Supporting Online Material for

Origin and Radiation of the Earliest Vascular Land Plants

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This PDF file includes:

Materials and Methods
SOM Text
References
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This PDF file includes:

Materials and Methods
Age dating
Spore descriptions
Discussion of trilete spore affinities
References and Notes

**Abstract**: Colonization of the land by plants most likely occurred in a step-wise fashion starting in the Mid-Ordovician. The earliest flora of bryophyte-like plants appears to have been cosmopolitan and dominated the planet, relatively unchanged, for some 30 million years. It is represented by fossilized dispersed cryptospores and fragmentary plant remains. In the Early Silurian, cryptospore abundance and diversity diminished abruptly as trilete spores appeared, became abundant and underwent rapid diversification. This change coincides approximately with the appearance of vascular plant megafossils and probably represents the origin and adaptive radiation of vascular plants. We have obtained a diverse trilete spore occurrence from the Late Ordovician suggesting that vascular plants originated and diversified earlier than previously hypothesized, in Gondwana, before migrating elsewhere and secondarily diversifying.

**Supporting Online Material**

**Material and methods**

Samples were taken directly from the borehole cores and care was taken to clean the core samples prior to processing to prevent contamination from modern material. An initial set of samples was processed at the University of Liège and samples were processed according to standard Liège palynological laboratory methods. Each sample was crushed and 10-25 grams were demineralised in HCl-HF. The residue was oxidized in HNO₃ and KClO₃ and sieved through a 10 µm mesh. A hot bath in 25 % HCl eliminated the remaining fine mineral particles. All samples were rinsed through a 10 µm mesh. Subsequently selected duplicate samples were independently processed at the University of Sheffield and prepared using
standard HCl-HF-HCl palynological acid maceration techniques followed by heavy liquid (zinc bromide) separation and sieving using a 10 μm mesh. The palynological characteristics of preparations from both laboratories were identical and included trilete spores and hilate spores.

Age dating

All thirty-seven core samples were productive yielding rich organic residues including palynomorphs that are abundant, well-preserved and of low thermal maturity. The palynomorphs included marine elements (abundant acritarchs and chitinozoans with rarer scolecodonts and graptolite fragments) and non-marine elements (land plant spores). Independent ages are provided by: (i) both chitinozoan and acritarch biostratigraphy; (ii) graptolite biostratigraphy in the upper units; (iii) lithostratigraphical recognition of Hirnantian (End Ordovician) glacial deposits.

The oldest section examined is from the upper part of the Qasim Formation, which is represented by a shallowing upward succession consisting of offshore shelf sandy silt-and mudstones (from which the oldest samples were collected) and overlain by sandstones of the ?Quwarah Member (S1). Two chitinozoan biozones are present in the Qasim Formation (S2). The oldest samples are interpreted as no younger than mid Katian (late Caradoc) in age (Fig. 1), based on the chitinozoans present and the absence of important stratigraphic markers for the late Katian and Hirnantian. The succeeding chitinozoan assemblage indicates that the uppermost Qasim sediments are late Katian in age (early-middle Ashgill). Acritarch biostratigraphy, consisting of diverse assemblages of acritarchs mainly of Katian age from throughout the Qasim Formation, is in agreement with that of chitinozoans (S3). The Qasim Formation is unconformably overlain by glacial advance and retreat facies of the Sarah Formation. To a certain extent this provides an independent lithostratigraphical age constraint in that these deposits are almost certainly produced by the Hirnantian (End Ordovician) glaciations. The Sarah Formation includes shallow-marine deposits (Baq’a Member) in its upper part (S4). The sediments of the Sarah Formation are characterised by Hirnantian acritarch species with reworked earlier Ordovician assemblages (S3). The Qalibah Formation (Qusaiba Member) overlies the Sarah Formation and consists of shelfal marine deposits of Early Silurian age based on graptolite and palynological evidence (S1,S5).
Spore descriptions

Seven taxa of trilete spores were identified. Two of the taxa are unornamented. Fig. 1B illustrates a laevigate, crassitate form (*Ambitisporites* sp.). Fig. 1H illustrates a laevigae form that lacks curvaturae (*Leiotriletes* sp.). The five other identified species are variously ornamented with verrucae, muri or grana. Figs 1C-F are ornamented crassitate forms that can be accommodated in the genus *Synorisporites*. Fig. 1C is a form with ill defined distal ornament, transitional between the laevigate crassitate form (Fig. 1B) and clearly ornamented forms (Fig. 1D). Fig. 1D has a distal ornament of faint verrucae. Fig. 1E has a distal ornament of round, low verrucae. Fig. 1F has a prominent trilete mark and a distal ornament of faint muri. Fig. 1G has a distal ornament of grana and can be accommodated in the genus *Aneurospora*. Fig. 1I is verging on patinate in structure and has a distal ornament of convoluted muri and may be assigned to the genus *Chelinospora*?

Discussion of the affinities of trilete spores

The appearance and adaptive radiation of hilate/trilete spores is usually considered to reflect the emergence of the vascular plant lineage. There is a clear correlation between the appearance and diversity increase in hilate/trilete spores and the diversification of vascular plants (and their immediate predecessors) during the Silurian. Jane Gray argued persuasively that trilete spores derived predominantly from vascular plants (*S6*). Extant basal vascular plant groups (non-seed plant ‘pteridophytes’), essentially the lycopsids and ferns, nearly all produce dissociated single spores that are in either the trilete or the more derived monolete form. Similarly, a survey of the vast record of fossil ‘pteridophytes’ preserving in situ spores demonstrates that they nearly all produced trilete or monolete spores (*S7*). This includes various stem group tracheophytes (protracheophytes) (*S8*-*S9*) such as *Aglaophyton* (*S10*). Furthermore, Gray demonstrated that trilete spores are rare among the extant ‘bryophyte’ groups (*S6*). They are produced by most hornworts, some mosses (sphagnales and some andreaeales, pottiales, funariales and isobryales) and rarely among the liverworts (some marchantiales). In many of these cases the spores are termed cryptotrilete because the trilete mark is merely a scar formed during ontogeny by contact with the other members of the tetrad and is not a functional germination suture(*S6*). True trilete marks are characterized by a suture and often associated lips, which are usually discernible in fossil trilete spores, including many
of those in the present study. Gray \((S6)\) estimates that only some 2.5% of extant ‘bryophyte’ genera produce trilete spores, and many of these are cryptotrilete forms.

It seems likely that the ancestral condition among embryophytes was to disperse meiotically produced spores united permanently in either tetrads or dyads. Dissociation of these units into individual trilete spores (from tetrads) or hilate spores (from dyads) \((S11-S12)\) is a more derived condition. This was probably adopted by either the polysporangiates (protracheophytes plus tracheophytes) or a clade consisting of the hornworts + polysporangiates. In either case it is clear that in a small number of examples, such as the sphagnales, trilete spores appear to have subsequently evolved independently due to convergence. Extant bryophyte groups probably began to diverge from the stem group embryophytes at the same time as vascular land plants were undergoing an adaptive radiation (Late Silurian-Devonian). Certainly key characters of extant bryophytes were acquired by at least the Mid-Devonian \((S13)\). It would appear that during this time many of the extant bryophyte groups also changed reproductive strategy with many plants adopting early tetrad disintegration and dispersal of individual spores. Some extant forms retain the ancestral permanent tetrad condition (e.g. Sphaerocarpales) \((S6)\). Many, such as most extant mosses, adopted very early tetrad dissociation (prior to spore wall formation) that results in individual inaperturate spores. Delay in tetrad dissociation may also result in cryptotrilete spores with non-functional trilete marks.

Further evidence that the appearance and diversification of hilate/trilete spores reflects vascular plants comes from the megafossil record (Fig. 1A). The earliest plants belonging to the vascular plant lineage, simple rhyniophytes of the \textit{Cooksonia}-type, are reported from the late Wenlock of Avalonia \((S14)\). These are more-or-less coincident with the appearance and diversification of hilate/trilete spores in this area \((S15-S16)\). Better preserved \textit{Cooksonia} from the Late Silurian (Pridoli) are demonstrably vascular plants with simple conducting tissues \((S17)\). These plants are exceptionally preserved by charcoalification \((S18)\) and retain exquisite anatomical detail including in situ spores \((S19)\). It is clear that these and other polysporangiate plants produced trilete spores \((S19)\), but occasionally hilate spores \((S11-S12)\). In situ trilete spores from different \textit{Cooksonia} species are either unornamented, have a simple verrucate-muornate ornament, or a more complex apiculate ornament. The in situ spores are similar to those reported herein from the Ordovician of Saudi Arabia.
Supplementary references


