sandstones (Ghaba-1: core 21, 1542.0–1545.3 m) belonging to the Hasirah Member (Safiq Formation: Haima Supergroup). This member comprises a sequence of shales deposited during a major marine transgression, overlain by coarser sediments interpreted as marginal marine and terrestrial deposits that accumulated during a continuously regressive phase that followed[8–9]. The fossils derive from the latter and are considered to be Late Ordovician (Caradoc) in age based on palynology[9] and sequence stratigraphy[8]. The fossil-bearing deposits yield non-marine palynomorph assemblages indicative of a Caradoc age. They are further constrained because they are sandwiched between distinct and regionally continuous shale units, representing the discrete marine incursions, which yield rich *in situ* marine palynomorph assemblages that are reliably dated[9], and are correlated with surface exposures dated using marine invertebrate megafossils[8].

Thirteen samples from core 21 were prepared by standard palynological acid maceration techniques, and sieved using a 10-μm mesh. The palynological residues are dominated by well preserved and thermally immature spores, although some samples contained rare marine elements (acritarchs, chitinozoans, scolecodonts). These data suggest a marginal marine setting, a predominantly non-marine environment with minor marine incursions, or an entirely non-marine sequence into which the rare marine elements are reworked.

Large quantities of three of the samples were top-sieved using 100-μm mesh in the hope of retrieving larger fragments of the spore producers. This yielded a total of 12 large (0.24–0.49 mm) plant fragments. These were examined using scanning electron microscopy (SEM) and then sectioned for transmission electron microscopy (TEM) analysis. The fragments consist of spore masses, some of which preserve part of the presumed sporangium covering. Some are disc-shaped with a relatively large area of covering preserved, and probably represent relatively complete contents of originally spherical sporangia. Individual spore masses all contain identical spores comprising naked tetrads, envelope-enclosed tetrads or naked dyads. Presumably these spore units were 'permanent' (that is, did not separate before dispersal) because separating tetrads and dyads are unknown before the latest Ordovician[3]. Late Silurian coprolites, consisting predominantly of spores, are similar in size and appearance to the described fossils[10]; however, they are easily distinguished because the coprolites are elongated, do not possess any form of covering, and usually contain a number of different spore types in addition to fragments of plant debris, such as cuticle.

Figure 1a–e illustrates a specimen containing naked permanent tetrads. The fragment measures 0.30 by 0.23 mm and it is estimated that it contains approximately 2,620 tetrads (that is, 10,480 single spores). The spore mass overlies part of the presumed sporangium covering (Fig. 1a arrow, c), which is 3.6 μm in thickness and appears entirely featureless on the surface and in cross-section. The specimen illustrated in Fig. 1f, g is 0.30 mm in diameter and contains an estimated 2,670 naked permanent tetrads. Here, however, a much greater area of covering (about 5,680 μm^2) is preserved. Again it is thin (about 2 μm) and entirely featureless. Figure 1h illustrates an envelope-enclosed permanent tetrad preserved in another sporangium. The envelope is clearly discernible and has a sculpture of fine muri.

TEM analysis of sections of the fragments revealed details of spore wall ultrastructure, in addition to evidence pertaining to the nature of the covering/spore mass contact. In two specimens, comprising naked permanent tetrads, the spore walls are entirely homogeneous. In one specimen (Fig. 2), comprising naked permanent dyads, exquisite spore wall ultrastructure is preserved. The walls are 0.72–1.01 μm in thickness. The bulk of the wall consists of a stacked series of continuous, parallel lamellae. The lamellae are a consistent 41–58 nm in thickness, and up to 11 lamellae are discernible in each wall. However, the lamellae become less clearly defined towards the inside and outside of the wall, and in some spores these parts appear homogeneous. It is likely that the entire wall was originally lamellated, but the lamellae have been obscured by sporopollenin accretion. Preservation of wall ultrastructure is fickle, and is dependent on both ontogenetic stage and subtle diagenetic effects. Although it

is likely that there is a genuine difference between homogeneous walls of naked permanent tetrads and lamellate wall of naked permanent dyads[11], these differences may relate to ontogeny and/or diagenesis.

Sections through the presumed sporangial covering were observed only in the specimen illustrated in Fig. 1a–e. The covering clearly overlies the outermost spores, and in fact penetrates into the gaps between them. The section of preserved covering is 1.6 μm in thickness. Unlike the spore walls that are homogeneous, it is extremely variable in nature. It is made up of convoluted 'swirls' of material, distinguishable because of their differing electron density. Also there are some small, irregularly shaped voids. The covering becomes less 'swirly' and more regular towards the outside. The swirly material possibly represents sporopollenin released as the tapetum lining the sporangial wall degenerated. Similar sporangium wall linings, interpreted as a residue of the tapetum, are reported in a number of extant plant groups[12], in addition to fossils of early land plants[13].

Ordovician dispersed spore assemblages consist of spores of unusual morphology that are termed cryptospores[14] (permanent tetrads, permanent dyads and monads that are either naked or enclosed within an envelope). They have been reported from throughout the globe, exhibiting surprisingly little temporal and spatial variation[3,15], and this uniformity suggests a worldwide cosmopolitan flora. The Oman dispersed spore assemblages provide further evidence for this hypothesis, as they are identical to coeval assemblages from elsewhere.

There are several lines of evidence indicating that Ordovician cryptospores derive from land plants[2–3]. First, the distribution of the spores is similar to that of extant spores/pollen (that is, they occur in non-marine sediments, and when found in marine sediments their abundance declines offshore). Second, they are similar in size and morphology to the spores of land plants. In fact, some of the permanent tetrads, including envelope-enclosed forms, closely resemble the spores of extant liverworts[2]. Third, wall ultrastructure in Ordovician cryptospores is varied, but in some morphotypes suggests affinities with extant liverworts[16]. Fourth, and rather intriguingly, some of the earliest known land plant mesofossils/megafossils from the latest Silurian to earliest Devonian contain spores similar in morphology to the Ordovician forms (for example, permanent tetrads and dyads)[17–20]. These cryptospore producers are of uncertain affinity, although some possess tantalizing bryophyte-like characteristics[17], and it is possible that they represent relic elements of the flora.

The *in situ* spores described herein are clearly identical to coeval dispersed forms proposed as the earliest evidence for land plants. The new fossils confirm that the spores were produced in large numbers in sporangia, providing further, convincing evidence that they derive from land plants. The ability to produce abundant sporopollenin-coated spores is considered to be an adaptation vital for colonization of the terrestrial environment[21]. It is clear that the sporangia were extremely small, but this is not unexpected. Sporangia of extant bryophytes are rarely more than 1 mm in diameter. It is calculated that a spherical sporangium of this size could contain 95,000 spore tetrads of similar size to the Ordovician forms. Indeed some of the Oman fossils contain a minimum of 7,450 tetrads. In recent years our search image for the earliest plants has begun to focus on very small plants, based on the small size of extant bryophytes and diminutive cryptospore producers recovered from the Late Silurian to Early Devonian (the so-called Lilliputian plants[13]). Clearly our discovery of fragments of very early plants, which suggest that the plants were miniscule, supports such an hypothesis.

Spore wall development is conserved and variable between

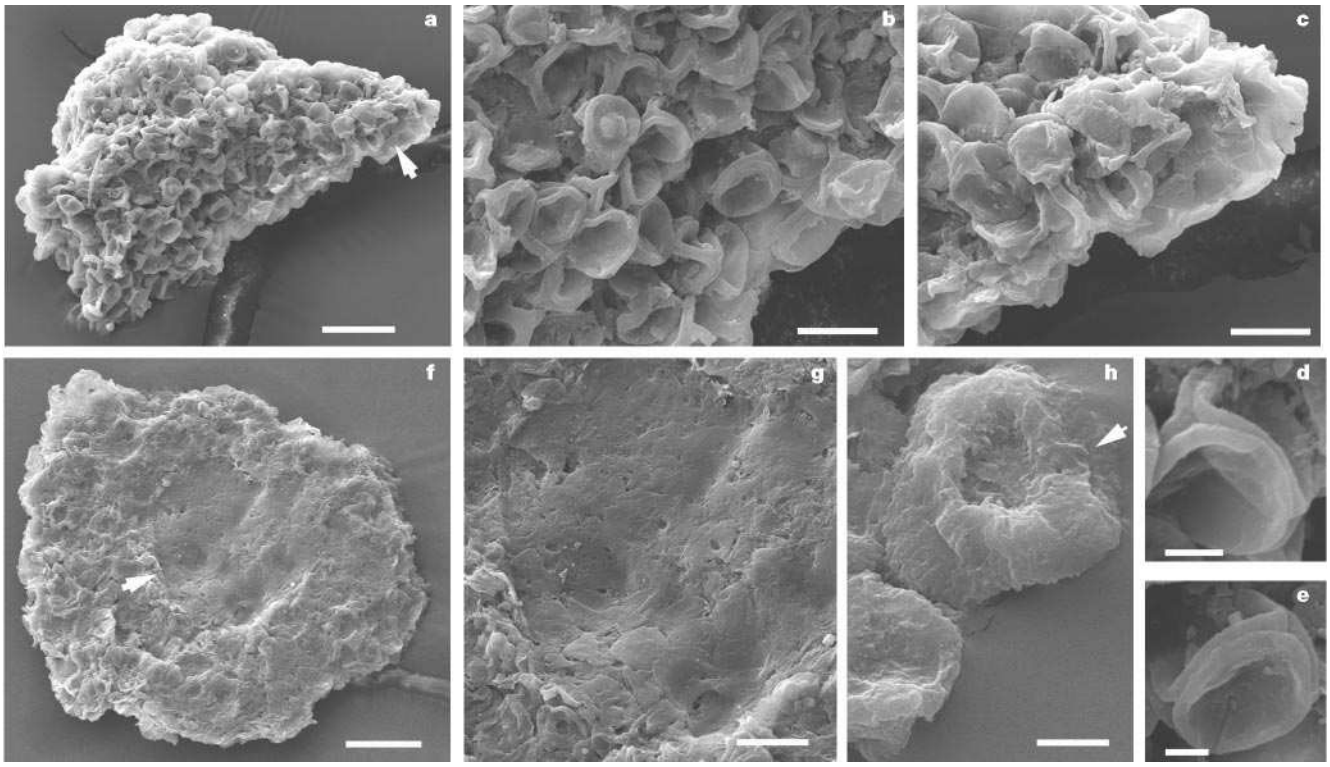

Figure 1 Fossil plant fragments from borehole Ghaba-1, core 21. **a**, Specimen CW47a. SEM of fragment of sporangium containing naked permanent tetrads. Note the presence of sporangium covering in the bottom right-hand corner (arrow). **b**, Close-up of **a** illustrating the spore contents. **c**, Close-up of **a** illustrating spores overlying the sporangium covering. **d**, **e**, Close-up of **a** illustrating individual spore tetrads. **f**, Specimen CW47f. SEM of relatively complete sporangium, with a large patch of sporangium covering preserved (arrow). **g**, Close-up of **f** illustrating the nature of the sporangium covering. **h**, Specimen CW47i. SEM of an envelope-enclosed permanent tetrad that is preserved in a fragmentary sporangium. Note the muri ornamenting the envelope (arrow). Scale bars: **a**, 50 μm; **b**, **c**, 20 μm; **d**, **e**, 5 μm; **f**, 75 μm; **g**, 30 μm; **h**, 10 μm.

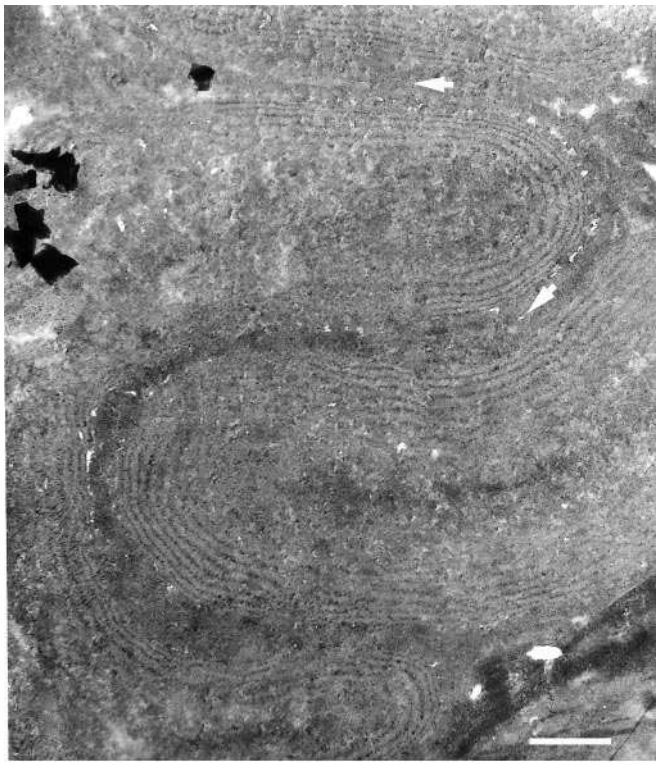

Figure 2 Specimen CW47c. TEM of part of a sporangium illustrating partial sections through three spores. The top arrow marks contact between the upper spore and the spore to the lower left. The bottom arrow marks contact between the spores to the lower left and lower right. Note the lamellate ultrastructure. Scale bar, 0.5 μm.

different plant groups, and hence the presence of exquisitely preserved spore wall ultrastructure provides further strong evidence of the affinities of these Ordovician plants. Lamellae are considered to be produced at some point during the ontogeny of virtually all land plant spores/pollen, and may be present in a variety of forms and positions in many mature spore/pollen walls. However, spores/pollen in which continuous, parallel-arranged lamellae are present at maturity throughout the walls are confined to extant liverworts[16,22], suggesting that the Ordovician plants from Oman may have liverwort affinities. This reinforces claims for liverwort affinities based on morphology of Ordovician spores, and conforms to land plant phylogenies that place liverworts as basal.

Recent phylogenetic analyses of land plants use morphological and/or molecular evidence, and although most are based solely on extant plants, some also incorporate evidence from fossils[23]. These analyses provide strong evidence that land plants are monophyletic, and that extant charophycean green algae are their sister group (that is, the earliest land plants evolved from aquatic green algal ancestors)[21]. It is generally agreed that the earliest divergent land plants were bryophytes, but there is some disagreement as to the exact relationship between the three extant bryophyte groups (liverworts, hornworts, mosses) and vascular plants. Most recent analyses indicate that the bryophytes are paraphyletic with respect to the vascular plants, with a moss/vascular plant sister group relationship, and either the liverworts or hornworts as the most primitive, early divergent, extant land plants[23–26].

If liverwort affinities are accepted for the Ordovician fossils, one unexpected finding is the absence of elaters. Elaters are elongate cells that occur interspersed with spores and facilitate their dispersal by hygroscopic movements. Among extant plants they occur in the liverworts and hornworts, but are absent from more derived members of the plant kingdom, possibly because the latter have evolved increasingly sophisticated sporangia with more effective spore dispersal mechanisms[24]. On the basis of recent phylogenies suggesting that either liverworts or hornworts are basal, and evidence that the earliest plants were liverwort-like, one might expect the earliest land plants to possess elaters. Indeed, studies of recent elaters suggest that they should survive in the fossil record[27]. However, elaters have only rarely been reported *in situ* in the fossil record, and never as dispersed microfossils. This indicates one of the following possibilities: they were only present in certain taxa (they have been secondarily lost in many liverworts); they are a post-Caradoc adaptation; they are not readily fossilized; or they are present but not discernible (perhaps they were attached at the point where the axis meets the sporangium, as is the case in certain extant bryophytes).

The new fossils from Oman shed light on a number of controversies concerning the origin of land plants and their fossil record. They provide convincing evidence that the spores purported to provide the earliest fossil evidence for land plants do indeed derive from land plants that produced sporangia containing abundant spores. Spore wall ultrastructure also provides further evidence for liverwort affinities, although the absence of elaters is intriguing. Finally, the fossils demonstrate the diminutive size of the plants, providing a search image for further discoveries. We predict that top-sieving of palynological residues of suitable age and lithology will produce further finds of the earliest land plants. □

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Inferring the palaeoenvironment of ancient bacteria on the basis of resurrected proteins

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Features of the physical environment surrounding an ancestral organism can be inferred by reconstructing sequences[1–9] of ancient proteins made by those organisms, resurrecting these proteins in the laboratory, and measuring their properties. Here, we resurrect candidate sequences for elongation factors of the Tu family (EF-Tu) found at ancient nodes in the bacterial evolutionary tree, and measure their activities as a function of temperature. The ancient EF-Tu proteins have temperature optima of 55–65 °C. This value seems to be robust with respect to uncertainties in the ancestral reconstruction. This suggests that the ancient bacteria that hosted these particular genes were thermophiles, and neither hyperthermophiles nor mesophiles. This conclusion can be compared and contrasted with inferences drawn from an analysis of the lengths of branches in trees joining proteins from contemporary bacteria[10], the distribution of thermophily in derived bacterial lineages[11], the inferred G+C content of ancient ribosomal RNA[12], and the geological record combined with assumptions concerning molecular clocks[13]. The study illustrates the use of experimental palaeobiochemistry and assumptions about deep phylogenetic relationships between bacteria to explore the character of ancient life.

This year marks the 40th anniversary of the observation by Pauling and Zuckerkandl that it should be possible to infer the sequences of ancient proteins by comparing the sequences of their descendants[14]. Some 25 yr were required, however, before their vision of resurrecting ancient proteins for study was first realized[1,2,4]. The properties of resurrected ancestral proteins have been used to correlate molecular behaviour with changing geology, ecology and physiology in mammals[15], analyse the evolution of substrate specificity in biomedically important proteases[5], and identify *in vitro* behaviours of proteins involved in inflammation and vision that are important to changing physiological function[8,9].

So far, however, experimental palaeobiochemistry has carried experimental scientists back in time only approximately 240 million years[8]. This has left untouched many of the most intriguing questions about ancient life. One of these relates to the role of thermophily in the history of life on Earth. Various models for environments in the Precambrian have suggested that the Earth was cold and covered with snow. Other models, inspired by the discovery of modern microorganisms that live at high temperatures, suggest that early bacteria may have been thermophiles, or possibly extreme thermophiles. Arguments based on indirect evidence, such as the lengths of branches of various trees, the G+C content of reconstructed ancestral rRNA, the possible cold temperature of early Earth, and the distribution of thermophily in contemporary taxa, have generated contradictory inferences.

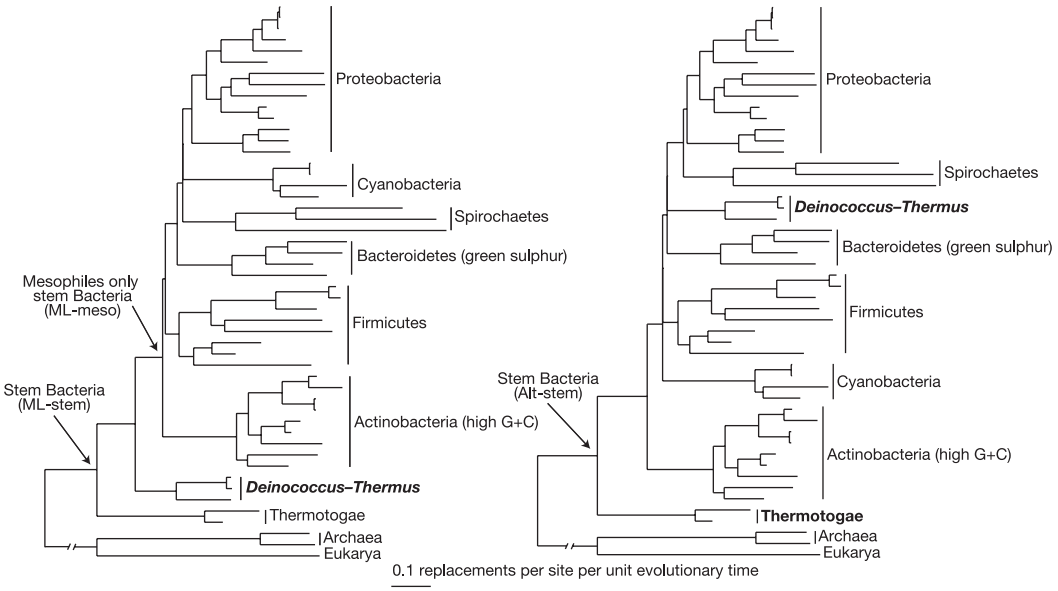

Figure 1 The two unrooted universal trees used to reconstruct ancestral bacterial sequences. Archaea and Eukarya serve to provide a node within the bacterial subtree from which ancient sequences can be inferred. Thermophilic lineages are highlighted in bold. Aquificaceae subfamily not shown. **a**, Maximum likelihood topology used to reconstruct the stem elongation factors from bacteria (ML-stem), or most recent common ancestor of bacteria, and the ancestral sequence for mesophilic lineages only (ML-meso). **b**, Alternative topology used to reconstruct the stem elongation factors from bacteria (Alt-stem).